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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
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OFFICE OF  
PREVENTION, PESTICIDES, AND  
TOXIC SUBSTANCES

MEMORANDUM

**SUBJECT:** Review of Daphnid Life Cycle Toxicity Study Submitted to Support  
Registration of Metsulfuron Methyl (122010)  
(DP Barcode D211883)

**FROM:** Anthony F. Maciorowski, Ph.D. *ACW 02/11/96*  
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**TO:** Robert Taylor  
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EEB has received and reviewed the daphnid life cycle toxicity test 72-4(b) (MRID # 434906-01 submitted by DuPont Company, Inc.. The study was submitted to support registration of metsulfuron methyl. The following is a brief summary of the review:

**CITATION:** Hutton, David G. 1989. Chronic toxicity of DPX-T6376-74 to *Daphnia magna*; performed by Haskell Laboratory, Newark, Delaware; submitted by DuPont de Nemours and Company, Inc.; Laboratory Report ID: HLR 833-88; MRID# 434906-01.

**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 fail to have a significant effect, 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.

EEB has recently reviewed an acute and several chronic daphnid studies submitted by DuPont. During these reviews, EEB has found recurring test design problems which render the results of the study questionable. EEB has identified some of these problems in the past

and a meeting with DuPont has held to discuss the problems. Unfortunately, the senior aquatic scientist could not be present at the meeting and not all issues were addressed. Listed below are areas of concern with DuPont daphnid testing methods, which EEB would like transmitted to DuPont so that they can respond to all our concerns.

- 1) Water quality characterization of the dilution water used in aquatic testing is required to be measured twice annually or whenever changes may occur, if the dilution water has been monitored and demonstrated to be of stable quality. The dilution water used in daphnid studies by Haskell Laboratory is "filtered fish tank water" (i.e., well water which has been aged and/or conditioned in tanks with fathead minnows. With the studies, DuPont provides measurements of water quality characteristics for their well water, but they have not submitted characterization of the dilution water taken from the fish tanks. Until a history of annual stability of this dilution water is available, Haskell should make monthly, or more frequent, analyses depending on whether changes in water quality might be expected. EEB recommends that Haskell draw all water necessary for a test into a holding tank and measure the water quality characteristics for each study until it is determined that the dilution water is stable.
- 2) EPA (1975) and ASTM (1980) have set recommended water quality levels for a number of pollutants (see Attachment). Haskell Laboratory consistently fails to measure dilution water characteristics on their well water at levels of detection recommended by EPA and ASTM for water quality. Levels of detection reported may be one to two orders of magnitude higher than necessary to assess water quality. EEB recommends that analytical methods be revised to permit assessment of water quality and that measurements be made monthly on well water in addition to the measurements on the dilution water specified above, in order to identify the contaminant(s) in the well water which prevent healthy daphnid cultures and testing. Once the contaminant(s) in the well water is/are identified, methods to remove them can be considered. In any event, Haskell Laboratory needs to improve the characterization of water quality measurements, so that the Agency can evaluate the acceptability of the dilution water for aquatic testing.
- 3) In daphnid life cycle studies, Haskell Laboratory used a static-renewal method which renewed the test solutions on Monday, Wednesday and Friday. It is appropriate to measure old and new test solutions every time the test solution is renewed, in order to verify test concentrations. Test concentrations were not measured before and after each renewal.
- 4) Water samples collected for chemical analyses for the test material are to be filtered with a 0.45 micron filter to remove alga, microorganisms and particulates prior to quantification of the test concentrations. Some chemicals sorb to surfaces of particulates and membranes and are not as readily available to the test organism as dissolved test materials. Prior to chemical analyses for the test material, all water samples should be filtered with a 0.45 micron filter, as is the normal procedure. In cases where dissolved chemicals may sorb to the filter during filtration, water samples can be centrifuged following standard EPA methods.

If you have questions, contact William Rabert (305-5610).

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