

2/6/2001

DATA EVALUATION RECORD
§ 72-1 -- ACUTE LC₅₀ TEST WITH A COLDWATER FISH

1. CHEMICAL: Benzyl Benzoate PC Code No.: 009501

2. TEST MATERIAL: Benzyl Benzoate Purity: 99.8%

3. CITATION:

Authors: Memmert, Ulrich

Title: Acute Toxicity of Benzyl Benzoate to Rainbow Trout (*Oncorhynchus mykiss*) in a 96-hour Flow-through Test

Study Completion Date: July 4, 2000

Laboratory: RCC Ltd., Environmental Chemistry & Pharamalytics Division, CH-4452 Itingen, Switzerland

Sponsor: Allergopharma Joachim Ganzer KG, Hermann-Korner-Strasse 52, D-21462 Reinbek, Germany

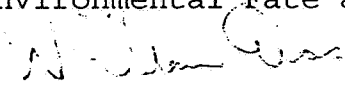
Laboratory Report ID: 752286

MRID No.: 452095-01

DP Barcode: D269516

4. REVIEWED BY: William Evans, Biologist
Ecological Effects Branch
Environmental Fate and Effects Division

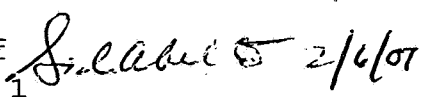
Signature:



Date:

2/7/01

5. APPROVED BY: Sid Abel, Acting Branch Chief
Environmental Review Branch 1
Environmental Fate and Effects Division



Signature:

Date:

6. STUDY PARAMETERS:

Age or Size of Test Organism: 5.5 cm; 1.8 g

Definitive Test Duration: 96 hours

Study Method: Flow-through

Type of Concentrations: Measured

7. CONCLUSIONS: This study significantly deviated from the EPA test protocols and has been classified as **Invalid**. The unacceptable deviations included the use of chlorinated tap water, inconsistent water temperature measurements, and high

water hardness levels.

Since this study is classified as Invalid and does not meet the guideline requirements for an acute freshwater fish toxicity test, toxicity values are not recorded.

Results Synopsis

LC₅₀: N/A

95% C.I.: N/A

NOEC: N/A

Probit Slope: N/A

8. ADEQUACY OF THE STUDY:

- A. **Classification:** Invalid.
- B. **Rationale:** Since this study was conducted using chlorinated tap water. Other significant deviations from the protocol included inconsistent water temperature measurements and high water hardness levels.
- C. **Repairability:** No

9. GUIDELINE DEVIATIONS:

1. The fish were acclimated one week prior to testing. They should have been acclimated for 14 days..
2. Local tap water was mixed with purified water to reduce hardness. Chlorine concentrations ranged from <0.008 to 0.01 mg/L. Chlorinated tap water must not be used for testing.
3. Total Hardness of 40 to 48 mg/L as CaCO₃ is preferred. Measured hardness was 143 mg/L as CaCO₃.
4. The flow rate meter systems should be calibrated before study and checked twice daily during test period. The flow water and dosing system were checked and controlled at least once on working days.
5. Biomass loading rate was not mentioned.
6. Temperature was measured prior to introduction of fish, then daily. Temperature should be measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C.

10. SUBMISSION PURPOSE:11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> Preferred species is the rainbow trout (<i>Oncorhynchus mykiss</i>)	<i>Oncorhynchus mykiss</i>
<u>Mean Weight</u> 0.5-5 g	1.8 ± 0.3 g (range 1.5 - 2.4 g)
<u>Mean Standard Length</u> Longest not > 2x shortest	5.5 ± 0.3 cm (range 5.0 - 6.0 cm)
<u>Supplier</u>	P. Hohler, Trout breeding Station Zeiningen, CH-4314 Zeiningen, Switzerland
All fish from same source?	Yes
All fish from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
<u>Acclimation Period</u> Minimum 14 days	Acclimated for one week prior to testing.
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A

Guideline Criteria	Reported Information
<u>Feeding</u> No feeding during the study	The fish were not fed during the test period.
<u>Pretest Mortality</u> < 3% mortality 48 hours prior to testing	< 1% mortality during the last 5 weeks prior to the test.

C. Test System

Guideline Criteria	Reported Information
<u>Source of dilution water</u> Soft reconstituted water or water from a natural source, not dechlorinated tap water	Local tap water mixed with purified water to reduce hardness. Chlorine concentrations ranged from <0.008 to 0.01 mg/L.
Does water support test animals without observable signs of stress?	Yes
<u>Water Temperature</u> 12°C	12°C
<u>pH</u> Prefer 7.2 to 7.6	7.4 - 7.6
<u>Dissolved Oxygen</u> Static: ≥ 60% during 1 st 48 hrs and ≥ 40% during 2 nd 48 hrs, flow-through: ≥ 60%	≥60% saturation throughout the test.
<u>Total Hardness</u> Prefer 40 to 48 mg/L as CaCO ₃	143 mg/L as CaCO ₃
<u>Test Aquaria</u> 1. <u>Material</u> : Glass or stainless steel 2. <u>Size</u> : Volume of 18.9 L (5 gal) or 30 x 60 x 30 cm 3. <u>Fill volume</u> : 15-30 L of solution	1. Glass 2. 54 x 29 x 38 cm 3. 48 liters

<u>Type of Dilution System</u> Must provide reproducible supply of toxicant	Flow-through
<u>Flow Rate</u> Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	A continuous flow rate of 6 volume exchange per day- The flow water and dosing system were checked and controlled at least once on working days.
<u>Biomass Loading Rate</u> Static: ≤ 0.8 g/L at $\leq 17^{\circ}\text{C}$, ≤ 0.5 g/L at $> 17^{\circ}\text{C}$; flow-through: ≤ 1 g/L/day	Not mentioned.
<u>Photoperiod</u> 16 hours light, 8 hours dark	16 hours light, 8 hours dark
<u>Solvents</u> Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests	Solvent: DMF Maximum conc.: 0.1 mL/L

D. Test Design

Guideline Criteria	Reported Information
<u>Range Finding Test</u> If $\text{LC}_{50} > 100$ mg/L with 30 fish, then no definitive test is required.	A range-finding test was performed., but not in compliance with GLP regulations.
<u>Nominal Concentrations of Definitive Test</u> Control & 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series	Five nominal test concentrations (0.25, 0.5, 1.0, 2.0, and 4.0 mg/L), a dilution water control and a solvent control were used.
<u>Number of Test Organisms</u> Minimum 10/level, may be divided among containers	10 fish/aquarium
Test organisms randomly or impartially assigned to test vessels?	Yes

Guideline Criteria	Reported Information
Biological observations made every 24 hours?	Yes,
<u>Water Parameter Measurements</u> 1. <u>Temperature</u> Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C 2. <u>DO and pH</u> Measured at beginning of test and every 48 h in the high, medium, and low doses and in the control	1. Temperature was measured prior to introduction of fish, then daily. 2. DO and pH were measured prior to the introduction of fish, then daily in each test vessel.
<u>Chemical Analysis</u> Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Mean measured concentrations ranged from 78 to 80% of nominal in highest test concentration of 4.0 mg/L and 92 to 108% in the 0.5 to 2.0 mg/L nominal concentrations. The lowest test concentration of 0.25 mg/L was not analyzed because it was well below LC ₅₀ and NOEC.

12. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
<u>Recovery of Chemical</u>	Ranged from 78 to 119% of nominal values in test media.
<u>Control Mortality</u> Not more than 10% control organisms may die or show abnormal behavior.	0% mortality in each control
Raw data included?	Yes

Guideline Criteria	Reported Information
Signs of toxicity (if any) were described?	Yes, signs of toxicity were observed in fish exposed to test concentrations \geq 2.0 ppm,

Mortality

Concentration (ppb)		Number of Fish	Cumulative Number Dead			
Nominal (ppb)	Mean Measured (ppb)		Hour of Study			
			24	48	72	96
Control	N/A	10	0	0	0	0
Solvent Control	N/A	10	0	0	0	0
0.25	Not reported	10	0	0	0	0
0.5	0.56	10	0	0	0	0
1.0	0.95	10	0	0	0	0
2.0	1.98	10	0*	10	10	10
4.0	3.19	10	0*	10	10	10

* Observed tumbling while swimming and fish lying on side or back on the bottom.

Other Significant Results: N/A

B. Statistical Results - based on nominal concentrations.

Method: Geometric mean

96-hr LC₅₀: 1.4 ppm

95% C.I.: 1.0 - 2.0 ppm

Probit Slope: N/A

NOEC: 1.0 ppm

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical results were not verified because this study is classified as invalid.

Parameter	Result
Binomial Test LC ₅₀ (C.I.)	N/A
Moving Average Angle LC ₅₀ (95% C.I.)	N/A
Probit LC ₅₀ (95% C.I.)	N/A
Probit Slope	N/A
NOEC	N/A

14. **REVIEWER'S COMMENTS:** Since this study was conducted using chlorinated tap water, this study is classified as **Invalid**. Other significant deviations from the protocol included inconsistent water temperature measurements and high water hardness levels.