

Data Evaluation Report on the acute toxicity of Hallcomid M-8-10 to Rainbow Trout**(*Oncorhynchus mykiss*)****PMRA Submission Number {.....}****EPA MRID Number 45369708****Data Requirement:**

PMRA DATA CODE {.....}

DRAFT COPY

EPA DP Barcode D284964

OECD Data Point {.....}

EPA MRID 45369708

EPA Guideline 72-1(d)

Test material: Hallcomid M-8-10**Purity:** 94.4%**Common name:** Hallcomid M-8-10**Chemical name:** IUPAC: Not reported

CAS name: Not reported

CAS No.: Not reported

Synonyms: Not reported

Primary Reviewer: Dana Worcester

Staff Scientist, Dynamac Corporation

Signature:**Date:** 6/9/03**QC Reviewer:** Teri Myers

Staff Scientist, Dynamac Corporation

Signature:**Date:** 6/9/03**Primary Reviewer:**

{EPA/OECD/PMRA}

Date:**Secondary Reviewer(s):**

{EPA/OECD/PMRA}

Date:**Reference/Submission No.****Company Code:****Active Code:****EPA PC Code:** 999999**Date Evaluation Completed:**

CITATION: Dorgerloh, M. 1993. Hallcomid M-8-10 - Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) in a Static Test. Unpublished study performed and sponsored by Bayer AG, Leverkusen, Germany and submitted by The C.P. Hall Company, Chicago, IL. Study No. E 280 0720-9. Experimental start date June 14, 1993 and experimental termination date June 18, 1993. Final report issued August 3, 1993.



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(*Oncorhynchus mykiss*)

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EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, Rainbow trout (*Oncorhynchus mykiss*) were exposed to Hallcomid M-8-10 at nominal concentrations of 0 (control), 5.00, 8.89, 15.8, 28.1, and 50.0 mg/L under static conditions. The test material, Hallcomid M-8-10 contains four ingredients; mean measured concentrations, determined for three of the isomers, averaged 88-95% of the nominal concentrations. Because measured concentrations do not represent all components of Hallcomid M-8-10, toxicity values are based on the nominal concentrations. After 96 hours of exposure, mortality was 100% in the 28.1 and 50.0 mg/L treatment groups and 0% in all other groups. Signs of toxicity were observed in the 15.8 mg/L treatment group and included fish lying on the bottom of the tank, tumbling during swimming, and change in coloration. The LC_{50} was 21.1 mg/L, which categorizes Hallcomid M-8-10 as slightly toxic to juvenile Rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis. The NOEC was reported to be 5.00 mg/L, because fish were lying on the bottom of the tank after 72 hours in the 8.89 mg/L treatment group.

This study is scientifically sound, but does not satisfy the guideline requirements for an acute toxicity study with the Rainbow trout [§72-1(d)] because measured concentrations were not reported. This study is classified as Supplemental.

Results Synopsis

Test Organism Size/Age (mean Weight or Length): 1.4 g and 5.0 cm (mean of fish at start of study)
Test Type (Flow-through, Static, Static Renewal): Static

96-Hour

LC_{50} : 21.1 mg/L 95% C.I.: 15.8 - 28.1 mg/L
NOEC: 5.00 mg/L
LOEC: 8.89 mg/L
Endpoints affected: Mortality and sublethal effects

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study was based on procedures outlined in the EPA Guideline §72-1 (1982); EEC Directive 79/831, C.1: Acute Toxicity for Fish, 1984; and OECD Guideline for the Testing of Chemicals, No. 203, Fish, Acute Toxicity Test, 1992. Deviations from U.S. EPA Guideline §72-1c included:

- The water hardness (40-60 mg $CaCO_3$ /L) was greater than recommended (40-48 mg $CaCO_3$ /L).
- The measured concentrations were provided for only three of the four active ingredient isomers. This deviation does not affect the validity of the study, but is significant and affects the acceptability of the study.

COMPLIANCE: Signed and dated GLP, Confidentiality, and Quality Assurance statements were provided.

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A. MATERIALS:

1. Test Material Hallcomid M-8-10

Description: Clear, light yellow liquid

Lot No./Batch No.: Pt. 233290307

Purity: 94.4%

Stability of Compound

Under Test Conditions: Not reported.

OECD requires water solubility, stability in water and light, pK_a , P_{ow} and vapor pressure of the test compound. OECD requirements were not reported.

Water Solubility: 1.7 g/L

Storage conditions of

test chemicals: Room temperature

2. Test organism:

Species: Rainbow Trout (*Oncorhynchus mykiss*)

Age at test initiation: Juvenile

Weight at study initiation: The weight fish measured at start of test averaged 1.4 ± 0.4 g

Length at study initiation: The length of 10 fish measured at start of test averaged 5.0 ± 0.5 cm

Source: Dr. Rosengarten, Georgsmarienhütte

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B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: No range finding study was conducted.

b) Definitive Study

Table 1. Experimental Parameters

Parameter	Details	Remarks	
		Criteria	
Acclimation period:	Fish were held ≥ 14 days prior to testing.		
Conditions: (same as test or not)	Same as test	<i>EPA requires: minimum 14 days; no feeding during test OECD requires minimum of 12 days.</i>	
Feeding:	Commercial fish diet (Brutfutter FB50) except during the 48 hours prior to testing.		
Health: (any mortality observed)	<3% mortality.		
Duration of the test	96-hour	<i>EPA/OECD requires: 96 hour</i>	
Test condition			
static/flow through	Static	<i>EPA: Must provide reproducible supply of toxicant, with a consistent flow rate of 5-10 vol/24 hours, and meter systems calibrated before study and checked twice daily during test period</i>	
Type of dilution system- for flow through method.	N/A		
Renewal rate for static renewal	N/A		
Aeration, if any	None	<i>EPA requires: no aeration; OECD permits aeration</i>	
<u>Test vessel</u>			
Material: (glass/stainless steel)	Glass		
Size:	32 x 36 x 38 cm		
Fill volume:	40 L		

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Parameter	Details	Remarks
		Criteria
		EPA requires: Size 19 L (5 gal) or 30 x 60 x 30 cm Fill volume: 15-30 L of solution
Source of dilution water	Reconstituted water	
		EPA 1975; Soft reconstituted water or water from a natural source, not dechlorinated tap water; OECD permits dechlorinated tap water.
<u>Water parameters:</u>		
Hardness	40-60 mg CaCO ₃ /L	The water hardness (40-60 mg CaCO ₃ /L) was greater than recommended (40-48 mg CaCO ₃ /L).
pH	7.4-8.0	
Dissolved oxygen	10.1-11.1 mg/L	Hardness and pH EPA requires hardness of 40-48 mg/L as CaCO ₃ and pH of 7.2-7.6; 8.0-8.3 for marine-stenohaline fishes; 7.7-8.0 for estuarine-euryhaline fishes; monthly range <0.8. OECD allows hardness of 10-250 mg/L as CaCO ₃ and pH between 6 and 8.5. Dissolved Oxygen <u>Renewal:</u> ≥60% during 1 st 48 hrs and ≥40% during 2 nd 48 hrs <u>Flow-through:</u> ≥60% through out test. OECD requires at least 80% saturation value. Temperature EPA requires 22 ± 1 °C for estuarine/marine. OECD requires range of 21 - 25 °C for bluegill and 13-17 °C for rainbow trout. Salinity 30-34 ‰ (parts per thousand) salinity, weekly range < 6 ‰ EPA water quality measured at beginning of test and every 48 hours
Total Organic Carbon	<2 mg/L	
Particulate Matter	Not reported	
Metals	See Appendix C, p. 20	
Pesticides	See Appendices D and E, pp. 21-22	
Chlorine	<0.01 mg/L	
Temperature	12 ± 1 °C	
{Salinity for marine or estuarine species}	N/A	
Intervals of water quality measurement	DO, pH, and temperature were determined daily.	
<u>Concentration of test material:</u> nominal:	5.00, 8.89, 15.8, 28.1, and 50 mg/L	Only concentrations for 3 of the 4 active ingredient isomers were measured.

measured:

Not reported

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Parameter	Details	Remarks
		Criteria
		<i>EPA/OECD requires: Control and five treatment levels. Each conc. should be 60% of the next highest conc., and should be in a geometric series</i>
Solvent (type, percentage, if used)	N/A	<i>EPA requires: Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests; OECD requires solvent, exceed 100 mg/L.</i>
<u>Number of fish/replicates:</u> negative control:	10 fish	<i>EPA: ≥ 10/concentration; OECD requires at least 7 fish/concentration</i>
solvent control:	N/A	
treated:	10 fish	
Biomass loading rate	less than 0.4 g fish/L	<i>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; OECD requires maximum of 1 g fish/L for static and semi-static with higher rates accepted for flow-through</i>
Lighting	16-hours light/8-hours dark	<i>EPA requires: 16 hours light/8 hours dark; OECD requires 12 -16 hours photoperiod.</i>
Feeding	Animals were not fed during testing.	<i>EPA/OECD requires: No feeding during the study</i>
Recovery of chemical	Not reported	
Level of Quantitation	Not reported	
Level of Detection	Not reported	
Positive control {if used, indicate the chemical and concentrations}	N/A	

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Parameter	Details	Remarks
		Criteria
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks/Criteria
Parameters measured including the sublethal effects/toxicity symptoms	Mortality and sublethal effects	
Observation intervals	4, 24, 48, 72 and 96 hours of exposure	(EPA/OECD requires: minimally every 24 hours)
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

After 96 hours of exposure, mortality was 100% in the 28.1 and 50.0 mg/L test groups and 0% in all other groups.

Table 3: Effect of Hallcomid M-8-10 mortality of Rainbow Trout (*Oncorhynchus mykiss*).

Treatment, mg/L, nominal conc.	No. of fish at start of study						
		0-24 Hours		48-72 Hours		96 Hours	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Negative control	10	0	0	0	0	0	0
5.00	10	0	0	0	0	0	0
8.89	10	0	0	0	0	0	0
15.8	10	0	0	0	0	0	0
28.1	10	9	90	10	100	10	100

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50.0	10	10	100	10	100	10	100
NOEC (mortality)	5.00 mg/L						
LC ₅₀ (95% C.I.)	21.1 mg/L (15.8 - 28.1 mg/L)						
Positive control, if used mortality: LC ₅₀ :	N/A	N/A	N/A	N/A	N/A	N/A	N/A

B. NON-LETHAL TOXICITY ENDPOINTS:

Signs of intoxication such as apathy, convulsions, irregular swimming and fish on the bottom were observed in the 5.00, 8.89, 15.8, and 28.1 mg/L treatment groups after 4 hours. After 96 hours, symptoms of intoxication were only observed in the 15.8 mg/L treatment group.

Table 4. Sub-lethal effect of Hallcomid M-8-10 on Rainbow Trout (*Oncorhynchus mykiss*).

Treatment, mg/L, nominal conc.				
	endpoint at 24 Hours	endpoint at 48 Hours	endpoint at 72 Hours	endpoint at 96 Hours
	% affected	% affected	% affected	% affected
Negative control	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected
5.00	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected
8.89	Fish mainly at the bottom (50%)	Fish mainly at the bottom (40%)	Fish mainly at the bottom (40%)	No abnormalities detected
15.8	Fish mainly at the bottom (100%)	Fish mainly at the bottom and irregular swimming (100%)	Fish mainly at the bottom, tumbling and irregular swimming (100%)	Fish mainly at the bottom, tumbling and coloration change (100%)
28.1	Laying on side and apathy (100%)	Mortality 100%	---	---
50.0	Mortality 100%	---	---	---
NOEC (sublethal)	5.00 mg/L			
LOEC (sublethal)	8.89 mg/L			

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Treatment, mg/L, nominal conc.				
	endpoint at 24 Hours	endpoint at 48 Hours	endpoint at 72 Hours	endpoint at 96 Hours
	% affected	% affected	% affected	% affected
EC ₅₀	Not determined			
Positive control, if used % sublethal effect: EC ₅₀ :	N/A	N/A	N/A	N/A

C. REPORTED STATISTICS:

Statistical Method: The 96-hour LC₅₀ value were determined using a computer program which using either moving average, binomial probability or probit..

96-HourLC₅₀: 21.1 mg/L 95% C.I.: 15.8-28.1

NOEC: 5.00 mg/L

LOEC: 8.89 mg/L

Endpoints affected: Mortality and sublethal effects

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The NOEC was determined visually, based on sublethal effects and the LC₅₀ was estimated using the binomial method via TOXANAL statistical software.

96-HourLC₅₀: 21.1 mg/L 95% C.I.: 15.8-28.1

NOEC: 5.00 mg/L

LOEC: 8.89 mg/L

Endpoints affected: Mortality and sublethal effects

E. STUDY DEFICIENCIES:

Measured concentrations were provided for only three of the four active ingredient isomers. As a result, toxicity values are based on nominal concentrations. This does not affect the validity of the study, but it affects the acceptability.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study author's. The LC₅₀ was 21.1 mg/L, which categorizes Hallcomid M-8-10 as slightly toxic to juvenile Rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis.

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The study author noted 5 fish showed signs of intoxication at 4 hours in the 5.00 mg/L treatment group. However, because this happened only at the beginning, it was not considered in the evaluation of the NOEC.

The test material, Hallcomid M-8-10 contains four ingredients; mean measured concentrations, determined for three of the isomers, averaged 88-95% of the nominal concentrations. Because measured concentrations do not represent all components of Hallcomid M-8-10, toxicity values are based on the nominal concentrations.

G. CONCLUSIONS:

This study is scientifically sound, but does not satisfy the guideline requirements for an acute toxicity study with the Rainbow trout [§72-1(d)] because measured concentrations were not reported. This study is classified as Supplemental. The LC₅₀ was 21.1 mg/L, which categorizes Hallcomid M-8-10 as slightly toxic to juvenile Rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis.

96-Hour

LC₅₀: 28.1 mg/L

95% C.I.: 15.8 28.1 mg/L

NOEC: 5.00 mg/L

LOEC: 8.89

Endpoints affected: Mortality and sublethal effects

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III. REFERENCES:

Brauhn, J.L., R.A. Schoettger, "Acquisition and Culture of Research Fish: Rainbow Trout, Fathead Minnows, Channel Catfish and Bluegill Sunfish". Environmental Protection Agency, Ecological Research Series EPA-660/3-75-011, May 1975.

Stephan, C.E. 1982. U.S.E.P.A., Environmental Research Laboratory, Duluth, MN. Personal Communication to Dr. Lowell Bahner, Chairman, ASTM Task Group on Calculating LC50.

Stephan, C.E. 1977. Methods for Calculating and LC50. In: Aquatic Toxicology and Hazard Evaluation, ASTM STP 634. F.L. Mayer and J.L. Hamelink, eds. American Society for Testing Materials, Philadelphia, PA. 65-84.

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APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

	EXPOSED	DEAD	DEAD	PROB.(PERCENT)
50	10	10	100	9.765625E-02
28.1	10	10	100	9.765625E-02
15.8	10	0	0	9.765625E-02
8.890001	10	0	0	9.765625E-02
5	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 15.8 AND 28.1 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 21.07082

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.