



PPG Industries, Inc. 4325 Rosanna Drive Allison Park, Pennsylvania 15101
Phone: 412 492-5308 FAX: 412 492-5611 Email: sabraham@ppg.com

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Washington, DC 20460-0001

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To Whom It May Concern:

PPG Industries Inc., (PPG) is submitting this information pursuant to Section 8(e) of TSCA.

PPG is submitting a report on a Daphnia Reproduction Test (21 Day, Semi-Static) study concerning **WRS-2390, CAS# 68227-46-3**, an isolated intermediate chemically converted in subsequent production of a resin that is a component of an electrodeposition coating. As a part of EPA's high production volume challenge program, toxicity studies were conducted on WRS-2390.

Enclosed you will find the full report, including summary, titled:

"Daphnia Reproduction Test (21 Day, Semi-Static)"

PPG provides our associates with labels and MSDS, which specify procedures for proper handling and disposal of this material including the use of personal protective equipment.

Please telephone me at [412 492-5308] if you have any questions.

Sincerely yours,

Stacie Abraham
Senior Product Safety Analyst
North America and European Regions
PPG Industries, Inc.



DHL# 30252895945
send to address:
Office of Pollution Prevention and Toxics
Ronald Reagan Complex EPA East Building CBIC 6428
1201 Constitution Avenue N.W.
Washington, DC 20460

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Inveresk Report Number 24048

WRS-2390TX
Daphnia Reproduction Test (21 Day, Semi-Static)

Authors:

T L Hargreaves
C R Kelly
C M Murphy

Sponsor:

PPG Industries Inc.
4325 Rosanna Drive
PO Box 9
Allison Park
Pennsylvania 15101
USA

Performing Laboratory:

Inveresk
Tranent
EH33 2NE
Scotland

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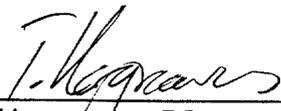
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Authentication

"I, the undersigned, hereby declare that this work was performed in accordance with the OECD Principles of Good Laboratory Practice as set forth by the United Kingdom Department of Health. The study was conducted according to the procedures herein described and this report represents a true and accurate record of the results obtained."



T L Hargreaves BSc
Study Director

03 May 2005

Date

Quality Assurance Statement

Inveresk Quality Assurance Unit conducted the following QA functions on this study.

<u>Date of QA Activity</u>	<u>Phase</u>	<u>Date of Report to Management/ Study Director</u>
25 February 2004	Protocol Review	26 February 2004
14 May 2004	Dose Preparation / Dosing / Addition of Test Organisms	19 May 2004
26 May 2004	Observations	02 June 2004
04 June 2004	Sample Preparation for Analysis/ Preparation of Samples for Chromatography	10 June 2004

This report is considered to describe accurately the procedures used in the study and the results obtained.

D. Allan
D Allan BSc
Quality Assurance

27 APR 05
Date

Personnel Involved

Study Director:

T L Hargreaves BSc
(28 July 2004 – Study completion)

C R Kelly BSc PhD
(Study initiation – 27 July 2004)

Head, Environmental Sciences:

B E Hall BSc PhD

Report Compilation:

C R Kelly BSc PhD
C M Murphy BSc

Scientific Assistance:

C R Kelly BSc PhD
C M Murphy BSc
A M Paterson BSc

Chemical Analysis:

A Hogg BSc
W Brown BSc
J MacDougall BSc

Statistical Analysis:

J Hummel MSc
G Hunter MSc

Quality Assurance:

D Allan BSc
A P C Lam MChem

1 Summary

The effect of WRS-2390TX on mortality, reproductive capacity and other signs of intoxication in *Daphnia magna* were investigated over a 21 day period. The study was conducted under semi-static conditions in accordance with OECD (1998) Guideline 211 and EC (2001) Method C20.

Based on the results of an acute toxicity (EC_{50}) test (conducted under Inveresk Study No. 803823) a reproduction test was conducted at 0.07, 0.22, 0.7, 2.2 and 7.0 mg/L WRS-2390TX, with an untreated control. These concentrations are equivalent to 0.050, 0.157, 0.498, 1.566 and 4.984 mg/L, corrected for test item purity (71.2%).

Ten *Daphnia* were exposed at each test concentration, with 20 for the untreated control. The *Daphnia* were <24 h old at test initiation and were housed singly for the duration of the test.

Samples were taken for chemical analysis at the beginning and end of 3 replacement periods over the duration of the test, on Days 0 and 3; 7 and 10; and 19 and 21 (for fresh and expired solutions respectively). The corresponding time weighted mean measured concentrations were 0.015, 0.083, 0.365, 1.369 and 4.653 mg/L, which is equivalent to 30.0, 52.9, 73.3, 87.4 and 93.4 % of the nominal concentrations (corrected for purity). All results are based on time weighted means.

The mean total reproduction for each treatment group at Day 21 was as follows:

Time Weighted Mean Concentration of WRS-2390TX (mg/L)	Mean Total Reproduction
Control	146
0.015	151
0.083	173
0.365	213
1.369	-
4.653	-

- All parent *Daphnia* dead.

The Day 7 EC_{50} for WRS-2390TX to *Daphnia* reproduction was estimated as 0.69 mg/L. As there were limited intermediate levels of effect, the probit model was not a good fit to the observed data, as such the estimated value should be regarded with caution and 95% confidence limits were not calculated.

As all parent daphnids were dead by Days 14 and 21 at the highest two concentrations and no reproduction effect was noted at 0.365 mg/L, the EC_{50} for these time points was estimated to be between 0.365 and 0.69 mg/L (*i.e.* between the NOEC and Day 7 EC_{50}).

The Day 21 No Observed Effect Concentration (NOEC) for WRS-2390TX to *Daphnia* reproduction was 0.365 mg/L, under the conditions of the test.



Water quality parameters remained within the following range over the duration of the test: pH 7.26-7.92; temperature 19.2-22.2°C and dissolved oxygen 75.0-98.4 % ASV (air saturation value).

2 Introduction

The effect of WRS-2390TX on mortality, reproductive capacity and other signs of intoxication in *Daphnia magna* were investigated over a 21 day period. The study was conducted under semi-static conditions in accordance with OECD (1998) Guideline 211 and EC (2001) Method C20.

The study was initiated on 17 February 2004 and was conducted at Inveresk, Tranent, EH33 2NE, Scotland. Experiments commenced on 14 May 2004 and were completed on 09 June 2004. For study completion date see date of Study Director's Authentication.

All data generated and recorded during this study, including a copy of the final report, will be stored in the Scientific Archives of Inveresk for 5 years after issue of the final report. After the 5 year period the Sponsor will be consulted regarding the disposal, transfer or continued storage of raw data.

3 Experimental Procedure

3.1 Test Item

Identification: WRS-2390TX
Appearance: Brown viscous liquid
Notebook No.: 02-181-126
CAS No.: 68227-46-3
Purity: 71.2%*
Receipt Date: 02 October 2003
Expiry Date: 15 November 2004
Storage: Under ambient temperature, in the dark.

* A specific purity was supplied by the Sponsor for this test item with a reference of Notebook No. 02-181-126.

The test item is a Propanoic acid, 2-hydroxy- compound with 3-[2-(dimethylamino)ethyl]1-(2ethylhexyl)(4-methyl-1,3-Phenylene)bis[carbamate] (1:1).

3.2 Test Species

Daphnia magna (water flea) were used for this study. They were cultured within the laboratory by acyclical parthenogenesis. Neonates used were <24 h old at study initiation.

3.3 Culture Medium

Daphnia cultures were maintained in a synthetic medium (Elendt M4). All testing was conducted using this medium (Appendix 2).

The total hardness and alkalinity was determined for each batch of medium used during the test (Appendix 7).

3.4 Food and Feeding Regime

Daphnia were fed on a diet of concentrated green algae (*Chlorella vulgaris*) for both culturing and testing. During the reproduction test *Daphnia* were fed daily at a rate of ca 0.1-0.2 mg C/parent *Daphnia*/day.

To determine feeding rates for the test a nomograph of Total Organic Carbon (TOC) vs. cell numbers was prepared. This allowed the TOC content of a sample of concentrated *Chlorella vulgaris* to be determined from a background cell count. (Figure 2).

3.5 Test Vessels

The test was conducted using glass beakers (ca 100 mL capacity) covered with perspex lids to minimise dust contamination and evaporative losses. Prior to test

initiation the beakers were acid washed and oven dried. Immediately prior to each use the beakers were rinsed with Elendt M4 medium.

3.6 Reproduction Test

Based on the results of an acute toxicity (EC_{50}) test (conducted under Inveresk Study No. 803823) a reproduction test was conducted at 0.07, 0.22, 0.7, 2.2 and 7.0 mg/L WRS-2390TX, with an untreated control. These concentrations are equivalent to 0.050, 0.157, 0.498, 1.566 and 4.984 mg/L, corrected for test item purity (71.2%).

Test solutions were prepared by parallel dilution of a 7 mg/L stock solution (the highest test concentration). This stock solution was prepared by adding *ca* 14 mg WRS-2390TX to Elendt M4 and bringing to volume in a 2 litre volumetric flask. The stock was then ultrasonicated for *ca* 10 min to aid dissolution.

Ten *Daphnia* were exposed at each test concentration, with 20 for the untreated control. The *Daphnia* were <24 h old at test initiation and were housed singly for the duration of the test. Each vessel contained *ca* 60 mL of test or control media, as appropriate.

The test was conducted under semi-static conditions, with media replacement conducted three times weekly (Monday, Wednesday and Friday).

3.7 Observations

All vessels were observed daily throughout the duration of the test for adult *Daphnia* health and neonate production. Neonates were discarded after counting. Males, where present, were discarded and discounted from the results.

Daphnia were recorded as immobile if no movement was observed within 15 s following gentle agitation of the test vessel.

3.8 Determination of WRS-2390TX in Test Solutions

Duplicate samples were taken for chemical analysis at the beginning and end of 3 replacement periods over the duration of the test; these were on Days 0 and 3; 7 and 10; and 19 and 21 (for fresh and expired solutions respectively). Samples for fresh solutions were taken from the test solutions immediately after preparation. The expired samples were taken from a pooled sample of all replicates.

Test solutions were analysed according to the procedures established and validated under Inveresk Study No. 342753.

Analyses were normally performed on the day of collection. In cases where this was not possible, samples were kept under suitable storage conditions until analysis took place.



3.9 Calculation of Time Weighted Mean Concentrations of WRS-2390TX in Test Solutions

As the concentration of WRS-2390TX in test solutions decreased over the duration of the renewal periods (Table 1), a time weighted mean was calculated as outlined in OECD Guideline 211. The time weighted mean is calculated so that the area under the curve is equal to the concentration decline curve. The area was calculated using the following formula:

$$\text{Area} = \frac{\text{Conc 0} - \text{Conc 1}}{\text{Ln}(\text{Conc 0}) - \text{Ln}(\text{Conc 1})} \times \text{Days}$$

Where:

Days	= Number of days in a renewal period
Conc 0	= The measured concentration at the start of each renewal period
Conc 1	= The measured concentration at the end of each renewal period
Ln(Conc 0)	= The natural logarithm of Conc 0
Ln(Conc 1)	= The natural logarithm of Conc 1

The time weighted mean is calculated by adding the areas from each of the renewal periods for a single concentration and dividing by the cumulative number of days covered by all the renewal period for that concentration. The time weighted mean is based only on those renewal periods for which analysis was conducted.

3.10 Statistical Analysis

Appendix 3 gives details of the statistical procedures performed.

3.11 Water Quality Parameters

Water quality parameters were measured at media change in a single replicate for each treatment group, prior to addition of the *Daphnia* (fresh media), and in expired media for all vessels (Appendix 5 and 6, respectively).

Water quality parameters were measured using a Sentron Argus X pH meter and a YSI 550A dissolved oxygen meter.

3.12 Environmental Control

Test vessels were maintained within a temperature-controlled laboratory with the aim of achieving a temperature in the range 18-22 °C (maximum range of 2 °C per vessel). In addition, temperature was monitored continuously throughout the test in a single control vessel (Appendix 8).

A light cycle of 16 h light and 8 h dark was in operation throughout the test. Artificial daylight fluorescent tubes provided illumination. Test vessels were not aerated during the test.

4 Results and Discussion

4.1 Reproduction Test

A summary of the analytical results is presented in Table 1, with a copy of the analytical method in Appendix 1. These indicate that the concentration of test item decreased over the period of media renewal. Samples were taken for chemical analysis at the beginning and end of 3 replacement periods over the duration of the test, on Days 0 and 3; 7 and 10; and 19 and 21 (for fresh and expired solutions respectively). The corresponding time weighted mean measured concentrations were 0.015, 0.083, 0.365, 1.369 and 4.653 mg/L, which is equivalent to 30.0, 52.9, 73.3, 87.4 and 93.4 % of the nominal concentrations (corrected for purity). All results are based on time weighted means.

All prepared test solutions were clear and colourless at preparation.

Table 2 shows observed immobilisation during the test. One daphnid each, from the control, 0.015 and 0.365 mg/L concentrations were observed to be immobilised during Days 7 to 10 during the test. No immobilisation was noted at the 0.083 mg/L concentration. All daphnids were immobilised on Day 14 for the 1.369 mg/L concentration, a day after the last reproductive cycle, combined test item and reproductive stress being the probable cause for the synchronised mortality. For the highest concentration of 4.653 mg/L, all daphnids were immobile by Day 4 (*ie* before reproduction started).

At test completion, 5.6, 14.3, 0, 16.7, 100 and 100 % immobilisation was observed in the control, 0.015, 0.083, 0.365, 1.369, and 4.653 mg/L treatment groups, respectively. The mortality for the parental daphnids was less than 20% by the end of the test for the control, 0.015, 0.083 and 0.365 mg/L treatment groups. Twenty per-cent is the maximum allowable control parental mortality stipulated by OECD Guideline 211, by inference it is therefore suggested that all mortality noted in test item treatments which is less than 20% can be regarded as natural susceptibility and not directly as an effect of the test item.

A summary of the observed reproduction during the test are presented in Table 3 and Figure 1, with the raw data in Appendix 4. No significant decrease in reproduction was noted for all surviving treatment groups.

Where the sporadic appearance of males was noted in the treatment groups, they were treated in accordance with the following recommendations of OECD Guideline 211; "If, in any replicate the parent animal dies during the test or turns out to be male, then the replicate is excluded from the analysis. The analysis will be based on a reduced number of replicates". Hence males, when identified (smaller size and more intense colouration), were discarded and discounted from the test results.

By Day 7 reproduction had been noted in all treatments, excluding the highest concentration of 4.653 mg/L, where all daphnids were dead. Table 3 shows the mean number of neonates with coefficient of variation. The coefficient of variation is greatest

in the highest surviving concentration of 1.369 mg/L, probably as a consequence of the majority of neonates first appearing on Day 8. This suggests that reproduction was delayed in this concentration, this however has limited relevance as all parental daphnids were dead by Day 14. From the Day 7 data, the EC₅₀ for WRS-2390TX to *Daphnia* reproduction was estimated as 0.69 mg/L. As there were limited intermediate levels of effect, the probit model was not a good fit to the observed data, as such the estimated value should be regarded with caution and 95% confidence limits were not calculated.

As all parent daphnids were dead by Days 14 and 21 at the highest two concentrations and no reproduction effect was noted at 0.365 mg/L, the EC₅₀ for these time points was estimated to be between 0.365 and 0.69 mg/L (*i.e.* between the NOEC and Day 7 EC₅₀).

The Day 21 No Observed Effect Concentration (NOEC) for WRS-2390TX to *Daphnia* reproduction was 0.365 mg/L, under the conditions of the test.

4.2 Environmental Conditions

Water quality parameters remained within the following range over the duration of the test: pH 7.26-7.92; temperature 19.2-22.3°C, dissolved oxygen 75.0-98.4 % ASV (air saturation value), (Table 4).

The hardness and alkalinity of the Elendt M4 used to prepare the test solutions were in the range 216-240 mg CaCO₃/L (hardness) and 60-95 mg CaCO₃/L (alkalinity).

Although the maximum temperature was briefly out-with acceptable limits this deviation did not have an impact on the outcome of the study (Appendix 8).

5 Conclusions

The Day 7 EC_{50} for WRS-2390TX to *Daphnia* reproduction was estimated as 0.69 mg/L. As there were limited intermediate levels of effect, the probit model was not a good fit to the observed data, as such the estimated value should be regarded with caution and 95% confidence limits were not calculated.

As all parental daphnids were dead by Days 14 and 21 at the highest two concentrations and no reproduction effect was noted at 0.365 mg/L, the EC_{50} for these time points was estimated to be between 0.365 and 0.69 mg/L (ie between the NOEC and Day 7 EC_{50}).

The Day 21 No Observed Effect Concentration (NOEC) for WRS-2390TX to *Daphnia* reproduction was 0.365 mg/L, under the conditions of the test.

6 *References*

EC (2001) 59/EC, Method for the Determination of Ecotoxicity, C.20, *Daphnia magna* Reproduction Test.

OECD Paris (1998), OECD Guideline for Testing of Chemicals, Test Guideline 211, *Daphnia magna* Reproduction Test.

7 Tables

Table 1 Measured Concentrations of WRS-2390TX

Nominal Concentration of WRS-2390TX (mg/L)	Renewal No.	Duration of Media Change (Days)	Measured Concentration (mg/L)		Time Weighted Mean Measured Concentration (mg/L)	% of Nominal*
			Fresh	Expired		
Control	1	3	ND	ND	ND	NA
	2	3	ND	ND		
	3	2	ND	ND		
0.07 (0.050*)	1	3	<LOQ (0.030)	<LOQ (0.020)	0.015	30.0
	2	3	<LOQ (0.019)	ND (0.0036)		
	3	2	<LOQ (0.026)	<LOQ (0.002)		
0.22 (0.157*)	1	3	0.126	0.095	0.083	52.9
	2	3	0.097	0.080		
	3	2	0.128	ND (0.0036)		
0.7 (0.498*)	1	3	0.425	0.355	0.365	73.3
	2	3	0.383	0.395		
	3	2	0.413	0.203		
2.2 (1.566*)	1	3	1.605	1.370	1.369	87.4
	2	3	1.330	1.175		
7.0 (4.984*)	1	3	4.895	4.425	4.653	93.4

* Corrected for test item purity

<LOQ = Below the limit of reliable quantification, (0.036 mg/L the lowest analytical standard prepared) values in parenthesis were extrapolated beyond the LOQ and should be treated with caution

ND = Not detected, a surrogate value of 1/10th LOQ (ie 0.0036 mg/L) was used to estimated time weighted means
NA = Not applicable

Table 2 Adult Immobilisation Observed During the Test

Time Weighted Mean Concentration of WRS-2390TX (mg/L)	Number of Immobile <i>Daphnia</i> *	% Immobilisation
Control	1/18	5.6
0.015	1/7	14.3
0.083	0/8	0
0.365	1/6	16.7
1.369	6/6	100
4.653	10/10	100

* Males discounted from calculation

Table 3 **Summary of Daphnia Reproduction**

Time Weighted Mean Concentration of WRS-2390TX (mg/L)	Mean Days to First Brood	Mean Number of Neonates Produced after 7 Days	Mean Number of Neonates Produced after 14 Days	Mean Number of Neonates Produced after 21 Days
Control	7.5	14 [38.8]	88 [5.2]	146 [6.3]
0.015	7.5	13 [71.2]	88 [10.5]	151 [14.8]
0.083	8.5	17 [41.0]	97 [7.0]	173 [8.3]
0.365	8.5	9 [77.8]	106 [7.1]	213 [9.0]
1.369	8.5	6 [155.0]	NA	NA
4.653	NA	NA	NA	NA

NA = Not applicable, all dead
[Coefficient of variation %]

Table 4 Water Quality Parameter Ranges for Fresh and Expired Media

a) Fresh Media

Time Weighted Mean Concentration of WRS-2390TX (mg/L)	pH		Dissolved Oxygen (% ASV)		Temperature (°C)	
	Min	Max	Min	Max	Min	Max
Control	7.38	7.79	89.0	98.4	20.2	22.2
0.015	7.54	7.78	88.1	97.6	20.2	22.2
0.083	7.55	7.81	89.9	96.4	20.4	22.3
0.365	7.54	7.83	89.0	95.8	20.4	22.3
1.369	7.53	7.84	90.3	95.9	20.4	21.7
4.653	7.60	7.60	91.8	93.7	21.3	21.4

b) Expired Media

Time Weighted Mean Concentration of WRS-2390TX (mg/L)	pH		Dissolved Oxygen (% ASV)		Temperature (°C)	
	Min	Max	Min	Max	Min	Max
Control	7.27	7.88	77.9	98.3	19.4	21.0
0.015	7.30	7.88	78.0	96.8	19.2	20.9
0.083	7.30	7.89	79.6	96.7	19.2	20.8
0.365	7.26	7.92	76.5	96.1	19.2	20.8
1.369	7.27	7.85	75.0	95.5	19.4	20.6
4.653	7.52	7.58	87.4	89.2	20.3	20.4

8 Figures

Figure 1 Mean Day 21 Cumulative Neonate Production with Standard Error

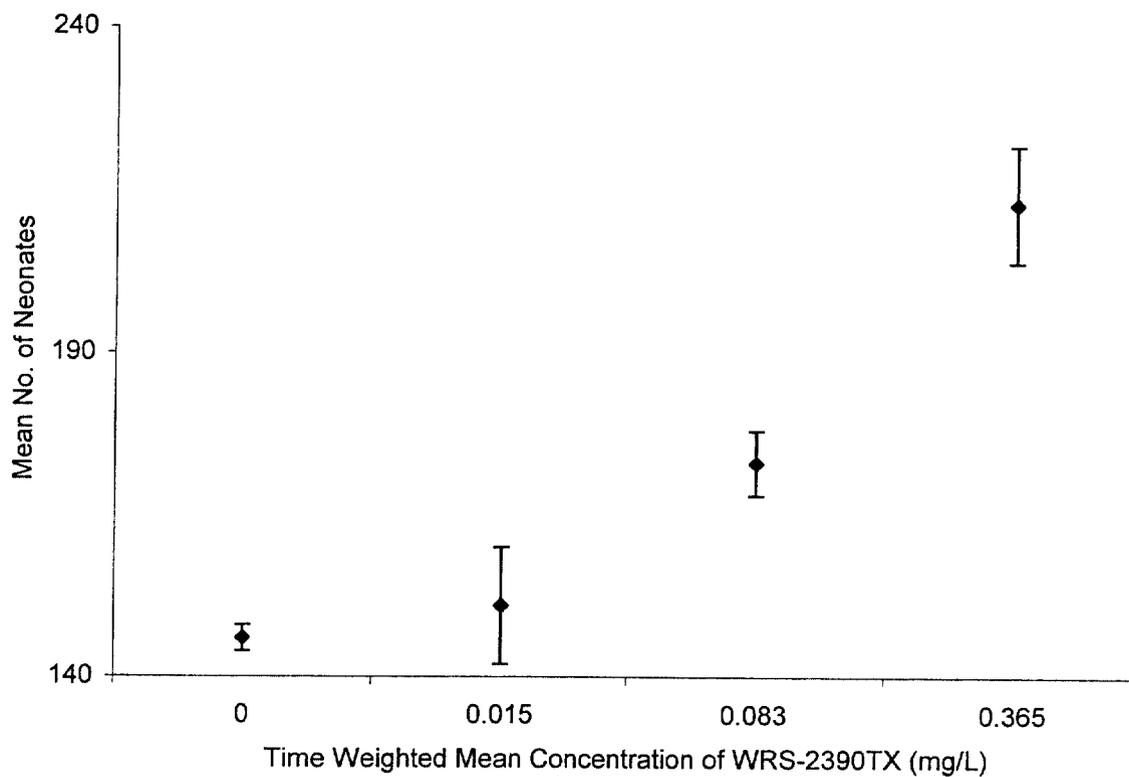
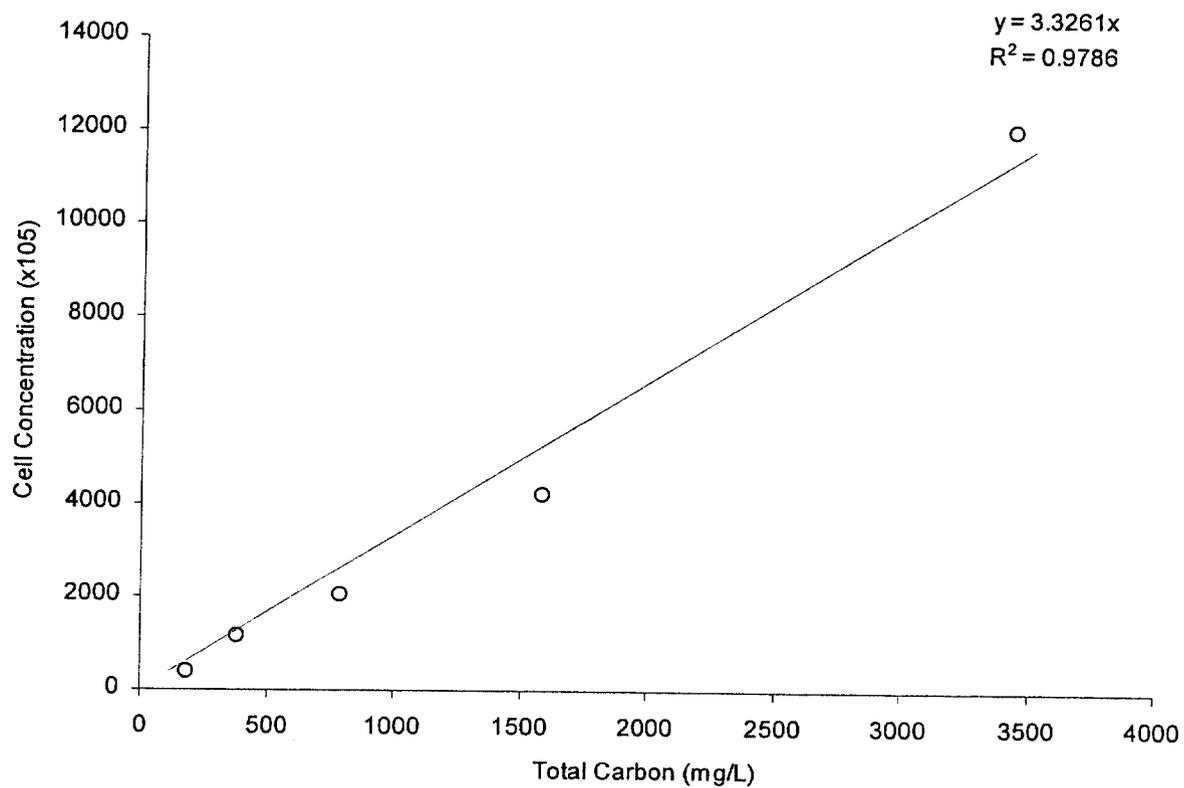


Figure 2 **Nomograph**



9 Appendices

Appendix 1 Analytical Method No. 4275

8 Appendix

Appendix 1 Analytical Method No. 4275

Assay: HPLC (Range 1.42 mg.l ⁻¹ to 0.036 mg.l ⁻¹)	Analytical Method No.: 4275
Test Item: WRS-2390	Date of Approval: 05 December 2003
	Approved: <i>A. Hoag</i>

I. Summary

Solutions of WRS-2390 are assayed by high performance liquid chromatography. Detection is by uv and external standardisation is used. Other instrumentation may be used providing adequate accuracy and precision can be achieved.

II. Reagents

- WRS-2390 (purity 71.2 %).
- Phosphoric acid.
- Milli Q water or equivalent grade.
- Acetonitrile, HPLC grade.
- Milli RO water or equivalent.

III. Apparatus

- LC Module 1, supplied by Waters or equivalent.
- Balance, 5 figure.
- Laboratory glassware.
- Volumetric glassware.
- Sonic bath.

IV. Chromatographic Conditions/Set Up

HPLC: Waters LC Module 1.
Column: YMC ODS-AM, 150 mm x 4.6 mm, 3 µm.
Column temperature: 40°C.
Mobile phase A: 0.1 % phosphoric acid in Milli Q water.
Mobile phase B: acetonitrile.
Detection: uv at 225 nm.
Injection volume: 150 µl.
Run time: 20 min.

Appendix 1
(continued) **Analytical Method No. 4275**

Appendix 1
(continued)

Flow rate: 1 ml.min⁻¹.
Needle wash: Milli Q water/acetonitrile (9:1, v/v).
Data collection: LabSystems Vax Multichrom 2 version 2.30b or other suitable data collection system.
Gradient timetable:

Time (min)	% Mobile Phase A	% Mobile Phase B	Curve
0	90	10	-
1	90	10	6
10	5	95	6
12	5	95	6
12.10	90	10	6
20	90	10	6

V. Preparation of Standard Curve

Accurately weigh ca 50 mg of WRS-2390 into a 100 ml volumetric flask then dissolve and make to volume using acetonitrile to give a ca 500 mg.l⁻¹ solution. Sonicate to aid dissolution. Dilute the ca 500 mg.l⁻¹ solution by adding 2.5 ml to a 50 ml volumetric flask and make to volume using 0.1% phosphoric acid in Milli Q water/acetonitrile (9:1, v/v) to give a ca 25 mg.l⁻¹ solution. Dilute this solution as detailed in the following table:

Standard Identity	Concentration of Stock Solution used (mg.l ⁻¹)	Volume Diluted (ml)	Final Volume (ml)	*Nominal Concentration (mg.l ⁻¹)
B1	25	4	50	1.42
B2	25	3	50	1.07
B3	25	2	50	0.71
B4	25	1	50	0.36
B5	25	1	100	0.18
B6	2	5	100	0.071
B7	2	2.5	100	0.036

* corrected for test item purity (71.2 %)

Make each standard to volume using 0.1% phosphoric acid in Milli Q water/acetonitrile (9:1, v/v).

Note: Alternative dilutions may be performed providing the sample final concentration is achieved.

Analyse each standard solution, in duplicate, according to the chromatographic conditions detailed in Section IV.

Appendix 1
(continued) **Analytical Method No. 4275**



Appendix 1
(continued)

VI. Preparation of Quality Control Sample

Accurately weigh ca 50 mg of WRS-2390 into a 100 ml volumetric flask then dissolve and make to volume using acetonitrile to give a ca 500 mg.l⁻¹ solution. Sonicate to aid dissolution. Dilute the ca 500 mg.l⁻¹ solution by adding 2.5 ml to a 50 ml volumetric flask and make to volume using 0.1% phosphoric acid in Milli Q water/acetonitrile (9:1, v/v) to give a ca 25 mg.l⁻¹ solution. Dilute this solution by adding 2 ml to a 50 ml volumetric flask and make to volume using 0.1% phosphoric acid in Milli Q water/acetonitrile (9:1, v/v) to give a ca 1 mg.l⁻¹ (0.71 mg.l⁻¹ purity corrected) solution.

Note: Alternative dilutions may be performed providing the sample final concentration is achieved.

Analyse the ca 1 mg.l⁻¹ (0.71 mg.l⁻¹ purity corrected) solution, in duplicate, according to the chromatographic conditions detailed in Section IV.

VII. Preparation of Aqueous Samples for Analysis

Aqueous solutions of WRS-2390 in Milli RO water or algae or daphnia media may be directly injected onto the analytical column and analysed according to the chromatographic conditions detailed in Section IV, providing the sample concentration is within the range of the standard curve detailed in Section V.

If required, aqueous solutions of WRS-2390 in Milli RO water or algae or daphnia media may be diluted to within the range of the standard curve detailed in Section V. using 0.1% phosphoric acid in Milli Q water/acetonitrile (9:1, v/v).

Note: The method validation indicated that direct injections of WRS-2390 in aqueous media resulted in ca 82 % recoveries from the nominal applied concentration. If possible, aqueous samples should be diluted using 0.1% phosphoric acid in Milli Q water/acetonitrile (9:1, v/v) prior to analysis. Otherwise the accuracy of the results should be quoted as 100 % ± 20 % of the found concentration.

The diluted samples should be analysed, in duplicate, according to the chromatographic conditions detailed in Section IV.

Appendix 2 Elendt M4 Medium

Elendt M4 medium was prepared as follows:

Separate stock solutions (I) of individual trace elements are prepared in deionised water. From these, a second single stock solution (II) is prepared containing all the trace elements.

Component	Stock Solution I (mg/L)	Volume of Stock Solution I used to prepare Stock Solution II (mL/L)
H ₃ BO ₃	57190	1.0
MnCl ₂ .4H ₂ O	7210	1.0
LiCl	6120	1.0
RbCl	1420	1.0
SrCl ₂ .6H ₂ O	3040	1.0
NaBr	320	1.0
Na ₂ MoO ₄ .2H ₂ O	1260	1.0
CuCl ₂ .2H ₂ O	335	1.0
ZnCl ₂	260	1.0
CoCl ₂ .6H ₂ O	200	1.0
KI	65	1.0
Na ₂ SeO ₃	43.8	1.0
NH ₄ VO ₃	11.5	1.0
Na ₂ EDTA.2H ₂ O	5000	20.0*
FeSO ₄ .7H ₂ O	1991	

* = The Na₂EDTA and FeSO₄ solutions are prepared separately, combined and autoclaved to produce Fe-EDTA. The volumes indicated of the combined solution are added to Stock Solution II.

A stock solution of vitamins is prepared in deionised water from the following components:

Vitamin	Concentration (mg/L)
Thiamine hydrochloride	750
Cyanocobalamine	10
Biotin	7.5

The media is prepared from the trace elements in Stock Solution II, the vitamins stock solution, and stock solutions in deionised water of individual macro-nutrients as indicated below:

Appendix 2
(continued) Elendt M4 Medium

Component	Stock Solution I (g/L)	Volume of Stock Solution I in Final Medium (mL/L)
Trace elements Stock Solution II	-	50
Vitamins Stock Solution	-	0.1
CaCl ₂ ·2H ₂ O	293.8	1.0
MgSO ₄ ·7H ₂ O	246.6	0.5
KCl	58.0	0.1
NaHCO ₃	64.8	1.0
Na ₂ SiO ₃ ·9H ₂ O	50.0	0.2
NaNO ₃	2.74	0.1
KH ₂ PO ₄	1.43	0.1
K ₂ HPO ₄	1.84	0.1

To avoid precipitation of salts when preparing the final medium, aliquots of the stock solutions are added to ca half the final volume of deionised water, then made up to the final volume.

Appendix 3 Statistical Analysis Report

STATISTICAL REPORT

Date: 26th July 2004
To: Craig Kelly
From: Gillian Hunter
Project: 804481

1. Statistical Methods

Following the OECD guideline, the total number of offspring per daphnia (reproduction numbers) at Day 7, Day 14 and Day 21 were analysed separately for homogeneity of variance using Levene's test⁽¹⁾ at a 1% significance level. If there was no evidence of heterogeneity of variance, reproduction numbers were analysed using analysis of variance (ANOVA) techniques⁽²⁾. Following the ANOVA, pairwise comparisons were performed between the control and each of the mean measured concentrations using a one-tailed Dunnett's test⁽³⁾ for a decrease at the 5% significance level.

If there was evidence of heterogeneity of variance, reproduction numbers were to be transformed using a log transformation. The transformed values were then to be re-analysed separately for homogeneity of variance using Levene's test⁽¹⁾ at a 1% significance level. If there was no evidence of heterogeneity of variance, the transformed total number of offspring per daphnia were to be analysed in an identical way to the untransformed values.

If the variability between concentrations was still heterogeneous after having tried the log transformation then a square root transformation was to be performed.

If the variability between concentrations was still heterogeneous after having tried the square root transformation then a non-parametric analysis of variance based on the ranked data was to be performed⁽⁴⁾. Following the non-parametric ANOVA, pairwise comparisons between the control and each of the concentrations was to be performed using a one-tailed Dunnett's test at the 5% significance level.

Based on the assumption of a linear dose-response relationship, the LOEC was defined to be the lowest concentration which is significantly lower than the control at the 5% significance level using a one-tailed Dunnett's test. However all concentrations above the LOEC must also be significantly lower than the control. From the LOEC, the NOEC was defined as the concentration immediately below the LOEC, which was not statistically lower than the control at the 5% significance level.

The EC₅₀ value was defined to be the concentration causing a 50% decrease in reproduction numbers. In order to calculate the EC₅₀ values, the mean reproduction numbers were calculated for each concentration at Day 7, Day 14 and Day 21, separately. Using Wadley's adjustment⁽⁵⁾, a probit transformation

Appendix 3 (continued) *Statistical Analysis Report*

was applied to the data. The probit transformed data was subjected to a regression procedure against logarithmically transformed concentrations of test material with a Newton-Raphson maximum likelihood iterative procedure being used to obtain parameter estimates⁽⁶⁾. From the fitted model the EC₅₀ value was estimated, together with 95% confidence intervals.

The goodness of fit of the probit model was checked via the Pearson chi-squared test statistic. A significant chi-squared test statistic indicated heterogeneity between the observed and expected % inhibition values. If the test statistic was statistically significant at the 1% level, the variances and covariances were adjusted by a heterogeneity factor. Following this, the confidence limits were not reported and the EC₅₀ value should be treated with caution.

The statistical package SAS (v8.2) was used for all statistical analysis.

2. Statistical Results

Data from male replicates were excluded from the statistical analyses. Additionally, data from replicates that were not alive at the timepoint of interest were excluded from the statistical analyses at that timepoint.

At Day 7, using Wadleys adjustment, the Pearson's chi-square test statistic was significant at the 1% level ($p=0.007$), therefore the estimate of the EC₅₀ of 0.69 mg/L should be treated with caution and no confidence intervals have been reported. At Day 14 and 21, using Wadleys adjustment, the data did not span 50%, and as a result it was not possible to estimate the EC₅₀ values at these timepoints.

For the estimate of the NOEC, at all timepoints, the homogeneity of variance assumption was satisfied at the 1% significance level using Levene's test, therefore all analyses were performed using untransformed data. The estimate of the NOEC was 0.365 mg/L for Day 7 and Day 14. For Day 21, there were no data for the 4.653 mg/L dose level, and there were no statistically significant decreases (versus control) at all other dose levels; therefore the estimate of the NOEC was ≥ 0.365 mg/L for Day 21.

A copy of all QC checks performed on data entry and all statistical analysis output have been included.

3. References

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Appendix 3
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Appendix 4 Number of Live Neonates Produced Daily per Surviving Female

a) Control

Replicate	Day																			Total
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21					
I	18	0	0	30	0	0	36	0	42	0	0	21	0	0	23	170				
II	19	0	0	35	0	0	45	0	0	37	0	0	18	0	0	154				
III	0	17	0	29	0	0	37	0	0	41	0	0	20	0	0	144				
IV	2	18	0	31	0	0	36	0	0	36	0	0	20	0	0	143				
V	Male																			
VI	20	0	0	30	0	0	40	0	0	29	0	0	19	0	0	138				
VII	10	6	0	33	0	0	37	0	0	41	0	0	19	0	0	146				
VIII	19	0	0	35	0	0	35	0	0	34	0	0	16	0	0	54				
IX	18	0	0	34	0	0	38	0	0	34	0	0	16	0	0	140				
X	17	0	0	38	0	0	37	0	0	33	0	0	16	0	0	141				
XI	18	0	0	29	0	0	35	0	0	31	0	0	16	0	0	129				
XII	16	0	0	32	0	0	40	0	28	10	0	0	20	0	0	146				
XIII	17	0	0	38	0	0	40	0	0	37	0	0	22	0	0	154				
XIV	19	0	0	28	0	0	45	0	0	42	0	0	23	0	0	157				
XV	Male																			
XVI	20	0	0	30	0	0	37	0	0	37	0	0	13	0	0	137				
XVII	20	0	0	30	0	0	38	0	0	36	0	0	21	0	0	145				
XVIII	14	1	0	31	0	0	39	0	0	40	0	0	22	0	0	147				
XIX	18	0	0	28	0	0	40	0	0	39	0	0	19	0	0	144				
XX	11	0	0	31	0	0	41	0	0	37	0	0	25	0	0	145				



Appendix 4
(continued) **Number of Live Neonates Produced Daily per Surviving Female**

b) 0.015 mg/L

Replicate	Day														Total	
	7	8	9	10	11	12	13	14	15	16	17	18	19	20		21
I	21	0	0	40	0	44	0	0	34	0	0	34	0	0	22	195
II	19	0	0	25	0	0	37	0	0	36	0	0	23	0	0	140
III	19	0	0	28	0	0	39	0	0	28	0	0	18	0	0	132
IV	0	11	0	29	0	0	39	0	0	37	0	0	33	0	0	149
V	0	11						Dead								11
VI								Male								
VII								Male								
VIII	21	0	0	30	0	0	38	0	0	36	0	0	19	0	0	144
IX	13	0	0	32	0	0	41	0	0	39	0	0	22	0	0	147
X								Male								

c) 0.083 mg/L

Replicate	Day														Total	
	7	8	9	10	11	12	13	14	15	16	17	18	19	20		21
I	20	0	0	34	0	0	46	0	41	0	0	26	0	0	29	196
II	19	0	0	34	0	0	47	0	0	39	0	0	24	0	0	163
III	21	0	0	32	0	0	48	0	20	18	0	0	21	0	0	160
IV	20	0	0	35	0	43	0	0	35	0	0	32	0	0	23	188
V	19	18	0	31	0	0	40	0	0	39	0	0	25	0	0	172
VI	0	0	0	34	0	46	5	0	41	0	0	31	0	0	27	184
VII	17	0	0	33	0	0	42	0	3	41	0	0	26	0	0	162
VIII								Male								
IX								Male								
X	20	0	0	32	0	0	43	0	0	43	0	0	22	0	0	160

**Appendix 4
(continued) Number of Live Neonates Produced Daily per Surviving Female**

d) 0.365 mg/L

Replicate	Day														Total	
	7	8	9	10	11	12	13	14	15	16	17	18	19	20		21
I	0	20	0	42	0	0	49	0	0	48	0	0	34	0	0	193
II	17	0	0	42	0	46	0	45	0	0	0	44	0	0	32	226
III	17	0	0	36	0	0	55	0	49	0	0	0	35	0	0	192
IV	17	0	0	38	0	0	56	0	47	0	0	0	32	0	42	232
V	15	0	0	46	0	47	0	39	0	0	0	47	0	0	15	222
VI	0	0	0	46	0	47	0	0	0	0	0	0	0	0	43	
VII																
VIII																
IX																
X																

e) 1.369 mg/L

Replicate	Day														Total	
	7	8	9	10	11	12	13	14	15	16	17	18	19	20		21
I	0	18	0	35	0	0	28	Dead								81
II	0	18	0	31	0	0	(16)	Dead								66
III	2	13	0	29	0	0	20	Dead								64
IV	0	11	0	32	0	0	36	Dead								79
V	20	0	0	29	0	0	10	Dead								59
VI	0	15	0	28	0	0	37	Dead								80
VII																
VIII																
IX																
X																

Note – no reproduction data are presented for the 4.653 mg/L treatment group as all parent *Daphnia* were dead by Day 4

Appendix 5 Water Quality Parameters – Fresh Media

Day	Parameter	Time Weighted Mean Concentration of WRS-2390TX (mg/L)							
		Control	0.015	0.083	0.365	1.369	4.653		
0	pH	7.61	7.64	7.64	7.63	7.63	7.60		
	Temperature (°C)	21.1	21.2	21.3	21.3	21.3	21.3		
	Dissolved Oxygen (%)	97.4	95.5	95.9	94.6	94.4	93.7		
3	pH	7.61	7.56	7.59	7.58	7.56	7.60		
	Temperature (°C)	22.1	21.5	21.7	21.7	21.7	21.4		
	Dissolved Oxygen (%)	95.1	93.5	89.9	90.1	91.5	91.8		
5	pH	7.79	7.75	7.74	7.77	7.84	-		
	Temperature (°C)	20.8	21.0	21.0	21.0	21.0	-		
	Dissolved Oxygen (%)	91.9	92.7	91.6	89.1	90.6	-		
7	pH	7.55	7.54	7.55	7.54	7.53	-		
	Temperature (°C)	21.0	21.2	21.1	21.1	21.0	-		
	Dissolved Oxygen (%)	89.0	88.1	90.1	89.0	90.3	-		
10	pH	7.38	7.54	7.61	7.61	7.62	-		
	Temperature (°C)	20.7	21.1	21.1	21.1	21.1	-		
	Dissolved Oxygen (%)	96.8	94.6	93.8	92.9	93.6	-		

- = Not Applicable

**Appendix 5
(continued) Water Quality Parameters – Fresh Media**

Day	Parameter	Time Weighted Mean Concentration of WRS-2390TX (mg/L)					
		Control	0.015	0.083	0.365	1.369	4.653
12	pH	7.62	7.62	7.62	7.62	7.61	-
	Temperature (°C)	20.2	20.2	20.4	20.4	20.4	-
	Dissolved Oxygen (%)	98.4	97.6	96.4	95.8	95.9	-
14	pH	7.65	7.61	7.63	7.64	7.63	-
	Temperature (°C)	21.0	21.1	21.0	21.0	21.0	-
	Dissolved Oxygen (%)	91.3	91.1	90.8	91.7	91.6	-
17	pH	7.53	7.70	7.74	7.72	-	-
	Temperature (°C)	21.3	21.1	21.0	21.0	-	-
	Dissolved Oxygen (%)	93.4	92.8	92.7	92.0	-	-
19	pH	7.70	7.78	7.81	7.83	-	-
	Temperature (°C)	22.2	22.2	22.3	22.3	-	-
	Dissolved Oxygen (%)	92.2	92.3	92.8	92.3	-	-

- = Not Applicable

Appendix 6 Water Quality Parameters – Expired Media

a) Control

Day	Parameter	Vessel Replicate											
		I	II	III	IV	V	VI	VII	VIII	IX	X		
3	pH	7.79	7.73	7.74	7.75	7.83	7.77	7.73	7.71	7.70	7.71	7.71	7.71
	Temperature (°C)	20.7	20.7	20.7	20.7	20.8	20.8	20.8	20.8	20.7	20.8	20.8	20.5
	Dissolved Oxygen (%)	94.1	92.3	91.3	93.8	94.8	92.8	93.5	92.2	92.5	92.3	92.3	92.3
5	pH	7.61	7.52	7.55	7.63	7.80	7.61	7.57	7.67	7.40	7.38	7.38	7.38
	Temperature (°C)	19.7	19.8	19.8	19.8	19.9	19.9	20.0	20.0	19.9	20.1	20.1	20.1
	Dissolved Oxygen (%)	82.8	84.7	84.1	83.3	87.3	86.1	82.1	84.4	82.8	82.1	82.1	82.1
7	pH	7.27	7.39	7.40	7.43	7.70	7.43	7.41	7.40	7.39	7.36	7.36	7.36
	Temperature (°C)	19.9	19.7	19.7	19.6	19.5	19.5	19.5	19.4	19.4	19.5	19.5	19.5
	Dissolved Oxygen (%)	81.1	82.7	84.0	85.9	91.3	84.4	84.7	84.8	85.3	84.4	84.4	84.4
10	pH	7.43	7.45	7.41	7.41	7.35	7.42	7.42	7.49	7.48	7.42	7.42	7.42
	Temperature (°C)	20.2	20.0	19.9	19.9	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8
	Dissolved Oxygen (%)	92.4	91.7	88.7	88.9	81.5	86.7	88.2	88.7	88.3	87.7	87.7	87.7
12	pH	7.35	7.37	7.40	7.40	-	7.40	7.41	-	7.41	7.41	7.41	7.41
	Temperature (°C)	20.0	19.6	19.7	19.7	-	19.7	19.7	-	19.7	19.7	19.9	19.9
	Dissolved Oxygen (%)	81.3	79.6	81.2	82.7	-	81.5	81.9	-	82.3	81.5	81.5	81.5
14	pH	7.34	7.41	7.41	7.42	-	7.45	7.44	-	7.46	7.45	7.45	7.45
	Temperature (°C)	21.0	20.9	20.8	20.8	-	20.6	20.7	-	20.7	20.8	20.8	20.8
	Dissolved Oxygen (%)	87.9	88.7	86.3	86.4	-	86.9	87.2	-	86.5	86.0	86.0	86.0
17	pH	7.34	7.52	7.53	7.55	-	7.56	7.56	-	7.54	7.57	7.57	7.57
	Temperature (°C)	20.2	20.1	20.1	20.1	-	20.1	20.1	-	20.2	20.3	20.3	20.3
	Dissolved Oxygen (%)	82.9	85.6	86.6	87.1	-	86.2	86.8	-	85.2	86.6	86.6	86.6
19	pH	7.50	7.49	7.49	7.49	-	7.52	7.48	-	7.51	7.48	7.48	7.48
	Temperature (°C)	19.7	19.7	19.7	19.7	-	19.7	19.7	-	19.8	19.9	19.9	19.9
	Dissolved Oxygen (%)	88.7	86.7	86.9	87.1	-	88.1	86.5	-	86.6	87.4	87.4	87.4
21	pH	7.36	7.37	7.38	7.37	-	7.38	7.36	-	7.37	7.38	7.38	7.38
	Temperature (°C)	20.6	20.7	20.8	20.9	-	20.9	20.9	-	21.0	21.0	21.0	21.0
	Dissolved Oxygen (%)	89.9	98.0	96.1	96.3	-	96.8	95.2	-	95.8	94.2	94.2	94.2

- = Not Applicable



**Appendix 6
(continued) Water Quality Parameters – Expired Media**

a) Control (continued)

Day	Parameter	Vessel Replicate													
		XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX				
3	pH	7.72	7.74	7.74	7.80	7.88	7.77	7.72	7.76	7.72	7.72	7.72	7.72	7.72	7.72
	Temperature (°C)	20.6	20.6	20.7	20.7	20.8	20.8	20.8	20.7	20.8	20.8	20.8	20.7	20.6	20.5
	Dissolved Oxygen (%)	91.8	93.9	92.0	93.8	96.1	93.4	92.8	93.8	93.8	92.8	92.2	93.8	92.2	92.7
5	pH	7.44	7.49	7.45	7.43	7.66	7.43	7.43	7.43	7.66	7.43	7.43	7.43	7.43	7.40
	Temperature (°C)	20.1	20.1	20.1	20.1	20.1	20.1	20.2	20.2	20.1	20.2	20.2	20.2	20.2	20.2
	Dissolved Oxygen (%)	84.5	87.2	83.5	83.0	85.5	80.8	83.4	83.0	85.5	83.4	81.9	83.0	81.9	83.0
7	pH	7.41	7.38	7.38	7.37	7.67	7.43	7.41	7.40	7.67	7.43	7.39	7.40	7.39	7.39
	Temperature (°C)	19.7	19.7	19.7	19.6	19.6	19.6	19.6	19.6	19.6	19.6	19.6	19.6	19.6	19.7
	Dissolved Oxygen (%)	84.8	82.1	84.0	83.5	90.1	85.1	85.8	83.8	90.1	85.8	84.4	83.8	84.4	84.4
10	pH	7.48	7.51	7.47	7.48	7.33	7.48	7.51	7.50	7.33	7.48	7.47	7.50	7.47	7.48
	Temperature (°C)	19.7	19.6	19.6	19.6	19.6	19.6	19.7	19.7	19.6	19.7	19.7	19.7	19.7	19.7
	Dissolved Oxygen (%)	89.7	90.3	88.7	88.2	77.9	86.4	88.3	87.7	77.9	86.4	86.6	87.7	86.6	87.5
12	pH	7.42	7.39	7.38	7.40	-	7.40	7.38	7.37	-	7.40	7.36	7.37	7.36	7.38
	Temperature (°C)	20.1	20.1	20.0	19.9	-	19.9	19.8	19.8	-	19.9	19.8	19.8	19.8	19.9
	Dissolved Oxygen (%)	82.0	81.3	82.4	82.8	-	82.6	82.3	82.9	-	82.6	81.7	82.9	81.7	82.7
14	pH	7.47	7.46	7.48	7.46	-	7.47	7.48	7.46	-	7.47	7.45	7.46	7.45	7.46
	Temperature (°C)	20.8	20.7	20.7	20.7	-	20.7	20.7	20.7	-	20.7	20.7	20.7	20.7	20.8
	Dissolved Oxygen (%)	84.4	85.7	86.0	86.2	-	85.6	86.5	86.0	-	85.6	86.4	86.0	86.4	87.0
17	pH	7.58	7.56	7.57	7.57	-	7.56	7.53	7.58	-	7.56	7.59	7.58	7.59	7.59
	Temperature (°C)	20.0	20.0	19.9	20.0	-	20.0	20.0	20.0	-	20.0	20.1	20.0	20.1	20.2
	Dissolved Oxygen (%)	87.5	85.6	85.9	86.1	-	85.7	86.1	86.2	-	85.7	86.6	86.2	86.6	86.9
19	pH	7.53	7.53	7.50	7.52	-	7.54	7.50	7.51	-	7.54	7.49	7.51	7.49	7.48
	Temperature (°C)	19.9	19.9	19.8	19.6	-	19.6	19.6	19.7	-	19.6	19.8	19.7	19.8	19.8
	Dissolved Oxygen (%)	87.0	87.5	87.1	87.0	-	84.7	88.2	87.9	-	84.7	85.9	87.9	85.9	87.0
21	pH	7.40	7.40	7.42	7.43	-	7.44	7.45	7.46	-	7.44	7.47	7.46	7.47	7.45
	Temperature (°C)	21.0	21.0	21.0	21.0	-	21.0	21.0	21.0	-	21.0	20.9	21.0	20.9	20.9
	Dissolved Oxygen (%)	95.7	94.9	95.8	94.8	-	98.3	94.7	96.0	-	98.3	94.8	96.0	94.8	94.2

- = Not Applicable

**Appendix 6
(continued)** **Water Quality Parameters – Expired Media**

b) 0.015 mg/L

Day	Parameter	Vessel Replicate									
		I	II	III	IV	V	VI	VII	VIII	IX	X
3	pH	7.74	7.77	7.80	7.81	7.82	7.87	7.85	7.74	7.77	7.88
	Temperature (°C)	20.5	20.5	20.5	20.6	20.6	20.6	20.6	20.6	20.6	20.5
	Dissolved Oxygen (%)	92.7	94.7	94.4	95.1	93.5	94.9	95.1	93.5	94.0	95.6
5	pH	7.48	7.46	7.42	7.42	7.46	7.72	7.74	7.56	7.48	7.72
	Temperature (°C)	20.1	20.0	19.9	19.9	19.9	19.9	19.9	19.9	19.9	20.1
	Dissolved Oxygen (%)	85.6	86.0	83.6	85.4	85.2	91.0	91.1	84.0	84.0	92.1
7	pH	7.36	7.37	7.39	7.43	7.41	7.68	7.71	7.43	7.40	7.70
	Temperature (°C)	19.7	19.7	19.5	19.4	19.3	19.3	19.2	19.3	19.3	19.4
	Dissolved Oxygen (%)	83.4	85.9	85.9	86.2	84.1	91.7	92.1	84.8	86.1	92.7
10	pH	7.51	7.51	7.54	7.48	7.60	7.71	7.44	7.53	7.51	7.40
	Temperature (°C)	19.7	19.6	19.6	19.6	19.6	19.7	19.7	19.7	19.7	19.7
	Dissolved Oxygen (%)	87.5	87.4	88.1	85.7	88.0	88.8	88.0	86.8	87.9	78.4
12	pH	7.30	7.39	7.38	7.39	-	-	-	7.38	7.37	-
	Temperature (°C)	20.0	20.0	19.9	19.9	-	-	-	19.9	19.9	-
	Dissolved Oxygen (%)	79.2	82.5	83.3	82.4	-	-	-	81.5	82.7	-
14	pH	7.41	7.45	7.46	7.45	-	-	-	7.45	7.47	-
	Temperature (°C)	20.7	20.7	20.6	20.6	-	-	-	20.6	20.7	-
	Dissolved Oxygen (%)	84.5	85.4	85.0	84.8	-	-	-	84.7	86.2	-
17	pH	7.57	7.58	7.56	7.55	-	-	-	7.54	7.54	-
	Temperature (°C)	20.1	20.0	19.9	19.9	-	-	-	20.0	20.0	-
	Dissolved Oxygen (%)	86.2	86.5	85.7	86.1	-	-	-	84.9	85.6	-
19	pH	7.45	7.48	7.49	7.47	-	-	-	7.51	7.49	-
	Temperature (°C)	20.0	19.9	19.8	19.7	-	-	-	19.7	19.8	-
	Dissolved Oxygen (%)	85.7	87.1	86.7	86.8	-	-	-	87.9	87.8	-
21	pH	7.38	7.43	7.44	7.43	-	-	-	7.45	7.45	-
	Temperature (°C)	20.8	20.8	20.8	20.9	-	-	-	20.9	20.9	-
	Dissolved Oxygen (%)	96.8	93.3	94.5	93.2	-	-	-	94.3	92.9	-

- = Not Applicable



**Appendix 6
(continued)** **Water Quality Parameters – Expired Media**

c) 0.083 mg/L

Day	Parameter	Vessel Replicate									
		I	II	III	IV	V	VI	VII	VIII	IX	X
3	pH	7.87	7.82	7.80	7.77	7.73	7.79	7.77	7.83	7.89	7.82
	Temperature (°C)	20.4	20.4	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.4
	Dissolved Oxygen (%)	96.7	96.5	94.0	94.2	93.7	94.2	94.7	96.0	96.6	95.4
5	pH	7.73	7.52	7.48	7.47	7.45	7.48	7.47	7.45	7.71	7.52
	Temperature (°C)	20.0	19.9	19.9	19.8	19.9	19.9	19.9	19.9	19.9	20.0
	Dissolved Oxygen (%)	91.2	87.0	82.0	86.5	84.9	86.6	85.7	86.1	90.9	86.6
7	pH	7.72	7.36	7.35	7.34	7.34	7.38	7.39	7.38	7.68	7.41
	Temperature (°C)	19.6	19.5	19.4	19.3	19.3	19.2	19.2	19.2	19.2	19.3
	Dissolved Oxygen (%)	92.4	83.7	83.4	83.5	83.2	82.9	85.7	86.1	91.4	84.7
10	pH	7.56	7.52	7.52	7.52	7.48	7.47	7.57	7.56	7.61	7.54
	Temperature (°C)	19.7	19.8	19.7	19.7	19.8	19.8	19.8	19.6	19.8	19.9
	Dissolved Oxygen (%)	86.2	87.0	87.7	87.9	88.9	87.8	90.2	89.0	86.0	87.1
12	pH	-	7.38	7.36	7.36	7.30	7.36	7.35	7.38	-	7.37
	Temperature (°C)	-	19.9	19.9	19.9	20.0	20.0	20.1	20.1	-	20.2
	Dissolved Oxygen (%)	-	81.9	82.0	82.2	79.6	82.2	82.1	81.8	-	83.5
14	pH	-	7.45	7.46	7.48	7.48	7.48	7.49	7.48	-	7.49
	Temperature (°C)	-	20.6	20.5	20.5	20.5	20.5	20.5	20.5	-	20.7
	Dissolved Oxygen (%)	-	84.3	85.6	86.0	85.3	85.5	86.0	85.9	-	86.9
17	pH	-	7.56	7.54	7.53	7.53	7.55	7.58	7.55	-	7.54
	Temperature (°C)	-	20.1	20.0	20.0	20.0	20.0	20.1	20.1	-	20.3
	Dissolved Oxygen (%)	-	84.1	84.6	84.3	85.0	84.9	85.5	84.5	-	84.7
19	pH	-	7.49	7.48	7.49	7.48	7.48	7.49	7.44	-	7.48
	Temperature (°C)	-	19.9	19.8	19.7	19.7	19.7	19.9	20.0	-	20.3
	Dissolved Oxygen (%)	-	85.9	86.7	86.7	87.3	87.2	86.0	85.4	-	86.7
21	pH	-	7.38	7.42	7.41	7.38	7.45	7.38	7.43	-	7.42
	Temperature (°C)	-	20.6	20.6	20.7	20.7	20.8	20.8	20.8	-	20.8
	Dissolved Oxygen (%)	-	92.3	92.7	91.5	92.3	94.1	93.5	93.7	-	93.6

- = Not Applicable

**Appendix 6
(continued) Water Quality Parameters – Expired Media**

d) 0.365 mg/L

Day	Parameter	Vessel Replicate									
		I	II	III	IV	V	VI	VII	VIII	IX	X
3	pH	7.87	7.81	7.85	7.86	7.75	7.77	7.89	7.80	7.79	7.92
	Temperature (°C)	20.4	20.4	20.4	20.4	20.4	20.5	20.5	20.5	20.4	20.4
	Dissolved Oxygen (%)	96.1	94.9	95.9	95.4	92.5	93.0	96.1	94.5	93.6	95.6
5	pH	7.77	7.50	7.75	7.72	7.50	7.49	7.73	7.50	7.74	7.49
	Temperature (°C)	20.1	20.0	19.9	19.8	19.9	19.9	19.9	19.9	19.9	20.0
	Dissolved Oxygen (%)	91.4	84.6	91.9	92.1	85.2	86.0	91.8	85.6	85.1	86.0
7	pH	7.67	7.51	7.69	7.71	7.45	7.44	7.67	7.45	7.41	7.69
	Temperature (°C)	19.7	19.6	19.5	19.4	19.3	19.3	19.2	19.2	19.2	19.3
	Dissolved Oxygen (%)	93.1	85.6	92.1	93.2	84.8	85.8	92.5	85.1	84.3	91.7
10	pH	7.89	7.53	7.39	7.41	7.45	7.47	7.37	7.46	7.43	7.46
	Temperature (°C)	19.9	19.9	19.8	19.7	19.8	19.8	19.8	19.8	19.8	19.9
	Dissolved Oxygen (%)	94.1	87.0	78.8	79.9	88.9	86.6	76.5	85.7	79.6	86.3
12	pH	-	7.38	-	-	7.34	7.34	-	7.33	-	7.33
	Temperature (°C)	-	20.1	-	-	20.1	20.1	-	20.1	-	20.1
	Dissolved Oxygen (%)	-	80.9	-	-	81.0	81.1	-	80.8	-	81.3
14	pH	-	7.45	-	-	7.45	7.48	-	7.47	-	7.47
	Temperature (°C)	-	20.6	-	-	20.5	20.5	-	20.5	-	20.6
	Dissolved Oxygen (%)	-	85.2	-	-	85.3	86.2	-	86.8	-	86.9
17	pH	-	7.56	-	-	7.55	7.59	-	7.58	-	7.55
	Temperature (°C)	-	20.3	-	-	20.3	20.2	-	20.2	-	20.2
	Dissolved Oxygen (%)	-	86.3	-	-	86.4	86.4	-	87.2	-	86.5
19	pH	-	7.45	-	-	7.45	7.43	-	7.44	-	7.46
	Temperature (°C)	-	20.3	-	-	20.2	20.1	-	20.1	-	20.2
	Dissolved Oxygen (%)	-	85.5	-	-	86.0	86.6	-	86.4	-	88.2
21	pH	-	7.42	-	-	7.35	7.34	-	7.31	-	7.26
	Temperature (°C)	-	20.8	-	-	20.8	20.4	-	20.8	-	20.8
	Dissolved Oxygen (%)	-	93.7	-	-	93.6	92.9	-	93.3	-	91.9

- = Not Applicable

**Appendix 6
(continued)** **Water Quality Parameters – Expired Media**
e) 1.369 mg/L

Day	Parameter	Vessel Replicate											
		I	II	III	IV	V	VI	VII	VIII	IX	X		
3	pH	7.84	7.81	7.81	7.81	7.80	7.79	7.84	7.85	7.85	7.84	7.85	7.84
	Temperature (°C)	20.3	20.4	20.4	20.4	20.4	20.4	20.4	20.4	20.4	20.4	20.4	20.3
	Dissolved Oxygen (%)	95.0	95.0	95.5	95.2	94.1	94.5	94.2	94.2	95.0	94.8	94.8	95.1
5	pH	7.56	7.57	7.60	7.62	7.60	7.59	7.69	7.73	7.75	7.75	7.74	7.74
	Temperature (°C)	20.1	20.1	20.0	20.0	20.1	20.1	20.1	20.1	20.1	20.2	20.2	20.2
	Dissolved Oxygen (%)	88.5	89.2	89.6	89.7	89.0	88.6	91.3	91.4	91.4	91.9	92.6	92.6
7	pH	7.50	7.47	7.48	7.49	7.46	7.48	7.64	7.68	7.74	7.74	7.72	7.72
	Temperature (°C)	19.9	19.9	19.8	19.6	19.6	19.5	19.4	19.4	19.4	19.4	19.5	19.5
	Dissolved Oxygen (%)	87.2	86.9	86.9	87.5	86.4	87.6	90.0	91.0	91.0	92.5	93.3	93.3
10	pH	7.55	7.40	7.47	7.40	7.36	7.39	7.31	7.35	7.42	7.42	7.35	7.35
	Temperature (°C)	20.0	20.0	20.0	20.0	19.9	19.9	19.9	19.9	19.9	19.9	19.9	20.0
	Dissolved Oxygen (%)	91.2	84.4	86.6	84.9	82.5	83.8	84.6	79.4	79.4	82.8	80.8	80.8
12	pH	7.28	7.30	7.27	7.31	7.31	7.29	-	-	-	-	-	-
	Temperature (°C)	20.2	20.2	20.2	20.2	20.3	20.3	-	-	-	-	-	-
	Dissolved Oxygen (%)	75.0	75.8	75.4	78.8	79.0	76.4	-	-	-	-	-	-
14	pH	7.79	7.78	7.77	7.77	7.74	7.65	-	-	-	-	-	-
	Temperature (°C)	20.6	20.5	20.5	20.5	20.5	20.5	-	-	-	-	-	-
	Dissolved Oxygen (%)	91.4	87.9	90.9	92.4	89.6	89.0	-	-	-	-	-	-
17	pH	-	-	-	-	-	-	-	-	-	-	-	-
	Temperature (°C)	-	-	-	-	-	-	-	-	-	-	-	-
	Dissolved Oxygen (%)	-	-	-	-	-	-	-	-	-	-	-	-
19	pH	-	-	-	-	-	-	-	-	-	-	-	-
	Temperature (°C)	-	-	-	-	-	-	-	-	-	-	-	-
	Dissolved Oxygen (%)	-	-	-	-	-	-	-	-	-	-	-	-
21	pH	-	-	-	-	-	-	-	-	-	-	-	-
	Temperature (°C)	-	-	-	-	-	-	-	-	-	-	-	-
	Dissolved Oxygen (%)	-	-	-	-	-	-	-	-	-	-	-	-

- = Not Applicable



**Appendix 6
(continued) Water Quality Parameters – Expired Media**

f) 4.653 mg/L

Day	Parameter	Vessel Replicate										
		I	II	III	IV	V	VI	VII	VIII	IX	X	
3	pH	7.58	7.54	7.55	7.53	7.53	7.53	7.53	7.52	7.53	7.53	7.53
	Temperature (°C)	20.3	20.3	20.3	20.3	20.4	20.4	20.4	20.4	20.4	20.4	20.3
	Dissolved Oxygen (%)	88.2	87.6	88.0	88.3	87.9	88.2	89.2	89.0	87.4	88.3	88.3
5	pH	-	-	-	-	-	-	-	-	-	-	-
	Temperature (°C)	-	-	-	-	-	-	-	-	-	-	-
7	Dissolved Oxygen (%)	-	-	-	-	-	-	-	-	-	-	-
	pH	-	-	-	-	-	-	-	-	-	-	-
	Temperature (°C)	-	-	-	-	-	-	-	-	-	-	-
10	Dissolved Oxygen (%)	-	-	-	-	-	-	-	-	-	-	-
	pH	-	-	-	-	-	-	-	-	-	-	-
	Temperature (°C)	-	-	-	-	-	-	-	-	-	-	-
12	Dissolved Oxygen (%)	-	-	-	-	-	-	-	-	-	-	-
	pH	-	-	-	-	-	-	-	-	-	-	-
	Temperature (°C)	-	-	-	-	-	-	-	-	-	-	-
14	Dissolved Oxygen (%)	-	-	-	-	-	-	-	-	-	-	-
	pH	-	-	-	-	-	-	-	-	-	-	-
	Temperature (°C)	-	-	-	-	-	-	-	-	-	-	-
17	Dissolved Oxygen (%)	-	-	-	-	-	-	-	-	-	-	-
	pH	-	-	-	-	-	-	-	-	-	-	-
	Temperature (°C)	-	-	-	-	-	-	-	-	-	-	-
19	Dissolved Oxygen (%)	-	-	-	-	-	-	-	-	-	-	-
	pH	-	-	-	-	-	-	-	-	-	-	-
	Temperature (°C)	-	-	-	-	-	-	-	-	-	-	-
21	Dissolved Oxygen (%)	-	-	-	-	-	-	-	-	-	-	-
	pH	-	-	-	-	-	-	-	-	-	-	-
	Temperature (°C)	-	-	-	-	-	-	-	-	-	-	-

- = Not Applicable

Appendix 7 Total Hardness and Alkalinity of Elendt M4 Used to Prepare Test Solutions

Day of Use	Batch Number	Total Hardness (mg CaCO ₃ /L)	Alkalinity (mg CaCO ₃ /L)
0	I	230	80
3	II	222	75
5	III	270	60
7	IV	235	95
10	V	234	80
12	VI	216	60
14	VII	216	60
17	VIII	230	70
19	IX	240	70

Appendix 8 Daily Minimum and Maximum Temperatures

Day	Minimum temperature (°C)	Maximum temperature (°C)
1	21.2	21.6
2	21.3	21.5
3	21.1	21.5
4	21.3	21.8
5	21.5	21.7
6	21.0	21.7
7	22.1	22.2
8	21.2	21.8
9	20.9	21.7
10	20.9	21.7
11	21.5	21.7
12	21.4	21.6
13	21.4	21.7
14	21.4	21.5
15	-	-
16	21.3	21.7
17	21.0	21.7
18	21.5	21.7
19	21.4	21.7
20	21.5	21.7
21	21.5	21.8

- = Temperature not recorded, in error

Appendix 9 GLP Certificate



**THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM**

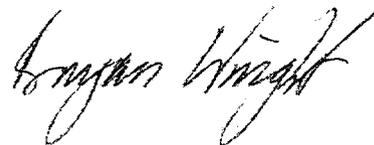
GOOD LABORATORY PRACTICE

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

LABORATORY	TEST TYPE
Inveresk Research International Ltd. Elphinstone Research Centre Tranent East Lothian EH33 2NE	Analytical Chemistry Clinical Chemistry Ecosystems Environmental Toxicity Environmental Fate Mutagenicity Phys/chem Tests Toxicology
Including	
Veterinary Clinical Trials Unit Talkin Brampton Cumbria CA8 1LE	Safety studies on Veterinary products
DATE OF INSPECTION	
13 April 2004	

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

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



Mr. Bryan J. Wright
Head, UK GLP Monitoring Authority