Corresponds to study #18 in Attachment A of transmittal memo on CBI
HERO ID:4731539

Perylimid

Testing the acute toxicity in the fish model
Zebra danio (brachydanio rerio)
over the course of 96 hours

Author

Dipl.-Ing Markert, Dr. Jung

Test end

July 1st, 1988

Testing laboratory

Pharma Research Toxicology and Pathology
Hoechst Corporation
PO box 80 03 20
6230 Frankfurt 80

Test number

88.0389

Statement

This experiment has been carried out in line with the principles of best laboratory practice. There were no unforeseen circumstances that might have affected the quality or integrity of the experiment presented here.

Test director: Dipl.-Ing. Markert

Industrial toxicology: Dr. Jung

GLP Statement

Hoechst Corporation
Pharma research
Unit GLP

July 20, 1988

Title: Perylimid
Testing the acute toxicity in the fish model
Zebra danio (brachydanio rerio)
over the course of 96 hours

Date: July 5, 1988

Test no: 88.0389

This test has been inspected regularly and the written, duly signed documentation has been presented to the directors of the test institutions and the test directors as follows:

Inspection	Report
April 7, 1988	April 7, 1988
June 27, 1988	June 27, 1988
July 19, 1988	July 20, 1988

Pharma Research
Unit GLP

[Illegible]

CONTENTS

1. SUMMARY

In testing the lethal effects of Perylimid on danio (Brachydanio rerio) over the course of 96 hours, according to the method described under item 4, there was no lethality at 5000 mg/l. The LO50 after 48 and 96 hours is therefore above 5000 mg/l.

2. PRELIMINARY REMARKS

The present determination of the acute fish toxicity of Perylimid has been carried out according to the procedure proposed by the "ad hoc working group of the German Federal Environment Agency for the development of ecotoxicological test procedures in aquatic systems" (adopted May 1, 1984)

"Lethal effects on the danio Barchydanio rerio"
(LCO, LC50, LC100, 48 to 96 HOURS)
Recommendation of the German Federal Environment Agency
for testing according to Para 4 Section 1 no. 6
of the regulations for registration documents and
test evidences according the German Chemicals Act
Texts 16/84, German Federal Environment Agency

And fulfils the requirements of the EU guideline

EU Guideline C 1 acute toxicity for fish
Guideline 84/449/EEC
Guideline of the Commission dates April 25, 1984
Concerning the sixth amendment of the guideline 67/548/EEC of the Council
on the Approximation of the Laws and Regulations for Categorization,
Packaging and Identification of Compounds Hazardous to Technical Progress

As well as of the OECD guideline

OECD guideline for testing of chemicals
203 Fish, Acute Toxicity Test,
Adopted: April 4, 1984

3. OVERSIGHT

Test type:	Acute toxicity in fish
Test no :	88.0389
Test compound:	Perylimid
Customer:	GB: D, Dept.: Farben Nord
Fish species:	Zebra danio Brachydanio rerio (HAMILTON-BUCHANAN)
Temperature:	22 ± 1 °C
Test system:	Static
Test duration:	96 hours
Test start:	June 27, 1988
Test end:	July 1, 1998
Test concentration:	0 / 5000 mg/l

R E S P O N S I B L E:

Test director:	Dipl.-Ing. Markert
Industrial toxicology:	Dr. Jung
Unitl GLP:	Ap. Harston
Testing and archiving unit:	Pharma Research Toxicology and Pathology HOECHST CORPORATION PO Box 80 03 20 6230 Frankfurt 80

4. MATERIAL AND METHODOLOGY

4.1 Test compound

Commercial name:	Perylimid
Chemical name:	Perylen-3, 4, 9, 10-tetracarbonaciddiimide
Product no:	EMGW 985
Application type:	Dyestuffs intermediate
Purity grade:	> 95%
Date of sample receipt:	January 25, 1988
Storage:	In a dark place at 20 °C, in fume hood
Appearance:	Purple powder
Mol mass:	390.3 g/mol
Melting point:	400 °C
Density:	> 1 kg/l
Water solubility:	670 mg/l (20 °C)

4.2 Testing organism

Fish species:	Zebra danio Brachydanio rerio (HAMILTON-BUCHANAN)
Origin:	West Aquarium 3422 Bad Lauterberg
Date of hatching:	February 15, 1988
Delivery date:	April 21, 1988

The conditioning took place over a period of at least 14 days prior to test start in diluted water (see item 4.4) under the following conditions:

Temperature:	22 ± 1 °C
Oxygen content:	≥ 80% of the saturation value
Lighting duration:	12 hours daily
Stocking density:	≤ 1 g fish / l water
Feeding:	Twice daily ad libidum
Food:	Tetra Min, Tetra Werke, Melle

Immediately prior to the start of the test, the body length of 10 fish was determined to represent the entire fish batch used. These animals were not used for the experiment.

		Body length in cm			
Batch no	Date	n	Variance	x	s
13c/L2	June 27, 1988	10	2.7 – 3.5	3.1	0.28

4.3 Test groups

Per test group, 10 fish were entered. As a result of orienting preliminary tests, the following concentration was being tested:

Start	Test pool nl.	Concentration (mg/l)
June 26, 1988	33 /11	0 / 5000

4.4 Dilution water

Reconstituted water according to ISO/DIS 7346/1 was used as diluted water.

The preparation occurred in a unit consisting of two steel pool with Hostalen^{R}-lining and a capacity of 1700 l each.

The dilution water prepared in this manner has been aired to the point of oxygen saturation. The pH value of 7.9 to 8.1 was measured prior to test start.

4.5 Test set-up

The test was carried out in a static system. The text containers calibrated to 10 litres consisted of glass (length 30 cm, width 22 cm, height 24 cm) and stood in a water bath made from Hostalit Z^{R} with a Plexiglas viewing window. The temperature of the water bath was thermostat controlled to 22 ± 1 ^{o}C. The pools were illuminated from above in a day-night rhythm of 12 hours each. Directly above the pools, illumination was at approx. 700 lux. The test pools were not aired throughout the duration of the test.

4.6 Creation of the test concentration

The test compound was added to the diluted water, homogenized in an ultrasound batch and an Ultra-Turrax and added to the test pool, while being stirred with a glass rod. After that, the content of the pool was stirred for approx. 2 hours with a KPG stirrer and a glass rod. The test concentration was claret-coloured with suspended particles. The concentration value does therefore not refer to dissolved matter, but to the amount of compound added to the test pool overall.

4.7 Carrying out the test

After preparation of the test concentration and measuring the water parameter, 10 fish were randomly put in each of the test and control pools. For the duration of the test, the fish were not fed. After 2-4, 24, 48 and 96 hours, the fish were checked, and any lethality and visible changes in appearance and behaviours were recorded. At the same time, water parameters were measured and recorded. The measuring methods are listed in the annex (page 13).

5. RESULTS

5.1 Lethality

In the concentration group with 5000mg/l and in the control group, no lethality occurred throughout the test duration.

5.2 Appearance and behaviour

Due to the claret coloration of the test water by the testing compound, the observation of symptoms was limited.

At 5000 mg/l, no changes in appearance and behaviour could be observed in comparison to the control group.

5.3 Water parameters

For the concentration group and the control, the following variations occurred throughout the test duration:

Parameter	Concentration Group	Control
pH value	6.9 – 7.4	7.4 – 7.9
Oxygen content (mg/l)	7.4 – 8.5	7.0 – 9.0
Temperature (°C)	21.1 – 22.9	21.1 – 22.8

The individual values must be taken from the annex (page 14).

DI Mar/KOE

Unit GLP

HOECHST AKTIENGESELLSCHAFT
Pharma Research
Toxicology and Pharmacology

Dipl.-Ing. Markert
Test director

Dr. Jung
Industrial toxicology

6. ANNEX

6.1 Methodology for determining the water parameters

Parameter	Unit	Method
Temperature	°C	Electronic measurement with oxygen measuring device OXI 196(WTW) and oxygen electrode EO 196-1.5 with integrated temperature probe (Wissenschaftlich Technische Werkstätten, Weilheim)
Oxygen content	mg/l	Electronic measurement with oxygen measuring device OXI 196(WTW) and oxygen electrode EO 196-1.5 with integrated temperature probe (Wissenschaftlich Technische Werkstätten, Weilheim)
Ph value	Mg/l	Electronic measurement with ph measuring device ph 763 (Knick) and single rod measuring chain (Schott Geräte GmbH, Hofheim)

6.2 Water parameters – individual values

6.2.1 ph value

Concentration (mg/l)	Test pool no	Test start (date)	Prior to test start	Ph value Hours after test start 0	2 – 4	24	48	72	96
0	33	6/27/88	7.9	7.9	7.8	7.7	7.5	7.4	7.4
5000	11	27/6/88	8.1	7.4	7.4	7.2	7.0	6.9	7.3

6.2.2 Oxygen content

Concentration (mg/l)	Test pool no	Test start (date)	Prior to test start	Oxygen content (mg/l) Hours after test start 0	2 – 4	24	48	72	96
0	33	6/27/88	9.3	9.0	8.5	7.5	7.5	7.0	7.1
5000	11	27/6/88	9.2	8.5	8.1	7.7	7.9	7.4	7.4
Oxygen saturation (100%) at 22 °C and local air pressure			8.8	8.8	8.8	8.8	8.7	8.7	8.7

6.2.3 Temperature

Concentration (mg/l)	Test pool no	Test start (date)	Prior to test start	Temperature °C Hours after test start 0	2 – 4	24	48	72	96
0	33	6/27/88	21.9	22.8	22.2	21.1	22.8	22.2	22.5
5000	11	27/6/88	22.0	22.9	22.2	21.1	21.6	21.7	21.7