5-29-96

DATA EVALUATION RECORD
AQUATIC INVERTEBRATE LIFE CYCLE TEST
GUIDELINE 72-4(B)

1. **CHEMICAL:** Metsulfuron-methyl PC Code No.: 122010

2. **TEST MATERIAL:** DPX-T6376-74 (H-17,452); 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) amino] carbonyl] amino] sulfonyl] Benzoic Acid, Methyl ester
Purity: 98.8 %

3. **CITATION**

Authors:	Hutton, David G.
Title:	Chronic toxicity of DPX-T6376-74 to *Daphnia magna*
Study Completion Date:	January 4, 1989
Laboratory:	Haskell Laboratory, Newark, Delaware
Sponsor:	E.I. du Pont de Nemours and Company, Inc.
Laboratory Report ID:	Du Pont HLR 833-88
MRID No.:	434906-01
DP Barcode:	D211883

4. **REVIEWED BY:** William S. Rabert, Biologist, EEB, EFED

Signature: William S. Rabert **Date:** 5/23/96

5. **APPROVED BY:** Harry Craven, Head of Section 4, EEB, EFED

Signature: Henry T. Craven **Date:** 5/29/96

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	*Daphnia magna*
Age of Test Organism:	< 21 hours old
Definitive Test Duration:	21 days
Study Method:	Static-renewal
Type of Concentrations:	Mean measured

7. **CONCLUSIONS:** This 21-day daphnid study is not scientifically sound, because the test results are erratic and the objective of this study to evaluate the chronic toxicity of this pesticide has not been achieved. The authors concluded that all statistically significant differences from controls were either erratic or biologically insignificant. If all endpoints failed to have a statistically significant effect, then the intent of the study has not been met. Also, test concentrations are not considered reliable for the following reasons: filtered fish tank water used in the study as the dilution water was not chemically characterized for contaminants which might affect or mask toxicity by sorbing test chemical; analytical measurements of old and new test concentrations were not made at each renewal; and test samples were not filtered (0.45 microns) prior to chemical analyses to remove sorbed test chemical, which is less

available to test organisms than dissolved chemicals.

NOEC: Not determined
LOEC: Not determined

LOEC's for specific effects:

Adult Survival:	77 mg ai/L (no mortality at 150 mg ai/L)
Number of Young:	77 mg ai/L (18 % reduction at 150 mg ai/L, but not statistically significant)
Growth (length):	Statistically significant reductions of 3 to 8 % at all test concentrations
(dry weight):	not measured

8. ADEQUACY OF THE STUDY

A. Classification: Invalid.

B. Rationale: The objective of the study to evaluate the chronic toxicity was not fulfilled (i.e., an NOEL was not attained). Measured test concentrations are questionable. Dilution water from fish aquarium used in the test was not chemically characterized for contaminants which might affect or mask toxicity. Soluble organic chemicals may affect availability of the test chemical to the test organism.

C. Repairability: Repairability is possible, if dissolved test levels and dilution water quality measurements are submitted, and an NOEC can be determined.

9. GUIDELINE DEVIATIONS

1. Raw data for measurements were not included for test temperature, pH, and dissolved oxygen.

2. Water quality measurements for dilution water from the fish tank were not reported. Dilution water from a fish aquarium may lack the water quality stability necessary for water characteristics (i.e., contaminants) for toxicity testing.

3. Levels of detection for contaminants in well water are too high to determine the acceptability or problems with well water quality for aquatic toxicity testing.

4. Test concentrations were measured only once weekly instead of prior to and after each renewal (3 renewals per week).

5. Water samples were not filtered prior to remove test material sorbed to particulates, that reduce availability to test organisms.

6. Dry weight measurements were not made for growth assessment. Dry weight is expected to be the most sensitive endpoint for this study. Unfortunately, the data rejection analysis excuses the absence of dry weight measurements, until guidance is formalized.

10 **SUBMISSION PURPOSE:** Test data to support registration.

11. **MATERIALS AND METHODS**

A. Test Organisms/Acclimation

Guideline Criteria	Reported Information
Species *Daphnia magna*	< 24 hours old
Source	Haskell Lab culture
Parental Acclimation Conditions Parental stock must be maintained separately from the brood culture in dilution water and under test conditions.	Parental stock and brood cultures appear to be the same. Culture water was the same dilution water: filtered fish tank water.
Parental Acclimation Period At least 21 days.	daphnid culture kept under test conditions: 20 °C
Age of Parental Stock At least 10-12 days old at the beginning of the acclimation period.	14 days old when young daphnids were collected.
Food Synthetic foods (trout chow), algae, or synthetic foods in combination with alfalfa yeast and algae.	Trout chow (Glencoe) and yeast (Fleischmann's)
Food Concentration 5 mg/L (dry wt.) of synthetic food or 10^8 cells/L of algae is recommended.	Not reported
Were daphnids in good health during acclimation period?	No abnormalities

B. Test System

Guideline Criteria	Reported Information

Test Water Unpolluted well or spring that has been tested for contaminants, or appropriate reconsti-tuted water (see ASTM for details).	Filtered fish tank water: Well water circulated through the fathead minnow culture tank and filtered with 0.8 micron filter.
Water Temperature 20°C ± 2°C. Must not deviate from 20°C by more than 5°C for more than 48 hours.	Raw data missing Target: 20 °C Mean: 19.9 °C Range: 19.4 to 22.2 °C
pH 7.6 to 8.0 is recommended. Must not deviate by more than one unit for more than 48 hours.	Raw data are missing for weekly measurements. A range of 7.1 to 7.7 was reported.
Total Hardness 160 to 180 mg/L as $CaCO_3$ is recommended.	Well water, not dilution water: 78 mg/L as $CaCO_3$
Dissolved Oxygen Renewal: must not drop below 50% for more than 48 hours. Flow-through: ≥ 60% throughout test.	Raw data are missing for weekly measurements. A range of 5.2 to 8.8 % saturation was reported.
Test Vessels or Compartments 1. Material: Glass, No. 316 stainless steel, or perfluorocarbon plastics 2. Size: 250 ml with 200 ml fill volume is preferred; 100 ml with 80 ml fill volume is acceptable.	Glass beakers 250 ml with 200 ml
Covers Renewal: Test vessels should be covered with a glass plate. Flow-through: openings in test compartments should be covered with mesh nylon or stainless steel screen.	Not reported
Type of Dilution System Must provide reproducible supply of toxicant. Intermittent flow proportional diluters or continuous flow serial diluters should be used.	N/A

Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.	N/A
Aeration Dilution water should be vigorously aerated, but the test tanks should not be aerated.	Not reported
Photoperiod 16 hours light, 8 hours dark.	16 hours: 8 hours
Solvents Not 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.	Solvent: N/A Maximum conc.: ___ ml/L.

C. **Test Design**

Guideline Criteria	Reported Information
Duration: 21 days	21 days
Nominal Concentrations Control(s) and at least 5 test concentrations; dilution factor not greater than 50%.	5, 10, 19, 38, 75 and 150 mg/L
Number of Test Organisms 22 daphnids/level; 7 test chambers should contain 1 daphnid each, and 3 test chambers should contain 5 daphnids each.	40 daphnids/conc.; 10 replicates of 4 daphnids each.
Test organisms randomly or impartially assigned to test vessels?	Stated as random, but method of randomization was not specified.
Renewal Parent daphnids in all beakers must be transferred to containers with fresh test solution (< 4 hours old) three times each week (e.g. every Monday, Wednesday and Friday).	The age of the fresh test solution was not reported. It was reported that the food was already in the test solution when the test organisms were added.

Guideline Criteria	Reported Information
Water Parameter Measurements 1. Dissolved oxygen must be measured at each concentration at least once a week. 2. pH, alkalinity, hardness, and conductance must be measured once a week in one test concentration and in one control. 3. Temperature should be monitored at least hourly throughout the test in one test chamber, and near the beginning, middle and end of the test in all test chambers.	Measured in fresh and old test solutions three times a week pH measured in fresh and old test solutions three times a week; alkalinity, hardness and conductance measured weekly Temperature monitored continuously with recording thermometer and daily on work days with mercury thermometer.
Chemical Analysis Needed if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used.	Chemical concentrations were measured weekly.

12. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control Mortality ≤ 30%	0 %
Did daphnids in each control produce at least 40 young after 21 days?	Yes (80 to 113)
Were no ephippia produced in any of the controls?	Not reported

Guideline Criteria	Reported Information
Data Endpoints - Survival of first-generation daphnids, - Number of young produced per female, - Dry weight (optional) and length (required) of each first generation daphnid alive at the end of the test, - Observations of other effects or clinical signs.	adult survival number of young/vessel number of young/female Body length None
Raw data included?	Data were included for adult survival, number of young, young/surviving adult, and length, but not test parameters.

Effects Data

Toxicant Concentration (mg/L)		No. (%) Dead or Immobile (21 Days)	No. of Young	Young per Female per Repro. Day	Total Length (mm)	Dry Weight (mg)
Nominal	Measured					
Control	--	0	402	n/a	3.9	**
Solvent Control	--	--	--	--	--	--
5	5.1	0	355	n/a	3.7*	**
10	11	2	381	n/a	3.8*	**
19	17	0	392	n/a	3.8*	**
38	39	0	417	n/a	3.8*	**
75	77	15*	304*	n/a	3.7*	**
150	150	0	328	n/a	3.6*	**

* Significantly different ($p < 0.05$) from controls.
** Dry weight measurements were not reported.

Toxicity Observations:

B. Statistical Results

Most sensitive endpoint: Growth (body length)

Endpoint	Method	NOEC	LOEC
Survival	Dunnett's Test	150 mg/L	> 150 mg/L
Reproduction	Dunnett's Test	150 mg/L	> 150 mg/L
Weight	Dunnett's Test	n/a	n/a
Length	Dunnett's Test	< 5.1 mg/L	5.1 mg/L

13. VERIFICATION OF STATISTICAL RESULTS

Most sensitive endpoint:

Endpoint	Method	NOEC	LOEC
Survival	N/A	N/A	N/A
Reproduction	N/A	N/A	N/A
Weight	N/A	N/A	N/A
Length	N/A	N/A	N/A

14. REVIEWER'S COMMENTS:

Statistically significant effects are erratic and generally are not dose-related. The authors conclude that the NOEC is > 150 ppm based on lack of dose-responses and minimal length differences compared to controls (< 3%). Actually the length differences range from 3 to 8 percent less than controls; it is unclear if any of these length differences are biologically significant. For organisms with an external skeleton, growth in length is a function of the number of molts. Dry weight which was not measured, is generally considered to be more responsive to environmental conditions than length. For this chemical, body weight is likely to be the most sensitive endpoint.

Adult survival was 100 percent at all test concentrations, except 11 mg/L (98 %) and significantly difference at 77 mg/L (85%). The first day appearance of young was Day

10 in all concentrations, except at 11 and 17 mg/L (Day 9.7) and 39 mg/L (Day 9.4). Reproduction reported as number of young was significantly different only at 77 mg/L (24 percent reduction); the 18 percent reduction in the number of young at 150 mg/L was not significant. Statistical analyses (P =, 0.05) show that the mean body length was reduced at all test concentrations compared to controls. Daphnids are an crustacea with an exoskeleton, hence body length is largely a function of the number of molts and not environmental stresses. Body weight is a much more sensitive endpoint for crustacea, because weight responses more readily affected by environmental pressures including chemical toxicity, especially in crustacea. No measurements on dry weight were reported for this study.

Statistically significant (P = 0.05) reductions in mean body lengths show a dose-response effect at test concentrations of 11, 17, 39, 77, and 150 mg/L. The reductions in body length ranged from 2.6 to 9.2 percent compared to controls. It is uncertain what biological these body length differences might represent.

Measured test concentrations are uncertain for the following reasons. 1) Water samples were measured only once per week (i.e., old and new solutions) despite the fact that the test solutions were renewed three times a week. Analyses of old and new test solutions at each renewal are necessary to characterize the exposure levels throughout the test. 2) The dilution water in the test was taken from a fathead minnow culture tank. While water quality measurements were made on the well water that went into the fathead aquarium, water quality analyses were not made on the dilution test actually used in the study as required. While the dilution water from the fish tank was filtered to remove particulates, dissolved organic chemicals can also sorb and/or mask the toxicity of test chemicals. Measurements of total organic carbon are required to indicate possible adsorption of test material by organic molecules in the dilution water. 3) When water samples were collected from test chambers, the samples were not filtered to remove particulates, such as algal cell, yeast, microbes, etc., as is the normal procedures to determine dissolved test concentrations.

In most cases, the detection levels used to measure for the presence of metals, toxic chemicals and pesticides in the well water were too high to identify contaminants of concern (see Attachment for recommended water quality criteria). Methylene chloride levels at 64 ug/L exceeds the criterion for total organochlorine pesticides at < 50 ng/L. On other occasions, concentrations of various pollutants have exceeded recommended concentrations for toxicity testing (e.g., iron and zinc). Du Pont should measure their well water with adequate chemical sensitivity to determine what pollutants are impacting their ability to use the well directly for aquatic testing. And when the problem chemicals are identified should use appropriate purification methods to eliminate the problem or find an alternate dilution water source.

The use of dilution water from a fish aquarium is not recommended. Stability in the water quality of dilution water in toxicity testing is essential. Dilution water quality measurements are required twice yearly, after the laboratory has demonstrated with monthly analyses that the water quality is stable. Du Pont has not submitted dilution water analyses for filtered fish tank water and it is doubtful that monthly measurements will demonstrate stable water quality for chemical characteristics. The description of the test solution renewal process indicates that an undefined level of food was added to the test solution before the daphnids were transferred. It is unclear how long the test solution was prepared before introducing the daphnids. The presence of organic matter in the test solution may sorb and affect the amount of test chemical readily available to the test organism.

In summary, inadequate scientific methods with respect to dilution water quality characteristics, sampling of test concentrations, and preparation of water samples for chemical analyses raise serious questions on the validity of test concentrations in this study. In addition, this study failed in its objective to evaluate the chronic toxicity of this pesticide. The author's discount all toxic effects as random and failing to show a dose-response relationship. Consequently, the study has failed to determine a NOEC/LOEC endpoint. EEB recommends that this study to be repeated, preferably without the use of filtered, fish tank water, and addressing all areas of concern raised in this review. Should Haskell Laboratory use filtered, fish tank water as the dilution water, measurements of water quality characteristics need to be made on the dilution water batch used for the test. EEB also requests that both dry weight and carapace length be measured as growth endpoints when the study is rerun, since growth appears to be the most sensitive endpoint for this pesticide.

Table 1. Recommended analytical detection limits for dilution water quality from freshwater and saltwater sources (measured twice yearly in constant water sources).

Water Quality Criteria	Specification
Specific Conductance [a]	< 1 micromho/cm
Particulate matter	< 20 mg/l
Chemical oxygen demand (COD)	< 5 mg/l
Total organic carbon	< 2 mg/l
Boron	< 100 ug/l
Fluoride	< 100 ug/l
Residual chlorine	< 3 ug/l
Un-ionized ammonia	< 1 ug/l
Aluminum	< 1 ug/l
Arsenic	< 1 ug/l
Chromium	< 1 ug/l
Cobalt	< 1 ug/l
Copper	< 1 ug/l
Iron	< 1 ug/l
Lead	< 1 ug/l
Nickel	< 1 ug/l
Zinc	< 1 ug/l
Cadmium	< 100 ng/l
Mercury	< 100 ng/l
Silver	< 100 ng/l
Total organophosphorus pesticides	< 50 ng/l
Total organochlorine pesticides plus polychlorinated biphenyls (PCBs) or organic chlorine	< 50 ng/l
	< 25 ng/l

[a] Measured for freshwater only

Source

ASTM. 1980. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. ASTM E 729-80. p. 272-296.

EPA. 1975. Methods for acute toxicity tests with fishes, macroinvertebrates, and amphibians. EPA-660/3-75-009. 61 p.