19) OPPTS 850.1035, 96 hour
Static acute toxicity with
(saltwater mysid), 454A-128

PFBS:
A 96-HOUR STATIC ACUTE TOXICITY TEST WITH THE SALTWTER MYSID (*Mysidopsis bahia*)

FINAL REPORT

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454A-128
3M Environmental Lab Project No. E00-1429

U.S. Environmental Protection Agency
Series 850 – Ecological Effects Test Guidelines
OPPTS Number 850.1035

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STUDY INITIATION DATE: October 6, 2000

STUDY COMPLETION DATE: March 21, 2001

Submitted to

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: 3M Corporation

TITLE: PFBS: A 96-Hour Static Acute Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia*)

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454A-128

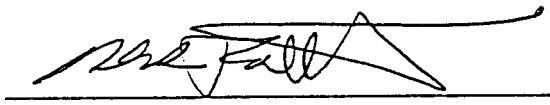
STUDY COMPLETION: March 21, 2001

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); and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984, with the following exceptions:

The test substance was not characterized in compliance with Good Laboratory Practices prior to its use in the study. However, subsequent GLP compliant characterization resulted in a purity similar to the original characterization purity.

The stability of the test substance under conditions of storage at the test site was not determined in accordance with Good Laboratory Practice Standards.

STUDY DIRECTOR:



Kurt R. Drottar
Senior Biologist

3/21/01
DATE

SPONSOR APPROVAL:

3/22/01
DATE

QUALITY ASSURANCE STATEMENT

This study was examined for 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); 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:

		DATE REPORTED TO:	
ACTIVITY:	DATE CONDUCTED:	STUDY DIRECTOR:	MANAGEMENT:
Test Substance Preparation	January 29, 2001	January 29, 2001	February 1, 2001
Matrix Fortification	January 29, 2001	January 29, 2001	January 31, 2001
Observations	February 2, 2001	February 2, 2001	February 8, 2001
Biological Data and Draft Report	February 15 and 16, 2001	February 16, 2001	February 16, 2001
Analytical Data and Draft Report	February 15 and 16, 2001	February 16, 2001	February 16, 2001
Final Report	March 21, 2001	March 21, 2001	March 21, 2001

Robert N. McGee
Quality Assurance Representative

March 21, 2001
DATE

REPORT APPROVAL

SPONSOR: 3M Corporation

TITLE: PFBS: A 96-Hour Static Acute Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia*)

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454A-128

STUDY DIRECTOR:

Kurt R. Drottar
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3/21/01
DATE

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Director, Aquatic Toxicology and
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3/21/01
DATE

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SANITIZED

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SUMMARY

SPONSOR:	3M Corporation
SPONSOR'S REPRESENTATIVE:	
LOCATION OF STUDY, RAW DATA AND A COPY OF THE FINAL REPORT:	Wildlife International, Ltd. Easton, Maryland 21601

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER:	454A-128
TEST SUBSTANCE:	Perfluorobutanesulfonate, Potassium Salt (PFBS)
STUDY:	PFBS: A 96-Hour Static Acute Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia*)
MEAN MEASURED TEST CONCENTRATIONS:	Negative Control; 32, 64, 127, 269, 554 and 1071 mg a.i./L
TEST DATES:	Experimental Start – January 29, 2001 Biological Termination – February 2, 2001 Experimental Termination – February 2, 2001
LENGTH OF TEST:	96 Hours

TEST ORGANISM:	Saltwater Mysid (*Mysidopsis bahia*)
SOURCE OF TEST ORGANISMS:	Wildlife International, Ltd. cultures Easton, Maryland 21601
AGE OF TEST ORGANISMS:	Juveniles

96-HOUR LC50:	372 mg a.i./L
95% CONFIDENCE LIMITS:	314 and 440 mg a.i./L
NO MORTALITY CONCENTRATION:	127 mg a.i./L
NO-OBSERVED-EFFECT-CONCENTRATION:	127 mg a.i./L

INTRODUCTION

This study was conducted by Wildlife International, Ltd. for 3M Corporation at the Wildlife International, Ltd. aquatic toxicology facility in Easton, Maryland. The in-life phase of the test was conducted from January 29, 2001 to February 2, 2001. Raw data generated by Wildlife International, Ltd. and a copy of the final report are filed under Project Number 454A-128 in archives located on the Wildlife International, Ltd. site.

OBJECTIVE

The objective of this study was to evaluate the acute toxicity of Perfluorobutanesulfonate, Potassium Salt (PFBS) to the saltwater mysid, *Mysidopsis bahia*, during a 96-hour exposure period under static test conditions.

EXPERIMENTAL DESIGN

Saltwater mysids were exposed to a geometric series of six test concentrations and a negative (saltwater) control. Two replicate test chambers were maintained in each treatment and control group, with 10 mysids in each test chamber for a total of 20 mysids per test concentration. Nominal test concentrations were selected in consultation with the Sponsor, and were based upon the results of an exploratory rangefinding toxicity test. Nominal test concentrations selected were 31, 63, 125, 250, 500 and 1000 mg active ingredient (a.i.)/L. Mean measured test concentrations were determined from samples of test water collected from each treatment and control group at the beginning of the test, at approximately 48 hours and at test termination.

The mysids were indiscriminately assigned to exposure chambers at test initiation. Observations of mortality and other clinical signs were made at approximately 4, 24, 48, 72, and 96 hours after test initiation. Cumulative percent mortality observed in the treatment groups was used to calculate LC50 values at 24, 48, 72 and 96 hours. The no mortality concentration and no-observed-effect-concentration (NOEC) were determined by visual interpretation of the mortality and clinical observation data.

MATERIALS AND METHODS

The study was conducted based on the procedures outlined in the protocol, "PFBS: A 96-Hour Static Acute Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia*)". The protocol was based on procedures outlined in U.S. Environmental Protection Agency Series 850 Ecological Effects Test Guidelines, OPPTS Number 850.1035 (1); U.S. Environmental Protection Agency, *Standard Evaluation Procedure, Acute Toxicity*

Test for Estuarine and Marine Organisms (2); and ASTM Standard E729-88a *Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians* (3).

Test Substance

The test substance was received from 3M Corporation on March 27, 2000 and was assigned Wildlife International, Ltd. identification number 5216. The test substance was described as a white powder. It was identified as Potassium Perfluorobutane Sulfonate from lot number 2. Information provided by the Sponsor indicated a purity of 97.90%, and an expiration date of March 2010. A subsequent revision of the certificate of analysis indicated a purity of 97.3% and an Expiration/Reassessment Date of January 17, 2002. The test substance was stored at ambient room temperature.

Preparation of Test Concentrations

Nominal test concentrations were 31, 63, 125, 250, 500 and 1000 mg a.i./L. All materials which came into contact with the test substance during preparation of test concentrations were constructed of plastic or stainless steel. A 7-L primary stock solution was prepared in dilution water at a concentration of 1000 mg a.i./L. The primary stock solution was mixed with an electric mixer for approximately 22 minutes to aid in the solubilization of the test substance. After mixing, the primary stock solution was proportionally diluted with dilution water to prepare 3-L of the other five concentrations. After mixing, 1500 mL of test solution was added to the two replicate test chambers for each treatment group. All test solutions appeared clear and colorless. Test concentrations were corrected for the original reported purity of the active ingredient in the test substance (97.9%).

Test Organism

The saltwater mysid, *Mysidopsis bahia*, was selected as the test species for this study. The saltwater mysid is representative of an important group of aquatic marine invertebrates and was selected for use in the test based upon past history of use and ease of culturing in the laboratory. Mysids used in the test were obtained as juveniles from cultures maintained by Wildlife International, Ltd., Easton, Maryland. The identification of the species was provided by the supplier of the original brood stock.

Adult mysids were held at approximately the same temperature as used during the test. During the holding and acclimation periods, the adults showed no signs of disease or stress. During the 14-day holding period preceding the test, water temperatures ranged from 24.6 to 25.9°C. The pH of the water ranged from 8.0 to 8.2, salinity ranged from 20 to 21‰ (parts per thousand) and dissolved oxygen ranged from 6.6 to 7.0 mg/L. Instrumentation used for water measurements is described in the *Environmental Conditions* section of this report. At test initiation, the juvenile mysids were carefully collected from the cultures and indiscriminately distributed one to two at a time into the test chambers until each chamber contained 10 mysids. The juvenile mysids were fed live brine shrimp (*Artemia* sp.) nauplii daily during the test to prevent cannibalism.

Test Apparatus

Test chambers were 2-L polyethylene buckets filled with 1500 mL of test solution. The depth of the test water in a representative test chamber was approximately 8.8 cm. Test chambers were impartially positioned in a temperature-controlled water bath set to maintain a temperature of 25±2°C. The water bath was covered with a plexiglass hood to reduce the potential for cross-contamination. The test chambers were labeled with the project number, test concentration and replicate.

Dilution Water

The water used for holding, acclimation and testing was natural seawater collected at Indian River Inlet, Delaware, and diluted to a salinity of approximately 20‰ with well water. Salinity and pH measurements taken during the four-week period immediately preceding the test are presented in Appendix 1.

The freshly-collected seawater was passed through a sand filter to remove particles greater than approximately 25 µm, and pumped into a 37,800-L storage tank. The filtered seawater then was diluted with freshwater from a well on the Wildlife International, Ltd. site and aerated with spray nozzles. Prior to use, the water again was filtered (0.45 µm) to remove microorganisms and particles. The results of periodic analyses performed to measure the concentrations of selected contaminants in saltwater used by Wildlife International, Ltd. are presented in Appendix 2.

Environmental Conditions

Lighting used to illuminate the cultures and test chambers during culturing and testing was provided by fluorescent tubes that emitted wavelengths similar to natural sunlight (Colortone® 50). A photoperiod of 16 hours of light and 8 hours of darkness was controlled with an automatic timer. A 30-minute transition period

of low light intensity was provided when lights went on and off to avoid sudden changes in lighting. Light intensity at test initiation was approximately 159 lux at the surface of the water. Light intensity was measured using a SPER Scientific Ltd. light meter.

Temperature was measured in each test chamber at the beginning and end of the test using a liquid-in-glass thermometer. Temperature also was measured continuously in one negative control replicate using a Fulscope ER/C Recorder. The target test temperature during the study was 25±2°C. Dissolved oxygen measurements were made on water samples collected from all replicate test chambers of each treatment and control at test initiation, and at approximately 24-hour intervals thereafter. Measurements of pH were made in alternating replicates of each treatment and the control group at test initiation and at approximately 24-hour intervals thereafter. Salinity was measured in the dilution water at test initiation.

Measurements of pH were made using a Fisher Accumet Model 915 pH meter, and dissolved oxygen was measured using a Yellow Springs Instrument Model 51B dissolved oxygen meter. Salinity was measured using a Bio-marine, Inc. Aquafauna refractometer.

Observations

Observations were made to determine the number of mortalities. The number of individuals exhibiting clinical signs of toxicity or abnormal behavior also were evaluated. Observations were made approximately 4, 24, 48, 72 and 96 hours after test initiation.

Statistical Analyses

The 24, 48, 72 and 96-hour LC50 values and the 95% confidence intervals were calculated when possible by probit analysis, the moving average method or binomial probability with non-linear interpolation (4,5,6) using the computer software of C.E. Stephan (7). In this study, the binomial method was used to calculate the 24-hour LC50 value and the probit method was used to calculate the 48, 72 and 96-hour LC50 values. The no mortality concentration and NOEC were determined by visual interpretation of the mortality and clinical observation data.

Analytical Chemistry

Water samples were collected at mid-depth from each replicate test chamber of each treatment and the control group at the beginning of the test, at 48 hours and at test termination to measure concentrations of the test

substance. The samples were collected in plastic scintillation vials and analyzed as soon as possible without storage. Analytical procedures used in the analysis of the samples are provided in Appendix 3.

RESULTS AND DISCUSSION

Measurement of Test Concentrations

Results of analyses to measure concentrations of PFBS in water samples collected during the test are presented in Table 1 and in the analytical chemistry report (Appendix 3). Nominal concentrations selected for use in this study were 31, 63, 125, 250, 500 and 1000 mg a.i./L. Samples collected at test initiation had measured values that ranged from 99 to 110% of nominal values. Measured values for samples taken at 48 hours ranged from 99 to 112% of nominal. Measured values for samples taken at 96 hours ranged from 102 to 112% of nominal. When measured concentrations of the samples analyzed at test initiation, approximately 48 hours and at test termination were averaged, the mean measured concentrations for this study were 32, 64, 127, 269, 554 and 1071 mg a.i./L. Mean measured concentrations were used in the calculation of LC50 values.

Observations and Measurements

Measurements of temperature, dissolved oxygen and pH are presented in Table 2. Temperatures were within the 25 ± 2°C range established for the test. Dissolved oxygen concentrations remained ≥ 6.5 mg/L (88% of saturation) throughout the test. Measurements of pH ranged from 8.0 to 8.3 during the test.

Daily observations of mortality and other clinical signs of toxicity observed during the test are shown in Table 3. Mysids in the negative control appeared normal and healthy during the test. Mysids in the 32, 64 and 127 mg a.i./L treatment groups also appeared normal and healthy during the test. After 96-hours of exposure, mortality in the 269, 554 and 1071 mg a.i./L treatment groups was 15, 90 and 100%, respectively. LC50 values and 95% confidence limits at 24, 48, 72 and 96 hours were calculated from the mortality data, and are shown in Table 4.

CONCLUSIONS

The 96-hour LC50 value for saltwater mysids (*Mysidopsis bahia*) exposed to Perfluorobutanesulfonate, Potassium Salt (PFBS) was 372 mg a.i./L. The 95% confidence limits were 314 and 440 mg a.i./L, and the slope of the concentration-response curve was 7.4. The 96-hour no mortality concentration and the NOEC were 127 mg a.i./L.

REFERENCES

1 **U.S. Environmental Protection Agency.** 1996. Series 850 – Ecological Effects Test Guideilnes (*draft*), OPPTS Number 850.1035: *Mysid Acute Toxicity Test.*

2 **U.S. Environmental Protection Agency.** 1985. *Standard Evaluation Procedure, Acute Toxicity Test for Estuarine and Marine Organisms (Shrimp 96-Hour Acute Toxicity Test).* Hazard Evaluation Division. Office of Pesticide Programs. EPA 540/9-85-010. Washington, D.C.

3 **ASTM Standard E729-88a.** 1994. *Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians.* American Society for Testing and Materials.

4 **Thompson, W.R.** 1947. *Bacteriological Reviews*, Vol. II, No. 2. Pp. 115-145.

5 **Stephan, C.E.** 1977. "Methods for Calculating an LC50", *Aquatic Toxicology and Hazard Evaluations.* American Society for Testing and Materials. Publication Number STP 634, pp 65-84.

6 **Finney, D.J.** 1971. *Statistical Methods in Biological Assay.* Second edition. Griffin Press, London.

7 **Stephan, C.E.** 1978. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. Personal communication.

Table 1

Summary of Analytical Chemistry Data

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Saltwater mysid, *Mysidopsis bahia*
Dilution Water: Filtered Saltwater

Nominal Test Concentration (mg a.i./L)	Replicate	Sampling Time (Hours)	Measured Concentration (mg a.i./L)	Mean Measured Concentration (mg a.i./L)	Percent of Nominal
Negative Control	A	0	<LOQ[1]	<LOQ	--
	B	0	<LOQ		
	A	48	<LOQ		
	B	48	<LOQ		
	A	96	<LOQ		
	B	96	<LOQ		
31	A	0	32.8	32	103
	B	0	33.4		
	A	48	31.7		
	B	48	32.4		
	A	96	32.4		
	B	96	31.9		
63	A	0	65.9	64	102
	B	0	64.1		
	A	48	63.9		
	B	48	63.5		
	A	96	65.1		
	B	96	64.3		
125	A	0	125	127	102
	B	0	124		
	A	48	125		
	B	48	124		
	A	96	132		
	B	96	131		
250	A	0	267	269	108
	B	0	264		
	A	48	277		
	B	48	267		
	A	96	270		
	B	96	266		
500	A	0	552	554	111
	B	0	541		
	A	48	558		
	B	48	558		
	A	96	560		
	B	96	556		
1000	A	0	1071	1071	107
	B	0	1070		

[1]The limit of quantitation (LOQ) was 10 mg a.i./L.

Table 2

Temperature, Dissolved Oxygen and pH of Water in the Test Chambers

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Saltwater mysid, *Mysidopsis bahia*
Dilution Water: Filtered Saltwater

Mean Measured Test Concentration (mg a.i./L)	Replicate	0 Hour[1]			24 Hours		48 Hours		72 Hours		96 Hours		
		Temp[2] (°C)	DO[3] (mg/L)	pH	DO (mg/L)	pH	DO (mg/L)	pH	DO (mg/L)	pH	Temp (°C)	DO (mg/L)	pH
Negative Control	A	24.5	7.4	8.0	6.8	--	6.5	8.1	6.6	--	24.7	6.5	8.1
	B	24.6	7.4	--	6.7	8.2	6.6	--	6.6	8.1	24.7	6.6	--
32	A	24.4	7.4	8.2	6.8	--	6.6	8.2	6.6	--	24.7	6.6	8.2
	B	24.4	7.6	--	6.8	8.3	6.6	--	6.6	8.1	24.7	6.6	--
64	A	24.5	7.5	8.2	6.8	--	6.6	8.2	6.6	--	24.7	6.6	8.2
	B	24.5	7.5	--	6.8	8.3	6.5	--	6.6	8.2	24.7	6.5	--
127	A	24.4	7.5	8.2	6.8	--	6.6	8.2	6.5	--	24.7	6.5	8.2
	B	24.5	7.6	--	6.8	8.3	6.6	--	6.6	8.2	24.7	6.5	--
269	A	24.5	7.6	8.2	6.8	--	6.6	8.2	6.6	--	24.5	6.5	8.2
	B	24.5	7.6	--	6.8	8.3	6.6	--	6.6	8.2	24.6	6.5	--
554	A	24.5	7.6	8.3	6.8	--	6.5	8.2	6.6	--	24.6	6.6	8.2
	B	24.4	7.6	--	6.8	8.3	6.6	--	6.6	8.2	24.6	6.6	--
1071	A	24.4	7.6	8.3	6.8	--	--[4]	--	--	--	--	--	--
	B	24.5	7.7	--	6.8	8.3	--[4]	--	--	--	--	--	--

[1]The 0-hour dilution water (negative control) measurement for salinity was 20‰.

[2]Temperature measured continuously during the test ranged from approximately 23.0 to 25.5°C.

[3]A dissolved oxygen concentration of 4.4 mg/L represents 60% saturation at 25°C in saltwater with a salinity of 20‰.

[4]Measurements discontinued due to 100% mortality.

Table 3

Cumulative Percent Mortality and Treatment-Related Effects

Sponsor: 3M Corporation
Test Substance: PFBS
Test Organism: Saltwater mysid, *Mysidopsis bahia*
Dilution Water: Filtered Saltwater

Mean Measured Test Concentration (mg a.i./L)	Replicate	No. Exposed	4 Hours			24 Hours			48 Hours			72 Hours			96 Hours		Cumulative Percent Mortality
			No. Dead[1]	Effects[2]	% Dead	No. Dead	Effects	% Dead	No. Dead	Effects	% Dead	No. Dead	Effects	% Dead	No. Dead	Effects	
Negative Control	A	10	0	10 AN	0	0	10 AN	0	0	10 AN	0	0	10 AN	0	0	10 AN	0
	B	10	0	10 AN		0	10 AN		0	10 AN		0	10 AN		0	10 AN	
32	A	10	0	10 AN	0	0	10 AN	0	0	10 AN	0	0	10 AN	0	0	10 AN	0
	B	10	0	10 AN		0	10 AN		0	10 AN		0	10 AN		0	10 AN	
64	A	10	0	10 AN	0	0	10 AN	0	0	10 AN	0	0	10 AN	0	0	10 AN	0
	B	10	0	10 AN		0	10 AN		0	10 AN		0	10 AN		0	10 AN	
127	A	10	0	10 AN	0	0	10 AN	0	0	10 AN	0	0	10 AN	0	0	10 AN	0
	B	10	0	10 AN		0	10 AN		0	10 AN		0	10 AN		0	10 AN	
269	A	10	0	10 AN	0	0	10 AN	0	0	10 AN	5	1	9 AN	15	1	9 AN	15
	B	10	0	10 AN		0	10 AN		1	9 AN		2	8 AN		2	8 AN	
554	A	10	0	10 AN	5	1	7AN, 1E, 1C	25	3	5AN, 2E	45	6	3C, 1E	65	10	–	90
	B	10	1	9 AN		4	6 AN		6	4 AN		7	3C		8	2C	
1071	A	10	1	8C, 1AN	5	10	–	100	10	–	100	10	–	100	10	–	100
	B	10	0	10C		10	–		10	–		10	–		10	–	

[1] Cumulative number of dead mysids.
[2] Observed Effects: AN = Appears Normal, C = Lethargic, E = Erratic Swimming

Table 4

LC50 Values

Sponsor:	3M Corporation
Test Substance:	PFBS
Test Organism:	Saltwater mysid, *Mysidopsis bahia*
Dilution Water:	Filtered Saltwater

Time	LC50 (mg a.i./L)	Lower 95% Confidence Limits (mg a.i./L)	Upper 95% Confidence Limits (mg a.i./L)	Statistical Method
24 Hours	661	554	1071	Binomial
48 Hours	540	450	643	Probit
72 Hours	441	362	533	Probit
96 Hours	372	314	440	Probit

Appendix 1

Salinity and pH of Saltwater Measured During the 4-Week Period Immediately Preceding the Test

Sponsor:	3M Corporation
Test Substance:	PFBS
Test Organism:	Saltwater mysid, *Mysidopsis bahia*
Dilution Water:	Filtered Saltwater

	Mean	Range
Salinity (‰)	20 (N = 4)	20 – 20
pH	8.1 (N = 4)	8.0 – 8.2

Appendix 2
Analyses of Pesticides, Organics and Metals in Wildlife International, Ltd. Saltwater[1]

Component	Measured Concentration	Component	Measured Concentration
Pesticides And Organics			
Aclonifen	<0.03 µg/L	Dichlorvos	<0.01 µg/L
Alachlor	<0.01 µg/L	Dicofol	<0.25 µg/L
Ametryn	<0.01 µg/L	Diethyltoluamide	<0.02 µg/L
Atrazine	<0.01 µg/L	Difenoconazole	<0.03 µg/L
Azinphos-ethyl	<0.04 µg/L	Dimethoate	<0.02 µg/L
Azinphos-methyl	<0.08 µg/L	Dimethomorph	<0.05 µg/L
Azoxystrobin	<0.25 µg/L	Disulfoton	<0.02 µg/L
Bifenthrin	<0.05 µg/L	DMST	<0.05 µg/L
Bioallethrin	<0.05 µg/L	Dodemorph	<0.01 µg/L
Bitertanol	<0.05 µg/L	Endosulfan-α	<0.01 µg/L
Bromacil	<0.05 µg/L	Endosulfan-β	<0.01 µg/L
Bromophos	<0.02 µg/L	Endosulfan-sulfte	<0.02 µg/L
Bromophos-ethyl	<0.02 µg/L	Epoxiconazole	<0.05 µg/L
Bromopropylate	<0.02 µg/L	Eptam	<0.02 µg/L
Bupirimate	<0.05 µg/L	Esfenvalerate	<0.02 µg/L
Carbaryl	<0.05 µg/L	Ethion	<0.05 µg/L
Carbofuran	<0.03 µg/L	Ethofumesate	<0.02 µg/L
Carboxin	<0.02 µg/L	Ethoprophos	<0.01 µg/L
Chlorfenvinphos	<0.02 µg/L	Etridiazole	<0.02 µg/L
Chloridazon	<0.05 µg/L	Etrimfos	<0.05 µg/L
Chlorpropham	<0.02 µg/L	Fenarimol	<0.05 µg/L
Chlorpyrifos	<0.01 µg/L	Fenchlorphos	<0.01 µg/L
Chlorpyrifos-methyl	<0.01 µg/L	Fenitrothion	<0.03 µg/L
Chlorothalonil	<0.04 µg/L	Fenoxycarb	<0.03 µg/L
Coumaphos	<0.02 µg/L	Fenpiclonil	<0.05 µg/L
Cyanazine	<0.05 µg/L	Fenpropathrin	<0.25 µg/L
Cyfluthrin	<0.05 µg/L	Fenpropimorph	<0.01 µg/L
Cypermethrin	<0.25 µg/L	Fenthion	<0.01 µg/L
Cyproconazole	<0.05 µg/L	Fenvalerate	<0.02 µg/L
Deltamethrin	<0.02 µg/L	Fluazifop-butyl	<0.02 µg/L
Demeton	<0.02 µg/L	Fluoroglycofen-ethyl	<0.02 µg/L
Demeton-O	<0.02 µg/L	Fluroxypyr-meptyl	<0.05 µg/L
Desethylatrazine	<0.01 µg/L	Flutolanil	<0.02 µg/L
Desisopropylatrazine	<0.02 µg/L	Fonophos	<0.01 µg/L
Desmetryn	<0.01 µg/L	Furalaxyl	<0.02 µg/L
Diazinon	<0.01 µg/L	Heptenophos	<0.02 µg/L
Dichlobenil	<0.01 µg/L	Imazalil	<0.01 µg/L
Dichloran	<0.03 µg/L	Iprodion	<0.05 µg/L
Dichlorbenzamide	<0.02 µg/L	Kresoxim-methyl	<0.02 µg/L
Dichlorfenthion	<0.01 µg/L	Lenacil	<0.05 µg/L
Dichlorfluanid	<0.03 µg/L	Lindane	<0.02 µg/L

[1]Analyses performed by TNO Nutrition and Food Institute on samples collected on October 14 and 15, 1999.

Appendix 2
Analyses of Pesticides, Organics and Metals in Wildlife International, Ltd. Saltwater[1]
Page 2

Pesticides And Organics			
Component	Measured Concentration	Component	Measured Concentration
Malathion	<0.02 µg/L	Methoxychlor	<0.01 µg/L
Metalaxyl	<0.05 µg/L	Metolachlor	<0.01 µg/L
Metamitron	<0.05 µg/L	Metribuzin	<0.02 µg/L
Metazachlor	<0.02 µg/L	Mevinphos	<0.01 µg/L
Methidathion	<0.02 µg/L	Nitrothal-Isopropyl	<0.05 µg/L
Paclobutazole	<0.05 µg/L	Pyrifenox-1	<0.01 µg/L
Parathion	<0.01 µg/L	Pyrifenox-2	<0.01 µg/L
Parathion-methyl	<0.01 µg/L	Pyrimethanil	<0.01 µg/L
Penconazole	<0.05 µg/L	Quizalofop-ethyl	<0.02 µg/L
Pendimethalin	<0.03 µg/L	Simazine	<0.01 µg/L
Permethrin-cis	<0.01 µg/L	Sulfotep	<0.02 µg/L
Permethrin-trans	<0.01 µg/L	Tebuconazole	<0.05 µg/L
Phosalone	<0.05 µg/L	Tebufenpyrad	<0.05 µg/L
Phosmet	<0.02 µg/L	Terbutryn	<0.01 µg/L
Phosphamidon-cis	<0.05 µg/L	Terbuthylazine	<0.01 µg/L
Pirimicarb	<0.01 µg/L	Tetrachlorvinphos	<0.01 µg/L
Pirimiphos-ethyl	<0.01 µg/L	Tetrahydroftalimide	<0.05 µg/L
Pirimiphos-methyl	<0.01 µg/L	Tetramethrin	<0.01 µg/L
Prochloraz	<0.02 µg/L	Thiabendazole	<0.05 µg/L
Procymidon	<0.01 µg/L	Thiometon	<0.04 µg/L
Prometryn	<0.01 µg/L	Tolclofos-methyl	<0.01 µg/L
Propachlor	<0.01 µg/L	Tolylfluanid	<0.04 µg/L
Propazine	<0.01 µg/L	Triadimefon	<0.05 µg/L
Propham	<0.02 µg/L	Triadimenol	<0.05 µg/L
Propiconazole	<0.05 µg/L	Triallate	<0.02 µg/L
Propoxur	<0.03 µg/L	Triazophos	<0.02 µg/L
Propyzamide	<0.02 µg/L	Trifluralin	<0.02 µg/L
Prosulfocarb	<0.02 µg/L	Vamidothion	<0.01 µg/L
Pyrazophos	<0.03 µg/L	Vinclozolin	<0.01 µg/L
Metals			
Magnesium	520 mg/L	Nickel	<11 µg/L
Sodium	4,400 mg/L	Copper	<7.0 µg/L
Calcium	181 mg/L	Zinc	<5.0 µg/L
Iron	<0.015 mg/L	Molybdenum	<3.0 µg/L
Potassium	3.6 mg/L	Silver	<2.0 µg/L
Aluminum	<0.02 mg/L	Cadmium	<1.0 µg/L
Manganese	<1.0 µg/L	Arsenic	0.7 µg/L
Beryllium	<2.0 µg/L	Mercury	<0.025 µg/L
Chromium	11.5 µg/L	Selenium	<0.5 µg/L
Cobalt	<2.0 µg/L		

[1]Analyses performed by TNO Nutrition and Food Institute on samples collected on October 14 and 15, 1999.

Appendix 3

THE ANALYSIS OF PFBS IN FILTERED SALTWATER

IN SUPPORT OF

WILDLIFE INTERNATIONAL, LTD. PROJECT NO.: 454A-128

REPORT APPROVAL

SPONSOR: 3M Corporation

TITLE: PFBS: A 96-HOUR STATIC ACUTE TOXICITY TEST with the SALTWATER MYSID (*Mysidopsis bahia*)

WILDLIFE INTERNATIONAL, LTD. PROJECT NO.: 454A-128

3M ENVIRONMENTAL LAB PROJECT NUMBER: E00-1429

PRINCIPAL INVESTIGATOR:

Raymond L. Van Hoven, Ph.D.
Scientist

03-21-01
DATE

MANAGEMENT:

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

3/21/01
DATE

Introduction

Saltwater samples were collected from a static acute aquatic toxicity study designed to determine the effects of PFBS (Perfluoro Butane Sulfonate, Potassium Salt) to the saltwater mysid (*Mysidopsis bahia*). This study was conducted by Wildlife International, Ltd. and identified as Project Number 454A- 128. The analyses of these water samples were performed at Wildlife International, Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). Samples were received for analysis on January 29, 31, and February 2, 2001. The submitted samples were prepared for analysis on each sample receipt day. Analyses were completed on the three sampling intervals on January 30, February 1, and February 2, 2001, respectively.

Analytical Standard

The analytical standard was received from 3M Environmental Technology and Safety Services on March 27, 2000, assigned Wildlife International, Ltd. Identification number 5216, and stored under ambient conditions. The analytical standard, a white powder, was identified as: Potassium Perfluorobutane Sulfonate Lot 2), expiration date: March 2010. The analytical standard was further identified with the 3M Environmental Laboratory test control and reference number TCR

The test substance had a reported purity of 97.90%. A subsequent revision of the certificate of analysis indicated a purity of 97.3% and an Expiration/Reassessment Date of January 17, 2002. The analytical standard was the same material and lot number as the test substance. The analytical standard was used to prepare calibration and matrix fortification samples.

Analytical Method

Water samples were analyzed according to the method entitled "Analytical Method Validation for the Determination of Perfluorobutane Sulfonate, Potassium Salt (PFBS) in Saltwater and Algal Media" (Wildlife International, Ltd. Project No. 454C-117). Samples were diluted in a 50% methanol : 50% NANOpure® water solution so that they fell within the calibration range of the PFBS methodology. Aliquots of the dilutions were transferred to autosampler vials and submitted for analysis by direct injection. Concentrations of PFBS in saltwater samples were determined by reverse-phase high performance liquid chromatography using a Hewlett-Packard Model 1100 High Performance Liquid Chromatograph (HPLC) interfaced with a Perkin-Elmer API 3000 mass spectrometer operated in selective ion monitoring (SIM) detection mode. The mass spectrometer was equipped with a Perkin-Elmer

TurboIonSpray ion source. Chromatographic separations were achieved using a Keystone PRISM RP column (30 mm × 1.5 mm, 3-µm particle size) fitted with a Keystone Javelin C_{18} Guard Cartridge (20 mm × 2 mm). The instrument parameters are summarized in Table 1 and a method flowchart is provided in Figure 1.

Primary and Secondary Stock Solutions.

All primary and secondary stock preparations were adjusted for the purity of the analytical standard (97.90%). A 10.0 mg a.i./mL primary stock solution of PFBS in methanol was prepared by weighing 1.024 g of the analytical standard and bringing to a final volume of 100 mL with methanol. Secondary stock solutions (1000, 100, 10.0, 1.00, and 0.100 mg a.i./L) of PFBS 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 10.0 mg a.i./L stock solution of PFBS in methanol. The calibration standards of PFBS, ranging in concentration from 0.0100 to 0.0500 mg a.i./L, were analyzed with each sample set. Five calibration standards (different concentrations) were analyzed with the samples. 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 the peak area responses versus the respective concentrations of the calibration standards. A typical calibration curve is presented in Figure 2. The concentration of PFBS in the samples was determined by substituting the peak area responses into the applicable linear regression equation. Representative ion chromatograms of low and high calibration standards are presented in Figures 3 and 4, respectively.

Limit of Quantitation

The method limit of quantitation (LOQ) for these analyses was set at 10.0 mg a.i./L calculated as the product of the lowest calibration standard analyzed (0.0100 mg a.i./L) and the dilution factor of the matrix blank samples (1000).

Matrix Blank and Fortification Samples

Three matrix blank samples were analyzed to determine possible interference. No interferences were observed at or above the LOQ during samples analyses (Table 2). A representative ion chromatogram of a matrix blank is presented in Figure 5.

Saltwater was either fortified with the appropriate PFBS stock solution in methanol (15.0 and 200 mg a.i./L), or directly fortified (*i.e.* without use of carrier solvent) with PFBS (1250 mg a.i./L). The fortified samples were analyzed concurrently with the test samples to determine the mean procedural recovery (Table 2). Sample concentrations were not corrected for the mean procedural recovery of 109%. A representative ion chromatogram of a matrix fortification is presented in Figure 6.

Example Calculations

Sample number 454A-128-9, nominal concentration of 250 mg a.i./L in saltwater.

First Initial Volume: 0.100 mL
First Final Volume: 10.0 mL
Second Initial Volume: 0.100 mL
Second Final Volume: 10.0 mL
Dilution Factor: 10000
PFBS Peak Area: 25861574

Calibration curve equation:
Slope: 713895744
Intercept: 6781268
Curve regression weighted 1/x

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

$$\text{PFBS (mg a.i./L) in sample} = \text{PFBS measured at instrument (mg a.i./L)} \times \text{dilution factor}$$

$$= \frac{25861574 - 6781268}{713895744} \times 10000$$

$$= 267$$

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

$$= \frac{267}{250} \times 100 = 107\%$$

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

RESULTS

Sample Analysis

Saltwater samples were collected from a static acute toxicity study with the saltwater mysid (*Mysidopsis bahia*) at test initiation, January 29, 2001 (Day 0), on January 31, 2001 (Day 2), and at test termination, February 2, 2001 (Day 4). The measured concentrations of PFBS in the samples collected at initiation of exposure of the test organisms (Day 0) ranged from 98.9 to 110% of the nominal concentrations. Samples collected at Day 2 had a measured concentration range of 99.4 to 112% of nominal values. Samples collected at test termination (Day 4) had a measured concentration range of 102% to 112% of nominal values (Table 3). A representative ion chromatogram of a test sample is shown in Figure 7.

Table 1

Typical HPLC/MS Operational Parameters

INSTRUMENT:	Hewlett-Packard Model 1100 High Performance Liquid Chromatograph with a Perkin-Elmer API 3000 Mass Spectrometer operated in Selective Ion Monitoring (SIM) Mode
ION SOURCE:	Perkin-Elmer TurboIonSpray
ANALYTICAL COLUMN:	Keystone PRISM RP (30 mm × 1.5 mm, 3-µm particle size)
GUARD COLUMN:	Keystone Javelin C_{18} cartridge (20 mm × 2 mm)
OVEN TEMPERATURE:	40°C
STOP TIME:	3.00 min
FLOW RATE:	200 µL/min
MOBILE PHASE:	25% NANOpure® Water with 0.1% Ammonium Formate: 75% Methanol
INJECTION VOLUME:	5.0 µL
PFBS PEAK RETENTION TIME:	Approximately 2.1 minutes
PFBS MONITORED MASS:	299.0 amu

Table 2

Matrix Blanks and Fortifications Analyzed Concurrently During Sample Analysis

Sample Number (454A-128-)	Sample Type	Concentrations of PFBS (mg a.i./L)		Percent Recovered[1]
		Fortified	Measured[1]	
MAB-1	Matrix Blank	0.00	<LOQ[2]	--
MAB-2	Matrix Blank	0.00	<LOQ	--
MAB-3	Matrix Blank	0.00	<LOQ	--
MAS-1	Matrix Fortification	15.0	16.4	109
MAS-4	Matrix Fortification	15.0	18.9	126[3]
MAS-7	Matrix Fortification	15.0	18.0	120
MAS-2	Matrix Fortification	200	218	109
MAS-5	Matrix Fortification	200	219	110
MAS-8	Matrix Fortification	200	214	107
MAS-3	Matrix Fortification	1250	1348	108
MAS-6	Matrix Fortification	1250	1306	105
MAS-9	Matrix Fortification	1250	1325	106
				Mean = 109
				Standard Deviation = 4.65
				CV = 4.26%
				N = 8

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

[2] The limit of quantitation (LOQ) was 10.0 mg a.i./L based upon the product of the lowest calibration standard analyzed (0.0100 mg a.i./L) and the dilution factor of the matrix blank samples (1000).

[3] Recovery outside 80-120% (suspect fortification or contamination). Sample not included in the statistical analysis of the data.

Table 3

Measured Concentrations of PFBS in Saltwater Samples from a Mysid Static Acute Toxicity Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-128-)	Sampling Time (Day)	PFBS Measured Concentration[1] (mg a.i./L)	Percent of Nominal[1]
0.0	1	0	< LOQ[2]	--
	2	0	< LOQ	--
	15	2	< LOQ	--
	16	2	< LOQ	--
	27	4	< LOQ	--
	28	4	< LOQ	--
31	3	0	32.8	106
	4	0	33.4	108
	17	2	31.7	102
	18	2	32.4	104
	29	4	32.4	105
	30	4	31.9	103
63	5	0	65.9	105
	6	0	64.1	102
	19	2	63.9	101
	20	2	63.5	101
	31	4	65.1	103
	32	4	64.3	102
125	7	0	125	100
	8	0	124	98.9
	21	2	125	100
	22	2	124	99.4
	33	4	132	106
	34	4	131	105

[1] Measured and Percent of Nominal values were calculated using MacQuan, version 1.6 software. Manual calculations may vary slightly.

[2] The limit of quantitation (LOQ) was 10.0 mg a.i./L based upon the product of the lowest calibration standard analyzed (0.0100 mg a.i./L) and the dilution factor of the matrix blank samples (1000).

Table 3 (Continued)

Measured Concentrations of PFBS in Saltwater Samples from a Mysid Static Acute Toxicity Test

Nominal Test Concentration (mg a.i./L)	Sample Number (454A-128-)	Sampling Time (Day)	PFBS Measured Concentration[1] (mg a.i./L)	Percent of Nominal[1]
250	9	0	267	107
	10	0	264	106
	23	2	277	111
	24	2	267	107
	35	4	270	108
	36	4	266	107
500	11	0	552	110
	12	0	541	108
	25	2	558	112
	26	2	558	112
	37	4	560	112
	38	4	556	111
1000	13	0	1071	107
	14	0	1070	107

[1] Measured and Percent of Nominal values were calculated using MacQuan, version 1.6 software. Manual calculations may vary slightly.

[2] The limit of quantitation (LOQ) was 10.0 mg a.i./L based upon the product of the lowest calibration standard analyzed (0.0100 mg a.i./L) and the dilution factor of the matrix blank samples (1000).

METHOD OUTLINE FOR THE ANALYSIS OF PFBS IN SALTWATER

Prepare matrix fortification samples in saltwater as follows: For target PFBS concentrations ≤ 200 mg a.i./L, fortify saltwater with the appropriate stock solution of PFBS. For target PFBS concentrations > 200 mg a.i./L, prepare by weighing the requisite amount of PFBS test substance on an analytical balance and transferring directly into a Class A volumetric flask partially filled with saltwater. Rinse weighing paper and the sides of the flask with repeat saltwater rinses. Swirl the flask to dissolve the test substance and then bring to final volume with saltwater. Sonicate as appropriate and mix with several repeat inversions. The matrix blank is unfortified saltwater.

↓

Prepare appropriate dilutions of study and QC samples to within the calibration range of the PFBS methodology: Partially fill Class A volumetric flasks with 50% methanol : 50% NANOpure® water dilution solvent. Add the appropriate volume of sample and bring to volume with dilution solvent. Perform secondary dilutions as necessary. Process matrix blank samples using the same dilution and aliquot volume as for the lowest fortification level. Mix well by several repeat inversions.

↓

Ampulate samples and submit for LCMS analysis.

Figure 1. Analytical method flowchart for the analysis of PFBS in saltwater.

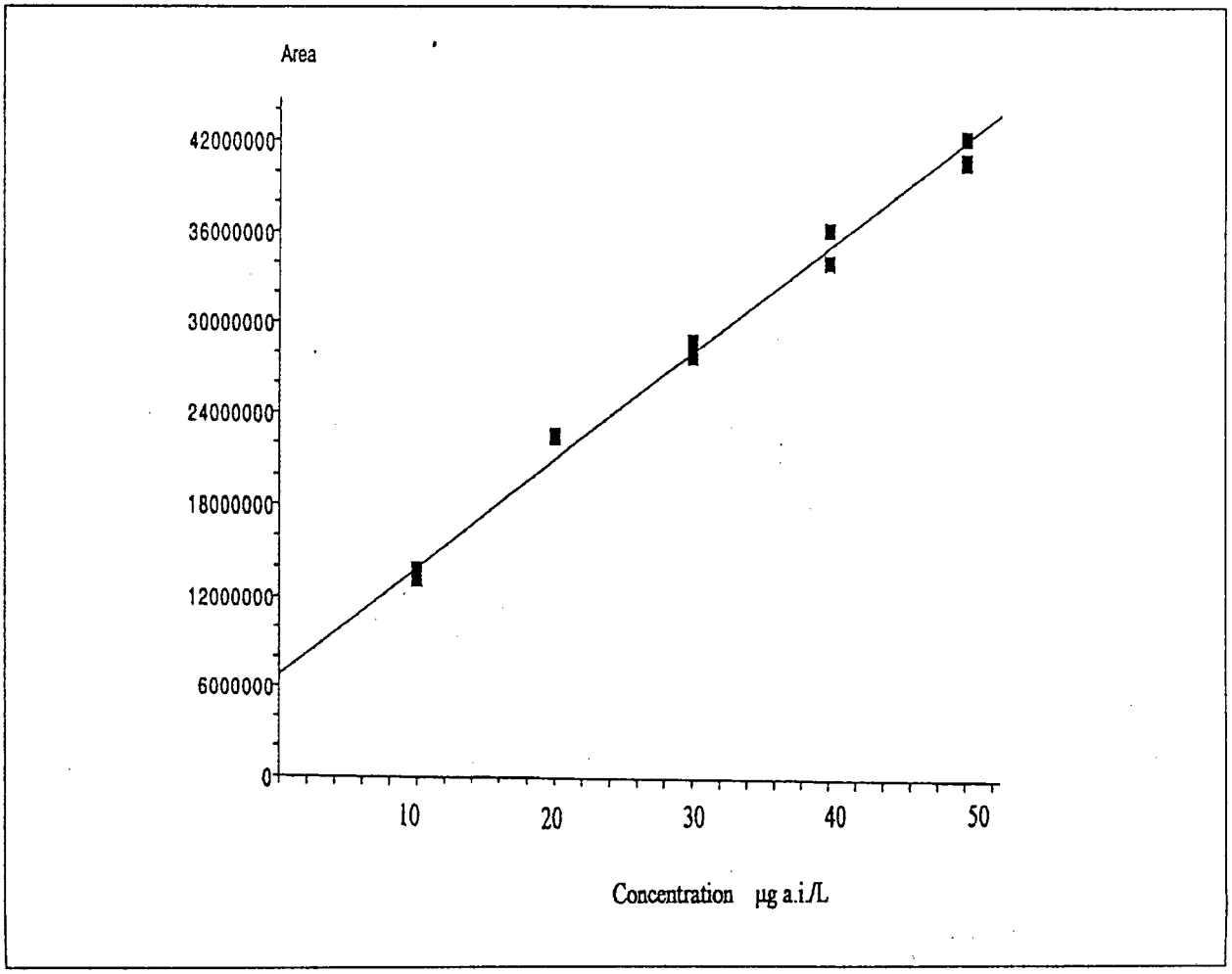

Figure 2. A typical calibration curve for PFBS. Slope = 713895744; Intercept = 6781268; r = 0.99625

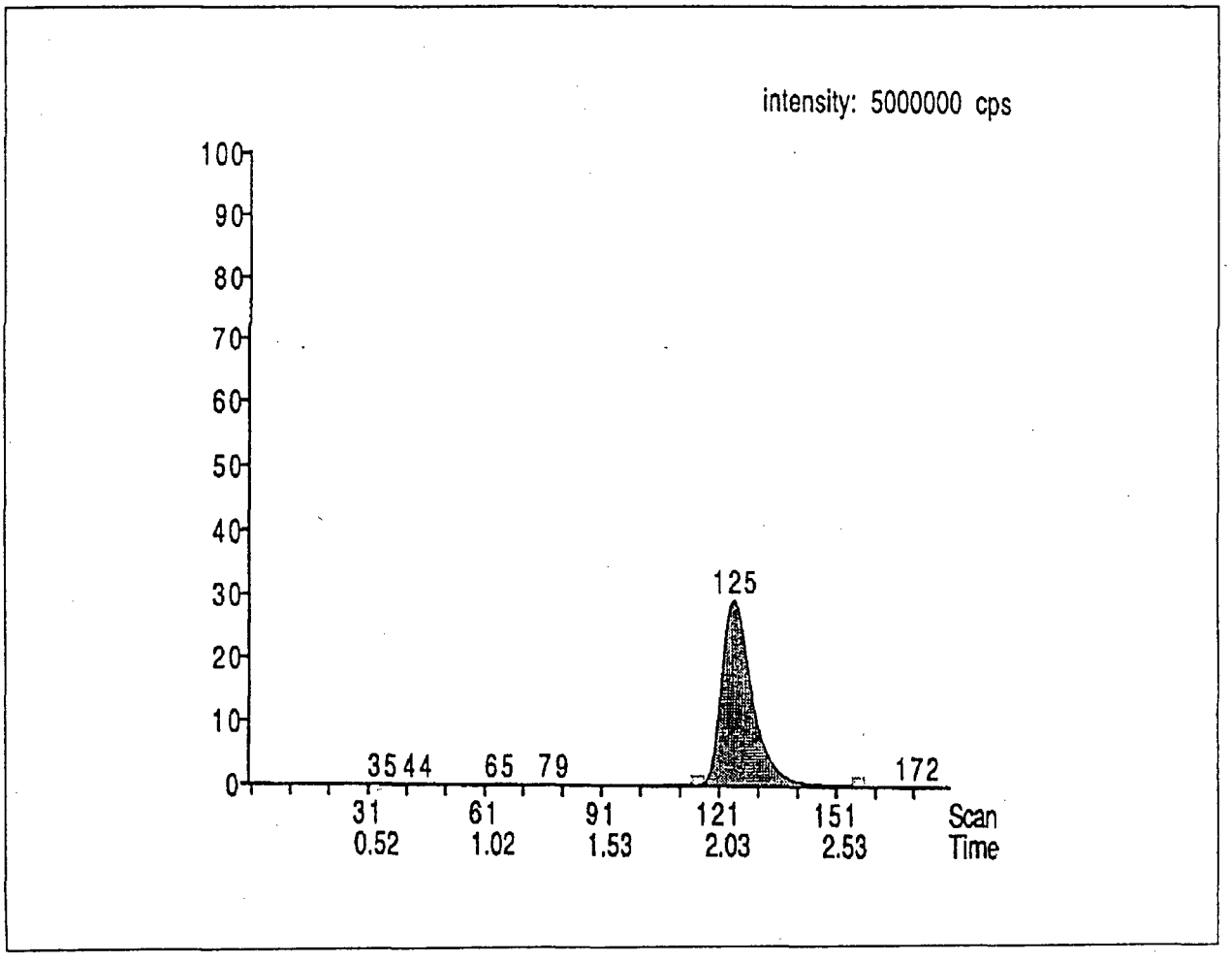

Figure 3. A representative ion chromatogram of a low-level (0.0100 mg a.i./L) PFBS standard.

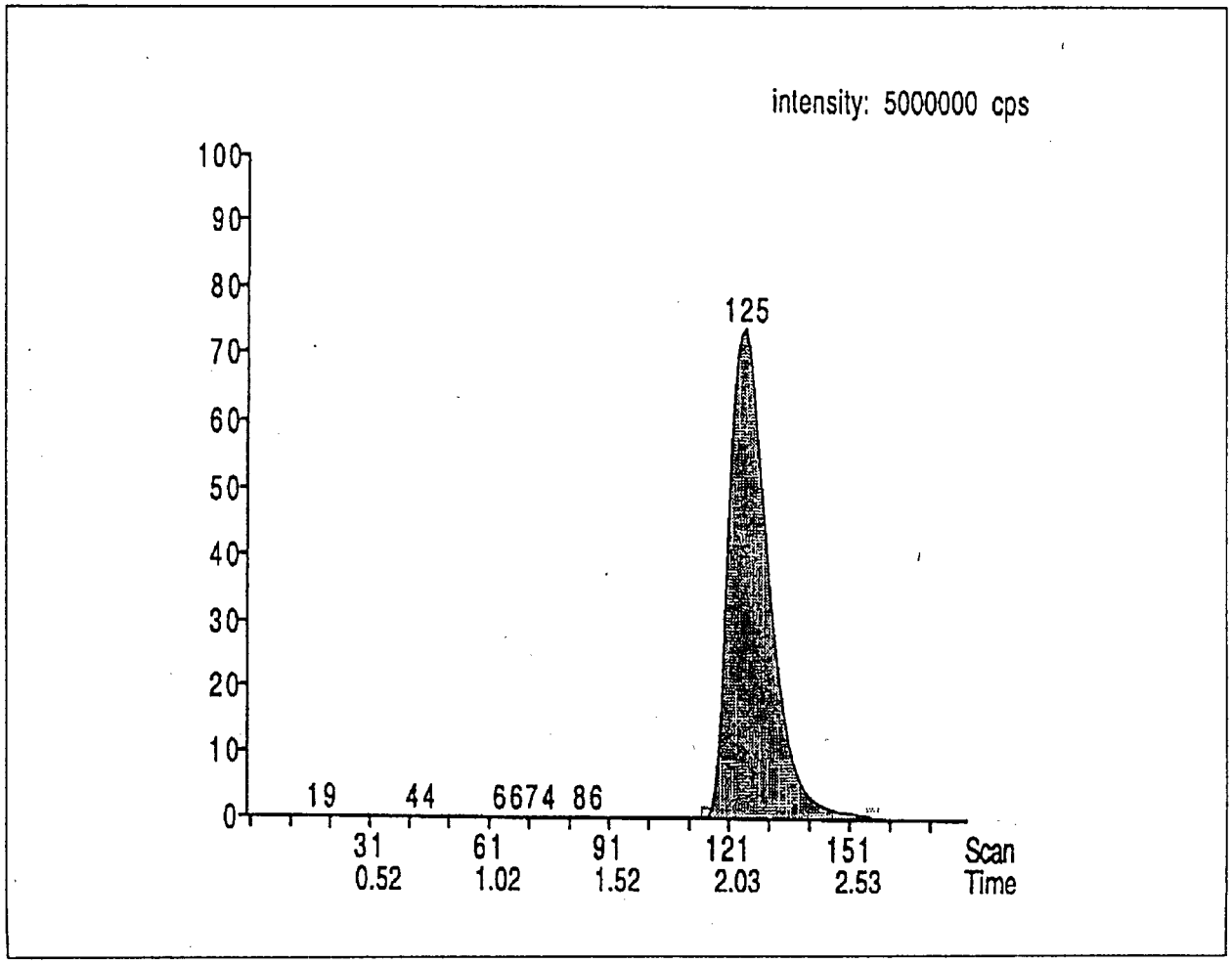

Figure 4. A representative ion chromatogram of a high-level (0.0500 mg a.i./L) PFBS standard.

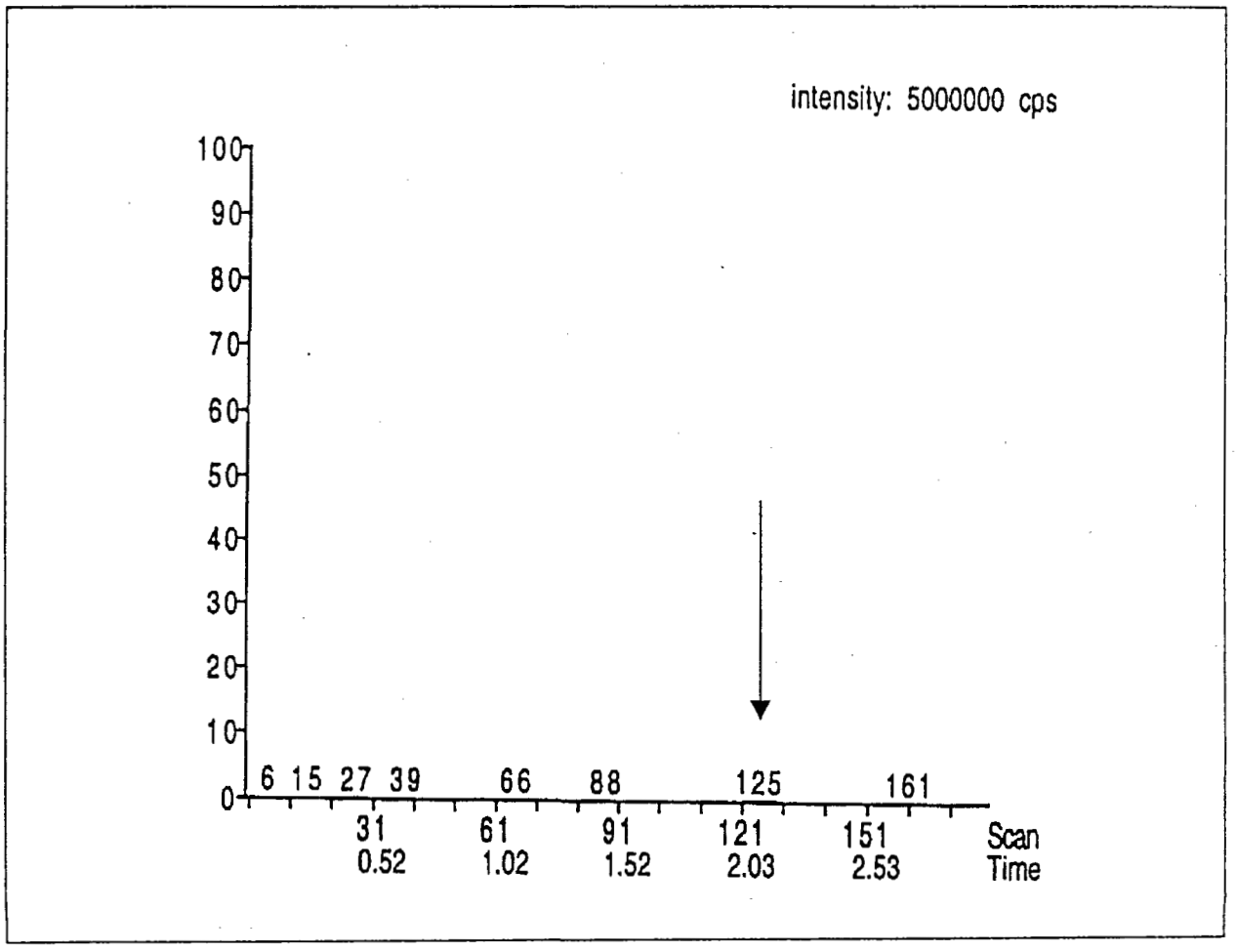

Figure 5. A representative ion chromatogram of a matrix blank sample (454A-128-MAB-1). The arrow indicates the retention time of PFBS.

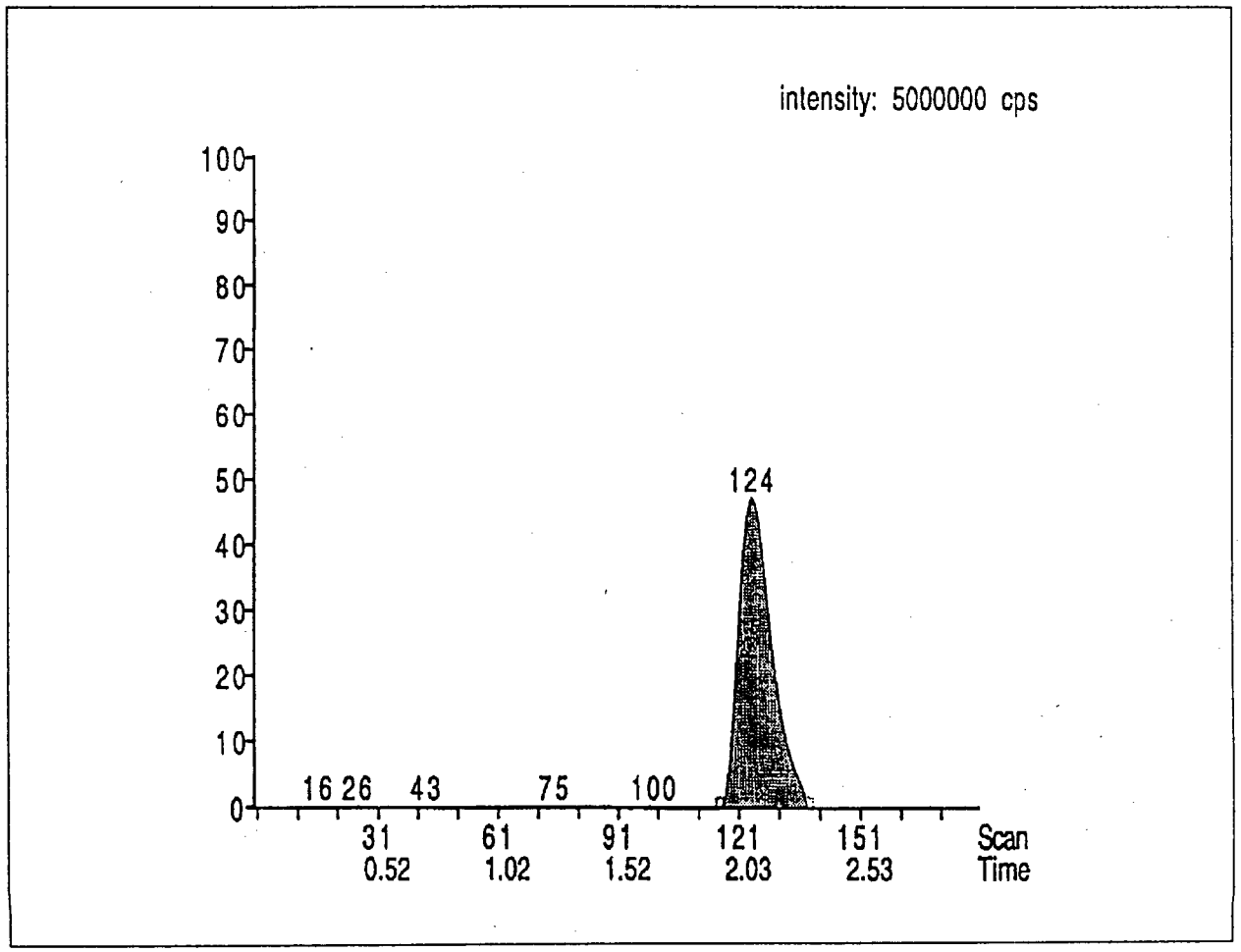

Figure 6. A representative ion chromatogram of a matrix fortification sample (454A-128-MAS-2, nominal PFBS concentration of 200 mg a.i./L, dilution factor = 10000x).

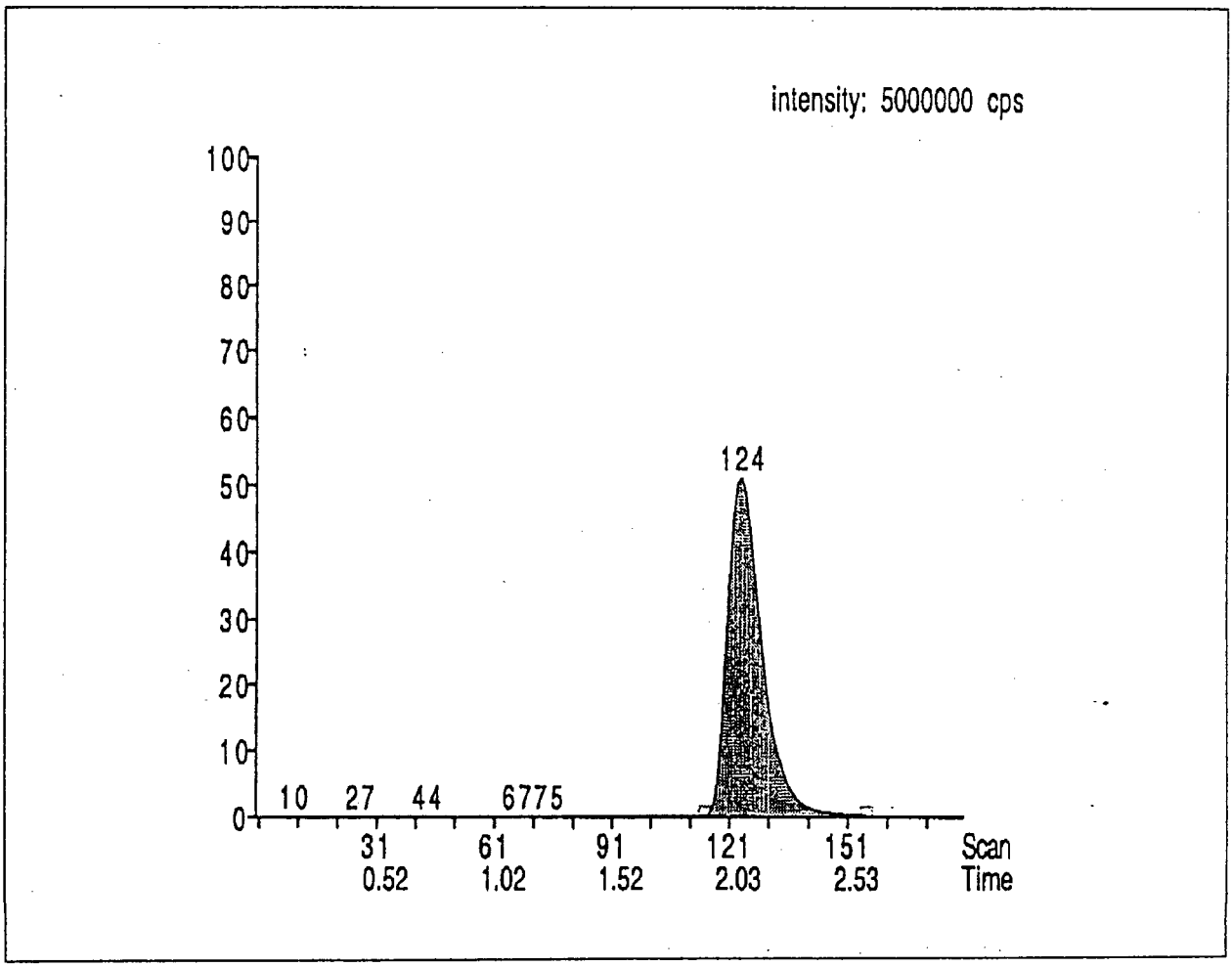

Figure 7. A representative ion chromatogram of a test sample (454A-128-9, nominal PFBS concentration of 250 mg a.i./L, dilution factor = 10000x).

Appendix 4

Changes to Protocol

This study was conducted in accordance with the approved Protocol with the following changes:

1. The protocol was amended to add the proposed experimental start and termination dates and test concentrations.

2. Water samples were not collected from the 1071 mg a.i./L treatment group on Day 2 of the test due to 100% mortality on Day 1.

Appendix 5

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. Willard B. Nixon, Ph.D., Manager, Analytical Chemistry
3. Cary A. Sutherland, Laboratory Supervisor
4. Raymond L. Van Hoven, Ph.D., Scientist
5. Kurt R. Drottar, Senior Biologist
6. Amy S. Blankinship, Biologist