

08 / 24 / 2005

**Data Evaluation Report on the Chronic Toxicity of Metolachlor Technical to Freshwater Invertebrates - Daphnia sp.**

PMRA Submission Number {.....}

EPA MRID Number 46322101

<b>Data Requirement:</b>	PMRA DATA CODE	
	EPA DP Barcode	D305788
	OECD Data Point	
	EPA MRID	46322101
	EPA Guideline	§72-4b
	OPPTS Guideline	850.1300

<b>Test material:</b>	Metolachlor Technical	<b>Purity:</b> 97.2%
<b>Common name</b>	Metolachlor	
<b>Chemical name:</b>	IUPAC: Not reported	
	CAS name: Not reported	
	CAS No.: Not reported	
	Synonyms: Not reported	

**Primary Reviewer:** Rebecca Bryan  
Staff Scientist, Dynamac Corporation

**Signature:**  
**Date:** 8/26/2004

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**Date:** 9/1/2004

**Primary Reviewer:** Paige Doelling Brown  
OPP/EFED/ERB-I

**Signature:**  
**Date:** 8/4/2005

**Secondary Reviewer(s):**  
{EPA/OECD/PMRA}

**Signature:**  
**Date:**

**Reference/Submission No.:**

**Company Code:**  
**Active Code:**  
**EPA PC Code:** 108801

**Date Evaluation Completed:**

**CITATION:** Palmer, S., Kendall, T. and Krueger, H. 2004. Metolachlor Technical: A Flow-Through Life-Cycle Toxicity Test with the Cladoceran (*Daphnia Magna*). Unpublished study performed by Wildlife International, Ltd., Easton, Maryland. Laboratory Study No. 568A-101A. Study sponsored by Metolachlor Task Force. Study initiated November 21, 2003 and completed July 8, 2004.

Study reviewed by contractor - original signature page lost. Replaced by this one 8/4/05

Paige Doelling Brown 8/4/05



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**Data Evaluation Report on the Chronic Toxicity of Metolachlor Technical to Freshwater Invertebrates -  
*Daphnia* sp.**

PMRA Submission Number {.....}

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**EXECUTIVE SUMMARY:**

The chronic toxicity of Metolachlor Technical to *Daphnia magna* was studied under flow-through conditions for 21 days. Daphnids were exposed to Metolachlor Technical at nominal concentrations of 0 (negative and solvent controls), 2.5, 5.0, 10, 20, and 40 ppm a.i. The mean-measured treatment concentrations were <1.00 (<LOQ, both controls), 2.4, 4.9, 9.4, 18, and 32 ppm a.i. Mean-measured concentrations were 90-98% of nominal for the test concentrations  $\leq$  32 ppm a.i. A surface slick and brown precipitate was observed in the 40 ppm a.i. test concentration, with measured concentration only 65% of nominal on the first day. Relevant endpoints occurred at lower concentrations, thus the low recovery in the 40 ppm a.i. test solution did not affect the experimental results.

After 21 days of exposure, cumulative mortality was 0% in the negative control, 10% in the solvent control, and 0, 0, 5, 95, and 100% in the mean-measured 2.4, 4.9, 9.4, 18, and 32 ppm a.i. groups, respectively. The 21-day  $LC_{50}$  (95% C.I.) was 12.4 ppm a.i. (11.3-15.3 ppm a.i.). The NOAEC for mortality was 9.4 ppm a.i.

Initial brood release was on Day 8 for the control groups and treatment groups at concentrations  $\leq$  9.4 ppm a.i., and between Days 8 and 9 in the 18 ppm a.i. treatment group. There was 100% adult mortality in the 32 ppm a.i. treatment group prior to onset of reproduction. Statistical analyses were not performed on time to first brood release data. The number of young per reproductive day averaged 9.23 for the negative control group and 9.45 for the solvent control group, compared to 8.72, 9.09, 6.65, and 2.92 for the 2.4, 4.9, 9.4, and 18 ppm a.i. test groups, respectively. The  $EC_{50}$  (95% C.I.) for reproduction was 14 ppm a.i. (12-15 ppm a.i.).

Terminal lengths averaged 4.9 mm for the negative and solvent control groups, compared to 4.9, 4.9, and 4.6 mm for the 2.4, 4.9, and 9.4 ppm a.i. test groups, respectively. Terminal dry weights averaged 0.83 mg for the negative control group and 0.85 mg for the solvent control group, compared to 0.85, 0.90, and 0.85 mg for the 2.4, 4.9, and 9.4 ppm a.i. test groups, respectively. There was a statistically significant difference in length for the 9.4 ppm a.i. treatment group, as compared to the controls. No statistically significant differences were noted for dry weight. The NOAEC growth is 4.9 ppm a.i., based on length. The LOAEC is 9.4 ppm a.i.

The study is scientifically sound and is classified as CORE. Mortality, reproduction, and growth (length) were affected by exposure to Metolachlor Technical. The NOAEC and LOAEC for the most sensitive endpoints, reproduction and growth (terminal length) were 4.9 and 9.4 ppm a.i., respectively.

**Results Synopsis:**

Test Organism Age (eg. 1<sup>st</sup> instar): <24 hours old (parental stock were 15 days old).

Test Type : Flow-through

**Mortality**

NOAEC: 9.4 ppm a.i.

LOAEC: 18 ppm a.i.

$LC_{50}$ : 12.4 ppm a.i.            95% C.I.: 10.3-15.3 ppm a.i.

Probit slope: N/A

**Mean # Young per Reproductive Day**

NOAEC: 4.9 ppm a.i.

LOAEC: 9.4 ppm a.i.

$EC_{50}$ : 14 ppm a.i.            95% C.I.: 12-15 ppm a.i.

Probit Slope: 3.91

**Length**

NOAEC: 4.9 ppm a.i.  
LOAEC: 9.4 ppm a.i.  
EC<sub>50</sub>: >9.4            95% C.I.: N/A  
Probit slope: N/A

**Dry weight**

NOAEC: 9.4 ppm a.i.  
LOAEC: >9.4 ppm a.i.  
EC<sub>50</sub>: >9.4            95% C.I.: N/A  
Probit slope: N/A

**Endpoints Affected:** Survival, reproduction, and growth (length)  
**Most Sensitive Endpoint:** Reproduction and growth (length)

**I. MATERIALS AND METHODS**

**GUIDELINES FOLLOWED:** The study protocol was based on procedures outlined in the U.S. Environmental Protection Agency Series 850-Ecological Effects Test Guidelines (draft) OPPTS Number 850.1300: *Daphnia Chronic Toxicity Test*; OECD Guidelines for Testing of Chemicals 211: *Daphnia magna* Reproduction Test; and ASTM Standard E1193-93 *Standard Guide for Conducting Life-Cycle Toxicity Tests with Daphnia magna*. Deviations from U.S. EPA FIFRA Guideline §72-4b include:

1. The health (including mortality) of the brood stock was not described.
2. The pH range (8.2-8.4) exceeded the recommended range (7.6-8.0).
3. The dilution water particulate matter and chlorine concentrations were not reported.
4. The study design differed from EPA guidance: in this flow-through study, 10 daphnid per test chamber were maintained, with 2 replicate compartments per concentration and control, i.e. 5 daphnids/replicate. EPA guidance recommends 22 daphnids/level for static renewal studies, where seven test chambers should contain one daphnid each (to collect data on survival, growth, and reproduction), and three test chambers should contain five daphnids each (to collect data on survival only).

These deviations did not affect the acceptability or validity of the study and the non-guideline experimental design was adequate for the assessment of all recommended endpoints.

**COMPLIANCE:**

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. This study was conducted in compliance with GLP regulations set forth by the U.S. Environmental Protection Agency (40 CFR Parts 160 and 792, 17 August 1989), OECD (ENV/MC/CHEM (98) 17), and Japan MAFF (1 October 1999)(p.3).

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**A. MATERIALS:**

**1. Test Material** Metolachlor Technical

**Description:** Red liquid

**Lot No./Batch No. :** 0207705

**Purity:** 97.2%

**Stability of Compound  
Under Test Conditions:**

Verified. The mean-measured recoveries (from day 0, 7, 14, and 21 samples) were 80-98% of nominal concentrations (Table 2, p. 23). Concurrent QC samples fortified at 2.00, 8.00, and 40.00 ppm a.i. had a recovery range of 97.6 to 102% of nominal (p. 15).

**Storage conditions of  
test chemicals:**

Ambient conditions.

*OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound). The OECD requirements were not reported.*

**2. Test organism:**

**Species:** *Daphnia magna*

**Age of the parental stock:** 15 days old (test daphnids were <24 hours old).

**Source:** In-house (Wildlife International, Ltd.) laboratory culture.

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**B. STUDY DESIGN:**

**1. Experimental Conditions**

a. Range-finding Study: A range-finding study was conducted to determine the definitive test concentrations. The results were not reported.

b. Definitive Study:

**Table 1: Experimental Parameters**

Parameter	Details	Remarks
		Criteria
<u>Parental acclimation:</u> Period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	Continuous (in-house culture) Same as test Mixture of yeast, Cerophyll®, trout chow, and a freshwater green alga ( <i>Selenastrum capricornutum</i> ). Not reported	
<u>Test condition:</u> static renewal/flow through: Type of dilution system- for flow through method. Renewal rate for static renewal	Flow-through Continuous-flow diluter N/A	The flow rate was 5 volume additions per day. Diluter operation was checked visually twice a day during the test and once at the end of the test. Rotameters were calibrated prior to test initiation and at weekly intervals during the test. <hr/> For flow-through study: consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.
Aeration, if any	Dilution water was aerated prior to testing. No aeration during testing.	<hr/> Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks should not be aerated.
Duration of the test	21 days	<hr/> EPA requires 21 days for static renewal

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Parameter	Details	Remarks
		<i>Criteria</i>
<u>Test vessel</u> Material: (glass/stainless steel)  Size: growth/reproduction test: survival test:  Fill volume: growth/reproduction test: survival test:	Teflon-lined, stainless steel aquaria.	Each test chamber (aquaria) contained two 300 mL glass beakers (6.5 cm diameter and 12 cm height) as test compartments (beakers). These test compartments had holes on opposite sides covered with nylon mesh screens. The water depth was 8.0 cm in the test compartment.
	25 L (aquaria)	
	same	
	22 L (aquaria)	1. <i>Material:</i> Glass, No. 316 stainless steel, or perfluorocarbon plastics 2. <i>Size:</i> 250 ml with 200 ml fill volume is preferred; 100 ml with 80 ml fill volume is acceptable. OECD requires parent animals be maintained individually, one per vessel, with 50 - 100 ml of medium in each vessel.
same		
Source of dilution water	Laboratory well water that was sand filtered, aerated, filtered again (0.45 µm), and UV-sterilized. Analysis for trace contaminants in report, and acceptable.	Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).

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Parameter	Details	Remarks
		Criteria
<p>Water parameters:</p> <p>Hardness pH Dissolved oxygen Temperature</p> <p>Total Organic Carbon Particulate matter Metals Pesticides Chlorine</p>	<p>124-132 mg/L as CaCO<sub>3</sub> 8.2-8.4 5.9-8.8 mg/L (≥66% saturation) 19.5-20.5°C</p> <p>&lt;1 mg C/L Not reported See Appendix 2, p. 34 &lt;LOD (Appendix 2, p. 33) Not reported</p>	<p>The pH range exceeded recommendations. This is not expected to affect results. The specific conductance of the dilution water ranged from 310-320 µmhos/cm and the alkalinity ranged from 180-184 ppm as CaCO<sub>3</sub> (Table 6, p. 27).</p>
<p>Interval of water quality measurements</p>	<p>The DO was measured in alternating test replicates at the beginning and end of the test, and approximately three times weekly during the test. The pH was measured in alternating test replicates at test initiation and weekly during the test. The water hardness was measured in alternating test replicates of negative control and highest test concentration at test initiation and weekly during the test.</p>	<p><i>EPA requires:</i> <b>hardness</b> 160 to 180 ppm a.i. as CaCO<sub>3</sub>; OECD requires &gt; 140 ppm a.i. as CaCO<sub>3</sub> <b>pH</b> 7.6 to 8.0 is recommended. Must not deviate by more than one unit for more than 48 hours. OECD requires pH range 6 - 9 and should not vary more than 1.5 units in any one test. <b>Dissolved Oxygen</b> <i>Renewal:</i> must not drop below 50% for more than 48 hours. <i>Flow-through:</i> ≥ 60% through out test. <b>Temperature</b> 20°C ± 2°C. Must not deviate from 20°C by more than 5°C for more than 48 hours. OECD requires range 18 - 22°C; temperature should not vary more than ± 2°C.</p> <p>OECD requires total organic carbon &lt; 2 ppm a.i.</p>

Parameter	Details	Remarks
		Criteria
<p><u>Number of organisms/replicates:</u></p> <p>For growth and reproduction:</p> <p>For survival test:</p>	<p>5 daphnids/replicate test compartments</p> <p>2 replicate test compartments/test chamber with 10 daphnids per chamber; 2 test chambers per level; 20 daphnids total per level.</p> <p>(Not differentiated; same test chambers as above)</p>	<p>Study did not follow recommended test design.</p> <hr/> <p><i>EPA requires 22 daphnids/level; 7 test chambers should contain 1 daphnid each, and 3 test chambers should contain 5 daphnids each.</i></p> <p><i>OECD requires minimum of 10 daphnids held individually for static tests. For flow-through tests, 40 animals divided into 4 groups of 10 animals at each test concentration.</i></p>
<p>Application rates nominal:</p> <p>measured:</p>	<p>0 (negative and solvent control), 2.5, 5.0, 10, 20, and 40 ppm a.i.</p> <p>Mean-measured: &lt;1.00 (&lt;LOQ, controls), 2.4, 4.9, 9.4, 18, and 32 ppm a.i.</p>	<p>Mean-measured concentrations are provided in Table 2, p. 23.</p> <hr/> <p><i>EPA requires control(s) and at least 5 test concentrations; dilution factor not greater than 50%. OECD requires at least 5 test concentrations in a geometric series with a separation factor not exceeding 3.2.</i></p>
<p>Solvent (type, percentage, if used)</p>	<p>DMF (dimethylformamide), 0.1 mL/L</p>	<hr/> <p><i>EPA requires: solvent to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests. Acceptable solvents are dimethylformamide, triethylene glycol, methanol, acetone and ethanol. OECD requires ≤ 0.1 ml/L</i></p>
<p>Lighting</p>	<p>16 hours of light, 8 hours of dark w/ a 30 minute transition period.</p>	<p>Light intensity was 205 Lux over one test vessel (p. 16).</p> <hr/> <p><i>EPA/OECD requires: 16 hours light, 8 hours dark.</i></p>



Parameter	Details	Remarks
		Criteria
Feeding	Feeding was performed 3x per daily for the first seven days and 4x per day until test termination; fed a mixture of yeast, Cerophyll®, trout chow, and a freshwater green alga ( <i>Selenastrum capricornutum</i> ).	
Recovery of chemical: Frequency of measurement: LOD: LOQ:	97.6-100% of nominal  Days 0, 7, 14, and 21  Not reported 1.00 ppm a.i.	Based on procedural recoveries of matrix samples fortified at 2.00, 8.00, and 40.0 ppm a.i. and analyzed concurrently with the sample analysis (Appendix 3.5 of the Analytical Report, p. 40).
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks
		Criteria
Data end points measured (list)	<ul style="list-style-type: none"> <li>- Survival and sublethal effects of first-generation daphnids</li> <li>- Length and dry weight of first-generation daphnids</li> <li>- Day of first brood</li> <li>- Number of young per reproductive day</li> </ul>	<p><i>EPA requires:</i></p> <ul style="list-style-type: none"> <li>- Survival of first-generation daphnids,</li> <li>- Number of young produced per female,</li> <li>- Dry weight (recommended) and length (required)* of each first generation daphnid alive at the end of the test,</li> <li>- Observations of other effects or clinical signs.</li> </ul> <p>*current requirement until the Agency provides specific guidance indicating otherwise (Pesticide Rejection Rate Analysis, p. 132).</p>

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Observation intervals	Mortality of first-generation daphnids was recorded daily and juvenile production was recorded three times per week (Monday, Wednesday, and Friday).	
Water quality was acceptable?	Yes	
Were raw data included?	Yes, sufficient.	
Other observations, if any	N/A	

**II. RESULTS AND DISCUSSION**

**A. MORTALITY:**

After 21 days of exposure, cumulative mortality was 0% in the negative control, 10% in the solvent control, and 0, 0, 5, 95, and 100% in the mean-measured 2.4, 4.9, 9.4, 18, and 32 ppm a.i. groups, respectively (Table 7, p. 28). The 21-day LC<sub>50</sub> (95% C.I.) was 13 ppm a.i. (11-15 ppm a.i.) (p. 20). The NOAEC and LOAEC values for mortality were 9.4 and 18 ppm a.i., respectively.

Table 1: Effect of Metolachlor Technical on Survival, Growth, and Reproduction of Daphnia sp.

Mean-Measured Treatment Concentrations (ppm a.i.) (Nominal Conc.)	Mortality (Dead or Immobile)		Mean Length (mm)	Mean Dry Weight (mg)	Reproduction (Mean # Live Young per Reproductive Day)
	No. Dead	%			
Negative control	0	0	4.9 ± 0.11	0.83 ± 0.06	9.23 ± 1.39
Solvent control	2	10	4.9 ± 0.13	0.85 ± 0.08	9.45 ± 1.19
Pooled Control	1	5	4.9 ± 0.11	0.84 ± 0.07	9.34 ± 1.20
2.4 (2.5)	0	0	4.9 ± 0.22	0.85 ± 0.08	8.72 ± 1.51
4.9 (5.0)	0	0	4.9 ± 0.08	0.90 ± 0.06	9.09 ± 0.64
9.4 (10)	1	5	4.6 ± 0.05**	0.85 ± 0.05	6.65 ± 0.90**
18 (20)	19	95*	- <sup>1</sup>	- <sup>1</sup>	2.92 ± 0.79**
32 (40)	20	100*	- <sup>1</sup>	- <sup>1</sup>	- <sup>1</sup>
NOAEC, ppm a.i. (nominal)	9.4		4.9	9.4	4.9
LOAEC, ppm a.i. (nominal)	18		9.4	N/A	9.4
MATC, ppm a.i. (nominal)	Not calculated		6.8	N/A	6.8
LC <sub>50</sub> /EC <sub>50</sub> (95% C.I.), ppm a.i. (nominal)	13 (11-15)		Not calculated	Not calculated	14 (12-15)

<sup>1</sup> Effects on mortality were significant (95-100% mortality).

\*Significantly lower than the pooled control (Fisher's Exact Test).

\*\* Significantly lower than the pooled control (Bonferroni t-test).

N/A-Not applicable due to the significant effects on mortality at the 18 and 32 ppm a.i.

**B. EFFECT ON REPRODUCTION AND GROWTH:**

Reproduction of young daphnids began on Day 8 for the control groups, and the mean-measured treatment groups at concentrations of ≤9.4 ppm a.i. and between Days 8 and 9 in the 18 ppm a.i. treatment group (Table 8, p. 29). Statistical analyses were not performed on time to first brood release data. By Day 7, 100% mortality had occurred in the 32 ppm a.i., prior to the onset of reproduction and this treatment group was not included in any endpoint statistical analyses with the exception of survival. The number of young per reproductive day averaged 9.23 for the negative control group and 9.45 for the solvent control group (9.34 for the pooled control), compared to 8.72, 9.09, 6.65, and 2.92 for the 2.4, 4.9, 9.4, and 18 ppm a.i. treatment groups groups. Reproduction (number of young per reproductive day) was statistically-reduced in the 9.4 and 18 ppm a.i. treatment groups (Bonferroni t-Test, α = 0.05) compared to the pooled controls. The EC<sub>50</sub> (95% C.I.) for reproduction was 14 ppm a.i. (12-15 ppm a.i.). The NOAEC and LOAEC values for reproduction were 4.9 and 9.4 ppm a.i., respectively.

Terminal length was significantly reduced (Bonferroni t-Test,  $\alpha = 0.05$ ) compared to the pooled controls at the 9.4 ppm a.i. level (statistical analyses not applicable at the 18 and 32 ppm a.i. levels; Table 10, p. 31). Terminal lengths averaged 4.9 mm for the negative and solvent control groups, compared to 4.9, 4.9, and 4.6 mm for the 2.4, 4.9, and 9.4 ppm a.i. test groups, respectively. Terminal dry weights averaged 0.83 and 0.85 mg for the negative and solvent control groups, compared to 0.85, 0.90, and 0.85 mg for the 2.4, 4.9, and 9.4 ppm a.i. test groups. The NOAEC for length was 4.9 ppm a.i. and the NOAEC for dry weight was 9.4 ppm a.i.

**C. REPORTED STATISTICS:**

The negative control and solvent control were compared using an appropriate statistical test (not specified). A pooled control was used in all comparisons to treatment groups. NOAEC and LOAEC values were determined using Chi-square and Fisher's Exact Tests for mortality, and ANOVA and Bonferroni's Test ( $\alpha = 0.05$ ) for reproduction and length endpoints (after confirming normality and homogeneity of variances). MATC values were determined as the geometric mean of the NOAEC and LOAEC values. EC<sub>50</sub> was determined based on reproduction and LC<sub>50</sub> was determined on mortality/immobility observed in the first-generation daphnids at test termination, actual statistical method was not reported. All statistical analyses were conducted using the computer program TOXSTAT or SAS software. Mean-measured concentrations were used for all calculations.

**Mortality**

NOAEC: 9.4 ppm a.i.  
LOAEC: 18 ppm a.i.  
LC<sub>50</sub>: 13 ppm a.i.                      95% C.I.: 11-15 ppm a.i.  
Slope: Not reported

**Mean # Young per Reproductive Day**

NOAEC: 4.9 ppm a.i.  
LOAEC: 9.4 ppm a.i.  
MATC: 6.8 ppm a.i.  
EC<sub>50</sub>: 14 ppm a.i.                      95% C.I.: 12-15 ppm a.i.  
Slope: Not reported

**Length**

NOAEC: 4.9 ppm a.i.  
LOAEC: 9.4 ppm a.i.  
MATC: 6.8 ppm a.i.  
EC<sub>50</sub>: Not determined                      95% C.I.: N/A

**Dry weight**

NOAEC: 9.4 ppm a.i.  
LOAEC: >9.4 ppm a.i.  
EC<sub>50</sub>: Not determined                      95% C.I.: N/A

**Endpoints Affected:** Survival, reproduction, and growth (length)  
**Most Sensitive Endpoint:** Reproduction and growth (length)

**D. VERIFICATION OF STATISTICAL RESULTS:**

NOAEC and LOAEC values for parental mortality were determined using Fisher's Exact test, with TOXSTAT statistical software. The LC50 value was determined using the moving average method with TOXANAL statistical software. After confirming normality and homogeneity of variances, reproduction data (number of live young produced per reproductive day) and terminal length data were assessed for treatment related effects using ANOVA and Bonferroni's test. The mean-measured 32 ppm a.i. treatment group was excluded from the reproduction analysis due to the 100% mortality prior to the onset of reproduction. The 18 and 32 ppm a.i. treatment groups were excluded from the terminal length analysis because of the statistically significant effects on survival. The above analyses were performed using TOXSTAT statistical software and mean-measured treatment concentrations. The negative and solvent control data were not significantly different, and were pooled.

**Mortality**

NOAEC: 9.4 ppm a.i.

LOAEC: 18 ppm a.i.

LC<sub>50</sub>: 12.4 ppm a.i.

95% C.I.: 10.3-15.3 ppm a.i.

Probit slope: N/A

**Mean # Young per Reproductive Day**

NOAEC: 4.9 ppm a.i.

LOAEC: 9.4 ppm a.i.

EC<sub>50</sub>: 14 ppm a.i.

95% C.I.: 12-15 ppm a.i.

Probit Slope: N/A

**Length**

NOAEC: 4.9 ppm a.i.

LOAEC: 9.4 ppm a.i.

EC<sub>50</sub>: >9.4

95% C.I.: N/A

Probit slope: N/A

**Dry weight**

NOAEC: 9.4 ppm a.i.

LOAEC: >9.4 ppm a.i.

EC<sub>50</sub>: >9.4

95% C.I.: N/A

Probit slope: N/A

**Endpoints Affected:** Survival, reproduction, and growth (length)

**Most Sensitive Endpoint:** Reproduction and growth (length)

**E. STUDY DEFICIENCIES:**

All deviations were considered minor and did not affect the validity or acceptability of the study.

**F. REVIEWER'S COMMENTS:**

The results of the reviewer's statistical were identical to those of the study authors with the exception of the 21-day LC<sub>50</sub> for mortality. The reviewer determined LC<sub>50</sub> value (12.4 ppm a.i.) was slightly less than that of the study author's (13.0 ppm a.i.), although 95% confidence intervals were the same. This may be due to slight differences in statistical methods. The reviewer determined LC<sub>50</sub> value is reported in the EXECUTIVE SUMMARY and CONCLUSION sections of this DER.

The study author noted that all test solutions appeared clear and colorless in the test chambers at test initiation and termination (p. 14). In the diluter mixing chambers, the nominal 2.5, 5.0, 10 and 20 ppm a.i. solutions appeared clear and colorless, with a slight surface slick evident in the 10 and 20 ppm a.i. solutions. The nominal 40 ppm a.i. solution appeared clear and colorless in the diluter mixing chamber, with a surface slick and brown precipitate on th bottom of the mixing cup. Analytical verification of the nominal 40 ppm a.i. treatment group on Day 0 indicated a recovery of 65.0% of nominal while the Day 14 (last day of test for this level due to 100% mortality) recovery was 92.7% of nominal. Overall, mean-measured concentration in the 40 ppm treatment group was 80% of nominal. Acceptable LC<sub>50</sub>, EC<sub>50</sub>, NOAEC, and LOAEC values were derived from lower concentrations with no solubility concerns.

**G. CONCLUSIONS:**

The study is scientifically sound and is classified as CORE. Mortality, reproduction, and growth (length) were affected by exposure to Metolachlor Technical. Reproduction and growth were the most sensitive endpoints. The NOAEC and LOAEC for the most sensitive endpoints were 4.9 and 9.4 ppm a.i., respectively. The LC<sub>50</sub> for Daphnia is 12.4 ppm, based on this study.

**Mortality**

NOAEC: 9.4 ppm a.i.

LOAEC: 18 ppm a.i.

LC<sub>50</sub>: 12.4 ppm a.i.

95% C.I.: 10.3-15.3 ppm a.i.

Probit slope: N/A

**Mean # Young per Reproductive Day**

NOAEC: 4.9 ppm a.i.

LOAEC: 9.4 ppm a.i.

EC<sub>50</sub>: 14 ppm a.i.

95% C.I.: 12-15 ppm a.i.

Probit Slope: 3.91

**Length**

NOAEC: 4.9 ppm a.i.

LOAEC: 9.4 ppm a.i.

EC<sub>50</sub>: >9.4

95% C.I.: N/A

Probit slope: N/A

**Dry weight**

NOAEC: 9.4 ppm a.i.

LOAEC: >9.4 ppm a.i.

EC<sub>50</sub>: >9.4

95% C.I.: N/A

Probit slope: N/A

**Endpoints Affected:** Survival, reproduction, and growth (length)

**Most Sensitive Endpoints:** Reproduction and growth (length)

**III. REFERENCES:**

- U.S. Environmental Protection Agency. 1996. Series 850-Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.1300: *Daphnid Chronic Toxicity Test*.
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- American Society for Testing and Materials Standard E1193-93. 1993. *Standard Guide for Conducting Life-Cycle Toxicity Test with Daphnia magna*. American Society for Testing and Materials. Philadelphia, PA.
- APHA, AWWA, WPCF. 1998. *Standard Methods for the Examination of Water and Wastewater*. 20<sup>th</sup> Edition, American Public Health Association. American Water Works Association. Water Pollution Control Federation, New York.
- West, Inc. and D.D. Gulley. 1996, TOXSTAT<sup>®</sup> Version 3.5. Western EcoSystems Technology, Inc. Cheyenne, Wyoming.
- The SAS System for Windows. 1999-2001. Version 8.2. SAS Institute, Inc., Cary, North Carolina.
- Stephan, C.E. 1978. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. Personal Communication.
- Finney, D.J. 1971. *Statistical Methods in Biological Assay*. Second edition. Griffin Press, London.

**APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:**

**Parental Mortality**  
 SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	20	1	
1	2.4	20	0	
2	4.9	20	0	
3	9.4	20	1	
4	18	20	19	*
5	32	20	20	*

**Parental mortality:**

LC50: Conc	Number Exposed	Number Dead	Percent Dead	Binomial Prob (Percent)
32	20	20	100	9.536742E-05
18	20	19	95	2.002716E-03
9.399999	20	1	5	2.002716E-03
4.9	20	0	0	9.536742E-05
2.4	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 9.399999 AND 18 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 13.00769

**RESULTS CALCULATED USING THE MOVING AVERAGE METHOD**

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMIT
4	4.544804E-02	12.41394	10.30857 TO 15.32879

Mean number of live young produced per reproductive day  
 File: 2101rd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	129.277	32.319	27.273
Within (Error)	19	22.516	1.185	
Total	23	151.793		

Critical F value = 2.90 (0.05,4,19)  
 Since F > Critical F REJECT Ho: All groups equal



**Data Evaluation Report on the Chronic Toxicity of Metolachlor Technical to Freshwater Invertebrates -  
Daphnia sp.**

PMRA Submission Number{.....}

EPA MRID Number 46322101

Mean number of live young produced per reproductive day  
File: 2101rd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	9.339	9.339		
2	2.4	8.718	8.718	0.932	
3	4.9	9.085	9.085	0.381	
4	9.4	6.650	6.650	4.033	*
5	18	2.915	2.915	9.636	*

Bonferroni T table value = 2.43 (1 Tailed Value, P=0.05, df=19,4)

Mean number of live young produced per reproductive day  
File: 2101rd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	8			
2	2.4	4	1.623	17.4	0.621
3	4.9	4	1.623	17.4	0.254
4	9.4	4	1.623	17.4	2.689
5	18	4	1.623	17.4	6.424

Mean number of live young produced per reproductive day  
File: 2101rd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	8	9.339	9.339	9.339
2	2.4	4	8.718	8.718	8.901
3	4.9	4	9.085	9.085	8.901
4	9.4	4	6.650	6.650	6.650
5	18	4	2.915	2.915	2.915

Mean number of live young produced per reproductive day  
File: 2101rd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	9.339				
2.4	8.901	0.656		1.73	k= 1, v=19
4.9	8.901	0.656		1.81	k= 2, v=19
9.4	6.650	4.033	*	1.84	k= 3, v=19
18	2.915	9.636	*	1.85	k= 4, v=19

s = 1.089

Note: df used for table values are approximate when v > 20.

**Data Evaluation Report on the Chronic Toxicity of Metolachlor Technical to Freshwater Invertebrates -  
Daphnia sp.**

PMRA Submission Number{.....}

EPA MRID Number 46322101

**Estimates of EC for mean number of live young%**

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	5.2	3.4	7.9	0.089	0.65
EC10	6.4	4.5	9.1	0.073	0.70
EC25	9.1	7.2	12.	0.049	0.79
EC50	14.	12.	15.	0.028	0.88

Slope = 3.91 Std.Err. = 0.680

Goodness of fit: p = 0.63 based on DF= 2.0 19.

2101RD : Mean number of live young produced per reproductive day

**Observed vs. Predicted Treatment Group Means**

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	8.00	9.34	9.20	0.138	100.	0.00
2.40	4.00	8.72	9.19	-0.468	99.8	0.163
4.90	4.00	9.08	8.82	0.269	95.8	4.18
9.40	4.00	6.65	6.75	-0.0952	73.3	26.7
18.0	4.00	2.92	2.90	0.0178	31.5	68.5

**Data Evaluation Report on the Chronic Toxicity of Metolachlor Technical to Freshwater Invertebrates -  
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PMRA Submission Number {.....}

EPA MRID Number 46322101

Terminal length  
File: 21011d Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	3	0.215	0.072	4.500
Within (Error)	16	0.255	0.016	
Total	19	0.470		

Critical F value = 3.24 (0.05,3,16)  
Since F > Critical F REJECT Ho:All groups equal

Terminal length  
File: 21011d Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	4.925	4.925		
2	2.4	4.900	4.900	0.323	
3	4.9	4.850	4.850	0.968	
4	9.4	4.650	4.650	3.550	*

Bonferroni T table value = 2.33 (1 Tailed Value, P=0.05, df=16,3)

Terminal length  
File: 21011d Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	8			
2	2.4	4	0.180	3.7	0.025
3	4.9	4	0.180	3.7	0.075
4	9.4	4	0.180	3.7	0.275

**Data Evaluation Report on the Chronic Toxicity of Metolachlor Technical to Freshwater Invertebrates -  
Daphnia sp.**

PMRA Submission Number {.....}

EPA MRID Number 46322101

Terminal length

File: 21011d

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	8	4.925	4.925	4.925
2	2.4	4	4.900	4.900	4.900
3	4.9	4	4.850	4.850	4.850
4	9.4	4	4.650	4.650	4.650

Terminal length

File: 21011d

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	4.925				
2.4	4.900	0.323		1.75	k= 1, v=16
4.9	4.850	0.970		1.83	k= 2, v=16
9.4	4.650	3.557	*	1.86	k= 3, v=16

s = 0.126

Note: df used for table values are approximate when v > 20.