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FINAL REPORT

ACUTE TOXICITY STUDY OF ISOPROTURON 50 SC IN EUROPEAN WELS

1994

DECLARATION OF THE STUDY DIRECTOR

This study was performed in accordance with the Protocol agreed upon by Sponsor, the O E C D Guideline for Testing of Chemicals, No.: 203 and the Principles of Good Laboratory Practice (O E C D, Paris, 1982).

I declare that this report constitutes a true record of the actions undertaken and the results obtained in this study.

Signature: _____ Date: 18 Oct. 1994

Dr. Béla Oláh
Study Director

According to the research and development Assignment between UNITED NATION INDUSTRIAL DEVELOPMENT ORGANISATION and TOXICOLOGICAL RESEARCH CENTRE Ltd. Acute Toxicity Study of ISOPROTURON 50 SC Test Substance on European wels has been performed.

To the best of our knowledge and belief this study was carried out in accordance to the conditions of the contract between UNITED NATION INDUSTRIAL DEVELOPMENT ORGANISATION (as Sponsor) and TOXICOLOGICAL RESEARCH CENTRE Ltd. (as Testing Facility) insisting on the GLP requirements lain down in "GOOD LABORATORY PRACTICE IN THE TESTING OF CHEMICALS" (OECD Paris 1982).

So far as can be reasonably established the methods described and the results given in this report accurately reflect the data produced during the study.

The final report has been checked up with raw data and confirmed as being in conformity GLP guidelines.

Signature: _____ Date: 28 Oct 1994

Dr. Erzsébet Béres
Director of Toxicology

QUALITY ASSURANCE STATEMENT

Study Code: 92/126-009H

Subject title: Acute Toxicity Study of ISOPROTURON 50 SC on European wels

Test substance: ISOPROTURON 50 SC

In compliance with the Principles of Good Laboratory Practice this study has been inspected, and this report audited by the Quality Assurance Unit. As far as 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 writing to the study director and to management. The dates of such inspections and of the report audit are given below:

Date inspections/audit	Date of report to Management and	Study Director
28 July 1994	28 July 1994	28 July 1994
01 Aug. 1994	03 Aug. 1994	01 Aug. 1994
21 Oct. 1994	21 Oct. 1994	21 Oct. 1994
28 Oct. 1994	28 Oct. 1994	28 Oct. 1994

Signature: A. Hámori Date: 28 Oct. 1994

Mrs. Andrea Hámori
/Head of QA/

STUDY TITLE	:	**ACUT TOXICITY STUDY OF ISOPROTURON 50 SC IN EUROPEAN WELS**
SPONSOR	:	**THE UNITED NATION INDUSTRIAL DEVELOPMENT ORGANIZATION** **Address: A-1400 Vienna P.O.Box 300** **Fax : 222 2308272**
STUDY PERFORMED BY	:	**TOXICOLOGICAL RESEARCH CENTRE Ltd.** **Address: H-8201 Veszprém, Szabadságpuszta P.O.B. 348. HUNGARY** **Phone : (88) 421-629, 421-711, 421-850** **Fax : (88) 429-237**
STUDY DIRECTOR	:	Dr Béla Oláh biologist and toxicologist
QUALITY ASSURANCE	:	Mrs. Andrea Hámori
TECHNICAL ASSISTANTS	:	Mrs. Krisztina Szabó Mrs. Erzsébet Napkori Mrs. Anita Forintos
PERFORMING LABORATORY	:	Hydrobiology Laboratory H-2441 Százhalombatta Vörösmarty u. 66 P.O.B.:47. HUNGARY
EXPERIMENT LEADER	:	Dr Bethen Pénzes biologist
START OF EXAMINATION	:	18. July 1994
END OF EXAMINATION	:	05. Aug. 1994
BASIS OF STUDY	:	OECD GUIDELINES FOR TESTING OF CHEMICALS (1981 and continuing series) OECD: Paris
OECD NUMBER OF STUDY	:	203

CONTENTS

1. SUMMARY

The LC_{50} value of the ISOPROTURON 50 SC test substance in acute toxicity study in European wels:

The 96h LC_{50} value:		31.24	mg/l
95% confidence limits (lower-upper):	28.89 -	34.32	mg/l

All signs of reaction and mortalities were recorded during the 96 hours /3 h, 6h, 24h, 48h, 72h, 96h/ observation period.
The maximum concentration causing no mortality within the period of this test : 21.0 mg/litre.

2. INTRODUCTION

Instead of at 19. Apr. 1993 performed study (wich was repeat, because in this study the election of concentrations were incorrect), we performed the new study at 18. July 1994. Because the supply of experimental animals is seasonal, we cannot start of study in an earlier time. The results of this new study show hereafter.
The objective of this study was to determine the LC_{50} value of the ISOPROTURON 50 SC test substance in acute toxicity test in European wels with static testing.

3. MATERIALS AND METHODS

3.1 TEST SUBSTANCE

3.1.1	Test Substance:	: ISOPROTURON 50 SC
	Active ingredient	: 51.0 w/v %
	Main effects	: herbicide
	Storage	: at room temperature
	Appearance	: white suspension
	Dilution	: The testing solution of test substance was maded directly, by puting of test substance, into the water of test aquarium (25 litre). The quantity of test substance for a test aquarium: 375, 450, 525, 625, 750 and 875 mg, by the planned concentration of test substance.

Analytical certificate	:	Responsibility of Sponsor
Batch No.	:	not supplied by the Sponsor

The test substance was administered in form suspension.

3.2 EXPERIMENTAL ANIMALS

Species	:	European wels (Silurus glanis)
Source	:	WARMWATER FISH HATCHERY Ltd. (Temperáltvizü Halszaporitó Gazdaság TEHAG Kft.) H-2440 Százhalombatta Vörösmarty út 68. HUNGARY
Justification of the strain	:	The European wels (Silurus glanis L.) is one of the convenience species for Acute Toxicity Study. They have a frequent occurence in the living waters.
Number of animals	:	There were 10 animals in each concentrations
Age of animals	:	one summer old
Acclimation time	:	16 days

3.3 TEST CONDITIONS

3.3.1. Dilution water

Cleaned and filtered Danube water (at Százhalombatta) 25 litre /testing tank.
It was aerated before the addition of the test substance (test substance was solved in the water of test aquarium)

Parameters of cleaned and filtered Danube water:
(date of analysis: 08:00. 1.Aug.94)

temperature	:	21.0	°C
pH	:	7.8	
solved oxygen	:	8.1	mg/l
oxygen saturation	:	93.3	%
total hardness	:	160.2	mg/l $CaCO_3$
phosphate	:	0.14	mg/l
nitrite	:	0.0	mg/l
nitrate	:	12.3	mg/l

ammonium	:	0.08	mg/l
ammonia	:	0.0	mg/l
sulphide	:	0.0	mg/l
hydrogensulphide	:	0.0	mg/l
oxygen requiement (KOI)	:	2.6	mg/l
conductivity	:	460.0	uS/cm

These circumstances were optimal for the test animals.

3.3.2 Testing liquid in the aquariums

Dissolved oxygen Concentration: It was checked at the beginning of the test and at each 24 hours in the control and a test aquarium with highest concent ration tested.
The range of dissolved oxygen concentration values:

6.20 - 8.00 mg/l

Oxygen saturation: It was checked at the beginning of the test and at each 24 hours in the control and a test aquarium with highest concentration tested.
The range of oxygen saturation values:

77.00 - 99.50 %

pH values: It was checked at the beginning of the test and at each 24 hours in the control and a test aquarium with highest concentration tested.
The range of pH values:

7.92 - 8.25

Global hardness: It was checked at the beginning of the test and at each 24 hours in the control and a test aquarium with highest concentration tested.
The range of Global hardness values:

160.2 - 142.4 $CaCO_3$ mg/l

Test temperature: It was checked at the beginning of the test and at each 24 hours in the control and a test aquarium with highest concentration tested.
The range of temperature values:

24.7 - 25.6 °C

Lighting periods: 15 hours daily
about 5.00 a.m. to 8.00 p.m.

Food and feeding: Before the test with mixed TAGGER FOOD and Tubifex (Tubifex rivolorum).
The TAGGER FOOD was produced by TAGGER Ltd. 8020 Graz, P.O.B.854 AUSTRIA.
The Tubifex was supplied by Zoltán Kutsera, Százhalombatta Irinyi u.15. HUNGARY.

Feeding frequency: Daily until 24 hours before the test was started. During the test, the animals were not fed.

Test tanks: Gummed glass-aquarium, volume 25 litre, (25 litre of testing solution, for 10 test animals)

3.4 ADMINISTRATION OF THE TEST SUBSTANCE

The dose levels used and results of testing in the range finding study:

	1.range finding study					2.range finding study		
dose level of test substance (nom.conc.) mg/l	4.00	6.00	9.20	14.00	22.00	24.00	31.00	40.00
Number of animals	3	3	3	3	3	3	3	3
Died animals	0	0	0	0	0	3	3	3

Dosage

The choice of the doses was made on the basis of the preliminary dose-range finding study.

The following dose levels were used in the main study:

dose level of test substance (mg/l) : 15.00 18.00 21.00 25.00 30.00 35.00
Control of the test : - control without test substance

The suspension of the test substance was made freshly and the fishes were treated during the morning hours (9,30 a.m.).

3.5 OBSERVATIONS

The observations of the fishes was made in the following intervals:
3, 6, 24, 48, 72 and 96 hours.

The number of dead animals and the percentage of mortality was determined once in each 24 hour.
The test conditions (pH, temperature, dissolved oxygen, oxygen saturation, global hardness) were checked at the beginning of the test and once in each 24 hour.
After the test all fishes was eradicated.

3.6 BODY WEIGHT

The body weight was recorded on the day 0 (the beginning of the main study).
Analytical ballance was used the weight of fishes. The weight of a water filled glass was determined firstly. Then 5 fishes were placed intc this glass. The gross weight of fishes was registered. After it, the nett weight of fishes was calculated.
On the basis of the above, the loading of the testing solution in the test-aquariums was established (g fish/litre testing solution).

3.7 STATISTICS

Statistical analysis was performed for the calculation of LC_{50} at 96 hours, by probit analysis, by SPSS PC+ software with 95 % confidence limits.

3.8 ARCHIVES

- study plan and any amendments
- all raw data
- sample of test substance
- study report and any amendment
- correspc .dence

are stored for five years in the archives of TRC Ltd. Hungary 8201 Veszprém, Szabadságpuszta P.O.B. 348 according to the OECD GLP and to the TRC's GLP regulation.

4. RESULTS

4.1 TEST CONDITIONS

4.1.1 The data of test conditions checked at the beginning of the test and once in each 24 hour, in the control (without test substance) aquarium.

	measuring (hours)				
	0	24	48	72	96
pH	7.92	8.21	7.95	8.10	8.25
temperature (°C)	24.70	25.50	25.30	25.60	25.60
solved oxygen (mg/l)	7.80	7.40	7.20	8.00	7.30
hardness (mg/l $CaCO_3$)	160.2	160.2	160.2	160.2	160.2
solved oxygen saturation (%)	95.70	91.90	89.20	99.50	90.80

4.1.2 The data of test conditions checked at the beginning of the test and once in each 24 hour, in the test aquarium with highest dosage level.

	measuring (hours)				
	0	24	48	72	96
pH	8.13	8.19	8.20	8.25	8.23
temperature (°C)	24.70	25.50	25.30	25.60	25.60
solved oxygen (mg/l)	7.90	6.20	7.00	7.90	7.50
hardness (mg/l $CaCO_3$)	142.4	142.4	160.2	160.2	160.2
solved oxygen saturation (%)	96.90	77.00	86.70	98.30	93.30

The dissolved oxygen concentration at the end of the test was >60 per cent of the air saturation value at the temperature used.

4.2 THE DOSE LEVELS USED AND NUMBER OF ANIMALS IN THE MAIN STUDY

dose level of test subst. (nom.conc.) mg/l	: 0	15.00	18.00	21.00	25.00	30.00	35.00
Number of animals/conc.	: 10	10	10	10	10	10	10

4.3 MORTALITIES

dose level (mg/l)	n	mortality rate 3h	6h	24h	48h	72h	96h
0.00	10	00/10	00/10	00/10	00/10	00/10	00/10
15.00	10	00/10	00/10	00/10	00/10	00/10	00/10
18.00	10	00/10	00/10	00/10	00/10	00/10	00/10
21.00	10	00/10	00/10	00/10	00/10	00/10	00/10
25.00	10	00/10	00/10	00/10	00/10	00/10	01/10
30.00	10	00/10	00/10	02/10	02/10	02/10	02/10
35.00	10	00/10	00/10	07/10	09/10	09/10	09/10

See the chart: "Changing of number of dead animals plotted against the concentrations" on page 1 of Appendix 1.
There was no mortality in the controls.

4.4 OBSERVATIONS

The movement the vital process of the fishes were observed.
By dosage 15.00 - 21.00 mg/l was not extraordinary behaviour or mortality by the fishes. But at the dose level of 30.00 mg/l, after 24 hours the survivier fishes condition was bad, with unbalance, this effect rise by the dose level of 35.00 mg/l, afteer 3 hours the survivier fishes condition was bad, with unbalance.

4.5 BODY WEIGHT

dose level of test subst. mg/l	measured weight of 10 fishes g	dose level of test subst. mg/l	measured weight of 10 fishes g
0.00 (C)	23.69 (0.95)*	25.00	22.68 (0.91)*
15.00	21.69 (0.87)*	30.00	23.76 (0.95)*
18.00	21.53 (0.86)*	35.00	22.00 (0.88)*
21.00	25.65 (1.03)*		

( )* : loading of testing aquarium (g fish/litre) testing solution

On the day of beginning of experiment (7:50 a.m. 1 aug.1994), the average weight of 10 fishes was 23.00 g/25 litre testing solution (average loading for one aquarium = 0.92 g fish/litre testing solution).

4.6 STATISTICAL ANALYSIS

Statistical analysis was performed, with the used concentrations, for the calculation of LC_{50} by probit analysis, by SPSS PC+ software.

The results of probit analysis are in page 2 of Appendix 1.

Under our experimental conditions:

The LC_{50} value of ISOPROTURON 50 SC test substance, by Acute toxicity study in European wels:

The 96h LC_{50} value: 31.24 mg/l
95% confidence limits (lower- upper): 28.89 - 34.32 mg/l

See the chart: "Changing of number of dead animals plotted against the concentrations" on page 1 of Appendix 1.

5. DISCUSSION

The LC50 value of the ISOPROTURON 50 SC test substance in acute toxicity study in European wels:

The 96h LC_{50} value:		31.24	mg/l
95% confidence limits (lower- upper):	28.89 -	34.32	mg/l

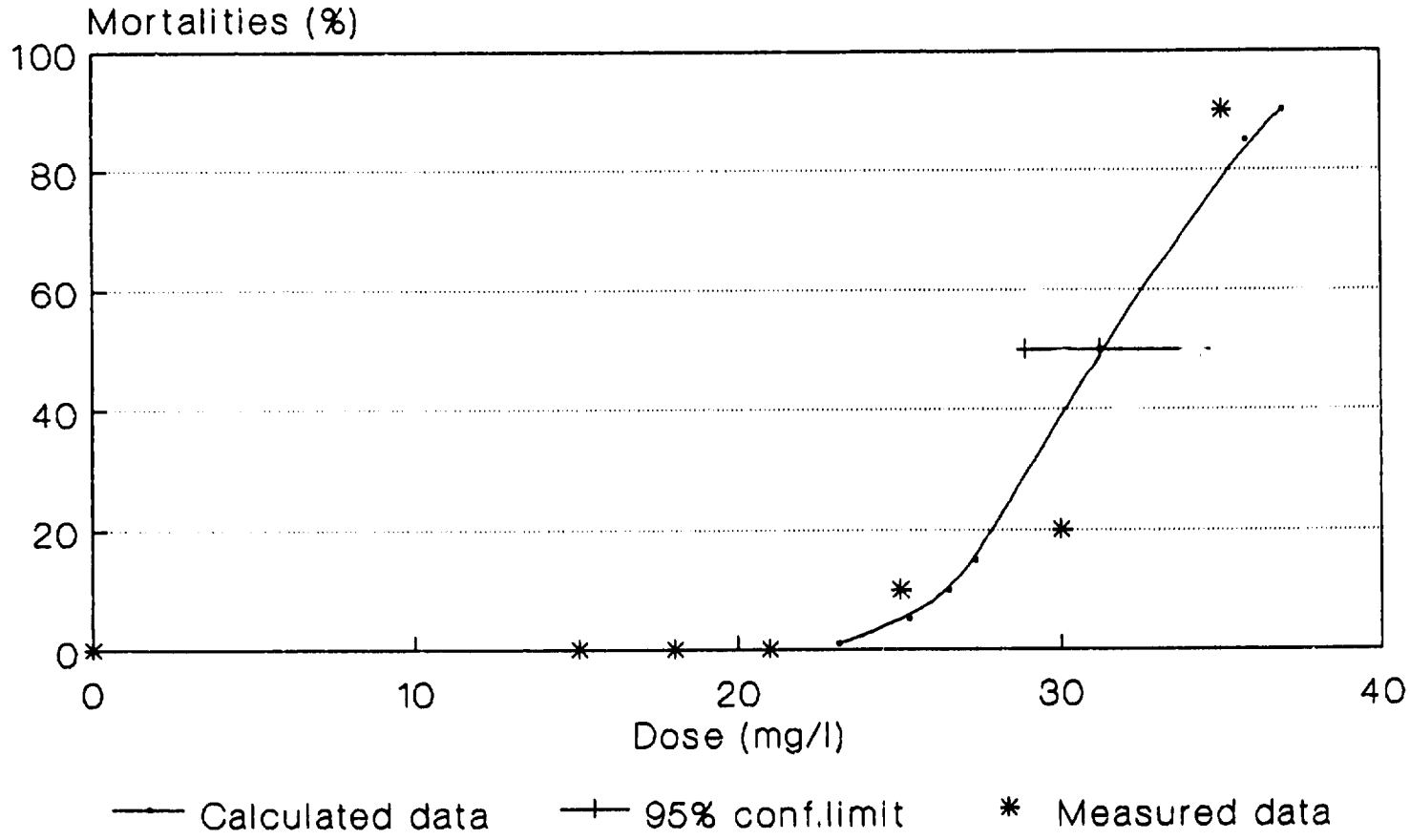
The maximum concentration causing no mortality within the period of this test : 21.0 mg/litre.
The minimum concentration causing 100 per cent mortality within the period of the test >35 mg/l (it was no pointed because one animal was survivor in the highest concentration).

A P P E N D I C E S

CONTENTS

DOSE - MORTALITY RESPONSE

Europen wels (silurus glanis L.)



STUDY No.: 92/126-009H

STUDY No. : 92/126-009N

TEST SUBSTANCE : ISOPROTURON 50 SC

TEST SYSTEM : European wels (Silurus glanis L.)

DATA SHEET FOR PROBIT ANALYSIS

GROUPS	DOSE (mg/l)	No. of ANIMALS TREATED	ANIMAL DIED	
			No.	PERCENT
0	0.00	10	0	0
1	15.00	10	0	0
2	18.00	10	0	0
3	21.00	10	0	0
4	25.00	10	1	10
5	30.00	10	2	20
6	35.00	10	9	90

Mortalities %	LC (mg/l)	95% Confidence Limits	
		Lower	Upper
1	23.1348	15.7305	26.0157
5	25.2627	19.1575	27.6841
10	26.4760	21.2308	28.6831
15	27.3273	22.7148	29.4292
50	31.2402	28.8914	34.3205
85	35.7133	32.9516	44.6352
90	36.8617	33.7554	47.8308

PROBIT = A + B*LOG C

A = -21.65613

B = 17.83361 ± 5.15664

CHI' = 2.737

P = 0.603

Since Goodnes-of-Fit Chi square is NOT significant, no heterogeneity factor is used in the calculation of confidence limits