INTRODUCTION

Verification samples were fortified and analyzed to evaluate the performance of a method for the analysis of iodomethane in freshwater and the stability of iodomethane in freshwater over a 96 hour period in a closed and open test system. The study was conducted by Wildlife International, Ltd. and identified as Project Number 443C-107. The analyses of the samples were performed at Wildlife International, Ltd. using High Performance Liquid Chromatography (HPLC) with UV detection. Verification samples were prepared and analyzed on January 15, 2001 and April 4, 2001. Stability samples were prepared and analyzed between December 11, 2000 and December 15, 2000. All raw data generated by Wildlife International, Ltd. and a copy of the final report are filed under Project Number 443C-107 in archives located on the Wildlife International, Ltd. site.

PURPOSE

The purpose of this study was to verify a method for measuring iodomethane in freshwater used at Wildlife International, Ltd. for proposed environmental effects studies and determine the stability of iodomethane in freshwater over a 96 hour period in a closed and open test system.

EXPERIMENTAL DESIGN

Freshwater was fortified at six different concentrations and analyzed based on a method provided by the Sponsor. Reagent and matrix blanks were analyzed concurrently to evaluate potential analytical interferences. Freshwater was also fortified at two different concentrations, stored in a closed and open test system, and analyzed at 0, 24, 48 and 96 hours. Calibration curves were prepared from external standards of iodomethane to determine the test substance concentrations in samples.

MATERIALS AND METHODS

Test Substance

The test substance was received from Tomen Agro, Inc. on November 13, 2000 and was assigned Wildlife International, Ltd. identification number 5445 upon receipt. The test substance, described as a liquid, was identified as: Iodomethane TM 425; Lot 007403; Batch 02; Drum 2: CAS No. 74-88-4. The test substance had a reported purity of 99.7% and an expiration date of May 4, 2002. The test substance was stored under ambient conditions.

Stock/Standards Preparation

Stock solution of iodomethane was prepared by accurately measuring 439 µl (corrected for specific gravity) of the test substance. The test substance was transferred to a 100-mL class A volumetric flask, and brought to volume using methanol. This primary stock solutions contained 10.0 mg/mL of iodomethane. The stock solutions were used to fortify the method verification samples.

Calibration standards containing iodomethane were prepared in freshwater and analyzed with each sample set. Preparation of the calibration standards was performed by diluting aliquots of a 0.0100, 1.00 or 10.0 mg/mL stock solution. The following shows the typical dilution scheme for each set of standards.

Calibration standards during stability analyses

Stock Concentration mg/mL	Aliquot (µl)	Final Volume (mL)	Standard Concentration (mg/L)
1.00	100	100	1.00
10.0	25.0	100	2.50
10.0	75.0	100	7.50
10.0	125	100	12.5
10.0	200	100	20.0
10.0	250	100	25.0

Calibration standards during verification analyses

Stock Concentration mg/mL	Aliquot (µl)	Final Volume (mL)	Standard Concentration (mg/L)
1.00	40.0	100	0.400
1.00	100	100	1.00
1.00	200	100	2.00
1.00	300	100	3.00
1.00	400	100	4.00
1.00	500	100	5.00

Stock Concentration mg/mL	Aliquot (µl)	Final Volume (mL)	Standard Concentration (mg/L)
0.0100	150	100	0.0150
0.0100	250	100	0.0250
0.0100	750	100	0.0750
0.0100	1000	100	0.100
0.0100	150	10.0	0.150
0.0100	250	10.0	0.250

Reagents and Solvents

All solvents used in the methods were of HPLC grade or equivalent. NANOpure® water (equivalent to ASTM Type II Designation D1193-91) was used (1).

Freshwater

The water used for the method verification was freshwater obtained from a well approximately 40 meters deep located on the Wildlife International, Ltd. site. The well water is characterized as moderately-hard water. The means and ranges of specific conductance, hardness, alkalinity and pH measurements of the well water during the four-week period immediately preceding the test are presented in Appendix I.

The well water was passed through a sand filter to remove particles greater than approximately 25 µm, and pumped into a 37,800-L storage tank and aerated with spray nozzles. Prior to use, the water again was filtered to 0.45 µm in order to remove microorganisms and fine particles. The results of periodic analyses performed to measure the concentrations of selected contaminants in well water used by Wildlife International, Ltd. are presented in Appendix II.

Analytical Method

The method used for the analysis of the method verification samples was based upon methodology provided by the sponsor. The analytical method consisted of the direct analysis of aqueous samples using HPLC with UV detection.

Concentrations of iodomethane in the samples were determined using high performance liquid chromatography (HPLC) with UV detection. The instrument, a Hewlett Packard Model 1090 or 1100 High Performance Liquid Chromatograph was equipped with a Hewlett-Packard Model 1100 Variable Wavelength or Jasco Model 975 Detector operated at 254 nm. Chromatographic separations were achieved using a YMC-Pack ODS AM column (150 mm x 4.6 mm, 3 µm particle size). Instrumental parameters for the analysis of iodomethane are summarized in Table 1 and a method flowchart is provided in Figure 1.

Calibration Curve, and Limit of Quantitation (LOQ)

Calibration standards of iodomethane, ranging in concentration from 0.0150 to 0.250 mg/L and 0.400 to 5.00 mg/L, were analyzed with the freshwater verification samples. Calibration standards of iodomethane, ranging in concentration from 1.00 to 25.0 mg/L, were also analyzed with the freshwater stability sample sets. Linear regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. A typical calibration curve of iodomethane is presented in Figure 2. The concentration of iodomethane in the samples was determined by substituting the peak area responses into the applicable linear regression equation. Representative chromatograms of low and high-level calibration standards are presented in Figures 3 and 4, respectively.

The method limit of quantitation (LOQ) for the method verification analysis of iodomethane in freshwater was set at 0.0150 or 0.400 mg/L, calculated as the product of the lowest calibration standard (0.0150 or 0.400 mg/L) and the dilution factor of the matrix blank sample (1.00). The method limit of quantitation (LOQ) for the stability analysis of iodomethane in freshwater was set at 1.00 or 2.50 mg/L, calculated as the product of the lowest calibration standard (1.00 or 2.50 mg/L) analyzed with the samples and the dilution factor of the 5.0 mg/L samples (1.00).

Reagent and Matrix Blanks

Concurrent with each series of matrix fortification samples, one reagent blank and/or one matrix blank were analyzed to determine possible interferences. No interferences were observed at or above the appropriate LOQ during the sample analyses (Table 2). A representative chromatogram of a reagent blank is presented in Figure 5. A representative chromatogram of a matrix blank for freshwater is presented in Figure 6.

Freshwater Method Verification Samples

Freshwater was fortified in triplicate at 0.0250, 0.200, 0.600, 2.00, 4.00 and 12.0 mg/L using a stock solution containing iodomethane in methanol. Samples fortified at 0.0250, 0.200, 0.600, 2.00, 4.00 and 12.0 mg/L yielded mean recoveries of 110, 97.6, 101, 101, 100 and 94.4%, respectively (Table 2). Representative chromatograms of low and high-level freshwater fortifications are presented in Figures 7 and 8.

Freshwater Stability Samples

The stability of iodomethane was evaluated in freshwater in two potential test system (closed and open) proposed for aquatic toxicity testing. Freshwater was fortified using a stock solution containing iodomethane in methanol at nominal concentrations of 5.00 and 50.0 mg/L. Concentrations of iodomethane in freshwater were determined by collecting and analyzing samples at approximately 0, 24, 48 and 96 hours (Table 3). The closed test system consisted of 100 ml serum bottles with Teflon® - faced septa and aluminum seals. Recoveries of iodomethane in freshwater for 0, 24, 48 and 96 hours in a closed system at 5.00 mg/L yielded percent recoveries of 110, 88.4, 71.4 and 89.0%, respectively. Recoveries of iodomethane in freshwater for 0, 24, 48 and 96 hours in a closed system at 50.0 mg/L yielded percent recoveries of 93.0, 77.2, 50.9 and 68.7%, respectively. The open test system consisted of 250 ml beakers. Iodomethane in freshwater for 0 hours in an open system at 5.00 and 50.0 mg/L yielded percent recoveries of 94.8 and 75.5%, respectively. The low recovery obtained at the higher concentration may reflect diminished solubility. Iodomethane in freshwater for 24, 48 and 96 hours in an open system at 5.00 or 50.0 mg/L was determined to be not detectable (<1.00 or 2.50 mg/L). The test systems were placed in a temperature controlled water bath with an initial temperature of 21.3°C.

Example Calculations

The analytical result and percent recovery for sample number 443C-107-VMAS-12, nominal concentration of 12.0 mg/L in freshwater, was calculated using the following equations.

$$\text{Iodomethane (mg/L) in sample} = \frac{\text{Peak area - (Y-intercept)}}{\text{Slope}} \times \text{Dilution factor}$$

Peak area = 48.62970
Y-intercept = -0.2312
Slope = 12.9990
Initial Volume (V_i): = 0.500 mL

Final Volume (V_f): = 1.50 mL
Dilution Factor (V_f/V_i): = 3.00

$$\text{Iodomethane (mg/L) in sample} = \frac{48.62970 + 0.2312}{12.9990} \times 3.00$$

$$= 11.3$$

$$\text{Percent of nominal concentration} = \frac{\text{Iodomethane in sample (mg/L)}}{\text{Iodomethane fortified conc. (mg/L)}} \times 100$$

$$\text{Percent of nominal concentration} = \frac{11.3}{12.0} \times 100$$

$$= 94.0\%$$

Table 1

Typical HPLC Operational Parameters

INSTRUMENT:	Hewlett-Packard Model 1090 or 1100 High Performance Liquid Chromatograph with a Hewlett-Packard 1100 Variable Wavelength or Jasco Model 975 Detector
ANALYTICAL COLUMN:	YMC-Pack ODS AM (150 mm x 4.6 mm, 3 µm particle size)
STOP TIME:	6 minutes
FLOW RATE:	1.00 mL/minute
OVEN TEMPERATURE:	40°C
MOBILE PHASE:	SOLVENT A: 40% 0.1% H_3PO_4 SOLVENT B: 60% CH_3CN
INJECTION VOLUME:	100 µL
IODOMETHANE RETENTION TIME:	~ 4.36 minutes
PRIMARY ANALYTICAL WAVELENGTH:	254 nm

FLOWCHART FOR THE ANALYSIS OF IODOMETHANE IN FRESHWATER

Prepare matrix fortification samples by adding appropriate volumes of freshwater to individual class A volumetric flasks. Fortify as required and bring to final volume with freshwater. Mix well. Prepare reagent and matrix blank samples using NANOpure® water and freshwater, respectively.

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Transfer an aliquot of each sample or dilution to autosampler vials and submit for analysis.

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Analyze samples by HPLC with UV detection.

Figure 1. Analytical method flowchart for the analysis of iodomethane in freshwater.