

# **3M Environmental Laboratory**

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## **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

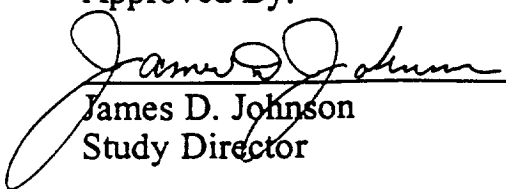
#### **Method Numbers and Revisions:**

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  $-20\pm 10^{\circ}\text{C}$ .

**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**

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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**

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**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**

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### **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<sup>-</sup> 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<sup>-</sup> 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.)**

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  
Madison, Wisconsin 53704

Laboratory Project Identification:

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 |          | Phase               | Date                          |                       |
|------------------|----------|---------------------|-------------------------------|-----------------------|
| From             | To       |                     | Reported to<br>Study Director | Date to<br>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              |

*Cecilia M. Danner*  
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<br>2. T-6051                                                                                                                                                |
| Sponsor                           | 3M<br>Toxicology Service<br>Medical Department<br>3M Center, Bldg. 220-2E-02<br>P.O. Box 33220<br>St. Paul, MN 55133-3220                                             |
| Sponsor's Representative          | John L. Butenhoff, PhD<br>3M<br>Toxicology Service<br>Medical Department<br>3M Center, Bldg. 220-2E-02<br>P.O. Box 33220<br>St. Paul, MN 55133-3220<br>(612) 733-1962 |
| Study Director                    | Steven M. Glaza<br>Hazleton Wisconsin, Inc.<br>P.O. Box 7545<br>Madison, WI 53707-7545<br>(608) 241-7292                                                              |
| Study Location                    | Hazleton Wisconsin, Inc.<br>Building No. 3<br>3802 Packers Avenue<br>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  
Study Coordinator

Patricia Padgham  
In-life Supervisor

Rose M. Bridge  
Report Supervisor

Quality Assurance

Sherry R. W. Petsel  
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Laboratory Animal Medicine

Cindy J. Cary, DVM  
Diplomate, ACLAM  
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Thomas E. Palmer, PhD  
Anatomical Pathologist

Jack Serfort/  
Deborah L. Pirkel  
Supervisors  
Necropsy

Anne Mosher  
Supervisor  
Pathology Data

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## 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.

| <u>Group</u> | <u>Test Material</u> | <u>Dose Level<br/>(mg/kg)</u> | <u>Number of Animals</u> |                |
|--------------|----------------------|-------------------------------|--------------------------|----------------|
|              |                      |                               | <u>Males</u>             | <u>Females</u> |
| 1 (Control)  | Sterile water        | 0 <sup>a</sup>                | 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:

| <u>Identification</u> | <u>Physical Description</u> |
|-----------------------|-----------------------------|
| 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

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taken and will be retained in a freezer set to maintain a temperature of  $-20^{\circ}\text{C} \pm 10^{\circ}$  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  $19^{\circ}$  to  $23^{\circ}\text{C}$ , a relative humidity of  $50\% \pm 20\%$ , 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  $\pm 2$  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:

| <u>Group</u> | <u>Test Material</u> | <u>Dose Level<br/>(mg/kg)</u> | <u>Number of Animals</u> |                |
|--------------|----------------------|-------------------------------|--------------------------|----------------|
|              |                      |                               | <u>Males</u>             | <u>Females</u> |
| 1 (Control)  | Sterile water        | 0 <sup>a</sup>                | 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<sup>2</sup>.

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<sup>2</sup> and the approximate rate of application was 0.006 mL/cm<sup>2</sup>.

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<sup>1</sup> 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  $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$  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  $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$ . 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

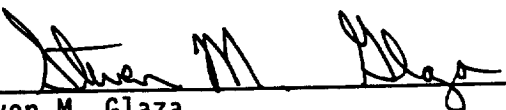
Individual animal pathology comments are presented in Table 4. There were no lesions observed in any of the animals.

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 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



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Steven M. Glaza  
Study Director  
Acute Toxicology

6-16-95  
Date

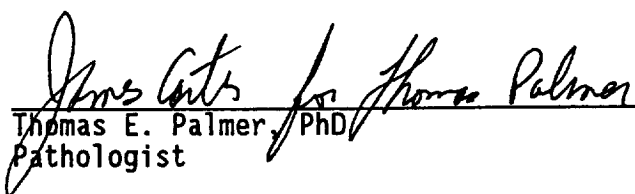
REFERENCE

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).

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.

  
Thomas E. Palmer, PhD  
Pathologist

6-16-95  
Date

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Table 1  
Individual and Mean Body Weights (g)

| Male                                                      |                              |       |       | Female           |                              |       |       |
|-----------------------------------------------------------|------------------------------|-------|-------|------------------|------------------------------|-------|-------|
| Animal<br>Number                                          | Random-<br>ization<br>Day -9 | Day   |       | Animal<br>Number | Random-<br>ization<br>Day -9 | Day   |       |
|                                                           |                              | 1     | 29    |                  |                              | 1     | 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<br>Number                         | Random-<br>ization<br>Day -9 | Day   |       | Animal<br>Number    | Random-<br>ization<br>Day -9 | Day   |       |
|                                          |                              | 1     | 29    |                     |                              | 1     | 29    |
|                                          |                              |       |       |                     |                              |       |       |
| Group 5 - T-6051 (10-cm x 10-cm Section) |                              |       |       |                     |                              |       |       |
| F52879                                   | 2,278                        | 2,398 | 2,923 | F52877 <sup>a</sup> | 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 |

<sup>a</sup> 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

| <u>Sex</u>                                                       | <u>Animal Number</u> | <u>Observation</u>  | <u>1-4 Hours</u> | <u>Day</u>   |          |               |
|------------------------------------------------------------------|----------------------|---------------------|------------------|--------------|----------|---------------|
|                                                                  |                      |                     | <u>(Day 1)</u>   | <u>2 - 7</u> | <u>8</u> | <u>9 - 29</u> |
| <u>Group 1 (Control) - Sterile Water for Injection (0 mg/kg)</u> |                      |                     |                  |              |          |               |
| Male                                                             | F52885               | Appeared normal     | ✓                | ✓            | ✓        | ✓             |
|                                                                  | F52873               | Appeared normal     | ✓                | ✓            | ✓        | ✓             |
|                                                                  | F52898               | Appeared normal     | ✓                | ✓            | ✓        | ✓             |
| Female                                                           | F52967               | Appeared normal     | ✓                | ✓            | ✓        | ✓             |
|                                                                  | F52878               | Appeared normal     | ✓                | ✓            | ✓        | ✓             |
|                                                                  | F52883               | Appeared normal     | ✓                | ✓            | ✓        | ✓             |
| <u>Group 2 - T-6054 (0.128 mg/kg)</u>                            |                      |                     |                  |              |          |               |
| Male                                                             | F52887               | Appeared normal     | ✓                | ✓            | ✓        | ✓             |
|                                                                  | F52893               | Appeared normal     | ✓                | ✓            | ✓        | ✓             |
|                                                                  | F52897               | Appeared normal     | ✓                | ✓            | ✓        | ✓             |
| Female                                                           | F52901               | Appeared normal     | ✓                | ✓            | ✓        | ✓             |
|                                                                  | F52876               | Appeared normal     | ✓                | ✓            | ✓        | ✓             |
|                                                                  | F52882               | Appeared normal     | ✓                | ✓            | ✓        | ✓             |
| <u>Group 3 - T-6054 (1.28 mg/kg)</u>                             |                      |                     |                  |              |          |               |
| 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

| <u>Sex</u>                                      | <u>Animal<br/>Number</u> | <u>Observation</u> | <u>1-4 Hours<br/>(Day 1)</u> | <u>Day</u>   |          |               |
|-------------------------------------------------|--------------------------|--------------------|------------------------------|--------------|----------|---------------|
|                                                 |                          |                    |                              | <u>2 - 7</u> | <u>8</u> | <u>9 - 29</u> |
| <u>Group 4 - T-6054 (12.8 mg/kg)</u>            |                          |                    |                              |              |          |               |
| Male                                            | F52886                   | Appeared normal    | ✓                            | ✓            | ✓        | ✓             |
|                                                 | F52880                   | Appeared normal    | ✓                            | ✓            | ✓        | ✓             |
|                                                 | F52899                   | Appeared normal    | ✓                            | ✓            | ✓        | ✓             |
| Female                                          | F52889                   | Appeared normal    | ✓                            | ✓            | ✓        | ✓             |
|                                                 | F52894                   | Appeared normal    | ✓                            | ✓            | ✓        | ✓             |
|                                                 | F52895                   | Appeared normal    | ✓                            | ✓            | ✓        | ✓             |
| <u>Group 5 - T-6051 (10-cm x 10-cm Section)</u> |                          |                    |                              |              |          |               |
| 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)

| <u>Dermal Reaction</u> | <u>Males</u>             |          |          |          | <u>Females</u>           |          |          |          |
|------------------------|--------------------------|----------|----------|----------|--------------------------|----------|----------|----------|
|                        | <u>Study Day</u>         |          |          |          | <u>Study Day</u>         |          |          |          |
|                        | <u>1</u>                 | <u>2</u> | <u>4</u> | <u>8</u> | <u>1</u>                 | <u>2</u> | <u>4</u> | <u>8</u> |
|                        | <u>Animal No. F52885</u> |          |          |          | <u>Animal No. F52967</u> |          |          |          |
| 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        |
|                        | <u>Animal No. F52873</u> |          |          |          | <u>Animal No. F52878</u> |          |          |          |
| 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        |
|                        | <u>Animal No. F52898</u> |          |          |          | <u>Animal No. F52883</u> |          |          |          |
| Erythema               | 0                        | 0        | 0        | 0        | 0                        | 0        | 0        | 0        |
| Edema                  | 0                        | 0        | 0        | 0        | 0                        | 0        | 0        | 0        |
| Atonia                 | 0                        | 0        | 0        | 0        | 0                        | 0        | 0        | 0        |
| Desquamation           | 0                        | 0        | 0        | 0        | 0                        | 0        | 0        | 0        |
| Coriaceousness         | 0                        | 0        | 0        | 0        | 0                        | 0        | 0        | 0        |
| Fissuring              | 0                        | 0        | 0        | 0        | 0                        | 0        | 0        | 0        |

Table 3 (Continued)  
Individual Dermal Irritation Scores

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

| <u>Dermal Reaction</u> | <u>Males</u>             |          |          |          | <u>Females</u>           |          |          |          |
|------------------------|--------------------------|----------|----------|----------|--------------------------|----------|----------|----------|
|                        | <u>Study Day</u>         |          |          |          | <u>Study Day</u>         |          |          |          |
|                        | <u>1</u>                 | <u>2</u> | <u>4</u> | <u>8</u> | <u>1</u>                 | <u>2</u> | <u>4</u> | <u>8</u> |
|                        | <u>Animal No. F52887</u> |          |          |          | <u>Animal No. F52901</u> |          |          |          |
| 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        |
|                        | <u>Animal No. F52893</u> |          |          |          | <u>Animal No. F52876</u> |          |          |          |
| 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        |
|                        | <u>Animal No. F52897</u> |          |          |          | <u>Animal No. F52882</u> |          |          |          |
| 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)

| <u>Dermal Reaction</u> | <u>Males</u>             |          |          |          | <u>Females</u>           |          |          |          |
|------------------------|--------------------------|----------|----------|----------|--------------------------|----------|----------|----------|
|                        | <u>Study Day</u>         |          |          |          | <u>Study Day</u>         |          |          |          |
|                        | <u>1</u>                 | <u>2</u> | <u>4</u> | <u>8</u> | <u>1</u>                 | <u>2</u> | <u>4</u> | <u>8</u> |
|                        | <u>Animal No. F52965</u> |          |          |          | <u>Animal No. F52884</u> |          |          |          |
| 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        |
|                        | <u>Animal No. F52891</u> |          |          |          | <u>Animal No. F52900</u> |          |          |          |
| 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        |
|                        | <u>Animal No. F52892</u> |          |          |          | <u>Animal No. F52968</u> |          |          |          |
| Erythema               | 0                        | 0        | 0        | 0        | 0                        | 0        | 0        | 0        |
| Edema                  | 0                        | 0        | 0        | 0        | 0                        | 0        | 0        | 0        |
| Atonia                 | 0                        | 0        | 0        | 0        | 0                        | 0        | 0        | 0        |
| Desquamation           | 0                        | 0        | 0        | 0        | 0                        | 0        | 0        | 0        |
| Coriaceousness         | 0                        | 0        | 0        | 0        | 0                        | 0        | 0        | 0        |
| Fissuring              | 0                        | 0        | 0        | 0        | 0                        | 0        | 0        | 0        |

Table 3 (Continued)  
Individual Dermal Irritation Scores

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

| <u>Dermal Reaction</u> | <u>Males</u>             |          |          |          | <u>Females</u>           |          |          |          |
|------------------------|--------------------------|----------|----------|----------|--------------------------|----------|----------|----------|
|                        | <u>Study Day</u>         |          |          |          | <u>Study Day</u>         |          |          |          |
|                        | <u>1</u>                 | <u>2</u> | <u>4</u> | <u>8</u> | <u>1</u>                 | <u>2</u> | <u>4</u> | <u>8</u> |
|                        | <u>Animal No. F52886</u> |          |          |          | <u>Animal No. F52889</u> |          |          |          |
| 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        |
|                        | <u>Animal No. F52880</u> |          |          |          | <u>Animal No. F52894</u> |          |          |          |
| 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        |
|                        | <u>Animal No. F52899</u> |          |          |          | <u>Animal No. F52895</u> |          |          |          |
| 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)

| <u>Dermal Reaction</u> | <u>Males</u>             |          |          |          | <u>Females</u>           |          |          |          |
|------------------------|--------------------------|----------|----------|----------|--------------------------|----------|----------|----------|
|                        | <u>Study Day</u>         |          |          |          | <u>Study Day</u>         |          |          |          |
|                        | <u>1</u>                 | <u>2</u> | <u>4</u> | <u>8</u> | <u>1</u>                 | <u>2</u> | <u>4</u> | <u>8</u> |
|                        | <u>Animal No. F52879</u> |          |          |          | <u>Animal No. F52877</u> |          |          |          |
| 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        |
|                        | <u>Animal No. F52963</u> |          |          |          | <u>Animal No. F52888</u> |          |          |          |
| 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        |
|                        | <u>Animal No. F52874</u> |          |          |          | <u>Animal No. F52966</u> |          |          |          |
| 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

| <u>Animal<br/>Number</u>                                         | <u>Sex</u> | <u>Test Day</u> |                   | <u>Necropsy Observation</u> |
|------------------------------------------------------------------|------------|-----------------|-------------------|-----------------------------|
|                                                                  |            | <u>Died</u>     | <u>Sacrificed</u> |                             |
| <u>Group 1 (Control) - Sterile Water for Injection (0 mg/kg)</u> |            |                 |                   |                             |
| 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.         |
| <u>Group 2 - T-6054 (0.128 mg/kg)</u>                            |            |                 |                   |                             |
| 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.         |
| <u>Group 3 - T-6054 (1.28 mg/kg)</u>                             |            |                 |                   |                             |
| 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

| <u>Animal<br/>Number</u>                        | <u>Sex</u> | <u>Test Day</u> |                   | <u>Necropsy Observation</u> |
|-------------------------------------------------|------------|-----------------|-------------------|-----------------------------|
|                                                 |            | <u>Died</u>     | <u>Sacrificed</u> |                             |
| <u>Group 4 - T-6054 (12.8 mg/kg)</u>            |            |                 |                   |                             |
| 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.         |
| <u>Group 5 - T-6051 (10-cm x 10-cm Section)</u> |            |                 |                   |                             |
| 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) |         | Dermal Appli-<br>cation Site | Bile<br>Volume (mL) |
|-----------------------------------------------------------|---------------|------------|---------|------------------------------|---------------------|
|                                                           |               | Liver      | Kidneys |                              |                     |
| 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

| <u>Sex</u>                                      | <u>Animal Number</u> | <u>Weight (g)</u> |                | <u>Dermal Appli-<br/>cation Site</u> | <u>Bile<br/>Volume (mL)</u> |
|-------------------------------------------------|----------------------|-------------------|----------------|--------------------------------------|-----------------------------|
|                                                 |                      | <u>Liver</u>      | <u>Kidneys</u> |                                      |                             |
| <u>Group 4 - T-6054 (12.8 mg/kg)</u>            |                      |                   |                |                                      |                             |
| 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                         |
| <u>Group 5 - T-6051 (10-cm x 10-cm Section)</u> |                      |                   |                |                                      |                             |
| 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

| <u>Protocol</u>                                                                                                                                                                                                                                    | <u>Actual Procedure</u>                                                                      |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|
| 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**  
W I S C O N S I N  
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

TP3016.AB  
Page 2

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<br>2. T-6051                                                                                                                                             |
| Sponsor                       | 3M Toxicology Service Medical<br>Department<br>3M Center, Bldg. 220-2E-02<br>P.O. Box 33220<br>St. Paul, MN 55133-3220                                             |
| Sponsor's Representative      | John L. Butenhoff, PhD<br>3M Toxicology Service Medical<br>Department<br>3M Center, Bldg. 220-2E-02<br>P.O. Box 33220<br>St. Paul, MN 55133-3220<br>(612) 733-1962 |
| Study Director                | Steven M. Glaza<br>Hazleton Wisconsin, Inc.<br>P.O. Box 7545<br>Madison, WI 53707-7545<br>(608) 241-7292                                                           |
| Study Location                | Hazleton Wisconsin, Inc.<br>Building No. 3<br>3802 Packers Avenue<br>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
- ☒ 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  $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$  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%  $\pm$  20%, and a 12-hour light/12-hour dark cycle.
  - (f) Acclimation  
At least 7 days
- (8) Selection of Test Animals  
Based on health and body weight according to HWI SOPs. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test. 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

| <u>Group</u> | <u>Test Material</u> | <u>Dose Level<br/>(mg/kg)</u> | <u>Number of Animals</u> |                |
|--------------|----------------------|-------------------------------|--------------------------|----------------|
|              |                      |                               | <u>Males</u>             | <u>Females</u> |
| 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  $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$ . 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 necropsied in each group will be collected and immediately placed in a freezer set to maintain a temperature of  $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$ . 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

TP3016.AB  
Page 10

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

*Hay Shad*

Representative  
Quality Assurance Unit  
Hazleton Wisconsin, Inc.

*12-13-94*

Date

(6329-133.protdisk2)

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:

| <u>Group</u> | <u>Test Material</u> | <u>Dose Level<br/>(mg/kg)</u> | <u>Number of Animals</u> |                |
|--------------|----------------------|-------------------------------|--------------------------|----------------|
|              |                      |                               | <u>Males</u>             | <u>Females</u> |
| 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

Amendment No. 1

HWI 6329-133  
Page 2

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<br>(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

Amendment No. 1

HWI 6329-133  
Page 3

PROTOCOL APPROVAL

John L. Butenhoff

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

1-19-95

Date

Steven M. Glaza

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

1-12-95

Date

Lacy Meda

Representative  
Quality Assurance Unit  
Hazleton Wisconsin, Inc.

1-12-95

Date

(6329-133.Am1.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

3M Toxicology Service  
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Contractor

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

Sponsor's Representative

John L. Butenhoff, PhD

Study Director

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  $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$ .

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).

Amendment No. 2

HWI 6329-133  
Page 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

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

2/15/95

Date

Steven M. Glaza

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

2-6-95

Date

Tracy Medel

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

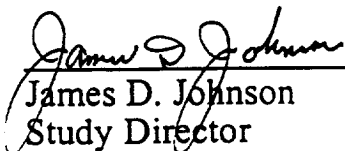
**Proposed Initiation Date:** July 25, 1995  
**Proposed Completion Date:** August 25, 1995

#### **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:**

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

 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),  $-20 \pm 10^{\circ}\text{C}$  (2.1.2, 2.1.4). Test and Control samples will be received according to AMDT-S-10-0.

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

**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

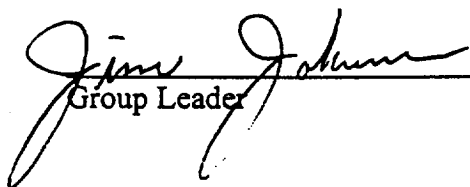
Revision Number: 0

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

## **1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES**

**1.1 Scope:** This method is for the operation of a Dohrmann DX2000 when it is used to extract fluoride from various matrices. The fluoride is typically collected in TISAB solution for analysis with an ion selective electrode.

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

**1.3 Matrices:** Biological tissues, particularly liver.

## **2.0 KEYWORDS**

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

## **3.0 PRECAUTIONS**

**3.1** Glassware and exhaust gases can be extremely hot.

**3.2** Glassware is fragile, broken glass may cause injuries.

**3.3** Pressurized gases, proper compressed gas handling practices required.

**3.4** Solvent based samples may flash, may need to allow them to dry down before starting run.

**3.5** Potential biohazards due to the biological matrices. Use appropriate personal protective equipment.

## **4.0 SUPPLIES AND MATERIALS**

**4.1** Compressed Oxygen, Hydrocarbon free, regulated to 30 PSI.

**4.2** Compressed Helium, High Purity Grade, regulated to 45 PSI.

**4.3** Quartz glass sample boat with Teflon™ tubing, Dohrmann 890-097 or equivalent.

**4.4** Quartz glass combustion tube, Reliance Glass G-9405-012 or equivalent.

**4.5** Orion 940999 Total Ionic Strength Adjustment Buffer (TISAB II ) or equivalent.

**4.6** Sample collection vials, HDPE.

**4.7** Milli-Q™ water

**4.8** Polystyrene pipettes.

**4.9** Activated Charcoal, E. Merck 2005 or equivalent.

**4.10** Hamilton Syringe or equivalent.

**4.11** Miscellaneous laboratory glassware

## **5.0 EQUIPMENT**

**5.1** Rosemount Dohrmann DX2000 Organic Halide Analyzer, modified for fluoride extraction.

**5.2** IBM compatible 386 or 486 computer.

**5.3** DX2000 software, version 1.00, modified for fluoride extraction.

**5.4** Excel Spreadsheet, version 5.0 or greater

## **6.0 INTERFERENCES**

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

## **7.0 SAMPLE HANDLING**

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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

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### 9.1 Typical Operating Conditions:

9.1.1 Combustion tube temperature = 950°C.

9.1.2 Oxygen and Helium flow = 50 cc/minute.

9.1.3 Vaporization/Drying time = 240 seconds.

9.1.4 Bake time = 300 seconds.

### 9.2 Start Up Procedure:

9.2.1 If the program is not started, start the EOX program on the PC.

9.2.2 Open the SYSTEM SETUP window.

9.2.3 Put the furnace module and the cell in the READY mode.

9.2.4 Close the SYSTEM SETUP window.

9.2.5 When the oven has reached the READY temperature, run the CLEAN BOAT program found in the CELL CHECK menu.

9.2.6 See AMDT-EP-3 for details of the Dohrmann software.

### 9.3 Sample Extraction Procedure:

9.3.1 Open the SAMPLE HATCH and place the sample in the BOAT. It may be necessary to mix approximately 50 mg of charcoal with the sample to aid combustion. If this is done, charcoal should also be mixed in while establishing the baseline and when generating the standard curve.

9.3.2 Close SAMPLE HATCH.

9.3.3 Add appropriate volume of TISAB solution or 1:1 TISAB:Milli-Q™ water mixture to a labeled sample collection vial. Typically 0.6 mL to 15 mL are used. For rabbit studies, use 1.0 or 2.0 mL of 1:1 TISAB:Milli-Q™ water mixture.

9.3.4 Place the vial so that the tip of the COMBUSTION TUBE is in the TISAB at least 0.25 inches. Gases released during pyrolysis must bubble through the TISAB.

9.3.5 Run the EOX-SOLIDS program found in the RUN menu.

9.3.6 When the EOX program is finished, remove the collection vial from the combustion tube.

9.3.7 If undiluted TISAB was used to collect the sample, add an equal volume of Milli-Q™ water to the TISAB to make 1:1 TISAB:Milli-Q™.

9.3.8 Rinse the end of the combustion tube with Milli-Q™ water and wipe with a KIMWIPE to remove any TISAB remaining on the tube.

9.3.9 Open the sample hatch and remove any remaining ash from the boat. Ash can be removed with a cotton tipped applicator or vacuumed out. It may be necessary to scrap particles off the bottom with a spatula or other similar device. A drop of Milli-Q™ water may be added to the boat to aid in the Clean Cycle.

9.3.10 Close the hatch.

9.3.11 Run the CLEAN BOAT program.

9.3.12 Sample is ready for analysis by ion selective electrode (AMDT-M-2).

### 9.4 Sample Calculations

9.4.1 Use the standard curve to calculate the sample value.

9.4.2 Sample Mass Recovered F (ug) = (TISAB vol in mL) x  $\frac{(\text{Orion reading in ppm} - \text{intercept})}{(\text{Slope})}$

## 10.0 VALIDATION

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### 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**

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None

## **13.0 REFERENCES**

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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**

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| <u>Revision</u><br><u>Number</u> | <u>Reason for Change</u> | <u>Revision</u><br><u>Date</u> |
|----------------------------------|--------------------------|--------------------------------|
|----------------------------------|--------------------------|--------------------------------|



# 3M Environmental Laboratory

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## Method

### Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

Method Identification Number: AMDT-M-2

Adoption Date: 10-4-95

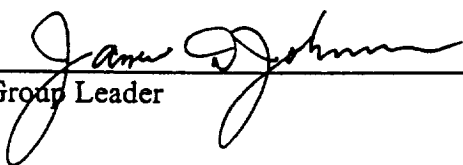
Revision Number: 0

Revision Date: None

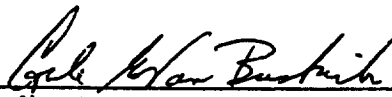
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Author: Rich Youngblom

Approved By:

  
Group Leader

10/3/95  
Date

  
Quality Assurance

10-4-95  
Date

Software: MS Word 5.1a

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

## **1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES**

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

**1.2 APPLICABLE COMPOUNDS:** Fluoride.

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

## **2.0 KEYWORDS**

**2.1** Fluoride, fluorine, ion selective electrode

## **3.0 PRECAUTIONS**

**3.1** No hazards identified with this method.

## **4.0 SUPPLIES AND MATERIALS**

**4.1** Orion 940999 Total Ionic Strength Adjustment Buffer II (TISABII) or equivalent.

**4.2** Orion Model 900001 electrode filling solution (AgCl) or equivalent.

**4.3** Orion 940907 100 ppm fluoride standard or equivalent.

**4.4** Milli-Q™ water or equivalent.

**4.5** Magnetic stir bars.

**4.6** Lab tissues.

**4.7** Sample collection vials.

**4.8** Plastic 100 mL volumetric flasks.

**4.9** Polystyrene pipettes.

**4.10** Miscellaneous laboratory glassware.

## **5.0 EQUIPMENT**

**5.1** Orion Model EA940 Expandable Ion Analyzer or equivalent.

**5.2** Orion Model 960900 Solid State Combination Fluoride electrode or equivalent.

**5.3** Magnetic Stir Plate.

**5.4** IBM compatible 386 or 486 computer (only needed if using Orion 3E software).

**5.5** Orion RS232 interface cable (only needed if using Orion 3E software).

**5.6** Microsoft Excel 5.0 (only needed if using Orion 3E software).

## **6.0 INTERFERENCES**

**6.1** It is recommended that the pH be at or near 6.0. A 1:1 mixture of TISAB and sample/Milli-Q™ water will generally bring sample to pH of 6.0.

**6.2** Sample temperature may effect fluoride measurement. It is recommended that the sample be at room temperature as the standards were when the meter was calibrated.

**6.3** The rate the samples are stirred at should be consistent with the rate the standards were stirred.

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

## **7.0 SAMPLE HANDLING**

---

7.1 No special handling necessary.

## **8.0 CALIBRATION AND STANDARDIZATION**

---

### **8.1 Preparation of Calibration Standards**

8.1.1 Measure 50 mL of TISAB II into 5 100 mL plastic volumetric flasks.

8.1.2 Label the flasks as 0.05, 0.1, 0.5, 1.0, and 1.5 ppm F-, along with the date and your initials.

8.1.3 Pipette 0.05, 0.1, 0.5, 1.0, and 1.5 mL of 100 ppm fluoride standard into the appropriately labeled flasks.

8.1.4 Add approximately 30 mL of Milli-Q™ water to each flask.

8.1.5 Shake the flasks to mix the solutions.

8.1.6 Eliminate air bubbles from the flasks by tipping the flasks on their sides and rolling the air in the flasks over the air bubbles.

8.1.7 Bring the volume in the flasks up to the 100 mL mark with Milli-Q™ water.

8.1.8 Invert and shake the flasks for the final mixing.

8.1.9 Record standards in Standards Log Book.

### **8.2 Calibration**

8.2.1 If necessary, remove tape from electrode filling hole.

8.2.2 Invert probe to wet top seal.

8.2.3 Eject a few drops of filling solution from bottom of electrode to wet lower seal.

8.2.4 Fill the electrode with filling solution.

8.2.5 The meter and the F- electrode are typically calibrated by direct measurement with no blank correction, using standards with concentrations of 0.05, 0.1, 0.5, 1.0, and 1.5 ppm F-, following the manufacturer's instructions.

8.2.6 Record the slope in the appropriate log book.

8.2.7 Clean the electrode by rinsing with Milli-Q™ water and wiping the sides down with lab tissues.

### **8.3 Storage Conditions for Standards**

8.3.1 Calibration standards are stored at room temperature.

## **9.0 PROCEDURES**

---

### **9.1 Calibration and Measurement, Standard method:**

9.1.1 The sample to be measured needs to be mixed with TISAB using the proportions recommended by the TISAB manufacturer.

9.1.2 Place a stir bar in the sample and place the sample on the stir plate.

9.1.3 Allow the sample to mix for a few seconds before inserting the electrode. When the electrode is inserted, make sure there are no air bubbles trapped under the electrode.

9.1.4 The sample should be the same temperature as the calibration standards and stirred at the same rate as the calibration standards.

9.1.5 When the readings have stabilized, record the reading in the appropriate log book.

## **9.2 Calibration And Measurement, Using Orion 3E Software:**

### **9.2.1 Calibration:**

9.2.1.1 Follow steps 8.2.1 to 8.2.4.

9.2.1.2 Press Function Key #8 (F8).

9.2.1.3 The computer screen will ask you to confirm the number of standards to be used, concentration of the standards, and whether or not a blank is to be included in the calibration. Make any necessary changes to the information presented and click on CONTINUE.

9.2.1.4 Place the electrode in the first standard on the stir plate and click on CONTINUE.

9.2.1.5 Observe the readings on the graphic display on the computer. When the readings have stabilized, press ACCEPT READING.

9.2.1.6 Repeat step 9.2.1.4 and 9.2.1.5 for the remaining standards.

9.2.1.7 After the final standard, the computer will display the slope of the curve, as well as the intercept and correlation. Record the slope, intercept, and correlation in the appropriate log book and click on CONTINUE. The calibration data is automatically copied to C:\Orion\Data\Calib.txt.

### **9.2.2 Data Spreadsheet:**

9.2.2.1 Select either NEW or OPEN from the FILE menu to open a new or existing spreadsheet to store data in.

9.2.2.2 Record the name of the spreadsheet used in the appropriate log book.

### **9.2.3 Fluoride Measurement:**

9.2.3.1 Follow steps 9.2.1 through 9.2.4

9.2.3.2 Enter the name of the sample in the appropriate place on the screen.

9.2.3.3 Click on the NEW SAMPLE button

9.2.3.4 When the readings have stabilized, click on the RECORD button and write the result in the appropriate log book.

## **10.0 VALIDATION**

---

### **10.1 Quality Control:**

### **10.2 Precision and Accuracy**

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

## **11.0 DATA ANALYSIS**

---

**11.1 Calculations** None necessary.

**11.2 Analyzing the Data** None necessary.

## **12.0 ATTACHMENTS**

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None

## **13.0 REFERENCES**

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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**

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| <u>Revision<br/>Number</u> | <u>Reason for Change</u> | <u>Revision<br/>Date</u> |
|----------------------------|--------------------------|--------------------------|
|----------------------------|--------------------------|--------------------------|

# 3M Environmental Laboratory

## Method

### Extraction of Fluorochemicals from Rabbit Livers

SOP Identification Number: AMDT-M-4

Adoption Date: 10-31-95

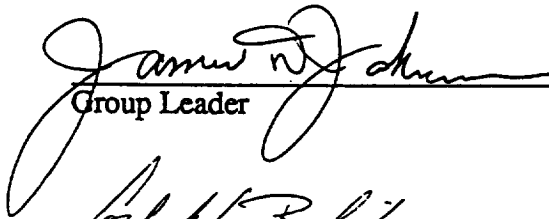
Revision Number: 0

Revision Date: None

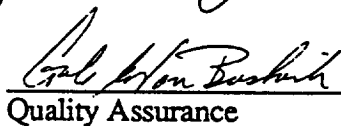
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Author: Dave Christenson/Cynthia Weber

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**

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- 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**

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

## **3.0 PRECAUTIONS**

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

## **4.0 SUPPLIES AND MATERIALS**

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### **4.1 Supplies**

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

### **4.2 Reagents**

- 4.2.1 Aqueous Ammonium Acetate (Aldrich), approx. 250 ppm: Prepare a 2500 ppm aqueous solution of ammonium acetate by adding 250 mg ammonium acetate to a 100 mL volumetric flask and dilute to volume with Milli-Q water. Dilute this solution 1:10 for a 250 ppm solution.
- 4.2.2 Sodium carbonate/Sodium Bicarbonate Buffer (J.T. Baker), ( $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ ) 0.25 M: Weigh 26.5 g of sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) and 21.0 g of sodium bicarbonate ( $\text{NaHCO}_3$ ) 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**

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- 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**

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- 6.1 There are no known interferences at this time.

## **7.0 SAMPLE HANDLING**

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

## **8.0 CALIBRATION AND STANDARDIZATION**

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### **8.1 Preparation of Internal Standards**

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

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

### **8.2 Prepare FC-95 Anion Standards**

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

### **8.3 Prepare Beef Liver Homogenate to Use for Standards**

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



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

| Working Standard<br>(Approximate Conc.) | uL  | Approximate final<br>concentration of<br>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 perfluorooctanesulfonate anion and the internal standard anion versus concentration of the perfluorooctanesulfonate anion.

## 8.6 Storage Conditions for Standards

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

## 8.7 Storage Conditions for Standards

8.7.1 Beef liver homogenates may be frozen after preparation.

# 9.0 PROCEDURES

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

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

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

9.4 Record the liver weight in the study note book.

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

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

## **10.0 VALIDATION**

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

## **11.0 DATA ANALYSIS**

- 11.1 None

## **12.0 ATTACHMENTS**

- 12.1 Worksheet AMDT-M-4

## **13.0 REFERENCES**

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

## **14.0 REVISIONS**

Revision

Number

Reason for Change

Revision

Date

**Worksheet AMDT-M-4**[illegible]

# 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

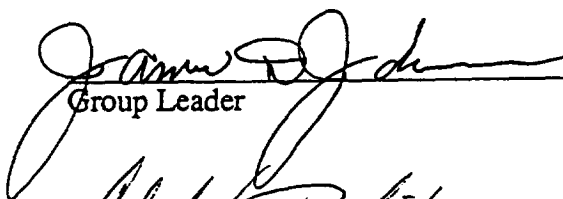
Revision Number: 0

Revision Date: None

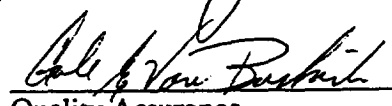
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Author: Dave Christenson/Cynthia Weber

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**

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- 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**

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

## **3.0 PRECAUTIONS**

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- 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**

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- 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**

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- 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**

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- 6.1 There are no known interferences at this time.

## **7.0 SAMPLE HANDLING**

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

## **8.0 CALIBRATION AND STANDARDIZATION**

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### **8.1 Preparation of Calibration Standards**

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

### **8.2 Calibration**

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

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

### **8.3 Storage Conditions for Standards**

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

### **8.4 Storage Conditions for Beef Liver Homogenates**

8.4.1 Beef liver homogenates may be frozen after preparation.

## **9.0 PROCEDURE**

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### **9.1 Initial Set-up**

9.1.1 Set software to "Operate on", Ion Mode ES<sup>-</sup>.

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  $\mu$ L/min. Observe droplets coming out of the tip of the probe. The pressure should be at 1700 to 1800 psi.

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

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

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

9.1.8 Go to the "Editor" page, and set Ionization Mode to ES<sup>-</sup>, 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  $\mu$ L 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  $\mu$ L

9.3.3.3 Set inject/sample = 2

9.3.3.4 Set Cycle time = 0

9.3.3.5 Name the file: Livers

9.3.3.6 Identify the tray used

9.3.3.7 Add the samples to Queue by pressing "Enter"

9.3.3.8 Press "Run" to start

## 10.0 VALIDATION

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### 10.1 Quality Control

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

### 10.2 Precision and Accuracy

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

### 10.3 Other Validation Parameters

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

## 11.0 DATA ANALYSIS

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### 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**

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None

## **13.0 REFERENCES**

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13.1 AMDT-EP-17

## **14.0 REVISIONS**

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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

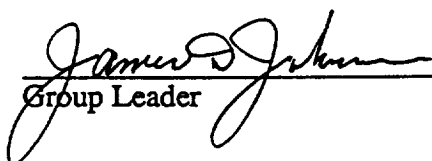
Revision Number: 0

Revision Date: None

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Author: Deb Wright / Cynthia Weber

Approved By:

  
Group Leader

10/5/95  
Date

  
Quality Assurance

9-27-95  
Date

Software: IBM MS Word, 6.0

Affected Documents: AMDT-EP-26, Operation and Maintenance of the Skalar Segmented Flow  
Analyzer

## 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 ( $\text{HNO}_3$ ) to 250 mLs of Milli-Q water. Bring the volume up to 1 L with Milli-Q water.

### 4.3 Standards

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

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

|         |     |   |
|---------|-----|---|
| 1.2 ppm | 1.2 | - |
| 1.5 ppm | 1.5 | - |

## **5.0 EQUIPMENT**

- 5.1 Skalar Segmented Flow Auto Analyzer Sans<sup>Plus</sup> 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**

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### **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

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### 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

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None

## 13.0 REFERENCES

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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 |          | Phase        | Date Inspection Reported to |                |
|------------------|----------|--------------|-----------------------------|----------------|
| From             | To       |              | Management                  | Study Director |
| 10/12/95         | 10/19/95 | Final Report | 10/19/95                    | 10/19/95       |

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<sup>-</sup> 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\***

|                          | $\mu\text{g}$   | Std. Dev. |
|--------------------------|-----------------|-----------|
| Control Group            | 16.2 $\pm$ 5.5  |           |
| 0.128 mg/kg dose (T6054) | 11.1 $\pm$ 2.0  |           |
| 1.28 mg/kg dose (T6054)  | 17.1 $\pm$ 2.6  |           |
| 12.8 mg/kg dose (T6054)  | 45.2 $\pm$ 20.5 |           |
| Fabric Exposure (T6051)  | 14.7 $\pm$ 2.7  |           |

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

| FC129 AB      |      | Actual   | Average  |         | Whole   | Total F- in |         |
|---------------|------|----------|----------|---------|---------|-------------|---------|
| ID            | %    | ppm F-   | ppm F-   | liver   | liver   | whole       | Dosage  |
| rcvry         |      | in liver | in liver | burned  | weight  | liver       | (mg/kg) |
| (W/W)         |      | (W/W)    | (W/W)    | (grams) | (grams) | (ug)        |         |
| 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    |             |         |

| FC129 AB    |     | Actual   | Average  |         | Whole   | Total F- in |         |
|-------------|-----|----------|----------|---------|---------|-------------|---------|
| ID          | %   | ppm F-   | ppm F-   | liver   | liver   | whole       | Dosage  |
| rcvry       |     | in liver | in liver | burned  | weight  | liver       | (mg/kg) |
| (W/W)       |     | (W/W)    | (W/W)    | (grams) | (grams) | (ug)        |         |
| 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    |             |         |

| FC129 AB       |            | Actual                      | Average                     |                            | Whole                      | Total F- in            |                   |
|----------------|------------|-----------------------------|-----------------------------|----------------------------|----------------------------|------------------------|-------------------|
| ID             | %<br>rcvry | ppm F-<br>in liver<br>(W/W) | ppm F-<br>in liver<br>(W/W) | liver<br>burned<br>(grams) | liver<br>weight<br>(grams) | whole<br>liver<br>(ug) | Dosage<br>(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                      |                            |                        |                   |

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



contains pages  
A-1 through A-12  
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 $\mu\text{g/g}^*$ | Total mass of liver g | Total amount of FC-95 per liver mg |
|----------------------------------------|----------|----------------|----------------|-----------------|---------------------------------|-----------------------|------------------------------------|
| Group 1:<br>Sterile Water<br>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:<br>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:<br>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:<br>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:<br>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 than 171 mg. The concentrations in the standard curve are therefore 5/4 larger than they should be. By multiplying the calculated concentration in the standard curve by 4/5, the correct result is obtained.

**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 $\mu\text{g/g}$ ** | Total mass of liver g | Total amount of FC-95 per liver mg |
|--------------------------|----------|----------------|----------------|-----------------|----------------------------------|-----------------------|------------------------------------|
| Group 2:<br>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:<br>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:<br>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 than 171 mg. The concentrations in the standard curve are therefore 5/4 larger than they should be. By multiplying the calculated concentration in the standard curve by 4/5, the correct result is obtained.

Replot

A-3

Method DLCLIV

Sample DLCLIV

Operator DLC

FILE: C40495B

Run date 05-09-1995 06:49:44 Version: 12

Printed on 05-09-1995 AT 06:49:56

Straight Line Fit forced through Origin.

Component #1

AREA  
R 132059  
E  
A

85693

47743

.4

1.2

AMOUNT

Component 1 =  
EXTERNAL STANDARD CALIBRATION  
AREA

| LEVEL | AMOUNT | AREA   |
|-------|--------|--------|
| 1     | 0.4000 | 47743  |
| 2     | 0.8000 | 85693  |
| 3     | 1.2000 | 132059 |

Y = SLOPE \* X + INTERCEPT

Area = 1.0988E+05 \* Amount + 0.0000E+00  
Amount = 9.1012E-06 \* Area + 0.0000E+00  
R squared = 0.9946

FILE: 040395D

Replot

Method DLCLIU

Sample DLCLIU

Operator DLC

A-4

INCE 6329-133

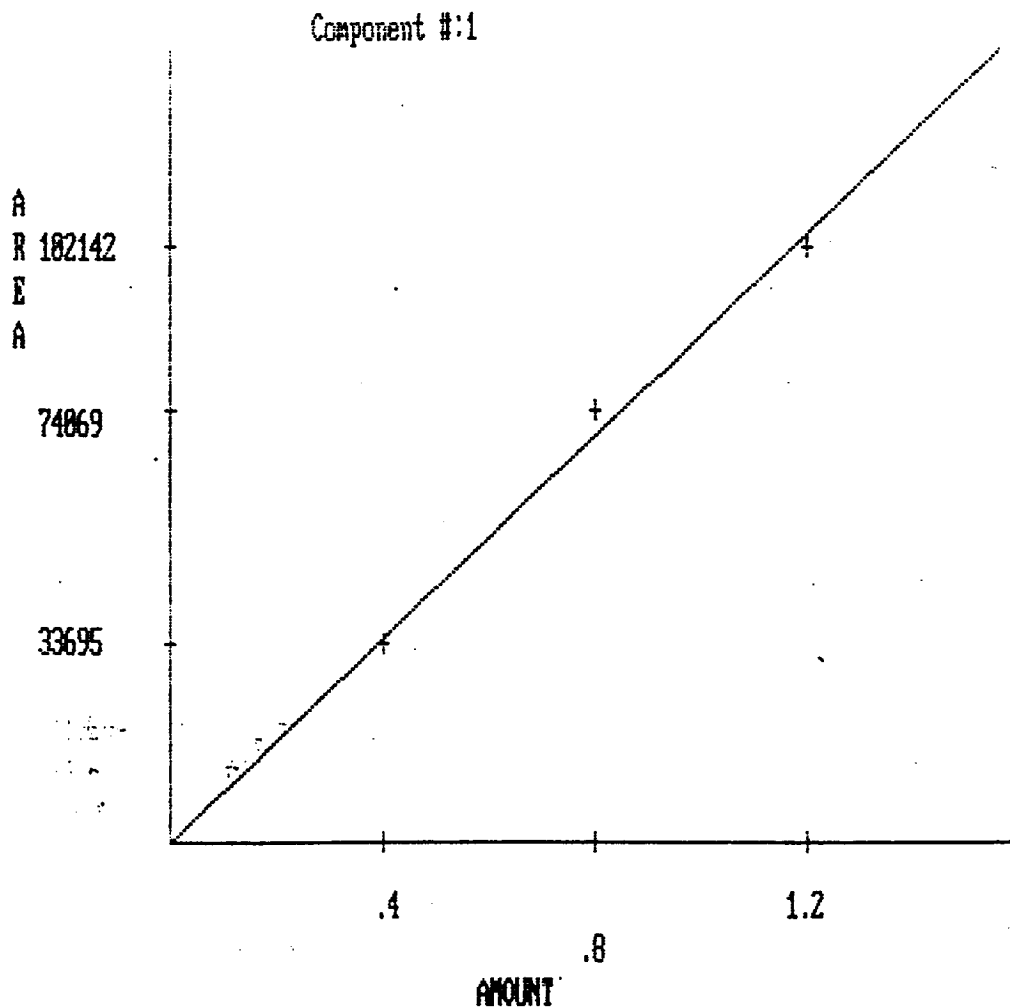
FC-129

Run date 05-09-1995 07:24:45 Version: 10

Printed on 05-09-1995 AT 07:24:56

Straight Line Fit forced through Origin.

Exact Copy of Original  
Initial DLC  
Date 6/22/95



Component 1 =  
EXTERNAL STANDARD CALIBRATION

| EVEL | AMOUNT | AREA   |
|------|--------|--------|
| 1    | 0.4000 | 33695  |
| 2    | 0.8000 | 74069  |
| 3    | 1.2000 | 102142 |

Y = SLOPE \* X + INTERCEPT

Area = 8.7189E+04 \* Amount + 0.0000E+00

Amount = 1.1469E-05 \* Area + 0.0000E+00

R squared = 0.9889

10/13/95  
ALC

A-9  
A-6

HWI 6329-133

FC-129

Exact Copy of Original

DLC

Initial

6/22/95

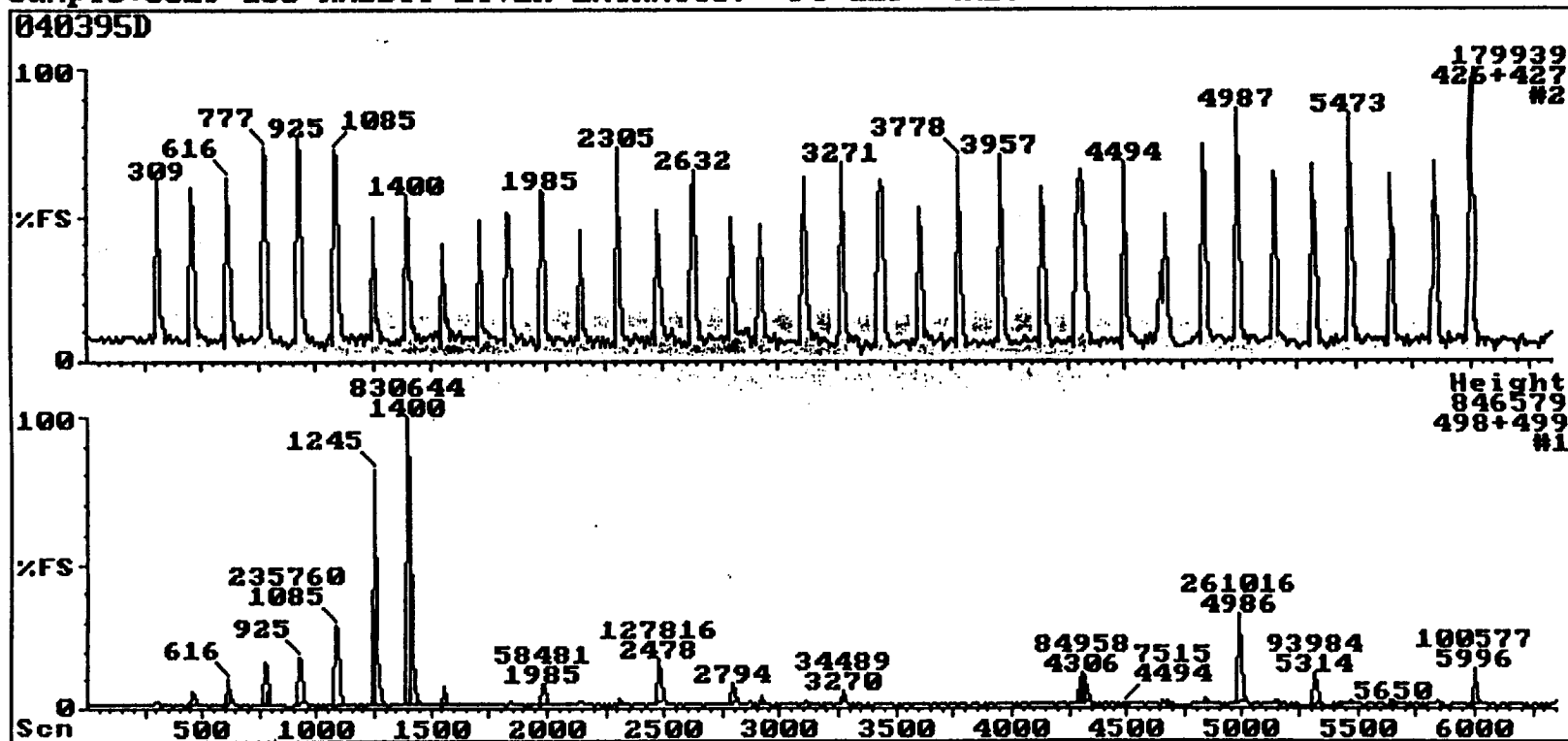
Date

File: 040395D

LAB-BASE - The MS Data System

03/04/1995 14:00

Sample: 6329-133 RABBIT LIVER EXTRACTS: FC-129 (AB)



10-13-11  
A-10  
A-7

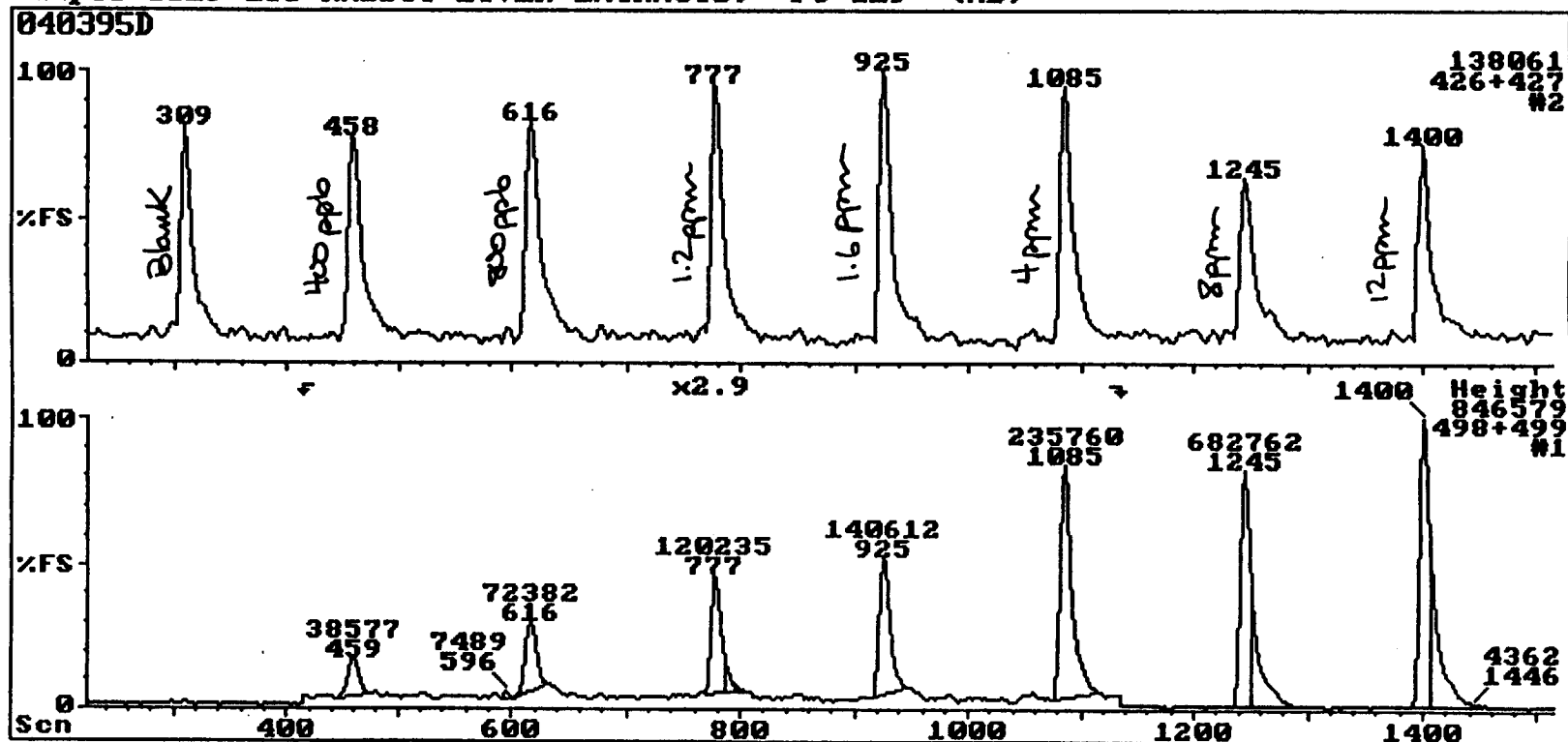
Beef liver extracts for std. curve

File:040395D

LAB-BASE - The MS Data System

03/04/1995 14:00

Sample:6329-133 RABBIT LIVER EXTRACTS; FC-129 (AB)



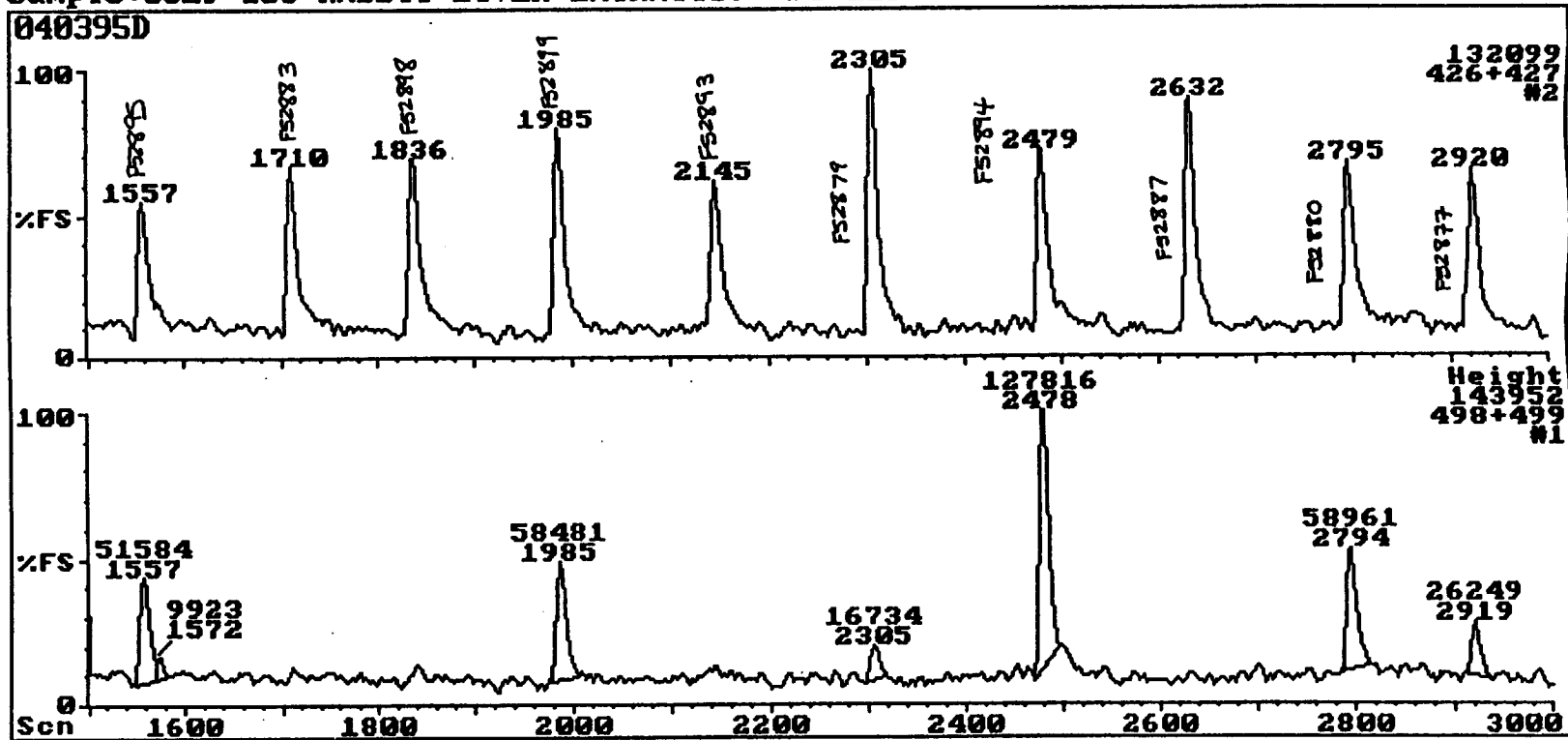
A-11  
A-8 10-18  
QAC

File:040395D

LAB-BASE - The MS Data System

03/04/1995 14:00

Sample:6329-133 RABBIT LIVER EXTRACTS; FC-129 (AB)



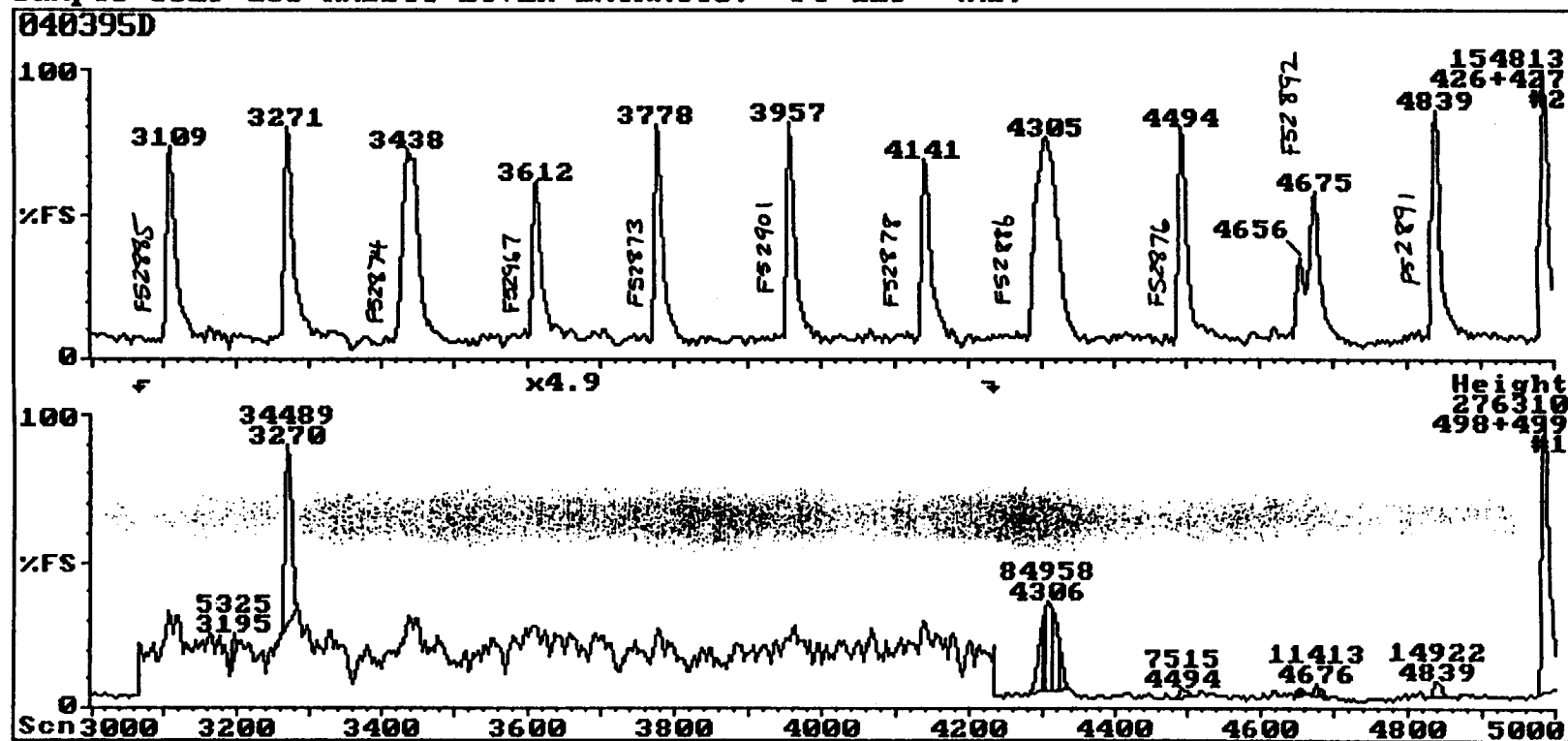
A-12  
A-9  
10-18-  
DUC

File:040395D

LAB-BASE - The MS Data System

03/04/1995 14:00

Sample:6329-133 RABBIT LIVER EXTRACTS; FC-129 (AB)





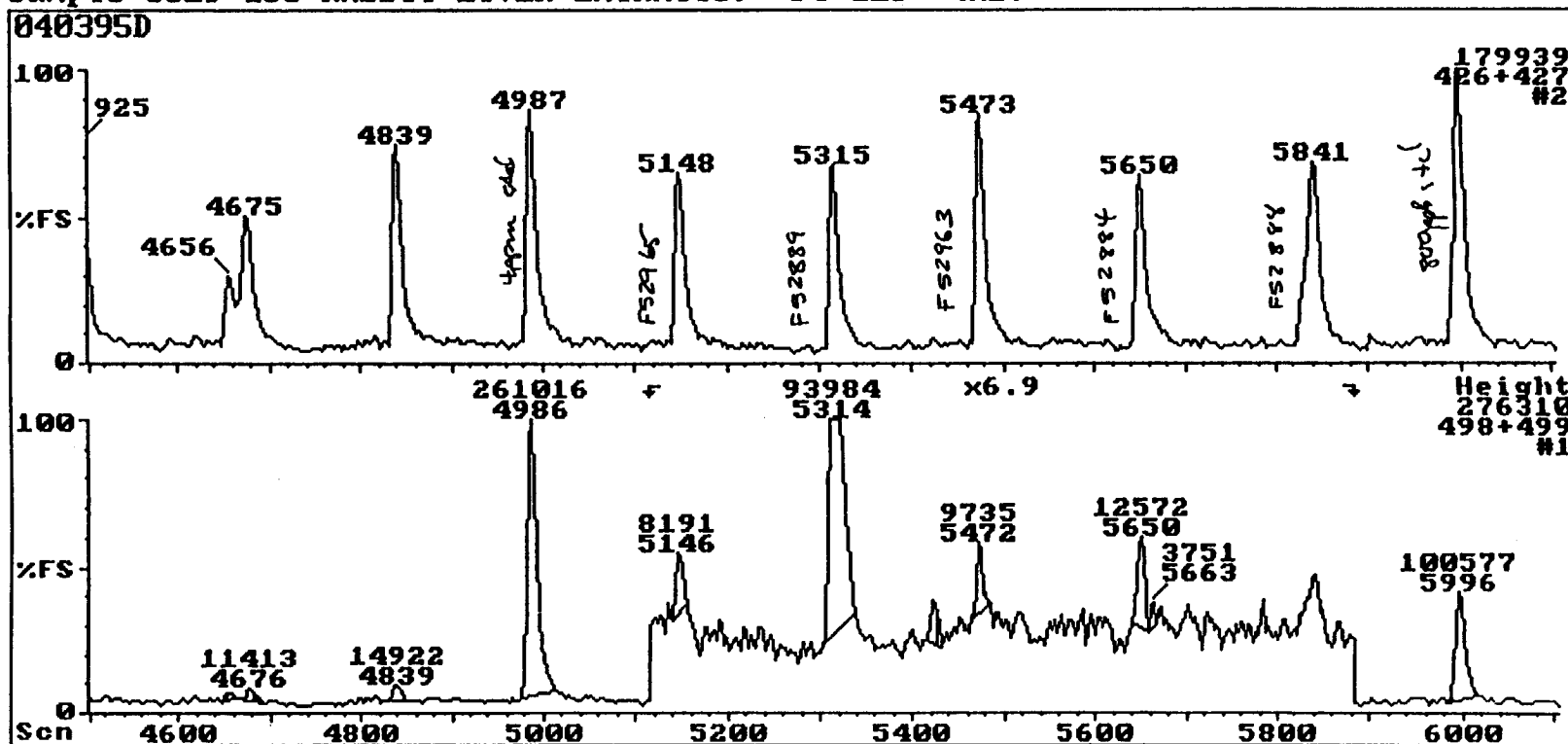
A-13  
R-10

File:040395D

LAB-BASE - The MS Data System

03/04/1995 14:00

Sample:6329-133 RABBIT LIVER EXTRACTS; FC-129 (AB)



10-14-95  
A-14  
A-11  
(DC)

HWT 6329-133 & 6329-143

Exact Copy of Original

DC

Initial

6/22/95

Date

6329-133 Animals Analyzed

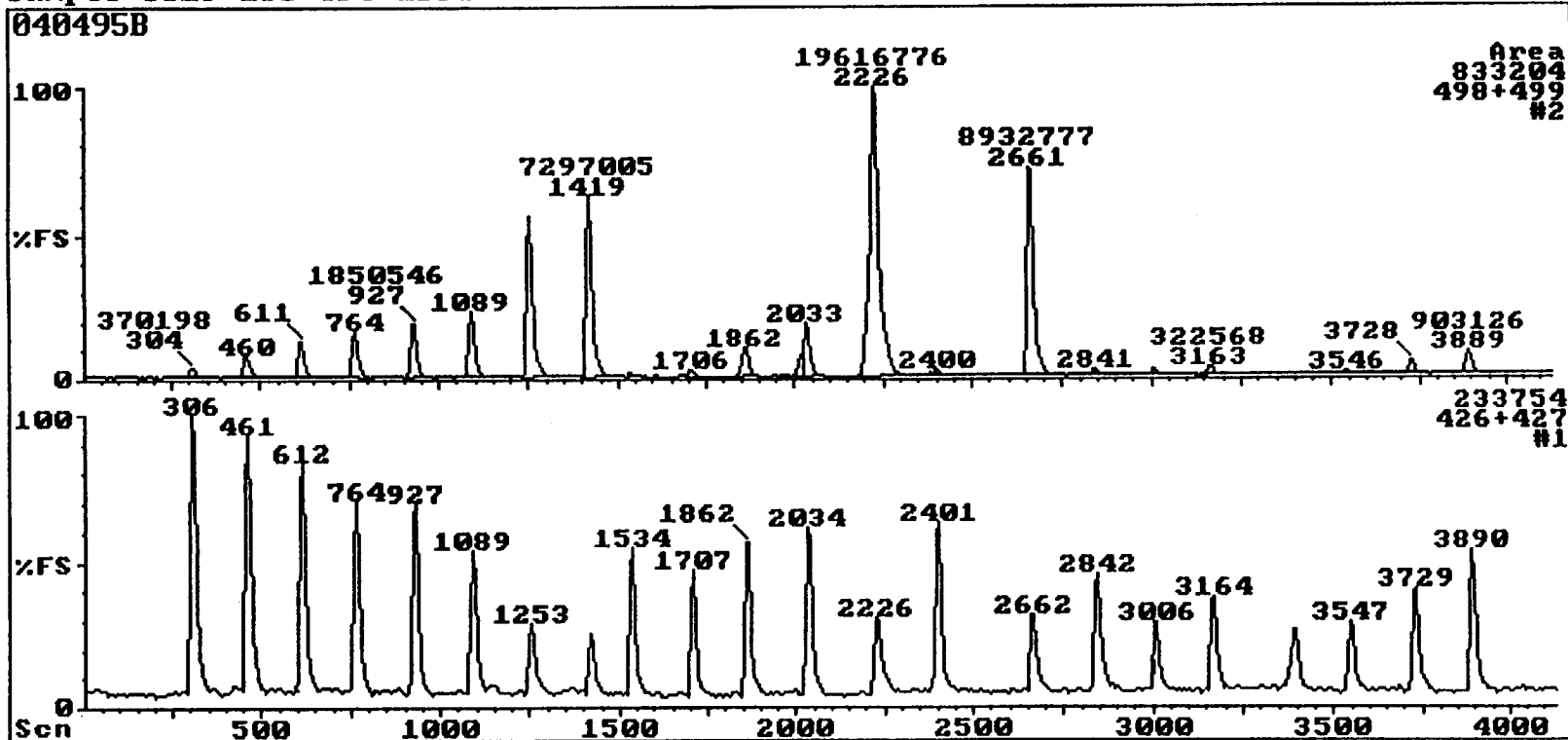
F52897  
F52900  
F52968  
F52882  
F52966

File:040495B

LAB-BASE - The MS Data System

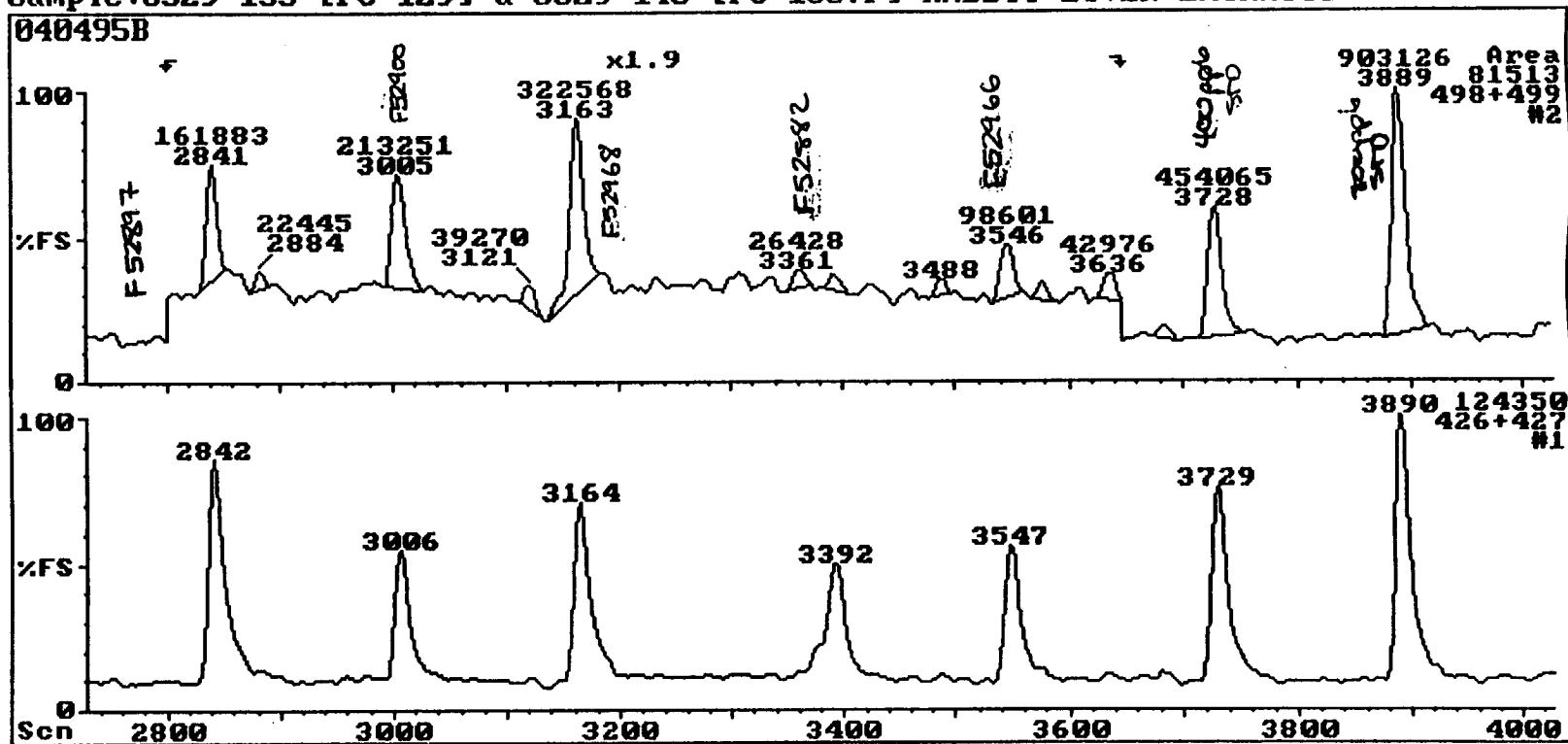
04/04/1995 10:07

Sample:6329-133 [FC-129] & 6329-143 [FC-1367F] RABBIT LIVER EXTRACTS



4-15  
4-12  
10-18-88  
DL

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



**9.11.3 Summary and raw data; ug F<sup>-</sup> 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 1/18/95  
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.

*Robert Wright*

**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- 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

|                                                  |          |      |     |        |      |      |         |    |    |
|--------------------------------------------------|----------|------|-----|--------|------|------|---------|----|----|
| <b>GROUP 3</b><br><b>Dose Level : 1.28 mg/kg</b> | 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 |    |
|                                                  | F52892-1 | 0.02 | 3.0 | 0.1322 | 0.43 | 0.20 | 68.7067 | 29 | 15 |
|                                                  | 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 |    |

**SUMMARY of 6329-133  
LIVER SAMPLES  
AMDT 013195.1**

|                                                                | Sample ID | Skalar Result (ppm) | DI-TISAB final vol (mL) | Qty Sample (mL or grams) | Actual ppm F- in Sample | Average Actual ppm F- | Total Tissue Wt. (grams) | Total F- per tissue (ppm) | Average Total F- per tissue |
|----------------------------------------------------------------|-----------|---------------------|-------------------------|--------------------------|-------------------------|-----------------------|--------------------------|---------------------------|-----------------------------|
| <b>GROUP 4</b><br><b>Dose Level : 12.8 mg/kg</b>               | 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                        |                             |
|                                                                | F52894-2  | 0.06                | 3.0                     | 0.1183                   | 1.43                    | 1.27                  | 73.9834                  | 105                       | 94                          |
|                                                                | 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                        |                             |
| <b>GROUP 5</b><br><b>Dose Level : 10.0 cm x 10.0 cm fabric</b> | 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                        |                             |
|                                                                | F52879-1  | 0.02                | 3.0                     | 0.1338                   | 0.34                    | 0.17                  | 89.1246                  | 30                        | 16                          |
|                                                                | 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                        |                             |

| Sample # | Sample ID | Skalar Standard (ppm) | Skalar Result (ppm) | % Recovery | DI-TISAB final vol (mL) | Qty Sample (mL or grams) | Actual ppm P <sub>o</sub> in Sample | Total Tissue Wt (grams) | Total P <sub>o</sub> per tissue (ppm) | mL FC-95 Solution Spiked | Conc. FC-95 Soln (ppm) | Mass Spiked (ug P <sub>o</sub> ) | Mass Recovered (ug P <sub>o</sub> ) | % 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                      |                                       |                          |                        |                                  |                                     |            |
| 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                       |                        |                                  |                                     |            |

Ambt 13195.1  
 HwI 6329-133  
 Juere



| 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 PC 95 Solution | Conc PC 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                  |            |                         |                         |                         |                         |                          |                   |                       |                     |                        |            |

| Sample # | Sample ID | Skalar Standard (ppm) | Skalar Result (ppm) | % Recovery | DI/TISAB final vol (mL) | Qty Sample (mL or gram) | Actual ppm P- in Sample | Total Tissue Wt (mg) | Total P- per tissue (ug) | mL PC-95 Solution Spiked | Conc PC-95 Soln (ppm) | Mass Spiked (ug P-) | Mass Recovered (ug P-) | % 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                  |            |                         |                         |                         |                      |                          |                          |                       |                     |                        |            |

POST 13195.1  
HWI 6329-13  
Jensen

| 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 F2) | Mass Recovered (ug F2) | % 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                   |                          |                       |                     |                        |            |

AND T 13195.1  
 HWI 6329-133  
 Juven

| Sample # | Sample ID | Skalar Standard (ppm) | Skalar Result (ppm) | % Recovery | DI TISAB final vol (mL) | Qty Sample (mL or gram) | Actual ppm P- in Sample | Total Tissue Wt (gram) | Total F- per tissue (ppm) | mL FC 95 Solution Spiked | Conc FC 95 Soln (ppm) | Mass Spiked (ug P-) | Mass Recovered (ug P-) | % 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      |                       |                     |            |                         |                         |                         |                        |                           |                          |                       |                     |                        |            |

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

DDW 7/15/95  
AMDT 13195.1  
HWI 6329-133  
Juno

### Fluoride 1.5

Calibration order = Inverse Logarithm

Slope : s = #.#####

Result =  $10^{\frac{x - c1}{s}}$   
x = corrected value of the sample  
c1 = corrected value of the concentration 1  
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<sup>2</sup> + 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

1995-03-28 17:30

OutPut of : 950328F1

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 : #.####

1995-03-28 17:30

OutPut of : 950328F1

Page 1 of 3

Fluoride 1.5  
Fluoride LPPM  
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  |
|     |     |              |     |        | 4  | 0.9756 |   | 2065 | 0    | 0    |
| 2   | d   | Drift        | 1   | 1.000  | 3  | 1.230  |   | 2096 | 2312 | 380  |
|     |     |              |     |        | 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 |
|     |     |              |     |        | 4  | 1.0106 |   | 2113 | 0    | 0    |
| 15  | w   | Wash         | 1   | 1.000  | 3  | 0.056  |   | 0    | 226  | 2661 |
|     |     |              |     |        | 4  | 0.0060 |   | 0    | 0    | 0    |

TV: 1.2 ppm

TV: 1.2 ppm

TV: 1.2 ppm



1995-03-28 17:30

OutPut of : 950328F1

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Fluoride 1.5  
Fluoride LPPM  
PPM

| Pos | Typ | Ident    | Dil | Weight | Ch | Result  | F    | Cor. | Valu | Time | Notes                                            |
|-----|-----|----------|-----|--------|----|---------|------|------|------|------|--------------------------------------------------|
| 16  | u   | BLK 1    | 1   | 1.000  | 3  | too e < | 14   | 240  | 2779 |      | Dehmen Sample<br>Skala @ 3x (100, 1045) Cr. 1.05 |
|     |     |          |     |        | 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 |      | 0.0399<br>ND                                     |
|     |     |          |     |        | 4  | 0.0133  | 33   | 0    | 0    |      | 0.0328<br>ND                                     |
| 30  | u   | F52884-2 | 1   | 1.000  | 3  | 0.061   | 34   | 256  | 5282 |      | 0.0405<br>ND                                     |
|     |     |          |     |        | 4  | 0.0135  | 34   | 0    | 0    |      | 0.02496<br>ND                                    |
| 31  | u   | F52884-3 | 1   | 1.000  | 3  | 0.061   | 34   | 256  | 5435 |      | 0.0405<br>ND                                     |
|     |     |          |     |        | 4  | 0.0135  | 34   | 0    | 0    |      | 0.0306<br>ND                                     |

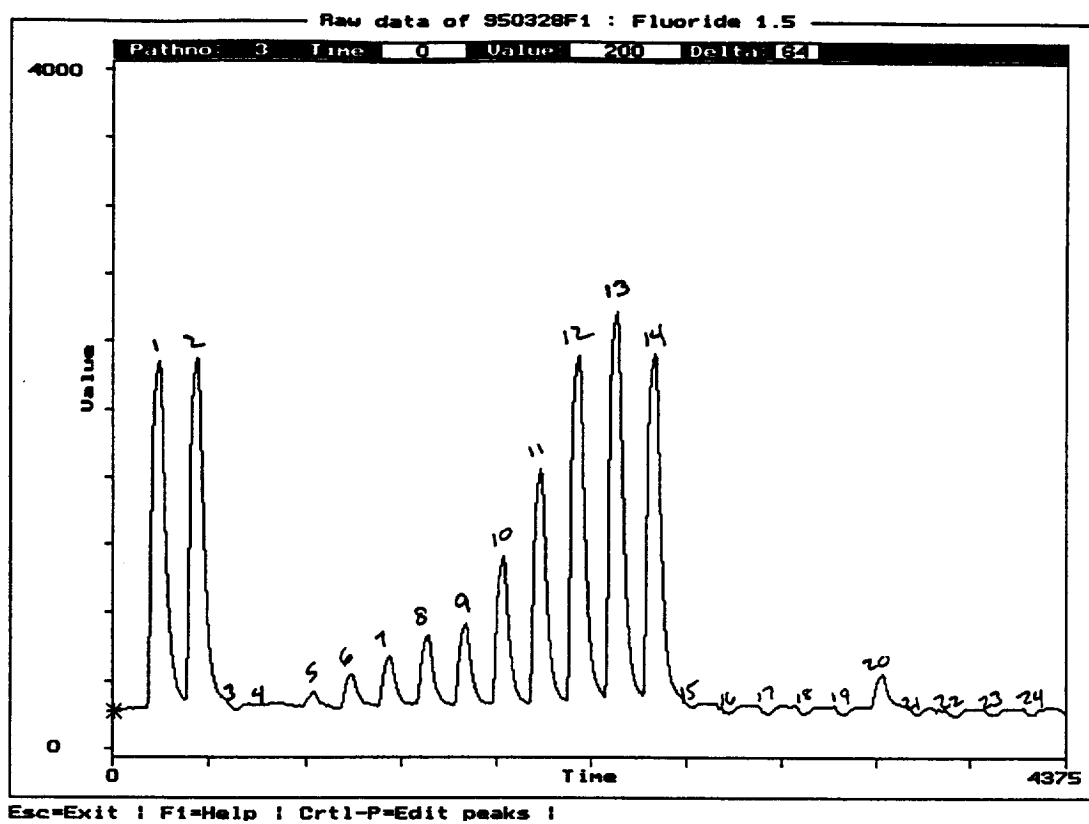
1995-03-28 17:30

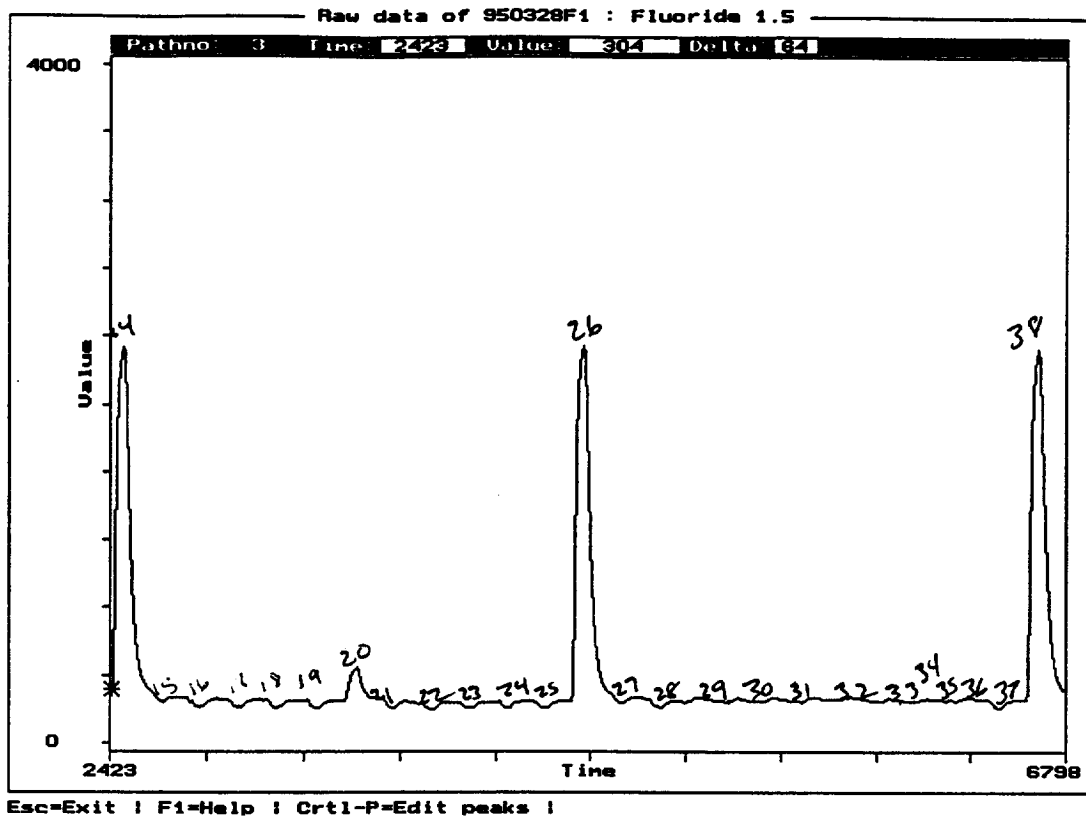
OutPut of : 950328F1

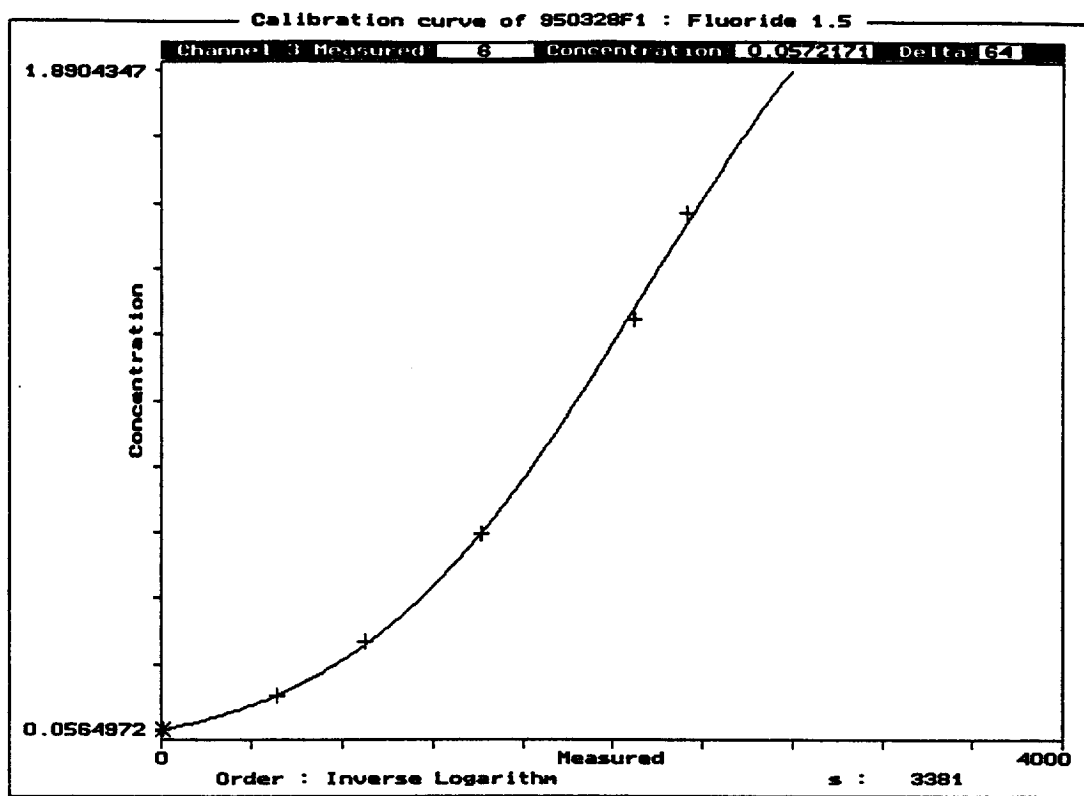
Page 3 of 3

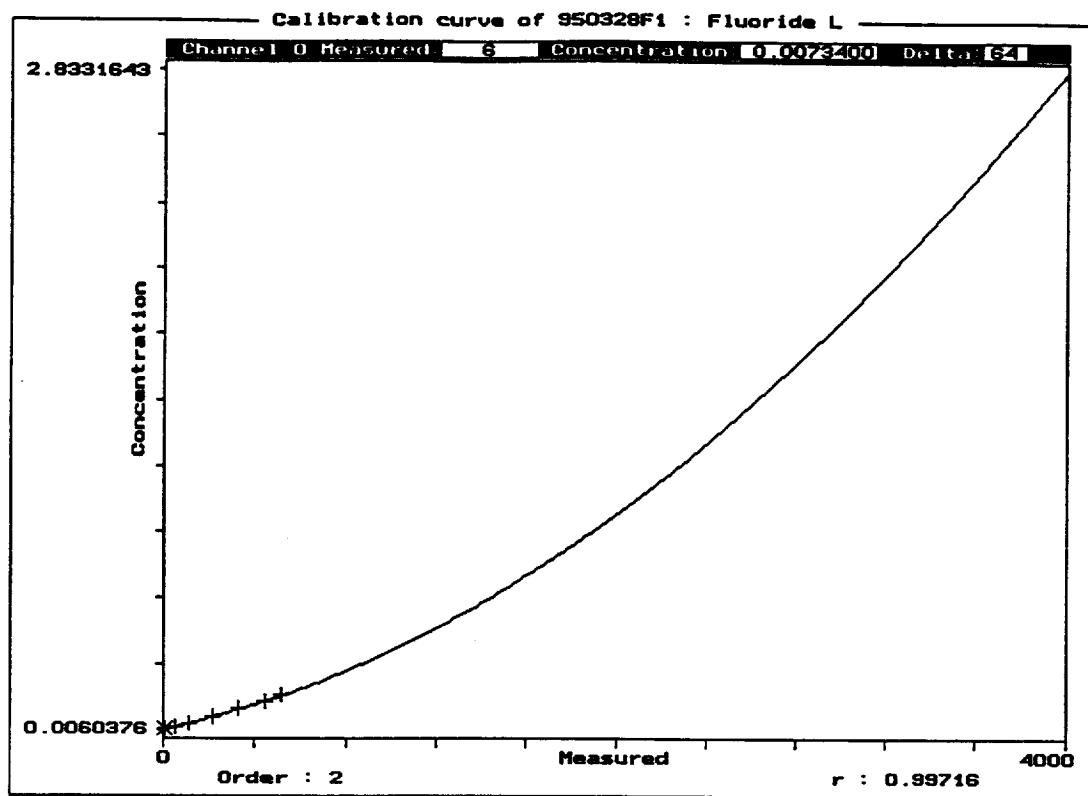
Fluoride 1.5  
Fluoride LPPM  
PPM

| Pos | Typ | Ident                              | Dil | Weight | Ch | Result  | F | Cor. | Valu | Time | 3 <sup>rd</sup><br>Dohren | Orion  |
|-----|-----|------------------------------------|-----|--------|----|---------|---|------|------|------|---------------------------|--------|
| 32  | u   | F52892-2                           | 1   | 1.000  | 3  | 0.061   |   | 38   | 260  | 5635 | ND                        | 0350   |
|     |     |                                    |     |        | 4  | 0.0144  |   | 38   | 0    | 0    | .0432                     | ND     |
| 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                     | .02275 |
|     |     |                                    |     |        | 4  | 0.0140  |   | 36   | 0    | 0    | ND                        | ND     |
| 35  | u   | F52963-2                           | 1   | 1.000  | 3  | 0.061   |   | 36   | 256  | 6159 | ND                        | .02157 |
|     |     |                                    |     |        | 4  | 0.0140  |   | 36   | 0    | 0    | .042                      | ND     |
| 36  | u   | F52963-3                           | 1   | 1.000  | 3  | 0.061   |   | 36   | 256  | 6333 | ND                        | .02001 |
|     |     |                                    |     |        | 4  | 0.0140  |   | 36   | 0    | 0    | .042                      | ND     |
| 37  | u   | <del>F52963-4</del><br>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    |                           |        |









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

DDW 7/18/95  
AMST 13.95.1  
HWI 6329-133  
Jwers

### Fluoride 1.5

Calibration order = Inverse Logarithm

Slope : s = #.####

Result =  $10^{\frac{x - c1}{s}}$   
x = corrected value of the sample  
c1 = corrected value of the concentration 1  
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<sup>2</sup> + 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           : #.####
```

1995-03-29 12:46

OutPut of : 950329A1

Page 1 of 3

Fluoride 1.5  
Fluoride LPPM  
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  |      |
|     |     |              |     |        | 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 |      |
|     |     |              |     |        | 4  | 1.1617 | 2103 | 0    | 0    |      |
| 15  | w   | Wash         | 1   | 1.000  | 3  | 0.061  |      | 0    | 256  | 2663 |
|     |     |              |     |        | 4  | 0.0084 |      | 0    | 0    | 0    |

TV=1.2 ppm

TV=1.2 ppm

1995-03-29 12:46

OutPut of : 950329A1

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Fluoride 1.5  
Fluoride L

PPM  
PPM

| Pos | Typ | Ident    | Dil | Weight | Ch | Result  | F | Cor. | Valu | Time | Notes                        |
|-----|-----|----------|-----|--------|----|---------|---|------|------|------|------------------------------|
| 16  | u   | BLK 1    | 1   | 1.000  | 3  | 0.100   |   | 255  | 512  | 2829 |                              |
|     |     |          |     |        | 4  | 0.0709  |   | 255  | 0    | 0    | 206 2127, 206 2127, 206 2127 |
| 17  | u   | BLK 2    | 1   | 1.000  | 3  | 0.070   |   | 68   | 321  | 3010 |                              |
|     |     |          |     |        | 4  | 0.0230  |   | 68   | 0    | 0    | 259 2009, 259 2009, 259 2009 |
| 18  | u   | BLK 3    | 1   | 1.000  | 3  | 0.067   |   | 48   | 300  | 3185 |                              |
|     |     |          |     |        | 4  | 0.0186  |   | 48   | 0    | 0    | 255 8, 255 8, 255 8          |
| 19  | u   | SPK 1    | 1   | 1.000  | 3  | 0.086   |   | 180  | 432  | 3357 |                              |
|     |     |          |     |        | 4  | 0.0503  |   | 180  | 0    | 0    | 257 2509, 257 2509, 257 2509 |
| 20  | u   | SPK 2    | 1   | 1.000  | 3  | 0.087   |   | 186  | 436  | 3533 |                              |
|     |     |          |     |        | 4  | 0.0519  |   | 186  | 0    | 0    | 251 2157, 251 2157, 251 2157 |
| 21  | u   | SPK 3    | 1   | 1.000  | 3  | 0.085   |   | 173  | 422  | 3711 |                              |
|     |     |          |     |        | 4  | 0.0485  |   | 173  | 0    | 0    | 250 2155, 250 2155, 250 2155 |
| 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    | 256 4, 256 4, 256 4          |
| 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    | 20504, 20504, 20504          |
| 29  | u   | F52965-3 | 1   | 1.000  | 3  | 0.065   |   | 34   | 274  | 5110 |                              |
|     |     |          |     |        | 4  | 0.0155  |   | 34   | 0    | 0    | 20465, 20465, 20465          |
| 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    |                              |

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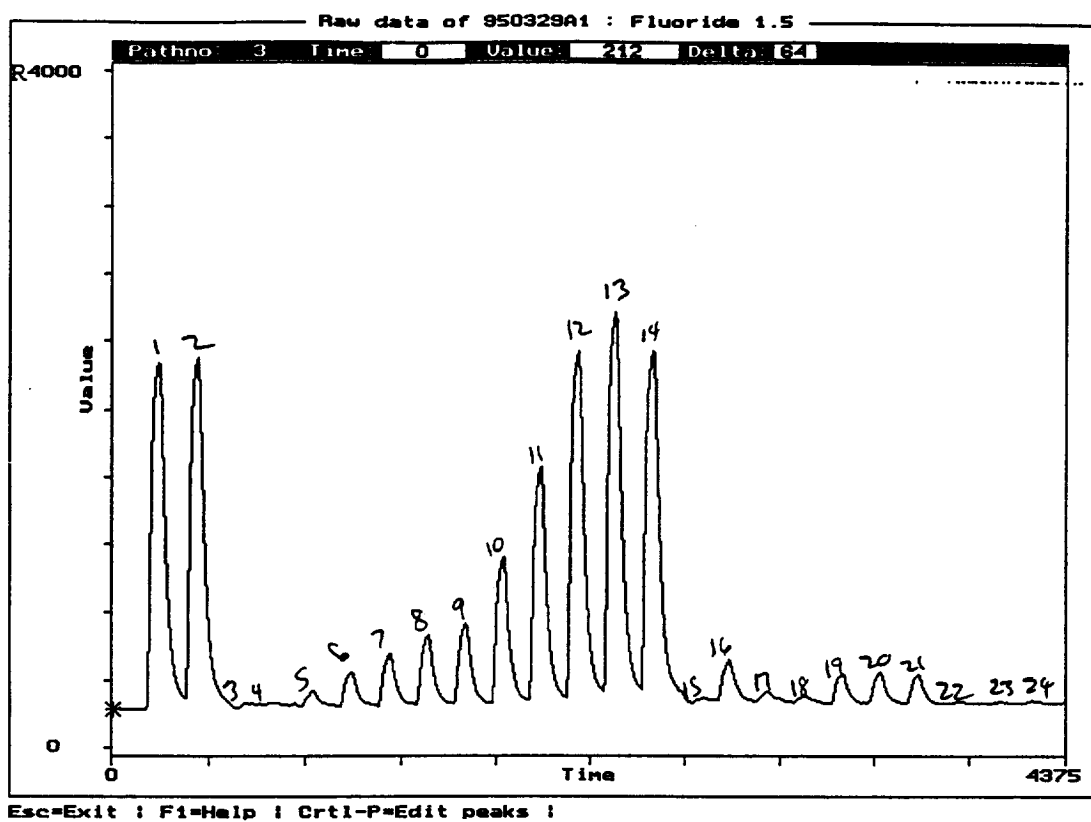
OutPut of : 950329A1

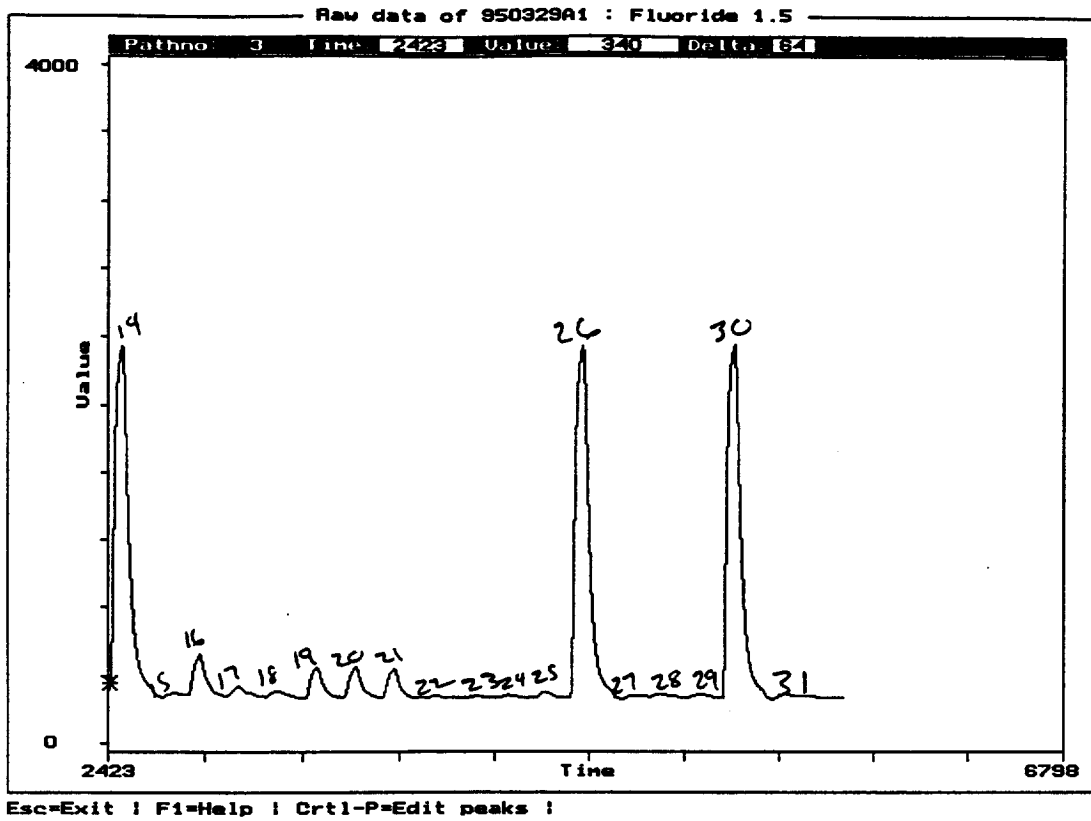
Page 3 of 3

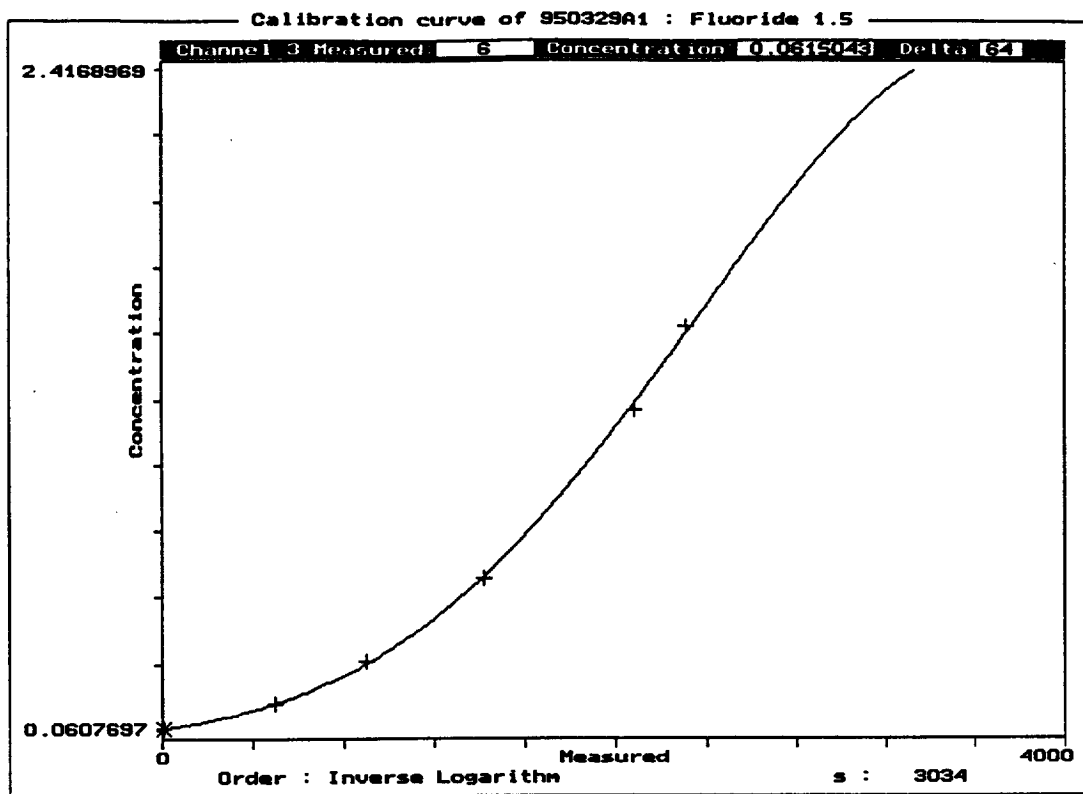
Fluoride 1.5  
Fluoride L

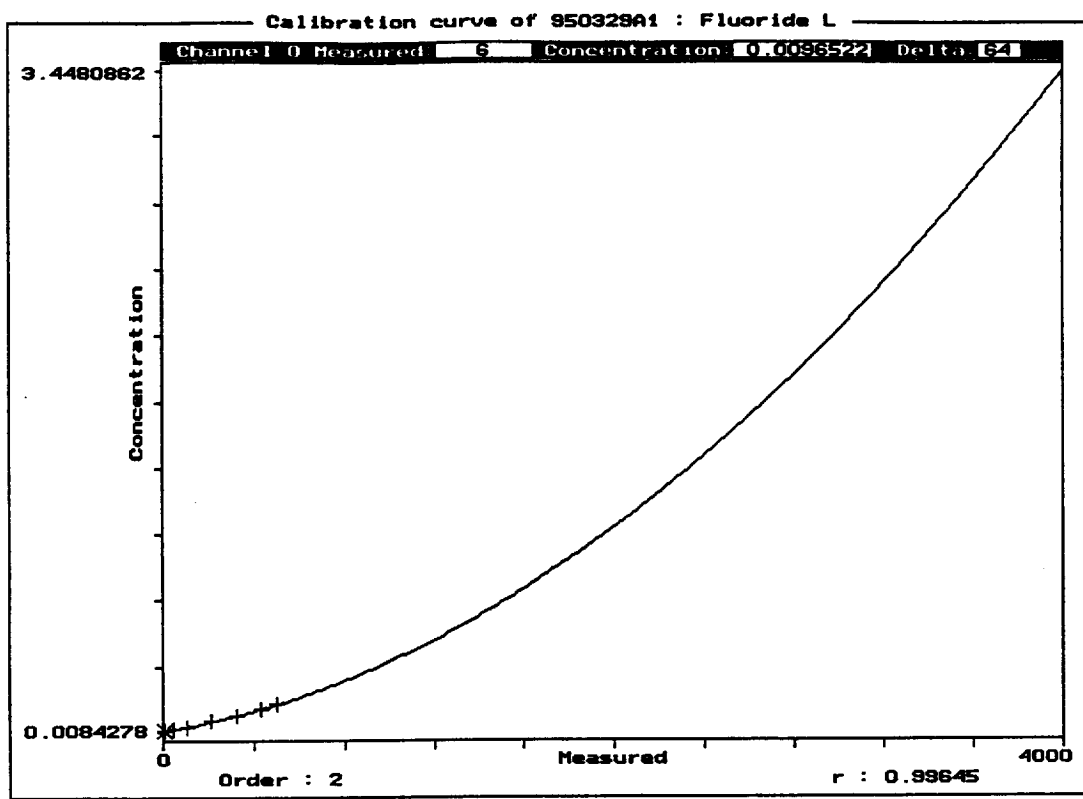
PPM  
PPM

| 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    |











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

ODW 7/12/95  
Am DT 1319 S.1  
HWT 6329-133  
Jues

### Fluoride 1.5

Calibration order = Inverse Logarithm

Slope : s = #.#####

Ö x - c1 ¢      x = corrected value of the sample  
° áááááá °      c1 = corrected value of the concentration 1  
Result = 10â s î      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 \* x² + 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

1995-03-30 10:59

OutPut of : 950330A1

```
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           : #.####
```

1995-03-30 10:59

OutPut of : 950330A1

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Fluoride 1.5  
Fluoride LPPM  
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    |

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OutPut of : 950330A1

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Fluoride 1.5  
Fluoride LPPM  
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    |

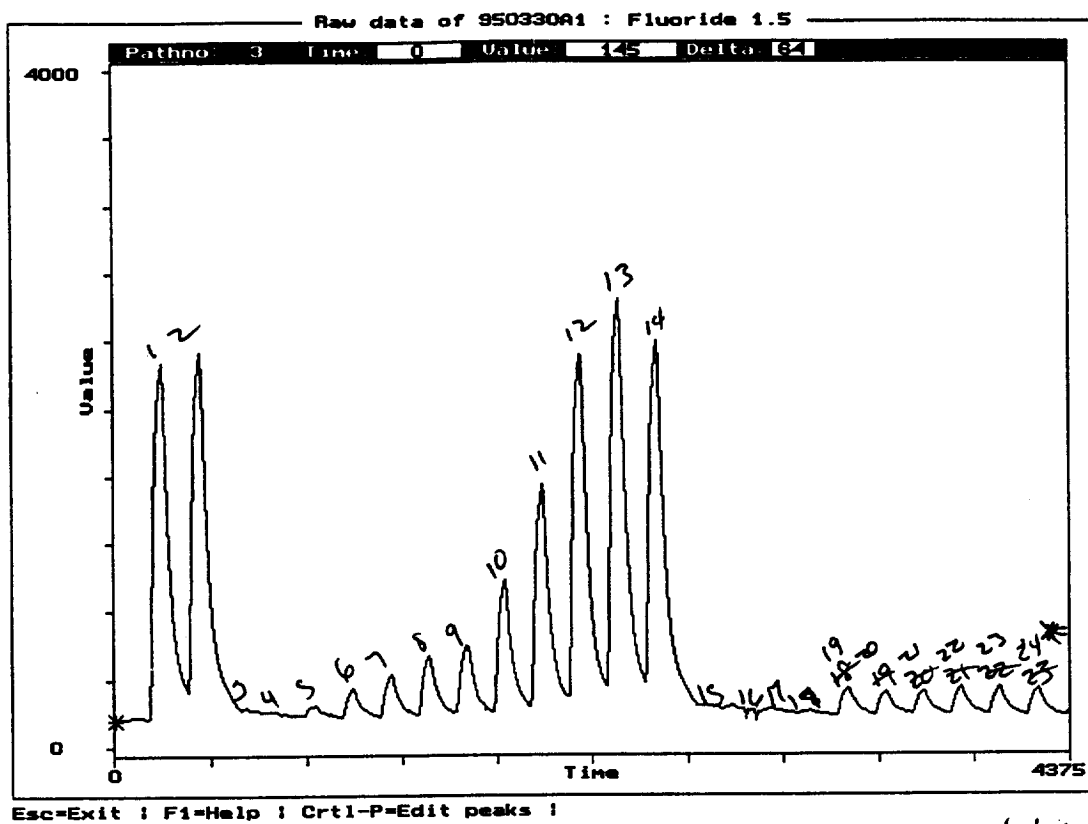
1995-03-30 10:59

OutPut of : 950330A1

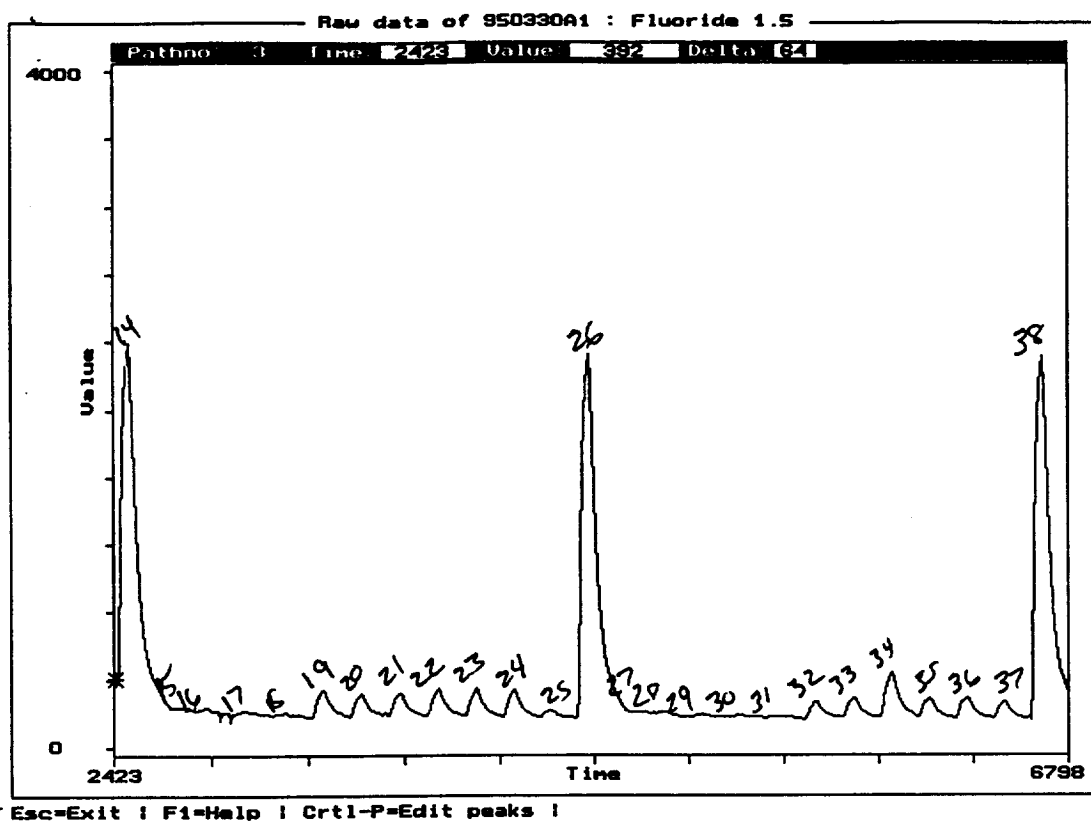
Page 3 of 3

Fluoride 1.5  
Fluoride LPPM  
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    |

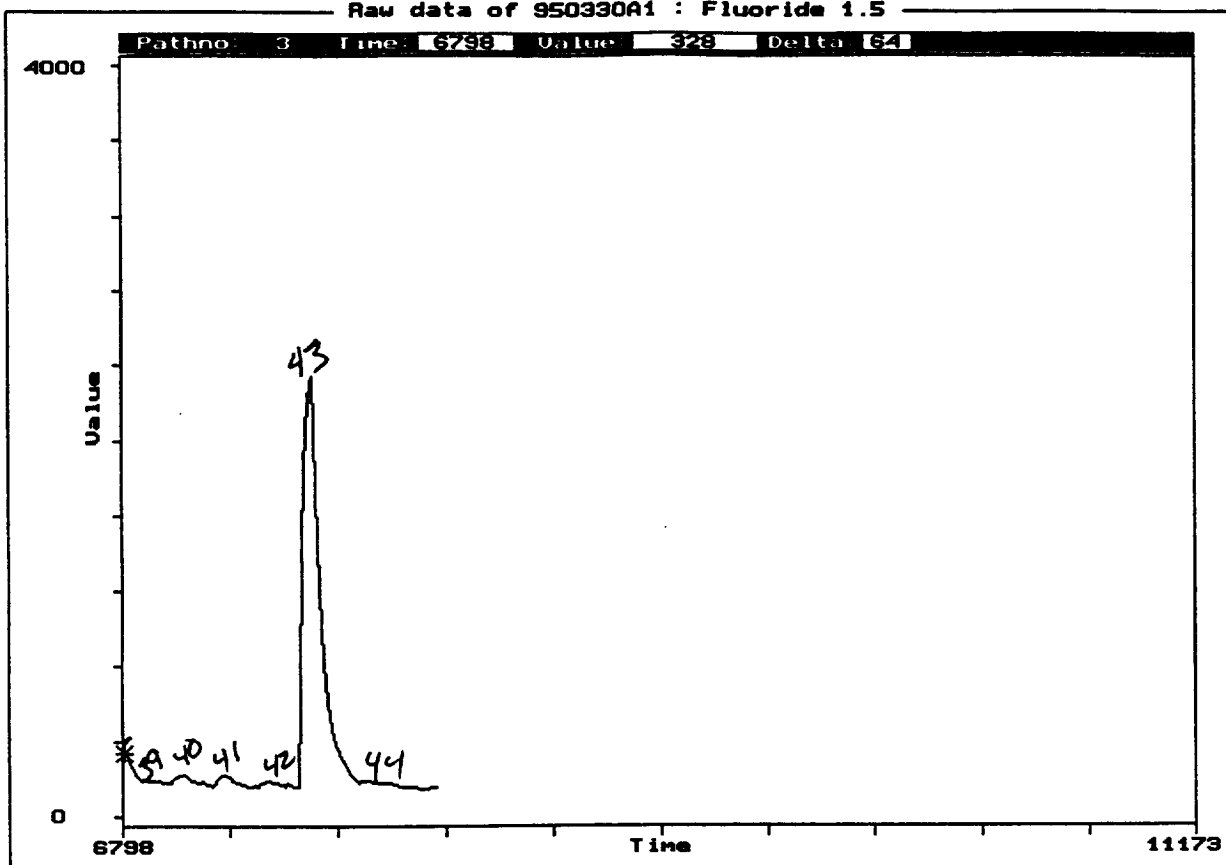


QOWTE 7/18/95





Raw data of 950330A1 : Fluoride 1.5



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

