Accession # 415068-01

DATA EVALUATION RECORD

1. **CHEMICAL:** 0,0-Dimethyl-0-4-nitrophenyl)thiophosphate. Shaughnessey No. 035601.

2. **TEST MATERIAL:** Parathion-methyl Technical. A brown liquid, 96% Active, Batch #233 690 479.

3. **STUDY TYPE:** Freshwater invertebrate chronic test. Species Tested: Daphnia magna.

4. **CITATION:** Heimbach, F. 1987. Influence of Parathion-methyl on the Reproduction of Water Fleas (Daphnia magna). Prepared by Bayer AG, Crop Protection-Research, Chemical Product Development and Environmental Biology, Institute for Environmental Biology, Leverkusen, Bayerwerk. Submitted by A/S Cheminova, Denmark. Accession No. 415068-01.

5. **REVIEWED BY:**

Mark R. Roberts
Wildlife Biologist
EFED/EEB

Signature: Mark R. Roberts
Date: 4.19.91

6. **APPROVED BY:**

Charles Lewis
Acting Head, Section III
EFED/EEB

Signature: Charles Lewis
Date: 4-23-91

7. **CONCLUSIONS:** The data submitted do not fulfill the Guideline requirements for a freshwater invertebrate chronic test. Based on adult survival, growth, and reproductive performance, the MATC of methyl parathion for Daphnia magna appears to be, at a minimum, > 178 ng ai/L and < 562 ng ai/L nominal concentrations. However, because of the semi-static study design, inconsistent measured concentrations, and questionable stability analyses, the actual concentrations tested could not be determined.

8. **RECOMMENDATIONS:** Submit a new flow-thru chronic invertebrate life-cycle study with appropriate measured concentration and stability data.

9. **BACKGROUND**: N/A.

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

11. **MATERIALS AND METHODS**:

A. **Test Animals**: Daphnia magna used in this test were obtained from The Federal Department of Health in Berlin. Instars 6 to 24 hours old were obtained from the parents of the stock culture. The test daphnids were fed a diet consisting of green algae (Scenedesmus subspicatus) as well as an aqueous suspension of a commercial toy fish food (trade name TETRAMIN).

B. **Test System**: The test was conducted in 100ml glass beakers covered with glass plates. A photoperiod of 16.8 hours of light: dark was provided. Test temperature was maintained at 20 $\pm$ 1°C.

Culture and test dilution water for the Daphnia magna was prepared by adding $CaCl_2$ x $2H_2O$ p.a. (0.08 mole/L), $MgSO_4$ x $7H_2O$ p.a. (0.02 mole/L), $NaHCO_3$ p.a. (0.03 mole/L), and KCl p.a. (0.003 mole/L) to deionized water. The water was characterized as having a pH range of 7.8 $\pm$ 0.2, total hardness range of 250 $\pm$ 25 mg/L as $CaCO_3$, and a conductivity of 580 $\pm$ 30 umhos/cm.

C. **Dosage**: 21-day life-cycle test. Test concentrations were selected on the basis of the results of a range finding test. The following nominal test concentrations were selected: 10, 18, 32, 56, 100, 178, and 562 ng ai/L (ppt).

D. **Design**: One first instar D. magna ($\leq$ 24 hours old) was placed into each test vessel with 20 individuals per replicate to initiate the test. After 7 days, the number of animals was reduced by randomized sampling to 10 females per replicate. These 10 female daphnids were then exposed semistatically to the respective test concentrations. Three times a week (Mondays, Wednesdays, and Fridays on days 2, 5, 7, 9, 12, 14, 16, and 19 after test initiation) the adult female daphnids were transferred into freshly prepared test medium. For this purpose, each daphnid was drawn into a blunt pipette and placed into a freshly prepared test vessel. At these transfers and on days 20 and 21, the number of dead and living offspring were counted and removed. Each time the test animals were transferred, the test

concentrations were prepared as follows: 10.4 mg of technical Parathion-methyl was dissolved in 1000 ml of test water (stock solution I) and 1 ml of this solution was then diluted to 1000 ml with test water (stock solution II). The appropriate amount of stock solution II (1.0, 1.8, 3.2, 5.6, 10, 17.8, and 56.2 ml) was then further diluted to 1000 ml to derive the actual testing solutions.

Prior to each transferral, the oxygen content and the pH value were measured in the freshly prepared test solutions of the control, highest and lowest test concentrations. After the 2-3 day exposure period, these values were once again measured at the control, highest and lowest concentrations. No actual measurements of total hardness, alkalinity, and specific conductivity of test solutions were taken.

Concentrations of Parathion-methyl in each test concentration were analytically measured twice during the test (i.e. on days 0 and 12). This was accomplished by preparing twice the amount necessary and shipping one-half of the preparation to the Institute for Residue Analysis. In addition to these test preparations, the stock solutions and a control were also submitted for analysis.

The test was evaluated 3 times a week at the times of transferral. On days 2, 5, 7, 9, 12, 14, 16, 19, 20, and 21 the surviving parental daphnids were counted, and after day 7, the offspring were also counted during each transfer. Additionally, the body lengths of the parent daphnids were measured on day 21.

E. **Statistics:** Two separate statistical evaluations of the data were provided, 1 in the original report, and 1 as an amendment.

Original Report: Total numbers of offspring per parent and body lengths of adults were analyzed by the R/s test to determine if a Gaussian distribution (condition of the t-test) could be assumed. When the results indicated that a Gaussian distribution could not be assumed, the U-test of Wilcoxon, Mann and Whitney (non-parametric) was used. All statistical conclusions were made at the 95% level of certainty.

Amended Report: The data were reanalyzed using the Kolmogorov-Smirnov one-sample test for goodness of fit to a normal distribution. If this condition was met, then different tests for homogeneity of variance (Bartlets test,

AVOVA) were used to determine significance. If the condition was not met, then the non-parametric method of Kruskal-Wallis was used.

12. **REPORTED RESULTS:**

The pH and oxygen saturation ranged from 7.98 to 8.91 and 96.0 to 105.8, respectively (Table 1). The number of surviving parent daphnids is summarized in Table 2. The average number of offspring produced per female was reported and summarized in Table 12.

Percent survival of parent offspring is presented in Table 3. On day 2 all concentrations suffered a certain loss (mean, 10%). As the same number of animals died in the untreated control, the result was not contributed to the test substance. No further mortality occurred after day 5 of exposure except at the highest concentration which accumulated to 40% mortality by test termination.

The number of offspring produced per female in each concentration is summarized in Tables 4-11.

Individual measurements of adult body lengths at test termination are given in Table 14.

Original Report Statistics:

Summary statistics for number of offspring produced per adult are provided in Table 12. Since a Gaussian distribution could not be assumed (R/s test; $p \geq 0.05$) the data were analyzed with the U-test. This analysis is presented in Table 13 and shows a significant decrease in number of offspring produced at the highest treatment level.

Statistical evaluation of adult daphnid body lengths is summarized in Tables 15 and 16. There was a significant reduction in adult body length at the highest concentration level (U-test; $p \leq 0.05$).

Amended Report Statistics:

Survival of parent daphnia was significantly affected at the highest concentration level (Chi-square 2X2 Contingency Table A1; $p = 0.025$). As significant survival effects were obtained at the highest concentration level, this concentration was excluded from statistical analyses of reproduction and growth data.

Reproductive data showed normal distribution as tested by the Kolmogorov-Smirnov goodness of fit test. Significant differences were not detected by ANOVA, Barletts test, or by the non-parametric method of Kruskal-Wallis (Tables A3, A4, A5).

Adult daphnid growth data was shown to fit a normal distribution. Therefore, ANOVA and Barletts test was the preferred method of analysis and showed no significant differences (Table A8).

Water residue analysis data is presented in Tables 17 and 18. Measured concentrations ranged from 90 to 160% of nominal concentrations.

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

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report, indicating that the study was conducted in accordance with the FIFRA Good Laboratory Practice Standards set forth in 40 CFR Part 160.

Original Report:
The highest concentration tested without effects (NOEC) was 178 ng ai/L (ppt) during the 21 day exposure period. The lowest concentration tested with effects (LOEC) was 562 ng ai/L (ppt).

Amended Report:
Results based on nominal concentrations:
NOEC = 178 ng ai/L
MATC = 316 ng ai/L
Results based on mean measured concentrations:
NOEC = 166 ng ai/L
MATC = 343 ng ai/L

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

A. **Test Procedure:** The report deviated from the SEP and ASTM guidelines as follows:

o The SEP states that prior to test initiation, 10-12 day old daphnids should be maintained for at least 21 days to ensure good health and test conditions are present. No acclimation data was provided in the study report.

o No information regarding temperature monitoring was reported. It was simply reported that temperature was maintained at 20 ± 1°C.

o No actual measurements of hardness, alkalinity, or conductivity of the test water were taken. The SEP states that these parameters must be analyzed at least once a week.

o The SEP recommends test vessels of 250 ml. Test vessels in this study were 100 ml glass beakers.

B. **Statistical Analysis:** Statistical analyses of the data were not recalculated by the reviewer because the computer printouts for all analyses were included in the report. Furthermore, both parametric and non-parametric evaluations conducted by the authors statistically confirmed a very visible decrease in all parameters at the highest concentration tested.

C. Discussion/Results: The analytical results provided in the report contain conflicting information. Table 17 suggests that the range of measured concentrations recovered from test samples is from 90 to 160% of nominal concentrations. However, as indicated in the attached summary of analytical results from the Institute for Residue Analysis (RA-546, File MTH120), this range is from 90 to 245%. Furthermore, the summary Table 17 provides mean measured results for the 100 ng ai/L treatment level whereas the information from the attached laboratory analysis does not show that the 100 ng ai/L treatment level was analyzed. The only analysis of the 100 ng ai/L treatment group confirmed in the attached report was the 2 analyses of stability measured after 72 hours of exposure. This stability data is essentially meaningless since the initial measured concentrations in these 2 replicates was not reported. New test media was prepared 8 separate times during this 21-day life cycle study. Although there were measured concentrations in most treatment dosages for 2 separate media preparations (n=12 or 14, depending on which table), the sample size is much too small and the results are much too variable to confirm actual concentrations of test material. With such minimal and variable data, it is not appropriate to calculate means and surmise at actual concentrations. Although most measured concentrations were in excess of the nominal values, stability information is certainly lacking. Therefore, the data submitted do not fulfill the Guideline requirements for a Daphnia magna chronic toxicity test.

Based on adult survival, growth, and reproductive performance, the MATC of methyl parathion for Daphnia magna appears to be at least $\geq$ 178 ng ai/L and $\leq$ 562 ng ai/L nominal concentrations. However, because of the semi-static study design, inconsistent measured concentrations, and questionable stability analyses, the actual concentrations tested could not be determined.

D. Adequacy of the Study:

(1) **Classification:** Supplemental.

(2) **Rationale:** Because of the semi-static study design, inconsistent measured concentrations, and questionable stability analyses, the actual concentrations tested could not be determined. Furthermore, data cited in section 14A of this review were either not recorded or reported.

(3) **Repairability:** No.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 4-9-91.

Methyl parathion DER (R2026406)

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