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RESEARCH TOXICOLOGY CENTRE S.p.A.

[redacted]

ACUTE TOXICITY STUDY IN Brachydanio rerio

SUMMARY REPORT

RTC Enquiry No.: 4923/1

Seen and approved by:

A. Nunziata
Responsible for Toxicological
Experimentation as authorized
by the Italian Ministry of Health

A. Marzoli
President

25

RTC Enquiry No.: 4923/1

COMPLIANCE STATEMENT

We, the undersigned, hereby declare that the following report is a true and faithful account of the procedures adopted and the results obtained in the performance of the study. The aspects of this study conducted by Research Toxicology Centre S.p.A. were performed in accordance with:

A. *"Good Laboratory Practice Standard"* of the U.S. Environmental Protection Agency, Code of Federal Regulations, 40, Part 160, U.S. Federal Register, Vol. 54, No. 158, 17th August 1989.

B. Decreto Legislativo 27 Gennaio 1992 n. 120 published in the Gazzetta Ufficiale della Repubblica Italiana 18 Febbraio 1992.

(Adoption of the Commission Directive of 18 December 1989 adapting to technical progress the Annex to Council Directive 88/320/EEC on the inspection and verification of Good Laboratory Practice (90/18/EEC)).

Daniela Gallo

D. Gallo, Chem. Pharm. Tech.D., Pharm.D.
(Study Director): Date : 18.12.95

J. Brightwell

J. Brightwell, Ph.D.
(Scientific Director): Date : 18.12.95

Contents

Table

1. OBJECTIVE

To assess the acute toxicity to fish of the test substance, [redacted] following exposure in water over a period of 96 hours. This allowed an indication of the hazard classification required by the classification, packaging and labelling of dangerous substances regulations.

2. STUDY DESIGN

The procedures used, summarised below, were designed to meet the requirement of the limit test for acute toxicity to fish using a semi-static system described in the Organisation for Economic Co-operation and Development (OECD) guidelines for the testing of chemicals No. 203, adopted on 17th July 1992. These methods are recognised by Commission Directive 92/69/EEC of 31st July 1992 (adapting to technical progress for the seventeenth time, Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances).

Brachydanio rerio 2.5 - 3.5 cm long were obtained from Euraquarium, Bologna, Italy on 15th September 1995. This batch was considered acceptable following the required acclimatization period.

Fish were fasted 24 hours before dosing commenced.

The study was performed using a single dose-level of the test substance at a nominal concentration of 100 mg/l in water.

The test substance (batch No. D6, purity 99%) was dissolved in, and thoroughly mixed with, the vehicle (Tween 80 at 10 % in distilled water) to give a stock solution. An appropriate volume of this solution was then added into a glass tank containing 7 litres of water drawn from the stock tanks (final concentration of 100 mg/l in the water).

Three groups each of seven fish were used. One group was exposed to the substance. A second group was designated a comparator control and exposed to the vehicle alone. The third group served as an untreated control.

Fish were exposed to the test substance for a period of 96 hours. A semi-static system was employed, changing the water at 48 hour intervals.

Fish were not fed during the test.

The water in the test tanks was aerated from 24 hours after the beginning of treatment up to the end of the study.

All tanks were inspected approximately 1, 2 and 4 hours after first dosing and at 24 hour intervals thereafter for a total of 96 hours. Water in the tanks was monitored for oxygen content, temperature and pH before the start of the test and daily thereafter (the exhausted water immediately prior to being changed and the fresh water to replenish the tanks). Effects on the behaviour of the fish were assessed at the same intervals.

The raw data and documentation generated during the course of this study will be retained at RTC for a period of least five years after which the Sponsor will be contacted for instructions regarding the despatch or disposal of the material .

3. RESULTS

No mortality occurred and no clinical signs or behavioural changes were observed in the fish. These negative data have not been reported.

Dissolved oxygen, temperature and pH were within accepted limits for the study (see Table 1).

4. CONCLUSIONS

The results of this study suggest that [redacted] has no adverse or toxic effect on Brachydanio rerio during an acute exposure of 96 hours at a concentration of 100 mg/l.

Evaluation of acute toxicity to fish according to Council Directive 93/21/EEC on classification, packaging and labelling of dangerous substances indicates that classification may not be required.

The study was carried out at:

Research Toxicology Centre S.p.A.
Via Tito Speri, 12
00040 Pomezia (Roma)
Italy

The study was carried out on behalf of:

Ausimont S.p.A.
Viale Lombardia, 20
20021 Bollate (Milano)
Italy

[redacted] : ACUTE TOXICITY STUDY IN Brachydanio rerio

TABLE 1 - Water quality assessment

STUDY NO.: 4923/1

UNTREATED CONTROL

	TIME (hours)					
PARAMETER	0	24	48 Exhausted	48 Fresh	72	96
Dissolved Oxygen (%)	96	98	99	100	79	82
Temperature (°C)	23	22	22	23	22	23
pH	8.3	8.2	7.8	8.3	8.2	7.8

VEHICLE CONTROL

	TIME (hours)					
PARAMETER	0	24	48 Exhausted	48 Fresh	72	96
Dissolved Oxygen (%)	97	60	99	99	74	87
Temperature (°C)	23	22	22	23	22	23
pH	8.3	7.6	8.0	8.2	8.2	8.1

TEST SUBSTANCE

	TIME (hours)					
PARAMETER	0	24	48 Exhausted	48 Fresh	72	96
Dissolved Oxygen (%)	99	61	99	100	80	88
Temperature (°C)	23	22	22	23	22	23
pH	7.5	7.5	7.7	7.5	8.0	8.1

0 = Before dosing
Fresh = Freshly formulated water
Exhausted = Exhausted water after a 48 hour period of use

30

[redacted]

ACUTE TOXICITY STUDY IN Daphnia magna

SUMMARY REPORT

RTC Enquiry No.: 4924/1

Seen and approved by:

A. Nunziata
Responsible for Toxicological
Experimentation as authorized
by the Italian Ministry of Health

A. Marzoli
President

Sede: Via Tito Speri, 12 - 00040 POMEZIA (Roma) - ITALIA Telefono: + 39.6.910951 - Fax: 06/9105737 - C.P. 15301 - 00143 Roma Eur Laurentino
Ufficio Milano: Via Cania, 26 - 20132 MILANO - ITALIA - Tel.: +39.2.26144686 - Fax: +39.2.26144778
Ufficio Parigi: Rue du Pont des Halles, 21 - Delta 109 - 94536 RUNGIS CEDEX (PARIS) - FRANCE - Tel.: +33.1.45609725 - Fax: +33.1.46879431
Capitale Sociale: 10.000.000.000 - C.C.I.A.A. n° 375376 - Reg. Soc. Trib. di Roma n° 2828/72 - Cod. Fisc.: 00653120584 - Partita IVA: 00920611001
Autoriz. G.L.P. : DL 120 del 27/1/92 - D.M. ex Art. 6 del D.P.R. n° 927/81 del 20/5/89

RTC Enquiry No.: 4924/1

COMPLIANCE STATEMENT

We, the undersigned, hereby declare that the following report is a true and faithful account of the procedures adopted and the results obtained in the performance of the study. The aspects of this study conducted by Research Toxicology Centre S.p.A. were performed in accordance with:

A. *"Good Laboratory Practice Standard"* of the U.S. Environmental Protection Agency, Code of Federal Regulations, 40, Part 160, U.S. Federal Register, Vol. 54, No. 158, 17th August 1989.

B. Decreto Legislativo 27 Gennaio 1992 n. 120 published in the Gazzetta Ufficiale della Repubblica Italiana 18 Febbraio 1992.

(Adoption of the Commission Directive of 18 December 1989 adapting to technical progress the Annex to Council Directive 88/320/EEC on the inspection and verification of Good Laboratory Practice (90/18/EEC)).

P. Ciliutti, Biol.D.
(Study Director): [signature] Date : 17.01.96

J. Brightwell, Ph.D.
(Scientific Director): [signature] Date : 17.01.96

[redacted] : Acute Toxicity Study in Daphnia magna

RTC Study No. : 4924/1

The study was carried out at : Research Toxicology Centre S.p.A.
Via Tito Speri, 12
00040 Pomezia (Roma)
Italy

The study was conducted on behalf of: Ausimont S.p.A.
Viale Lombardia, 20
20021 Bollate (Milano)
Italy

Contents

REDACTED AS TO TRADE NAMES

Page

Tables

1. OBJECTIVE

To assess the acute toxicity to daphnia of the test substance, [redacted] following exposure in water over a period of 48 hours. This allows hazard classification of the test substance to be carried out as required by the classification, packaging and labelling of dangerous substances regulations.

2. STUDY DESIGN

The procedures used, summarised below, were designed to meet the requirement of the limit test for acute toxicity to Daphnia using a static system described in the Organisation for Economic Co-operation and Development (OECD) guidelines for the testing of chemicals No. 202, adopted on 4th April 1984. These methods are recognised by Commission Directive 92/69/EEC of 31st July 1992 (adapting to technical progress for the seventeenth time, Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances).

Daphnids, less than 24 hours old, of normal appearance and viability were obtained from our parthenogenetic colony on 29 November 1995.

The study was performed using a single dose-level of the test substance at a nominal concentration of 100 mg/l in the medium.

The test substance (batch No. D6, purity 99%) was dissolved in, and thoroughly mixed with, the vehicle (Tween 80 at 10 % in distilled water) to give a stock solution. Appropriate volumes of this solution were then added to four glass beakers containing 100 ml Elendt M7 medium (final concentration of 100 mg/l in the medium).

Three groups each of twenty daphnids were used. One group was exposed to the substance. A second group was designated a comparator control and exposed to the vehicle alone. The third group served as an untreated control.

Daphnids were exposed to the test substance for a period of 48 hours. A static system was employed.

Daphnids were not fed during the test.

All beakers were inspected at 24 hour intervals. Medium in the beakers was monitored for oxygen content, temperature and pH at the beginning and at the end of the test.

The raw data and documentation generated during the course of this study will be retained at RTC for a period of least five years after which the Sponsor will be contacted for instructions regarding the despatch or disposal of the material .

3. RESULTS

No mortality occurred in the test substance group or in the control groups.

Dissolved oxygen, temperature and pH were within accepted limits for the study (see Table 1).

4. CONCLUSIONS

The results of this study indicate that [redacted] has no adverse or toxic effect on Daphnia magna during an acute exposure of 48 hours at a concentration of 100 mg/l.

Evaluation of acute toxicity to daphnia according to Council Directive 93/21/EEC on classification, packaging and labelling of dangerous substances indicates that classification may not be required.

The study was carried out at:

Research Toxicology Centre S.p.A.
Via Tito Speri, 12
00040 Pomezia (Roma)
Italy

The study was carried out on behalf of:

Ausimont S.p.A.
Viale Lombardia, 20
20021 Bollate (Milano)
Italy

[redacted] : ACUTE TOXICITY STUDY IN Daphnia magna

TABLE 1 - Medium quality assessment

STUDY NO.: 4924/1

UNTREATED CONTROL

PARAMETER	TIME (hours) 0 Beaker N°. 1A	1B	1C	1D	48 Beaker N°. 1A	1B	1C	1D
Dissolved Oxygen (%)	79.4	81.6	81.4	81.8	95.0	95.0	93.0	94.0
Temperature (°C)	20.1	20.1	20.1	20.1	20.1	20.3	20.7	20.6
pH	8.75	8.79	8.74	8.62	8.28	8.33	8.32	8.33

VEHICLE CONTROL

PARAMETER	TIME (hours) 0 Beaker N°. 2A	2B	2C	2D	48 Beaker N°. 2A	2B	2C	2D
Dissolved Oxygen (%)	82.0	82.1	82.3	82.4	90.0	90.0	90.0	90.0
Temperature (°C)	20.1	20.1	20.1	20.1	20.6	20.8	20.7	20.7
pH	8.31	8.46	8.46	8.47	8.12	8.21	8.26	8.30

TEST SUBSTANCE

PARAMETER	TIME (hours) 0 Beaker N°. 3A	3B	3C	3D	48 Beaker N°. 3A	3B	3C	3D
Dissolved Oxygen (%)	83.0	83.2	82.8	82.0	89.0	90.0	90.0	90.0
Temperature (°C)	20.1	20.1	20.1	20.1	20.6	20.7	20.6	20.7
pH	8.26	8.16	7.96	8.07	8.20	8.18	8.16	8.15

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[redacted]

ALGAL GROWTH INHIBITION TEST
IN Selenastrum capricornutum

SUMMARY REPORT

RTC Study No.: 4925/1

Seen and approved by:

A. Nunziata
Responsible for Toxicological
Experimentation as authorized
by the Italian Ministry of Health

A. Marzoli
President

Sede: Via Tito Speri, 12 - 00040 POMEZIA (Roma) - ITALIA Telefono: + 39.6.910951 - Fax: 06/9105737 - C.P. 15301 - 00143 Roma Eur Laurentino
Ufficio Milano: Via Carnia, 26 - 20132 MILANO - ITALIA - Tel.: +39.2.26144686 - Fax: +39.2.26144778
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Autoriz. G.L.P. : DL 120 del 27/1/92 - D.M. ex Art. 6 del D.P.R. n° 927/81 del 20/5/89

RTC Study No.: 4925/1

COMPLIANCE STATEMENT

We, the undersigned, hereby declare that the following report is a true and faithful account of the procedures adopted and the results obtained in the performance of the study. The aspects of this study conducted by Research Toxicology Centre S.p.A. were performed in accordance with:

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(Adoption of the Commission Directive of 18 December 1989 adapting to technical progress the Annex to Council Directive 88/320/EEC on the inspection and verification of Good Laboratory Practice (90/18/EEC)).

P. Ciliutti, Biol.D.
(Study Director) : Date : 08 Feb 1996

J. Brightwell, Ph.D.
(Scientific Director) : Date : 8.02.96

[redacted] : Algal Growth Inhibition Test in Selenastrum capricornutum

RTC Study No. : 4925/1

The study was carried out at : Research Toxicology Centre S.p.A.
Via Tito Speri, 12
00040 Pomezia (Roma)
Italy

The study was conducted on behalf of: Ausimont S.p.A.
Viale Lombardia, 20
20021 Bollate (Milano)
Italy

Contents

REDACTED AS TO TRADE NAMES

Page

1. OBJECTIVE

To determine the effects of the growth of the unicellular green alga Selenastrum capricornutum following treatment with [redacted] in the medium over a period of 72 hours. This allowed a hazard classification of the test substance to be carried out as required by the classification, packaging and labelling of dangerous substances regulations.

2. STUDY DESIGN

The procedures used, summarised below, were designed to meet the requirement of the limit test for algal inhibition using a static system described in the Organisation for Economic Co-operation and Development (OECD) guidelines for the testing of chemicals No. 201 , adopted on 7th June 1984. These methods are recognised by Commission Directive 92/69/EEC of 31st of July 1992 (adapting to technical progress for the seventeenth time, Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances).

The study was performed using a single dose-level of the test substance at a nominal concentration of 100 mg/l in Gorham's medium.
In addition, a comparator control was exposed to the solvent alone and an additional control remained untreated.

On the day of treatment, subcultures of Selenastrum capricornutum cells at a density of 0.3 x 10^5/ml were prepared in glass conical flasks containing Gorham's medium to give a final volume of 100 ml.
Algal suspensions were obtained from pre-cultures growing in exponential phase.
Three replicate cultures were prepared for each test point.

The test substance (batch no. D6, purity 99%) was dissolved in the solvent (10% Tween 80 in distilled water) to give a stock solution. Appropriate volumes of this solution were then added to each culture containing the test substance.

Cells were exposed to the test substance for a period of 72 hours using a static system.

The cell density of each culture was determined 24, 48 and 72 hours after the beginning of the test. Data are presented in Table 1.

Medium in the flasks was monitored for pH at the beginning and at the end of the exposure period. Data are presented in Table 2.

The raw data and documentation generated during the course of this study will be retained at RTC for a period of at least five years after which the Sponsor will be contacted for instructions regarding the despatch or disposal of the material.

3. RESULTS

A mean reduction of 32.9% over the control value of the cellular growth was observed in the cultures treated with the test substance.

4. CONCLUSIONS

A limit test carried out with the substance [redacted] at the concentration of 100 mg/l resulted in 32.9% inhibition of the cellular growth. A full test may be required to define a precise EC_{50}, but the results of the test do suggest that classification of [redacted] as harmful to aquatic organisms may not be required.

5. TABLES 1 - 2

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TABLE 1.1 - Cell densities and mean values

STUDY NO.: 4925/1

Treatment	Culture code	Cell densities (10^6 cells/ml)	Mean (10^6 cells/ml)
24 HOURS			
Untreated control	1A	0.22	
	1B	0.22	0.23
	1C	0.24	
Solvent control	2A	0.12	
	2B	0.16	0.15
	2C	0.17	
Test substance	3A	0.10	
	3B	0.10	0.12
	3C	0.15	

TABLE 1.2 - Cell densities and mean values

STUDY NO.: 4925/1

Treatment	Culture code	Cell densities (10^6 cells/ml)	Mean (10^6 cells/ml)
48 HOURS			
Untreated control	1A	0.68	
	1B	0.99	0.85
	1C	0.89	
Solvent control	2A	0.62	
	2B	0.54	0.57
	2C	0.54	
Test substance	3A	0.29	
	3B	0.32	0.31
	3C	0.32	

TABLE 1.3 - Cell densities and mean values

STUDY NO.: 4925/1

Treatment	Culture code	Cell densities (10^6 cells/ml)	Mean (10^6 cells/ml)
72 HOURS			
Untreated control	1A	1.48	
	1B	1.64	1.53
	1C	1.47	
Solvent control	2A	1.89	
	2B	1.31	1.54
	2C	1.41	
Test substance	3A	1.14	
	3B	1.31	1.23
	3C	1.24	

TABLE 2 - pH of treatment medium

STUDY NO.: 4925/1

Treatment	Culture code	Beginning of treatment	End of treatment
Untreated control	1A	8.18	10.19
	1B	8.16	10.16
	1C	8.16	10.58
Solvent control	2A	8.04	10.09
	2B	8.10	10.19
	2C	8.12	10.08
Test substance	3A	7.71	10.07
	3B	7.72	10.23
	3C	7.79	10.71