[redacted]

REPORT

[redacted] Acute Toxicity to Rainbow Trout

[redacted]:	[redacted]
Version ID:	Final Report
Issue date:	13 June 2014

Sponsor and Test Facility Details

Sponsor

[redacted]

Test facility

[redacted]

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[redacted]

Compliance with Good Laboratory Practice

[redacted] Acute Toxicity to Rainbow Trout

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

The UK Good Laboratory Practice Regulations (Statutory Instrument 1999 No. 3106, as amended by Statutory Instrument 2004 No. 994).

OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17.

EC Commission Directive 2004/10/EC of 11 February 2004 (Official Journal No L 50/44).

These principles of Good Laboratory Practice are accepted by the Regulatory Authorities of the United States of America and Japan on the basis of Intergovernmental Agreements.

No claim of compliance is made for the results of analysis of the diluent water supply.

[redacted]

13 June 2014
Date

[redacted]

Quality Assurance Statement

[redacted] Acute Toxicity to Rainbow Trout

The following inspections and audits have been carried out in relation to this study:

Study Phase	Date(s) of Inspection	Date of Reporting to Study Director and Management
Protocol Audit	02 Aug 2013	02 Aug 2013
	29 Aug 2013	29 Aug 2013
Protocol Amendment No.1	14 Feb 2014	14 Feb 2014
Report Audit	28 Mar 2014 - 01 Apr 2014	01 Apr 2014

Process based inspections: At or about the time this study was in progress inspections of procedures employed on this type of study were carried out. These were conducted and reported to appropriate Company Management as indicated below:

Process Based Inspections	Date(s) of Inspection	Date of Reporting to Management
Fish Transfer	05 Dec 2013	05 Dec 2013
Experimental set-up, dose formulation and sampling	28 Feb 2014	03 Mar 2014
Study Management & conduct	28 Feb 2014	03 Mar 2014
Observations	20 Mar 2014	20 Mar 2014
Study Management & conduct	20 Mar 2014	20 Mar 2014

In addition, an inspection of the facility where this study was conducted was carried out on an annual basis. These inspections were reported to Company Management.

[redacted]

13 June 2014

[redacted]

Contributing Scientists

[redacted] Acute Toxicity to Rainbow Trout

Study management

Aquatic Ecotoxicology and Biodegradation

[redacted] BSc
Study Director

Summary

A study was performed to assess the acute toxicity of [redacted] to rainbow trout (*Oncorhynchus mykiss*) under semi-static conditions with daily renewal of the media.

The study was conducted in accordance with EC Methods for Determination of Ecotoxicity, Annex to Commission Regulation (EC) No 440/2008, Part C, Method 1 "Acute Toxicity For Fish" and the OECD Guideline for Testing of Chemicals No. 203 "Fish, Acute Toxicity Test" (1992).

A group of ten juvenile fish was exposed to a water accommodated fraction (WAF) of [redacted] prepared from an aqueous mixture with an initial nominal loading rate of 100 mg/L. The test medium was prepared by the direct addition of the test substance to the diluent water. The aqueous mixture was stirred for approximately 24 hours in the dark and then left to stand for approximately 24 hours in the dark before the aqueous phase (WAF) was removed and used as the test medium.

As a result of the low aqueous solubility of [redacted] (<0.004 mg/L), it was not possible to develop a suitable method of analysis with sufficient sensitivity to monitor the exposure concentration employed in this study. Therefore, following agreement with the Sponsor, no supporting achieved concentration analysis was undertaken during this study.

Observations of the fish were made after approximately 2, 4, 24, 48, 72 and 96 hours of exposure. No mortality or sub-lethal effects were observed. A LL_{50} could not be calculated but this must be greater than 100 mg/L (nominal), and the "no observed effect loading rate" (NOELR) was 100 mg/L (nominal), the nominal loading rate employed in this test.

1. Introduction

This study was designed to assess the acute toxicity of [redacted] to rainbow trout (*Oncorhynchus mykiss*) under semi-static conditions.

The study was conducted in accordance with EC Methods for Determination of Ecotoxicity, Annex to Commission Regulation (EC) No 440/2008, Part C, Method 1 "Acute Toxicity For Fish" and the OECD Guideline for Testing of Chemicals No. 203 "Fish, Acute Toxicity Test" (1992).

The protocol was approved by the Study Director on 23 August 2013. The experimental phase of the study was conducted between 26 February 2014 and 11 March 2014.

The study was conducted at [redacted]

Information provided by the Sponsor indicated that [redacted] was insoluble in water (<0.004 mg/L). It was not possible to develop a suitable method of analysis with sufficient sensitivity to monitor the exposure concentrations employed in the test, consequently, no supporting analysis was undertaken.

2. Test Substance

Name:	[redacted]
Chemical Name:	[redacted]
Use:	[redacted]
Molecular Formula:	[redacted]
Molecular Weight:	[redacted]
Physical State:	Pale yellow powder
Source:	Sponsor
Batch Number:	OF1211
Purity:	99.9%
Storage:	Room temperature in the dark
Expiry Date:	30 April 2014
Date sample received:	28 January 2013
Certificate of analysis:	Appendix 1

3. Experimental Procedure

3.1 Test species

3.1.1 Name

Rainbow trout (*Oncorhynchus mykiss*).

3.1.2 Source

The fish were supplied by a commercial fish farm in the UK and were reared at the farm from eggs that hatched in October 2013.

3.1.3 Acclimatisation

The stock of fish was obtained from the supplier on 07 January 2014 and they were held in an aerated supply of diluent water under flow-through conditions until use. During the 14-day period immediately before the definitive test, temperatures remained within the range 12.6 to 12.8°C, pH values within the range 7.61 to 8.18, dissolved oxygen concentrations within the range 90 to 99% air saturation value (ASV) and total hardness within the range 147 to 182 mg/L as $CaCO_3$.

The fish were fed daily with an amount of commercial fish food (TROUW (UK) Ltd; Nutra Fry 02) equivalent to 1% of the total wet-weight of fish in the holding tank. No food was given during the 24-hour period immediately before exposure or during the exposure period itself. No medication was given during the holding period, and no mortality was recorded during the 14 days before the definitive test.

The size of the fish used in the definitive test was determined by weighing and measuring a sample of ten fish taken at random from the holding tank on 07 March 2014; their mean total length was 5.91 cm and their mean wet weight was 2.64 g.

3.2 Diluent water

The water used to hold the fish and for the study was laboratory tap water, dechlorinated and softened by passage through a water purification system. It was passed through a high grade activated carbon filter to remove chlorine and any organic contaminants. A proportion of the supply then passed through a water softener before final reverse osmosis treatment to produce a highly purified water supply. The two grades of dechlorinated water were then remixed to give a supply with the desired water hardness. This water was then held in an intermediate tank where it was equilibrated to the test temperature and gently aerated before being supplied to the holding and test areas. Typical water quality characteristics of the diluent supply are given in Appendix 2.

3.3 Test substance preparation

The method of preparation used during the definitive test was based on the results of a range finding test.

Duplicate batches of the test medium were prepared each day by adding the test substance (3 g) to dilution water (*ca.* 25 L) in an aspirator (30 L), and then made up to volume whilst magnetically stirring. The bulk was stirred for 24 hours in the dark, a syphon was added to each and then left to stand for 24 hours in the dark. An aliquot (*ca.* 1L) of the WAF was removed via the syphon tube and discarded, after which the mid-vessel content was syphoned into each fish tank, to give 20 L of water accommodated fraction (WAF) and this was used as test medium with a nominal loading rate of 100 mg/L as [redacted]

3.4 Exposure conditions

3.4.1 Experimental design

A range finding test was followed by a definitive test with a single test concentration (limit test), plus a diluent water control group.

In the definitive test, five fish were placed at random into glass aquarium containing the prepared control or test medium in duplicate. Each vessel contained 20 litres of medium to a depth of 18 cm. This provided an initial static loading of 0.658 g bodyweight/litre.

3.4.2 Test concentrations

The range finding test employed water accommodated fraction (WAF) with nominal loading rates of 1, 10 and 100 mg/L. No mortality or treatment-related effects were observed after 96 hours. Based on this result, the definitive (limit) test employed a single WAF with a nominal loading rate of 100 mg/L as [redacted]

3.4.3 Medium renewal

The fish were exposed to the control or test conditions for a period of 96 hours with daily batch renewal of the media to ensure the maintenance of satisfactory environmental conditions.

3.4.4 Stability of test concentrations

As the test material was insoluble in water (<0.04 mg/L), it was not possible to develop a suitable method of analysis with sufficient sensitivity to monitor the exposure concentrations employed in the test, consequently, no supporting analysis was undertaken.

3.4.5 Environmental conditions

Treatment and control groups were maintained at 15 ± 2°C throughout the exposure period and constant to within ± 1°C during the study. The temperature of the water in the diluent water control vessel was continuously monitored during the study.

Supplementary aeration was provided via narrow bore glass tubes. A photoperiod of 16 hours light: 8 hours dark was maintained, with periods of subdued lighting at the beginning and end of each light phase. Daily records of temperature, pH and dissolved oxygen were kept for each control and test vessel together with measurements of total hardness for selected vessels at 0 hours. The fish were not fed during the 96 hour exposure period.

3.5 Criteria of effect

The criteria of death employed in this study were (i) absence of respiratory movement and (ii) absence of response to physical stimulation of the caudal peduncle.

In addition to observations on mortality at approximately 2, 4, 24, 48, 72 and 96 hours, subjective assessments were also made on the incidence and type of any sub-lethal effects compared with control fish.

3.6 Evaluation of data

The "no observed effect concentration" (NOEC) was derived by direct inspection of the data for lethal and treatment-related-effects. An incidence rate of more than one affected fish out of ten is considered to be significant.

3.7 Protocol deviations

The following deviation from protocol occurred:

During the acclimatisation period (14 days before commencement of the test) the total hardness range was 147 to 182 mg/L as $CaCO_3$. The total hardness of the dilution medium used for the test was within acceptable range.

There was no mortality observed in the holding tank during the acclimatisation period, therefore it is considered that the slight increase in hardness observed had no effect on the survival of the fish or suitability for study.

This deviation was considered not to have affected the integrity or validity of the study.

4. Maintenance of Records

This report has been compiled from original data, which will be stored, together with a copy of the final report, a copy of the protocol and amendments in [redacted] Archives, [redacted]

Records will be retained for a minimum period of at least one year from the date of issue of the final report. At the end of the one year retention period the Sponsor will be contacted and advice sought on the future requirements. Under no circumstances will any item be discarded without the Sponsor's knowledge.

[redacted] will retain the Quality Assurance records relevant to this study for twenty years, and a copy of the final report in its archives indefinitely.

5. Results

5.1 Mortality and observations

Observations of the fish were made after approximately 2, 4, 24, 48, 72 and 96 hours of exposure. No mortality or sub-lethal effects were observed. Therefore, a LL_{50} could not be calculated but this must be greater than 100 mg/L (nominal loading rate), and the "no observed effect loading rate" (NOELR) was 100 mg/L (nominal), the nominal loading rate employed in the test.

5.2 Environmental parameters

The measurements of water quality (temperature, pH, concentrations of dissolved oxygen and total hardness) are summarised in Table 1; they remained within acceptable limits throughout the study.

The test medium was colourless.

6. Conclusions

After 96 hours of exposure of rainbow trout (*Oncorhynchus mykiss*) to [redacted] as a water accommodated fraction (WAF) with a nominal loading rate of 100 mg/L, no mortality or treatment-related effects were observed. Therefore the LL_{50} value could not be calculated but must be >100 mg/L. The "no observed effect loading rate" (NOELR) was 100 mg/L, under the conditions of the test.

Table 1 Environmental parameters

Nominal loading rate [redacted] (mg/L)	Temperature°C		pH		Dissolved oxygen % ASV		Total hardness mg/L as $CaCO_3$
	Min	Max	Min	Max	Min	Max	0 hours
Control	13.7	14.6	8.10	8.32	97	103	166
Control	13.6	14.7	8.16	8.32	97	105	160
100	14.0	15.2	8.15	8.39	94	102	-
100	14.0	15.6	8.22	8.46	96	101	162

ASV Air saturation value (100% ASV = 9.09 mgO_2/L at 20ºC).
Continuous monitoring of control vessel media temperature = 12.4 to 14.4°C

Appendix 1 Certificate of analysis

[redacted]

[redacted]

CERTIFICATE OF ANALYSIS

17 April, 2013

Name of Sample	[redacted]
Chemical Name	[redacted]
CAS No.	-
Lot No.	[redacted]
Purity	99.9% (measured by HPLC)
Impurity	[redacted]
Date of Analysis	16th November, 2012
Expiry date	30th April, 2014

[redacted]

Appendix 2 Typical water quality characteristics of the diluent supply

Diluent water comprised filtered dechlorinated tap water blended with tap water that had been softened and subsequently treated by reverse osmosis to a hardness of 150-200 mg/L as $CaCO_3$. Analysis of samples taken in September and October 2013 gave the following results:

Colony count 37°C	3 / 1 mL
Coliform bacteria/ 100mL	0 / 100 mL
E. coli	0 / 100 mL
	µg/L
Organochlorine pesticides	<1.24
Organophosphorus pesticides	<3.30
Polychlorinated biphenyls	<0.007
	mg/L
Aluminium	<0.0200
Ammonium (as NH_4)	<0.01
Arsenic	<0.0020
Boron	0.0278
Cadmium	<0.0025
Calcium	56
Carbon (total organic)	0.65
Chloride	13.5
Residual disinfectant (total chlorine)	<0.02
Residual disinfectant (free chlorine)	<0.02
Chromium	<0.0020
C.O.D.	<3.0
Cobalt	<0.0020
Copper	0.0249
Fluoride	0.00017
Iron	0.0805
Lead	0.00053
Magnesium	3.36
Mercury	<0.00006
Nitrate (as NO_3)	1.46
Nitrite (as NO_2)	<0.05
Nickel	<0.002
Phosphorus	<0.10
Potassium	1.24
Silver	0.00174
Sodium	8.110
Tin	<0.002
Suspended solids	<2.0
Zinc	0.0186
Conductivity (20°C)	378 µS/cm
Turbidity	<2.0 FTU

FTU Formazin turbidity unit.

No claim of compliance is made for the above data.

Appendix 3 Compliance statements

DH Department of Health

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE IN ACCORDANCE WITH DIRECTIVE 2004/9/EC

TEST FACILITY

[redacted]

TEST TYPE(S)

Analytical/Clinical Chemistry
Environmental Fate
Environmental Toxicity
Ecosystems
Phys.Chem. Testing
Residue studies
Mutagenicity
Toxicology

DATE OF INSPECTION
18th – 20th June 2012

An inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK Good Laboratory Practice Compliance Monitoring Programme.

This statement confirms that, on the date of issue, the UK Good Laboratory Practice Monitoring Authority were satisfied that the above test facility was operating in compliance with the OECD Principles of Good Laboratory Practice.

This statement constitutes a Good Laboratory Practice Instrument (as defined in the UK Good Laboratory Practice Regulations 1999).

14/9/12

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority

MHRA

[redacted]

Department of Health

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE IN ACCORDANCE WITH DIRECTIVE 2004/9/EC

TEST FACILITY

[redacted]

TEST TYPE(S)

Analytical/Clinical Chemistry
Environmental Fate
Environmental Toxicity
Ecosystems
Phys.Chem. Testing
Residue studies
Mutagenicity
Toxicology

DATE OF INSPECTION **05 to 07 February 2014**

An inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK Good Laboratory Practice Compliance Monitoring Programme.

This statement confirms that, on the date of issue, the UK Good Laboratory Practice Monitoring Authority were satisfied that the above test facility was operating in compliance with the OECD Principles of Good Laboratory Practice.

This statement constitutes a Good Laboratory Practice Instrument (as defined in the UK Good Laboratory Practice Regulations 1999).

24/4/14

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority

MHRA