Data Evaluation Report on the acute toxicity Hallcomid M-8-10 to Fathead Minnow (*Pimephales promelas*)

PMRA Submission Number {.....} EPA MRID Number 45369709

Data Requirement:

PMRA DATA CODE {.....}
EPA DP Barcode D284964
OECD Data Point {.....}
EPA MRID 45369709
EPA Guideline: 72-1(d)

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Test material: Hallcomid M-8-10
Common name: Hallcomid M-8-10
Chemical name: IUPAC: Not reported
CAS name: Not reported
CAS No.: Not reported
Synonyms: Not reported

Purity: 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: Bowman, J.H. 1991. Acute Toxicity of Hallcomid® M-8-10 to Fathead Minnow (*Pimephales promelas*). Unpublished study performed by ABC Laboratories, Inc. Columbia, MO. Study sponsored by The C.P. Hall Company, Chicago, IL. Laboratory Project No. #38937. Experimental start date January 21, 1991 and experimental termination date February 2, 1991. Final report issued August 30, 1991.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, fathead minnow (*Pimephales promelas*) were exposed to Hallcomid M-8-10 at nominal concentrations of 0 (negative control), 1.8, 3.2, 5.6, 10, 18, and 32 mg/L under static conditions. Mean-measured concentrations were not reported. After 96 hours of exposure, mortality was 0% in the control, 0% in the 1.8, 3.2, 5.6, and 10 mg/L treatment groups, 40% in the 18 mg/L treatment group and 100% in the 32 mg/L treatment group. The LC_{50} was 19 mg/L. After 96 hours, signs of toxicity were observed in the 5.6, 10 and 18 mg/L treatment groups.

This study is scientifically sound, but it does not satisfy the guideline requirements for an acute toxicity study with freshwater fish [§72-1(d)] because the purity of the test material was not reported. This limits the utility of the results for risk assessment and, as a result, the study is classified as INVALID. If the purity is provided, this study may be upgraded to Supplemental status (test solutions were not analytically determined and fathead minnow is not a recommended species for acute toxicity testing).

Results Synopsis

Test Organism Size/Age (mean Weight or Length): 0.21 ± 0.03 g, 2.4 ± 0.1 cm
Test Type (Flow-through, Static, Static Renewal): Static

96-Hour–INVALID study; results not reported
LC_{50}: N/A 95% C.I.: N/A
NOEC: N/A
LOEC: N/A
EC_{50}: N/A 95% C.I.: N/A
Endpoint(s) affected:

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the U.S. EPA-TSCA, 40 CFR, Part 797, Guideline 797.1400. Deviations from §72-1(d) included:

1. The purity of the test substance was not reported. The purity of the test substance is mandatory for a risk assessment and should be provided for the potential upgrade of the classification of this study.

2. The test material concentrations that fish were exposed to were not analytically determined in this study.

3. The acclimation period (48 hours) was shorter than recommended by EPA (14 days).

4. The dilution water was harder (180 mg/L as $CaCO_3$) than recommended by EPA (40-48 mg/L as $CaCO_3$).

5. Fathead minnow is not an EPA-recommended species for acute toxicity testing.

COMPLIANCE: Signed and dated GLP (TSCA 40 CFR 792), Confidentiality, and Quality Assurance statements were provided.

A. MATERIALS:

1. Test Material Hallcomid M-8-10

Description: Yellow liquid

Lot No./Batch No. : Not reported

Purity: Not reported

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. Several OECD requirements were not reported.

Storage conditions of test chemicals: Room temperature.

2. Test organism:

Species: Fathead minnow (*Pimephales promelas*)

Age at test initiation: Juvenile

Weight at test initiation: The weight of the 10 fish measured at test termination averaged 0.21 ± 0.3g

Length at test initiation: The length of the 10 fish measured at test termination averaged 2.4 ± 0.1cm

Source: In-house culture

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: A range finding study was conducted at nominal concentrations of 1, 10, 32, and 100 mg/L.

b) Definitive Study:

Table 1 . Experimental Parameters

Parameter	**Details**	**Remarks**
		Criteria
Acclimation period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	48 hours prior to testing. Same as test Brine shrimp (*Artemia sp.*), *Daphnia magna*, and rotifers and/or commercial feed. Not reported	*EPA requires: minimum 14 days; no feeding during test OECD requires minimum of 12 days.*
Duration of the test	96 hours	*EPA/OECD requires: 96 hours*
Test condition static/flow through Type of dilution system- for flow through method. Renewal rate for static renewal	 Static N/A N/A	*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*
Aeration, if any	None	*EPA requires: no aeration; OECD permits aeration*
Test vessel		
Material: (glass/stainless steel) Size: Fill volume:	Glass 5 gallon 15 L	

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	Hard well water mixed with demineralized well water	
		*EPA 1975; Soft reconstituted water or water from a natural source, **not** dechlorinated tap water;* *OECD permits dechlorinated tap water.*
Water parameters: Hardness	180 mg/L as $CaCO_3$	
pH	7.8-8.3	
Dissolved oxygen	5.3-8.9 mg/L (65-106% saturation)	***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*** *Renewal: ≥60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs* *Flow-through: ≥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	<1 mg/L	
Particulate Matter	0.8 mg/L (suspended solids)	
Metals	See Table 1, p. 11	
Pesticides	See Table 1, p. 11	
Chlorine	Not reported	
Temperature	22-23°C	
{Salinity for marine or estuarine species}	N/A	
Intervals of water quality measurement	DO, pH, and temperature were determined daily.	

Parameter	Details	Remarks
		Criteria
Concentration of test material: nominal: measured:	1.8, 3.2, 5.6, 10, 18, and 32 mg/L Not reported	
		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
Solvent (type, percentage, if used)	N/A	
		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.
Number of fish/replicates: negative control: solvent control: treated:	10 fish N/A 10 fish	
		EPA: ≥10/concentration; OECD requires at least 7 fish/concentration
Biomass loading rate	0.14 g fish/L (instantaneous)	
		Static: ≤0.8 g/L at ≤17°C, ≤0.5 g/L at >17°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
Lighting	16-hours light/8-hours dark	
		EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod.
Feeding	Animals were not fed during testing.	
		EPA/OECD requires: No feeding during the study
Recovery of chemical Level of Quantitation Level of Detection	Not reported Not reported Not reported	

Parameter	Details	Remarks / Criteria
Positive control {if used, indicate the chemical and concentrations}	N/A	
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	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 32 mg/L treatment group, 40% in the 18 mg/L treatment group and 0% in all other groups.

Table 3: Effect of Hallcomid M-8-10 on mortality of Fathead Minnow (*Pimephales promelas*).

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
1.8	10	0	0	0	0	0	0
3.2	10	0	0	0	0	0	0
5.6	10	0	0	0	0	0	0
10	10	0	0	0	0	0	0
18	10	4	40	4	40	4	40
32	10	10	100	10	100	10	100
NOEC (mortality)	3.2 mg/L						
LC_{50} (95% C.I.)	19 mg/L (10 - 32 mg/L)						
Positive control, if used mortality: LC_{50}:	N/A						

B. NON-LETHAL TOXICITY ENDPOINTS:

After 96 hour, signs of intoxication such as loss of equilibrium, surfacing, dark discoloration, labored respiration, and twitching was observed in the 5.6, 10, and 18 mg/L treatment groups.

Table 4. Sub-lethal effects of Hallcomid M-8-10 on Bluegill (*Lepomis macrochirus*).

Treatment, mg/L, measured and (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
1.8	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected
3.2	No abnormalities detected	No abnormalities detected	No abnormalities detected	Loss of mobility 1fish
5.6	Twitching (1)	Twitching (1)	Twitching (1)	Labored respiration (1)
10	On bottom, quiescent (4); twitching (2)	On bottom (2); surfacing (2); on bottom, quiescent, discoloration (2)	Discoloration, on bottom (1); surfacing (2); discoloration, (1); surfacing, quiescent (1)	Surfacing (2); surfacing , labored respiration (2); on bottom (2)
18	Loss of equilibrium, erratic swimming, labored respiration (1); on bottom, loss of equilibrium, quiescent, labored respiration (5)	Discoloration, on bottom, labored respiration, quiescent (4); discoloration, on bottom, loss of equilibrium, labored respiration (2)	On bottom (2); surfacing (2); discoloration, on bottom, labored respiration (2)	Erratic swimming (2); surfacing, quiescent (1); surfacing, labored respiration (3)
32	100% Mortality	---	---	---
NOEC	3.2 mg/L			
LOEC	Not reported			
EC_{50}	19 mg/L			
Positive control, if used % sublethal effect: EC_{50}:	Not reported			

C. REPORTED STATISTICS:

Statistical Method: The LC_{50}: was calculated using the binomial method.

96-Hour
LC_{50}: 19 mg/L 95% C.I.: 10 - 32 mg/L
NOEC: 3.2 mg/L
LOEC: 5.6 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 LC_{50} was estimated using the binomial method via TOXANAL statistical software. Because this study is classified as INVALID, the results are not reported in the Executive Summary and Conclusions sections.

96-Hour
LC_{50}: 19.4 mg/L 95% C.I.: 10-32 mg/L
NOEC: 3.2 mg/L
LOEC: 5.6 mg/L
Endpoints affected: Mortality and sublethal effects

E. STUDY DEFICIENCIES:

The purity of the test material was not reported. This information is mandatory for a risk assessment (US EPA Pesticide Reregistration Rejection Rate Analysis: Ecological Effects, p. 64). Furthermore, the test concentrations that fish were exposed to were not analytically determined and fathead minnow is not a US EPA-recommended species for acute toxicity testing. If the purity is reported, this study may be upgraded to Supplemental.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study author's. However, because the test substance purity was not reported, this study is classified as INVALID and the results are not reported in the Executive Summary and Conclusions sections.

The study was conducted in compliance with EPA GLP standards under the Toxic Substances Control Act (40 CFR 792).

G. CONCLUSIONS:

This study is scientifically sound, but it does not satisfy the guideline requirements for an acute toxicity study with freshwater fish [§72-1(d)] because the purity of the test material was not reported. This limits the utility of the results for risk assessment and, as a result, the study is classified as INVALID. If the purity is provided, this study may be upgraded to Supplemental status (test solutions were not analytically determined and fathead minnow is not a recommended species for acute toxicity testing).

96-Hour–INVALID study; results not reported
LC_{50}: N/A 95% C.I.: N/A
NOEC: N/A
LOEC: N/A
EC_{50}: N/A 95% C.I.: N/A
Endpoint(s) affected:

III. REFERENCES:

Committee on Methods for Toxicity Tests with Aquatic Organisms (C.E. Stephan, Chairman). 1975. Methods for Acute Toxicity Tests with Fish, Macroinvertabrates and Amphibians. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-009, April 1975. 61 p.

American Public Health Association. 1980. Standard Methods for the Examination of Water and Wastewater. 15th ed. Washington, DC. 1134 p.

Brauhn, J.L, R.A. Schoettger, "Aquisitation 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. 45 p.

Stephan, C.E., K.A. Busch, R. Smith, J. Burke and R.W. Andrew. 1978. A computer program for calculating LC_{50}. U.S. Environmental Protection Agency, Duluth, MN. Pre-publication manuscript, August, 1978.

Stephan, C.E. 1977. Methods for Calculating and LC_{50}. P. 65-84. In: F.L. Mayer and J.L. Hamelink, eds. Aquatic Toxicology and Hazard Evaluation. ASTM Special Technical Publication 634. ASTM, Philadelphia.

U.S. Environmental Protection Agency. 1989. Toxic Substances Control; Good Laboratory Practice Standards; Final Rule (40 CFR, Part 792). Federal Register, Vol. 54, No. 158:34043-34050.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

32	10	10	100	9.765625E-02
18	10	4	40	37.69531
10	10	0	0	9.765625E-02
5.6	10	0	0	9.765625E-02
3.2	10	0	0	9.765625E-02
1.8	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 10 AND 32 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 19.36234

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.