

US EPA ARCHIVE DOCUMENT

12/12/1996

MRID No. 439335-02

**DATA EVALUATION RECORD**  
**§ 72-2 -- ACUTE EC<sub>50</sub> TEST WITH A FRESHWATER INVERTEBRATE**

- 1. **CHEMICAL:** Benzyl Benzoate PC Code No.: 009501
- 2. **TEST MATERIAL:** Benzyl Benzoate Purity: ≥99%

3. **CITATION:**  
Authors: V. Wüthrich  
Title: 48-Hour Acute Toxicity of Benzyl Benzoate to *Daphnia magna* (OECD-Immobilization Test)  
Study Completion Date: February 20, 1995  
Laboratory: RCC Umweltchemie Ag, Itingen, Switzerland  
Sponsor: Allergopharma Joachim Ganzer KG, Reinbeck bei Hamburg, Germany  
Laboratory Report ID: 370776  
MRID No.: 439335-02  
DP Barcode: D224064

- 4. **REVIEWED BY:** Rosemary Graham Mora, M.S., Environmental Scientist, KBN Engineering and Applied Sciences, Inc.

**Signature:**  **Date:** 5/9/96

**APPROVED BY:** Pim Kosalwat, Ph.D., Senior Scientist, KBN Engineering and Applied Sciences, Inc.

**Signature:** P. Kosalwat **Date:** 5/9/96

- 5. **APPROVED BY:**

**Signature:**  **Date:** 12/17/96

- 6. **STUDY PARAMETERS:**

**Scientific Name of Test Organism:** *Daphnia magna*  
**Age of Test Organism:** <24 hours  
**Definitive Test Duration:** 48 hours  
**Study Method:** Static-Renewal  
**Type of Concentrations:** Mean measured

- 7. **CONCLUSIONS:** This study is not scientifically sound and does not meet the guideline requirements for an acute toxicity test using *Daphnia magna*. The 48-hour EC<sub>50</sub> was 2.44 ppm which classifies benzyl benzoate as moderately toxic to *Daphnia magna*. The NOEC could not be determined.

**Results Synopsis**

48-Hour EC<sub>50</sub>: 2.44 ppm  
NOEC: Not determined.

95% C.I.: 2.00-2.99 ppm  
Probit Slope: 5.34

**8. ADEQUACY OF THE STUDY:**

**A. Classification:** Invalid

**B. Rationale:** 1) Volume of test solutions was not the same in all test vessels, depending upon test concentrations and the sample volume needed for chemical analysis.  
3) The loading factor was extremely high (1 daphnid in at least 2 ml).  
2) Test solution temperature was not monitored during the test.

**C. Repairability:** No

**9. GUIDELINE DEVIATIONS:**

1. The test system was not fully described (i.e., the size of the test vessels and the volume of test solution were not reported).
2. The loading factor was one daphnid/a minimum of 2 ml of test solution (5-10 times higher than recommended).
3. The water temperature was not monitored and the room temperature was variable ranging from 18.5 to 22.5°C.
4. It is not clear from the report whether food was withheld from the test organisms during the test period as required by the guidelines.
5. Data from two tests were pooled for the calculation of EC<sub>50</sub>.

**10. SUBMISSION PURPOSE:**

**11. MATERIALS AND METHODS:**

**A. Test Organisms**

Guideline Criteria	Reported Information
<b><u>Species</u></b> Preferred species is <i>Daphnia magna</i>	<i>Daphnia magna</i>
<b>All organisms are approximately the same size and weight?</b>	Not Reported.
<b><u>Life Stage</u></b> Daphnids: 1 <sup>st</sup> instar (<24 h). Amphipods, stoneflies, and mayflies: 2 <sup>nd</sup> instar. Midges: 2 <sup>nd</sup> & 3 <sup>rd</sup> instar.	<24 hours
<b><u>Supplier</u></b>	In-house cultures
<b>All organisms from the same source?</b>	Yes

**B. Source/Acclimation**

Guideline Criteria	Reported Information
<b><u>Acclimation Period</u></b> Minimum 7 days	Daphnid cultures were maintained under conditions similar to those used in the tests.
<b>Wild caught organisms were quarantined for 7 days?</b>	N/A
<b>Were there signs of disease or injury?</b>	Not reported.
<b>If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?</b>	N/A
<b><u>Feeding</u></b> No feeding during the study.	Not reported.
<b><u>Pretest Mortality</u></b> No more than 3% mortality 48 hours prior to testing.	N/A

**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.	Reconstituted water medium used for testing was Elendt's M7 medium prepared using bidistilled water.
<u>Does water support test animals without observable signs of stress?</u>	Yes
<u>Water Temperature</u> Daphnia: 20°C Amphipods and mayflies: 17°C Midges and mayflies: 22°C Stoneflies: 12°C	Not reported. Room temperature was 18.5-22.5°C.
<u>pH</u> Prefer 7.2 to 7.6.	7.4-8.3
<u>Dissolved Oxygen</u> Static: ≥ 60% during 1 <sup>st</sup> 48 h and ≥ 40% during 2 <sup>nd</sup> 48 h, flow-through: ≥ 60%.	≥81% saturation
<u>Total Hardness</u> Prefer 40 to 48 mg/L as CaCO <sub>3</sub> .	250 mg/L as CaCO <sub>3</sub>
<u>Test Aquaria</u> 1. <u>Material:</u> Glass or stainless steel. 2. <u>Size:</u> 250 mL (daphnids and midges) or 3.9 L (1 gal). 3. <u>Fill volume:</u> 200 mL (daphnids and midges) or 2-3 L.	1. Glass 2. Not reported. 3. At least 20 ml; volume dependent upon the test concentrations and the sample volume needed for the chemical analysis.
<u>Type of Dilution System</u> Must provide reproducible supply of toxicant.	Static renewal with solutions renewed every 24 hours.
<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.	N/A

Guideline Criteria	Reported Information
<b>Biomass Loading Rate</b> Static: $\leq 0.8$ g/L at $\leq 17^\circ\text{C}$ , $\leq 0.5$ g/L at $> 17^\circ\text{C}$ ; flow- through: $\leq 1$ g/L/day.	1 daphnid in at least 2 ml.
<b>Photoperiod</b> 16 hours light, 8 hours dark.	16 hours light, 8 hours dark
<b>Solvents</b> Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests.	0.01% ethanol

**D. Test Design**

Guideline Criteria	Reported Information
<b>Range Finding Test</b> If $EC_{50} > 100$ mg/L, then no definitive test is required.	The results of a range-finding test indicated little or no effect at 0.01-1.0 ppm and 95- 100% immobility at 5, 10, and 50 ppm.
<b>Nominal Concentrations of Definitive Test</b> Control & 5 treatment levels; a geometric series with each concentration being at least 60% of the next higher one.	Dilution water control, solvent control, and 6 nominal test concentrations (0.1, 0.5, 1.0, 2.0, 4.0, and 8.0 ppm)
<b>Number of Test Organisms</b> Minimum 20/level, may be di- vided among containers.	10 daphnids per vessel, 2 vessels per level
<b>Test organisms randomly or impartially assigned to test vessels?</b>	Not reported.

<p><b><u>Water Parameter Measurements</u></b></p> <p>1. <u>Temperature</u> Measured continuously or, if water baths are used, every 6 h, may not vary &gt; 1°C.</p> <p>2. <u>DO and pH</u> Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control.</p>	<p>1. Room temperature was measured continuously. Water temperature was not measured.</p> <p>2. DO and pH were measured at the beginning and end of each 24-hour period.</p>
<p><b><u>Chemical Analysis</u></b> 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</p>	<p>Chemical analysis of the test solutions was performed on samples collected at 0 and 24 hours.</p>

**12. REPORTED RESULTS:**

Guideline Criteria	Reported Information
<b>Quality assurance and GLP compliance statements were included in the report?</b>	Yes
<b><u>Control Mortality</u></b> Static: ≤10% Flow-through: ≤5%	0%
<b><u>Percent Recovery of Chemical</u></b>	70.1-83.9% of nominal
<b>Raw data included?</b>	Yes

Mortality Results are from Test 1 only.

Concentration (ppm)		Number of Organisms	Cumulative Number Immobile	
Nominal	Mean Measured		Hour of Study	
			24	48
Control	<0.1	20	0(1)*	0(1)*

Concentration (ppm)		Number of Organisms	Cumulative Number Immobile	
Nominal	Mean Measured		Hour of Study	
			24	48-hr % mortal
Solvent Control	<0.1		0	10
0.1	N/D	2.39 **	1	60
0.5	0.39	0.71 **	0	1 - 0
1.0	0.71	See analysis	0	15
2.0	1.5	Sublethal	0	3 - 70
4.0	3.2	lethal	7	14 - 100
8.0	6.7		14	20

\* = One daphnid was accidentally killed and not included in percentage mortality.

\*\* = All 10 daphnids in one replicate beaker were immobile due to turbid solution, not considered treatment-related.

N/D = Not determined.

Other Significant Results: Four definitive tests were conducted during the course of this study. Test 1 is summarized in the table above. "Since the test solution turned turbid in one of the two test vessels of the 0.5 mg/L concentration, and caused 100% immobility, a second test was performed with test concentrations in the range from 0.05 to 1.0 mg/L." Test 2 had unacceptable control immobility, therefore, the test was repeated as Test 3. Test 3 also had unacceptable control immobility, and the test was repeated as Test 4.

B. Statistical Results The authors combined results from tests 1 and 4 for the calculation of EC<sub>50</sub>.

Method: Logit model

48-hr EC<sub>50</sub>: 2.39 ppm  
Probit Slope: Not reported.

95% C.I.: 1.47-4.87 ppm  
NOEC: 0.051 ppm

13. **VERIFICATION OF STATISTICAL RESULTS:** The reviewer determined the EC<sub>50</sub> using only data from the four highest test concentrations of Test 1 since the lowest test concentration was not measured and the immobile data from the next highest concentration were invalid due to turbid test solution.

Parameter	Result
Binomial Test EC <sub>50</sub> (C.I.)	2.46 (1.5-6.7)
Moving Average Angle EC <sub>50</sub> (95% C.I.)	2.40 (1.99-2.94)
Probit EC <sub>50</sub> (95% C.I.)	2.44 (2.00-2.99)
Probit Slope	5.34
NOEC	Not determined.

14. **REVIEWER'S COMMENTS:** The test system was not fully described. In particular, the details of the size of the test vessels and the amount of test solution used in each vessel are unclear. The author states that "10 daphnids were exposed to the test article dispersed in at least 20 ml test medium. The volume of the test medium was dependent upon the test concentrations and the sample volume needed for the analytics." This statement implies that the volume of test solution at each test level was different and that the solution volume was below the recommended 200 mL. Physical conditions to which organisms are exposed must be uniform across treatments to support the integrity of an experiment.

In addition, water temperature was not measured and the reported room temperature (18.5-22.5°C) was highly variable.

This study is not scientifically sound and does not fulfill the guideline requirements. The 48-hour EC<sub>50</sub> for *Daphnia magna* exposed to benzyl benzoate was 2.44 ppm which classifies the test material as moderately toxic. The study is classified as **Invalid**.

\*\*\*\*\*

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
6.7	20	20	100	9.536742E-05
3.2	20	14	70	5.765915
1.5	20	3	15	.1288414
.71	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 1.5 AND 6.7 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 2.457335

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.0513501	2.403732	1.986162	2.938915

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.150772	1	.8043928

SLOPE = 5.336811  
 95 PERCENT CONFIDENCE LIMITS = 3.264561 AND 7.409061

LC50 = 2.44188  
 95 PERCENT CONFIDENCE LIMITS = 1.995696 AND 2.9864

LC10 = 1.41174  
 95 PERCENT CONFIDENCE LIMITS = .9280114 AND 1.76706

\*\*\*\*\*