

US EPA ARCHIVE DOCUMENT

(11-29-94)

DATA EVALUATION RECORD

- 1. CHEMICAL: S-Methoprene
Shaughnessey No. 105401
- 2. TEST MATERIAL: S-Methoprene technical; Batch No. 542-71;
90% active ingredient; a dark, amber-colored liquid.
- 3. STUDY TYPE: 154-9. Freshwater Invertebrate Static Acute
Toxicity Test. Species Tested: *Daphnia magna*.
- 4. CITATION: D. C. Suprenant. 1985. Acute Toxicity of S-
Methoprene Technical to Daphnids (*Daphnia magna*). Project
No. 10828.0884.6102.110. Prepared by Springborn Bionomics,
Inc. Aquatic Toxicology Laboratory, Wareham, MA. Submitted
by Sandoz Agro, Inc., Denton Dr., TX., EPA MRID No.
43163301.
- 5. REVIEWED BY:

Joanne S. Edwards, M.S. Entomologist Ecological Effects Branch Environmental Fate and Effects Division (7507C)	Signature: <i>Joanne S. Edwards</i> Date: 11/22/94
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- 6. APPROVED BY:

Leslie W. Touart, Ph.D. Supervisory Biologist Ecological Effects Branch Environmental Fate and Effects Division (7507C)	Signature: <i>L. L. T.</i> Date: 11/29/94
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- 7. CONCLUSIONS: This study is classified supplemental due to
too few test organisms per concentration and lack of
reporting of residues of s-methoprene at the end of the
study. Based on measured concentrations, the study author
reported a 48-hour LC₅₀ for s-methoprene of 360 ppb (95%
C.I.= 210 - 550).
- 8. RECOMMENDATIONS: N/A.
- 9. BACKGROUND: Miscellaneous study submitted for review. The
EEB has an acceptable invertebrate acute toxicity study on
file (1991 Methoprene RED; LeBlanc (1975), MRID 00010388;
48-hour LC₅₀ 89 ppb).
- 10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

- A. Test Animals: *Daphnia magna* neonates (<24 hours old) were obtained from in-house cultures. The daphnids were cultured in the same dilution water and at the same temperature as used in the test. Daphnids were fed a solution of green algae (Ankistrodesmus sp. or Selenastrum sp.) and yeast suspension daily.
- B. Test System: The static test was conducted using 250 ml glass beakers. Test solution depth was 6.2 cm with a surface area of 33 cm². Three control beakers contained the same dilution water, and three control beakers contained the maximum amount of DMF (0.5 ml/l) present in any test solution.

A 16-hour light photoperiod was provided. Light intensity at the test solution surface was 5-10 hectolux and was provided by florescent lighting.

The culture water was prepared by fortifying well water and filtering it through a carbon filter and an Amberlite XAD-7 resin. At test initiation, the water was characterized as having a conductivity range of 400-600 μ mhos/cm, a pH range of 7.9-8.3, a temperature of 20 \pm 1°, a hardness range of 160-180 mg/l as CaCO₃, and an alkalinity range of 110-130 mg/l as CaCO₃. The dissolved oxygen concentration was greater than 60% of saturation. The solutions were not aerated.

Preliminary testing showed that \leq 0.45 mg/l s-methoprene technical contained a surface film of insoluble material. The daphnids exposed to these solutions died within 24 hrs (probable cause was physical effects of entrapment, e.g. desiccation, high levels of insoluble material). Therefore, the definitive test solutions were prepared using only the water soluble portion of s-methoprene technical. The primary stock solution was prepared by mixing s-methoprene technical with dimethyl-formamide (DMF). A 1.5 ml aliquot of the stock solution was added to the dilution water and stirred for one hour, and then left undisturbed for one hour. A 2.5 l of the solution was transferred via the bottom drain to a second glass bottle. This solution was left undisturbed for 24 hours and then 2 l were removed via the bottom drain for use as the primary stock solution. The test solutions were made by adding appropriate volume of the stock solution to the dilution water to total 10000 ml.

- C. **Dosage:** Forty-eight-hour, static toxicity test. Based on preliminary testing showing that exposure solutions of ≤ 0.45 ppm of s-methoprene technical containing a surface film of insoluble test material, a method was employed (see discussion above) to separate the water soluble portion of s-methoprene. Nominal concentrations were 1.0, 0.60, 0.36, 0.22, 0.13, and 0.078 mg/l.
- D. **Design:** Five daphnids (≤ 24 hours) per replicate/three replicates per concentration. The daphnids were impartially distributed into the glass beakers within 20 minutes of test solution preparation. Mortalities were recorded at 24 and 48 hours of exposure. Biological observations and observations of physical characteristics were made at 0, 24, and 48 hours. The daphnids were not fed during the test.

The dissolved oxygen concentration (DO) and pH were measured in one replicate vessel of the high, middle and low treatment levels and the solvent control and control at the beginning of the test and at 48 hours. The temperature was measured in one replicate beaker of the control at 0, 24, and 48 hours of exposure.

Test solution samples were collected from each test chamber at 0 hours for analysis of s-methoprene technical. The concentration in the samples was determined using gas liquid chromatography with flame ionization detection.

- E. **Statistics:** The authors employed a program the Stephan (1982) computer program to calculate the 48 hour LC_{50} value.

12. **REPORTED RESULTS:** The measured concentrations were 0.97, 0.55, 0.32, 0.21, 0.12, and 0.064 mg/l (Table 1, attached), and ranged from 82-97% of their nominal values. The measured concentrations were used for the LC_{50} determination.

Data on mortalities and biological observations are provided in Table 2. There was no mortality observed in the solvent control or dilution water control group throughout the test. By test termination, mortality in both the 0.97 and 0.55 ppm treatment groups was 100%. In the next lower group, (0.32 ppm), mortality was 33%. Several of the surviving daphnids were reported as lethargic and on the bottom of the test vessel, while several other were reported as at the test solution's surface. One daphnid was reported as lethargic and on the bottom of a vessel in test group 0.21 ppm.

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

The 48-hour LC₅₀ value based on measured concentrations was 0.36 ppm (95% C.I.= 0.21-0.55).

A 1985 Quality Assurance statement was included in the report, indicating that the study was conducted in accordance with protocols, SEPs and pertinent EPA Good Laboratory Practices. A statement was included in the report (signed by the Senior Registration Specialist/Sandoz Agro., Inc.) indicating that the Sandoz does not know whether the study was conducted in accordance with 40 CFR Part 160.

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

A. Test Procedure: The test procedures deviated from the SEP as follows:

First instar *Daphnia magna* used in tests should be from the fourth or later broods of a given parent. The authors did not indicate which brood was the source of the test animals.

There was only one measured test concentration per treatment level analyzed for s-methoprene. Residues found at the beginning and end of the study should be reported.

It was not indicated if the beakers were covered during the study to prevent evaporation.

Temperature was measured in one replicate beaker of the control at 0, 24, and 48 hours of exposure. It should have been recorded continuously.

Chemical characteristics of the water were not provided.

A minimum of 20 daphnids/level is recommended. There were only 15/treatment level employed in this study.

A hardness range of 160-180 mg/l as CaCO₃ was reported. The SEP recommends a range of 40 to 48 mg/l as CaCO₃.

Only an EC₅₀ cannot be calculated because the study authors failed to adequately record the biological observations (e.g. several lethargic daphnids were noted, instead of \bar{X} number of daphnids were reported as lethargic).

- B. **Statistical Analysis:** The reviewer used EPA's Toxanal computer program to determine the 48-hour EC₅₀ value. The results confirmed those of the authors' (see attached printout).
- C. **Discussion/Results:** This study is classified as supplemental since the number of test organisms per treatment level (15) was lower than the minimum number recommended for this study (20). Since an inadequate number of test organisms per test level can produce unreliable results, this study is classified supplemental. In addition, the concentrations of methoprene should also have been measured at the end of the study. In this study there was only one test concentration per treatment level analyzed at 0 hour.
- D. **Adequacy of the Study:**
- (1) **Classification:** Supplemental.
 - (2) **Rationale:** The number of test organisms per treatment level was lower than the minimum number recommended for this study. Samples should have been analyzed both at the beginning and at the end of the study.
 - (3) **Repairability:** No.
15. **COMPLETION OF ONE-LINER FOR STUDY:** No

jedwards methoprene acute oral daphnid

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.97	15	15	100	3.051758E-03
.55	15	15	100	3.051758E-03
.32	15	5	33.33334	15.08789
.21	15	0	0	3.051758E-03
.12	15	0	0	3.051758E-03
.064	15	0	0	3.051758E-03

THE BINOMIAL TEST SHOWS THAT .21 AND .55 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 .3555731

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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The material not included contains the following type of information:

- Identity of product inert ingredients.
- Identity of product impurities.
- Description of the product manufacturing process.
- Description of quality control procedures.
- Identity of the source of product ingredients.
- Sales or other commercial/financial information.
- A draft product label.
- The product confidential statement of formula.
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