

CASE GS0097 CHLOROTHALONIL

PM 400 6-24-83 08/03/82

CHEM 081901

Chlorothalonil (tetrachloroisophthalon

BRANCH EEE DISC 40 TOPIC 05103043

FORMULATION

FICHE/MASTER ID RI 0 CHL 05 CONTENT CAT

Surprenant, D., L. Altshal, G. LeBlanc and J. Mastone. 1981. The chronic toxicity of t-117-11 to the water flea (Daphnia magna). Research report submitted by Diamond Shamrock Corp. Accession # 071097.

SUBST, CLASS = S,

DIRECT RVN TIME = (MH) START-DATE END DATE

REVIEWED BY: Daniel Rieder  
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Data Evaluation Record

1. Chemical: Tetrachloroisophthalonitrile  
(chlorothalonil)
2. Formulation: Analytical (99.8% a.i.)
3. Citation: Surprenant, D., L. Altshal, G. Leblanc and J. Mastone.  
1981. The chronic toxicity of T-117-11 to the water flea  
(Daphnia magna). Research report submitted to Diamond Shamrock  
Corp., Plainessville, Ohio, by EG&G Bionomics, Wareham, Mass.  
Report No. FW-81-10-1031. Registration No. 677-313.  
Accession No. 071097.
4. Reviewed By: John J. Bascietto  
Wildlife Biologist  
EEB/HED
5. Date Reviewed: Nov. 3, 1982
6. Test Type: Chronic toxicity - Aquatic Invertebrate  
life-cycle.

A. Test species: Daphnia magna (water flea)

7. Reported Results:

MATC for this compound is >50 <100 ug/l (nominal). Numbers of offspring produced per female is significantly reduced at 100 ug/l during the first generation. Numbers of offspring produced per female is significantly enhanced at 100 ug/l in the second generation.

8. Reviewer's Conclusions:

The MATC for this compound is >39 <79 ug/l  
(analytical).

In stating their conclusions from the data the authors stated exactly what was observed but neglected to qualify their conclusions with very important material facts. Because of very severe acute mortality, reproduction with survival is basically precluded at the 100 ug/l exposures for both generations. The authors should have interpreted their numerical results in light of the episode involving the

substitution of F<sub>1</sub> controls to act as P<sub>2</sub> generation at 100 ug/l (because F<sub>1</sub> - 100 ug/l daphnids were all dead). This significantly altered the experimental conditions and invalidates comparisons with the first generation. At 100 ug/l the "second" generation had no previous exposure history (because they were previously controls).

Regarding the enhancement of production of offspring, while it is true that significantly more were produced at 100 ug/l in the "second" generation, as compared to controls, no significant difference in production of F<sub>2</sub> offspring was observed with respect to the F<sub>1</sub> controls. This would cast doubt on a causal link between toxicant concentration and "enhancement" of production of offspring. Other factors, like the increased availability of food due to acute mortality, could be invoked to explain the observed increase in production of F<sub>2</sub> at 100 ug/l.

The study is scientifically sound and fulfills the requirement for an aquatic invertebrate life-cycle study.

## Materials / Methods

### A. Test Procedures:

Stock solutions of T-117-11 (toxicant) were prepared on test days 0, 10, 22 and 32 in acetone.

Test methodology was based on EPA's 1975 publication - "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians" (EPA 660/3-75-009; 1975) and Diamond Shamrock protocol "chronic toxicity study in *Daphnia magna* with T-117-11" (protocol document # 447-STX-81-0006 -000) (appended to the lab report). Selection of the test concentrations was based on a range-finding acute toxicity study as per EPA approved protocol.

Diluent water was that recommended as "reconstituted fresh water", as per EPA 660/3-75-009 (pH = 7.8 - 8.2).

Chronic procedure - A 200 ml Mount & Brungs proportional diluter, calibrated to provide 50% dilutions, delivered the diluent water and toxicant to test aquaria. These were glass battery jars with 1.75 liter volume. Test solutions drained from aquaria through 40 mesh screen to retain daphnids. Five toxicant concentrations were employed. One solvent control (acetone  $\leq 46$   $\mu$ l/l) and one fresh water control were also employed. All treatments and controls were run in triplicate. Test solutions were delivered to aquaria at five volumes per day.

D.O. and water temperature were monitored every week day. Total hardness, alkalinity, specific conductance and pH were monitored weekly. Photoperiod was 16 hrs light/8 hrs darkness (fluorescent).

Initial toxicant concentrations of all levels tested were analytically determined four days prior to test, as well as during test - i.e., weekly on two replicates (200 ml samples per replicate).

Daphnids were cultured at EG&G Bionomics in same reconstituted fresh water as used for diluent. Twenty *D. magna* ( $< 24$  hrs. old) were randomly assigned to each test aquarium. Adult ( $P_1$ ) survival was determined weekly. Offspring production was checked each weekday from day 7 through 21 of each generation. On day 21, twenty (20)  $F_1$  offspring from each test vessel were retained and used to initiate the second generation (i.e., they became  $P_2$ ). Daphnids were fed a fish food suspension and unicellular algae ( $2-5 \times 10^7$  cells/ml). Food was introduced into aquaria at 2 ml fish food + 1 ml algae three times daily on weekdays and once on weekends. Test chambers were rinsed with diluent water, followed by replacement of original test solution, whenever survival and reproduction were assessed.

### B. Statistical Analysis

The author's used Stephan's computer program (same as EEB's) to estimate  $LC_{50}$ 's. Weekly survival data were ARC SIN transformed and the determinations of cumulative production of offspring per female were subjected to analysis

of variance (ANOVA). Dunnett's procedure was used if  $p=0.05$  was obtained by the ANOVA, to determine which treatments varied from controls. The MATC was defined as the "maximum amount.... which would not elicit an adverse response..... during the chronic toxicity test".

## 10. Results -

### Chemistry - (See Table I)

Measured concentrations (analytical) of toxicant ranged from 54-79% of nominal throughout exposure (results reported in nominal concentrations).

Water quality parameters varied minimally between treatment levels throughout exposure (Table II).

### Biological

Acute Phase - Mortality in Range - finder test

TABLE III

| Nominal concentration<br>(ug/l) | Percent mortality |                 |
|---------------------------------|-------------------|-----------------|
|                                 | 24-hr.            | 48-hr.          |
| Control (0)                     | 0                 | 0               |
| Solvent control (0)             | 0                 | 0               |
| 66                              | 0                 | 0               |
| 110                             | 0                 | 47              |
| 180                             | 67 <sup>a</sup>   | 93 <sup>a</sup> |
| 300                             | 100               | 100             |
| 500                             | 100               | 100             |

<sup>a</sup> Daphnids Lethargic

24-HR  $LC_{50}$  + 95% c.i. = 180 (110-300) ug/l

48-HR  $LC_{50}$  + 95% c.i. = 120 (99-140) ug/l

Select 100, 50, 25, 12, and 6.2 ug/l as Chronic test concentrations.

Chronic Phase -

### 1st Generation ( $P_1$ & $F_1$ )

TABLE IV shows that daphnid survival at 100 ug/l was significantly reduced during week 1, with continued decline in survival throughout the test.

Reduced survival on Day 21 at 25 ug/l was not considered treatment related because:

- 1) it was comparable to survival of solvent control
- 2) the effect was not observed of the next higher treatment level, i.e., 50 ug/l

so that, all concentrations  $\leq$  50 ug/l had survival comparable to controls.

Table V shows that cumulative number of offspring produced per female was significantly reduced at 100 ug/l as compared to both controls. A similar reduction in this parameter at 50 ug/l on days 17 and 18 was observed but it should be noted that this parameter, (at 50 ug/l) was comparable to that of the freshwater control on days 17 and 18.

#### 2nd Generation (P<sub>2</sub> & F<sub>2</sub>)

Since only one (1) daphnid survived as a P<sub>1</sub> at 100 ug/l, an insufficient number of offspring (F<sub>1</sub>) were produced by this individual on test day 21. Thus no F<sub>1</sub> generation existed on day 21 to use as the P<sub>2</sub> for the second generation exposure at 100 ug/l. The author's instead used excess F<sub>1</sub> daphnids from the control vessels on day 21. Table VI shows that survival of daphnids exposed to 100 ug/l was again significantly reduced as compared to controls during the first week, and subsequently. Survival of daphnids exposed to  $<100$  ug/l was comparable to controls.

No adverse effects on offspring production at any level were observed (Table VII). Surviving daphnids exposed to 100 ug/l produced significantly more offspring per female than controls by day 42. The authors speculate that this maybe due to increased availability of food because of the decreased number of daphnids due to acute effects [N.B. - the second generation's exposure was initiated with older daphnids ( $< 72$  hrs old)]. At 100 ug/l the P<sub>2</sub> had not been previously by exposed, as had daphnid parents at the lower levels ].

The author's stated that the "MATC" for this compound is  $> 50 < 100$  ug/l. The highest "no effect" level observed during chronic exposure, i.e. 50 ug/l is virtually the same as that observed during acute exposure (66 ug/l).

#### 11. Reviewer's Evaluation

A. Test Procedure: procedures used were based on accepted EPA protocols and or acceptable Diamond Shamrock protocol as noted earlier. The major problems in procedure are:

- 1) use of 72-hr old daphnids to initiate the 2nd generation exposure - this may invalidate comparisons with offspring production from the 1st generation exposure, which used the  $< 24$ -hr old 1st instars to initiate exposure (24-hr 1st instars are preferred and recommended).

- 2) the use of survivors from control vessels of generation #1 to use as the P<sub>2</sub> generation at 100 ug/l invalidates the results (for comparative purposes) at this level because the exposure history is completely different. This is especially important because the report indicates (p. 11 - also known as p. 000049) that reproduction was enhanced at 100 ug/l during the 2nd generation.

- B. Statistical Analysis - acute studies used EEB's Stephan's program. Validation of chronic data was unnecessary as the raw data clearly indicated significant differences as discussed by the authors. Second generation results cannot be used for comparison so these were not tested with respect to the first generation (see below).

C. Results

Since actual levels producing adverse effects are 50 to 79% of the nominal concentrations reported, the actual MATC range is reduced to:

(actual) MATC >39 <79 ug/l

LC<sub>50</sub>'s would be extrapolated down to:

(actual) 24-hr LC<sub>50</sub> = 142.2 ug/l

(actual) 48-hr LC<sub>50</sub> = 94.8 ug/l

Adverse reproductive effects can be expected to occur at the adjusted MATC of >39 <79 ug/l. "No effect" levels are expected to be: 39.5 for chronic; 52.1 for acute (ug/l).

The results of the second generation reproduction cannot be used to indicate lack of a reproductive effect at 100 ug/l and also may not be used to attribute enhancement of reproduction to the treatment as was inferred (former point) and stated (latter point) by the authors. EEB rejects these claims for reasons partially indicated in the "Reviewer's Evaluation" of test procedures, A-2 (above). Clearly these claims are exaggerated because the basis of comparison, i.e., the parental generation exposure, is completely lacking at the beginning of the second generation for the 100 ug/l treatment group. Also production per P<sub>2</sub> female at 100 ug/l was about the same as that for P<sub>1</sub> control females.

Parsimony requires us to conclude that acute effects at 100 ug/l are so severe, that exposure of daphnids to these levels would preclude reproduction of viable offspring. No further reproductive information would be gained by repeating this study.

D. Conclusions

1. Category: Core
2. Rationale: Fulfills the Guidelines requirement for an aquatic invertebrate life-cycle study
3. Repair: N/A