

DATA EVALUATION RECORD

1. **CHEMICAL:** Dazomet. Shaughnessey Number: 035602.
2. **TEST MATERIAL:** Busan 1059 (Thion); 3,5-dimethyl-tetrahydro-1,3,5-2H-thiadiazine-2-thione; Lot No. PP801-177; 99% active ingredient; a white powder.
3. **STUDY TYPE:** 72-1. Freshwater Fish Acute Flow-Through Toxicity Test. Species Tested: Rainbow Trout (*Oncorhynchus mykiss*).
4. **CITATION:** Ward, G.S. 1992. Busan 1059 (Thion): Acute Toxicity to Rainbow Trout, *Oncorhynchus mykiss*, Under Flow-Through Test Conditions. Study ID No. J9203002c. Performed by Toxikon Environmental Sciences, Jupiter, FL. Submitted by Buckman Laboratories International, Inc., Memphis, TN. EPA MRID No. 426552-01.
5. **REVIEWED BY:**

Rosemary Graham Mora, M.S. Associate Scientist KBN Engineering and Applied Sciences, Inc.	Signature: <i>Rosemary Graham Mora</i> Date: <i>11 May 93</i>
--	--
6. **APPROVED BY:**

Louis M. Rifici, M.S. Associate Scientist KBN Engineering and Applied Sciences, Inc.	Signature: <i>Louis M Rifici</i> Date: <i>5/11/93</i>
Henry T. Craven, M.S. Supervisor, EEB/EFED USEPA	Signature: <i>C.E.L. 5-18-93</i> <i>H.T. Craven</i> Date: <i>8/5/93</i>
7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an acute toxicity test using freshwater fish. Based on mean measured concentrations, the 96-hour LC₅₀ for rainbow trout exposed to Busan 1059 was 16.2 mg ai/l which classifies Busan 1059 as slightly toxic to *Oncorhynchus mykiss*. The NOEC was 12.1 mg ai/l.
8. **RECOMMENDATIONS:** N/A.
9. **BACKGROUND:**
10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.



11. MATERIALS AND METHODS:

- A. Test Animals: Rainbow trout were obtained from a commercial supplier in Hampton, NH, and held in the laboratory in freshwater for 7 weeks. The holding water had a hardness and alkalinity of 60-68 and 15-25 mg/l as CaCO₃, respectively, and a temperature of 10.9-11.8°C. Fish were fed throughout the holding period except for 48 hours prior to test initiation when food was withheld.

The fish had a mean standard length of 39 ±2 mm (range of 33-43 mm) and a mean wet weight of 0.73 ±0.17 g (range of 0.37-1.03 g).

- B. Test System: The test was conducted under flow-through conditions using a proportional diluter. Test vessels were 24-l glass tanks with automatic siphons which provided a maximum solution depth of 13 cm and solution volume of 15 l. The diluter system provided 8.7 volume additions per day. Test tanks were randomly positioned in a waterbath.

The test was conducted under fluorescent lighting on a 16-hour light (intensity of 392-621 lux) and 8-hour dark photoperiod. Thirty-minute dawn/dusk simulation periods were provided.

The dilution water was carbon-treated Town of Jupiter freshwater. During the test, the dilution water had a conductivity of 415-420 μmhos/cm and a hardness and alkalinity of 52-60 and 21-23 mg/l as CaCO₃, respectively. The dilution water was vigorously aerated prior to use.

The stock solution (200,000 mg ai/l) was prepared daily by combining 20.2 g of test material with dimethyl formamide (DMF) to a final volume of 100 ml.

99.01% active ingredient

*200,000
20.2 g / 100 ml
= 99.01%*

- C. Dosage: Ninety-six-hour, flow-through test. Based on the results of preliminary testing, five nominal concentrations (5.2, 8.6, 14, 24, and 40 mg/l) were selected for this study. A dilution water control and a solvent control was also included. The concentration of solvent in the solvent control and all exposure solutions was 200 μl/l.

"The solvent concentration maintained during this test exceeded the recommended guidelines for testing because

of solubility problems observed and measured at 100 μ l DMF/l in a previous test. Although the solubility of thion in water was reported by Buckman Laboratories to be approximately 2000 mg/l, this concentration could not be achieved in the cold ($\sim 12^{\circ}\text{C}$) test water with mixing time limited by the cycling rate. The test system cycling rate was maximized to prevent degradation of the test substance. Thion solubility in the solvent, DMF, was approximately 240 g/l." ↗

- D. **Design:** The test was initiated when 20 fish were impartially added to each test chamber (i.e., 20 fish/treatment). The biomass loading rate was 0.11 g/l/day. Fish were not fed during the test.

Observations of mortality and abnormal effects were recorded daily. Dead fish were removed at each observation. Dissolved oxygen concentration (DO) and pH were measured daily in each treatment. Temperature was continuously monitored in the control chamber and in the waterbath.

Analytical determination of Busan 1059 using high performance liquid chromatography was performed on samples of each test solution collected at 0, 48, and 96 hours.

- E. **Statistics:** The 96-hour LC_{50} and its 95% confidence interval were calculated using a computer program by Wheat (1989).

12. **REPORTED RESULTS:** Mean measured concentrations were 4.22, 6.78, 12.1, 21.7, and 35.4 which represent 79-90% of nominal concentrations (Table 1, attached).

By test termination, no mortality was observed in the controls or at concentrations ≤ 12.1 mg ai/l (Table 2, attached). Total mortality was observed in the remaining exposure concentrations. Based on mean measured concentrations, the 96-hour LC_{50} was 16.2 mg ai/l with a 95% confidence interval of 12.1-21.7 mg ai/l. The NOEC was 12.1 mg ai/l.

During the test period, the test solutions had a pH range of 7.1-7.7, a temperature range of 11.5-13.6 $^{\circ}\text{C}$, a DO of ≥ 7.8 mg/l ($\geq 72\%$ of saturation).

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

No conclusions, other than those presented above, were made by the author.

Statements of quality assurance and good laboratory practice compliance were included in the report, indicating that the study was conducted in accordance with EPA Good Laboratory Practice Regulations (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 dilution water was dechlorinated municipal water. The use of dechlorinated water is discouraged because removal of chlorine is rarely complete and residual chlorine can be toxic to aquatic organisms. The residual chlorine in the dilution water was not reported as being measured. However, for this study, the use of dechlorinated water probably did not affect the results of the study, since no control mortality or sublethal effects were observed.

The concentration of solvent in the exposure solutions was 0.2 ml/l; the SEP recommends a solvent concentration ≤ 0.1 ml/l for flow-through studies.

The age of the test organisms was not reported.

- B. Statistical Analysis: The reviewer used EPA's Toxanal computer program to calculate the 96-hour LC_{50} (95% confidence interval) and obtained the same results as the author (printout, attached).
- C. Discussion/Results: This study is scientifically sound and meets the guideline requirements for an acute toxicity test using freshwater fish. Based on mean measured concentrations, the 96-hour LC_{50} for rainbow trout exposed to Busan 1059 was 16.2 mg ai/l which classifies Busan 1059 as slightly toxic to *Oncorhynchus mykiss*. The NOEC was 12.1 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, 30 April 1993.

Table 1. Measured Concentrations of Busan 1059 (Thion) During a 96-Hour Exposure of Rainbow Trout, Oncorhynchus mykiss, Under Flow-Through Conditions

Nominal Concentration (mg/L; ppm)	Measured Concentration (mg/L)				Percent of Nominal
	0 Hr	48 Hr	96 Hr	Mean	
Control	<0.12	<0.12	<0.12	<0.12	---
Sol. Control	<0.12	<0.12	<0.12	<0.12	---
5.2	4.54	3.14	4.98	4.22	81
8.6	7.35	5.04	7.96	6.78	79
14	13.1	9.30	14.0	12.1	86
24	24.3	16.3	24.5	21.7	90
40	39.3	27.3	39.8	35.4	89

QC Matrix Spikes					
MS 1	12.4	12.1	12.3	12.2	98
MS 2	12.3	12.1	12.0		

MS = Matrix spike. The matrix spike consisted of test substance in dilution water. The spike concentration was 12.4 mg/L and was conducted in duplicate.

Table 2. Mortality of Rainbow Trout, Oncorhynchus mykiss,
 Exposed to Busan 1059 (Thion) under Flow-Through Test
 Conditions

Mean Measured Concentration (mg/L; ppm)	Cumulative Number Dead (Percent Mortality)							
	24 Hour		48 Hour		72 Hour		96 Hour	
Control	0	(0)	0	(0)	0	(0)	0	(0)
Sol. Control	0	(0)	0	(0)	0	(0)	0	(0)
4.22	0	(0)	0	(0)	0	(0)	0	(0)
6.78	0	(0)	0	(0)	0	(0)	0	(0)
12.1	0	(0)	0	(0)	0	(0)	0	(0)
21.7	4	(20)	4	(20)	20	(100)	20	(100)
35.4	20	(100)	20	(100)	20	(100)	20	(100)

Zomet Rainbow Trout Rosemary Graham Mora

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
35.4	20	20	100	9.536742E-05
21.7	20	20	100	9.536742E-05
12.1	20	0	0	9.536742E-05
6.78	20	0	0	9.536742E-05
4.22	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 12.1 AND 21.7 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 16.20401.

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.
