Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Hydrogen Cyanamide

MRID #: 448047-01

Matrix: Water

Analysis: HPLC/FLD

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ANALYTICAL METHOD VERIFICATION FOR THE DETERMINATION OF HYDROGEN CYANAMIDE RESIDUES IN SALTWATER

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 248C-101

FINAL REPORT

AUTHORS:

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STUDY INITIATION DATE: March 11, 1998

STUDY COMPLETION DATE: August 31, 1998

Submitted to

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STATEMENT OF NO CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA section 10(d)(1)(A), (B), or (C).

Company: SKW Trostberg, AG (Typed Name)

Company Agent: Jane M. Miller (Typed Name)

Title: Consultant to SKW Trostberg

Signature: Jane M Miller Date: Sept 10, 1998

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: SKW Trostberg AG

TITLE: Analytical Method Verification for the Determination of Hydrogen Cyanamide Residues in Saltwater

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 248C-101

STUDY COMPLETION: August 31, 1998

This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Part 160, 17 August 1989; OECD Principles of Good Laboratory Practice, OCDE/GD (92) 32, Environment Monograph No. 45, Paris 1992; and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984.

PRINCIPAL INVESTIGATOR:

Timothy Zee Kendall
Timothy Zee Kendall, M.S.
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August 31, 1998
DATE

STUDY DIRECTOR:

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Winfried Gettmann
SKW Trostberg AG

Sept. 10, 1998
DATE

SPONSOR:

Sponsor

SEPT. 10, 1998
DATE

Applicant

Sept. 10, 1998
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 Part 160, 17 August 1989; OECD Principles of Good Laboratory Practice, OCDE/GD (92) 32, Environment Monograph No. 45, Paris 1992; 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:
Sample Preparation	May 4, 1998	June 2, 1998	June 2, 1998
Draft Report and Data	May 29, 1998	June 2, 1998	June 2, 1998
Revised Draft Report	July 24, 1998	July 29, 1998	July 29, 1998
Final Report	August 31, 1998	August 31, 1998	August 31, 1998

Susan L. Coleman

Susan L. Coleman, B.A.
Senior Quality Assurance Representative

8-31-98

DATE

REPORT APPROVAL

SPONSOR: SKW Trostberg AG

TITLE: Analytical Method Verification for the Determination of Hydrogen Cyanamide Residues in Saltwater

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 248C-101

STUDY DIRECTOR:

Winfried Gettmann
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Sept. 10, 1998
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8/31/98
DATE

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SUMMARY

SPONSOR:	SKW Trostberg AG
CONTACT:	Winfried Gettmann
LOCATION OF STUDY AND COPIES OF THE RAW DATA AND FINAL REPORT:	Wildlife International Ltd. Easton, Maryland 21601

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER:	248C-101
TEST SUBSTANCE:	Aqueous Hydrogen Cyanamide (50% w/w)
STUDY:	Analytical Method Verification for the Determination of Hydrogen Cyanamide Residues in Saltwater
FORTIFIED TEST CONCENTRATIONS:	0.402, 4.02, 40.2 and 402 mg/L
TEST DATES:	Experimental Start - May 4, 1998 Experimental Termination - May 7, 1998

TEST SYSTEM:	Filtered Saltwater

SUMMARY:	Filtered saltwater samples were fortified with aqueous hydrogen cyanamide (50% w/w) to reflect nominal concentrations of 0.402, 4.02, 40.2 and 402 mg/L. The samples and standards were derivatized, filtered and analyzed by high performance liquid chromatography using fluorescence detection. Recoveries of aqueous hydrogen cyanamide (50% w/w) at 0.402, 4.02, 40.2 and 402 mg/L yielded mean percent recoveries of 104, 103, 102 and 105 %, respectively, with an overall mean recovery of 104 ± 1.91% (CV = 1.84). Reagent and matrix blanks, reflecting unfortified NanoPure® water and filtered saltwater respectively, were devoid of interfering components.

INTRODUCTION

Verification samples were fortified and analyzed to evaluate the performance of a method for the analysis of aqueous hydrogen cyanamide (50% w/w) in filtered saltwater solutions. The study was conducted by Wildlife International Ltd. and identified as Project Number 248C-101. The analyses of the filtered saltwater samples were performed at Wildlife International Ltd. using high performance liquid chromatography (HPLC) with fluorescence detection. Fortification samples were prepared and analyzed between May 4, 1998 and May 7, 1998. All original raw data generated by Wildlife International Ltd. and a copy of the final report are filed under Project Number 248C-101 in archives located on the Wildlife International Ltd. site.

PURPOSE

The purpose of this study was to verify a method for measuring the test substance residues in saltwater used by Wildlife International Ltd. in support of environmental fate and/or effects studies.

EXPERIMENTAL DESIGN

Filtered saltwater was fortified at four different concentrations and analyzed based on a method supplied by the Sponsor. Reagent and matrix blanks were analyzed to evaluate possible analytical interferences. A calibration curve was prepared from standard solutions to determine the test substance sample concentrations.

MATERIALS AND METHODS

Test Substance

The test substance was received from SKW Trostberg AG on March 27, 1998 and was assigned Wildlife International Ltd. identification number 4416 upon receipt. The test substance was described as a liquid and was identified as: Aqueous Hydrogen Cyanamide (50% w/w); CYANAMID L 500; CH. NR.: 807601 with an expiration date of March, 1999. The test substance had a reported purity of 50.8%. The test substance was stored under refrigerated conditions.

Analytical Standard

The analytical standard was received from SKW Trostberg AG on March 27, 1998 and was assigned Wildlife International Ltd. identification number 4417 upon receipt. The analytical standard was described as a clear semisolid and was identified as: Cyanamid F1000: Lot No.: 031002. The analytical standard had a reported purity of 100.3% and was stored under refrigerated conditions.

Reagents and Solvents

Solvents/Reagents	Grade	Purity	Supplier
Potassium tetraborate tetrahydrate	ACS	99%	Aldrich Chem. Co.
4-chloro-7-nitrobenzofuran (NBD-Cl)	ACS	98%	Aldrich Chem. Co.
Hydrochloric acid	ACS	5.0 N	Lab Chem
Potassium dihydrogenphosphate	ACS	99.99%	Aldrich Chem. Co.
o-Phosphoric acid	ACS	85%	Mallinckrodt
Methanol	HPLC	99.9+%	Burdick & Jackson
NanoPure® water	eq. to ASTM Type II		Barnstead

Dilution Water

The water used for testing was prepared at Wildlife International Ltd. from natural seawater collected at Indian River Inlet, Delaware. The freshly-collected seawater was passed through a sand filter to remove particles greater than approximately 25 μm, diluted to a salinity of approximately 20‰ with filtered well water, and pumped into a 37,800-L storage tank where it was aerated with spray nozzles. Prior to delivery to the test system, the 20 ppt water again was filtered (0.2 μm) to remove microorganisms and particles. Salinity and pH measurements taken during the four-week period immediately preceding the test are presented in Appendix I. The results of periodic analyses performed to measure the concentrations of selected contaminants in saltwater used by Wildlife International Ltd. are presented in Appendix II.

Analytical Method

The analytical method used for the processing and analysis of aqueous hydrogen cyanamide (50% w/w) in filtered saltwater was developed at Wildlife International, Ltd. Samples, diluted as necessary,

and calibration standards were derivatized in the following manner. Two milliliters of each standard and test solution were transferred to 15-mL graduated centrifuge tubes to which 1.00 mL of (0.37M) potassium tetraborate solution was added. The solution was mixed by vortex, then 2.00 mL of methanolic (0.025M) NBD-CL solution was added and the contents of the tube were vortexed again. Solutions were then heated in a heating block at 80°C for 30 minutes. One milliliter of 1.2M HCl was added to each solution and mixed by vortex. Solutions were then filtered through a PTFE Acrodisc 0.45 μm filter into vials for HPLC analysis.

Concentrations of aqueous hydrogen cyanamide (50% w/w) in the calibration standards and aqueous samples were determined by high performance liquid chromatography using a Hewlett-Packard Model 1090 High Performance Liquid Chromatograph (HPLC) equipped with a fluorescence detector operated at an excitation wavelength of 470 nm and an emission wavelength of 530 nm. Chromatographic separations were achieved using a Zorbax Phenyl analytical column (250 mm x 4.6 mm I.D., 5 μm particle size). The instrument parameters are summarized in Table 1. A method flow chart is provided in Figure 1.

Calibration Curve, Limit of Detection and Limit of Quantitation

Calibration standards, ranging in concentration from 0.100 to 1.00 mg a.i./L, were analyzed with the verification sample set. Linear regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. A representative calibration curve is presented in Figure 2. The concentration of aqueous hydrogen cyanamide (50% w/w) in the samples was determined by substituting the peak area responses into the linear regression equation. Representative chromatograms of low and high calibration standards are shown in Figures 3 and 4, respectively.

The method limit of detection (LOD) for the method verification analyses was defined as a peak reflecting three times the signal-to-background ratio. The background peak height of a matrix blank was 3.95536. The peak height of a low (0.100 mg a.i./L) calibration standard was 106.18207 (signal). The signal-to-background ratio was 26.84. Calculated as three times the standard concentration divided by the signal-to-background ratio, the LOD was 11.2 μg a.i./L.

The method limit of quantitation (LOQ) for the method verification analyses was set at 0.100 mg a.i./L as determined by the product of the lowest standard (0.100 mg a.i./L) and the dilution factor of the matrix blanks (1.00). This LOQ was equivalent to 0.197 mg/L as product.

Reagent and Matrix Blanks

Along with the series of fortification samples analyzed for the method verification, three reagent (prepared in NanoPure® water) and three matrix (prepared in filtered saltwater) blanks were analyzed to determine possible interferences. No interferences were observed at or above the LOQ (0.197 mg/L) during the sample analyses (Table 2). Representative chromatograms of reagent and matrix blank samples are presented in Figures 5 and 6, respectively.

Method Verification Samples

During the method verification, filtered saltwater matrix was fortified in triplicate at 0.402, 4.02, 40.2 and 402 mg/L using stock solutions containing aqueous hydrogen cyanamide (50% w/w) in NanoPure® water. Samples fortified at 0.402, 4.02, 40.2 and 402 mg/L yielded mean recoveries of 104, 103, 102 and 105%, respectively (Table 2). Representative chromatograms of low and high-level matrix fortifications are presented in Figures 7 and 8, respectively.

Example Calculations

Sample number 248C-101-VMAS-12, nominal concentration of 402 mg/L in filtered saltwater.

Initial Volume: 0.200 mL
Final Volume: 50.0 mL
Dilution Factor: 250
Peak Area: 11089.5
Purity: 50.8%

Calibration curve equation.
Slope: 12994.30
Intercept: -104.89114

$$\text{Aq. HC}^1 \text{ (mg a.i./L) at instrument} = \frac{\text{Peak area - (Y-intercept)}}{\text{Slope}}$$

$$= (11089.5 + 104.89114) / 12994.30 = 0.8615 \text{ mg a.i./L}$$

$$\text{Aq. HC}^1 \text{ (mg/L) in sample} = \frac{\text{Aq. HC}^1 \text{ (mg a.i./L) at instrument x Final volume}}{\text{(Initial volume x Purity)}}$$

$$= (0.8615 \text{ mg a.i./L x } 50.0 \text{ mL}) / (0.200 \text{ mL x } 0.508) = 424 \text{ mg/L}$$

$$\text{Percent of Nominal Concentration} = \frac{\text{Aq.HC}^1 \text{ (mg/L) in sample}}{\text{Aq. HC}^1 \text{ (mg/L) fortified}} \times 100$$

$$= (424 \text{ mg/L} / 402 \text{ mg/L}) \times 100 = 105\%$$

1 Aq. HC = Aqueous hydrogen cyanamide (50% w/w)

CONCLUSIONS

The method for the analysis of aqueous hydrogen cyanamide (50% w/w) in filtered saltwater was verified for concentrations ranging from 0.402 to 402 mg/L. The mean recoveries ranged from 100 to 106% of nominal concentrations, with an overall mean recovery of 104 ± 1.91% (cv = 1.84).

Table 1

Typical HPLC Operational Parameters

INSTRUMENT:	Hewlett-Packard Model 1090 High Performance Liquid Chromatograph with a Jasco FP-920 Fluorescence Detector.
ANALYTICAL COLUMN:	Zorbax Phenyl Column (250 x 4.6 mm, 5 μm particle size)
STOP TIME:	20 minutes
FLOW RATE:	1.00 mL/min
OVEN TEMPERATURE:	45°C
MOBILE PHASE:	Solvent A: 0.01M Potassium dihydrogenphosphate at pH 3.6 Solvent B: Methanol

Time	Solvent A	Solvent B
0.00	70%	30%
2.00	70%	30%
13.00	50%	50%
13.01	40%	60%
16.00	40%	60%
16.01	70%	30%
20.00	70%	30%

INJECTION VOLUME:	50 μL
AQUEOUS HYDROGEN CYANAMIDE(50% w/w) RETENTION TIME:	Approximately 12.0 to 12.4 minutes
EXCITATION WAVELENGTH:	470 nm
EMISSION WAVELENGTH:	530 nm
GAIN:	x 100

Table 2

Method Verification Recoveries of Aqueous Hydrogen Cyanamide (50% w/w) in Filtered Saltwater

Sample		Concentration (mg/L)		Percent Recovery[2]	Overall Mean Measured (mg/L)	Overall Mean Percent Recovery
Number (248C-101-)	Type	Fortified	Measured[1]			
VREB-1	Reagent Blank	0.0	<0.197	--		
VREB-2	Reagent Blank	0.0	<0.197	--		
VREB-3	Reagent Blank	0.0	<0.197	--	--	--
VMAB-1	Matrix Blank	0.0	<0.197	--		
VMAB-2	Matrix Blank	0.0	<0.197	--		
VMAB-3	Matrix Blank	0.0	<0.197	--	--	--
VMAS-1	Matrix Fortification	0.402	0.421	105		
VMAS-2	Matrix Fortification	0.402	0.419	104		
VMAS-3	Matrix Fortification	0.402	0.416	104	0.419	104
VMAS-4	Matrix Fortification	4.02	4.05	101		
VMAS-5	Matrix Fortification	4.02	4.18	104		
VMAS-6	Matrix Fortification	4.02	4.23	105	4.15	103
VMAS-7	Matrix Fortification	40.2	40.3	100		
VMAS-8	Matrix Fortification	40.2	41.4	103		
VMAS-9	Matrix Fortification	40.2	41.1	102	40.9	102
VMAS-10	Matrix Fortification	402	424	106		
VMAS-11	Matrix Fortification	402	424	106		
VMAS-12	Matrix Fortification	402	424	105	424	105
					Totals: N = 12 Mean = 104 S.D. = 1.91 CV = 1.84	

[1] The limit of quantitation (LOQ) was calculated to be 0.197 mg/L based on the product of the lowest standard (0.100 mg a.i./L) and the dilution factor of the matrix blanks (1.00) times 1.97 (to convert to equivalent product).

[2] Results were generated using Excel 4.0 in the full precision mode. Manual calculation of percent recoveries may differ slightly.

METHOD OUTLINE FOR THE PROCESSING OF AQUEOUS HYDROGEN CYANAMIDE (50% w/w) IN FILTERED SALTWATER

Prepare calibration standards in filtered saltwater using volumetric flasks and gas-tight syringes.

↓

Prepare recovery samples in filtered saltwater using volumetric flasks and gas-tight syringes. The reagent blank will be unfortified NanoPure® water; the matrix blank will be unfortified filtered saltwater. Dilute samples as necessary with filtered saltwater.

↓

DERIVATIZATION STEPS (Samples and Standards)

↓

Add 1.0 mL of 0.37M potassium tetraborate solution to a 15-mL centrifuge tube.

↓

Add 2.0 mL of the standard solution or test solution to the 15-mL centrifuge tube. Vortex/mix.

↓

Add 2.0 mL of methanolic 0.025M NBD-CL solution to each 15-mL centrifuge tube and vortex/mix.

↓

Heat the centrifuge tubes using a heating block at 80°C for 30 minutes.

↓

Add 1.0 mL of 1.2 M HCl solution. Vortex/mix.

↓

Filter the solutions through an Acrodisc PTFE 0.45 μm filter into HPLC vials.

↓

Analyze using HPLC equipped with fluorescence detection.

Figure 1. Analytical method flow chart for the analysis of aqueous hydrogen cyanamide (50% w/w) in filtered saltwater.

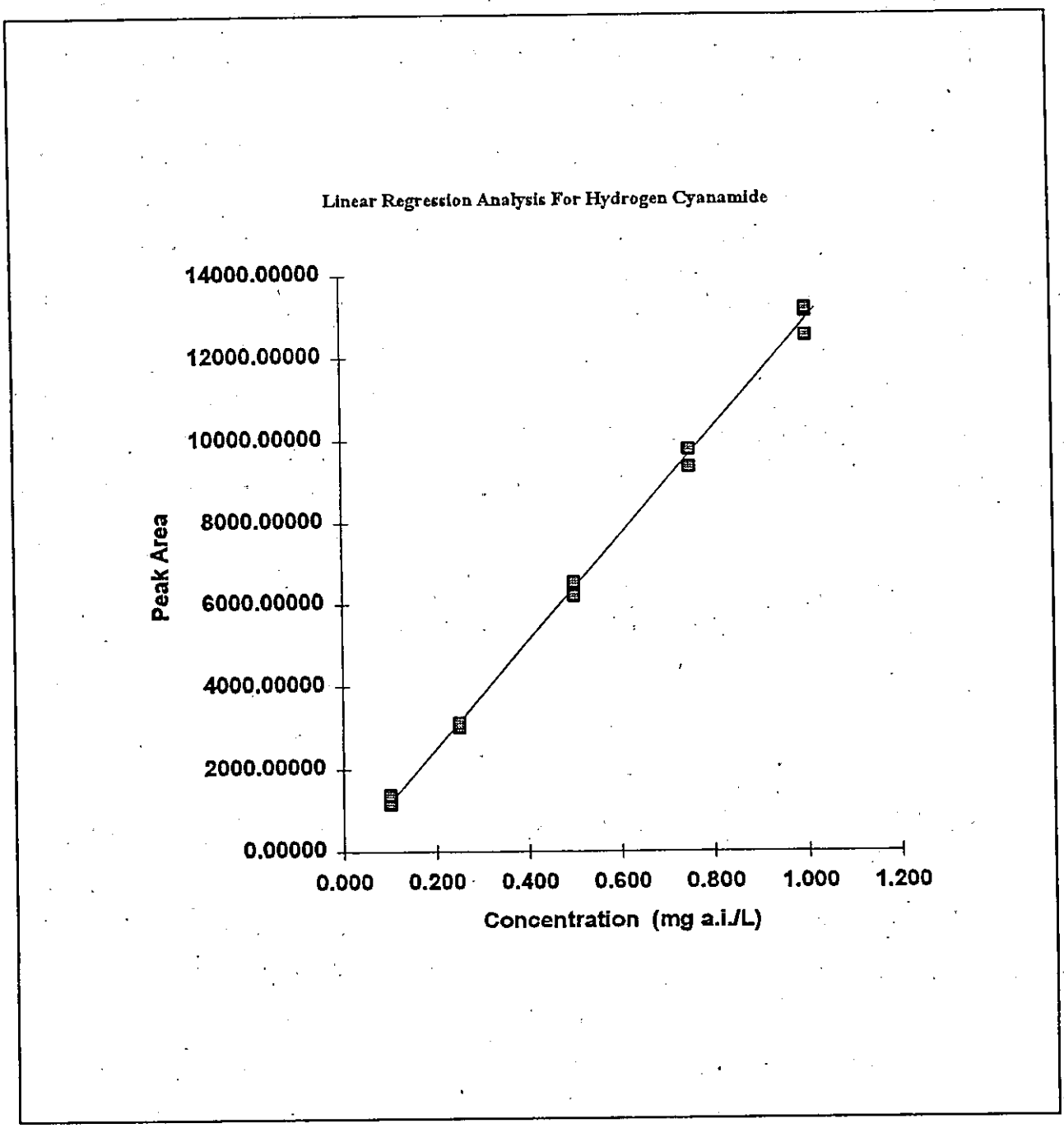

Figure 2. A representative calibration curve for Cyanamid F1000. Slope = 12994.30; Intercept = -104.89114; $r^2 = 0.9980$.

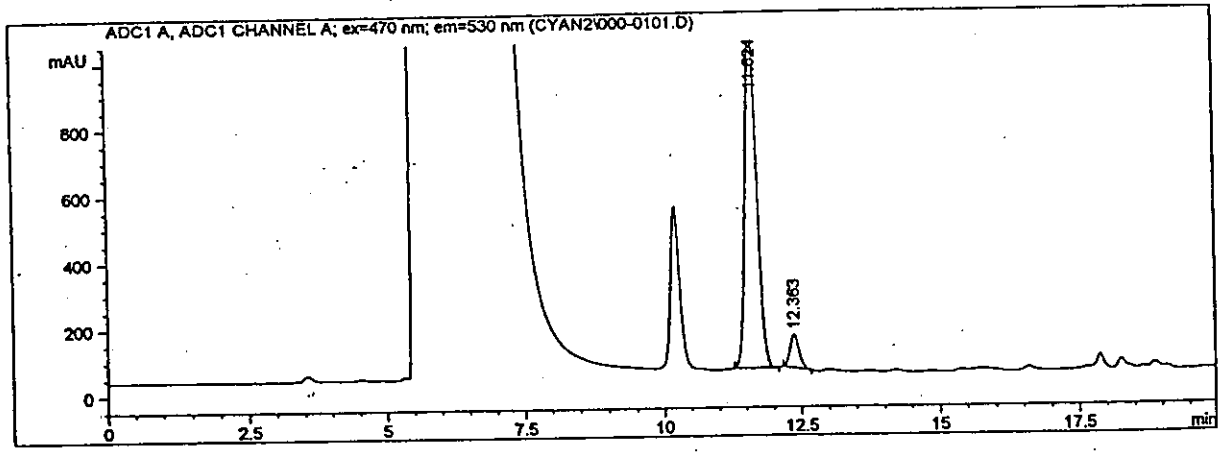

Figure 3. A representative chromatogram of a low-level (0.100 mg a.i. Cyanamid F1000/L) standard.

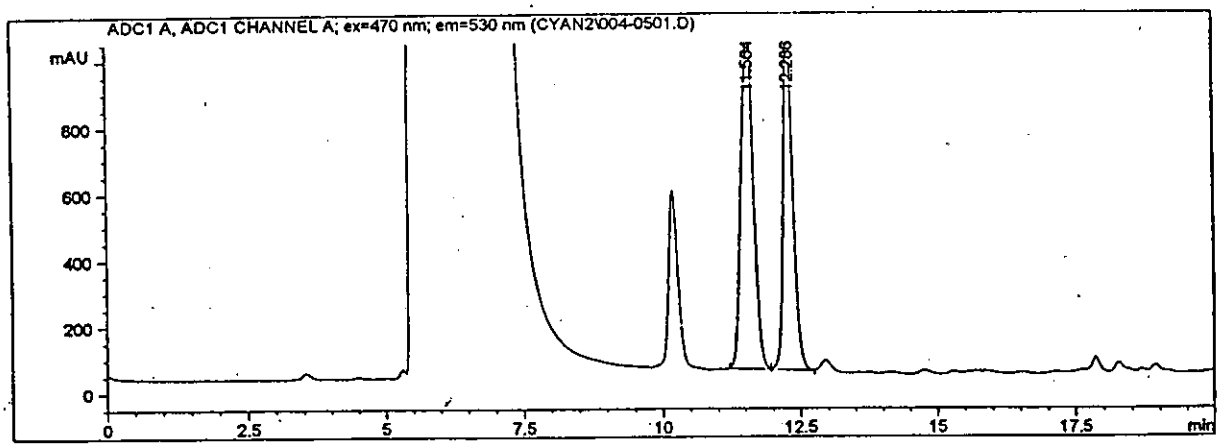

Figure 4. A representative chromatogram of a high-level (1.00 mg a.i. Cyanamid F1000/L) standard.

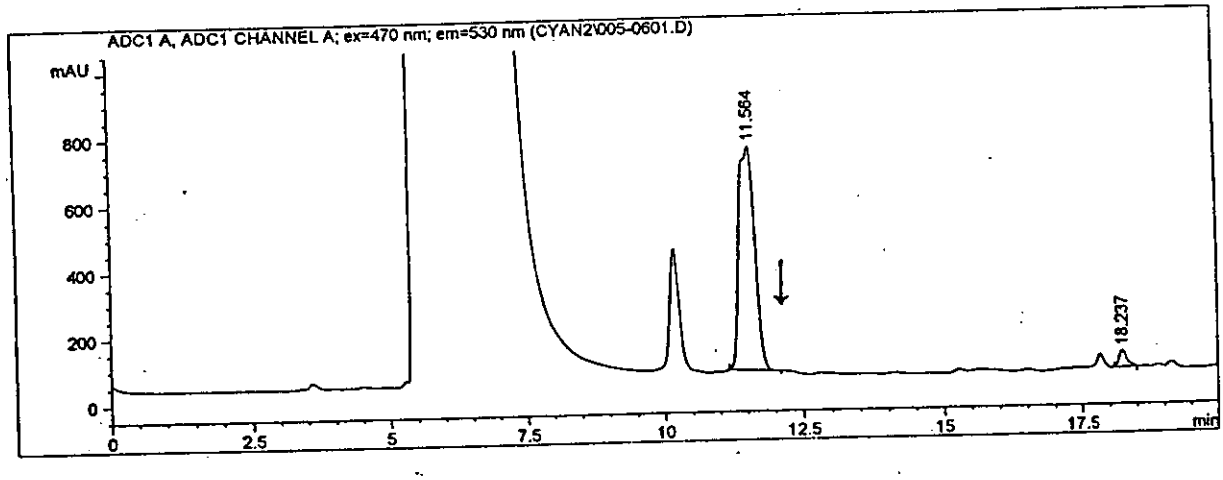

Figure 5. A representative chromatogram of a NanoPure® water reagent blank (248C-101-VREB-1). Arrow indicates the retention time of aqueous hydrogen cyanamide (50% w/w) peak.

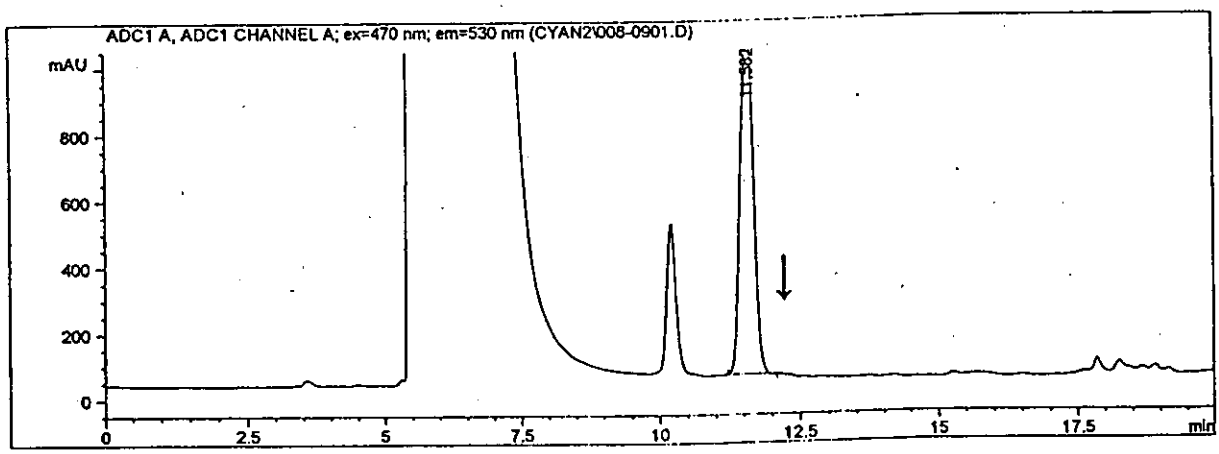

Figure 6. A representative chromatogram of a filtered saltwater matrix blank (248C-101-VMAB-1). Arrow indicates the retention time of aqueous hydrogen cyanamide (50% w/w) peak.

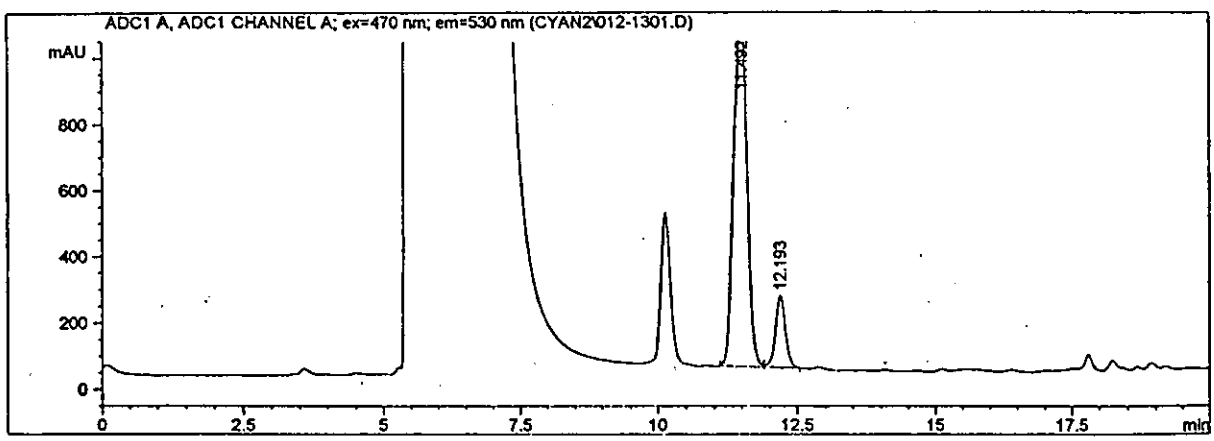

Figure 7. A representative chromatogram of a low-level filtered saltwater matrix fortification (248C-101-VMAS-1), 0.402 mg/L.

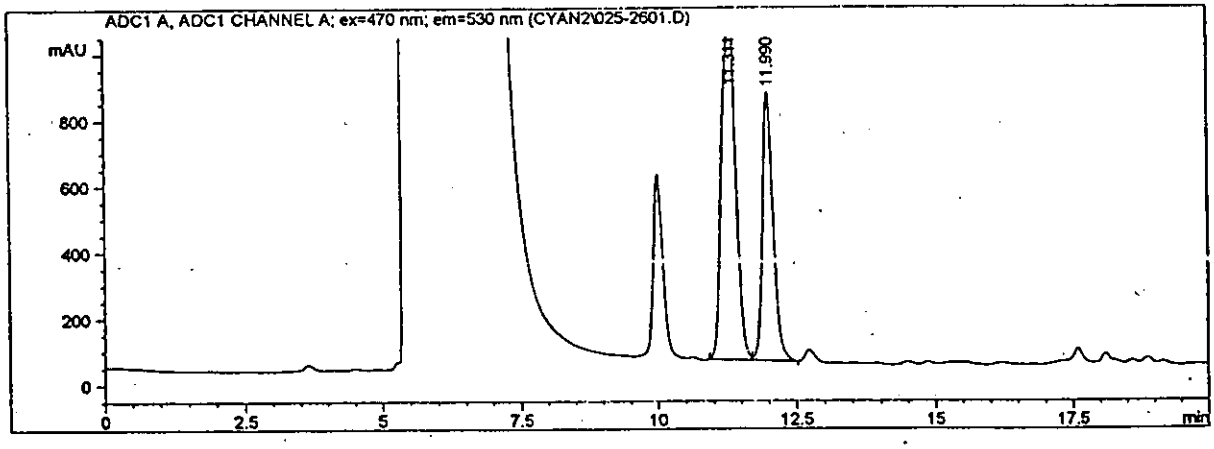

Figure 8. A representative chromatogram of a high-level filtered saltwater matrix fortification (248C-101-VMAS-12), 402 mg/L.

APPENDIX I

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

Sponsor:	SKW Trostberg AG
Test Substance:	Aqueous Hydrogen Cyanamide (50% w/w)
Dilution Water:	Filtered Saltwater

	Mean	Range
Salinity (ppt)	20 (N = 4)	20 - 20
pH	8.1 (N = 4)	8.1 - 8.2

APPENDIX II

Analyses of Pesticides, Organics, Metals and Other Inorganics in Wildlife International Ltd. Filtered Saltwater[1]

ANALYSIS	MEASURED CONCENTRATION		
Miscellaneous Measurements			
Total Dissolved Solids		23,500	mg/L
Ammonia Nitrogen	<	0.050	mg/L
Total Organic Carbon[2]	<	1.0	mg/L
Total Cyanide	<	10.0	µg/L
Organochlorines and PCBs			
Aldrin	<	0.005	µg/L
Alpha BHC	<	0.005	µg/L
Beta BHC	<	0.005	µg/L
Delta BHC	<	0.005	µg/L
Gamma BHC (Lindane)	<	0.006	µg/L
Chlordane	<	0.025	µg/L
DDD, pp'	<	0.006	µg/L
DDE, pp'	<	0.005	µg/L
DDT, pp'	<	0.008	µg/L
Dieldrin	<	0.005	µg/L
Endosulfan, A	<	0.005	µg/L
Endosulfan, B	<	0.005	µg/L
Endosulfan Sulfate	<	0.018	µg/L
Endrin	<	0.010	µg/L
Endrin Aldehyde	<	0.005	µg/L
Heptachlor	<	0.005	µg/L
Methoxychlor	<	0.007	µg/L
Heptachlor Epoxide	<	0.005	µg/L
Toxaphene	<	0.500	µg/L
PCB-1016	<	0.260	µg/L
PCB-1221	<	0.260	µg/L
PCB-1232	<	0.260	µg/L
PCB-1242	<	0.720	µg/L
PCB-1248	<	0.720	µg/L
PCB-1254	<	0.720	µg/L
PCB-1260	<	0.720	µg/L
Metals and Other Inorganics			
Aluminum[3]	<	100	µg/L
Arsenic[3]	<	25.0	µg/L
Beryllium[3]	<	0.50	µg/L
Cadmium[3]	<	1.0	µg/L
Calcium[3]		235	mg/L
Chromium[3]	<	2.0	µg/L
Cobalt[3]	<	1.0	µg/L
Copper[3]	<	20.0	µg/L
Iron[3]	<	100	µg/L
Lead[3]	<	10.0	µg/L
Magnesium[3]		760	mg/L
Manganese[3]	<	4.0	µg/L
Mercury	<	0.20	µg/L
Molybdenum[3]	<	2.0	µg/L
Nickel[3]	<	20.0	µg/L
Potassium[3]		277	mg/L
Selenium[3]	<	25.0	µg/L
Silver[3]	<	1.0	µg/L
Sodium[3]		6,010	mg/L
Zinc[3]	<	20.0	µg/L

[1] Analyses performed by QST Environmental, Gainesville, Florida for samples collected on November 3 through November 7, 1997.
[2] Analyses performed by Wildlife International Ltd. for the sample collected on November 5, 1997.
[3] Analyses performed by Wildlife International Ltd. for samples collected on November 5 through 7, 1997.

APPENDIX III

Changes to Protocol

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

1. The proposed experimental start and termination dates, test concentrations, test substance and receipt date, and analytical standard were added by amendment.
2. The LOQ and LOD were clarified by amendment.
3. The ASTM type of NanoPure® water was corrected by amendment.
4. The Sponsor's method was added by amendment.
5. The LOD was further clarified by amendment.
6. The test concentrations were incorrectly amended based on a 50% purity and corrected to a 50.8% purity by deviation.

APPENDIX IV

Personnel Involved in the Study

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

1. Willard B. Nixon, Ph.D., Manager, Analytical Chemistry
2. Timothy Z. Kendall, M.S., Supervisor
3. David Leffingwell, B.S., Chemist