SafePharm Laboratories

PFHA:

ACUTE TOXICITY TO RAINBOW TROUT
(*Oncorhynchus mykiss*)

SPL PROJECT NUMBER: 1742/018

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QUALITY ASSURANCE REPORT

This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress.

This report has been audited by Safepharm Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

	02 December 2003	Standard Test Method Compliance Audit
	12 July 2004	Test Material Preparation
	12 July 2004	Test System Preparation
	05 July 2004	Exposure
	20 July 2004	Assessment of Response
	06, 27 July 2004	Chemical Analysis
§	31 August 2004	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

..... DATE: 22 DEC 2004

For Safepharm Quality Assurance Unit*

***Authorised QA Signatures:**

Head of Department:	JR Pateman CBiol MIBiol DipRQA AIQA FRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	JV Johnson BSc MRQA; G Wren ONC MRQA

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106 as amended by SI 2004/0994)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 2004/9/EC and 2004/10/EC.

These international standards are acceptable to the Regulatory agencies of the following countries: Australia, Austria, Belgium, Canada, the Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Republic of Korea, Luxembourg, Mexico, The Netherlands, New Zealand, Norway, Poland, Portugal, Slovenia, South Africa, Spain, Sweden, Switzerland, Turkey, the United Kingdom, and the United States of America.

This report fully and accurately reflects the procedures used and data generated.

..... Date: 17 DEC 2004

P M Wetton BSc
Study Director

The analytical data presented in this report were compiled by me or under my supervision and accurately reflect the data obtained.

..... Date: 17 DEC 2004

Dr J McKenzie CChem MRSC
Head of Analytical Services

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SUMMARY

Introduction. A study was performed to assess the acute toxicity of the test material to rainbow trout (*Oncorhynchus mykiss*). The method followed that described in the OECD Guidelines for Testing of Chemicals (1992) No 203, "Fish, Acute Toxicity Test" referenced as Method C.1 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC).

Methods. Following a preliminary range-finding test fish were exposed, in two groups of seven, to an aqueous solution of the test material, at a single concentration of 100 mg/l for a period of 96 hours at a temperature of approximately 14°C under semi-static test conditions. The number of mortalities and any sub-lethal effects of exposure in each test and control vessel were determined 3 and 6 hours after the start of exposure and then daily throughout the test until termination after 96 hours.

Results. The 96-Hour LC_{50} based on nominal test concentrations was greater than 100 mg/l and correspondingly the No Observed Effect Concentration was 100 mg/l.

It was considered unnecessary and unrealistic to test at concentrations in excess of 100 mg/l.

Analysis of the test preparations at 0, 24 and 96 hours showed measured test concentrations to range from 101% to 104% of nominal and so the results are based on nominal test concentrations only.

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ACUTE TOXICITY TO RAINBOW TROUT (*Oncorhynchus mykiss*)

1. INTRODUCTION

This report contains a description of the methods used and results obtained during a study to investigate the acute toxicity of the test material to rainbow trout. The method followed the recommendations of the OECD Guidelines for Testing of Chemicals (1992) No 203 "Fish, Acute Toxicity Test" referenced as Method C.1 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC).

Rainbow trout is a freshwater fish representative of a wide variety of natural habitats, and can therefore be considered as an important non-target organism in freshwater ecosystems.

The range-finding test was conducted between 5 May 2004 and 9 May 2004 and the definitive test between 26 July 2004 and 30 July 2004.

2. TEST MATERIAL AND EXPERIMENTAL PREPARATION

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	PFHA
Description	:	colourless liquid
Chemical name	:	perfluorohexanoic acid
Purity	:	99%
Batch numbers	:	C15009601 used in the range-finding test and validation of method of analysis
	:	00102 used in the definitive test and chemical analysis of test samples
Dates received	:	12 January 2004
	:	05 July 2004
Storage conditions	:	room temperature in the dark

The integrity of supplied data relating to the identity, purity and stability of the test material is the responsibility of the Sponsor.

2.2 Experimental Preparation

For the purpose of the definitive test the test material was dissolved directly in dechlorinated tap water.

An amount of test material (2000 mg) was dissolved in dechlorinated tap water with the aid of shaking by hand for approximately 1 minute and the volume adjusted to 1 litre to give a 2000 mg/l stock solution. The entire volume of the 2000 mg/l stock solution was dispersed in a final volume of 20 litres of dechlorinated tap water and stirred using a flat bladed mixer for approximately 1 minute to give the 100 mg/l test concentration.

Each of the stock solutions was inverted several times to ensure adequate mixing and homogeneity.

The concentration and stability of the test material in the test preparations were verified by chemical analysis at 0 (fresh media), 24 (old media) and 96 hours (old media) (see Appendix 1).

3. METHODS

3.1 Test Species

The test was carried out using juvenile rainbow trout (*Oncorhynchus mykiss*). Fish were obtained from Brow Well Fisheries Limited, Hebden, near Skipton, Yorkshire, UK and maintained in-house since 14 July 2004. Fish were maintained in a glass fibre tank with a "single pass" water renewal system. Fish were acclimatised to test conditions from 14 July 2004 to 26 July 2004. The lighting cycle was controlled to give a 16 hours light and 8 hours darkness cycle with 20 minute dawn and dusk transition periods.

The water temperature was controlled at approximately 14°C with a dissolved oxygen content of greater than or equal to 9.1 mg O_2/l. These parameters were recorded daily. The stock fish were fed commercial trout pellets which was discontinued approximately 24 hours prior to the start of the definitive test. There was zero mortality in the 7 days prior to the start of the test and the fish had a mean standard length of 4.4 cm (sd = 0.1) and a mean weight of 1.07 g (sd = 0.08) at the end of the definitive test. Based on the mean weight value this gave a loading rate of 0.37 g bodyweight/litre.

The diet and diluent water are considered not to contain any contaminant that would affect the integrity and outcome of the study.

3.2 Test Water

The test water used for both the range-finding and definitive tests was the same as that used to maintain the stock fish.

Laboratory tap water was dechlorinated by passage through an activated carbon filter (Purite Series 500) and partly softened (Elga Nimbus 1248D Duplex Water Softener) giving water with a total hardness of approximately 100 mg/l as $CaCO_3$. After dechlorination and softening the water was passed through a series of computer controlled plate heat exchangers to achieve the required temperature. Typical water quality characteristics for the tap water as supplied, prior to dechlorination and softening, are given in Appendix 2.

3.3 Procedure

3.3.1 Range-finding test

The test concentration to be used in the definitive test was determined by a preliminary range-finding test.

In the range-finding test fish were exposed to a series of nominal test concentrations of 1.0, 10 and 100 mg/l. The test material was dissolved directly in water.

An amount of test material (1000 mg) was dissolved in dechlorinated tap water with the aid of shaking by hand for approximately 1 minute and the volume adjusted to 500 ml to give a 2000 mg/l stock solution. A further amount of test material (2000 mg) was dissolved in dechlorinated tap water with the aid of shaking by hand for approximately 1 minute and the volume adjusted to 1 litre to give a 2000 mg/l stock solution. Aliquots (10 and 100 ml) of the initial 2000 mg/l stock solution and the entire volume of the second 2000 mg/l stock solution were each separately dispersed in a final volume of 20 litres of dechlorinated tap water and stirred using a flat bladed mixer for approximately 1 minute to give the 1.0, 10 and 100 mg/l test concentrations respectively.

Each of the stock solutions was inverted several times to ensure adequate mixing and homogeneity.

In the range-finding test 3 fish were added to each 20 litre test and control vessel and maintained at approximately 14°C in a temperature controlled room with a photoperiod of 16 hours light and

The control group was maintained under identical conditions but not exposed to the test material.

Data from the control group was shared with similar concurrent studies.

Each vessel was covered to reduce evaporation. After 3, 6, 24, 48, 72 and 96 hours any mortalities or sub-lethal effects of exposure were determined by visual inspection of the test fish.

3.3.2 Definitive test

Based on the results of the range-finding test a "Limit test" was conducted at a concentration of 100 mg/l to confirm that at the maximum concentration given in the OECD/EEC Test Guidelines, no mortalities or sub-lethal effects of exposure were observed.

3.3.2.1 Preparation of the test material

For the purpose of the definitive test the required amount of test material was added to each test vessel using the method described in Section 2.2.

3.3.2.2 Exposure conditions

As in the range-finding test 20 litre glass exposure vessels were used for each test concentration. At the start of the test seven fish were placed in each test vessel at random, in the test preparations. The test vessels were then covered to reduce evaporation and maintained at approximately 14°C in a temperature controlled room with a photoperiod of 16 hours light and 8 hours darkness with 20 minute dawn and dusk transition periods for a period of 96 hours. The test vessels were aerated via narrow bore glass tubes. The fish were not individually identified and received no food during exposure.

The control group was maintained under identical conditions but not exposed to the test material.

A semi-static test regime was employed in the test involving a daily renewal of the test preparations to ensure that the concentrations of the test material remained near nominal and to prevent the build up of nitrogenous waste products.

Any mortalities and sub-lethal effects of exposure were recorded at 3, 6, 24, 48, 72 and 96 hours after the start of exposure. The criteria of death were taken to be the absence of both respiratory movement and response to physical stimulation.

3.3.2.3 Physico-chemical measurements

The water temperature, pH and dissolved oxygen concentrations were recorded daily throughout the test. The measurements at 0 hours, and after each test media renewal at 24, 48 and 72 hours, represent those of the freshly prepared test preparations while the measurements taken prior to each test media renewal, and on termination of the test after 96 hours, represent those of the used or 24-Hour old test preparations. The pH was measured using a WTW pH 320 pH meter, the dissolved oxygen concentration was measured using a YSI 550 dissolved oxygen meter and the temperature was measured using a Hanna Instruments HI 93510 digital thermometer.

3.3.2.4 Verification of test concentrations

Water samples were taken from the control and each replicate test vessel at 0 (fresh media), 24 (old media) and 96 hours (old media) for quantitative analysis.

Duplicate samples and samples at 24 (fresh media), 48 and 72 hours (fresh and old media) were taken and stored at approximately -20°C for further analysis if necessary.

The method of analysis, stability, recovery and test preparation analyses are described in Appendix 1.

3.3.2.5 Evaluation of data

An estimate of the LC_{50} values was given by inspection of the mortality data.

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archives for five years, after which instructions will be sought as to further retention or disposal.

5. RESULTS

5.1 Range-finding Test

Cumulative mortality data from the exposure of rainbow trout to the test material during the range-finding test are given in Table 1. There were no sub-lethal effects of exposure during the range-finding test.

The results showed no mortalities at the test concentrations of 1.0, 10 and 100 mg/l.

Based on this information, a single test concentration, in duplicate, of 100 mg/l was selected for the definitive test. This experimental design conforms to a "Limit test" to confirm that at the maximum test concentration given in the OECD/EEC Test Guidelines, no mortalities or sub-lethal effects of exposure were observed.

5.2 Definitive Test

5.2.1 Mortality data

Cumulative mortality data from the exposure of rainbow trout to the test material during the definitive test are given in Table 2.

There were no mortalities in 14 fish exposed to a test concentration of 100 mg/l for a period of 96 hours. Inspection of the mortality data gave the following results:

Time (h)	LC_{50} (mg/l)	95% Confidence limits (mg/l)
3	> 100	-
6	> 100	-
24	> 100	-
48	> 100	-
72	> 100	-
96	> 100	-

The results of the definitive test showed the highest test concentration resulting in 0% mortality to be greater than or equal to 100 mg/l, the lowest test concentration resulting in 100% mortality to be greater than 100 mg/l and the No Observed Effect Concentration (NOEC) to be 100 mg/l. The No Observed Effect Concentration is based upon zero mortalities and the absence of any sub-lethal effects of exposure at this concentration (Section 5.2.2).

It was considered unnecessary and unrealistic to test at concentrations in excess of 100 mg/l.

5.2.2 Sub-lethal effects

There were no sub-lethal effects of exposure observed in 14 fish exposed to a test concentration of 100 mg/l for a period of 96 hours.

5.2.3 Observations on test material solubility

The test preparations were observed to be clear, colourless solutions throughout the duration of the test.

5.2.4 Physico-chemical measurements

The results of the physico-chemical measurements are given in Appendix 3. Temperature was maintained at approximately 14°C throughout the test. While there were no treatment related differences for oxygen concentration, the pH of the fresh media in the replicates R_1 and R_2 of the 100 mg/l test concentration were observed to be lower than the control throughout the test.

The pH of the control group was observed to vary between 7.5 and 8.3. This variation was considered not to affect the validity or integrity of the test given that no mortalities or adverse reactions to exposure were observed in the control group and the Test Guideline states that the pH should not vary by more than 1 unit.

5.2.5 Verification of test concentrations

Analysis of the test preparations at 0, 24 and 96 hours (see Appendix 1) showed measured test concentrations to range from 101% to 104% of nominal and so it was considered justifiable to estimate the LC_{50} values in terms of the nominal test concentrations only.

6. CONCLUSION

The acute toxicity of the test material to the freshwater fish rainbow trout (*Oncorhynchus mykiss*) has been investigated and gave a 96-Hour LC_{50} of greater than 100 mg/l. Correspondingly the No Observed Effect Concentration was 100 mg/l.

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Table 1 Cumulative Mortality Data in the Range-finding Test

Nominal Concentration (mg/l)	Cumulative Mortality (Initial Population = 3)					
	3 Hours	6 Hours	24 Hours	48 Hours	72 Hours	96 Hours
Control	0	0	0	0	0	0
1.0	0	0	0	0	0	0
10	0	0	0	0	0	0
100	0	0	0	0	0	0

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Table 2 Cumulative Mortality Data in the Definitive Test

Nominal Concentration (mg/l)	Cumulative Mortality (Initial Population =7)						% Mortality
	3 Hours	6 Hours	24 Hours	48 Hours	72 Hours	96 Hours	96 Hours
Control	0	0	0	0	0	0	0
100 R_1	0	0	0	0	0	0	0
100 R_2	0	0	0	0	0	0	0

R_1 and R_2 = Replicates 1 and 2

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Appendix 1 Verification of Test Concentrations

1. METHOD OF ANALYSIS

1.1 Introduction

The test material concentration in the test samples was determined by high performance liquid chromatography (HPLC) using an external standard. The test material gave a chromatographic profile consisting of a single peak.

The method was developed by the Department of Analytical Services, Safepharm Laboratories Limited.

1.2 Sample Preparation

A volume of test sample was diluted with methanol to give a final theoretical concentration of 1.0 mg/l.

1.3 Standards

Standard solutions of test material were prepared in methanol at a nominal concentration of 1.0 mg/l.

1.4 Procedure

The standards and samples were analysed by HPLC using the following conditions:

HPLC System	:	Agilent Technologies 1100 MSD, incorporating autosampler and workstation
Mass selective detector		
Source	:	electrospray
Fragmentation energy	:	50 volts
Polarity	:	negative
Mode	:	single ion mode with 269 amu, 313 amu and 314 amu
Gas temperature	:	275°C

Appendix 1 (continued) Verification of Test Concentrations

Drying gas	:	11 litre/minute
Nebuliser pressure	:	40 psi
Capillary voltage	:	2000 volts
Gain	:	1
Column	:	Luna C18, 5 µ, (250 x 4.6 mm id)
Column temperature	:	30°C
Mobile phase	:	methanol:0.1% ammonium acetate (90:10, v/v)
Flow rate	:	0.5 ml/min
Injection volume	:	5 µl
Retention time	:	approximately 5 minutes

2. VALIDATION

2.1 Linearity

A range of standard solutions covering 0.10 to 2.0 mg/l (10% to 200% of the working standard concentration) was analysed.

Linearity was confirmed (correlation factor, $R^2 = 0.9967$) ranging from 0 to 2.0 mg/l.

The results are presented graphically on page 17.

2.2 Recoveries

Preliminary test samples, accurately fortified at a known concentration of test material, were prepared and analysed.

The recovery samples were prepared by direct addition of the test material to a sample of test medium.

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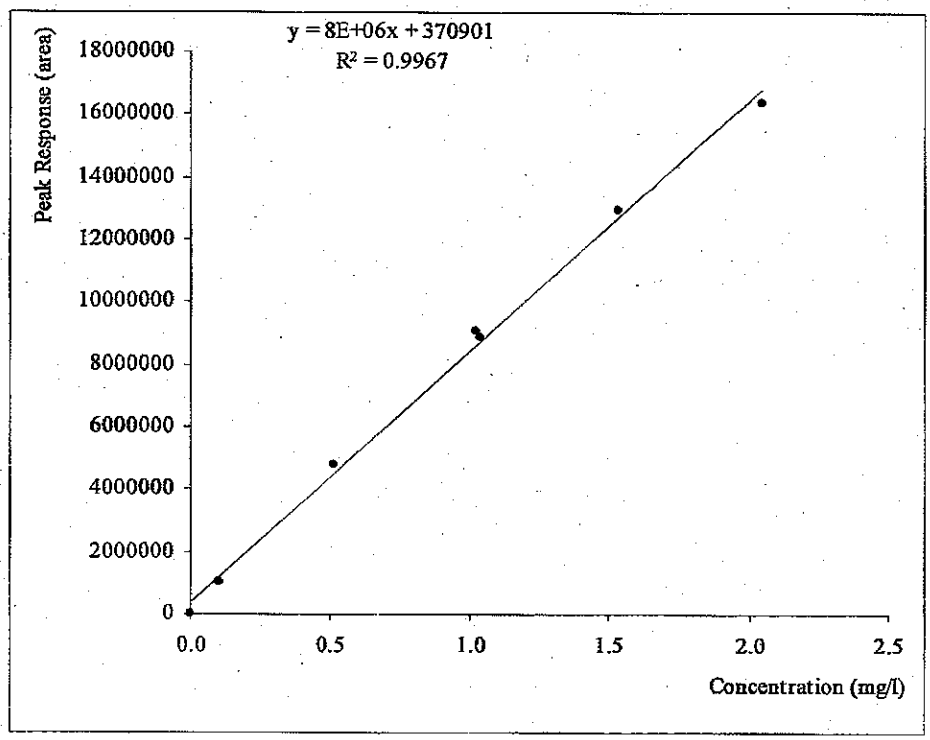
Appendix 1 (continued) Verification of Test Concentrations

Linearity of Detector Response



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Appendix 1 (continued) Verification of Test Concentrations

Fortification (mg/l)	Recoveries		
	(mg/l)	(%)	Mean %
112	110	98	98
112	108	97	

The method has been considered to be sufficiently accurate for the purposes of this test. The test sample results have not been corrected for recovery.

2.3 Limit of Quantitation

The limit of quantitation has been assessed down to 0.56 mg/l.

3. STABILITY

Preliminary test samples were prepared, analysed initially and then after storage in sealed glass vessels at ambient temperature in light and dark conditions for approximately 24 hours (equivalent to the period of medium renewal). In addition a test sample was tested for stability without prior mixing (sonication) of the test sample bottle to assess for losses due to adsorption and/or insolubility.

Nominal concentration (mg/l)	100
Concentration found initially (mg/l)	109
Concentration found after storage in light conditions (mg/l)	115
Expressed as a percent of the initial concentration	106
Concentration found after storage in dark conditions (mg/l)	113
Expressed as a percent of the initial concentration	104
Concentration found after storage in dark conditions (mg/l) – unsonicated sample	112
Expressed as a percent of the initial concentration	103

The test samples have been shown to be stable in the test medium.

The unsonicated stability vessel showed no evidence of insolubility or adherence to glass.

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Appendix 1 (continued) Verification of Test Concentrations

4. RESULTS

Sample	Nominal Concentration (mg/l)	Concentration Found (mg/l)	Expressed as a Percent of the Nominal Concentration (%)
0 Hours	Control	<LOQ	-
	100 R_1	104	104
	100 R_2	104	104
24 Hours	Control	<LOQ	-
	100 R_1	103	103
	100 R_2	103	103
96 Hours	Control	<LOQ	-
	100 R_1	101	101
	100 R_2	102	102

5. DISCUSSION

The detection system was found to have acceptable linearity. The analytical procedure had acceptable recoveries of test material in test medium. A method of analysis was validated and proven to be suitable for use.

The test material was stable in the test medium for the period of medium renewal.

LOQ = Limit of quantitation

R_1 –R_2 = Replicates 1-2

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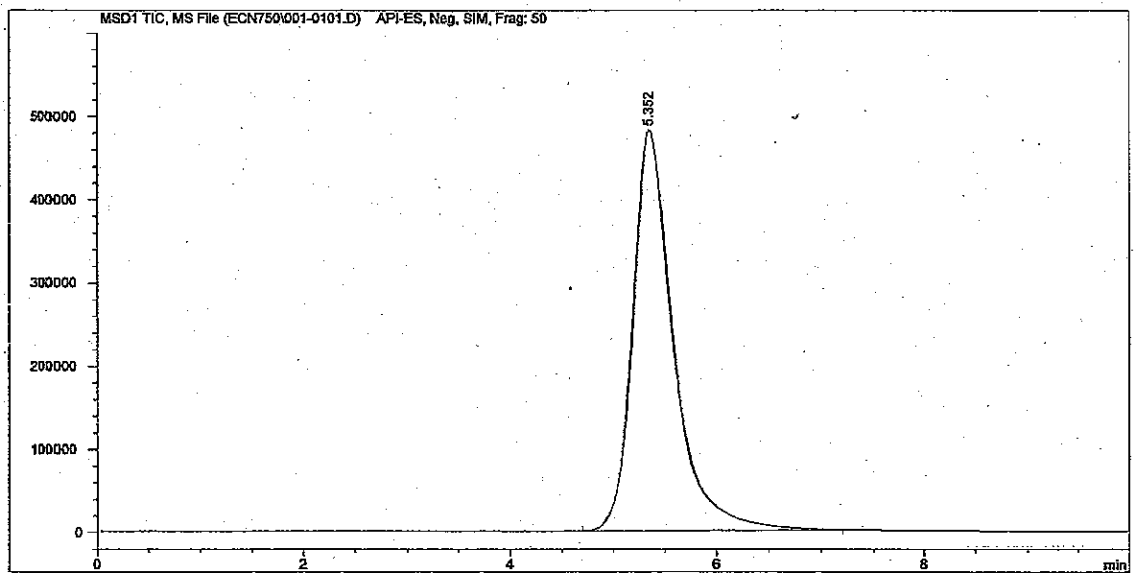
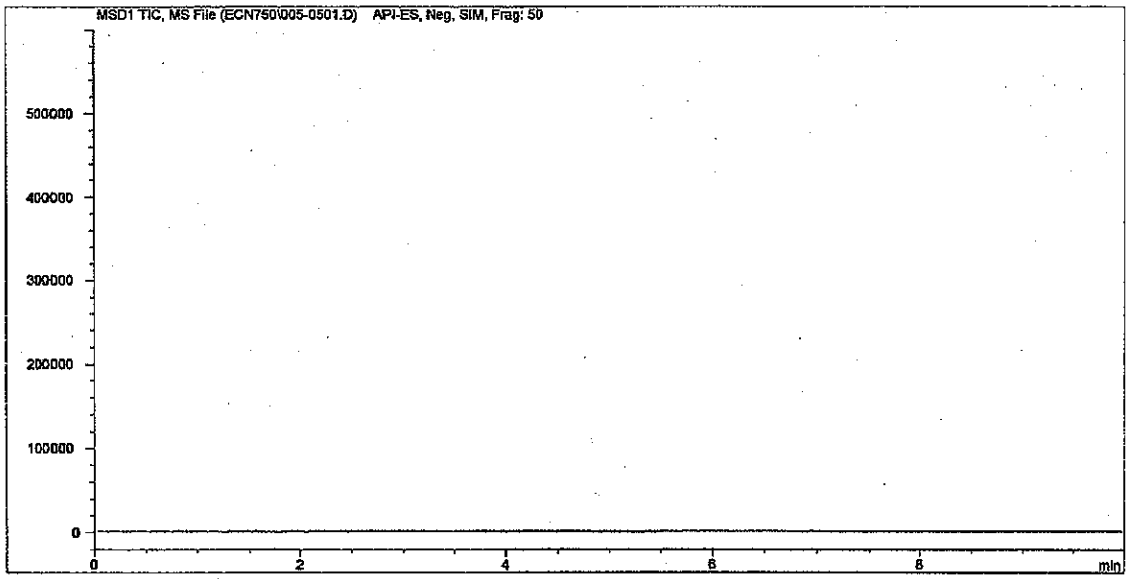
Appendix 1 (continued) Verification of Test Concentrations

6. TYPICAL CHROMATOGRAPHY



Standard 1.0 mg/l



Control Sample

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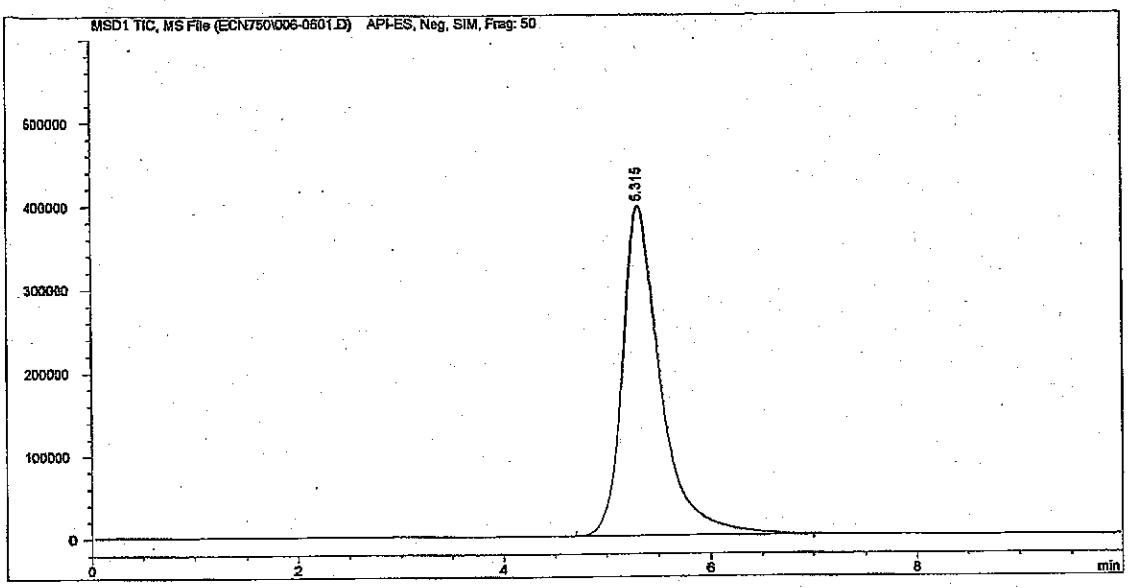
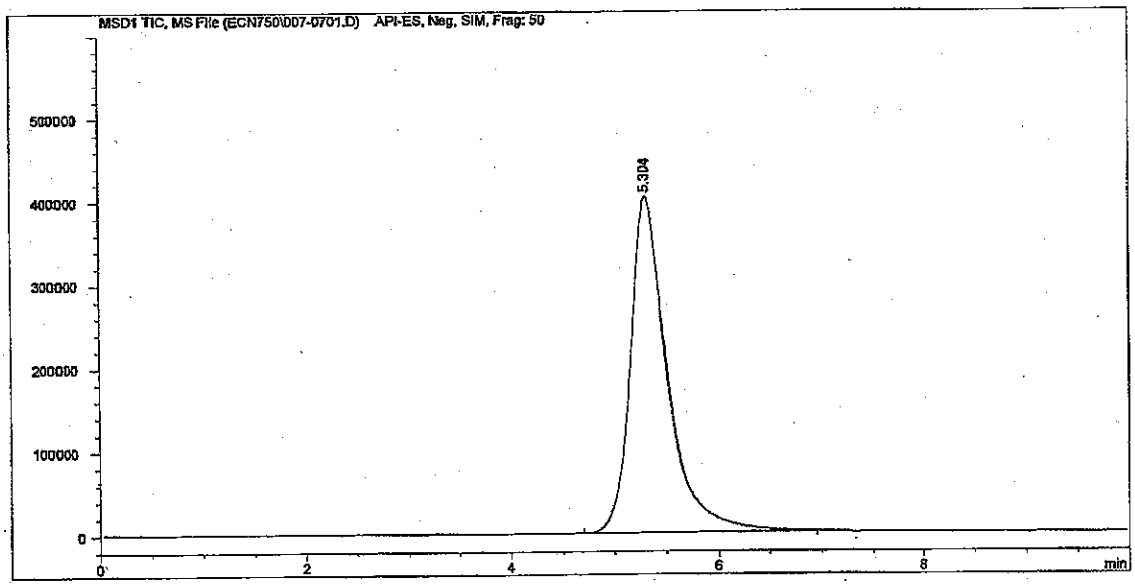
Appendix 1 (continued) Verification of Test Concentrations



Test Sample 100 mg/l R_1



Test Sample 100 mg/l R_2

$R_1 - R_2$ = Replicates R_1 and R_2

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Appendix 2 Typical Water Quality Characteristics

WATER SUPPLY ZONE SINFIN CHELLASTON SHARDLOW

REPORTING PERIOD: 01/01/2003 TO 31/12/2003

PARAMETER	NO. OF SAMPLES PLANNED PER ANNUM	NO. OF SAMPLES TAKEN IN YEAR	PCV RELAXED	NO. OF SAMPLES CONTRAVENING PCV	% OF SAMPLES CONTRAVENING PCV	CONCENTRATION OR VALUE (ALL SAMPLES)			
						MIN	MEAN	MAX	UNITS
QUALITATIVE TASTE	60	60		0	0.000	0.000	0.000	0.000	
QUALITATIVE ODOUR	60	60		0	0.000	0.000	0.000	0.000	
CONDUCTIVITY	60	61	>1500	0	0.000	359.000	439.164	610.000	µS/cm
TURBIDITY	36	36	>4.0	0	0.000	<0.200	<0.200	1.000	NTU
TEMPERATURE	60	61	>25.0	0	0.000	5.400	12.887	22.400	deg C
pH	6	6	9.5	0	0.000	7.100	7.617	8.00	pH unit
NITRATE	6	6	>50.00	0	0.000	9.800	12.283	14.600	mg NO_3/l
NITRITE	6	6	>0.10	0	0.000	<0.010	<0.010	<0.010	mg NO_2/l
NITRATE NITRITE CALCULAT	6	6		0	0.000	<1.000	<1.000	<1.000	
AMMONIUM	6	6	>0.50	0	0.000	<0.050	<0.050	<0.050	mg NH_4/l
IRON	36	36	>200	2	5.556	<20.000	55.000	250.000	µg Fe/l
ALUMINIUM	6	6	>200	0	0.000	<20.000	<20.000	62.000	µg Al/l
QUANTITATIVE TASTE	6	6	>3	0	0.000	0.000	0.000	0.000	
QUANTITATIVE ODOUR	6	6	>3	0	0.000	0.000	0.000	0.000	
MANGANESE	6	6	>50	0	0.000	<5.000	<7.000	28.000	µg Mn/l
COLOUR	6	6	>20	0	0.000	<2.000	<2.000	<2.000	mg/l Pt/C
CHLORODIBROMOMETHANE	4	4		0	0.000	5.800	9.700	12.100	µg/l
BROMODICHLOROMETHANE	4	4		0	0.000	8.200	13.125	18.800	µg/l
CHLOROFORM	4	4		0	0.000	12.900	18.850	30.800	µg/l
TRIHALOMETHANES	4	4	>100.0	0	0.000	31.100	43.475	63.600	µg/l
TETRACHLOROMETHANE	4	4	>3.0	0	0.000	<0.100	<0.100	<0.100	µg/l
TRICHLOROETHENE	4	4	>30.0	0	0.000	<1.000	<1.000	<1.000	µg/l
TETRACHLOROETHENE	4	4	>10.0	0	0.000	<1.000	<1.000	<1.000	µg/l
COPPER	1	1	>3000.0	0	0.000	<100.000	<100.000	<100.000	µg Cu/l
LEAD	1	1	>50	0	0.000	<1.000	<1.000	<1.000	µg Pb/l
ZINC	1	1	>5000.0	0	0.000	37.000	37.000	37.000	µg Zn/l
SIMAZINE	1	1	>0.100	0	0.000	<0.010	<0.010	<0.010	µg/l
ATRAZINE	1	1	>0.100	0	0.000	<0.010	<0.010	<0.010	µg/l
PESTICIDES	1	1	>0.50	0	0.000	<0.010	<0.010	<0.010	µg/l
POLYCYCLIC AROMATIC HYDR	1	1	>0.200	0	0.000	0.750	0.075	0.075	µg/l
INDENO (1,2,3-CD) PYRENE	1	1		0	0.000	<3.000	<3.000	<3.000	ng/l
BENZO 3,4 PYRENE	1	1	>10.0	0	0.000	<1.000	<1.000	<1.000	ng/l
BENZO 3,4 FLUORANTHENE	1	1		0	0.000	<2.000	<2.000	<2.000	ng/l
BENZO 11,12 FLUORANTHENE	1	1		0	0.000	<2.000	<2.000	<2.000	ng/l
BENZO 1,12 PERYLENE	1	1		0	0.000	<2.000	<2.000	<2.000	ng/l
FLUORANTHENE	1	1		0	0.000	75.000	75.000	75.000	ng/l
BROMOFORM	4	4		0	0.000	1.400	1.800	2.000	µg/l
CHLORIDE	1	1	>400	0	0.000	27.000	27.000	27.000	mg Cl/l
SULPHATE	1	1	>250	0	0.000	73.000	73.000	73.000	mg SO_4/l
CALCIUM	1	1	>250	0	0.000	51.000	51.000	51.000	mg Ca/l
MAGNESIUM	1	1	>50.0	0	0.000	8.000	8.000	8.000	mg Mg/l
SODIUM	1	1	>150	0	0.000	28.000	28.000	28.000	mg Na/l
POTASSIUM	1	1	>12.0	0	0.000	3.000	3.000	3.000	mg K/l
OXIDISABILITY	1	1	>5.0	0	0.000	1.100	1.100	1.100	mg O_2/l
TOTAL ORGANIC CARBON	1	1		0	0.000	1.700	1.700	1.700	mg C/l
BORON	1	1	>1000	0	0.000	<50.000	<50.000	<50.000	µg B/l
SURFACTANTS	1	1	>200	0	0.000	<20.000	<20.000	<20.000	µg/l
PHOSPHORUS	1	1	>2200	0	0.000	1950.000	1950.000	1950.000	µg P/l
FLUORIDE	1	1	>1500	0	0.000	304.000	304.000	304.000	µg F/l
BARIUM	1	1	>1000	0	0.000	89.000	89.000	89.000	µg Ba/l
SILVER	1	1	>10.0	0	0.000	<0.300	<0.300	<0.300	µg Ag/l
ARSENIC	1	1	>50	0	0.000	<1.000	<1.000	<1.000	µg As/l
CADMIUM	1	1	>5.0	0	0.000	<0.500	<0.500	<0.050	µg Cd/l
CYANIDE	1	1	>50	0	0.000	<5.000	<5.000	<5.000	µg Cn/l
CHROMIUM	1	1	>50	0	0.000	<1.000	<1.000	<1.000	µg Cr/l
MERCURY	1	1	>1.0	0	0.000	<0.100	<0.100	<0.100	µg Hg/l
NICKEL	1	1	>50	0	0.000	2.000	2.000	2.000	µg Ni/l
ANTIMONY	1	1	>10.0	0	0.000	<1.000	<1.000	<1.000	µg Sb/l
SELENIUM	1	1	>10.0	0	0.000	<1.000	<1.000	<1.000	µg Se/l
ALKALINITY	1	1		0	0.000	113.000	113.000	113.000	mg HCO_3/l
HARDNESS TOTAL	1	1		0	0.000	65.000	65.000	65.000	mg Ca/l
ENTEROCOCCI CONFIRMED	4	4		0	0.000	0.000	0.000	0.000	No 100 ml
COLIFORMS CONFIRMED	60	60	>0	0	0.000	0.000	0.000	0.000	No 100 ml
E.COLI CONFIRMED	60	60	>0	0	0.000	0.000	0.000	0.000	No 100 ml
COLONY COUNT AT 37°C	52	52		0	0.000	0.000	29.038	1430.00	No 1 ml
COLONY COUNT AT 21°C	52	52		0	0.000	0.000	61.885	2340.00	No 1 ml
CHLORINE FREE	60	60		0	0.000	0.010	0.129	0.370	mg/l
CHLORINE TOTAL	60	60		0	0.000	0.030	0.221	0.540	mg/l

PCV = Prescribed Concentration or Value

PFHA: ACUTE TOXICITY TO RAINBOW TROUT

Appendix 3 Physico-Chemical Measurements

Nominal Concentration (mg/l)	Time (Hours)											
	0 Hours (Fresh Media)				24 Hours (Old Media)				24 Hours (Fresh Media)			
	pH	mg O_2/l	%ASV*	T°C	pH	mg O_2/l	%ASV*	T°C	pH	mg O_2/l	%ASV*	T°C
Control	7.5	9.4	91	13.5	8.0	10.1	98	13.6	7.6	9.3	89	13.2
100 R_1	7.0	9.5	92	13.5	7.9	9.8	95	13.6	7.1	9.2	88	13.3
100 R_2	7.0	9.5	92	13.5	7.9	9.9	96	13.6	7.1	9.1	87	13.4

Nominal Concentration (mg/l)	Time (Hours)											
	48 Hours (Old Media)				48 Hours (Fresh Media)				72 Hours (Old Media)			
	pH	mg O_2/l	%ASV*	T°C	pH	mg O_2/l	%ASV*	T°C	pH	mg O_2/l	%ASV*	T°C
Control	8.3	10.3	100	13.6	7.5	8.1	77	13.1	8.2	10.0	97	13.8
100 R_1	8.2	10.3	100	13.8	7.0	8.3	79	13.1	8.2	10.1	98	13.8
100 R_2	8.1	10.1	98	13.9	7.0	8.1	77	13.2	8.1	9.9	96	14.0

Nominal Concentration (mg/l)	Time (Hours)							
	72 Hours (Fresh Media)				96 Hours (Old Media)			
	pH	mg O_2/l	%ASV*	T°C	pH	mg O_2/l	%ASV*	T°C
Control	7.5	8.0	78	13.5	8.2	10.0	97	14.1
100 R_1	7.0	8.1	77	13.4	8.0	10.0	97	14.1
100 R_2	7.0	8.1	78	13.5	8.0	10.0	97	14.3

*ASV = Dissolved oxygen concentration expressed as a percentage of Air Saturation Value
R_1 and R_2 = Replicates 1 and 2

Appendix 4 Statement of GLP Compliance in Accordance with Directive 88/320/EEC

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY
SafePharm Limited
Shardlow Business Park,
London Road,
Shardlow,
Derbyshire,
DE72 2GD

TEST TYPE
Analytical/Clinical
Chemistry
Environmental tox.
Environmental fate
Mutagenicity
Phys./Chem. tests
Toxicology

DATE OF INSPECTION

2nd December 2002

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

Roger G. Alexander
13/2/03

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority