

# **Joint Exhibit 86**



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OFFICE OF CHEMICAL SAFETY  
AND POLLUTION PREVENTION

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Date: March 20, 2014

**MEMORANDUM**

**Subject:** DCPA (Chlorthal-dimethyl): Review of Study Protocols for Determining Chronic Toxicity to Sediment-Dwelling Estuarine/Marine and Freshwater Organisms

**To:** Jill Bloom, Risk Manager Reviewer  
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EFED reviewed the following test protocols:

- “DCPA (Chlorthal Dimethyl) – Protocol for Conducting a 28-Day Toxicity Test Exposing Estuarine Amphipods (*Leptocheirus plumulosus*) to a Test Substance Applied to Sediment Following EPA Test Methods” by Smithers Viscient (DP 413319).
- “DCPA (Chlorthal Dimethyl) – Protocol for Conducting a 42-Day Toxicity Test Exposing Freshwater Amphipods (*Hyalella azteca*) to a Test Substance Applied to Sediment Under Static-Renewal Conditions Following EPA Test Methods” by Smithers Viscient (DP 413320).
- “DCPA (Chlorthal Dimethyl) – Protocol for Conducting a Life-Cycle Toxicity Test Exposing Midges (*Chironomus dilutus*) to a Test Substance Applied to Sediment Under Static-Renewal Conditions Following EPA Test Methods” by Smithers Viscient (DP 413321).

These protocols were submitted in response to the data call-in (DCI) issued as part of Registration Review. For the chronic sediment toxicity protocol reviews, the responses to the protocols reflects comments received from EFED’s Aquatic Biology Technical Team (ABTT).

Based on the ABTT comments, EFED recommends additional detail is added to the protocols to help ensure study acceptability. However, we anticipate the protocols to be adequate once revisions that address EFED's concerns and comments, as described below, are submitted. Revised protocols are not required, but the final report should take into consideration EFED's comments and recommendations.

**Chronic Sediment Toxicity Protocols; the following comments are applicable to all three of the sediment protocols (and comments specific for each protocol follow):**

**Section 2.1.3:** EFED recommends that the concentration/volume of the acetone to be used is provided and any changes or additions to the protocol from the addition of a solvent be described (*e.g.*, whether the range-finding test will include a solvent control).

**Section 2.2.3:** The protocols state that the source of test organisms will be from an in-house culture or a reputable supplier. EFED recommends including details of history/origin of the colony, presence of mortality, and general health of the colony. This information ensures reliability of the results associated with any studies conducted with these species.

**Section 2.3.4:** The submitted protocols state that "Periodic analysis of representative samples of the overlying water source will be conducted...to ensure the absence of potential toxicants..." EFED recommends that the revised protocols identify the frequency of this analysis and the most recent analysis prior to test initiation and conclusion should be submitted with the study report.

**Section 2.5.2:** The submitted protocols state that, "an appropriate sized sediment sample will be removed...for determination of sediment concentrations." EFED recommends that the revised protocols should identify the sample size needed for determination of sediment concentrations and ensure that an identical sample size is used in the treatment and control replicates.

**Section 2.5.3:** EFED recommends that conductivity, hardness, and alkalinity should not vary more than about 10% and pH by more than 1 pH unit. If fungal or bacterial growth is observed in test vessels from the feeding levels, more frequent ammonia measurements than described in the test protocols may be appropriate.

**Section 2.5.4:** EFED recommends that the pH and ammonia concentration in pore water be measured at test initiation, *mid-test*, and at test termination. Similarly, the sediment Eh should be measured at test initiation, *mid-test*, and at test termination. These measurements may be made from the separate chemistry replicates resembling the biological replicates and containing organisms and receiving food used to provide the required volume for chemical analysis.

**Section 3.1:** The protocols state: "All concentration-effect relationships will be based on measured concentrations of test substance in sediment." EFED recommends that the concentration-effect relationships also be based on measured concentrations of the test substance in pore water.

**Comments on “DCPA (Chlorthal Dimethyl) – Protocol for Conducting a 28-Day Toxicity Test Exposing Estuarine Amphipods (*Leptocheirus plumulosus*) to a Test Substance Applied to Sediment Following EPA Test Methods” by Smithers Viscient (DP 413319)**

**Section 2.2.1:** EFED notes that some labs have reported that using larger organisms (0.4-0.6 mm) at test initiation has led to improvements in control performance.

**Section 2.3.3:** Please confirm in the protocol that the five replicates (G through K) to be maintained for the purpose of chemical analysis and monitoring water quality in the pore water will contain the *same* numbers of individuals as the other replicates (A through F) that will be used for evaluating biological responses. Replicates for analytical measurements should contain organisms to allow for better replication of the same test conditions as the biologically monitored test organisms (replicates A through F). It is not necessary for the replicate used for chemical analysis on Day 0 to contain test organisms.

**Section 2.4.4:** The submitted protocol states three subsets of 20 individuals will be weighed at test initiation. In order to quantify the variability in amphipod size at test initiation and compare it to amphipod size at test termination, EFED recommends that the Day 0 growth measurement should be based on the same number of biological replicates used in the definitive test.

**Reference:**

United States Environmental Protection Agency (U.S. EPA). 2001. Method for Assessing the Chronic Toxicity of Marine and Estuarine Sediment-Associated Contaminants with the Amphipod *Leptocheirus plumulosus*. EPA 600/R-01/020. March 2001. Office of Research and Development.

**Comments on “DCPA (Chlorthal Dimethyl) – Protocol for Conducting a 42-Day Toxicity Test Exposing Freshwater Amphipods (*Hyalella azteca*) to a Test Substance Applied to Sediment Under Static-Renewal Conditions Following EPA Test Methods” by Smithers Viscient (DP 413320)**

**Section 1.0, last sentence:** Since the OSCPP draft 850.1770 chronic sediment toxicity guidelines were never officially released, we recommend that the text referring to the draft 850.1770 protocol be removed. If desired, you could add text that indicates the protocol reflects the latest discussions on protocol modifications with OPP/EFED scientists.

**Section 2.2.4:** As part of the process to finalize the OCSPP 850 guidelines for chronic sediment toxicity testing of aquatic invertebrates, EFED is consulting with the U.S. EPA Office of Research and Development and other government scientists associated with development of the 2000 and 2001 Agency-wide test guidelines. The nature of this consultation is to ensure that the latest science and 'lessons learned' over the past decade of sediment toxicity testing using the 2000 and 2001 guidelines can be reflected in the forthcoming OCSPP 850 guidelines, which are based on the earlier Agency-wide guidelines.

With respect to chronic sediment testing with *Hyalella azteca*, there has been some indication that the recommended diet (1 ml YCT) might lead to sub-optimal growth or reproduction. Increased feeding rates and/or enhanced diets may improve test organism growth and

reproduction, but the exact nature of the interaction among diet, overlying water source, and sediment source for optimizing *H. azteca* growth and reproduction is still being evaluated. The registrant is encouraged to consult with the Agency should issues concerning test organism performance arise over the course of testing.

EFED recommends adding the following language: “Records of feeding rates and the appearance of the sediment surface each day should be maintained.”

**Section 2.5.5:** As a part of the conduct of this study, please consider the following guidance for enumeration of amphipods: “A consistent amount of time should be taken to examine sieved material for recovery of test organisms (e.g., 5 min/replicate). Laboratories should demonstrate that their personnel are able to recover an average of at least 90% of the organisms from whole sediment” (Section 14.3.7.3 of U.S. EPA, 2000).

**Section 2.5.6:** If growth is determined by measurement of organism dry weight, EFED recommends adding the following to the acceptability criteria:

- The average dry weight of *H. azteca* in negative and solvent controls was > 0.15 mg/individual.

**Reference:**

USEPA. 2000. Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates, Second Edition, EPA 600/R-99/064. March 2000. Office of Research and Development.

**Comments on “DCPA (Chlorthal Dimethyl) – Protocol for Conducting a Life-Cycle Toxicity Test Exposing Midges (*Chironomus dilutus*) to a Test Substance Applied to Sediment Under Static-Renewal Conditions Following EPA Test Methods” by Smithers Viscient (DP 413321)**

**Section 1.0, last sentence:** Since the OSCPP draft 850.1760 chronic sediment toxicity guidelines were never officially released, we recommend that the text referring to the draft 850.1760 protocol be removed. If desired, you could add text that indicates the protocol reflects the latest discussions on protocol modifications with OPP/EFED scientists.

**Section 2.2.1:** (First instar larvae < 24h to 4 days); The USEPA 2000 guidance specifies that < 24-h organisms be used to initiate the test. However, EFED understands that some labs (including that of the original test developer) are finding improvements in test performance by starting with older organisms within the first instar. In order to minimize variability in the growth and reproduction measurements, please specify the maximum range in organism age that will be used to initiate testing (e.g., EFED recommends that range in test organism age be no more than 24 hours). If organisms older than 24 hours are used, EFED recommends the timing of the larval growth measurements be consistent with the organism age as specified in EPA 2000 (e.g., organism age between 20 and 21 days). This also ensures consistent application of the performance criterion for growth. EFED also recommends at this time that organisms used to initiate testing be kept to within the first instar.

**Section 2.5.6:** The submitted protocols state that surviving midge larvae from each replicate will be pooled and placed together. EFED recommends following the guidance from section 15.3.8.3.1 of EPA 600/R-99/064 that surviving larvae are kept separated by replicate for weight measurements. EFED also recommends that a consistent amount of time should be taken to examine sieved material for recovery of test organisms (*e.g.* 5 min/replicate).

**Section 2.5.8:** EFED recommends that the presence of secondary egg masses be recorded whenever this occurs in the reproductive/oviposit chambers for each treatment level and control. EFED agrees with the protocol that these egg masses should not be counted for egg numbers or used to determine hatch.

**Section 2.5.11:** The study protocols state that individual treatment levels or controls will be terminated if no additional emergence occurs for at least 7 days **OR** greater (typically between 50 and 65 days) or all treatment levels plus controls will be terminated on a single day between test day 55 and test day 65. Ten days is a large range in test duration; EFED requests that the laboratory identify in the final protocol what additional specific criteria will be used to determine the end of the test study.

**Section 2.5.12:** EFED recommends adding the following as acceptability criteria to the elements already identified in the study protocols:

- Tests age should be consistent among test chambers.
- The mean emergence rate was  $\geq 50\%$  in both negative control and solvent control, if a solvent vehicle was used.

**Reference:**

USEPA. 2000. Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates, Second Edition, EPA 600/R-99/064. March 2000. Office of Research and Development.