

US EPA ARCHIVE DOCUMENT

DATA EVALUATION RECORD

1. CHEMICAL: Parathion (ethyl)
2. TEST MATERIAL: Parathion (ethyl)
3. STUDY TYPE: §72-4 Aquatic Invertebrate life-cycle
4. CITATION:

Author: Kühn, R., Pattard, M., Pernak, K., Winter, A.
 Title: Results of the Harmful Effects of Water
 Pollutants to *Daphnia magna* in the 21 Day
 Reproduction Test, WAT. RES. Vol. No. 4 pp.
 501-510

Laboratory Report #: N/A

Any Other Study #: N/A


Sponsor: None (from open literature)

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5. REVIEWED BY:

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Signature: 

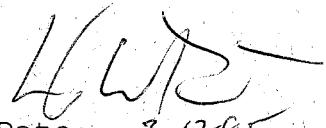
Environmental Fate and Effects Division (H7507 C)

Date:

February 16, 1995

6. APPROVED BY:

Les Touart, Chief, Section 1
 Ecological Effects Branch

Signature: 

Environmental Fate and Effects Division (H7507C)

Date:

3-17-95

7. CONCLUSION

For parathion, this study does not meet the guidelines, however, may provide some useful information. The study did not address the required endpoints, such as, the following:

1. production of young by first generation daphnids at various times for each treatment
2. the dry weight of first generation daphnids at the end of the test
3. the length of first generation daphnids
4. purity of test material
5. Tap water was used rather than well water or reconstituted water.



The parathion test results were 0.002 mg/L for the 24 hour EC₅₀ and 2 ppt for the 21 day NOEC based on parent mortality, reproduction rate, and appearance of first offspring. See attached copy of table showing results for the other chemicals.

8. RECOMMENDATIONS

N/A

9. BACKGROUND

This study investigated 73 "environmentally relevant substances". This review was prompted by the Special Review of parathion.

10. MATERIALS AND METHODS

A. Test Organisms:

Guideline Criteria	Reported Information
Species (Scientific Name)	<i>Daphnia magna</i>
All organisms should be approximately the same size and weight.	separated using seive sizes 0.315 mm DIN and 0.630 mm DIN
Immature organism should be used. Daphnids 1 st (<24hrs). Amphipods, stoneflies, and mayflies in 2 nd instar; midges 2 nd & 3 th instar	"at most 24 hours old"
Supplier	culture maintained since 1978
All organisms from same source (yes or no)	yes
Other Comments	None

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Maintain 10-12 days old culture for at least 21 days to insure good health	laboratory's own culture

daphnids ≤24 hrs old from 21 day old culture	N/A
Ten - 250 ml beakers (200 mls of test solution) are used each toxicant concentration: a) 7 beakers @ each conc. with 1 daphnid each for collection of survival, growth, & reproduction; b) 3 beakers @ each conc. will contain 5 daphnids each for collection of data on survival only.	1 animal/50 ml, 5 animals/250 ml container - in the case of volatile substances 250 ml wide-neck bottles with ground glass stoppers were used otherwise 400 ml beakers were used.
Assignment of daphnids random?	not reported
1) Synthetic food 2) Synthetic foods with alfalfa yeast and algae 3) Algae	Tetra-Hauptfutter and activated sludge were used as feed.
<u>Renewal</u> Monday, Wednesday, Friday Count live & dead. All parents counted & transferred. 7 beakers 1 parent/beaker, count and discard offspring both live & dead.	Transferred on monday, wednesday and friday offspring counted and total number for each vessel recorded.
<u>Duration</u> stop testing on 21 st day, count parents & indivisually measured to the nearest 0.01 mm from the apex of the helmet to the base of the spine. Count the # of young, both alive & dead, in each beaker.	21 days, no length measurements were made at the end of the study. The # young were counted.
<u>Test Rejection</u> 1. 30% of controls die 2. Must produce 40 after 21 days 3. Production of ephippia in controls 4. Temp. Δ5°C <>20°C in 48 hrs 6. pH Δ 1 unit after 48 hrs	"Parent animal mortality" after 21 days was 7.1% in beakers and 9.1% in bottles. reproduction rate per parent was 88.8 for beakers and 68 for the bottles. Production of ephippia not reported. Temperature area was set at thermostatically at 25±1°C. "based on 8.0±0.2 -lower than 7.0..."

<p><u>Data endpoints</u> Survival of 1st F, production of young, dry weight of 1st F</p>	<p>The endpoint was the highest concentration level, which did not differ (NOEC) from the control, and the lowest, which did differ from the control in respect to parental mortality reproduction rate, and appearance of first offspring.</p>
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C. Test System:

Guideline Criteria	Reported Information
<p>Describe source of dilution water (prefer soft reconstituted water) o Test for pesticides o Hardness 160 to 180 mg/L as CaCO₃</p>	<p>Reconstituted water 11.76 g CaCl₂·2H₂O(A.R.)/1 litre deionized water 4.93 g MgSO₄·7H₂O(A.R.)1 litre deionized water 2.59 g NaHCO₃ (A.R.)/1 litre deionized water 0.23 g KCL (A.R.)/1 litre deionized water dilution water was diluted with 10% tap water.</p>
<p>Water Temperature Life-cycle @ 20±2°C</p>	<p>20 °C</p>
<p>pH 7.6-8.0</p>	<p>8.0 ± 0.2</p>
<p>Dissolved Oxygen Dilution H₂O should be aerated to 90 or 100% Test tanks should not be. D.O.should not be >50% for any 48 hrs period</p>	<p>Water was aerated up to water saturation level.</p>
<p>Total hardness (160 to 180 mg/L as CaCO₃ well water)</p>	<p>Not Reported</p>
<p>Test Aquaria 1. Material (glass or stainless steel or perfluorocarbon plastics) 2. 250 ml glass beakers 200mls are typical 3. Glass plates prevent evaporation</p>	<p>400 ml beakers with 250 ml useful capacity with watch glass covers and 250 ml glass wide-neck bottles with ground glass stoppers for volatile substances.</p>

Type of Dilution System (Reproducible supply of toxicant)	Static renewal
Photoperiod (16 L & 8 D) Intensity 400 to 800 Lux (37 to 74 footcandles)	9 L & 15 D
Solvents Do not exceed 0.5 ml/L for static tests. Acceptable Solvents Dimethylformamide Triethylene glycol Methanol Acetone Ethanol	10mg ethanol/100ml or 100 mg/L
Other Comments	

D. Test Design:

Guideline Criteria	Reported Information
<u>Range Finding Test</u> (LC ₅₀ >100 mg/L with 30 fish, no definitive test required.)	24hr EC ₅₀ 0.002 mg/l
<u>Definitive Test</u>	
Nominal Concentrations (control+5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be geometric series)	dilution series ratio 1:2 11 treatment levels
Controls (Minimum control mortality; static 10%; flow-through 5%)	parent control mortality 7.1% in beakers: 9.1% in bottles.
Number of Test Organisms; (Minimum 20/level can be divided among containers)	20 daphnids per level 1/50ml in 250 ml per vessel, 4 vessels per level
All organisms must be randomly assigned to test vessels. (yes or no, describe if no)	Not reported

<p>Water Parameter Measurements</p> <p>1. Temperature - record every 6 hrs; >1°C.</p> <p>2. D.O. beginning, 48 hrs, end for control high, medium, and low dose.</p> <p>3. pH beginning, 48 hrs, end for control, high, medium, and low dose.</p>	<p>oxygen and pH were measure on transfer days- Mondays, Wednesdays, and Fridays</p> <p>pH range 8 to 7</p> <p>Oxygen 69% and 58% in beakers and bottles, respectively, at the end of the test.</p>
<p>Chemical Analysis (needed if aeration, volatile, insoluble, precipitate, not steel or glass, known to adsorb, and flow-through) (yes or no)</p>	<p>Samples were taken twice from selected concentration levels of the test series during the test period: 1st sampling took place on one of the transfer days before the 7th day (no offspring had appeared; 2nd between 16th and 21st day.</p>
Other Comments	

11. REPORTED RESULTS:

Guideline Criteria	Reported Information
Mean Measured Concentrations (report conc.)	Not reported
Recovery of Chemical (% recovery)	Not reported
Mortality & Observations (Describe observations & attach mortality tables)	Only NOEC level, 24h EC ₀ and 24 EC ₅₀ reported
Author's Comments	

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

"The test was conducted in accordance with the "Provisional Procedure: extended toxicology test with *Daphnia magna* determination of NOEC for reproduction rate, mortality, and the time of the first appearance of offspring; 21 d)" as of 1 January 1984, issued as the "Recommendation of the Federal Environmental Agency on the Performance of Testing according to Section 5, para 1.No. 3 of the Regulation on Application Documents and Evidence under the Chemicals Act" (Federal Environmental Agency)."

13. REVIEWER'S DISCUSSION AND INTERPRETATION

A. Test Procedure:

The following items did not meet the guideline criteria:

1. Instead of the 7 and 3 beakers (7 with 1 daphnid and 3 with 5 each. Five daphnids per 250 ml of water for each of 4 beakers or bottles were used.
2. Activated sludge in addition to a synthetic food were used. Activated sludge is not mentioned in the guidelines.
3. Tap water was used rather than well water or reconstituted water.
4. The daphnids were not measured for length.
5. Photoperiod was 9 hours light and 15 hours dark versus the required 16 hours light and 8 hours dark.
6. Mean measured concentration was not reported.
7. Production of young by first generation daphnids at various times for each treatment
8. The dry weight of first generation daphnids at the end of the test.
9. The length of first generation daphnids at the end of the test.
10. The test material was not described as to whether it was technical grade or purity.
11. Production of ehippa was not reported.

B. Statistical Analysis

Guideline Criteria	Reported Information
ANOVA and Dunnett's	Student's t-test and U-test
Other Comments	The statistical analysis was not verify the results for every level test was not available.

C. Discussion/Results:

This is a test from the open literature designed to determine the NOEC mortality of the parent animals, appearance of first offspring and the reproduction rate. The guidelines require these additional items:

1. production of young by first generation daphnids at various times for each treatment
2. the dry weight of first generation daphnids at the end of the test.
3. It is desirable to that data on the length of first generation daphnids also be provided.
4. Purity of test material.
5. Tap water was used rather than well water or reconstituted water.
6. The results for each test concentration were not reported. Therefore, the statistical analysis were not verified.

Hence, the study was not designed to provide the same information as a study designed to meet the guideline requirements.

D. Adequacy of the Study:

1. Classification: Supplemental
2. Rational: (See list of items above)
3. Reparability: No, it would be impossible to compensate for the items listed above.

14. COMPLETION DATE OF ONE-LINER FOR STUDY: yes 2-28-94

Table 1—continued

Tested substances	Acute <i>Daphnia</i> test		21 d <i>Daphnia</i> reproduction test			Dilution ratio	Test vessel
	24 h EC ₅₀ (mg l ⁻¹)	24 h EC ₂₅ (mg l ⁻¹)	Nominal value (mg l ⁻¹)	21 d NOEC Minimum value (mg l ⁻¹)	Most sensitive parameter		
Aldehydes							
Chloroacetaldehyde (50% sol.)	6.6	15.0	5.0	0.38	A, B and C	0.08–10 mg l ⁻¹	1:2
Salicylaldehyde	0.90	3.1	0.63		B	0.04–5 mg l ⁻¹	1:2
Ketones							
Methyl isobutyl ketone	930	3682	78	7.8–39	B	20–2500 mg l ⁻¹	1:2
Amines							
Ethylenediamine	3.5	14	0.16		B	0.02–5 mg l ⁻¹	1:2
Trichloroamine	1530	2038	16	0.004	A	8–1000 mg l ⁻¹	1:2
Aniline	0.10	0.90	0.16	0.1	B	0.1–316 µg l ⁻¹	1:√10
2,3-Dimethylaniline	1.6	10	0.16	0.01	B	0.02–2.5 mg l ⁻¹	1:2
3,4-Dimethylaniline	0.20	2.9	0.16		B	0.016–2.0 mg l ⁻¹	1:2
<i>o</i> -Tolidine	1.5	4.8	1.3		B and C	0.04–5.0 mg l ⁻¹	1:2
Chloroamine T	2.7	0.16	0.020		A and B	0.005–0.6 mg l ⁻¹	1:2
Niclosamide†	0.058						
Aromatic nitro compounds							
Nitrobenzene	19	60	13	2.6	B	1.6–200 mg l ⁻¹	1:2
2-Nitroanisole	37	65	13		A, B and C	0.8–100 mg l ⁻¹	1:2
4-Nitroanisole	9.3	15	3.2		A and B	0.8–25 mg l ⁻¹	1:2
4-Nitrophenol	3.0	8.0	2.0		A and B	0.08–10 mg l ⁻¹	1:2
2,4-Dinitrophenol (picric acid)	4.8	7.0	5.0	0.060	B and C	0.063–8 mg l ⁻¹	1:2
2,4,6-Trinitrophenol (10% water)	31	85	0.16	0.020	A and B	0.63–80 mg l ⁻¹	1:2
2,6-Dinitrotoluene (10% water)	11	38	2.3		B	0.02–2.5 mg l ⁻¹	1:2
2,4-Dinitrotoluene (10% water)	13	52	2.3		B and C	1.13–72 mg l ⁻¹	1:2
2-Nitro- <i>p</i> -cresol	19	2.3	1.3			0.02–2.5 mg l ⁻¹	1:2
4,6-Dinitro- <i>o</i> -cresol	1.5						1:√10
Phenols							1:√10
2,4,6-Trimethylphenol	1.8	3.4	0.10	0.04	B and C	0.3–3162 mg l ⁻¹	1:2
3,5-Dimethoxyphenol	1.0	14	0.10		C	0.003–10 mg l ⁻¹	1:2
2-Amino-4-methylphenol	0.49	3.3	0.25		A	0.03–4 mg l ⁻¹	1:2
<i>p</i> -Cresol	2.5	4.9	1.0		A	0.003–10 mg l ⁻¹	1:2
Aromatic compounds							
Toluene	53	84	2.0	1.0	A	0.5–62.5 mg l ⁻¹	1:2
Azobenzene	2.5	~50	0.023	0.009	B	0.023–3 mg l ⁻¹	1:2
1,3,5-trimethylbenzene (mesitylene)	40		2.0	0.4	B	0.125–16 mg l ⁻¹	1:2
Halogenated aromatics							
Perchlorocyclopentadienyl	0.19	0.21	0.009	0.30	A and C	0.6–75 µg l ⁻¹	1:2
2-Chlorophenol	2.0	6.3	0.50		B	0.063–8 mg l ⁻¹	1:2
4-Chlorophenol	3.7	8.6	0.63		C	0.08–5 mg l ⁻¹	1:2
2-Bromophenol	4.8	13	0.32	0.22		0.08–10 mg l ⁻¹	1:2

2-Bromoaniline	2.9	9.0	0.080	B	0.08-20 mg l ⁻¹	1:2	†
2-Chloroaniline	1.4	6.0	0.032	A, B and C	0.001-3.16 mg l ⁻¹	1:√10	
3-Chloroaniline	0.40	1.9	0.013	B and C	6.3-800 µg l ⁻¹	1:2	
4-Chloroaniline	0.10	13	0.010	B and C	0.1-316 µg l ⁻¹	1:√10	
3,4-Dichloroaniline	0.14	6.0	0.012	B	6-1536 µg l ⁻¹	1:2	
2,4-Dichlorophenol	2.8	3.9	0.32	B	0.08-10 mg l ⁻¹	1:2	†
1,2-Dichlorobenzene	1.0	1.7	0.63	A, B and C	0.08-20 mg l ⁻¹	1:2	†
1,3-Dichlorobenzene	5.5	7.0	0.80	B	0.1-12.5 mg l ⁻¹	1:2	†
1,4-Dichlorobenzene	1.5	3.2	0.50	C	0.03-4 mg l ⁻¹	1:2	†
1-Chloro-2-nitrobenzene	5.0	12	4.0	A, B and C	0.125-16 mg l ⁻¹	1:2	†
1-Chloro-4-nitrobenzene	3.3	15	0.32	B	0.08-20 mg l ⁻¹	1:2	†
2-Chlorotoluene	9.0	20	0.27	B and C	0.07-17.5 mg l ⁻¹	1:2	†
4-Chloro-2-nitrotoluene	7.7	12	0.32	A	0.08-10 mg l ⁻¹	1:2	†
4-Chloro-3-nitrotoluene	5.5	9.7	0.50	B	0.063-8.0 mg l ⁻¹	1:2	†
2-Chloro-6-nitrotoluene	2.0	4.0	0.63	A, B and C	0.04-5.0 mg l ⁻¹	1:2	†
4-Chloro-3-methylphenol (4-chloro- <i>m</i> -cresol)	1.9	4.4	1.3	B	0.04-5.0 mg l ⁻¹	1:2	†
1,2,3-Trichlorobenzene	1.4	>2.0	0.63	C	0.02-2.5 mg l ⁻¹	1:2	†
Heterocyclic hydrocarbons							
Quinoline	51	76	0.80	B	0.4-50 mg l ⁻¹	1:2	

*Where no details are given, test was conducted in an open vessel.

†Nominal concentration was calculated on the basis of the result of chemical analysis.

‡Test in closed vessel.

A—parent animal mortality, B—reproduction rate, C—appearance of first offspring.

The 24 h EC₅₀ and the 24 h EC₃₀ (referred to the nominal value) for the acute *Daphnia* test.

The NOEC as referred to the nominal value and, in addition, the minimum value of the test concentration range, the dilution ratio, the most sensitive parameter and the type of vessel used in the 21 d *Daphnia* reproduction test.

From data on the NOEC and the dilution ratios, it was possible to identify the lowest concentration tested where an effect of the substance could be observed.

Table 2 lists the substances according to their harmful effects (as referred to the nominal value) beginning with the most toxic. The minimum value was also given. In the case of 3 of the 73 toxic substances, the NOEC values in the concentration range comprising 3 logs were between 0.000001 and 0.001 mg l⁻¹, i.e. for ethyl parathion, bis(tri-*n*-butyltin)oxide and for the active ion Cd(II). The NOEC values for 13 of the substances tested were in the concentration range comprising 2 logs, i.e. 0.001- <0.1 mg l⁻¹: potassium dichromate and nickel acetate—as referred to Cr(VI) or Ni(II)—aniline, 2- and 3- and 4-chloroaniline, 3,4-dichloroaniline, 2,4-dimethylaniline, 2-bromoaniline, azobenzene, 2,4-dinitrotoluene, niclosamide and perchlorocyclopentadiene.

In the case of 23 substances, an NOEC was determined in the concentration range 0.1- <1 mg l⁻¹. Of the 73 tested substances, the NOEC for more than half was below 1 mg l⁻¹, the first observed effect under 2 mg l⁻¹.

When compared in terms of position isomerism, 4-chloroaniline was found to have an NOEC 3 times lower than 2-chloroaniline, 3,4-dimethylaniline an NOEC 10 times lower than 2,3-dimethylaniline and *p*-chlorobenzene an NOEC 12 times lower than in the *o*-position.

Table 2 reveals a higher toxicity of phthalates with increasing alkyl chain length. In comparison with phthalic acid diethyl ester, the NOEC of phthalic acid diallyl ester was 4 times lower; it was 13 times lower in the case of phthalic acid dibutyl ester.

Table 3 shows the nominal concentrations obtained for the 24 h EC₅₀ and the 21 d NOEC by substance groups. The nominal concentrations had to be given as no results of chemical analysis were available for the 24 h EC₅₀. For each tested substance, the statistically confirmed 24 h EC₅₀ and NOEC values were related to each other whereby a substance concentration of NOEC = 1 was used. This table shows that out of the 73 substances tested, the NOEC values for 3 substances were more than 3 logs, for 13 substances more than 2 logs, for 10 substances 50-90 times, for 24 substances 10-40 times and for 14 substances 50-9 times lower than the values for the 24 h EC₅₀ of the corresponding substance. According to these findings, the ratio NOEC to 24 h EC₅₀ varied between 1:10³ and 1:2 and was dependent on