LAB Research Ltd.

Address: 8200 Veszprém, Szabadságpuszta

Phone: +36 88 545 300

Fax: +36 88 545 301

FINAL REPORT

ACUTE TOXICITY TEST WITH

Ethylene Dibromide Industrial (EDB)

ON RAINBOW TROUT

(Oncorhynchus mykiss)

Date of Final Report: 01 October 2010

STUDY CODE: 10/112-009HR

STATEMENT OF THE STUDY DIRECTOR

This study has been performed in accordance with the study plan the OECD Guidelines for Testing of Chemicals (No. 203, 17 July 1992) and Directive 92/69/EEC, Annex Part C, C.1 and the EPA Health Effects Test Guidelines (OPPTS 850.1075) and the Principles of Good Laboratory Practice Regulations as specified by national Hungarian GLP Regulations (9/2001.(III.30.) EüM-FVM joint decree of the Minister of Health and the Minister of Agriculture and Regional Development) which corresponds to the OECD GLP, ENV/MC/CHEM(98)17.

I the undersigned declare that this report constitutes a true record of the actions undertaken and the results obtained in the course of this study. By virtue of my dated signature I accept the responsibility for the validity of the data and the following conclusion drawn from them:

Under the conditions of this acute fish toxicity study on rainbow trout (*Oncorhynchus mykiss*) the observed and calculated endpoints for the effect of Ethylene Dibromide Industrial (EDB) were the followings:

The 24h LC_{50} value:	**4.68 mg test item/L** (with 95 % conf. limits: 3.35 – 7.33)
The 48h LC_{50} value:	**1.86 mg test item/L** (with 95 % conf. limits: 1.56 – 2.15)
The 72h LC_{50} value:	**1.56 mg test item/L** (with 95 % conf. limits: 1.29 – 1.89)
The 96h LC_{50} value:	**1.13 mg test item/L** (with 95 % conf. limits: 0.88 – 1.40)
96h LC_{100} value:	**1.46 mg test item/L**
The 96h No-Observed Effect Concentration (NOEC):	**0.86 mg test item/L**
The 96h Lowest Observed Effect Concentration (LOEC):	**1.46 mg test item/L**

Signature: _____ Date: 01 October 2010

István Ágh M.Sc.
Study Director

STATEMENT OF THE MANAGEMENT

According to the conditions of the research and development agreement between CHEMTURA CORPORATION (as Sponsor) and LAB Research Ltd. (as Testing Facility) „Acute toxicity test with Ethylene Dibromide Industrial (EDB) on rainbow trout (*Oncorhynchus mykiss*)" has been performed in compliance with the study plan and the Principles of Good Laboratory Practice.

Signature: _____ Date: 01 Oct. 2010

Christopher Banks, DABT.
Managing Director

QUALITY ASSURANCE STATEMENT

Study Code: 10/112-009HR

Study Title: Acute toxicity test with Ethylene Dibromide Industrial (EDB) on rainbow trout (*Oncorhynchus mykiss*)

Test Item: Ethylene Dibromide Industrial (EDB)

This study has been inspected, and this report audited by the Quality Assurance Unit in compliance with the Principles of Good Laboratory Practice. As far as it can be reasonably established the methods described and the results incorporated in this report accurately reflect the raw data produced during this study.

All inspections, data reviews and the report audit were reported in written form to the study director and to management. The dates of such inspections and of the report audit are given below:

Date of Inspection	Phase(s) Inspected/Audited	Date of report to	
		Management	Study Director
03 August 2010	Study Plan	03 August 2010	03 August 2010
09 August 2010	Treatment	09 August 2010	09 August 2010
06 September 2010	Analytical Report	06 September 2010	06 September 2010
13 September 2010	Draft Report	13 September 2010	13 September 2010
01 October 2010	Final Report	01 October 2010	01 October 2010

Signature: Fábiánné Makovi Éva Date: 01 October 2010

Éva Makovi-Fábián B.Sc.
On behalf of QAU

GENERAL INFORMATION

STUDY TITLE	:	Acute toxicity test with Ethylene Dibromide Industrial (EDB) on rainbow trout (*Oncorhynchus mykiss*)
TEST ITEM	:	Ethylene Dibromide Industrial (EDB)
SPONSOR	:	CHEMTURA CORPORATION Address: 199, Benson Road, Middlebury, Connecticut 06749 USA
TEST FACILITY	:	LAB Research Ltd. Address: H-8200 Veszprém, Szabadságpuszta Phone: 36 88 545-300 Fax: 36 88 545-301
STUDY DIRECTOR	:	István Ágh M.Sc.
QUALITY ASSURANCE	:	Ramóna Heiderné Grób B.Sc. Éva Makovi-Fábián B.Sc.
RESPONSIBLE PERSON FOR ANALYTICAL MEASUREMENTS	:	Zsolt Sárvári M.Sc.
TECHNICAL STAFF	:	Ecotoxicological staff
STATISTICAL DATA PROCESSING	:	Ágnes Móricz
STUDY PLAN	:	03 August 2010
START OF EXPERIMENT	:	09 August 2010
END OF EXPERIMENT	:	13 August 2010
DRAFT REPORT	:	13 September 2010

BASIS OF STUDY:

- OECD Guideline for Testing of Chemicals, No. 203, "Fish, Acute Toxicity Test", adopted July 17, 1992
- EPA Health Effects Test Guidelines, OPPTS 850.1075
- Commission Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restrictions of Chemicals (REACH), Annex Part C, C.1 (published in the Official Journal of the European Union L 142 of 31 May 2008)

CONTENTS

		Page
1.	**SUMMARY**	**7**
2.	**INTRODUCTION**	**8**
3.	**MATERIALS AND METHODS**	**8**
3.1.	TEST ITEM AND CONTROLS AND VEHICLE	8
3.2.	EXPERIMENTAL ANIMALS	9
3.3.	TEST CONDITIONS	10
3.4.	DESCRIPTION OF THE TEST PROCEDURE	10
3.5.	STATISTICAL EVALUATION	11
3.6.	ANALYTICAL MEASUREMENTS	11
3.7.	ARCHIVES	12
3.8.	VALIDITY CRITERIA OF THE STUDY	12
3.9.	DISTRIBUTION OF THE FINAL REPORT	12
3.10.	DEVIATION FROM THE STUDY PLAN	13
3.11.	REFERENCES	13
4.	**RESULTS**	**14**
4.1.	VALIDITY	14
4.2.	CONCENTRATION OF THE TEST ITEM	14
4.3.	MORTALITY DATA	15
4.4.	BODY WEIGHT	16
5.	**CONCLUSION**	**17**

APPENDICES	**18**	
Data of test conditions	1	of Appendix 1
Symptoms of fish	1 - 3	of Appendix 2
Graphical presentation of mortality	1	of Appendix 3
Analytical investigations of main study	1 - 3	of Appendix 4
Copy of the GLP Certificate	1	of Appendix 5

1. SUMMARY

The acute toxicity of Ethylene Dibromide Industrial (EDB) was assessed with Acute Fish Toxicity Test on rainbow trout (*Oncorhynchus mykiss*) under static conditions over an exposure period of 96 hours.

Based on the results of the preliminary test and main test performed under the study code 10/112-009H, six different test concentrations in a geometric series (factor 1.7) and one control was tested in the main experiment.

The nominal concentrations of Ethylene Dibromide Industrial (EDB) used in the main experiment were: 1.4, 2.4, 4.1, 6.9, 11.8 and 20 mg/L. The corresponding calculated test item concentrations (based on the analytical measurements) were: 0.50, 0.86, 1.46, 2.09, 3.18 and 7.16 mg/L.

Because the analysed concentrations deviated more than 20 percent from the nominal concentration throughout the test, the biologically results are based on the measured test item concentrations.

All achievable validity criteria were met during this study.

Under the conditions of this acute fish toxicity study on rainbow trout (*Oncorhynchus mykiss*) the observed and calculated endpoints for the effect of Ethylene Dibromide Industrial (EDB) were the followings:

The 24h LC_{50} value: **4.68 mg test item/L** (with 95 % conf. limits: 3.35 – 7.33)

The 48h LC_{50} value: **1.86 mg test item/L** (with 95 % conf. limits: 1.56 – 2.15)

The 72h LC_{50} value: **1.56 mg test item/L** (with 95 % conf. limits: 1.29 – 1.89)

The 96h LC_{50} value: **1.13 mg test item/L** (with 95 % conf. limits: 0.88 – 1.40)

96h LC_{100} value: **1.46 mg test item/L**

The 96h No-Observed Effect Concentration (NOEC): **0.86 mg test item/L**

The 96h Lowest Observed Effect Concentration (LOEC): **1.46 mg test item/L**

2. INTRODUCTION

The objective of this study was to determine the median lethal concentration (LC_{50} value) of the test item in acute toxicity test rainbow trout (*Oncorhynchus mykiss*).

Fish were exposed in a static test to aqueous test media, containing the test item at different concentration.

The test method of application and the test species rainbow trout (*Oncorhynchus mykiss*) are recommended by the test guidelines.

3. MATERIALS AND METHODS

3.1. TEST ITEM AND CONTROLS AND VEHICLE

3.1.1. Test Item

Name:	Ethylene Dibromide Industrial (EDB)
Chemical name:	1,2-Dibromoethane
Batch No.:	510100003
Active component:	>99.94 % 1,2-Dibromoethane (CAS 106-93-4)
Description:	clear to amber liquid
Manufacture date:	February 2010
Expiry date:	February 2011
Storage:	room temperature;15-25°C (humidity 50 % ± 20)
Safety Precautions:	see Safety Data Sheet
Manufacturer:	Chemtura Manufacturing UK Limited
Address:	Tenax Road, Trafford Park Manchester United Kingdom M17 1WT

The test item of a suitable chemical purity was supplied by the Sponsor. All precautions required in the handling and disposal of the test item were outlined by the Sponsor. These documents are part of the raw data. Identification of the test item was performed in the Central Dispensary of LAB Research Ltd. on the basis of the information provided by the Sponsor (name, batch number, appearance and color).

3.1.2. Dilution and Preparation of Testing Solutions

Because the test item could not be directly dissolved in the test medium (aquarium water), the test item solutions used in the experiment were prepared as follows:

A stock solution (nominally 160 mg/L) was prepared by mechanical dispersion one day before the start of the test. This solution was shaken for about 24 hours. After shaking the test solutions were prepared by appropriate diluting of the stock solution (see Table 1).

Table 1: Preparation of test solutions from stock solution

Nominal concentration (mg/L)	**Amount of stock solution** (mL)	**Amount of aquarium water** (mL)
20	2500	q.s. ad 20000
11.8	1471	q.s. ad 20000
6.9	865	q.s. ad 20000
4.1	509	q.s. ad 20000
2.4	299	q.s. ad 20000
1.4	176	q.s. ad 20000

q.s. ad = quantum sufficiat ad (a sufficient quantity to make)

3.1.3. Untreated Control

The dilution water (circulated, filtered and cooled tap water) was used without of addition of the test item.

3.2. EXPERIMENTAL ANIMALS

Species:	Rainbow trout *(Oncorhynchus mykiss)*
Source:	HOITSY & RIEGER Kft., Trout Farm, Miskolc-Lillafüred, Hungary
Justification of species:	The Rainbow trout *(Oncorhynchus mykiss)* is one of the convenient species for acute fish toxicity test.
Number of animals:	seven fish per test group
Body length of animals:	5.8 – 7.1 cm
Food and Feeding:	The fish were not fed during the test
Acclimatisation:	>12 days
Animal health:	Fish were bred in a well-known fish farm, under certified disease- and parasite-controlled conditions, so the fish were healthy. There was no mortality of the population for seven days before the test.

3.3. TEST CONDITIONS

3.3.1. Equipment and test vessels

Normal laboratory equipments and the followings were used for determination of the parameters of the test:

- pH meter
- thermometer
- oxygen meter
- balance
- apparatus for temperature control
- aquaria with total capacity of 25 litres
- orbital shaker

3.3.2. Environmental test conditions

The water temperature, oxygen concentration and pH were measured at the start of the test and daily thereafter in each test aquarium.

The test temperature was between 13.8 and 15.1 °C.

The dissolved oxygen concentration was in the range of 92 – 96 % of the air saturation value at the temperature used.

The pH was in the range of 8.18 – 8.38.

The hardness of the dilution water was determined as 225 mg/L (as $CaCO_3$).

The light-dark cycle during the test was 16 hours light and 8 hours darkness.

The test conditions above are detailed in Appendix 1.

3.4. DESCRIPTION OF THE TEST PROCEDURE

The test duration was 96 hours. One aquarium was used for test groups and for the control group respectively. Each aquarium comprised 7 fish and 20 litre test solution. The animals were not fed during the test. The loading of the test aquaria was less than 1.0 g fish/L test solution at the start of the experiment.

3.4.1. Concentration Levels Investigated in the Main Test

Six concentrations arranged in a geometric series (factor 1.7) and one control group was tested in the main test. The choice of the test item concentrations was based on the results of the range-finding test and the main test performed earlier under the study code: 10/112-009H.

The nominal concentrations of test item were: 1.4, 2.4, 4.1, 6.9, 11.8 and 20 mg/L.

The corresponding calculated concentrations (based on the analytical measurements) were: 0.50, 0.86, 1.46, 2.09, 3.18 and 7.16 mg/L.

The test results are based on the calculated test item concentrations. The analytically measured test item concentrations are detailed in Appendix 4.

3.4.2. Observations

The observations of fish were carried out in the following intervals: at 3, 6, 24, 48, 72 and 96 hours. Mortality and any sub-lethal effect were determined in each observation period.

The test conditions (pH, temperature, oxygen saturation) were examined daily during the test.

The body weights of the fish were recorded before the introduction of fish. The body weight of 7 fish per aquarium was registered. The loading of the aquaria was calculated on the basis of these body weights (g fish/litre testing liquid).

3.5. STATISTICAL EVALUATION

The LC_{50} values were calculated by Probit analysis with 95 % confidence limits using using SPSS PC+ software (based on the calculated geometric mean concentrations).

The NOEC and LOEC and the LC_{100} values were determined directly from the raw data.

3.6. ANALYTICAL MEASUREMENTS

Concentration of Ethylene Dibromide Industrial (EDB) in the test solutions was determined at the beginning and at the end* of the study.

Three samples were taken from the test solutions and one sample was taken from the control solution.

The samples were analysed by an HPLC-UV method.

* As soon as each fish died in the test group, samples were taken from this aquarium and stored in a freezer until analysis at the end of the study.

3.7. ARCHIVES

The study documents:

- study plan,
- all raw data,
- sample of test item,
- study report, and any amendments,
- correspondence

are stored according to the Hungarian GLP and to the LAB Research Ltd. SOP-s in the archives of LAB Research Ltd. 8200 Veszprém, Szabadságpuszta, Hungary.

After the retention time has elapsed all the archived materials listed above will be returned to the Sponsor or retained for a further period if agreed by a contract. Otherwise the materials will be discarded.

3.8. VALIDITY CRITERIA OF THE STUDY

- Constant conditions should be maintained in this procedure.
- The mortality in the controls should not exceed 10 % at the end of the test (or one fish if less than ten are used). In case of limit test, no mortality should be occurred.
- The dissolved oxygen concentration (throughout the test) has to be at least 60 % of air saturation value.
- The concentration of the test item should be at least 80 % of the nominal concentration throughout the test. If the deviation from the nominal concentration is greater than 20%, results should be based on the measured concentration.

3.9. DISTRIBUTION OF THE FINAL REPORT

Sponsor: 1x copy, bound
1x copy, unbound
1x PDF file

Archive: 1x original, bound

3.10. DEVIATION FROM THE STUDY PLAN

Concerning:	Date of the Draft Report
According to the Study Plan:	not later than 03 September 2010
Deviation:	13 September 2010
Reason for this change:	Unscheduled delay
Presumed Effect on the Study:	None

3.11. REFERENCES

1. OECD Principles of Good Laboratory Practice, adopted by Council on 26th November 1997 [C(97)186/Final], Environment Directorate, Organisation for Economic Co-operation and Development, Paris 1998.
2. Hungarian Good Laboratory Practice Regulations: 9/2001 (III. 30) EÜM-FVM joint decree of the Minister of Health and the Minister of Agriculture and Regional Development which corresponds to the OECD GLP, 1997
3. Commission Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restrictions of Chemicals (REACh), Annex Part C, C.1 (published in the Official Journal of the European Union L 142 of 31 May 2008)
4. EPA Guideline 712-C-96-118: OPPTS 850.1075, "Fish Acute Toxicity Test, Freshwater and Marine" April 1996
5. OECD Guideline for Testing of Chemicals, Section 2, No. 203, "Fish, Acute Toxicity Test", adopted July 17, 1992.
6. Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures, section 3.1.2 Media preparation methods, Direct addition. OECD Series on Testing and Assessment No. 23, Paris September 2000.

4. RESULTS

4.1. VALIDITY

The achievable validity criteria were within acceptable limits and therefore the study can be considered as valid.

4.2. CONCENTRATION OF THE TEST ITEM

The following nominal concentrations were used in the main study: 1.4, 2.4, 4.1, 6.9, 11.8 and 20 mg/L.

The corresponding calculated test item concentrations (based on the analytical measurements) were: 0.50, 0.86, 1.46, 2.09, 3.18 and 7.16 mg/L.

Since there was greater than 20% difference between the start and end values, the geometric mean was calculated for the three highest concentrations (6.9, 11.8 and 20 mg/L nominal). The concentrations were below the Limit of Quantification (LOQ) in the three lowest test groups at the end of the test, therefore these concentrations were extrapolated from the calculated geometric mean value of the highest examined concentration level (7.16 mg/L calculated).

The biological results of the test are based on the calculated exposure concentrations.

The measured concentrations were significantly lower than the nominal at the start (after ~24h shaking) and particularly at the end of the test.

The possible reason for the quick decline of the concentrations could be that the test material is likely to be volatile. This could be supported by the results of the stability test in aqueous solutions at room temperature (see: “Validation of the Analytical Method for the Determination of Ethylene Dibromide Industrial (EDB)”; LAB code: 10/112-316AN) where the concentration was constant during the storage period in a well capped glass flask with minimal headspace.

The concentration decrease may be additionally explained by hydrolysis, because the hydrolysis of the test substance can increase at higher or lower pH from neutral (see: “Determination of the Hydrolysis of Ethylene Dibromide Industrial (EDB) as a Function of pH”; LAB code: 10/112-336AN). The pH of the present test was in the range of 8.18 – 8.38 during the exposure (see Appendix 1; Table 7).

Table 2: Concentration Levels in the main test

Nominal concentrations (mg/mL)		**Control**	**1.4**	**2.4**	**4.1**	**6.9**	**11.8**	**20**
Measured concentrations (mg/L)	**Start**	n.d.	0.74	1.05	1.97	2.97	5.6	9.5
	End	n.d.	BQL	BQL	BQL	1.47	1.81	5.4
Calculated concentrations (mg/L)		**0**	**Extrapolated values**			**Geometric mean**		
			0.50	**0.86**	**1.46**	**2.09**	**3.18**	**7.16**

n.d. – not detectable

BQL: below quantification limit

Methods and results of test item concentration analysis are described in Appendix 4.

4.3. MORTALITY DATA

Cumulative mortality data from the exposure of rainbow trout to the test material during the definitive test are given below in Table 3.

Table 3: Cumulative mortality data in the definitive test

Nominal concentrations (mg/L)	**Calculated concentrations** (mg/L)	**Cumulative mortality** (initial population = 7 fish)					
		3h	**6h**	**24h**	**48h**	**72h**	**96h**
Control	**0.00**	0	0	0	0	0	0
1.4	**0.50**	0	0	0	0	0	0
2.4	**0.86**	0	0	0	0	0	0
4.1	**1.46**	0	0	0	0	2	7
6.9	**2.09**	0	0	0	6	7	–
11.8	**3.18**	0	0	0	7	–	–
20	**7.16**	0	0	7	–	–	–

4.4. SYMPTOMS OF FISH

The symptoms observed during the test are listed in Appendix 2.

4.5. BODY WEIGHT

The body weight of 7 fish was weighed at the start of the test. The measured and calculated data are listed below in Table 4.

Table 4: Measured and calculated data of body weight

Nominal concentrations (mg/L)	Calculated concentrations (mg/L)	Measured weight of 7 fish (g)	Calculated mean weight of 1 fish (g)	Loading of testing aquarium (g fish/l testing liquid)
Control	**0.00**	18.44	2.63	0.92
1.4	**0.50**	19.42	2.77	0.97
2.4	**0.86**	20.00	2.86	1.00
4.1	**1.46**	18.76	2.68	0.94
6.9	**2.09**	19.43	2.78	0.97
11.8	**3.18**	19.13	2.73	0.96
20	**7.16**	18.84	2.69	0.94

There was no considerable difference observed concerning body weights between the groups.

5. CONCLUSION

The acute toxicity of Ethylene Dibromide Industrial (EDB) was assessed with Acute Fish Toxicity Test on rainbow trout (*Oncorhynchus mykiss*) under static conditions over an exposure period of 96 hours.

Because the analysed concentrations deviated more than 20 percent from the nominal concentration throughout the test, the biologically results are based on the measured test item concentrations.

All achievable validity criteria were met during this study.

Under the conditions of this acute fish toxicity study on rainbow trout (*Oncorhynchus mykiss*) the observed and calculated endpoints for the effect of Ethylene Dibromide Industrial (EDB) were the followings:

The 24h LC_{50} value: **4.68 mg test item/L** (with 95 % conf. limits: 3.35 – 7.33)

The 48h LC_{50} value: **1.86 mg test item/L** (with 95 % conf. limits: 1.56 – 2.15)

The 72h LC_{50} value: **1.56 mg test item/L** (with 95 % conf. limits: 1.29 – 1.89)

The 96h LC_{50} value: **1.13 mg test item/L** (with 95 % conf. limits: 0.88 – 1.40)

96h LC_{100} value: **1.46 mg test item/L**

The 96h No-Observed Effect Concentration (NOEC): **0.86 mg test item/L**

The 96h Lowest Observed Effect Concentration (LOEC): **1.46 mg test item/L**

A P P E N D I C E S

APPENDIX 1

DATA OF TEST CONDITIONS

Table 5: Temperature (°C)

Nominal concentrations (mg/L)	Calculated concentrations (mg/L)	Measuring				
		0h	24h	48h	72h	96h
Control	**0.00**	14.8	15.0	15.1	14.7	14.9
1.4	**0.50**	13.9	14.7	14.7	14.6	13.9
2.4	**0.86**	13.8	14.8	14.9	14.2	14.2
4.1	**1.46**	14.0	14.8	14.9	14.4	14.0
6.9	**2.09**	14.2	14.8	14.7	14.2	14.3
11.8	**3.18**	14.0	14.7	14.8	–	–
20	**7.16**	14.3	14.8	–	–	–

Table 6: Dissolved oxygen concentration (%)

Nominal concentrations (mg/L)	Calculated concentrations (mg/L)	Measuring				
		0h	24h	48h	72h	96h
Control	**0.00**	94	93	94	94	93
1.4	**0.50**	95	94	95	93	92
2.4	**0.86**	93	93	94	93	94
4.1	**1.46**	93	94	94	95	96
6.9	**2.09**	94	94	95	95	95
11.8	**3.18**	95	94	95	–	–
20	**7.16**	94	93	–	–	–

Table 7: pH values

Nominal concentrations (mg/L)	Calculated concentrations (mg/L)	Measuring				
		0h	24h	48h	72h	96h
Control	**0.00**	8.27	8.24	8.25	8.30	8.32
1.4	**0.50**	8.29	8.24	8.24	8.29	8.32
2.4	**0.86**	8.29	8.26	8.29	8.25	8.26
4.1	**1.46**	8.23	8.23	8.21	8.30	8.38
6.9	**2.09**	8.22	8.30	8.28	8.32	8.32
11.8	**3.18**	8.26	8.21	8.22	–	–
20	**7.16**	8.18	8.20	–	–	–

APPENDIX 2

SYMPTOMS OF FISH

Table 8

Nominal concentration:	**Control**					
Symptoms	**Numbers of fish / Observation time**					
	3h	**6h**	**24h**	**48h**	**72h**	**96h**
Swimming problems	0	0	0	0	0	0
Localisation near to the surface of the water	0	0	0	0	0	0
Localisation near the bottom of the aquarium	0	0	0	0	0	0
Cumulative mortality	0	0	0	0	0	0

Table 9

Nominal concentration:	**1.4 mg/L**					
Symptoms	**Numbers of fish / Observation time**					
	3h	**6h**	**24h**	**48h**	**72h**	**96h**
Swimming problems	0	0	0	0	0	0
Localisation near to the surface of the water	0	0	0	0	0	0
Localisation near the bottom of the aquarium	0	0	0	0	0	0
Cumulative mortality	0	0	0	0	0	0

Table 10

Nominal concentration:	**2.4 mg/L**					
Symptoms	**Numbers of fish / Observation time**					
	3h	**6h**	**24h**	**48h**	**72h**	**96h**
Swimming problems	0	0	0	0	0	0
Localisation near to the surface of the water	0	0	0	0	0	0
Localisation near the bottom of the aquarium	0	0	0	0	0	0
Cumulative mortality	0	0	0	0	0	0

Table 11

Nominal concentration:	4.1 mg/L					
Symptoms	**Numbers of fish / Observation time**					
	3h	**6h**	**24h**	**48h**	**72h**	**96h**
Swimming problems	0	0	0	3	1	-
Localisation near to the surface of the water	0	0	0	1	0	-
Localisation near the bottom of the aquarium	0	0	0	1	0	-
Cumulative mortality	0	0	0	0	2	7

Table 12

Nominal concentration:	6.9 mg/L					
Symptoms	**Numbers of fish / Observation time**					
	3h	**6h**	**24h**	**48h**	**72h**	**96h**
Swimming problems	0	0	0	1	-	-
Localisation near to the surface of the water	0	0	0	1	-	-
Localisation near the bottom of the aquarium	0	0	0	0	-	-
Cumulative mortality	0	0	0	6	7	-

Table 13

Nominal concentration:	11.8 mg/L					
Symptoms	**Numbers of fish / Observation time**					
	3h	**6h**	**24h**	**48h**	**72h**	**96h**
Swimming problems	0	0	3	-	-	-
Localisation near to the surface of the water	0	0	0	-	-	-
Localisation near the bottom of the aquarium	0	0	0	-	-	-
Cumulative mortality	0	0	0	7	-	-

Table 14

Nominal concentration:	20 mg/L					
Symptoms	**Numbers of fish / Observation time**					
	3h	**6h**	**24h**	**48h**	**72h**	**96h**
Swimming problems	0	0	-	-	-	-
Localisation near to the surface of the water	0	0	-	-	-	-
Localisation near the bottom of the aquarium	0	0	-	-	-	-
Cumulative mortality	0	0	7	-	-	-

APPENDIX 3

GRAPHICAL PRESENTATION OF MORTALITY

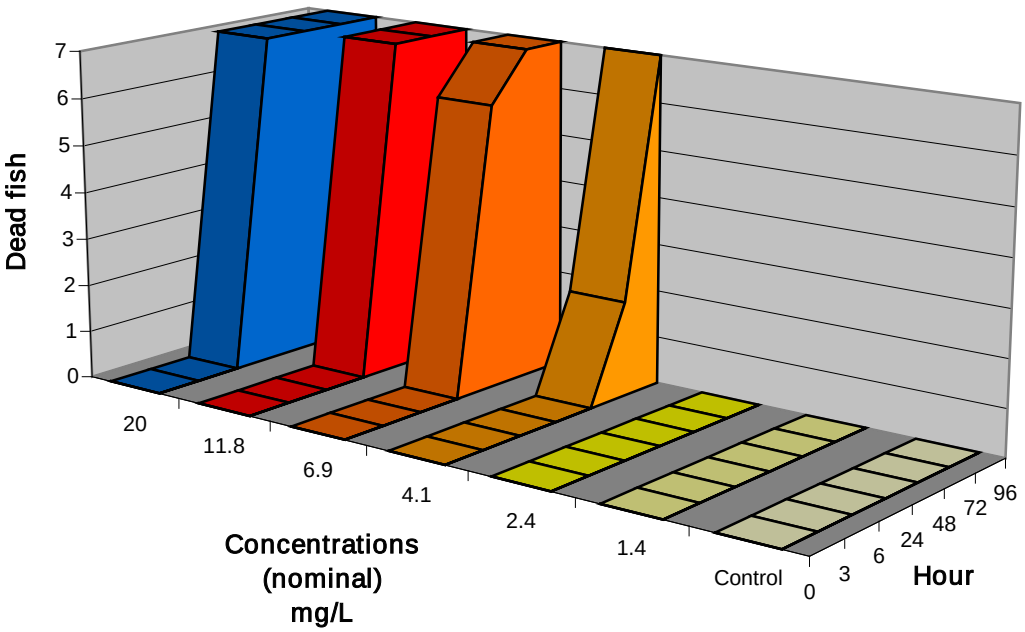

Figure 1: Cumulative mortality in each group

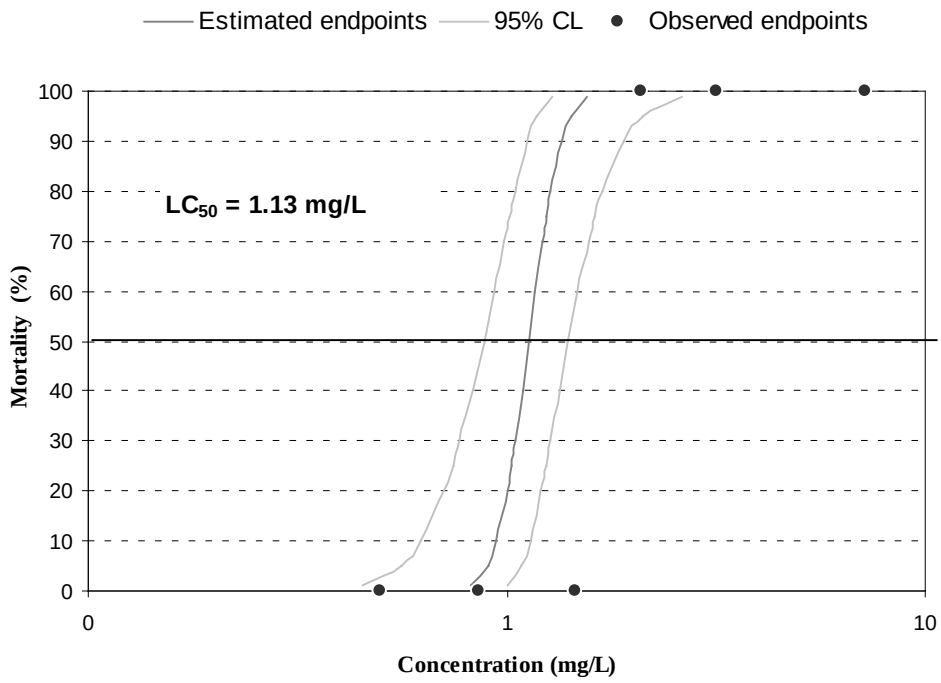

Figure 2: The Concentration / Response curve (96h)

APPENDIX 4

ANALYTICAL INVESTIGATIONS OF MAIN STUDY (METHODS AND RESULTS)

1. Principle of the Analytical Method

Concentration of Ethylene Dibromide Industrial (EDB) in the test solutions was determined at the beginning and at the end of the study.

Three samples were taken from the test solutions and one sample was taken from the control solution.

The samples were analysed by an HPLC-UV method.

2. Equipment and Chemicals

2.1. Apparatus

HPLC system:	Merck-Hitachi LaChrom HPLC system: D-7000 Interface, No.: 1442-122 L-7100 HPLC pump, No. : 1516-030 L-7200 Autosampler, No.: 1406-005 L-7400 UV Detector, No.: 1502-017 L-7360 Column Oven, No.: 00107295 L 7614 Degasser, No.: 14412YA0500
Balance:	BP221S Sartorius, No.: 11809117
Ultrasonic bath:	Elmasonic S300H, ELMA, No.: 010890105
Water purification system:	MILLIPORE, DIRECT Q3, FOMNO 7334I
Refrigerator:	Zanussi, No.: ZLKI-262

2.2. Materials

Name:	Ethylene Dibromide Industrial (EDB)
Chemical name:	1,2-Dibromoethane
Batch No.:	510100003
Active component:	>99.94 % 1,2-Dibromoethane (CAS 106-93-4)
Description:	clear to amber liquid
Manufacture date:	February 2010
Expiry date:	February 2011
Storage:	room temperature, protected from light
Safety Precautions:	see Safety Data Sheet

Manufacturer: Chemtura Manufacturing UK Limited

Address: Tenax Road, Trafford Park
Manchester
United Kingdom
M17 1WT

Other materials:

Ultra pure water (ASTM Type I): prepared by Direct-Q 3 system, Millipore

Acetonitrile: HPLC grade, Fisher Chemical Batch: 0959537

3. Method for the analysis of Ethylene Dibromide Industrial (EDB) content

Samples were analysed by the HPLC-UV method detailed below.

Column:	ACE 5 C18 150 x 4.6 mm, 5µm No.: A78425
Mobil Phase:	ACN : water = 1:1
Flow:	1.0 ml/min.
Injection volume:	20 µl
Temperature:	25 °C
Detector:	UV at 207 nm
Retention time:	5.5 min ± 0.5 min

4. Results of the Method Validation

Table 1: Method validation results (Study code: 10/112-316AN)

Selectivity	No interfering component was observed
Reinjection repeatability (9 injections)	CV% < 2 %
Linear range	0.5 – 50 µg/ml
Limit of Detection	0.2 µg/ml
Limit of Quantification	0.5 µg/ml
Recovery (2 and 100 mg/l)	78 and 87 %
Accuracy	13 and 22 %
Precision	2.5 and 3.2 %
Stability of the samples	At least 51 hours in the autosampler
Stock solution stability	At least 14 days at 5 ± 3 °C
Stability of the test item in Fish Test Medium at room temperature (2 and 100 mg/l)	86 and 95 % after four days

5. Measured data

5.1. Calibration

Table 2: Data of the regression lines at the start and the end of the study

Date of measurement	Analytical occasion	Constant	X Coefficient	R. Squared
09 August 2010	Start of the study	-135	3956	0.9999
13 August 2010	End of the study	645	3442	0.9990

5.2. Results of the Analysis

Table 3: Measured concentrations with the 95% confidence intervals

Sample code	Measured concentrations at the start		Measured concentrations at the end	
	mg/L	Percentage of the nominal	mg/L	Percentage of the nominal
Control	Not detected	-	Not detected	-
1.4 mg/L	0.739±0.07	53	BQL	-
2.4 mg/L	1.05 ± 0.13	44	BQL	-
4.1 mg/L	1.97 ± 0.07	48	BQL	-
6.9 mg/L	2.97 ± 0.55	43	1.47 ± 0.88	21
11.8 mg/L	5.60 ± 0.32	47	1.81 ± 1.35	15
20 mg/L	9.50 ± 0.08	47	5.40 ± 3.59	27

Analyst

04 Oct. 2010

Date

APPENDIX 5

COPY OF THE GLP CERTIFICATE

ORSZÁGOS GYÓGYSZERÉSZETI INTÉZET
National Institute of Pharmacy

H-1051 Budapest, Zrinyi u. 3.
Mail: 1372 P.O. Box 450.
Phone: +36 1 8869-300
Fax: +36 1 8869-460
E-mail: ogyi@ogyi.hu

Budapest, 20th December 2008
No: 38625/48/2007
Our ref.: Szilvia Karsai
Subject: GLP Certificate

GOOD LABORATORY PRACTICE (GLP) CERTIFICATE

Based on the Inspection report and the discussion of follow up activities it is hereby certified that the test facility

LAB Research Ltd.
H-8201 Veszprém, Szabadságpuszta, Hungary

is able to carry out **Physical-chemical testing, Toxicity studies, Mutagenicity studies, Environmental toxicity studies on aquatic and terrestrial organisms, Studies on behaviour in water, soil and air; bioaccumulation, Bioanalytical, Analytical and clinical chemistry testing** compliance with the Principles of GLP (Good Laboratory Practice).

Date of the inspection: **13-22 October 2008.**

This GLP Certificate is valid for 2 years.

Zsuzsanna Szepezdi, Ph. D.
Director-General