DATA EVALUATION RECORD

1. **CHEMICAL:** Methyl Bromide.
Shaughnessey No. 053201.

2. **TEST MATERIAL:** Methyl Bromide; Lot No. 6RL4; 99.87% purity; a pressurized liquid.

3. **STUDY TYPE:** 72-2. Freshwater Invertebrate Static Acute Toxicity Test. Species Tested: *Daphnia magna*.

4. **CITATION:** Drottar, K.R. and J.P. Swigert. 1993. Methyl Bromide: A 48-hour Static Acute Toxicity Test with the Cladoceran (*Daphnia magna*). Laboratory Project No. 264A-102B. Prepared by Wildlife International Ltd., Easton, MD. Submitted by Chemical Manufacturers Association, Washington, DC. EPA MRID No. 429329-01.

5. **REVIEWED BY:**

Nicole U. Jurczyk, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Nicole U. Jurczyk
Date: 2/18/94

6. **APPROVED BY:**

Rosemary Graham Mora, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Rosemary Graham Mora
Date: 2/18/94

James J. Goodyear, Ph.D.
Project Officer, EEB/EFED
USEPA

Signature: Henry T. Craven
Date: 4/18/94

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an acute freshwater invertebrate toxicity study. Based on mean measured concentrations, a 48-hour EC_{50} of 2.6 mg ai/l classifies methyl bromide as moderately toxic to *Daphnia magna*. The NOEC was 1.2 mg ai/l.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** *Daphnia magna* were obtained from in-house cultures. The adult daphnids were fed a mixture of yeast, Cerophyll®, trout chow, and a suspension of *Selenastrum capricornutum*. During the 14-day holding period preceding the test, the water had a temperatures of 20.0-20.8°C, a pH of 8.2-8.9, and a dissolved oxygen concentration (DO) of 7.4-8.4 mg/l.

The daphnids in the cultures were in good health and showed no signs of disease or stress. Neonates were obtained for testing by transferring individual adult daphnids to dilution water 24 hours prior to test initiation. First instar larvae (<24 hours old) from at least three different adults were chosen for the test.

B. **Test System:** The test chambers were 100-ml (nominal) serum bottles filled with approximately 125 ml of test solution with no head space. The test chambers were sealed with 20 mm Teflon® faced septa to prevent volatilization of the test substance. The test chambers were indiscriminantly positioned in a temperature-controlled environmental chamber set to maintain a temperature of 20±1°C. All surfaces that came in contact with the test substance were constructed of nylon, Teflon®, glass, stainless steel, or silicone.

The environmental chamber was maintained on a 16-hour daylight photoperiod (light intensity of 968 lux at solution surface) with 30-minute dawn and dusk simulations.

Medium-hard well water that had been aerated and filtered (0.25 µm) was used as dilution water. A typical batch of dilution water had a mean hardness of and alkalinity of 144 and 183 mg/l as $CaCO_3$, respectively, and a mean conductivity of 345 µmhos/cm, and a mean pH of 8.3.

The test solutions were prepared by adding the methyl bromide directly to the dilution water. All samples were prepared using either pure methyl bromide gas or gas diluted with gaseous nitrogen (25% methyl bromide in nitrogen). The test substance was transferred into an evacuated Tedlar bag where it vaporized and filled the bag. The needle of a gas-tight syringe was introduced into the septum of the Tedlar bag and a known volume of methyl bromide gas was removed. The gas was transferred to the test chamber by piercing the

septum of the 100-ml serum bottle that was filled with a known weight of water. The gas was bubbled into the water through the syringe needle.

C. **Dosage:** Forty-eight-hour static test. Based on results of preliminary tests, five nominal concentrations (1.3, 2.2, 3.6, 6.0, and 10 mg ai/l), and a dilution water control were selected for the definitive test.

D. **Design:** Each test concentration and the control had 4 replicate test chambers. Neonate daphnids were collected indiscriminantly one or two at a time from the cultures and transferred to 25-ml plastic cups. The daphnids were then transferred 5 at a time into the test containers below the air/water interface using a wide-bore disposable pipette. Each chamber had 5 daphnids, for a total of 20 individuals per concentration or control. The daphnids were not fed during the test. Observations of mortality and sublethal responses were made at 2, 24, and 48 hours after test initiation.

Water quality measurements for DO, pH, and temperature were made in a surrogate test chamber (replicate E of each treatment and control) at test initiation. At test termination, water quality measurements were made in the actual test chambers (replicates A,B,C, and D). Temperature was also measured continuously in an additional surrogate negative control replicate. Hardness, alkalinity, and conductivity were measured in the dilution water at test initiation.

Samples (2 ml) were taken at test initiation and termination for determination of the concentrations of the test material in the water. The samples were removed from the test chambers by gas-tight syringe and were transferred to autosampler vials for analysis.

E. **Statistics:** The binomial method was used to evaluate the EC_{50} an 95% confidence interval (CI) at 24 and 48 hours. The no mortality/immobility concentration was determined by inspection of the mortality/immobility and clinical observation data.

12. **REPORTED RESULTS:** The mean measured concentrations of were 1.2, 2.2, 3.5, 5.8, and 9.8 mg ai/l which represent 92-100% of nominal values (Table 1, attached).

No mortality or sublethal effects were observed in the control group or the lowest test concentration (Table 3, attached). Mortality at the remaining concentrations ranged from 15 to 100%.

The 48-hour EC_{50} value for daphnids exposed to methyl bromide was 2.6 mg ai/l (95% CI of 2.2-3.5 mg ai/l). The no mortality/immobility concentration was 1.2 mg ai/l.

During the test, the test solutions had a pH of 8.5-8.6, a DO range of 8.0-8.6 mg/l, and a temperature of 20.2-21.4°C.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The authors made no conclusions.

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report, indicating that the studies were conducted in accordance with 40 CFR Part 160.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedures were generally in accordance with the SEP, except for the following:

The brood number of the test daphnids was not reported. Neonate daphnids should come from the fourth or later brood of a given parent.

B. Statistical Analysis: The reviewer used EPA's Toxanal program to verify the EC_{50}. The results are the same as those of the authors (see attached printout).

C. Discussion/Results: This study is scientifically sound and meets the guideline requirements for an acute freshwater invertebrate toxicity study. Based on mean measured concentrations, the 48-hour EC_{50} was 2.6 mg ai/l which classifies methyl bromide as moderately toxic to *Daphnia magna*. The NOEC was 1.2 mg ai/l.

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. COMPLETION OF ONE-LINER: Yes, January 26, 1994.

Table 1
Summary of Analytical Chemistry Data

Sponsor: Methyl Bromide Industry Panel
Chemical Manufacturers Association
Test Substance: Methyl Bromide
Test Organism: Cladoceran, *Daphnia magna*
Dilution Water: Well Water

Nominal Concentration (mg a.i./L)	Replicate	Measured Concentration (mg a.i./L) Day 0	Measured Concentration (mg a.i./L) Day 2	Mean Measured Concentration (mg a.i./L)
Negative Control	A	ND[1]	ND	--
	B	ND	ND	
	C	ND	ND	
	D	ND	ND	
1.3	A	1.22[2]	1.17	1.2
	B	1.21	1.14	
	C	1.12	1.15	
	D	1.31	1.15	
2.2	A	2.30	2.09	2.2
	B	2.39	2.20	
	C	2.34	2.14	
	D	2.22	2.15	
3.6	A	3.26	3.29	3.5
	B	3.78	3.31	
	C	3.88	3.47	
	D	3.88	3.47	
6.0	A	6.15	5.60	5.8
	B	6.08	5.36	
	C	5.86	5.73	
	D	6.01	5.59	
10	A	10.4	8.72	9.8
	B	10.6	9.12	
	C	10.2	9.79	
	D	10.1	9.78	

[1] Not detected; peak area response was less than the lowest calibration standard (0.47 mg methyl bromide/L).
[2] Values are the calculated means of the two injections for each replicate.

Table 3
Cumulative Percent Mortality and Treatment-Related Effects[1]

Sponsor: Methyl Bromide Industry Panel
Chemical Manufacturers Association
Test Substance: Methyl Bromide
Test Organism: Cladoceran; Daphnia magna
Dilution Water: Well Water

Mean Measured Concentration (mg a.i./L)	Replicate	Daphnia/ Replicate	2 Hours			24 Hours			48 Hours			Percent Immobile and Dead
			Effects	Number Immobile	Number Dead	Effects	Number Immobile	Number Dead	Effects	Number Immobile	Number Dead	
Negative Control	A	5	5 AN	0	0	5 AN	0	0	5 AN	0	0	
	B	5	5 AN	0	0	5 AN	0	0	5 AN	0	0	0%
	C	5	5 AN	0	0	5 AN	0	0	5 AN	0	0	
	D	5	5 AN	0	0	5 AN	0	0	5 AN	0	0	
1.2	A	5	5 AN	0	0	5 AN	0	0	5 AN	0	0	
	B	5	5 AN	0	0	5 AN	0	0	5 AN	0	0	0%
	C	5	5 AN	0	0	5 AN	0	0	5 AN	0	0	
	D	5	5 AN	0	0	5 AN	0	0	5 AN	0	0	
2.2	A	5	5 AN	0	0	5 AN	0	0	4AN; 1C	0	0	
	B	5	5 AN	0	0	5 AN	0	0	4 AN	1	0	15%
	C	5	5 AN	0	0	5 AN	0	0	4 AN	0	1	
	D	5	5 AN	0	0	5 AN	0	0	4 AN	1	0	
3.5	A	5	5 AN	0	0	5 AN	0	0	--	-	5	
	B	5	5 AN	0	0	5 AN	0	0	--	-	5	100%
	C	5	5 AN	0	0	5 AN	0	0	--	-	5	
	D	5	5 AN	0	0	5 AN	0	0	--	-	5	
5.8	A	5	5 AN	0	0	--	3	2	--	-	5	
	B	5	5 AN	0	0	--	2	3	--	-	5	100%
	C	5	5 AN	0	0	--	2	3	--	-	5	
	D	5	5 AN	0	0	--	-	5	--	-	5	
9.8	A	5	5 AN	0	0	--	-	5	--	-	5	
	B	5	5 AN	0	0	--	-	5	--	-	5	100%
	C	5	5 AN	0	0	--	-	5	--	-	5	
	D	5	5 AN	0	0	--	-	5	--	-	5	

[1] Observed Effects: AN = Appears Normal; C = Lethargy.

NICOLE JURCZYK METHYL BROMIDE DAPHNIA MAGNA 01-26-94

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
9.8	20	20	100	9.536742E-05
5.8	20	20	100	9.536742E-05
3.5	20	20	100	9.536742E-05
2.2	20	3	15	.1288414
1.2	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 2.2 AND 3.5 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 2.589783

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.

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Ecological Effects Branch One-Liner Data Entry Form

Chemical Methyl Bromide Shaughnessy No. 053701 Pesticide Use Unknown

INVERTEBRATE ACUTE TOXICITY	% AI	EC_{50} (95%CL) SLOPE	HRS/ TYPE	NOEC	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1. Daphnia magna (< 24 hrs. old)	99.8%	2.6 mg ai/L (2.2 - 3.5 mg ai/L) Combined	48 - hour Static	1.2 mg ai/L	1993/1994	42938201 Core	WLI*	N.T
2.								
3.								
4.								
5.								
6.								
7.								

COMMENTS: * WLI = Wildlife International, Ltd.
Results based on mean measured concentrations.