US EPA ARCHIVE DOCUMENT

Data Evaluation Report on the acute toxicity of Iodomethane (TM-425) to Rainbow Trout (*Oncorhynchus mykiss*)
PMRA Submission Number {.....} EPA MRID Number 45593714

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	D280800
OECD Data Point	Mortality
EPA MRID	45593714
EPA Guideline	72-1

Test material: Iodomethane (TM-425)
Common name: Iodomethane (TM-425)
Chemical name: IUPAC: Not reported
CAS name: Not reported
CAS No. Not reported
Synonyms: Not reported

Purity: 99.7%

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation

Signature: Rebecca Bryan
Date: 3/28/02

QC Reviewer: Teri Myers, Ph.D.
Staff Scientist, Dynamac Corporation

Signature: Teri Myers
Date: 3/28/02

~~**Primary**~~ Secondary **Reviewer:** James Felkel
{EPA/OECD/PMRA}

Date: 9/17/02 J. Felkel

~~**Secondary**~~ Other **Reviewer(s):** {.....}
{EPA/OECD/PMRA}

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Active Code {.....} [For PMRA]
EPA PC Code 000011

Date Evaluation Completed: {dd-mmm-yyyy}

CITATION: Drottar K.R., T.Z. Kendell, and H.O. Krueger. 2002. Iodomethane (TM-425): A 96 -Hour Static Renewal Acute Toxicity test with the Rainbow Trout *(Oncorhynchus mykiss).* Unpublished study performed by Wildlife International, Ltd., Easton, Maryland and sponsored by Arvesta Corporation, San Francisco, California. Completed January 10, 2002.

2008815

EXECUTIVE SUMMARY:

In a 96-h acute toxicity study, rainbow trout (*Oncorhynchus mykiss*) were exposed to iodomethane (TM-425) at mean measured concentrations of 0.62, 0.97, 1.5, 2.3, 3.4, and 5.1 mg/L under static renewal conditions. Nominal concentrations were 0.80, 1.2, 1.8, 2.7, 4.1, and 6.1 mg/L. The 96-h LC_{50} was 1.33 mg /L. As a result, iodomethane (TM-425) is categorized as moderately toxic to rainbow trout in accordance with the classification system of the U.S. EPA. The NOEC was 0.62 mg/a.i./L, based on mortality and sublethal effects. During the 96 hours of treatment, sublethal effects included lethargy and lying on the bottom of the test chambers.

This toxicity study is classified as Core. It is scientifically sound and fulfills the guideline requirements for an acute toxicity test with freshwater fish (72-1).

Results Synopsis

Test Organism Size/Age (mean Weight or Length): Wet weight(termination) = 0.24 g; Length(termination) =3.3 cm; Juveniles.

Test Type (Flowthrough, Static, Static Renewal): Static Renewal
LC_{50}: 1.33 mg/L 95% C.I.: 1.12-1.56 mg/L
NOEC: 0.62 mg/L Probit Slope: N/A
EC_{50}: Not reported
Endpoint(s) Affected: Mortality and sublethal effects

I. MATERIALS AND METHODS REPORTED

GUIDELINE FOLLOWED: This study design was reported to be based on OECD Guideline for Testing of Chemicals No. 203, OPPTS 850.1075, and Japan MAFF, 12 Nousan No. 8147 (p. 10).

The following deviations from OPP guidelines (72-1) and SEP were noted by reviewer(s):

1) The age, weight, and length of fish at test initiation were not reported. At test termination, weights of fish were less than recommended by US EPA guidelines. The study authors reported that smaller fish were used to reduce the loading rate in the closed bottle test system, which was necessary because of the high volatility of Iodomethane.

2) The test vessel and fill volume was smaller than US EPA recommends (3.8 L vs. 19 L; 3.8 L vs. 15-30 L).

3) Water hardness and pH were greater than US EPA recommends (131 vs. 40-48 mg/L and 7.9-8.6 vs. 7.2-7.6).

Fish size and fill volume were due to the closed test system, which in turn was needed due to Iodomethane volatility. Water quality deviations are not considered sufficient to prevent Core status (Pers. Com., M. Rexrode and R. Pisigan, EFED).

COMPLIANCE: Signed and dated GLP and Quality Assurance statements were provided. A Confidentiality statement was not provided.

A. MATERIALS:

1. Test Material Iodomethane, TM-425

Description: Liquid

Lot No./Batch No. : 007403/02

Purity: 99.7% a.i.

Stability of Compound Under Test Conditions: Test concentrations were measured at 0 and 72 hours for fresh media and at 24 and 96 hours for expired media. The mean recovery of the test substance from samples of fresh and expired solutions ranged from 78-85% of the nominal concentrations (Table 1), showing that they were relatively stable under test conditions.

(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Storage conditions of test chemicals: Ambient room temperature

2. Test organism:

Species: Rainbow Trout (*Oncorhynchus mykiss*)
EPA requires a coldwater species (preferably rainbow trout *Oncorhynchus mykiss*) and a warmwater species (preferably bluegill sunfish *Lepomis macrochirus*).
OECD allows choice of species at discretion of testing laboratory.
Age at test initiation: Juveniles
Weight at study initiation: not reported, test termination mean =0.24 g
EPA requires: mean 0.5 - 5 g
Length at study initiation: test termination mean = 3.3 cm; range = 2.9-3.8 cm
EPA requires: Longest not > 2x shortest; OECD requires 2.0 ± 1.0 cm for bluegill and 5.0 ± 1.0 cm for rainbow trout
Source: Thomas Fish Company, Anderson, California

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study

A range-finding test was not reported.

b) Definitive Study

Table 1. Experimental Parameters

Parameter	Reported Details	Remarks Criteria
Acclimation: period: conditions: (same as test or not) Feeding: Health: (any mortality observed)	19 days Same as test. Not fed 48 hours prior to test initiation; not fed during exposure No mortality observed prior to test initiation	*EPA requires: minimum 14 days; no feeding during test OECD requires minimum of 12 days.*
Duration of the test	96-hour	*(EPA/OECD requires: 96 hour)*
Test condition static/flow through	Static renewal	
Type of dilution system- for flow through method.	N/A	
Renewal rate for static renewal	Daily	

Parameter	Reported Details	Remarks Criteria
		(EPA requires: Must provide reproducible supply of toxicant) *(EPA requires: Consistent flow rate of 5-10 vol/24 hours, 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 jars with Teflon®-lined lids. 3.8 L 3.8 L; jars were completely filled with test solution to minimize headspace, due to the high volatility of Iodomethane.	The test vessel size and fill volume were smaller than required (both 3.8 L). *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 Quality:	Filtered laboratory well water.	*(EPA 1975; Soft reconstituted water or water from a natural source, **not** dechlorinated tap water); OECD permits dechlorinated tap water.*

Parameter	Reported Details	Remarks Criteria
Water parameters: Hardness pH Dissolved oxygen Total Organic Carbon Particulate Matter Metals Pesticides Chlorine	 140 mg $CaCO_3$/L* (0-hr) 7.9-8.6* 7.4-10.6 mg/L* (≥65%) 1.2 mg/L* Not reported. Analysis p. 48. <0.25 µg/L Not reported	Water hardness and pH were greater than required (131 vs. 40-48 mg/L and 7.9-8.6 vs. 7.2-7.6).
Temperature {Salinity for marine or estuarine species} Intervals of water quality measurement	12.3-13.1°C N/A DO and pH were measured daily. Temperature was measured in the control aquarium continuously. *Table 2 (pp. 22-23)	*(EPA hardness: 40 - 48 mg as $CaCO_3$/L; OECD allows 10 -250 mg as $CaCO_3$/L)* *(EPA pH: 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 pH 6.0 - 8.5* (EPA Dissolved Oxygen: *Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60%); OECD requires at least 80% saturation value.* *(EPA temperature: estuarine/marine: 22 ± 1 °C* *OECD requires 21 - 25°C for bluegill and 13 - 17°C for rainbow trout* *(EPA salinity: 30-34 ‰ (parts per thousand) salinity, weekly range < 6 ‰)* *(EPA water quality: measured at beginning of test and every 48 hours)*
Number of replicates/groups: control: solvent control: treated ones:	 2 N/A 2	*(EPA/OECD requires: Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geo-metric series)*

Parameter	Reported Details	Remarks Criteria
Number of organisms per replicate /groups: control: solvent control: treated ones:	 7 N/A 7	*(EPA: ≥ 10/concentration); OECD requires at least 7 fish/concentration*
Biomass loading rate	0.44 g bodyweight/L	*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*
Test concentrations: nominal: measured:	 0.80, 1.2, 1.8, 2.7, 4.1, and 6.1 mg/L 0.62, 0.97, 1.5, 2.3, 3.4, and 5.1 mg/L	Measured concentrations are the average of old and new test solutions.
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.*
Lighting	16:8	*(EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod.*
Feeding	Not fed 48 hours prior to study or during exposure	*EPA/OECD requires: No feeding during the study*
Recovery of chemical Level of Quantitation Level of Detection	78-85% 0.200 mg/L Not reported.	
Positive control {if used, indicate the chemical and concentrations}	N/A	

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

2. Observations:

Table 2: Observations

Criteria	Reported Details	Remarks/Criteria
Parameters measured including the sublethal effects/toxicity symptoms	Mortality and sublethal effects	
Observation intervals	Mortality was measured at 3, 6, 24, 48, 72 and 96 hours of exposure	*(EPA/OECD requires: minimally every 24 hours)*
Were raw data included?	No	
Other observations, if any		

II. REPORTED RESULTS and DISCUSSION:

A. MORTALITY:

Following 96 hours of treatment, mortality was 0%, 7.1%, 64%, 100%, 100%, and 100% in the 0.62, 0.97, 1.5, 2.3, 3.4, and 5.1 mg/L treatment groups, respectively. There was no mortality in the control group.

Table 3: Effect of Iodomethane (TM-425) on Mortality of Rainbow Trout (*Oncorhynchus mykiss*).

Treatment (mg/L) measured and nominal conc.[1]	No. of fish at start of study	Observation period									
		Day 0 (3 and 6 hrs)		Day 1		Day 2		Day 3		Day 4	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Control (dilution water only)	14	0	0	0	0	0	0	0	0	0	0
0.62 (0.80)	14	0	0	0	0	0	0	0	0	0	0
0.97 (1.2)	14	0	0	0	0	0	0	0	0	1	7.1
1.5(1.8)	14	0	0	0	0	0	0	0	0	9	64
2.3 (2.7)	14	0	0	0	0	0	0	9	64	14	100
3.4 (4.1)	14	0	0	0	0	1	7.1	14	100	14	100
5.1(6.1)	14	0	0	0	0	14	100	14	100	14	100
NOEC (mortality)	0.62 mg/L										
LC_{50}	1.4 mg/L (0.235-1.40 mg/L)										
Positive control, if used mortality: LC_{50}:	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

[1] Nominal values are presented in parentheses.

B. NON-LETHAL TOXICITY ENDPOINTS:

Following 24 hours of treatment, all fish in the 5.1 mg/L treatment group appeared lethargic. By 48 hours, most fish in the 2.3 mg/L treatment group appeared lethargic and the remaining live fish in this concentration and in the 3.4 mg/L concentration were lying on the bottom of the test chambers. By 72 hours, one fish appeared lethargic in the 0.97 mg/L treatment group and all living fish at this and higher concentrations exhibited sublethal effects (lethargy and lying on the bottom of the chambers). After 96 hours of treatment, one fish in the 0.97 mg/L treatment group and al living fish in the 1.5 mg/L treatment group were lying on the bottom of the chamber.

Table 4. Sublethal Effect of Iodomethane (TM-425) on Rainbow Trout (*Oncorhynchus mykiss*).

Treatment (mg /L) measured and nominal concentrations	endpoint at 3 and 6 hrs	endpoint at Day 1	endpoint at Day 2	endpoint at Day 3	endpoint at Day 4
	% affected	% affected	% affected	% affected	% affected
Control (dilution water only)	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected
0.62 (0.80)	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected
0.97 (1.2)	No abnormalities detected	No abnormalities detected	No abnormalities detected	7% lethargic	7% lying on bottom
1.5(1.8)	No abnormalities detected	No abnormalities detected	No abnormalities detected	93% lethargic 7% lying on bottom	36% lying on bottom
2.3 (2.7)	No abnormalities detected	No abnormalities detected	79% lethargic 14% lying on bottom	36% lying on bottom	100% Mortality
3.4 (4.1)	No abnormalities detected	No abnormalities detected	93% lying on bottom	100% Mortality	100% Mortality
5.1(6.1)	No abnormalities detected	100% lethargic	100% Mortality	100% Mortality	100% Mortality

(Table continues, next page)

Treatment (mg /L) measured and nominal concentrations	**endpoint at 3 and 6 hrs**	**endpoint at Day 1**	**endpoint at Day 2**	**endpoint at Day 3**	**endpoint at Day 4**
	% affected	**% affected**	**% affected**	**% affected**	**% affected**
NOEC (sublethal)	0.62mg/L				
LOEC (sublethal)	Not reported				
EC_{50}	Not reported				
Positive control, if used % sublethal effect: EC_{50}:	N/A	N/A	N/A	N/A	N/A

C. REPORTED STATISTICS:

The study author reported that the EC_{50} value was calculated using the computer program of C.E. Stephan, which provides output for the probit analysis, the moving average method, and binomial probability with nonlinear interpolation. In this study, the 96-hour LC_{50} value was estimated using the probit method. The criteria for establishing the no-observed effect level was based on direct inspection of the data.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The LC_{50} was estimated using the moving average angle method via Toxanal software. The NOEC was determined visually.

LC_{50}: 1.33 mg/L 95% C.I.: 1.12-1.56 mg/L
NOEC: 0.62 mg/L Probit Slope: N/A
EC_{50}: Not reported
Endpoint(s) Affected: Mortality and sublethal effects

E. STUDY DEFICIENCIES:

The age, weight, and length of fish at test initiation were not reported. At test termination, average fish weight was less than half of the minimum initial fish weight recommended by US EPA guidelines (0.5 g). The study authors reported that smaller fish were used to reduce the loading rate in the closed bottle test system, which was necessary because of the high volatility of Iodomethane. In addition to this deviation, the test vessel and fill volume were substantially smaller and the test water was harder and more alkaline than recommended by US EPA.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were nearly identical to the study authors'. The reviewer chose a different method (moving average angle) than the study authors (probit method) to estimate the LC_{50} value. The reviewer selected the output from the moving average angle method because it provided the narrowest 95% confidence interval estimate. The 96-h LC_{50} was 1.33 mg /L. As a result, iodomethane (TM-425) is categorized as moderately toxic to rainbow trout in accordance with the classification system of the U.S. EPA. The NOEC was 0.62 mg/a.i./L, based on mortality and sublethal effects.

G. CONCLUSIONS:

This toxicity study is classified as Core. It is scientifically sound and fulfills the guideline requirements for an acute toxicity test with a freshwater fish, rainbow trout (72-1). The 96-h LC_{50} was 1.33 mg/L. As a result, iodomethane (TM-425) is categorized as moderately toxic to rainbow trout in accordance with the classification system of the U.S. EPA.

LC_{50}: 1.33 mg/L 95% C.I.: 1.12-1.56 mg/L
NOEC: 0.62 mg/L Probit Slope: N/A
EC_{50}: Not reported
Endpoint(s) Affected: Mortality and sublethal effects, such as lethargy and lying on bottom

III. REFERENCES:

Organization for Economic Cooperation and Development. 1992. *Fish, Acute Toxicity Test.* OECD Guidelines for Testing of Chemicals. Guideline 203. Paris.

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