INTRODUCTION

The objectives of this study were to validate methodology for the determination of Chloropicrin in three water types (ground, drinking and surface).

To determine the validity of the analytical method, it was necessary to determine:

- Recovery
- Accuracy
- Precision (repeatability)
- Linearity
- Specificity
- Limit of quantitation
- Limit of detection

The study was designed and performed in accordance with the following regulatory guidelines:

Directive 91/414/EEC as amended by Directive 96/46/EC, SANCO/3029/99 rev. 4 of 11 July 2000 and SANCO/825/00 rev. 7 of 17 March 2004.

The protocol was signed by the Study Director and Huntingdon Life Sciences Management on 19 December 2006, by the Sponsor's Monitoring Scientist on 21 December 2006 and the Sponsor's Representative on 28 December 2006.

The study was undertaken at Huntingdon Life Sciences, Eye between 7 February 2007 and 13 February 2007.

Some standard solutions used in this study have been shared with other GLP studies being performed simultaneously for the same sponsor. This is considered to have no effect on the integrity or validity of this study.

Huntingdon Life Sciences shall retain all the experimental raw data, the signed original protocol and any amendments thereto, and the signed final report from this study in its archive for a period of five years from the date of issue of the final report. After such time, the sponsor will be contacted and their advice sought on the return, disposal or further retention of the study data.

MATERIALS

TEST SUBSTANCE

Common name:	Chloropicrin
Chemical name (IUPAC):	Trichloronitromethane
Structure:	Cl_3C-NO_2
Molecular formula	CCl_3NO_2
Molecular weight	164.4
Appearance:	Colourless liquid
Storage conditions:	Room temperature
Batch number:	002-199A
Receipt date:	16 January 2007
Supplier:	Trinity Manufacturing, Ltd
Purity:	99.9%
Expiry date:	14 November 2008

Control matrices

The drinking water was obtained from a tap in the Residue Analysis Department, the ground water was obtained from a source in the Huntingdon area by Anglian Water and the surface water was obtained from Diss Mere, Diss, Norfolk. Upon receipt the water samples were allocated a unique Huntingdon Life Sciences, Residue Analysis Department identification number. The surface water was characterised in a separate study and is presented in the following table:

Parameter	Found Value
pH	7.7
Dissolved Oxygen (analysed on the date of sampling)	8.08 mgO_2/L* at 13.3 °C
Conductivity	569 µS/cm
Alkalinity	180 mg/L as $CaCO_3$
Total Hardness	180 mg/L as $CaCO_3$
Total Organic Carbon	15.2 mgC/L
Dissolved Organic Carbon	12.7 mgC/L

*reflects an air saturation value (ASV) for dissolved oxygen at the Mere water temperature (considered to be *ca* 9.1°C at the time of sampling).

METHOD

General principle

The method for the determination of residues of Chloropicrin in water comprised liquid: liquid partition using hexane prior to quantitation by gas chromatography using electron capture detection (GC-ECD). Confirmatory analysis was performed by gas chromatography with mass spectrometric detection (GC-MS).

Materials	Grade (or equivalent)	Manufacturer
Acetone	Certified	Fisher Scientific UK
Hexane	Distilled	Fisher Scientific UK

Local COSHH assessments contain full details on the safe handling of chemicals and reagents. Organic solvents are used in this procedure. All steps requiring the handling and transfer of these materials are performed in a fully functional ventilated laboratory fume hood or equivalent well ventilated area in accordance to local safety regulation.

Test substance solutions

An appropriate amount of Chloropicrin was accurately weighed and dissolved in acetone to give a stock standard solution. Appropriate dilutions of the stock standard solutions were made with acetone to give fortification standard solutions.

The fortification solutions were progressively diluted with hexane to produce a series of instrument calibration solutions in the ranges 0.5 to 100 ng/mL.

Procedures

A sub-sample (20 mL) of water was measured into a polypropylene tube (50 mL) and the sample fortified at this stage if required. An aliquot (2 mL) of hexane was added and the sample shaken on a mechanical shaker for 30 minutes. The sample was centrifuged at 3500 rpm for 5 minutes before aliquots of the upper hexane phase were taken for quantitation by GC-ECD and confirmatory analysis by GC-MS.

Instrumentation

GC-ECD conditions

Instrument:	Hewlett Packard 6890 gas chromatograph with electron capture detector (ECD)
Column:	Zebron ZB – 50 (30 m x 530 µm x 1 µm film thickness (Phenomonex)
Injector temperature:	200°C
Detector temperature:	260°C
Oven programme:	40°C held for 2 min then ramped at 10°C/min to 80°C and then ramped at 30°C/min to 200°C
Anode purge:	6 mL/min
Carrier:	7 mL/min
Make up (nitrogen):	Approximately 53 mL/min
Injection volume:	1 µL
Retention times:	approximately 4.3 min
LOQ	0.1 µg/L
LOD	0.5 ng/mL (equivalent to 0.05 µg/L in water)

GC-MS conditions

Instrument:	Varian 1200
Ionisation mode:	CI-
Ion monitoring details:	*m/z* 119 (although other ions were produced this ion gave the cleanest chromatogram with the necessary sensitivity).
Column:	RTX-5ms (30 m x 0.25 mm x 0.25 µm film thickness)
Carrier gas:	Helium
Flow rate:	1 mL/min
Injector temperature:	200°C
Oven programme:	40°C held for 3 min then ramped at 10°C/min to 60°C and then ramped at 30°C/min to 200°C
Injection volume:	1 µL
Retention time:	Approximately 4 minutes
LOQ	0.1 µg/L
LOD	0.5 ng/mL (equivalent to 0.05 µg/L in water)

Calculation of results

Test samples were quantified using the following equation:

$$\text{Residue found } (\mu g/L) = x \times \frac{1}{M} \times D$$

Where x (residue concentration in final solution) was calculated using the linear regression

$$y = m x + c \qquad \text{where } x \text{ (concentration in ng/mL)} = \frac{y - c}{m}$$

c = intercept
m = slope
y = peak area of sample
M = matrix concentration (mL/mL)
D = dilution factor

Example calculation of Chloropicrin detected in untreated surface water fortified at 0.1 µg/L (06/00/6160 F0.1 A – GC-ECD analysis). The primary data for this sample is presented in Table 9, Appendix 1.

Linear regression $y = m x + c$

$$321.3 = 359.1x - 9.363$$

where $y = 321.3$
m = 359.1
c = - 9.363

Therefore, concentration of Chloropicrin (x) $= \frac{321.3 + 9.363}{359.1} = 0.921$ ng/mL

Matrix concentration = 10 mL/mL
Dilution factor = 1

Chloropicrin detected (µg/L) $= \frac{0.921 \text{ ng/mL} \times 1}{10 \text{ mL/mL}} = 0.0921$ µg/L

Recovery $= \frac{0.0921 \text{ µg/L} \times 100\%}{0.1 \text{ µg/L}} = 92\ \%$