

PFOS: AN ACUTE TOXICITY STUDY WITH THE EARTHWORM IN AN ARTIFICIAL SOIL
SUBSTRATE

FINAL REPORT

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454-111

Environmental Laboratory Project Number U2723

OECD Guideline 207

AUTHORS:

Anne B. Sindermann
John R. Porch
Henry O. Krueger, Ph.D.
Raymond L. Van Hoven, Ph.D.

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STUDY COMPLETION DATE: May 10, 2002

SUBMITTED TO:

3M
Environmental Laboratory
935 Bush Avenue
St. Paul, Minnesota 55106

Wildlife International, Ltd.

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

- 2 -

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: 3M

TITLE: PFOS: An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454-111

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This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Parts 160 and 792, 17 August 1989; OECD Principles of Good Laboratory Practice, ENV/MC/CHEM (98) 17, Paris, 1998; and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984.

STUDY DIRECTOR:

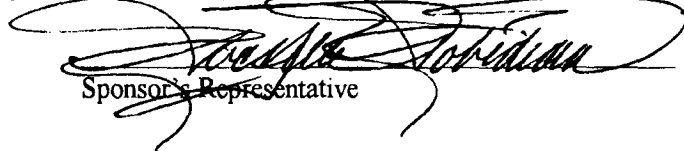


John R. Porch
Supervisor, Non-Target Plants and Insects

10 May 02

Date

SPONSOR APPROVAL:



Sponsor's Representative

5/14/02

Date

- 3 -

QUALITY ASSURANCE STATEMENT

This study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, 40 CFR Parts 160 and 792, 17 August 1989; OECD Principles of Good Laboratory Practice, ENV/MC/CHEM (98) 17, Paris, 1998; and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984. The dates of all inspections and audits, and the dates that any findings were reported to the Study Director and Laboratory Management were as follows:

ACTIVITY:	DATE CONDUCTED:	DATE REPORTED TO:	
		STUDY DIRECTOR:	MANAGEMENT:
Test Substance Preparation	November 2, 2001	November 2, 2001	November 5, 2001
Test Substance Preparation	November 19, 2001	November 19, 2001	November 20, 2001
Observations - Day 7	November 27, 2001	November 27, 2001	November 28, 2001
Matrix Fortifications	November 27, 2001	November 27, 2001	November 28, 2001
Body Weights - Day 14	December 4, 2001	December 4, 2001	December 5, 2001
Analytical Data, Draft Report	January 11, 2002	January 14, 2002	January 14, 2002
Raw Data, Draft Report	January 10 and 11, 2002	January 11, 2002	January 16, 2002
Final Report	May 6, 2002	May 6, 2002	May 10, 2002

James H. Coleman
James H. Coleman
Quality Assurance Representative

5-10-02
Date

- 4 -

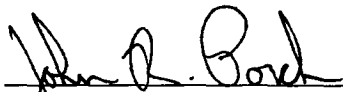
REPORT APPROVAL

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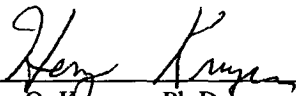


John R. Porch
Supervisor, Non-Target Plants and Insects

10 May 02

Date

WILDLIFE INTERNATIONAL, LTD. MANAGEMENT:



Henry O. Krueger, Ph.D.
Director, Aquatic Toxicology and Non-Target Plants

5/10/02

Date

TABLE OF CONTENTS

Title Page	1
Good Laboratory Practice Compliance Statement	2
Quality Assurance Statement	3
Report Approval	4
Table of Contents	5
Summary	7
Introduction	8
Objective	8
Experimental Design	8
Materials and Methods	9
Test Substance	9
Test Organism	9
Artificial Soil and Test Soils Preparation	9
Analytical Sampling	10
Analytical Testing	11
Test Chambers	11
Physical Properties of Test Soils	11
Environmental Conditions	11
Observations	12
Body Weights	12
Reference Toxicity Test	12
Statistical Analyses	12
Results and Discussion	13
Environmental Conditions	13
Physical Properties of Test Soils	13
Observations	13
Statistical Analyses	14
Reference Toxicity Test	14
Sample Analysis	15
Conclusions	15

TABLE OF CONTENTS

- Continued -

References	16
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TABLES

Table 1.	Moisture, pH _w , and Temperature of Test Soils	17
Table 2.	Cumulative Mortality and Observations of Earthworms Exposed to PFOS in an Artificial Soil Substrate.....	18
Table 3.	Average Body Weights of Earthworms Exposed to PFOS in an Artificial Soil Substrate.....	19
Table 4.	Day 14 Measured Values for PFOS in Earthworm Tissue	20

APPENDICES

Appendix 1.	Personnel Involved in the Study	21
Appendix 2.	Changes to the Study Protocol	22
Appendix 3.	Artificial Soil and Test Soils Preparation	23
Appendix 4.	Analytical Chemistry	25

- 7 -

SUMMARY

SPONSOR: 3M Corporation

TITLE : PFOS: An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454-111

TEST DATES:
Study Initiation: October 17, 2001
Experimental Start (OECD): November 2, 2001
Experimental Start (EPA): November 20, 2001
Experimental Termination: December 4, 2001
Study Completion: May 10, 2002

TEST ORGANISM : Earthworm (*Eisenia fetida*)

AGE OF TEST ORGANISM : Adult (with clitellum)

SOURCE OF TEST ORGANISM : Worm Man's Worm Farm
R & K Trading Co.
116 Pergola Ave.
Monroe Township, New Jersey 08831

MEAN MEASURED TEST CONCENTRATIONS:

Negative Control
77.0 mg a.i./ kg
141 mg a.i./ kg
289 mg a.i./ kg
488 mg a.i./ kg
1042 mg a.i./ kg

RESULTS:

7-Day LC50: 398 mg a.i./ kg
7-Day NOEC: 289 mg a.i./ kg
7-Day LOEC: 488 mg a.i./ kg
14-Day LC50: 373 mg a.i./ kg
14-Day NOEC: 77 mg a.i./ kg
14-Day LOEC: 141 mg a.i./ kg

- 8 -

INTRODUCTION

Wildlife International, Ltd. conducted this study for 3M at the Wildlife International, Ltd. toxicology facility in Easton, Maryland. The in-life portion of this test was conducted from November 19, 2001 to December 4, 2001. Raw data generated at Wildlife International, Ltd. and a copy of the final report are filed under Project Number 454-111 in archives located on the Wildlife International, Ltd. site. Key personnel involved in the conduct or management of the study are listed in Appendix 1.

OBJECTIVE

The objective of this study was to determine the acute effects of Perfluorooctanesulfonate, Potassium Salt (hereafter referred to as PFOS) on earthworms during a 14-day exposure period in an artificial soil substrate.

EXPERIMENTAL DESIGN

Earthworms (*Eisenia fetida*) were exposed to a geometric series of five concentrations of PFOS in soil. A control group was maintained concurrently in soil without the addition of PFOS. This route of administration was selected because it was representative of the natural exposure of earthworms to chemicals.

Four replicate test chambers were maintained in each treatment and control group, with 10 worms in each test chamber. Observations of mortality and clinical signs were conducted twice within the first four hours of test initiation, and then on Day 7 and Day 14. The mortality results were analyzed with the moving average method to determine an LC50 value.

At Wildlife International, Ltd., reference toxicity tests with a reference toxicant, chloroacetamide, are conducted periodically to assess the sensitivity of the test species and test procedures. These studies are conducted under separate protocols, as independent studies. A summary of the results from the most current reference toxicity test is presented in this report.

MATERIALS AND METHODS

The study was conducted based upon the procedures outlined in the protocol, "PFOS: An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate." The protocol was based upon procedures outlined in the Organization for Economic Cooperation and Development (OECD) Guideline No. 207, *Guideline for Testing of Chemicals, Earthworm, Acute Toxicity Tests (1)*. Changes to the protocol are listed in Appendix 2.

Test Substance

The test substance used was received from 3M on October 29, 1998 and assigned Wildlife International, Ltd. identification number 4675. The test substance was a white powder identified by the Sponsor as FC-95, lot 217. Information from the Sponsor indicated the purity of the test substance to be 86.9% with an expiration date of August 31, 2006. The test substance was stored under ambient conditions.

Test Organism

Adult earthworms (*Eisenia fetida*) were obtained from Worm Man's Worm Farm, Monroe Township, New Jersey. Approximately 18.5 hours prior to the test, 280 worms were selected and placed in a container of prepared artificial soil substrate adjusted to moisture content of approximately 33% by weight, for the acclimation period. On the day of test initiation, the worms were rinsed briefly with deionized water and indiscriminately distributed by pairs into groups of 10 worms each. Each group of worms was weighed then placed on the soil surface in the appropriate test chamber. The worms were not fed during testing.

Artificial Soil and Test Soils Preparation

The artificial soil was prepared in bulk by blending 70% sand, 20% kaolin clay and 10% sphagnum peat (Appendix 3). The pH of the bulk soil prior to hydration was adjusted to 6.0 using calcium carbonate. The bulk artificial soil was stored in a sealed container under ambient conditions until used to prepare the test soils.

- 10 -

Test soils were prepared by stirring the test substance into a portion of bulk soil before mixing it with the remainder of the soil and deionized water (Appendix 3). Sufficient deionized water was added to the dry artificial soil to achieve a moisture content of approximately 33% by weight. Test soil components were mixed for a total of 20 minutes to ensure a homogeneous mix. Negative control soil was prepared in the same manner as test soil, but without the addition of test substance. Seven hundred fifty grams of prepared soil were added to each of four test chambers for the control group, with the exception of the 625 mg a.i./kg concentration in which no more than 650 g would fit into the test chambers.

Test concentrations were adjusted for the purity of PFOS, which was reported to be 86.9% by the Sponsor. Therefore, test concentrations and the LC50 values are reported as milligrams of test substance active ingredient per kilogram of soil on a dry weight basis (mg a.i./kg). Test soils were prepared and held overnight at approximately $20 \pm 2^\circ\text{C}$ prior to adding worms to allow the soil to become less fluid. The target nominal concentrations were 78.1, 156, 313, 625, and 1250 mg a.i./kg dry soil. Mean measured concentrations at the time of soil preparation were 77.0, 141, 289, 488, and 1042 mg a.i./kg soil.

Analytical Sampling

During the test, samples of the experimental soils were collected to determine the homogeneity and stability of the test substance in the artificial soil and to verify test concentrations. Samples were collected at soil preparation to determine homogeneity, measure test concentrations, and establish Day 0 values for evaluating stability over the course of the exposure period. On Day 14, samples were collected from one replicate of the control and two replicates of each test concentration to assess stability of the test substance under test conditions.

At test termination samples of tissue from the earthworms in each treatment group were analyzed to determine the concentration of PFOS in the worm tissue from each treatment group. All surviving worms from each treatment group were placed on moistened filter paper for approximately 24 hours to purge gut contents. The entire mass of worms collected from each treatment group was composited, euthanized by freezing and stored frozen until analyzed.

Analytical Testing

Chemical analysis of the soil and worm samples was performed using HPLC/MS/MS methodology (Appendix 4).

Test Chambers

The test chambers were one-liter Nalgene® beakers covered with plastic wrap which was perforated for air exchange. All test chambers were identified with the project number, test concentration and replicate.

Physical Properties of Test Soils

Soil temperature was measured in one replicate of each treatment and control group at test initiation and termination using a hand-held thermometer. Moisture content and pH measurements were made on soil samples collected from each batch of soil prepared for treatment and control groups at test initiation. An additional soil moisture content measurement was made after test initiation to verify the soil moisture. At test termination, samples for moisture content and pH measurements were collected from one replicate of each control and treatment group. Measurements of pH were made on suspensions of soil and deionized water using a waterproof hand-held pH meter, and are reported as pH_w values. Soil moisture content was determined by measuring the initial weight of the soil sample, then weighing the soil sample after drying for at least 24 hours at approximately 105°C. The percent moisture was calculated using the following formula:

$$\% \text{ Moisture} = [(\text{wet weight} - \text{dry weight}) \div \text{wet weight}] * 100$$

Environmental Conditions

During the test, the worms were maintained in an environmental chamber set to maintain a temperature of approximately $20 \pm 2^{\circ}\text{C}$. Air temperature was measured at least once daily in the environmental chamber. The photoperiod during the test was 24 hours of continuous light per day provided by overhead fluorescent bulbs. The target light intensity during the test was approximately 400 to 800 lux, and was verified on Day 7 of the test.

- 12 -

Observations

At test initiation, the worms were placed on the surface of the soil in each test chamber and were observed for burrowing behavior. On Day 7 and Day 14 of the test, the contents of each test chamber were removed to determine the number of surviving worms. All surviving worms were observed for behavioral or pathological abnormalities. On Day 7, following observations, test soil was returned to the test chambers and the worms were placed on the soil surface in order to observe burrowing behavior. On Day 14, following observations and body weight determination, surviving worms were placed on moist filter paper in clean test chambers and allowed to purge their gut contents for approximately 24 hours. The worms were then euthanized by freezing and held prior to analytical testing.

Body Weights

On Day 0, group weights for all replicates were collected prior to being placed in the test chambers. On Day 14, all surviving worms were removed from each replicate test chamber and weighed. Prior to weighing, worms were rinsed with deionized water and blotted dry on paper towels. Group body weights were measured from each test chamber, and average individual body weights were calculated.

Reference Toxicity Test

A reference toxicity test was conducted under a separate protocol to determine the LC50 value for earthworms exposed to the reference toxicant, chloroacetamide, in the soil (2). The test was conducted under conditions similar to those used in this test, and with earthworms from the same source, to monitor the techniques used and sensitivity of the test population. The worms were exposed to chloroacetamide in the soil at nominal concentrations of 7.5, 15, 30 and 60 mg a.i./kg dry soil.

Statistical Analyses

The LC50 value and 95% confidence interval were calculated using the computer program of C.E. Stephan (3). The program was designed to calculate the LC50 value and the 95% confidence interval by probit analysis, moving average method or the binomial probability method (4,5,6). In this study, the 7-day LC50 value was calculated using the binomial probability method and the 14-Day

- 13 -

LC50 value was calculated using the moving average method. Mean body weights at test initiation and test termination as well as the mean change in body weight during the test were determined for each treatment and control group. Treatment group means were compared to control means with Dunnett's test ($\alpha=0.05$) using SAS Version 8 (7) in order to determine if significant differences from the control occurred.

RESULTS AND DISCUSSION

Environmental Conditions

Air temperature in the environmental chamber was within the desired range of approximately $20 \pm 2^\circ\text{C}$, remaining at $22\text{--}23^\circ\text{C}$ throughout the test. The worms were maintained under continuous lighting at an average intensity of 627 ± 83.9 lux, with a range over the surface of the test chambers of 494-775 lux.

Physical Properties of Test Soils

Measurements of soil pH_w , temperature and moisture content at test initiation and test termination are presented in Table 1. Soil pH_w ranged from 6.9 to 8.1 at test initiation and from 8.3 to 8.4 at test termination. Soil temperature was 22°C , within the desired range of $20 \pm 2^\circ\text{C}$ at test initiation and at test termination. At test termination the 1042 mg a.i./kg Replicate A soil temperature was 25°C . It was suspected that the Replicate A chamber was placed too close to a light ballast and was not indicative of the soil temperature for that treatment group. Replicate B was measured and found to be 22°C . Soil moisture content during the test ranged from 29.5 to 33.9% at test initiation and from 31.4 to 34.5% at test termination, indicating that there was little change in soil moisture content during the test.

Observations

The data from weekly observations of the worms for mortality and other signs of toxicity are presented in Table 2. There were no mortalities in the negative control group during the 14-day test. All control worms were normal in appearance and behavior throughout the test period. On Day 7 there was high mortality in the 488 and 1042 mg a.i./kg treatment groups. On Day 14 there was some

- 14 -

mortality in the 141 and 289 mg a.i./kg treatment groups and very high mortality in the 488 mg a.i./kg group and complete mortality in the 1042 mg a.i./kg group.

Worms in the 488 and 1042 mg a.i./kg treatment groups exhibited an aversion to the soil during observations of burrowing behavior on Day 0. On day 0 after one hour, some worms in the 488 mg a.i./kg group were not burrowed, and in the 1042 mg a.i./kg group many remained on the soil surface. After two hours, all but one worm in the 488 mg a.i./kg group had burrowed but in the 1042 mg a.i./kg group most worms remained out of the soil. On Day 7, all surviving worms burrowed into the soil within 15 minutes. On Day 7, worms found dead in the 1042 mg a.i./kg group were on the side of the test chamber not in the soil.

Statistical Analyses

A 7-Day LC50 estimation of 398 mg a.i./kg with 95% confidence limits of 289 and 488 mg a.i./kg soil was determined using the binomial probability method, since the data did not fit either the probit method or moving average method. A 14-Day LC50 was determined using the moving average method since the data did not fit the probit method. The moving average method was acceptable except for the indication that the confidence limits may be too close due to the lower mortality in the 289 mg a.i./kg soil than in the 141 mg a.i./kg soil treatment group. Therefore, the 14-day LC50 estimation for earthworms exposed to PFOS in an artificial soil substrate was determined to be 373 mg a.i./kg soil with 95% confidence limits of 316 and 440 mg a.i./kg.

After seven days of exposure the no-observed-effect-concentration, NOEC, was 289 mg a.i./kg soil and the lowest-observed-effect-concentration, LOEC, was 488 mg a.i./kg soil. The 14-Day NOEC was 77 mg a.i./kg soil and the LOEC was 141 mg a.i./kg soil.

Average individual body weights, and the change in body weight from test initiation to test termination, were calculated from the Day 0 and Day 14 replicate measurements (Table 3). A slight loss in body weight from test initiation to test termination was expected in both the control and treatment groups since the worms were not fed during the test. Mean body weight at test termination

- 15 -

as well as the mean loss in body weight among worms in the treatment groups were statistically significant ($p < 0.05$) at 488 mg a.i./kg dry soil compared to the control group.

Table 4 contains the 14 day measured values for PFOS in earthworm tissue.

Reference Toxicity Test

The 14-day LC50 value for earthworms exposed to the reference substance, chloroacetamide, in an artificial soil substrate was approximately 19.4 mg a.i./kg dry soil with a 95% confidence interval of 15 and 30 mg a.i./kg dry soil (2). These results are consistent with those observed in previous studies, and verify the adequacy and consistency of the methods used in this study (454-111).

Sample Analysis

Chemical analysis of test soils and earthworm tissue was conducted at Wildlife International, Ltd. and is reported in Appendix 4.

CONCLUSIONS

The 7-day LC50 estimation for earthworms exposed to PFOS in an artificial soil substrate was determined to be 398 mg a.i./kg. The 7-day NOEC was determined to be 289 mg a.i./kg and the 7-day LOEC was determined to be 488 mg a.i./kg. The 14-day LC50 estimation for earthworms exposed to PFOS in an artificial soil substrate was determined to be 373 mg a.i./kg. The 14-day NOEC was determined to be 77 mg a.i./kg and the LOEC was 141 mg a.i./kg.

REFERENCES

- 1 **OECD Guideline 207.** 1984. *Guideline for Testing of Chemicals, Earthworm, Acute Toxicity Tests.* Organization for Economic Cooperation and Development.
- 2 **Wildlife International, Ltd.** 2000. *Chloroacetamide: An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate.* Project No. 100R-105.
- 3 **Stephan, C.E.** 1978. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. Personal Communication.
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- 5 **Thompson, W.R.** 1947. *Bacteriological Reviews.* Vol. II, 2:115-145.
- 6 **Stephan, C.E.** 1977. Methods for Calculating an LC50. *Aquatic Toxicology and Hazard Evaluations.* American Society for Testing and Materials. Pub. No. STP 634:65-84.
- 7 **SAS Institute, Inc.** 1999. SAS/STAT User's Guide, Version 8. Cary, NC, SAS Institute, Inc.

- 17 -

Table 1

Moisture, pH_w, and Temperature of Test Soils¹

Mean Measured Concentration (mg a.i./kg)	Test Initiation Moisture Content ² (%)	Test Initiation pH _w ³	Test Initiation Temperature ³ (°C)	Test Termination Moisture Content (%)	Test Termination pH _w	Test Termination Temperature (°C)
Negative Control	33.9	6.9	22	32.1	8.3	22
77.0	31.9	7.9	22	31.4	8.4	22
141	29.5	8.1	22	31.5	8.3	22
289	31.4	8.1	22	33.0	8.3	22
488	31.0	8.1	22	32.3	8.3	22
1042	32.5	8.0	22	34.5	8.3	25 ⁴

¹Measurements taken from one replicate test chamber at each concentration.²Measurements taken from prepared soil two days after preparation.³Measurements taken from prepared soil on Day 0.⁴Measurement of soil temperature in Replicate B was 22°C.

- 18 -

Table 2

**Cumulative Mortality and Observations of Earthworms Exposed to PFOS
in an Artificial Soil Substrate**

Mean Measured Concentration (mg a.i./kg)	Rep.	Day 7		Day 14		Replicate Percent Mortality	Group Percent Mortality
		Number Dead ¹	Effects ²	Number Dead	Effects		
Negative Control	A	0/10	10 AN	0/10	10 AN	0	0
	B	0/10	10 AN	0/10	10 AN	0	
	C	0/10	10 AN	0/10	10 AN	0	
	D	0/10	10 AN	0/10	10 AN	0	
77.0	A	0/10	10 AN	0/10	10 AN	0	0
	B	0/10	10 AN	0/10	10 AN	0	
	C	0/10	10 AN	0/10	10 AN	0	
	D	0/10	10 AN	0/10	10 AN	0	
141	A	0/10	10 AN	2/10	8 AN	20	7.5
	B	0/10	10 AN	0/10	10 AN	0	
	C	0/10	9 AN, 1T+S	1/10	9 AN	10	
	D	0/10	10 AN	0/10	10 AN	0	
289	A	0/10	10 AN	0/10	10 AN	0	2.5
	B	0/10	10 AN	0/10	10 AN	0	
	C	0/10	10 AN	0/10	8 AN, 2 R	0	
	D	0/10	10 AN	1/10	9 AN	10	
488	A	9/10	1 AN	10/10	—	100	95
	B	8/10	2 AN	8/10	2 T + R	80	
	C	10/10	—	10/10	—	100	
	D	9/10	1 AN	10/10	—	100	
1042	A	10/10	—	10/10	—	100	100
	B	10/10	—	10/10	—	100	
	C	10/10	—	10/10	—	100	
	D	10/10	—	10/10	—	100	

¹ Mortality data are presented as the cumulative number dead per number exposed.

² Observed Effects: AN = normal in appearance and behavior, T=thin, S=soft, R=reduced reaction to mechanical stimuli

- 19 -

Table 3

Average Body Weights of Earthworms Exposed to PFOS in an Artificial Soil Substrate

Mean Measured Concentration (mg a.i./kg)	Replicate	Average Earthworm Body Weights (g)		
		Day 0	Day 14	Total Change
Negative Control	A	0.29	0.21	-0.08
	B	0.23	0.19	-0.04
	C	0.23	0.18	-0.05
	D	0.26	0.20	-0.06
	Mean $\pm$ Std. Dev.	0.25 $\pm$ 0.029	0.20 $\pm$ 0.013	-0.06 $\pm$ 0.017
77.0	A	0.25	0.20	-0.05
	B	0.25	0.19	-0.06
	C	0.26	0.20	-0.06
	D	0.25	0.18	-0.07
	Mean $\pm$ Std. Dev.	0.25 $\pm$ 0.005	0.19 $\pm$ 0.010	-0.06 $\pm$ 0.008
141	A	0.26	0.21	-0.05
	B	0.27	0.17	-0.10
	C	0.23	0.14	-0.09
	D	0.26	0.20	-0.06
	Mean $\pm$ Std. Dev.	0.26 $\pm$ 0.017	0.18 $\pm$ 0.031	-0.07 $\pm$ 0.024
289	A	0.27	0.17	-0.10
	B	0.25	0.17	-0.08
	C	0.23	0.16	-0.07
	D	0.25	0.18	-0.07
	Mean $\pm$ Std. Dev.	0.25 $\pm$ 0.016	0.17 $\pm$ 0.007	-0.08 $\pm$ 0.014
488	A	0.24	—	—
	B	0.25	0.08	-0.17
	C	0.23	—	—
	D	0.24	—	—
	Mean $\pm$ Std. Dev.	0.24 $\pm$ 0.008	0.08 $\pm$ — ¹	-0.17 $\pm$ — ²
1042	A	0.25	—	—
	B	0.25	—	—
	C	0.24	—	—
	D	0.25	—	—
	Mean $\pm$ Std. Dev.	0.25 $\pm$ 0.005	— $\pm$ —	— $\pm$ —

¹ Body weight was statistically different from ($p < 0.05$) the control groups using Dunnett's test.² Change in body weight was statistically significant ($p < 0.05$) at test concentration when compared to the control groups using Dunnett's test.

- 20 -

Table 4

Day 14 Measured Values for PFOS in Earthworm Tissue

Sample Number (454-111-)	Nominal Exposure	PFOS Concentration (mg a.i./kg)	Mean Measured (mg a.i./kg)
		Measured ¹	
W-1	0.0	< LOQ ²	--
W-2A	78.1	189	195
W-2B	78.1	201	
W-3A	156	209	203
W-3B	156	196	
W-4A	313	240	252
W-4B	313	263	
W-5	625	1105	--

¹ Measured values were calculated using MacQuan, version 1.6 software. Manual calculations may vary slightly.

² The method limit of quantitation (LOQ) in earthworm tissue was 50.0 mg a.i./kg on a wet-weight basis and was calculated as the product of the lowest calibration standard (0.000500 mg a.i./L) and the weight/volume dilution factor of the matrix blank sample (100,000 L/kg).

- 21 -

Appendix 1

Personnel Involved in the Study

The following key Wildlife International, Ltd. personnel were involved in the conduct or management of this study:

1. Henry O. Krueger, Ph.D., Director, Aquatic Toxicology and Non-Target Plants
2. John R. Porch, Supervisor, Non-Target Plants and Insects
3. Anne B. Sindermann, Senior Biologist
4. Raymond L. Van Hoven, Ph.D., Scientist, Analytical Chemistry

- 22 -

Appendix 2

Changes to the Study Protocol

This study was conducted according to the approved study protocol with the following changes:

1. Test soils were prepared, and analytical samples collected, one day prior to test initiation.
2. An additional soil moisture measurement was made.
3. Soil moisture for the 156 mg a.i./kg level was 29.5%, slightly below approximately 33%.
4. Test soil for the 625 mg a.i./kg level weighed 650 g rather than 750 g.
5. Earthworms used for the study weighed less than between 300 and 600 mg.
6. Earthworms were conditioned for approximately 18.5 hours rather than 24.
7. The soil temperature in Replicate A of the 1250 mg a.i./kg treatment group was 25°C at test termination, exceeding the 20±2°C range specified in the protocol.

- 23 -

Appendix 3**Artificial Soil and Test Soils Preparation****Artificial Soil Preparation**

The artificial soil was prepared in bulk by mixing the following constituents in a soil mixer for approximately 20 minutes:

quartz sand	31.500 kg
kaolin clay	9.000 kg
sphagnum peat	4.500 kg
calcium carbonate	1.125 kg

Test Soil Preparation

A total of approximately 4 kg (wet weight) of test soil with a calculated soil moisture content of approximately 33% was prepared for the negative control and each of the treatment groups. Test substance concentrations were based on the dry weight portion of the soil. The calculated dry weight of 4 kg of prepared soil, with a moisture content of 33%, was 2.680 kg. To account for the 4% moisture content typically present in the bulk soil stored in ambient conditions, 1208 mL of water were added to 2680 g. of bulk soil minus the weight of test substance. Amounts of test substance, bulk soil and water were as follows:

Nominal Weights and Volumes to Prepare Test Soils				
Concentration (mg a.i./kg test soil)	Required Amount of Test Substance (g)	Actual Amount of Test Substance (g)	Bulk Soil (g)	Water (mL)
Negative control	---	---	2680.0	1208
78.1	0.241	0.2410	2679.8	1208
156	0.482	0.4819	2679.5	1208
313	0.965	0.9650	2679.0	1208
625	1.927	1.9270	2678.1	1208
1250	3.854	3.8541	2676.2	1208

The appropriate amount of bulk soil was weighed into a tared Hobart® mixer bowl. Approximately a handful of dry soil was removed to a Nalgene® beaker and the measured amount of test substance added and mixed in with a metal spatula, after using dry soil to clean test substance off

- 24 -

of the weigh boat. The soil and test substance mixture was added to the Hobart bowl. 1208 mL of deionized water was added and the soil mixed for 5 minutes. After the 5-minute mix, the sides of the bowl were scraped and the soil was mixed for an additional 15 minutes. Prepared test soil, 750 g for each replicate, except for the 625 mg a.i./kg concentration, which received 650 g, was placed into test chambers labeled with concentration and replicate. Negative control soil was prepared in the same manner, without the addition of test substance, and 750 g was placed into each negative control test chamber.

- 25 -

Appendix 4

THE ANALYSIS OF PFOS IN ARTIFICIAL SOIL AND EARTHWORM TISSUE

IN SUPPORT OF

WILDLIFE INTERNATIONAL, LTD. PROJECT NO.: 454-111

- 26 -

REPORT APPROVAL

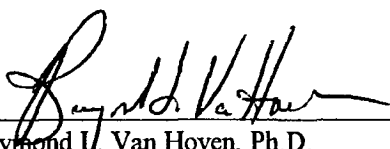
SPONSOR: 3M

TITLE: PFOS: AN ACUTE TOXICITY STUDY WITH THE EARTHWORM IN AN
ARTIFICIAL SOIL SUBSTRATE

WILDLIFE INTERNATIONAL, LTD. PROJECT NO.: 454-111

3M ENVIRONMENTAL LAB PROJECT NUMBER: U2723

PRINCIPAL INVESTIGATOR:

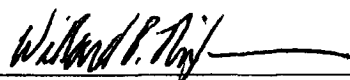


Raymond L. Van Hoven, Ph.D.
Scientist

5-10-02

DATE

MANAGEMENT:



Willard B. Nixon, Ph.D.
Director, Analytical Chemistry

5/10/02

DATE

Introduction

This study was conducted by Wildlife International, Ltd. for 3M at the Wildlife International, Ltd. analytical chemistry facility in Easton, Maryland. Samples were collected for the determination of Perfluorooctanesulfonate, Potassium Salt (PFOS) in artificial soil and earthworm tissue and were analyzed using high performance liquid chromatography with triple quadrupole mass spectrometric detection (HPLC/MS/MS). Samples were prepared and analyzed between November 27 and 28, 2001 and December 13, 2001. Raw data generated by Wildlife International, Ltd. and a copy of the final report are filed under Project Number 454-111 in archives located on the Wildlife International, Ltd. site.

Analytical Methodology**Artificial Soil**

Submitted artificial soil samples were analyzed for PFOS according to the validated procedures documented in WIL study number 454C-120. The entire submitted soil sample was blended for approximately two minutes prior to sub-sampling the requisite 10-g aliquot for the analytical determination. The artificial soil sub-samples were extracted with methanol, agitated for a minimum of 30 minutes on a gyratory shaker table at approximately 250 rpm and then vacuum filtered with qualitative filter paper. The filtrate was then transferred to a 200-mL volumetric flask and brought to volume with methanol. Approximately 20 milliliters of each sample was transferred to a separate vial and centrifuged for approximately 5 minutes at 2000 rpm. Dilutions into the calibration range of the HPLC/MS/MS methodology were performed with a solution of 50% methanol (HPLC grade, 99.9+%) and 50% NANOpure[®] water. Samples were then analyzed by direct injection. Concentrations of PFOS were determined by reverse-phase high performance liquid chromatography using a Hewlett-Packard Model 1100 High Performance Liquid Chromatograph (HPLC) with a Perkin-Elmer API 3000LC Mass Spectrometer equipped with a Perkin-Elmer TurboIonSpray ion source. Chromatographic separations were achieved using a Keystone Betasil C₁₈ column (50 mm x 2.0 mm, 3- μ m particle size) fitted with a Keystone Javelin C₁₈ guard column (20 mm x 2.0 mm). A method flowchart is provided in Figure 1 and the instrument parameters are summarized in Table 1.

Earthworm Tissue

Submitted earthworm tissue samples were analyzed for PFOS according to methodology developed at Wildlife International, Ltd. Approximately one gram of each earthworm tissue sample was weighed directly into tared scintillation vials to which 10.0 milliliters of methanol extraction solvent were added. The samples were concurrently extracted and homogenized using a tissue shredder (Janke and Kunkel) followed by sonic dismembration. The sample extracts were centrifuged and diluted into the calibration range of the HPLC/MS/MS methodology. Dilutions were performed with a solution of 50% methanol (HPLC grade, 99.9+%) and 50% NANOpure® water. Samples were then analyzed by direct injection HPLC/MS/MS as described previously. A method flowchart is provided in Figure 2.

Primary and Secondary Stock Solutions

All primary and secondary stock preparations were adjusted for the purity of the test substance (86.9%). A 10.0 mg a.i./mL primary stock solution of PFOS in methanol was prepared by weighing 1.1508 g of the test substance and bringing to a final volume of 100 milliliters with methanol. Secondary stock solutions (1000, 100, 10.0, 1.00, and 0.100 mg a.i./L) of PFOS in methanol were prepared by serial volumetric dilution from the primary stock.

Calibration Standards and Calibration Curves

Calibration standards were prepared in 50:50 methanol: NANOpure® water by appropriate dilutions of the 1.00 mg a.i./L stock solutions of PFOS in methanol. Calibration standards of PFOS, ranging in concentration from 1.00 to 10.0 µg a.i./L, were analyzed with each artificial soil sample set and calibration standards of PFOS, ranging in concentration from 0.500 to 5.00 µg a.i./L, were analyzed with the earthworm tissue sample set. The same and most prominent peak response for PFOS was utilized to monitor PFOS in all calibration and study samples. No attempt was made to quantify PFOS on the basis of individual isomeric components. The calibration standard series was injected at the beginning and end of each run, and one standard was injected, at a minimum, after every five samples. Linear regression equations were generated using peak area responses versus the respective concentrations of the calibration standards. A typical calibration curve for PFOS in artificial soil and earthworm tissue is presented in Figure 3 and Figure 4, respectively. Representative ion

- 29 -

chromatograms of low and high calibration standards for the artificial soil are presented in Figures 5 and 6, respectively and representative ion chromatograms of low and high calibration standards for PFOS in earthworm tissue are presented in Figure 7 and 8, respectively. The concentrations of PFOS in the samples were determined by substituting peak area responses into the applicable linear regression equation. Concentrations of PFOS in the samples were calculated on a dry weight (soil samples) or wet weight (earthworm tissue) basis using the at-instrument PFOS concentrations and the appropriate weight/volume dilution and/or moisture correction factors.

Matrix Blanks and Matrix Fortifications

Artificial Soil

Selected 20.0-g aliquots of artificial soil were fortified with the appropriate stock solutions of PFOS prepared in methanol using a gas-tight syringe. The fortified soils were then homogenized using a Waring blender for approximately 2 minutes. A 10-g aliquot of each homogenized fortified soil sample was weighed into a tared weigh boat and transferred to an 8-oz. French-square bottle for extraction. The matrix blanks were unfortified artificial soil.

Earthworm Tissue

A 1.00-g aliquot of negative control earthworm tissue was fortified with the 1000 mg a.i./L secondary stock solution of PFOS prepared in methanol using a gas-tight syringe. A matrix blanks was prepared with unfortified negative control earthworm tissue.

Limits of Quantitation

Artificial Soil

The method limit of quantitation (LOQ) in artificial soil was 29.3 mg a.i./kg on a dry-weight basis and was calculated as the product of the lowest calibration standard (0.00100 mg a.i./L), the weight/volume dilution factor of the matrix blank samples (20,000) divided by the percent solids for the test system soil (68.3%).

- 30 -

Earthworm Tissue

The method limit of quantitation (LOQ) in earthworm tissue was 50.0 mg a.i./kg on a wet-weight basis and was calculated as the product of the lowest calibration standard (0.000500 mg a.i./L) and the weight/volume dilution factor of the matrix blank sample (100,000 L/kg).

Example CalculationsExample Calculations

Sample number 454-111-5, nominal concentration of 78.1 mg a.i./kg in artificial soil.

First Initial Weight: 10.0 g

First Final Volume: 200 mL

Second Initial Volume: 0.0500 mL

Second Final Volume: 50.0 mL

Dilution Factor: 20000

Mean Soil Moisture Content: 31.7%

Percent Solids: 68.3%

LCMS Dilution Factor: $20000/0.683 = 29283$

PFOS Peak Area: 23914

Calibration curve equation:

Slope: 8981195

Intercept: 1011.0532

$$\text{PFOS (mg a.i./kg) measured at instrument} = \frac{\text{peak area} - (\text{y-intercept})}{\text{slope}}$$

PFOS (mg a.i./kg) in sample = PFOS measured at instrument (mg a.i./kg) $\times$ LCMS dilution factor

$$= \frac{23914 - 1011.0532}{8981195} \times 29283$$

$$= 74.67$$

$$\text{Percent of Nominal Concentration} = \frac{\text{PFOS (mg a.i./Kg) in sample}}{\text{PFOS (mg a.i./Kg) nominal}} \times 100$$

$$= \frac{74.67}{78.1} \times 100 = 95.6\%$$

Calculated with HPLC/MS/MS instrument software: MacQuan, version 1.6.

RESULTS

Matrix blank samples for each matrix type were analyzed to determine possible interferences. No interferences were observed at or above the corresponding LOQ (Tables 2 and 3). Representative ion chromatograms of artificial soil and earthworm tissue matrix blank samples are presented in Figures 9 and 10, respectively. Matrix fortification samples for artificial soil had recoveries ranging from 75.6% to 103.2% of the nominal concentrations with a mean recovery of 87.3% of nominal (SD=11.2; CV=12.8). A representative ion chromatogram of an artificial soil matrix fortification is presented in Figure 11. The earthworm tissue matrix fortification (Figure 12) had a recovery of 86.6% of the nominal concentration.

Artificial soil samples collected at Day 0 from the 78.1 mg a.i./kg, 156 mg a.i./kg, 313 mg a.i./kg, 625 mg a.i./kg and 1250 mg a.i./kg treatment levels had mean measured PFOS concentrations of 77.0 mg a.i./kg (SD=13.6; CV=17.7%, n=6), 141 mg a.i./kg (n=2), 289 mg a.i./kg (n=2), 488 mg a.i./kg (n=2), and 1042 mg a.i./kg (SD=201; CV=19.3%, n=6), respectively (Table 4). These values corresponded to mean percent recoveries of 98.6%, 90.4%, 92.3%, 78.1% and 83.4% of the nominal concentrations with an overall mean percent recovery of 89.6% (SD=15.3; CV=17.1%, n=18). A representative ion chromatogram of an artificial soil test sample is presented in Figure 13.

Artificial soil samples collected at Day 14 from the 78.1 mg a.i./kg, 156 mg a.i./kg, 313 mg a.i./kg, 625 mg a.i./kg and 1250 mg a.i./kg treatment levels had mean measured PFOS concentrations of 80.6 mg a.i./kg (n=2), 143 mg a.i./kg (n=2), 305 mg a.i./kg (n=2), 634 mg a.i./kg (n=2) and 1094 mg a.i./kg (n=2), respectively (Table 5). These values corresponded to mean percent recoveries of 103%, 91.7%, 97.4%, 101% and 87.5% of the nominal concentrations with an overall mean percent recovery of 96.3% (SD=10.7; CV=11.2%, n=10).

Earthworm tissue analyzed on Day 14 had mean measured concentrations of 195, 203 and 252 mg a.i./kg for nominal exposure levels of 78.1, 156, and 313 mg a.i./kg, respectively (Table 6). The measured value (1105 mg a.i./kg) for the 625 mg a.i./kg exposure level was suspect due to the limited sample size (3 worms, approximately 0.2 g total weight) available from this treatment group. A representative ion chromatogram of an earthworm tissue test sample is presented in Figure 14.

- 32 -

REFERENCES

1. **American Society for Testing and Materials.** 1991. Standard Specification for Reagent Water. D1193-91, ASTM Section II Water and Environmental Technology, Vol. 11.01:45-47.

- 33 -

Table 1

Typical HPLC/MS Operational Parameters

INSTRUMENT:	Hewlett-Packard Model 1100 High Performance Liquid Chromatograph with a Perkin-Elmer API 3000 Mass Spectrometer equipped with a Perkin-Elmer TurboIonSpray ion source. Operated in multiple ion reaction monitoring (MRM) mode.
ION SOURCE:	Perkin-Elmer TurboIonSpray
ANALYTICAL COLUMN:	Keystone Betasil C ₁₈ (50 mm × 2.0 mm, 3-μm particle size)
GUARD COLUMN:	Keystone Javelin C ₁₈ cartridge (20 mm × 2 mm)
OVEN TEMPERATURE:	40°C
STOP TIME:	5.00 min
FLOW RATE:	250 μL/min
MOBILE PHASE:	30% NANOpure® Water with 0.1% Formic Acid: 70% Methanol
INJECTION VOLUME:	5.0 μL
PFOS PEAK RETENTION TIME:	Approximately 4 minutes
PFOS MONITORED MASS:	499.0 amu → 99.1 amu

- 34 -

Table 2

Artificial Soil Matrix Blanks and Fortifications Analyzed Concurrently During Sample Analysis

Number (454-111-)	Sample Type	PFOS Concentration (mg a.i./kg)		Percent Recovered ¹	Mean Measured (mg a.i./kg)	Mean Percent Recovery
		Fortified	Measured ¹			
MAB - 1	Soil Matrix Blank	0.0	< LOQ ²	--	--	--
MAB - 2	Soil Matrix Blank		< LOQ ²	--	--	--
MAS - 1	Soil Matrix Fortification	36.6	28.5	77.7	28.9	79.0
MAS - 4	Soil Matrix Fortification		29.3	80.1		
MAS - 2	Soil Matrix Fortification	586	565	96.4	549	93.7
MAS - 5	Soil Matrix Fortification		533	90.9		
MAS - 3	Soil Matrix Fortification	1464	1503	103	1305	89.1
MAS - 6	Soil Matrix Fortification		1106	75.6		
Overall Mean =				87.3		
Standard Deviation =				11.2		
CV =				12.8		
N =				6		

¹ Measured and Percent Recovered values were calculated using MacQuan, version 1.6 software. Manual calculations may vary slightly.

² The method limit of quantitation (LOQ) in artificial soil was 29.3 mg a.i./kg on a dry-weight basis and was calculated as the product of the lowest calibration standard (0.00100 mg a.i./L), the weight/volume dilution factor of the matrix blank samples (20,000) divided by the percent solids for the test system soil (68.3%).

- 35 -

Table 3

Earthworm Tissue Matrix Blank and Fortification Analyzed Concurrently During Sample Analysis

Sample		PFOS Concentration (mg a.i./kg)		Percent Recovered ¹
Number (454-111-)	Type	Fortified	Measured ¹	
WMAB – 1	Worm Tissue Matrix Blank	0.0	< LOQ ²	--
WMAS – 1	Worm Tissue Matrix Fortification	100	86.6	86.6

¹ Measured and Percent Recovered values were calculated using MacQuan, version 1.6 software. Manual calculations may vary slightly.

² The method limit of quantitation (LOQ) in earthworm tissue was 50.0 mg a.i./kg on a wet-weight basis and was calculated as the product of the lowest calibration standard (0.000500 mg a.i./L) and the weight/volume dilution factor of the matrix blank sample (100,000 L/kg).

- 36 -

Table 4

Day 0 Recoveries for PFOS in Artificial Soil

Sample		PFOS Concentration (mg a.i./kg)		Mean Measured (mg a.i./kg)	Mean Percent Recovery
Number (454-111-)	Nominal Concentration	Measured ¹	Percent Recovered ¹		
1	0.0	< LOQ ²	--	--	--
2	78.1	74.6	95.5	$\bar{x}$ = 77.0 SD = $\pm$ 13.6 CV = 17.7%	98.6
3	78.1	72.7	93.1		
4	78.1	104	133		
5	78.1	74.7	95.6		
6	78.1	69.4	88.9		
7	78.1	66.5	85.1		
8	156	144	92.1	141	90.4
9	156	138	88.4		
10	313	312	99.6	289	92.3
11	313	266	85.0		
12	625	526	84.1	488	78.1
13	625	449	71.9		
14	1250	1152	92.1	$\bar{x}$ = 1042 SD = $\pm$ 201 CV = 19.3%	83.4
15	1250	906	72.4		
16	1250	894	71.5		
17	1250	930	74.4		
18	1250	1405	112		
19	1250	966	77.3		
Overall Mean =			89.6		
Standard Deviation =			15.3		
CV =			17.1%		
N =			18		

¹ Measured and Percent Recovered values were calculated using MacQuan, version 1.6 software. Manual calculations may vary slightly.

² The method limit of quantitation (LOQ) in artificial soil was 29.3 mg a.i./kg on a dry-weight basis and was calculated as the product of the lowest calibration standard (0.00100 mg a.i./L), the weight/volume dilution factor of the matrix blank samples (20,000) divided by the percent solids for the test system soil (68.3%).

- 37 -

Table 5

Day 14 Recoveries for PFOS in Artificial Soil

Number (454-111-)	Sample Nominal Concentration	PFOS Concentration (mg a.i./kg)		Mean Measured (mg a.i./kg)	Mean Percent Recovery
		Measured ¹	Percent Recovered ¹		
20	0.0	< LOQ ²	--	--	--
21	78.1	74.9	95.9	80.6	103
22	78.1	86.2	110.4		
23	156	143	91.4	143	91.7
24	156	142	91.1		
25	313	302	96.5	305	97.4
26	313	308	98.3		
27	625	532	85.1	634	101
28	625	735	118		
29	1250	1059	84.7	1094	87.5
30	1250	1129	90.4		
Overall Mean =			96.3		
Standard Deviation =			10.7		
CV =			11.2%		
N =			10		

¹ Measured and Percent Recovered values were calculated using MacQuan, version 1.6 software. Manual calculations may vary slightly.

² The method limit of quantitation (LOQ) in artificial soil was 29.3 mg a.i./kg on a dry-weight basis and was calculated as the product of the lowest calibration standard (0.00100 mg a.i./L), the weight/volume dilution factor of the matrix blank samples (20,000) divided by the percent solids for the test system soil (68.3%).

- 38 -

Table 6

Day 14 Measured Values for PFOS in Earthworm Tissue

Number (454-111-)	Sample Nominal Exposure	PFOS Concentration (mg a.i./kg)	
		Measured ¹	Mean Measured (mg a.i./kg)
W-1	0.0	< LOQ ²	--
W-2A	78.1	189	195
W-2B	78.1	201	
W-3A	156	209	203
W-3B	156	196	
W-4A	313	240	252
W-4B	313	263	
W-5	625	1105	--

¹ Measured values were calculated using MacQuan, version 1.6 software. Manual calculations may vary slightly.

² The method limit of quantitation (LOQ) in earthworm tissue was 50.0 mg a.i./kg on a wet-weight basis and was calculated as the product of the lowest calibration standard (0.000500 mg a.i./L) and the weight/volume dilution factor of the matrix blank sample (100,000 L/kg).

- 39 -

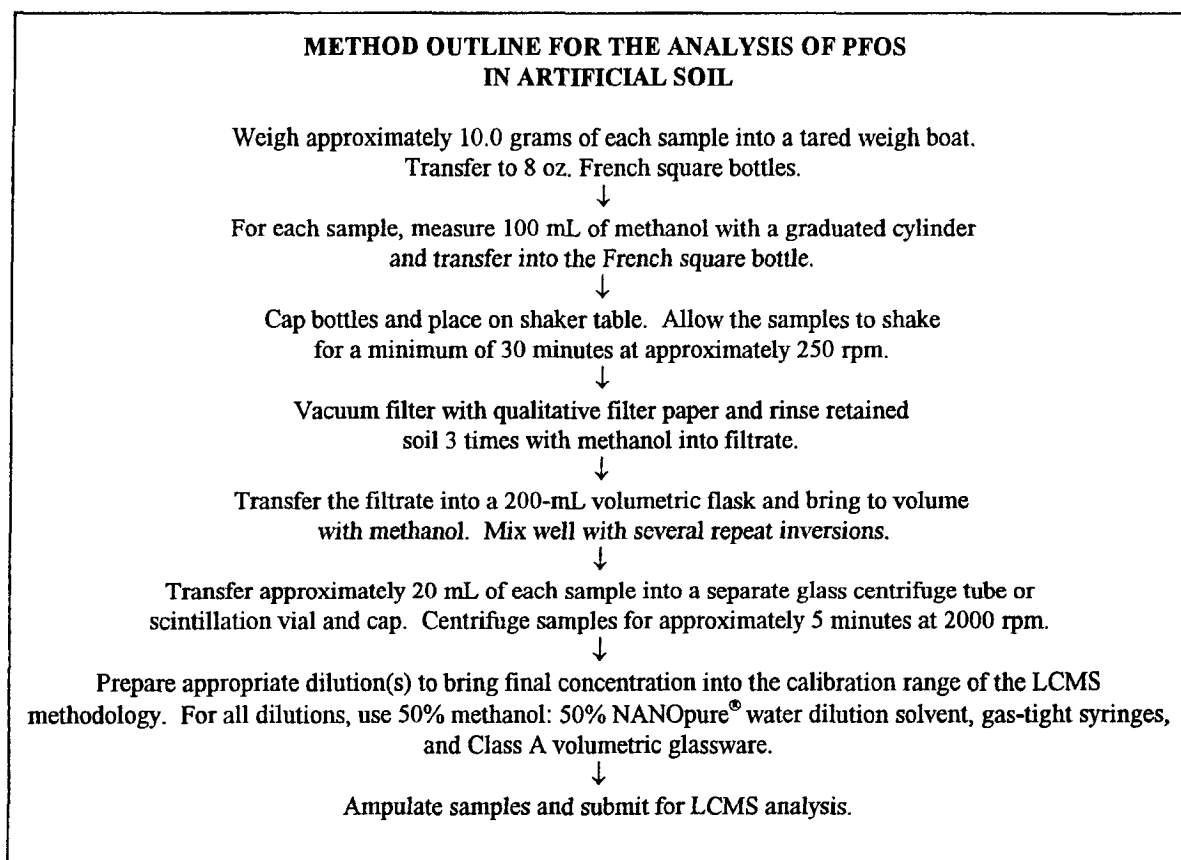


Figure 1. Analytical method flowchart for the analysis of PFOS in artificial soil.

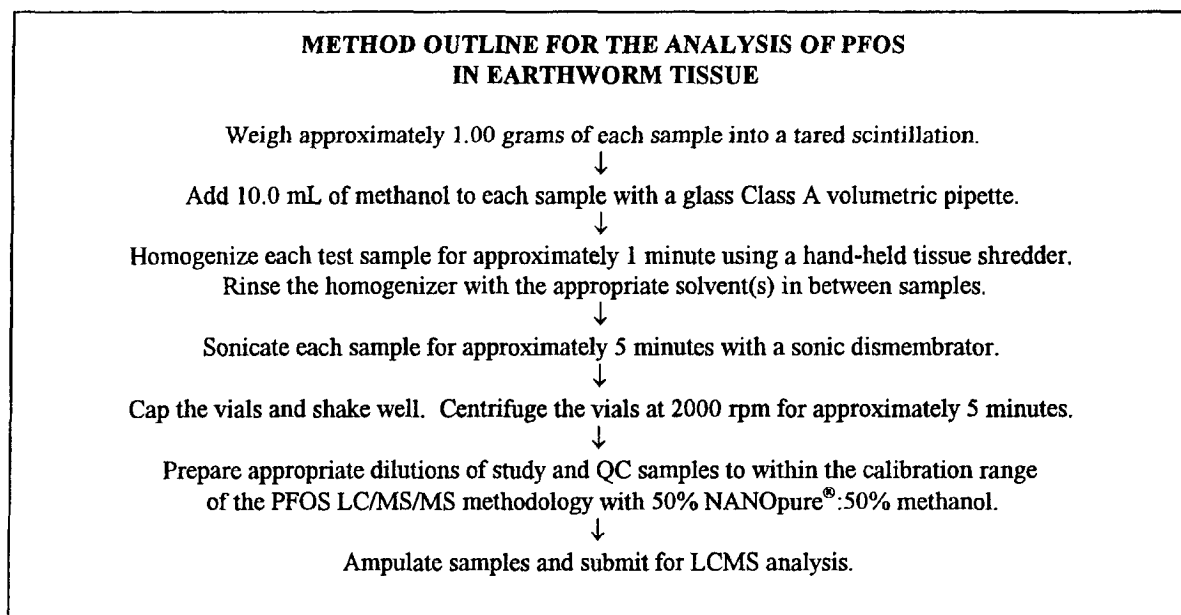


Figure 2. Analytical method flowchart for the analysis of PFOS in earthworm tissue.

- 41 -

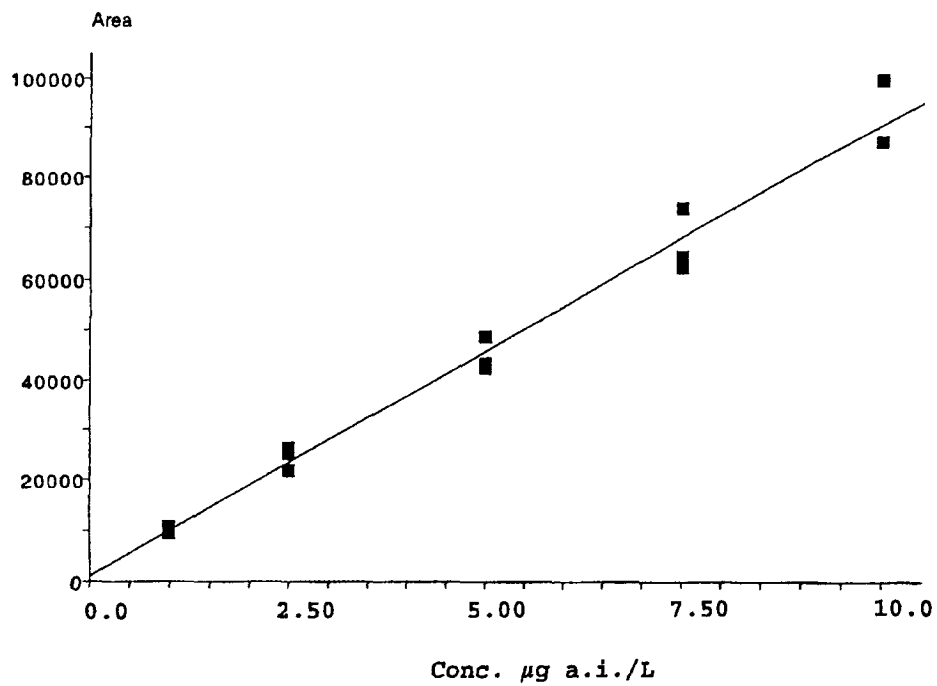


Figure 3. A typical calibration curve for PFOS (artificial soil). Slope = 8981195; Intercept = 1011.0532; $r = 0.994$.

- 42 -

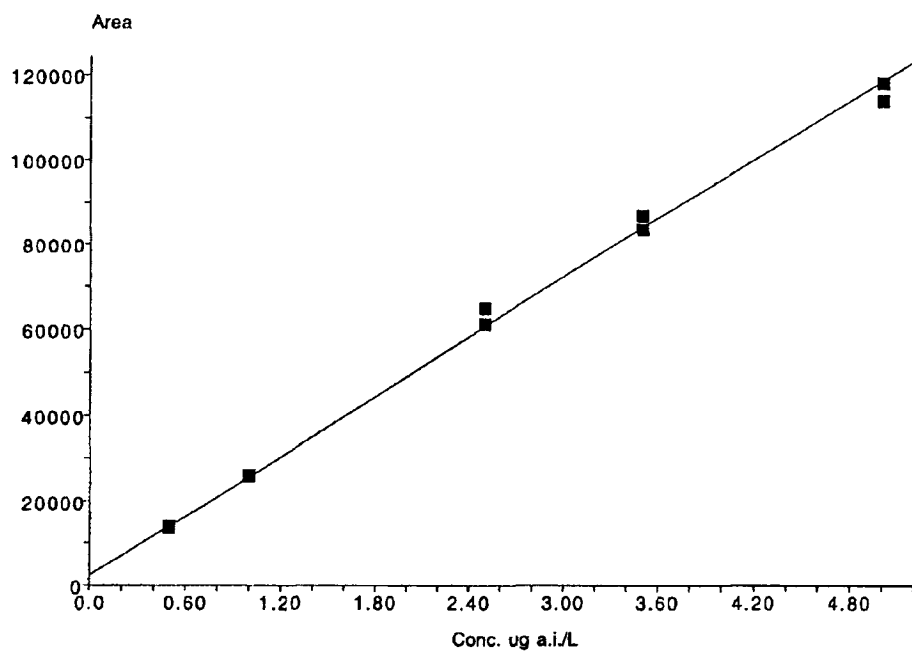


Figure 4. A typical calibration curve for PFOS (earthworm tissue). Slope =23387; Intercept =2337.9114; $r=0.999$.

- 43 -

PFOS_1 STD 0.00100 mg a.i./L
4675A-011D-16

Thu, Nov 29, 2001 05:52

4.98 in 1 period

PFOS

No Internal Standard

Use Area

1: 4.97 MRM, 298 scans

499.0->99.1

Noise Thres. 30.0

Quant Thres. 1.0

Min. Width 3

Mult. Width 6

Base. Width 40

RT Win. (secs) 20

Smooth 1

Expected RT 4.04

Area 10695

Height 1148

Start Time 3.67

End Time 4.34

Integration Width 0.67

Retention Time 4.00

Integration Type A - BB

intensity: 10000 cps

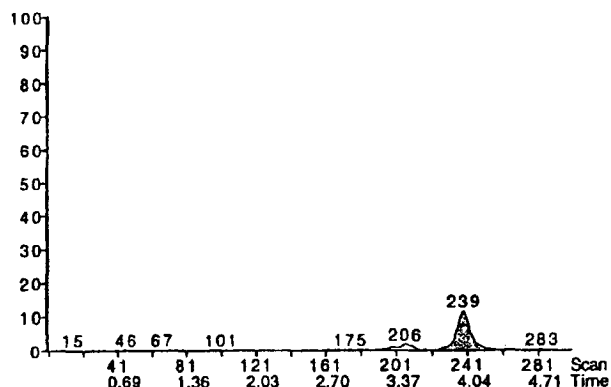


Figure 5. A representative ion chromatogram of a low-level (0.00100 mg a.i./L) PFOS standard (artificial soil).

- 44 -

PFOS_5 STD 0.0100 mg a.i./L Thu, Nov 29, 2001 08:16
4675A-011D-20

4.98 in 1 period
PFOS
No Internal Standard
Use Area

1: 4.97 MRM, 298 scans
499.0->99.1
Noise Thres. 30.0
Quant Thres. 1.0
Min. Width 3
Mult. Width 6
Base. Width 40
RT Win. (secs) 20
Smooth 1
Expected RT 4.04
Area 99915
Height 10171
Start Time 3.70
End Time 4.37
Integration Width 0.67
Retention Time 3.99
Integration Type A - VB

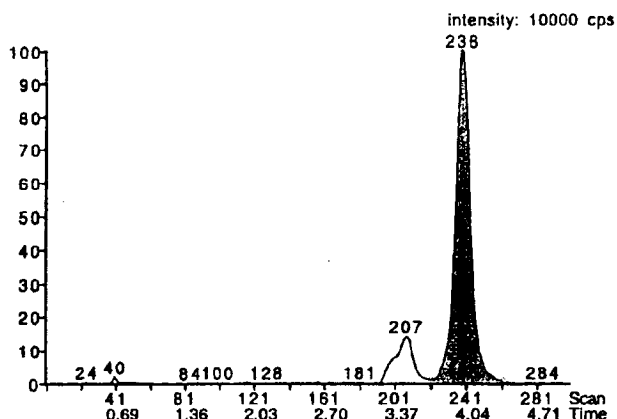


Figure 6. A representative ion chromatogram of a high-level (0.0100 mg a.i./L) PFOS standard (artificial soil).

- 45 -

PFOS_1 STD 0.500 ug a.i./L Fri, Dec 14, 2001 12:43
4675A-011D-6

4.98 in 1 period

PFOS

No Internal Standard

Use Area

1: 4.97 MRM, 298 scans

499.0->99.1

Noise Thres. 30.0

Quant Thres. 1.0

Min. Width 3

Mult. Width 6

Base. Width 40

RT Win. (secs) 20

Smooth 1

Expected RT 4.05

Area 13949

Height 1470

Start Time 3.77

End Time 4.41

Integration Width 0.64

Retention Time 4.05

Integration Type A - BB

intensity: 15000 cps

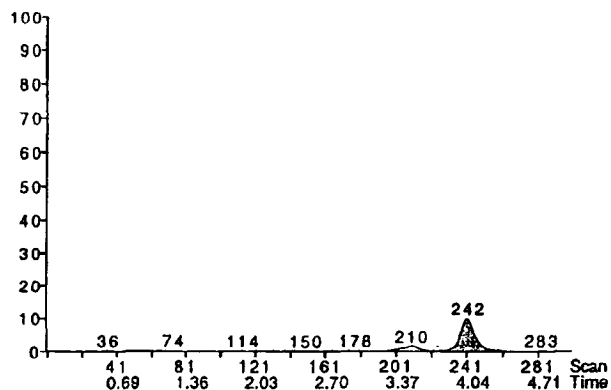


Figure 7. A representative ion chromatogram of a low-level (0.000500 mg a.i./L) PFOS standard (earthworm tissue).

- 46 -

PFOS_5 STD 5.00 ug a.i./L Fri, Dec 14, 2001 13:07
4675A-011D-10

4.98 in 1 period

PFOS

No Internal Standard

Use Area

1: 4.97 MRM, 298 scans

499.0->99.1

Noise Thres. 30.0

Quant Thres. 1.0

Min. Width 3

Mult. Width 6

Base. Width 40

RT Win. (secs) 20

Smooth 1

Expected RT 4.05

Area 118405

Height 12241

Start Time 3.70

End Time 4.29

Integration Width 0.59

Retention Time 3.94

Integration Type A - VB

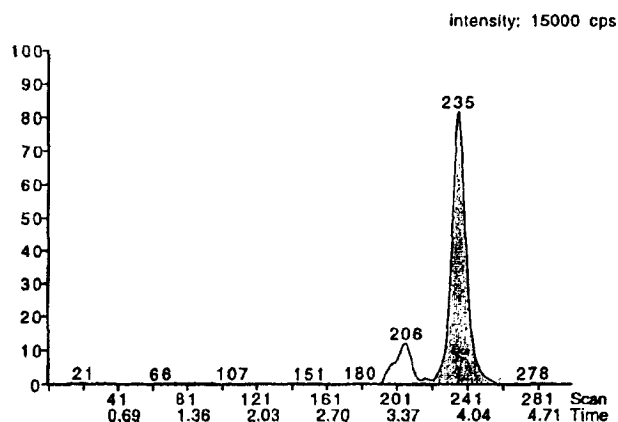


Figure 8. A representative ion chromatogram of a high-level (0.00500 mg a.i./L) PFOS standard (earthworm tissue).

- 47 -

PFOS_7 MAB-1 Thu, Nov 29, 2001 06:28
454-111-

4.98 in 1 period

PFOS

No Internal Standard

Use Area

1: 4.97 MRM, 298 scans

499.0->99.1

Noise Thres. 30.0

Quant Thres. 1.0

Min. Width 3

Mult. Width 6

Base. Width 40

RT Win. (secs) 20

Smooth 1

Expected RT 4.04

Area 0

Height 0

Start Time 0.00

End Time 0.00

Integration Width 0.00

Retention Time 0.00

Integration Type

intensity: 10000 cps

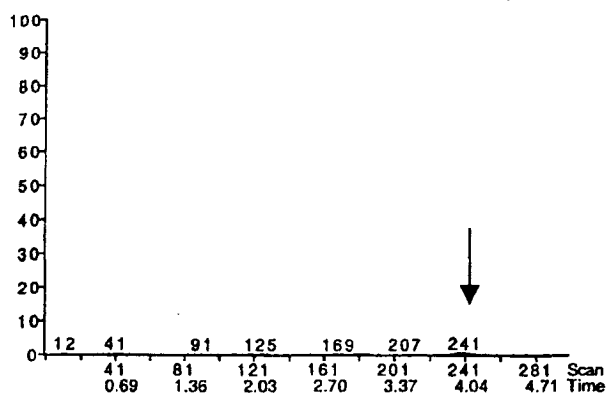


Figure 9. A representative ion chromatogram of an artificial soil matrix blank sample (454-111-MAB-1). The arrow indicates the approximate retention time of PFOS.

- 48 -

PFOS_7 WMAB-1 Fri, Dec 14, 2001 13:20
454-111-

4.98 in 1 period

PFOS

No Internal Standard

Use Area

1: 4.97 MRM, 298 scans

499.0->99.1

Noise Thres. 30.0

Quant Thres. 1.0

Min. Width 3

Mult. Width 6

Base. Width 40

RT Wln. (secs) 20

Smooth 1

Expected RT 4.05

Area 0

Height 0

Start Time 0.00

End Time 0.00

Integration Width 0.00

Retention Time 0.00

Integration Type

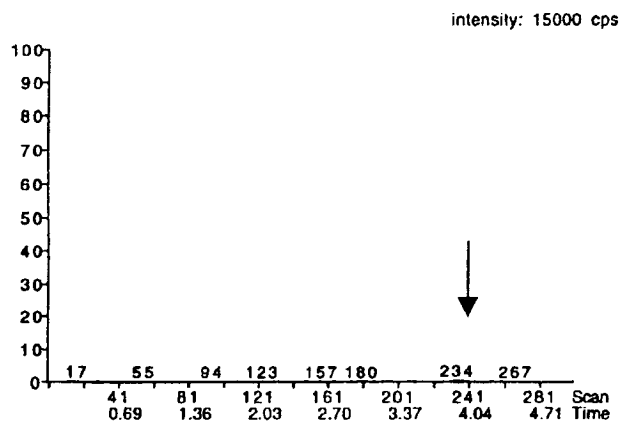


Figure 10. A representative ion chromatogram of an earthworm tissue matrix blank sample (454-111-WMAB-1). The arrow indicates the retention time of PFOS.

- 49 -

PFOS_9 MAS-2 Thu, Nov 29, 2001 06:40
454-111-

4.98 in 1 period
PFOS
No Internal Standard
Use Area
1: 4.97 MRM, 298 scans
499.0->99.1
Noise Thres. 30.0
Quant Thres. 0.5
Min. Width 3
Mult. Width 6
Base. Width 40
RT Win. (secs) 20
Smooth 1
Expected RT 4.04
Area 35671
Height 3641
Start Time 3.69
End Time 4.36
Integration Width 0.67
Retention Time 4.00
Integration Type A* - VB

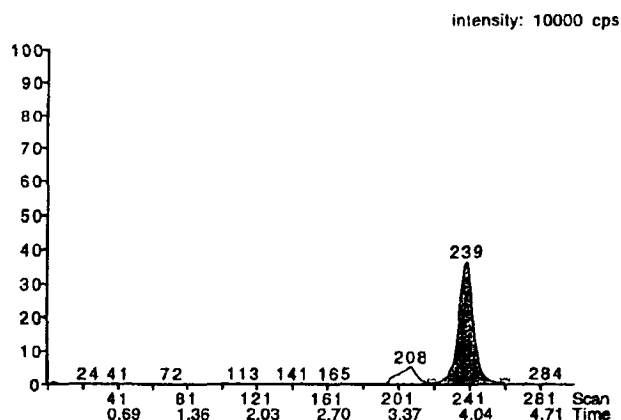


Figure 11. A representative ion chromatogram of an artificial soil matrix fortification sample (454-111-MAS-2, nominal PFOS concentration of 586 mg a.i./kg, dilution factor = 146413x).

- 50 -

PFOS_8 WMAS-1 Fri, Dec 14, 2001 13:26
454-111-

4.98 in 1 period

PFOS

No Internal Standard

Use Area

1: 4.97 MRM, 298 scans

499.0->99.1

Noise Thres. 30.0

Quant Thres. 1.0

Min. Width 3

Mult. Width 6

Base. Width 40

RT Win. (secs) 20

Smooth 1

Expected RT 4.05

Area 22590

Height 2367

Start Time 3.70

End Time 4.34

Integration Width 0.64

Retention Time 3.94

Integration Type A - VB

intensity: 15000 cps

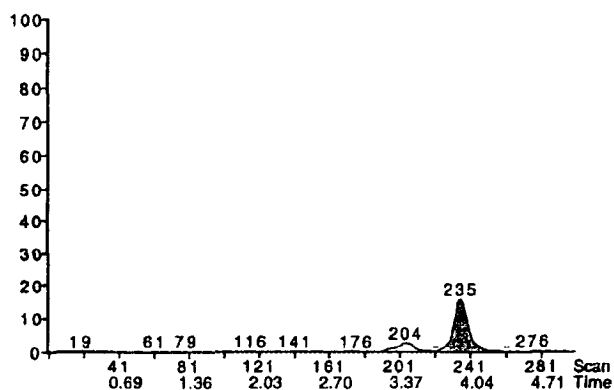


Figure 12. A representative ion chromatogram of an earthworm tissue matrix fortification sample (454-111-WMAS-1, nominal PFOS concentration of 100 mg a.i./kg, dilution factor = 100000x).

- 51 -

PFOS_13 2 Thu, Nov 29, 2001 07:04
454-111-

4.98 in 1 period
PFOS
No Internal Standard
Use Area

1: 4.97 MRM, 298 scans
499.0->99.1
Noise Thres. 30.0
Quant Thres. 1.0
Min. Width 3
Mult. Width 6
Base. Width 40
RT Win. (secs) 20
Smooth 1
Expected RT 4.04
Area 23883
Height 2478
Start Time 3.69
End Time 4.36
Integration Width 0.67
Retention Time 3.99
Integration Type A-BB

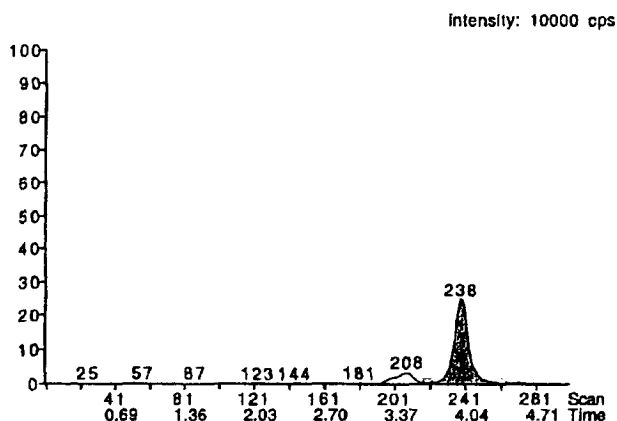


Figure 13. A representative ion chromatogram of an artificial soil test sample (454-111-2, nominal PFOS concentration of 78.1 mg a.i./kg, dilution factor = 29283x).

- 52 -

PFOS_10 W-2A Fri, Dec 14, 2001 13:38
454-111-

4.98 in 1 period

PFOS

No Internal Standard

Use Area

1: 4.97 MRM, 298 scans

499.0->99.1

Noise Thres. 30.0

Quant Thres. 1.0

Min. Width 3

Mult. Width 6

Base. Width 40

RT Win. (secs) 20

Smooth 1

Expected RT 4.05

Area 46647

Height 4953

Start Time 3.70

End Time 4.37

Integration Width 0.87

Retention Time 3.94

Integration Type A - BB

Intensity: 15000 cps

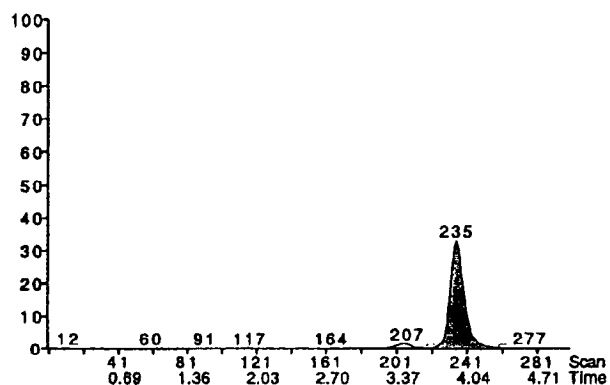


Figure 14. A representative ion chromatogram of an earthworm tissue test sample (454-111-W-2A, dilution factor = 100000x).