# Exhibit 60

AMVAC

December 15, 2014

Jill Bloom
U.S. Environmental Protection Agency
Document Processing Desk (DCI/PRD)
Office of Pesticide Programs (7508P)
2777 South Crystal Drive,
Arlington, VA 22202

Re: DCPA GDCI Data Call In (Order Number: GDCI-0798701-1140)
Chemical # PC Code: 078701 CAS #: 1861-32-1
Chronic Sediment Study Information and Proposed timelines for Guideline No: ss-1069, ss-1066, and ss-1072.

Dear Ms. Bloom:

Welcome back to working on the DCPA Registration Review DCI. Per Mr. Matthew Manupella's email dated December 5, 2014 the contact person has changed and as directed I will send the subject correspondence to your attention until further notice.

This submission includes information and proposed timelines regarding the Chronic Sediment studies in response to the Generic Data Call-In (GDCI) Notice dated January 31, 2013, more specifically the "DCPA (Chlorthal-dimethyl): Review of Study Protocols for Determining Chronic Toxicity to Sediment-Dwelling Estuarine/Marine and Freshwater Organisms" dated March 20, 2014, which AMVAC received October 20, 2014.

On April 29, 2013, Amvac Chemical Corporation (AMVAC) responded to EPA's Generic Data Call-In (GDCI) Notice for Dacthal dated January 31, 2013. At that time, three protocols were provided to the Agency for chronic sediment toxicity testing of *Chironomus dilutes* (ss-1069), *Hyalella azteca* (ss-1066), and *Leptocheirus plumulosus* (ss-1072). On October 20, 2014, we received the Agency response concerning the study protocols named. Immediately following, we contacted our performing laboratory Smithers Viscient and they scheduled two of the studies at their earliest timing. Both the *Chironomus dilutes* and *Hyalella azteca* studies are scheduled to be initiated in April of 2015. Final reports are anticipated to be complete and submitted to EPA by June 15, 2016.

Included with this document are Smithers Viscient's response to issues raised by the EPA in their review of the three protocols (Attachment I), study schedules for these two studies (Attachment II) and revised protocols (Attachments III and IV). Smithers Viscient has also discussed with us the need for additional method development time for establishing a rugged *Leptocheirus plumulosus* study. They have provided us with an update of their method development efforts and plans for proceeding (Attachment V). The laboratory anticipates that they will be able to address the current technical challenges that they are facing and AMVAC proposes to update the Agency by March 31, 2015 concerning the progress made at the laboratory and a schedule for this final sediment study.

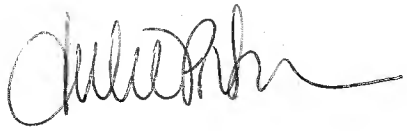
Please inform us if you have questions concerning our plan for addressing these three GDCI studies.

20141215jcp01.dcpa.us

For additional reference, the enclosed spreadsheet (Attachment VI) titled "Status of DCPA Registration Review DCI" dated December 15, 2014 has been updated. Please feel free to contact me at 949-221-6104 or email juliep@amvac-chemical.com if you have any questions or need further information.

Enclosures: Attachment I – EPA review including Smithers Viscient comments
Attachment II – Smithers Viscient letter regarding timelines for ss-1069 and ss-1066
Attachment III – Revised ss-1069 Protocol
Attachment IV – Revised ss-1066 Protocol
Attachment V – Smithers Viscient letter regarding ss-1072
Attachment VI – Status of DCPA Registration Review DCI

Best regards,



Julie Porter
Regulatory Product Manager

# Attachment I

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

**PC Code**: 078701
**DP Barcode**: 413319, 413320, and 413321
**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
Kevin Costello, Branch Chief
Risk Management and Implementation Branch 2
Pesticide Re-Evaluation Division (7508P)
Office of Pesticide Programs

**From:** Christina Wendel, Biologist Christina Wendel 03/20/14
Environmental Risk Branch 2
Environmental Fate and Effects Division (7507P)

**Through:** Brian Anderson, Branch Chief
Jean Holmes, DVM, MPH, Risk Assessment Process Leader (RAPL) Jean E Holmes 3/20/14
N.E. Federoff, Wildlife Biologist
Environmental Risk Branch 2
Environmental Fate and Effects Division (7507P)
Office of Pesticide Programs

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). 1

**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. 2

**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. 3

**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. 4

**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. 5

**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. 6

**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. 7

# Summary of Comments on DCPA Chronic Sediment Protocol Reviews_SMV RESPONSES.pdf

## Page: 2

Number: 1 Author: cpicard Subject: Sticky Note Date: 11/5/2014 7:56:13 AM
Changes made to clearly explain that a solvent control will be part of the study design. We also explain that amount of solvent will be equivalent across treatment levels/solvent control. It is difficult to give the exact volume without know the concentrations we will be testing at in definitive exposure. The exact volume added will be included in the report. Will add that solvent control will be part of range finding exposure also.

Number: 2 Author: cpicard Subject: Sticky Note Date: 11/5/2014 7:58:13 AM
Some general information about observations made of test organisms prior to testing were added. Typically, your control performance (meeting acceptability criteria) demonstrates that the population of organisms is acceptable for testing.

Number: 3 Author: cpicard Subject: Sticky Note Date: 11/5/2014 10:48:54 AM
Added that monitoring is done twice per year and that latest analysis will be added to report.

Number: 4 Author: cpicard Subject: Sticky Note Date: 11/5/2014 8:01:09 AM
Amount of sediment needed for analysis is dependent on concentration tested, analytical methodology etc. Consequently, exact amount of sediment needed will not be known prior to testing but will be in raw data.

Number: 5 Author: cpicard Subject: Sticky Note Date: 11/7/2014 12:00:38 PM
Water quality is expected to and will change over the course of these long term exposures as the overlying water is renewed, biological activity increases and food is added. Ammonia measurements are appropriate for this testing as sediment have historically supported testing. No protocol change needed.

Number: 6 Author: cpicard Subject: Sticky Note Date: 11/5/2014 8:03:27 AM
Measurements listed in protocol are sufficient to characterize the overlying and pore water quality throughout the exposure. Water quality listed in comments is excessive and typically utilized for field collected sediments where there may be differences in water quality based on individual sediment properties.

Number: 7 Author: cpicard Subject: Sticky Note Date: 11/5/2014 8:04:35 AM
Statement added to protocol that endpoints will also be based on measured pore water concentrations.

**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. [1]

**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. [2]

**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. [3]

**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. [4]

**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

# Page: 3

Number: 1 Author: cpicard Subject: Sticky Note Date: 11/5/2014 11:58:08 AM
Protocol allows for flexibility in size of organisms at initiation as currently written.

Number: 2 Author: cpicard Subject: Sticky Note Date: 11/5/2014 12:00:33 PM
Clarified in protocol.

Number: 3 Author: cpicard Subject: Sticky Note Date: 11/5/2014 12:16:16 PM
Weight of 60 organisms is sufficient to capture variability and is equivalent to what is used at government research facilities (ACOE). No change is needed.

Number: 4 Author: cpicard Subject: Sticky Note Date: 11/5/2014 11:24:15 AM
Change made and guidance removed from protocol.

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." [1]

**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). [2]

**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. [3]

**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. [4]

**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. [5]

# Page: 4

Number: 1 Author: cpicard Subject: Sticky Note Date: 11/5/2014 11:30:15 AM

Feeding regime has been modified since originally submitting this protocol for review and has yielded positive results to date. No change needed.

Observations of test system are made daily and explained elsewhere in the study protocol.

Number: 2 Author: cpicard Subject: Sticky Note Date: 11/5/2014 11:31:10 AM

Observations at test termination outlined in facility records as an SOP. These procedures are sufficient in obtaining accurate numbers of surviving amphipods.

Number: 3 Author: cpicard Subject: Sticky Note Date: 11/5/2014 11:35:14 AM

As length will be used to assess growth, this comments is not applicable.

Number: 4 Author: cpicard Subject: Sticky Note Date: 11/5/2014 8:06:50 AM

Change made and guidance removed from protocol.

Number: 5 Author: cpicard Subject: Sticky Note Date: 11/5/2014 8:11:33 AM

Smithers has conducted this method successfully using <24 hour old larvae as well as first instar larvae (1-4 days old). Government scientist from the USGS and EPA research facilities typically use older organisms to initiate (1-4) days old because the resulting biological data is less variable using older organisms. We agree with this based on our experience and suggest conducting the exposure with 1-4 day old organism with all organisms being within a 1 day range in age at initiation of the test. No change needed.

**Section 2.5.6:** The submitted protocols state that surviving midge larvae from each replicate will be pooled and place 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). 1

**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. 2

**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. 3

**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. 4
- The mean emergence rate was ≥50% in both negative control and solvent control, if a solvent vehicle was used. 5

**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.

# Page: 5

Number: 1 Author: cpicard Subject: Sticky Note Date: 11/5/2014 8:13:02 AM

Minor change made to clarify to state that midge larvae are pooled by replicate for weight measurement.

We can clarify that a consistent amount of time will be used to examine each replicate in the protocol. This is outlined in our SOP for terminating test vessels.

Number: 2 Author: cpicard Subject: Sticky Note Date: 11/5/2014 8:13:34 AM

Minor change made to clarify that secondary egg masses will be recorded as unsuccessful mating but not included in reproductive data.

Number: 3 Author: cpicard Subject: Sticky Note Date: 11/5/2014 8:14:34 AM

In order to facilitate scheduling, we typically terminate studies between day 55 and 65. Change made to toghten this range and edit the protocol to state that the exposure will definitively be terminated between day 60 and 65. This is long enough to capture sufficient data to asses emergence and reproductive endpoints.

Number: 4 Author: cpicard Subject: Sticky Note Date: 11/5/2014 8:15:44 AM

Inherent in protocol but acceptability criteria in protocol has been expanded to include this information.

Number: 5 Author: cpicard Subject: Sticky Note Date: 11/5/2014 8:16:18 AM

The emergence rate acceptability criteria of ≥50% is already referenced in this section.

# Attachment II

SMITHERS VISCIENT

November 7, 2014

To Whom It May Concern,

In May 2013, Smithers Viscient was contracted by AMVAC to perform chronic sediment toxicity testing with *Chironomus dilutus* (Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates, 2nd Edition", test method 100.5), *Hyalella azteca* (Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates, 2nd Edition", test method 100.4) and *Leptocheirus plumulosus* (Methods for Assessing the Chronic Toxicity of Marine and Estuarine Sediment-Associated Contaminants with the Amphipod *Leptocheirus plumulosus*) using dacthal.

Protocols were issued by Smithers Viscient and were sent to the agency for review. Comments were received from the regulators at the end of October 2014. Attached are the revised protocols as well as responses to EPA comments. In the responses, it is indicated where revisions have been made and, if no revisions was made, justification was given as to why no revision was necessary. Since originally submitting these protocols for review in May 2013, the protocols have evolved and we have worked with senior scientists at the EPA to draft a protocol that is acceptable to generate the appropriate data for risk assessment purposes.

Assuming that protocols are finalized by the end of 2014, a general schedule for the freshwater exposures is as follows:

***Hyalella azteca* and *Chironomus dilutus* Chronic:**

Start preliminary testing: Early April 2015
Terminate preliminary testing: Late June 2015
Start definitive testing: Mid October 2015
Terminate definitive testing: Late December 2015
Draft report: Late April 2016
Final report: Within three weeks of receiving comments from study monitor

For the chronic marine sediment exposure with *Leptocheirus plumulosus*, the EPA is aware of the ongoing pilot testing at Smithers Viscient focused on developing a more robust methodology. Attached is a detailed synopsis of the pilot work done to date and plans for future work.

If you have any questions or would like additional information, please contact me.

Very truly yours,
Smithers Viscient, LLC

Christian Picard
Senior Research Biologist

# Attachment III

SMITHERS VISCIENT

# TEST PROTOCOL

| | | |
|---|---|---|
| **Title:** | | 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 |
| **Data Requirement(s):** | | EPA Test Methods |
| **Test Substance(s):** | Name: | Dacthal Technical |
| | Purity: | 99.3% |
| | Batch or Lot #: | 120904-1 |
| **Analytical Standard:** | Name: | Dacthal Analytical Standard |
| | Purity: | 99.7% |
| | Batch or Lot #: | 10026-21-1 |
| **Study Sponsor:** | | AMVAC Chemical Corporation |
| **Address:** | | 4695 MacArthur Court, Suite 1200<br>Newport Beach, CA 92660 |
| **Study Monitor:** | | Dick Freedlander, Ph.D. |
| **Email / Phone Number:** | | DickF@amvac-chemical.com/949-260-1200 |
| **Sponsor Protocol/Project No. (when applicable):** | | NA |
| **Testing Facility:** | | Smithers Viscient<br>790 Main Street<br>Wareham, Massachusetts 02571 |
| **Study Director:** | | Christian R. Picard |
| **Smithers Viscient Study No.:** | | 11857-6110 |
| **Test Concentrations:** | | |
| **Proposed Experimental Dates** | | |
| | Start: | |
| | Termination: | |

**Sponsor Approval** _____ **Date** _____

**Study Director Signature** _____ **Study Initiation Date** _____



Smithers Viscient LLC
www.smithersviscient.com
790 Main Street | Wareham, MA 02571 | p 508.295.2550
2900 Quakenbush Road | Snow Camp, NC 27349 | p 336.376.0141

**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**

## 1.0 OBJECTIVE

The purpose of this test will be to determine the impact of a test substance to the life-cycle of the sediment-dwelling midge (*Chironomus dilutus*), under static-renewal conditions. The exposure duration is approximately 65 days or less. The study will assess the impact of the test substance on the survival, growth, emergence and reproduction of midges. The methods described in this protocol are designed to meet the testing requirements in the EPA document entitled "Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates, 2nd Edition", test method 100.5 (U.S. EPA 2000) and reflects the latest revisions based on discussions with regulatory scientists.

## 2.0 MATERIALS AND METHODS

### 2.1 Chemical System

#### 2.1.1 Test Substance

Upon arrival at Smithers Viscient, all test substances and reference substances will be received by the Test Material Center. Records will be maintained in accordance with GLP requirements, and a Chain-of-Custody established. The condition of the external packaging of the test substance will be recorded and any damage noted. The packaging will be removed, the primary storage container inspected for leakage or damage, and the condition recorded. Any damage will be reported to the Sponsor and/or manufacturer.

Each sample will be given a unique sample ID number and stored under the conditions specified by the Sponsor or manufacturer. The following information should be provided by the Study Sponsor, if applicable: test substance lot or batch number, test substance purity, water solubility (pH and temperature of solubility determination), vapor pressure, storage stability, methods of analysis of the test substance in water, MSDS, and safe handling procedures, and a verified expiration or reanalysis date.

#### 2.1.2 Test Substance Concentration Selection

Test substance concentrations will be based on the results of a preliminary range-finding test in consultation with the study sponsor. The objective of the preliminary exposure is to assess approximate level of toxicity and may be conducted prior to finalizing the protocol under non-GLP conditions. For the definitive test, the range of concentrations will be selected to determine a No-Observed-Effect Concentration (NOEC) and a Lowest-Observed-Effect Concentration (LOEC) based on the lethal and sublethal endpoints. If possible, based on the concentrations selected, an EC50 for sublethal endpoints and LC50 for lethal endpoints will also be calculated. A minimum of five test concentrations and a negative control will be used in the definitive test. A negative control consists of overlying water and sediment without the test substance or solvent.

The ratio for two adjacent test concentrations will be between 1.5 and 3.2 for definitive testing.

### 2.1.3 Solvent Control

An organic solvent (acetone) will be used as a carrier to solubilize the test substance. The solvent volume utilized will remain constant across the test concentration series. A solvent control will be included in the test (range-finding and definitive test) and will consist of sediment plus the equivalent volume of solvent used during the application of the test solutions to the sediment. An appropriate volume of each solvent stock will be added to silica sand and the solvent will be allowed to evaporate prior to mixing sand/test substance with the sediment, thereby minimizing the amount of solvent in the exposure system.

### 2.1.4 Application of Test Substance to Sediment

The appropriate amount of test substance will be removed from the test material container for dosing the exposure system (e.g., weighed on an analytical balance or volumetrically measured with a calibrated pipette). A Chemical Usage Log will be maintained in which the amount, the date, the intended use and the user's initials will be recorded each time the test substance is used. The amount of test substance will be applied to the sediment according to the following formula:

$$\text{Sediment Concentration (e.g., mg/kg)} = \frac{\text{T.S.}}{\text{S.A.} \times \text{D.W.}}$$

where:

| | | |
|---|---|---|
| T.S. | = | test substance (e.g., mg) |
| S.A. | = | sediment amount (kg) |
| D.W. | = | (percent dry weight of sediment) ÷ 100 |

The test substance will be applied by the following method:

A jar-rolling technique will be used to apply the test substance to the sediment (Ditsworth et al., 1990). If a solvent is utilized, the test substance will be applied to the sediment for each treatment level by directly adding the appropriate amount of test substance in a solvent stock solution to a small sample (i.e., 50 grams) of fine silica sand. The 50 grams of sand will be mixed thoroughly with a metal spatula for approximately 2 minutes. The solvent will be allowed to slowly and completely evaporate off for at least 20 minutes prior to mixing the sand into the appropriate amount of sediment. The sand containing the test substance and the appropriate weight of sediment (e.g., 3 kg wet weight) will be added to a glass jar and rolled for four hours at approximately 15 rpm on a rolling mill. Following the initial four hours of rolling, the jars will be stored upright under complete darkness at approximately 2-8 °C. The sediments will be allowed to equilibrate for at most a 30 ± 3 day period in the refrigerator. Once a week during the equilibration period and prior to addition into the replicate exposure vessels, the jars will be mixed on the rolling mill for approximately two hours to ensure the sediment is homogenous.

The exact equilibration period used in testing will be dependent upon the results of a trial equilibration study conducted in conjunction with the testing program. The results of this equilibration study will be presented in the final report of this study. However, this equilibration trial is considered a separate pilot study and not conducted under this protocol or GLP conditions.

## 2.2 Test System

### 2.2.1 Species

The dipteran midge, *Chironomus dilutus*, will be used to conduct the toxicity test. First instar larvae (< 24 hours to 4 days old with all organisms being within a 1 day range in age at initiation of the test.) will be used to initiate the exposure.

### 2.2.2 Justification of Species

Midges (*Chironomus dilutus*) will be used for several reasons. The larvae are sediment dwellers, widely distributed throughout North America, and are considered a reasonable representative of aquatic benthic invertebrates (Adams et al., 1985). The organism is easily cultured and has a relatively short life cycle (approximately 30 days at 25 °C), making it suitable for toxicity tests.

### 2.2.3 Origin

The midge larvae used to initiate the exposure will be obtained from in-house cultures at Smithers Viscient. In-house cultures are maintained on a daily basis and monitored for specific indicators of population health such as time to emergence and oviposition, as well as indicators of poor health such as larval mortality or delayed development. Prior to test initiation, newly laid egg masses will be isolated from the midge cultures and will be held in laboratory well water at the approximate test temperature (approximately 23 ± 2 °C). Egg masses will be checked daily for hatch and development. Hatch of the eggs should be complete approximately 72 hours after egg masses have been produced. Larvae which are released from egg masses which exhibit poor hatching (less than approximately 80% based on visual assessment) will not be used. First instar larvae to be used in the exposure will be pooled post hatch and prior to initiation. A description of the holding conditions and health assessments of the isolated egg masses and larvae prior to test initiation will be documented in the raw data.

### 2.2.4 Feeding

During the test, midges will be fed a finely ground flaked fish food suspension (4.0 mg/mL) on a daily basis. During the test, each replicate test vessel will be fed 1.5 mL of the 4.0 mg/mL flaked fish food suspension. A sample of the food source will be biannually analyzed using U.S. EPA standard methods (U.S. EPA, 1997) by GeoLabs, Inc., Braintree, Massachusetts, in accordance with Smithers Viscient's standard operating procedures, for the presence of pesticides, PCBs and selected toxic metals.

### 2.2.5 Handling

Wide-bore pipets will be used to transfer the midges, taking care to minimize possible stress due to handling. Midges that are damaged or dropped during transfer will not be used.

## 2.3 Physical System

### 2.3.1 Sediment

Artificial (formulated) sediment will be used in the exposure. The artificial sediment will be prepared based on the OECD 218 guideline (OECD, 2004) and will be prepared as follows:

a. 5 % (dry weight) sphagnum moss peat: no visible plant remains, air dried and finely ground. Peat will be soaked in laboratory well water for at least 5 days. Calcium carbonate ($CaCO_3$) will be added to adjust the pH of the peat mixture to 5.5 to 6.0.
b. 20 % (dry weight) kaolin clay (with kaolinite content, if possible, of >30%).
c. 75 % (dry weight) industrial sand (with >50% of the particles between 50 and 200 microns).
d. Laboratory well water will be added during mixing to obtain a homogeneous sediment batch.
e. Organic carbon content of the final mixture will be approximately 2% ± 0.5% and is to be adjusted by the use of appropriate amounts of peat and sand, according to a and c. Slight excursions from 2% ± 0.5% organic carbon content of the formulated sediment are common based on variability in the peat component. The batch of sediment used in the exposure will be evaluated as acceptable for use by the study director prior to testing.

The dry constituents are blended together in the correct proportions and mixed thoroughly in a large scale laboratory mixer (e.g. Hobart mixer). The artificial sediment will be characterized for total organic carbon (TOC) content, percent sand, silt, clay (particle size distribution) and percent water holding capacity by Agvise Laboratories, Northwood, North Dakota. A pH measurement of the sediment may also be made by either Agvise Laboratories, Northwood, North Dakota or at Smithers Viscient.

Periodic analysis of representative samples of the artificial sediment will be conducted using U.S. EPA standard methods (U.S. EPA, 1997) by GeoLabs, Inc., Braintree, Massachusetts, in accordance with Smithers Viscient standard operating procedures, to ensure the absence of potential toxicants, including pesticides, PCBs and selected toxic metals, at concentrations which may be harmful to the test organisms.

### 2.3.2 Test Vessels

The test vessels used in the static-renewal test will be 300-mL vessels. Each test vessel will have notches or slots cut on the top edge of the vessel and will be covered with 40-mesh nylon screen to allow for drainage during the renewal of overlying water drainage. Each vessel will contain 100 mL (an approximate 4-cm layer) of sediment and 175 mL of overlying water. The overlying/sediment volume will thus be maintained at approximately 275 mL. The mean wet weight of sediment added to all test vessels will

be determined by randomly selecting three replicate test vessels from each treatment level and control and weighing the mass of sediment added to each vessel on test day – 1. The test vessels will be labeled to identify the treatment/control, study number, and the replicate designation. On approximately test day 18, emergence traps will be placed over the test vessels to trap emergent flies for the remainder of the test. Emergence traps may be placed on the vessels earlier in the test if observations of emergence are evident. The emergence traps consist of a 3.5-cm tall Plexiglas tube (inside diameter of 6-cm) covered on the top with wide mesh Nitex screen.

### 2.3.3 Replication and Control of Bias

Twenty replicates will be included with each test concentration and the solvent control (if a solvent control is included in the study design). Twenty-three replicates will be included in the negative control. Twelve replicates (A through L) will be used to evaluate the biological response of the test organisms. Four of the replicates will be used for organism survival and growth measurements and the remaining eight replicates will be used for monitoring midge emergence. Four additional replicate vessels (M through P) will be established on test day 10 for production of auxiliary males during the emergence phase of the test but will not be included in any of the other biological observations. Replicate vessels Q through T of each concentration and the controls will be maintained for the purpose of analytical measurements. The last three negative control replicates (U through W) will be maintained for the purpose of measuring representative pore water quality characteristics (pore water ammonia and pH). Each replicate vessel for monitoring the biological response (replicates A through L) will contain twelve individuals, a total of 144 midges per concentration or control. The additional analytical and pore water quality replicates will be maintained under the same conditions as the biological replicates. These additional replicates will contain test organisms with the exception of the replicates being sacrificed at test initiation for analytical/water quality measurements. All the additional analytical/water quality replicates will not be included in the biological observations for the study.

Midges (first instar) will be added impartially to the test vessels. The test will be initiated when all applicable vessels contain twelve first instar midge larvae.

In addition, the position of the water distribution systems and the replicate test vessels under each water distribution system will be assigned in the water bath randomly.

### 2.3.4 Overlying Water

The overlying water source consists of unadulterated water from a 100-meter bedrock well supplemented on demand with untreated Town of Wareham well water, and will be characterized as soft water with an approximate total hardness of <180 mg/L as $CaCO_3$. Total hardness, total alkalinity, pH and conductivity of the overlying water source will be monitored weekly at a central location in the laboratory to assure that these parameters are within the normal acceptable ranges. These measured ranges during the conduct of the exposure will be transcribed and included in the raw data and report. Total hardness and alkalinity will be determined according to *Standard Methods for the Examination of Water and Wastewater* (APHA, 2005). Twice per year, analysis of representative samples of the overlying water source will be conducted using U.S. EPA standard methods (U.S. EPA, 1997) by GeoLabs, Inc., Braintree, Massachusetts, in accordance

with Smithers Viscient's standard operating procedures, to ensure the absence of potential toxicants, including pesticides, PCBs and selected toxic metals, at concentrations that may be harmful to the test organisms. Results of the most recent analysis will be includes as an appendix in the study report.

### 2.3.5 Renewal of Overlying Water

During the long-term study, the overlying water will be renewed by adding two volume additions (i.e., 350 mL) per day using an intermittent delivery system in combination with a calibrated water-distribution system (Zumwalt et al., 1994). The intermittent delivery system will be calibrated to provide 1 liter of water per cycle to the water-distribution system, which subsequently provides 50 mL of water (no test material present) per cycle to each replicate test vessel. The water delivery system cycles 7 times per day and provides 2 volume additions every 24 hours. Delivery of two volume replacements per day is generally sufficient to provide consistent and acceptable water quality characteristics throughout the duration of the study (typically 60 to 65 days). However, in the event that dissolved oxygen levels drop to unacceptable levels, the cycle rate may be increased to increase dissolved oxygen levels.

The calibration of the renewal system will be checked prior to test initiation and at test termination. If there is any indication during the test that the renewal system calibration has changed (e.g., delivery system malfunction or unexplained differences in dissolved oxygen concentration or temperature in the test vessels), calibration of the necessary renewal system components will be checked. During the test, the renewal system will be visually inspected at least twice daily. A complete check of the water delivery system will be made once daily.

## 2.4 Test Conditions

### 2.4.1 Temperature

Water temperature of the overlying water will be maintained at 23 ± 1 ºC by conducting the test in a temperature-controlled room or water bath maintained at the appropriate test temperature. Temperature will be monitored continuously in an auxiliary vessel housed in the same water bath as the test vessels using a thermometer. Readings of minimum and maximum temperatures will be recorded daily.

### 2.4.2 Lighting

The test will be conducted in a light controlled laboratory. The test will be illuminated to a light intensity of 100 to 1000 lux using fluorescent bulbs. The light intensity will be measured once during the test. A 16-hour light, 8-hour dark photoperiod will be maintained with an automatic timer.

### 2.4.3 Dissolved Oxygen

The total dissolved oxygen should be > 2.5 mg/L for the duration of the test. If dissolved oxygen levels fall below 2.5 mg/L, the cycle rate of the water-renewal system may be increased (e.g., 2 to 4 volume replacements per day) to increase the dissolved oxygen levels. Aeration, with oil free air, will be initiated as a final option to maintain the

dissolved oxygen concentration above 2.5 mg/L. If aeration is required, it will be applied to all replicates within all the treatment level/control group(s).

### 2.4.4 Test Vessel Preparation and Test Initiation

The day before test initiation (day -1) the treated sediments and control sediment(s) will be added to the replicate test vessels and the overlying water will be added. The water will be added gently to prevent re-suspension of the sediment layer in the water column. This allows the sediment and water to equilibrate prior to addition of the test organisms. On test day 9 of the exposure, four additional replicate test vessels (replicates M through P) will also be established and placed under the renewal system. These four additional vessels will be established for each treatment level and control for auxiliary male production.

The test will be initiated (Day 0) when all applicable vessels contain twelve first instar midge larvae (144 per test concentration and control). On test day 10 of the exposure, the four replicate vessels established for auxiliary male production will be initiated when all vessels contain twelve first instar midge larvae. Data from the replicates used to produce auxiliary males will not be collected or included in the assessment of endpoints listed in Section 3.1.

## 2.5 Sampling And Observations

### 2.5.1 Measurement of Test Substance Concentration

After application and mixing of the test substance with the sediment and prior to division into the individual replicate exposure vessels (i.e., during the equilibration period), a sample of treatment and control bulk sediment will be taken from each treatment level and control for determination of test substance concentrations. Three sediment quality control samples will also be analyzed with the bulk sediment samples. These quality control samples will be prepared at the time of sampling and will be handled and analyzed along with the bulk sediment samples. In addition, the stock solutions used to dose the sediment will be sampled and analyzed at the approximate time of dosing.

At test initiation (test day 0), approximately test day 20 and test termination, one sample from the overlying water, pore water and sediment of each treatment and control will be removed and analyzed for test substance concentration. The ability to accurately measure aqueous concentrations during the study will be based on the limit of detection of the methodology employed, concentrations of test substance in the aqueous samples, volume of aqueous samples produced from the test vessels and multiple other factors. A single day, at or near completion of midge emergence will be selected for analytical measurements of all treatment levels and controls for this study. Six quality control (QC) samples (three aqueous and three sediment samples) will be prepared at each sampling interval and stored and analyzed with the set of study samples. The sediment QC samples will be prepared in the test sediment at test substance concentrations similar to the treatment level range. The aqueous QC samples will be prepared in laboratory well water at relevant concentrations that can be utilized to demonstrate the accuracy of the analytical method. Results of these analyses indicate the accuracy of the analytical method used for measuring test substance concentration at each sampling period. If applicable, the analytical methods utilized in the testing will be validated by Smithers Viscient at the expected nominal concentration range prior to test initiation.

### 2.5.2 Sampling Procedures

The entire volume of overlying water will be removed and the appropriate volume collected for analysis from each test vessel by carefully decanting or pipetting. Pore water samples will be collected by removing the entire sediment sample (approximately 100 mL of sediment) from the test vessel and centrifuging for 15 to 30 minutes at a rate of approximately 10,000 *g*. Following centrifuging, the pore water will be decanted or removed by pipette for analysis. Following removal of the pore water from the sediment sample, an appropriate sized sediment sample will be removed from the centrifuge tube with a metal spatula and mixed thoroughly prior to determination of sediment concentrations.

### 2.5.3 Water Quality Measurements

At test initiation, test day 10 (initiation of the male auxiliary replicates), approximately test day 20 and test termination, temperature, dissolved oxygen (DO) concentration and pH will be measured in the overlying water and recorded for each test vessel (replicates A through L on days 0, 10 and approximately day 20 and the eight remaining replicates at test termination). On the remaining test days, temperature and dissolved oxygen will be measured and recorded daily in one alternating replicate of each control/treatment level. Measurement of temperature, dissolved oxygen and pH in the overlying water column will be made with probes and readings will be recorded in the water column approximately 1 to 2 cm above the sediment surface in each vessel. Total hardness, alkalinity, conductivity and total ammonia concentration will be determined in the overlying water at test initiation, test day 10 (initiation of the male auxiliary replicates), approximately test day 20 and test termination in a sample from each treatment level and control. Samples of the overlying water collected for total hardness, alkalinity, conductivity and total ammonia concentration will consist of a composite sample of water from multiple replicates and will be collected by pipette from approximately 1 to 2 cm above the sediment surface in each vessel.

### 2.5.4 Pore Water Quality Measurements

Representative pore water quality measurements (ammonia and pH) will be made throughout the exposure by sampling control replicates U through W. These measurements will be made on test days 0, approximately day 20, and at test termination. Pore water will be collected as described in section 2.5.2.

In using formulated sediment, pore water quality variables are relatively consistent across treatments, and those variables that could affect organism performance are mitigated. The aforementioned measurements will be considered representative of all treatment groups, as well as the controls, and will be used to establish that the sediment was suitable for testing during the exposure.

### 2.5.5 Biological Observations – Midge Observations

Observations of dead organisms (larvae or pupae) on the sediment surface, organism behavior (e.g., sublethal effects) and characteristics of sediment/overlying water will also be observed and recorded daily.

### 2.5.6 Biological Observations – Midge Survival and Growth

Prior to determination of organism survival and growth, four of the twelve replicate test vessels will be randomly selected. Midge survival and growth will be determined in these four designated test vessels of each treatment level and control on approximately test day 20 by sieving the sediment through a ≤425 µm mesh net or sieve to remove all surviving midges. Any immobile organisms isolated from the sediment surface or sieved sediment should be considered dead. Any pupae or adult midges encountered on or prior to survival and growth determination will be incorporated into the assessment of survival. The growth of surviving midge larvae only, as ash-free dry weight (AFDW), in each of these four replicates will also be recorded on approximately test day 20. All surviving midge larvae from each replicate vessel will be pooled per replicate and then placed in an ashed weighing tin. The weighing tins and pooled larvae will then be dried at 70 to 90 °C for approximately 24 ± 2 hours. The weighing tins containing the pooled dried larvae will then be cooled to room temperature and weighed on a calibrated analytical balance to the nearest 0.01 mg. After dry weights are obtained, the pooled dried larvae will be ashed in an oven at 550 ± 50 °C for approximately 2 hours. The ashed tins will then be cooled to room temperature and weighed on a calibrated analytical balance to the nearest 0.01 mg.

### 2.5.7 Biological Observations – Midge Percent Emergence and Emergence Rate

Starting on approximately test day 17, the number of male and female midges emerged from each replicate test vessel will be observed and recorded daily. The timing of these observations on emergence is dependent on the timing of initial emergence. Complete emergence occurs when a midge has shed the pupal exuvia completely and escapes the surface tension of the water. If a midge has shed the pupal exuvia but the midge has not escaped the surface tension, it will die within 24 hours. Therefore, midges that have shed the exuviae but not escaped the surface tension will be observed in the vessel for an additional 24 hours; if the midge has not escaped the surface tension after 24 hours, it will be recorded as a dead midge. The calculated response measure will be the proportion of the initial larvae that achieve complete emergence.

The mean male and female emergence rate per vessel is calculated according to the following calculation:

$$\text{Mean emergence rate} = \sum_{i=1}^{m} (F_i \bullet X_i) \div N_e$$

where:

| | | |
|---|---|---|
| m | = | maximum number of inspection intervals |
| i | = | index of inspection interval |
| $F_i$ | = | number of midges emerged in the inspection interval i |
| $N_e$ | = | total number of midges emerged until the end of the study (= $\Sigma F_i$) |

$X_i$ = emergence rate of the midges emerged in the interval i

$$X_i = 1 / \left[ day_i - \left( L_i / 2 \right) \right]$$

where:

$day_i$ = inspection day (days since application of test substance)

$L_i$ = length of inspection interval i (i = 1 day)

### 2.5.8 Biological Observations – Adult Midge Collection for Reproduction

Starting on approximately test day 17, the male and female midges emerged from each replicate test vessel will be collected daily using a collector dish and placed in reproductive/oviposit chambers. The timing of adult midge collection for emergence will be dependent on the timing of initial emergence. Reproductive/oviposit chambers consist of a 3.5-cm tall Plexiglas tube (inside diameter of 6 cm) covered on the top with wide mesh Nitex screen placed on top of a 100 x 20-mm petri dish. Once the adult midges are placed in the reproductive/oviposit chambers, approximately 50 mL of laboratory well water will be added to the petri dish. Male and female adult midges from each treatment level will be held individually until sufficient numbers are available to pair male/female adults in a male: female ratio of 1:1. The acceptability of this ratio to produce sufficient reproductive data has previously been confirmed by regulators and historical data. Survival of individual adults (male and female) will be recorded daily until death at which time the days until death will be calculated. Auxiliary males will be used to mate female midges towards the end of the female emergence period as male midges typically start to emerge 5 to 7 days prior to female midges. Each male may be used for mating with females from corresponding treatment levels for up to 5 days. Males may be used for breeding with more than one new emergent female from corresponding treatment levels. Males from a different replicate within the same treatment level may be paired with females of replicates where no males have emerged.

### 2.5.9 Biological Observations – Monitoring Reproduction

The reproductive/oviposit chambers for each treatment level and control will be checked daily for dead adults and egg masses. Dead adults will be removed daily. Female adults (paired with a male) will generally oviposit within 1 to 3 days and the days until oviposition will be recorded. Females generally will lay a single primary egg mass. Sometimes a second, generally smaller egg mass may be laid. These second egg masses are prone to fungus and poor viability and will not be counted for egg numbers or used to determine hatch but will be recorded in the raw data.

### 2.5.10 Biological Observations – Egg Counts and Hatch Determination

The number of eggs produced in each primary egg mass laid by females in each treatment level and control will be will be counted the day the egg mass is laid. The ring method will be used to determine the number of eggs in each egg mass. Five rings of eggs in each egg mass will be selected at about equal distances along the length of the egg mass. The number of eggs will then counted in these five rings. The mean number of eggs per ring will then be multiplied times the number of rings in the egg mass to estimate the total number of eggs. Egg masses will then be incubated in approximately 20 mL of laboratory well water in 30-mL plastic cups at the approximate test temperature (23 $\pm$ 1 °C). Typical hatch occurs within 2 to 6 days after the egg mass is laid. The number of unhatched eggs will be counted following 6 days of incubation post oviposition. Unhatched eggs either remain in the gelatinous mass or are distributed on the bottom of the incubation cup. Hatching success will be determined by subtracting the number of unhatched eggs from the original estimate of egg numbers from that egg mass.

### 2.5.11 Biological Observations – Termination of the Test

All treatment levels plus controls will be terminated on a single day between test day 60 and 65. This is a sufficient amount of time to collect data on midge emergence, development and reproduction for which to assess toxicity. At time of termination, water quality measurements will be performed on each treatment level plus controls.

### 2.5.12 Acceptability Criteria

The following criteria will be used to assess the acceptability of the exposure:

- The exposure was started with first instar midge larvae.

- All organisms in the exposure were from the same source.

- All test vessels were identical and contained the same amount of sediment and overlying water.

- A negative control group and a solvent control was included in the exposure.

- The test organism survival at the time of larval survival determination was ≥70% in both negative control group and solvent control group.

- The mean larval weight at the time of larval growth determination was ≥0.48 mg/surviving organism as AFDW in both negative control group and solvent control group.

- The mean percent emergence was ≥50% in both negative control and solvent control groups.

- The mean number of eggs/egg mass was ≥800 or the mean percent hatch was ≥80% in both negative control and solvent control groups.

## 3.0 ENDPOINT CALCULATIONS AND STATISTICAL ANALYSIS

### 3.1 Endpoints

The endpoints used for determination of significant effects by statistical evaluation are outlined in the table below:

| Lethal | Sublethal | | |
|---|---|---|---|
| Survival | Growth | Emergence | Reproduction |
| Larvae/Pupae/Adults (approximately day 20) | Larval Ash-Free Dry Weight (approximately day 20) | Total Percent Emergence | Primary Egg Masses per Mated Female |
| | | Male and Female Emergence Rate | Eggs per Primary Egg Mass |
| | | Male and Female Midge Days To Death After Complete Emergence | Eggs per Mated Female |
| | | | Egg Hatchability |
| | | | Time to Oviposition |

All concentration-effect relationships will be based on measured concentrations of test substance in the sediment. In addition, concentration-effect relationships may also be based on measured concentrations of test substance in the pore water if requested by the sponsor and regulatory scientists. All statistical analyses will be performed using the Comprehensive Environmental Toxicity Information System™ (CETIS, Ives, 2013).

### 3.2 Statistical Methods (Determination of LOEC/NOEC)

If a solvent is used as a carrier for the test substance, a t-Test ($p \leq 0.05$) will be used to compare the results (survival, growth and reproduction) of the solvent control to the negative control data. All the remaining statistical analyses will be performed comparing the treatment data to the negative control data per current EPA guidance.

The data will be tested for normality and homogeneity of variance using the appropriate qualifying test. If the data passes these two tests, then a parametric method will be used to evaluate the results of test. If the data fails the test for normality or homogeneity of variance, then a non-parametric method will be used to evaluate the results of the test. The Lowest-Observed-Effect-Concentration (LOEC) is defined as the lowest test concentration that shows a statistically significant effect ($p \leq 0.05$) and the No-Observed-Effect-Concentration (NOEC) is the highest test concentration that shows no statistically significant difference from the control.

### 3.3 Transformations

Transformation of data will be performed with data representing endpoint estimates obtained as a proportion (e.g., survival or percent emergence). Prior to analyzing data of this type, the observed proportion in each vessel will be transformed by using an angular transformation (arcsine square-root).

### 3.4 LC50 Calculation

The LC50 is the estimated concentration of the test substance that causes mortality of 50% of the test organisms when compared to the control data. If applicable, a computer program will be used to estimate the LC50 value using an appropriate method.

### 3.5 EC50 Calculation

The EC50 is the estimated concentration of the test substance that produces a 50% reduction in growth or reproduction of the test organisms when compared to the control data. If applicable, a computer program will be used to estimate the EC50 values using an appropriate method.

## 4.0 RECORDS TO BE MAINTAINED

Records to be maintained will include, but will not be limited to, correspondence and other documents relating to the interpretation and evaluation of data as well as all raw data and documentation generated as a result of the study.

## 5.0 REPORTING

The raw data generated at Smithers Viscient will be peer-reviewed and the final report will be reviewed by the Study Director. All values will be reported to various levels of significance depending on the accuracy of the measuring devices employed during any one process. The Quality Assurance Unit will inspect the final report to confirm that the methods, procedures, and observations are accurately and completely described, that the reported results accurately and completely reflect the raw data generated at Smithers Viscient and to confirm adherence with the study protocol. A single copy of the draft report will be submitted to the Sponsor for review. The report will be finalized according to standard operating procedures. The final report will meet the formatting requirements of EPA's PR Notice 2011-3. All reports will include, but will not be limited to, the following information:

- The report and project numbers from Smithers Viscient and Sponsor study number (if any).

- Laboratory and site, dates of testing and personnel involved in the study, i.e., Program Coordinator (if applicable), Study Director and Principal Investigator.

- Identification of the test substance which may include chemical name, additional designations (e.g., trade name), chemical designation (CAS number), empirical formula, molecular structure, manufacturer, lot or batch number, water solubility, vapor pressure,

degree of purity of test substance (percent test chemical) (Sponsor supplied, if available).

- Characterization and origin of the overlying water source.
- Characterization, percent organic carbon, and preparation of the sediment.
- Scientific name of the test organisms, source, age and culturing information.
- Test container volume, sediment and water volume, number of replicates used per concentration, and number of midges used per treatment.
- Description of exposure system and application of test substance to sediment.
- Test temperatures, dissolved oxygen concentration, and pH; and photoperiod and light intensity used, as well as conductivity, total ammonia, total alkalinity and total hardness measured.
- Definition of criteria used to determine the sublethal effects, and general observations on non-quantifiable effects.
- Summary of lethal and sublethal test endpoints, in tabular form. These endpoints include survival and growth on approximately test day 20, emergence data (total percent emergence, emergence rate for males and females, days to death after complete emergence by gender) and reproduction (number of eggs per primary egg mass, number of primary egg masses per mated female, number of eggs per female, egg hatchability and time to oviposition).
- Description or reference (or inclusion as an appendix) to chemical and statistical procedures applied.
- Analytical results of test concentration measurements and QC samples.
- If applicable, means and standard deviations of measured concentrations of the test compound, as well as nominal test concentrations.
- The EC50/LC50 with 95 percent confidence limits, for test endpoints of midges, if possible.
- The Lowest-Observed-Effect Concentration (LOEC) tested based on statistical analyses.
- The No-Observed-Effect Concentration (NOEC) tested based on statistical analyses.
- Good Laboratory Practice (GLP) compliance statement signed by the Study Director.
- Date(s) of Quality Assurance reviews, and dates reported to the Study Director and management, signed by the Quality Assurance Unit.
- Location of protocol, raw data and final report.

## 6.0 PROTOCOL CHANGES

All amendments to the approved protocol must be documented in writing and signed by both the Study Director and the Sponsor's contact or representative. Protocol amendments and deviations must include the reasons for the change and the predicted impact of the change on the results of the study, if any.

## 7.0 GOOD LABORATORY PRACTICES

All test procedures, documentation, records and reports will comply with the Organization of Economic Co-operation and Development's (OECD) Good Laboratory Practices as set forth under the OECD Guidelines for the Testing of Chemicals and the U.S. Environmental Protection Agency's Good Laboratory Practices as set forth under the Federal Insecticide, Fungicide and Rodenticide Act (40 CFR, Part 160).

## 8.0 REFERENCES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# Attachment IV

SMITHERS VISCIENT

# TEST PROTOCOL

**Title:** 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

**Data Requirement(s):** EPA Test Methods

**Test Substance(s):**
Name: Dacthal Technical
Purity: 99.3%
Batch or Lot #: 120904-1

**Analytical Standard:**
Name: Dacthal Analytical Standard
Purity: 99.7%
Batch or Lot #: 10026-21-1

**Study Sponsor:** AMVAC Chemical Corporation
**Address:** 4695 MacArthur Court, Suite 1200
Newport Beach, CA 92660

**Study Monitor:** Dick Freedlander, Ph.D.
**Email / Phone Number:** DickF@amvac-chemical.com/949-260-1200
**Sponsor Protocol/Project No. (when applicable):** NA

**Testing Facility:** Smithers Viscient
790 Main Street
Wareham, Massachusetts 02571

**Study Director:** Christian R. Picard

**Smithers Viscient Study No.:** 11857-6111

**Test Concentrations:**

**Proposed Experimental Dates**
Start:
Termination:

_____ _____
**Sponsor Approval** **Date**

_____ _____
**Study Director Signature** **Study Initiation Date**



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**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**

## 1.0 OBJECTIVE

The purpose of this test will be to determine the impact of a test substance to the freshwater, sediment-dwelling amphipod (*Hyalella azteca*), under static-renewal conditions for 42 days. The study will assess the impact of the test substance on the survival, growth and reproduction of the amphipods. The methods described in this protocol are designed to meet the testing requirements in the EPA document entitled "Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates, 2nd Edition", test method 100.4 (U.S. EPA 2000) and reflects the latest revisions based on discussions with regulatory scientists.

## 2.0 MATERIALS AND METHODS

### 2.1 Chemical System

#### 2.1.1 Test Substance

Upon arrival at Smithers Viscient, all test substances and reference substances will be received by the Test Material Center. Records will be maintained in accordance with GLP requirements, and a Chain-of-Custody established. The condition of the external packaging of the test substance will be recorded and any damage noted. The packaging will be removed, the primary storage container inspected for leakage or damage, and the condition recorded. Any damage will be reported to the Sponsor and/or manufacturer.

Each sample will be given a unique sample ID number and stored under the conditions specified by the Sponsor or manufacturer. The following information should be provided by the Study Sponsor, if applicable: test substance lot or batch number, test substance purity, water solubility (pH and temperature of solubility determination), vapor pressure, storage stability, methods of analysis of the test substance in water, MSDS, and safe handling procedures, and a verified expiration or reanalysis date.

#### 2.1.2 Test Substance Concentration Selection

Test substance concentrations will be based on the results of a 42-day preliminary range-finding test in consultation with the study sponsor. The objective of the preliminary exposure is to assess approximate level of toxicity and may be conducted prior to finalizing the protocol under non-GLP conditions. For the definitive test, the range of concentrations will be selected to determine a No-Observed-Effect Concentration (NOEC) and a Lowest-Observed-Effect Concentration (LOEC) based on the survival, growth and reproduction of the amphipods. If possible, based on the concentrations selected, an LC50 for survival and EC50 for growth/reproduction will also be calculated. A minimum of five test concentrations and a negative control will be used. A negative control consists of overlying water and sediment without the test substance or solvent. The ratio for two adjacent test concentrations will be between 1.5 and 3.2.

### 2.1.3 Solvent Control

An organic solvent (acetone) will be used as a carrier to solubilize the test substance. The solvent volume utilized will remain constant across the test concentration series. A solvent control will be included in the test (range-finding and definitive test) and will consist of sediment plus the equivalent volume of solvent used during the application of the test solutions to the sediment. An appropriate volume of each solvent stock will be added to silica sand and the solvent will be allowed to evaporate prior to mixing sand/test substance with the sediment, thereby minimizing the amount of solvent in the exposure system.

### 2.1.4 Application of Test Substance to Sediment

The appropriate amount of test substance will be removed from the test material container for dosing the exposure system (e.g., weighed on an analytical balance or volumetrically measured with a calibrated pipette). A Chemical Usage Log will be maintained in which the amount, the date, the intended use and the user's initials will be recorded each time the test substance is used. The amount of test substance will be applied to the sediment according to the following formula:

$$\text{Sediment Concentration (e.g., mg/kg)} = \frac{\text{T.S.}}{\text{S.A.} \times \text{D.W.}}$$

where:

| | | |
|---|---|---|
| T.S. | = | test substance (e.g., mg) |
| S.A. | = | sediment amount (kg) |
| D.W. | = | (percent dry weight of sediment) ÷ 100 |

The test substance will be applied by the following method:

A jar-rolling technique will be used to apply the test substance to the sediment (Ditsworth et al., 1990). ). If a solvent is utilized, the test substance will be applied to the sediment for each treatment level by directly adding the appropriate amount of test substance in a solvent stock solution to a small sample (i.e., 50 grams) of fine silica sand. The 50 grams of sand will be mixed thoroughly with a metal spatula for approximately 2 minutes. The solvent will be allowed to slowly and completely evaporate off for at least 20 minutes prior to mixing the sand into the appropriate amount of sediment. The sand containing the test substance and the appropriate weight of sediment (e.g., 3 kg wet weight) will be added to a glass jar and rolled for four hours at approximately 15 rpm on a rolling mill. Following the initial four hours of rolling, the jars will be stored upright under complete darkness at approximately 2-8 °C. The sediments will be allowed to equilibrate for at most a 30 ± 3 day period in the refrigerator. Once a week during the equilibration period and prior to addition into the replicate exposure vessels, the jars will be mixed on the rolling mill for an approximately two hours to ensure the sediment is homogenous.

The exact equilibration period used in testing will be dependent upon the results of a trial equilibration study conducted in conjunction with the testing program. The results of this

equilibration study will be presented in the final report of this study. However, this equilibration trial is considered a separate pilot study and not conducted under this protocol or GLP conditions.

## 2.2 Test System

### 2.2.1 Species

The freshwater invertebrate, *Hyalella azteca*, is the species used in this test. Test organisms will be 7 to 8 days old at initiation of the test and will be obtained from in house cultures or a reputable outside supplier. If amphipods are obtained from in house cultures test organisms will be obtained by removing adult amphipods from main culture tanks and placing them in 9.5-liter aquaria with approximately 8 L of water, 8 to 9 days prior to test initiation. On the following day, young produced by these isolated adults will then be removed from the isolation tanks and pipetted into 1-L beakers containing 0.80 liters of water until test initiation. If test organisms are obtained from an outside supplier, they will be received and maintained at Smithers Viscient in 1-L beakers containing 0.80 liters of water for a minimum of 48 hours prior to testing. Amphipods will not be used if > 10% mortality is observed during the 48 hours prior to test initiation and all amphipods used in the exposure will be obtained from a consistent source.

### 2.2.2 Justification of Test System

The characteristics which make this test organism suitable for this toxicity test are their relative ease of handling, their sensitivity to a variety of chemical substances, and the extensive data base for this common freshwater invertebrate species.

### 2.2.3 Origin

*Hyalella azteca* will be obtained from cultures at Smithers Viscient or from an outside supplier. At Smithers Viscient, the main culture of amphipods will be held in 38-L glass aquaria (containing approximately 31 L of culture water) under flow-through conditions. Water used to culture the amphipods is similar to the overlying water used during the 42-day test. Culture water will be maintained at approximately 23 ± 2 °C.

### 2.2.4 Feeding

While being maintained in the culture prior to the test, adult and juvenile amphipods will be fed once daily. Isolated offspring will be fed a combination of Yeast, Cerophyl® (or equivalent) and flaked fish food suspension (YCT). In addition, a small amount of unicellular green algae *Ankistrodesmus falcatus* and flaked food suspension may be added to vessels holding isolated offspring at the start of the isolation period as a supplemental food source. During testing, 1.5 mL of YCT suspension will be added daily to each test vessel, as well as an additional 0.5 mg of ground flake fish food in an aqueous suspension. If food collects on the sediment surface during testing, feeding may be suspended for one or more days. Overfeeding may cause a drop in dissolved oxygen. If applicable, feeding will be suspended in all treatment groups and the controls. Feeding will be suspended until there is no observations of food collecting on the sediment surface or if dissolved oxygen concentrations increase (>2.5 mg/L).

A sample of the food source will be periodically analyzed using U.S. EPA standard methods (U.S., EPA, 1997) by GeoLabs, Inc., Braintree, Massachusetts, in accordance with Smithers Viscient's standard operating procedures, for the presence of pesticides, PCBs and selected toxic metals.

### 2.2.5 Handling

Wide-bore pipets will be used to transfer the amphipods, taking care to minimize possible stress due to handling. Amphipods that are damaged or dropped during transfer will not be used.

## 2.3 Physical System

### 2.3.1 Sediment

Artificial (formulated) sediment will be used in the exposure. The artificial sediment will be prepared based on the OECD 218 guideline (OECD, 2004) and will be prepared as follows:

a. 5 % (dry weight) sphagnum moss peat: no visible plant remains, air dried and finely ground. Peat will be soaked in laboratory well water for at least 5 days. Calcium carbonate ($CaCO_3$) will be added to adjust the pH of the peat mixture to 5.5 to 6.0.
b. 20 % (dry weight) kaolin clay (with kaolinite content, if possible, of >30%).
c. 75 % (dry weight) industrial sand (with >50% of the particles between 50 and 200 microns).
d. Laboratory well water will be added during mixing to obtain a homogeneous sediment batch.
a. Organic carbon content of the final mixture will be approximately 2% $\pm$ 0.5% and is to be adjusted by the use of appropriate amounts of peat and sand, according to a and c. Slight excursions from 2% $\pm$ 0.5% organic carbon content of the formulated sediment are common based on variability in the peat component. The batch of sediment used in the exposure will be evaluated as acceptable for use by the study director prior to testing.

The dry constituents are blended together in the correct proportions and mixed thoroughly in a large scale laboratory mixer (e.g. Hobart mixer). The artificial sediment will be characterized for total organic carbon (TOC) content, percent sand, silt, clay (particle size distribution) and percent water holding capacity by Agvise Laboratories, Northwood, North Dakota. A pH measurement of the sediment may also be made by either Agvise Laboratories, Northwood, North Dakota or at Smithers Viscient.

Periodic analysis of representative samples of the artificial sediment will be conducted using U.S. EPA standard methods by GeoLabs, Inc., Braintree, Massachusetts, in accordance with Smithers Viscient standard operating procedures, to ensure the absence of potential toxicants, including pesticides, PCBs and selected toxic metals, at concentrations which may be harmful to the test organisms.

### 2.3.2 Test Vessels

The test vessels used in the static-renewal test will be 300-mL beakers. Each test vessel will have notches or slots cut on the top edge of the vessel and will be covered with

40-mesh nylon screen to allow for drainage during the renewal of overlying water. Each vessel will contain 100 mL (an approximate 4-cm layer) of sediment and 175 mL of overlying water. The overlying/sediment volume will thus be maintained at approximately 275 mL. The mean wet weight of sediment added to all test vessels will be determined by randomly selecting three replicate test vessels from each treatment level and control and weighing the mass of sediment added to each vessel on test day –1. The test vessels will be labeled to identify the treatment/control, study number, and the replicate designation.

### 2.3.3 Replication and Control of Bias

Fifteen replicates will be included with each test concentration and solvent control. Eighteen replicates will be included in the negative control. Twelve replicates (A through L) will be used to evaluate the biological response of the test organisms. Replicate vessels M through O of each concentration and the controls will be maintained for the purpose of analytical measurements. The last three negative control replicates (P through R) will be maintained for the purpose of measuring representative pore water quality characteristics (pore water ammonia and pH). Each replicate vessel for monitoring the biological response will contain ten individuals, a total of 120 amphipods per concentration or control. The additional replicates will be maintained under the same conditions as he biological replicates. These additional replicates will contain test organisms with the exception of the replicates being sacrificed at test initiation for analytical measurements. All the additional replicates will not be included in the biological observations for the study. Amphipods will be added impartially to an intermediate test beaker by adding no more than two amphipods to each vessel until all beakers contain two amphipods. This procedure will be repeated until each beaker contains ten amphipods. The test will be initiated when each intermediate beaker of amphipods is added to each respective test vessel. In addition, the position of the water distribution systems and the replicate test vessels under each water distribution system will be assigned in the water bath randomly.

### 2.3.4 Overlying Water

The overlying water consists of unadulterated water from a 100-meter bedrock well supplemented on demand with untreated Town of Wareham well water, and will be characterized as soft water with an approximate total hardness of <180 mg/L as $CaCO_3$. Total hardness, total alkalinity, pH and conductivity of the overlying water source will be monitored weekly at a central location in the laboratory to assure that these parameters are within the normal acceptable ranges. These measured ranges during the conduct of the exposure will be transcribed and included in the raw data and report. Total hardness and alkalinity will be determined according to *Standard Methods for the Examination of Water and Wastewater* (APHA, 2005). Twice per year, analysis of representative samples of the overlying water source will be conducted using U.S. EPA standard methods (U.S. EPA, 1997) by GeoLabs, Inc., Braintree, Massachusetts, in accordance with Smithers Viscient's standard operating procedures, to ensure the absence of potential toxicants, including pesticides, PCBs and selected toxic metals, at concentrations that may be harmful to the test organism. Results of the most recent analysis will be includes as an appendix in the study report.

### 2.3.5 Renewal of Overlying Water

During the 42-day study, the overlying water will be renewed by adding two volume additions (i.e., 350 mL) per day using an intermittent delivery system in combination with a calibrated water-distribution system (Zumwalt et al., 1994). The intermittent delivery system will be calibrated to provide 1 liter of water per cycle to the water-distribution system, which subsequently provides approximately 50 mL of water (no test material present) per cycle to each replicate test chamber. The water delivery system cycles approximately 7 times per day, providing 2 volume additions every 24 hours. Delivery of two volume replacements per day is sufficient to provide consistent and acceptable water quality characteristics throughout the duration of the 42-day exposure.

The calibration of the renewal system will be checked prior to test initiation and at test termination. If there is any indication during the test that the renewal system calibration has changed (e.g., renewal system malfunction or unexplained differences in dissolved oxygen concentration or temperature in the test vessels), calibration of the necessary renewal system components will be checked. During the test, the renewal system will be visually inspected at least twice daily.

## 2.4 Test Conditions

### 2.4.1 Temperature

Water temperature of the overlying water will be maintained at 23 ± 1 °C by conducting the test in a temperature-controlled room or water bath maintained at the appropriate test temperature. Temperature will be monitored continuously in an auxiliary vessel housed in the same water bath as the test vessels using a thermometer. Readings of minimum and maximum temperatures will be recorded daily.

### 2.4.2 Lighting

The test will be conducted in a light controlled laboratory. The test will be illuminated to a light intensity of 100 to 1000 lux using fluorescent bulbs. The light intensity will be measured once during the test. A 16-hour light, 8-hour dark photoperiod will be maintained with an automatic timer.

### 2.4.3 Dissolved Oxygen

The total dissolved oxygen should be > 2.5 mg/L for the duration of the test. If dissolved oxygen levels fall below 2.5 mg/L, the cycle rate of the water-renewal system may be increased (e.g., 2 to 4 volume replacements per day) to increase the dissolved oxygen levels. Aeration, with oil free air, will be initiated at a rate of approximately 1 bubble per second as a contingency to maintain the dissolved oxygen concentration above 2.5 mg/L. If it becomes necessary to initiate aeration in any one vessel, aeration will be administered to each replicate of each concentration and control as well.

### 2.4.4 Test Initiation

The day before test initiation (day -1) the treated sediments and control sediment(s) will be added to the replicate test vessels and the overlying water will be added. The water will be

added gently to prevent re-suspension of the sediment layer in the water column. This allows the sediment and water to equilibrate prior to addition of the test organisms.

Amphipods (7-8 days old) will be removed from the cultures. The test will be initiated (day 0) when all applicable vessels contain ten amphipods (120 per test concentration and control).

## 2.5 Sampling And Observations

### 2.5.1 Measurement of Test Substance Concentration

After application and mixing of the test substance with the sediment and prior to division into the individual replicate exposure chambers (i.e., during the equilibration period), a sample of treatment and control bulk sediment will be taken from each treatment level and control for determination of test substance concentrations. Three sediment quality control samples will also be analyzed with the bulk sediment samples. These quality control samples will be prepared at the time of sampling and will be handled and analyzed along with the bulk sediment samples. In addition, the stock solutions used to dose the sediment will be sampled and analyzed at the approximate time of dosing.

At test initiation, mid-test and test termination of the sediment exposure phase (i.e. test days 0, 14 and 28), one sample from the overlying water, pore water and sediment of each treatment and control will be removed and analyzed for test substance concentration. The ability to accurately measure aqueous concentrations during the study will be based on the limit of detection of the methodology employed, concentrations of test substance in the aqueous samples, volume of aqueous samples produced from the test vessels and multiple other factors. Six quality control (QC) samples (three aqueous and three sediment samples will be prepared at each sampling interval and stored and analyzed with the set of study samples. The QC samples will be prepared in test sediment at test substance concentrations similar to the treatment level range. The aqueous QC samples will be prepared in laboratory well water at relevant concentrations that can be utilized to demonstrate the accuracy of the analytical method. Results of these analyses indicate the accuracy of the analytical method used for measuring test substance concentration at each sampling period. If applicable, the analytical method will be validated by Smithers Viscient at the expected nominal concentration range prior to test initiation.

### 2.5.2 Sampling Procedures

The entire volume of overlying water will be removed and the appropriate volume collected for analysis from each test vessel by carefully decanting or pipetting. Pore water samples will be collected by removing the entire sediment sample (approximately 100 mL of sediment) from the test vessel and centrifuging for 15 to 30 minutes at a rate of at least 10,000 *g*. Following centrifuging, the pore water will be decanted or removed by pipette for analysis. Following removal of the pore water from the sediment sample, an appropriate sized sediment sample will be removed from the centrifuge tube with a metal spatula and mixed thoroughly prior to determination of sediment concentrations.

### 2.5.3 Water Quality Measurements

At test initiation (test day 0), test day 28, test day 29 and test termination (test day 42), temperature, dissolved oxygen (DO) concentration and pH will be measured in the

overlying water and recorded for each test vessel (replicates A through L at test initiation and test day 28 and the remaining eight vessels set up for biological observations on test days 29 and 42). On the remaining test days, temperature and dissolved oxygen will be measured and recorded in one alternating replicate each day. Measurement of temperature, dissolved oxygen and pH in the overlying water column will be made with probes and readings will be recorded in the water column approximately 1 to 2 cm above the sediment surface in each vessel. Total hardness, alkalinity, conductivity and total ammonia concentration will be determined in the overlying water at test initiation, test day 28, test day 29 and test termination (test day 42) in a composite sample of each treatment level and control. Samples of the overlying water collected for total hardness, alkalinity, conductivity and total ammonia concentration will consist of a composite sample of water from multiple replicates and will be collected by pipette from approximately 1 to 2 cm above the sediment surface in each vessel.

### 2.5.4 Pore Water Quality Measurements

Representative pore water quality measurements (ammonia and pH) will be made throughout the exposure by sampling control replicates P through R. These measurements will be made on test days 0, 14, and 28. Pore water will be collected as described in section 2.5.2.

In using formulated sediment, pore water quality variables are relatively consistent across treatments, and those variables that could affect organism performance are mitigated. The aforementioned measurements will be considered representative of all treatment groups, as well as the controls, and will be used to establish that the sediment was suitable for testing during the exposure.

### 2.5.5 Biological Observations

Daily observations of organism behavior (e.g., sublethal effects) and characteristics of sediment and overlying water will also be observed and recorded daily.

Survival and growth (length per adult) of the amphipods will be determined in each of four randomly selected replicate test vessels of each test concentration and control on test day 28 by sieving the sediment to remove all surviving amphipods. For all replicates, the sediment may be sieved in two separate aliquots with most of the amphipods typically being found on the sediment surface. Immobile or missing adults should be considered dead. The number of surviving adults and young will be recorded. A consistent amount of time should be taken to examine each replicate for surviving amphipods. The adults from the four selected replicates will be preserved for up to two weeks in a sugar formalin solution prior to images for the determination of amphipod length being taken. The growth of amphipods in these replicates will be determined by measuring body length from the base of the first antenna to the tip of the third uropod along the curve of the dorsal surface to the nearest 0.01 mm using an image analyzer.

The amphipods in the remaining eight replicates will also be removed on day 28 by sieving and the number of surviving adult and young amphipods will be recorded. The surviving adult amphipods from each replicate will be placed in 300-mL water-only test vessels containing a 3 cm x 3 cm piece of nylon Nitex screen. Each test vessel will have notches or slots cut on the top edge of the vessel and will be covered with 40-mesh Nitex® screen

to allow for drainage during the renewal of overlying water. On test days 35 and 42 the surviving adults and young in each beaker will be removed and counted. A consistent amount of time should be taken to examine each replicate for surviving amphipods. On day 35 the surviving adults will be returned to their respective vessels after enumeration. On day 42 the surviving adults will be preserved in sugar formalin. Surviving adult males will be identified by the enlarged second gnathopod, and the numbers of males and females will be recorded. In addition, the number of gravid females recovered on test day 42 in each replicate will also be recorded. The number of females recovered on test day 42 will be utilized to calculate all reproductive endpoints as offspring per female. Reproduction will be expressed as the total number of young recovered on days 28, 35, and 42 per adult female amphipod. Images for the determination of amphipod length may be taken of preserved amphipods within two weeks of test termination by utilizing the length measurement procedure described in the previous paragraph.

### 2.5.6 Acceptability Criteria

The following criteria will be used to assess the acceptability of the exposure:

- Test organisms were 7- to 8-days old at test initiation.

- Test organisms were all from the same source.

- A negative-control sediment treatment was included in the test and an appropriate solvent control treatment was included in the exposure.

- All test vessels were identical and/or contained approximately the same amount of sediment and overlying water.

- The mean survival of *H. azteca* on Day 28 was ≥80% in the negative control and in the solvent control.

- The mean length of *H azteca* on Day 28 was ≥3.2 mm in the negative control and in the solvent control.

- Reproduction by day 42 was ≥2 young per surviving female in the negative control and in the solvent control.

## 3.0 STATISTICAL ANALYSES

### 3.1 Endpoints

The endpoints used for determination of significant effects by statistical evaluation are outlined in the table below:

| Lethal | Sublethal | |
|---|---|---|
| Survival | Growth | Reproduction |
| Adults (day 28, 35 and 42)<br>Adults - Male:Female | Length Per Adult (day 28 and 42) | Cumulative Offspring Per Female (day 28-35)<br>Cumulative Offspring Per Female (day |

| | | |
|---|---|---|
| Ratio (day 42) | | 28-42) |

Survival and growth at test day 28 and cumulative reproduction at test day 42 are defined as the primary endpoints. All other endpoints are defined as supplemental endpoints. All concentration-effect relationships will be based on measured concentrations of test substance in the sediment. In addition, concentration-effect relationships may also be based on measured concentrations of test substance in the pore water if requested by the sponsor and regulatory scientists. All statistical analyses will be performed using the Comprehensive Environmental Toxicity Information System™ (CETIS, Ives, 2013).

### 3.2 Statistical Methods (Determination of LOEC/NOEC)

If a solvent is used as a carrier for the test substance, a t-Test ($p \leq 0.05$) will be used to compare the results (survival) of the solvent control to the negative control group. All the remaining statistical analyses will be performed comparing the treatment data to the negative control group data per current EPA guidance.

The data will be tested for normality and homogeneity of variance using an appropriate qualifying test. If the data passes these two tests, then a parametric method will be used to evaluate the results of test. If the data fails the test for normality and homogeneity of variance, then a non-parametric method will be used to evaluate the results of the test. The Lowest-Observed-Effect-Concentration (LOEC) is defined as the lowest test concentration that shows a statistically significant effect and the No-Observed-Effect-Concentration (NOEC) is the highest test concentration that shows no statistically significant difference from the control.

### 3.3 Transformations

Transformation of data will be performed with data representing endpoint estimates obtained as a proportion (e.g., survival). Prior to analyzing data of this type, the observed proportion in each vessel will be transformed by using an angular transformation (arcsine square-root).

### 3.4 LC50 Calculation

The LC50 is the estimated concentration of the test substance in sediment that produces 50% mortality in the test population of amphipods at a given interval when compared to the control. If applicable, a computer program will be used to estimate LC50 using an appropriate method.

### 3.5 EC50 Calculation

The EC50 is the estimated measured concentration of the test substance that produces a 50% reduction in growth or reproduction of the test organisms when compared to the control. If applicable, a computer program will be used to estimate the EC50 values using an appropriate method.

## 4.0 RECORDS TO BE MAINTAINED

Records to be maintained will include, but will not be limited to, correspondence and other documents relating to the interpretation and evaluation of data as well as all raw data and documentation generated as a result of the study.

## 5.0 REPORTING

The raw data generated at Smithers Viscient will be peer-reviewed and the final report will be reviewed by the Study Director. All values will be reported to various levels of significance depending on the accuracy of the measuring devices employed during any one process. The Quality Assurance Unit will inspect the final report to confirm that the methods, procedures, and observations are accurately and completely described, that the reported results accurately and completely reflect the raw data generated at Smithers Viscient and to confirm adherence with the study protocol. A single copy of the draft report will be submitted to the Sponsor for review. The report will be finalized according to Standard Operating Procedures. The final report will meet the formatting requirements of EPA's PR Notice 2011-3. All reports will include, but will not be limited to, the following information:

- The study number from Smithers Viscient and Sponsor Study number (if any).
- Laboratory and site, dates of testing and personnel involved in the study, i.e., Program Coordinator (if applicable), Study Director and Principal Investigator.
- Identification of the test substance which may include chemical name, additional designations (e.g., trade name), chemical designation (CAS number), empirical formula, molecular structure, manufacturer, lot or batch number, water solubility, vapor pressure, degree of purity of test substance (percent test chemical) (Sponsor supplied, if available).
- Characterization and origin of the overlying water.
- Characterization, percent organic carbon, and preparation of the sediment.
- Scientific name of the test organisms, source, age and culturing information.
- Test container volume, sediment and water volume, number of replicates used per concentration, and number of amphipods used per treatment.
- Description of exposure system and application of test substance to sediment.
- Test temperatures, dissolved oxygen concentration, and pH; and photoperiod and light intensity used, as well as conductivity, total ammonia, total alkalinity and total hardness measured.
- Observations of insolubility of the test substance, including the test levels and when observed.

- Definition of criteria used to determine the sublethal effects, and general observations on non-quantifiable effects.

- Number of surviving amphipods, length, reproduction and number of males and females recovered in each treatment at each applicable observation period, in tabular form.

- Description or reference (or inclusion as an appendix) to chemical and statistical procedures applied.

- Analytical results of test concentration measurements and QC samples.

- If applicable, means and standard deviations of measured concentrations of the test compound, as well as nominal test concentrations.

- The 28-, 35- and 42-day LC50 with 95 percent confidence limits for survival of amphipods, if applicable.

- EC50 with 95 percent confidence limits for growth/reproduction, if applicable.

- The Lowest-Observed-Effect Concentration (LOEC) tested based on statistical analyses.

- The No-Observed-Effect Concentration (NOEC) tested based on statistical analyses.

- Good Laboratory Practice (GLP) compliance statement signed by the Study Director.

- Date(s) of Quality Assurance reviews, and dates reported to the Study Director and management, signed by the Quality Assurance Unit.

- Location of the protocol, raw data and final report.

## 6.0 PROTOCOL CHANGES

All amendments to the approved protocol must be documented in writing and signed by both the Study Director and the Sponsor's contact or representative. Protocol amendments and deviations must include the reasons for the change and the predicted impact of the change on the results of the study, if any.

## 7.0 GOOD LABORATORY PRACTICES

All test procedures, documentation, records and reports will comply with the Organization of Economic Co-operation and Development's (OECD) Good Laboratory Practices as set forth under the OECD Guidelines for the Testing of Chemicals and the U.S. Environmental Protection Agency's Good Laboratory Practices as set forth under the Federal Insecticide, Fungicide and Rodenticide Act (40 CFR, Part 160).

## 8.0 REFERENCES


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# Attachment V

**Chronic Sediment Toxicity Test with *Leptocheirus plumulosus*: Continued Difficulties and Concerns**

Smithers Viscient Laboratories
Wareham, MA

**Update on Method Development and Pilot Testing – Nov 7, 2014**

SMV has been conducting pilot testing since September 2013 in order to improve the consistency of the *Leptocheirus plumulosus* chronic testing method. SMV has compared organism performance with a focus on improving survival using different sediments, water and feeding regimes. SMV has determined that the locally collected natural sediment used historically with some success for this testing is no longer suitable to generate acceptable results. In addition, SMV has yet to develop formulated sediment that is suitable for use in this testing. Developing suitable formulated marine sediment for chronic amphipod testing may take significant trial and error as there is not much information in the literature regarding this topic. Consequently, SMV attempted to find a new source of sediment that would be suitable for this testing. SMV was able to contact an environmental consulting firm in Washington that could collect and ship Sequim Bay sediment. This sediment was used as control sediment in the original method development by the EPA and has been used with some success by government laboratories to assess the toxicity of field collected sediment samples.

This plan was communicated to the EPA in a conference call on November 26, 2013. The EPA provided information on laboratories that are conducting these chronic exposures successfully. However, these laboratories were conducting exposures with field collected sediment samples as opposed conducting dosed sediment testing under GLP conditions. Overall, the information provided by the EPA was helpful and did support the decision by SMV to focus on the sediment source as the key component to conducting an acceptable exposure. Unfortunately, the survival data generated by the method validation pilot with Sequim Bay sediment was also highly variable and the same pattern of delayed mortality was observed in many replicates. Further pilot testing early in 2014 examining flaked fish food source also did not resolve the poor survival issues. In conclusion, the same issues with delayed mortality of adult organisms persisted when sediment and food source were different from those historically used at SMV.

SMV discussed these testing issues with technical staff at the US Army Engineer Research and Development Center (ERDC) in Vicksburg as this facility has had some success with the test method. Both laboratories coordinated an interlaboratory study designed to examine the effects of control sediment source, nutritional quality of food utilized and organism source on organism performance. This study terminated on April 11, 2014. The interlaboratory study was conducted according to the standard guidance with the following multifactorial experiment design:

| Treatment # | Replicates | Sediment | Food | Organisms Source |
|---|---|---|---|---|
| 1 | 7 | Sequim Bay Control | Tetramin | ERDC |
| 2 | 7 | Sequim Bay Control | Tetramin | Chesapeake Cultures |
| 3 | 7 | Southern LA Control | Tetramin | ERDC |

| | | | | |
|---|---|---|---|---|
| 4 | 7 | Southern LA Control | Tetramin | Chesapeake Cultures |
| 5 | 7 | Sequim Bay Control | Sera Vipan | ERDC |
| 6 | 7 | Sequim Bay Control | Sera Vipan | Chesapeake Cultures |
| 7 | 7 | Southern LA Control | Sera Vipan | ERDC |
| 8 | 7 | Southern LA Control | Sera Vipan | Chesapeake Cultures |

Upon completion of the interlaboratory exposure, survival continued to be low/variable in all groups tested at SMV while the ACOE observed survival of >84% in all groups. None of the factors tested (sediment, organism source and food type) seem to be significantly driving the variability in the survival data. Michael Bradley (Senior Biologist at SMV) visited the ACOE facility during the initiation and maintenance of this interlaboratory test in order to observe laboratory techniques. No significant differences were noted in regards to techniques between our two laboratories. Methods remained consistent between labs with the only exception being light intensity. It came to our attention that the ACOE conducts their chronic exposures at a lower light intensity (approximately 200 lux) than referenced in the current guidance (500-1000 lux). We have been conducting our exposures according to the guidance with regards to light intensity. Looking at our historical data, there was some other anecdotal evidence that suggests light intensity may affect long term survival.

SMV conducted another pilot test (terminated on May 23) to examine survival under lower, more controlled light conditions and generated acceptable survival data of >95% with low variability amongst replicates. SMV conducted a similar pilot that terminated on July 9 in order to verify that light intensity and lighting conditions may be indirectly or directly effecting survival over the course of a chronic exposure. Unfortunately, the results of the second lighting pilot did not yield acceptable survival data and results were highly variable. Consequently, SMV is not confident with moving forward with the chronic testing we have on the schedule at this time.

Upon review of all the data collected from pilot testing to date by SMV senior scientists, the overall general trend of highly variable survival suggests that there may be issues with latent toxicity at the replicate level as opposed to issues with the main variables of the test system (i.e. sediment, organism population, food source etc). If sediment was not acceptable to support survival or a population of organisms was unhealthy, suppression in survival would be observed across all replicates. However, SMV has observed survival ranging from <10% to 100% within the same test group in many of the pilot tests. The general trend of adult amphipod mortality in the last 7-10 days of the exposure also supports the hypothesis of latent toxicity in individual replicates.

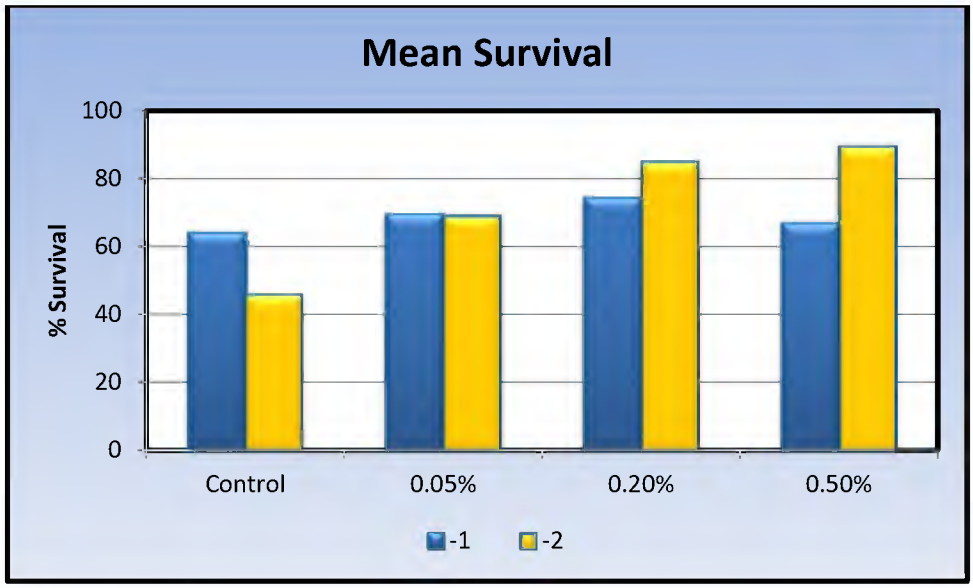
A possible cause for this mortality is glassware contamination. However, SMV has omitted this as a probable cause as the glassware used for this testing has been handled in the same manner as glassware used in other testing across our facility and similar issues with survival have not been observed in testing with other organisms. SMV believes that the latent toxicity could be coming from nitrite toxicity that is a function a variable bacterial population present in each replicate. Replicates may be building up a specific bacterial community toward the later stages of the 28 day test that convert the ammonium present in the vessel to nitrite. However, completion of nitrification process may not be possible if certain replicates lack the bacteria that further transform nitrite into nitrate leading to a buildup of nitrite in a replicate which could cause toxicity in adult amphipod toward the later

stages of the exposure. The imbalance in a bacterial population may be due to the inherent bacterial population in the sediment or overlying water utilized in testing.

This issue of latent toxicity due to variability in the bacterial population within each replicate is beyond the scope in which the current EPA guidance document will be helpful in rectifying the issue. SMV will continue to put this testing on hold until further pilot testing can be conducted to determine if nitrite toxicity is an issue and if other test conditions are exacerbating this toxicity. SMV plans to take an approach similar to a Toxicity Identification Evaluation in order to verify if nitrite toxicity in replicates is the cause of the observed delayed mortality.

SMV has since completed two identical pilot studies since this last communication to investigate the nitrogen cycle issues described in the above mentioned statement. These pilots included a number of treatments with one such treatment being the use of overlying water inoculated with nitrifying bacteria. In both pilots, this treatment yielded survival in the 71-77% range with some moderate variability (SD = 10-20%). Control replicates and other treatment groups exhibited results similar to those historically observed with significant mortality toward the latter stages of the exposure with high variability among replicates. These results seem to demonstrate that the addition of the nitrifying bacteria had a positive impact on survival but this treatment needs to be further investigated to consistently achieve >80% survival with lower variability.

A second set of identical pilot exposures were terminated later in October 2014. The objective of the latest pilot exposures was to investigate the addition of nitrifying bacteria to the overlying water at different concentrations/rates in an attempt to further improve the survival data. A control was set up with no addition of nitrifying bacteria to the overlying water while the treatments consisted of adding nitrifying bacteria at 0.05%, 0.20% and 0.50% of the overlying water volume. Survival results were as follows for the two pilot exposures:




While the data is still inconsistent across the pilots, the results of the second pilot exposure demonstrate a clear increase in survival with a theoretical increase in the nitrifying bacteria population. This evidence suggests that further pilot work focusing on the bacterial population of the overlying water and the nitrogen cycling within the test system is needed to improve consistency of the survival results. SMV will be presenting much of this pilot work at the upcoming NASETAC meeting in Vancouver and hope to discuss the ongoing issues with this methodology with research scientists outside of our organization. SMV is currently targeting early 2015 to restart

chronic sediment testing with *Leptocheirus plumulosus*. Once testing is restarted, the backlog of studies can be prioritized and the schedule can be better assessed for each individual studies.

# Attachment VI

Status of DCPA Registration Review DCI

EPA Science Reviews:

7/30/2014: Received review dated 7/7/2014 - "DCPA: HED Response to (12 Month) Comments on the Residue Chemistry Requirements of the Generic Data Call-In (GDCI-0798701-1140)"
7/31/2014: Received review dated 10/23/2013 - "DCPA: HED Response to Comments on the Residue Chemistry Requirements of the Generic Data Call-In (GDCI-0798701-1140)"
10/20/2014: Received review dated 3/20/2014 – "DCPA (Chlorthal-dimethyl): Review of Study Protocols for Determining Chronic Toxicity to Sediment-Dwelling Estuarine/Marine and Freshwater Organisms"
10/21/2014: Received review dated 11/19/2013 – "DCPA: HED Review of the Comparative Thyroid Toxicity Study Protocols"

| Guideline | Guideline Description | Test Substance | Due | Response Code | Submission Information | Current Status |
|---|---|---|---|---|---|---|
| Environmental Fate Data Requirements | | | | | | |
| 835.1230 | Adsorption/desorption | TPA | 12 months | 4 | 4/29/2013 – Promised to submit existing data.<br>1/29/2014 – Submitted data (MRID 49307517). | Waiting for response from EPA |
| 835.1230 | Adsorption/desorption | TPA | 12 months | 5 | 4/29/2013 – Request to upgrade cited study MRID 41648805 with existing data.<br>1/29/2014 – Submitted justification. | Waiting for response from EPA |
| 835.1240 | Leaching | TPA | 12 months | 6 | 4/29/2013 – Cited existing study MRID 44082601. | Waiting for response from EPA |
| 835.2120 | Hydrolysis | TPA | 12 months | 6 | 4/29/2013 – Cited existing study MRID 114648. | Waiting for response from EPA |

| Guideline | Guideline Description | Test Substance | Due | Response Code | Submission Information | Current Status |
|---|---|---|---|---|---|---|
| 835.4100 | Aerobic soil metabolism | TPA | 24 months | 4 | 4/29/2013 - Promised to submit existing data.<br>(NOTE - Incorrectly noted on DCI response as: 1, 4. Should be 4 as stated within 4/29/2013 submission.)<br>1/29/2014 – Submitted data (MRID 49307516) | Waiting for response from EPA |
| 835.4200 | Anaerobic soil metabolism | TPA | 24 months | 6 | 4/29/2013 - Cited existing study MRID 114651. | Waiting for response from EPA |
| 835.4300 | Aerobic Aquatic metabolism | DCPA | 24 months | 4 | 4/29/2013 - Promised to submit existing data.<br>1/29/2014 – Submitted data (MRID 49307515). | Waiting for response from EPA |
| 835.4300 | Aerobic aquatic metabolism | TPA | 24 months | 9 | 4/29/2013 - Submitted Waiver - Defer once review completed on Parent study | Waiting for response from EPA |
| 835.4400 | Anaerobic aquatic metabolism | TPA | 24 months | 9 | 4/29/2013 - Submitted Waiver - Cited EFED document | Waiting for response from EPA |
| 835.6100 | Terrestrial field dissipation | DCPA | 24 months | 6 | 4/29/2013 - Cited existing study MRID 44082601 | Waiting for response from EPA |
| 835.6100 | Terrestrial field dissipation | TPA | 24 months | 6 | 4/29/2013 - Citing Existing data MRID 44082601 | Waiting for response from EPA |

| Guideline | Guideline Description | Test Substance | Due | Response Code | Submission Information | Current Status |
|---|---|---|---|---|---|---|
| Nontarget Plant Protection Data Requirements | | | | | | |
| 850.4100 | Tier I Plant tox - Seedling Emergence | DCPA | 12 months | 1 | 4/29/2013 - Developing data. 1/29/2014 – Submitted data (MRID 49307513). | Waiting for response from EPA |
| 850.4100 | Tier I Plant tox - Seedling Emergence | TPA | 12 months | 9 | 4/29/2013 - submitted Waiver - Defer once review completed on Parent study. | Waiting for response from EPA |
| 850.4150 | Tier I Plant tox - Vegetative Vigor | DCPA | 12 months | 1 | 4/29/2013 - Developing data. 1/29/2014 – Submitted data (MRID 49307506). | Waiting for response from EPA |
| 850.4150 | Tier I Plant tox - Vegetative Vigor | TPA | 12 months | NA | 4/29/2013 - Noted on submission that this is not required. | NA |
| 850.4400 | Tier I/II Plant tox (Lemna spp.) | DCPA | 12 months | 1 | 4/29/2013 - Developing data. 1/29/2014 – Submitted data (MRID 49307509). | Waiting for response from EPA |
| 850.4400 | Aquatic vascular plant growth - Lemna spp. Tiers II | TPA | 12 months | 9 | 4/29/2013 - submitted Waiver - Defer once review completed on Parent study. | Waiting for response from EPA |
| 850.4500 | Algal tox test, Tier I/II | DCPA | 12 months | 1 | 4/29/2013 - Developing data. 1/29/2014 - Three studies included in January submission (MRID 49307508), (MRID 49307504), and (MRID 49307507). MRID 41054829 received approval in DER dated 10/17/1990 for fourth required species. | Waiting for response from EPA |
| 850.4500 | Algal tox test, Tier I/II | TPA | 12 months | 9 | 4/29/2013 - submitted Waiver - Defer once review completed on Parent study. | Waiting for response from EPA |

| Guideline | Guideline Description | Test Substance | Due | Response Code | Submission Information | Current Status |
|---|---|---|---|---|---|---|
| Residue Chemistry Data Requirements for Food Uses | | | | | | |
| 860.1300 | Nature of the residue: poultry | DCPA | 24 months | 7 | 4/29/2013 - Waiver submitted to delete alfalfa.<br>7/31/2014 - Received EPA HED review dated 10/23/2013 stating waiver possibly accepted, but need additional data regarding 860.1900 for confirmation.<br>9/24/2014 - AMVAC submitted justification submission for 860.1900 | Waiting for response from EPA |
| 860.1340 | Residue analytical method: livestock commodities | DCPA | 24 months | 7 | 4/29/2013 - Waiver submitted to delete ruminant commodities.<br>7/31/2014 - Received EPA HED review dated 10/23/2013 stating waiver possibly accepted, but need additional data regarding 860.1900 for confirmation.<br>9/24/2014 - AMVAC submitted justification submission for 860.1900 | Waiting for response from EPA |
| 860.1380 | Storage stability | DCPA | 24 months | 5 | 4/29/2013 - Request to upgrade study - Citing several studies.<br>1/29/2014 – Provided justification.<br>7/7/2014 – EPA HED review: Requirement satisfied.<br>7/31/2014 - Received EPA HED review dated 10/23/2013 acknowledging AMVAC's intention to submit additional data. | Guideline Satisfied |

| Guideline | Guideline Description | Test Substance | Due | Response Code | Submission Information | Current Status |
|---|---|---|---|---|---|---|
| 860.1480 | Meat/milk/ poultry/eggs: ruminants | DCPA | 24 months | 7 | 4/29/2013 - Waiver submitted to delete alfalfa, white potatoes, and peas. 7/31/2014 - Received EPA HED review dated 10/23/2013 stating waiver possibly accepted, but need additional data regarding 860.1900 for confirmation. 9/24/2014 - AMVAC submitted justification submission for 860.1900 | Waiting for response from EPA |
| Terrestrial and Aquatic Nontarget Organisms Data Requirements | | | | | | |
| 850.1010 | Acute tox, freshwater invertebrates | DCPA | 12 months | 4 | 4/29/2013 – Promised to submit existing data. 1/29/2014 – Submitted data (MRID 49307514). | Waiting for response from EPA |
| 850.1010 | Acute tox, freshwater invertebrates | TPA | 12 months | 4 | 4/29/2013 – Promised to submit existing data. 1/29/2014 – Submitted data (MRID 49307514). | Waiting for response from EPA |
| 850.1025 | Acute tox, oyster (shell deposition) | DCPA | 12 months | 1 | 4/29/2013 - Developing data. 1/29/2014 – AMVAC unintentionally omitted in submission (100-AQU-024). 10/29/2014 – Submitted Data (MRID 49500701). | Waiting for response from EPA |
| 850.1025 | Acute tox, oyster (shell deposition) | TPA | 12 months | 9 | 4/29/2013 - submitted Waiver - Defer once review completed on Parent study. | Waiting for response from EPA |
| 850.1035 | Acute tox, mysid | DCPA | 12 months | 1 | 4/29/2013 - Developing data. 1/29/2014 – Submitted data (MRID 49307505). | Waiting for response from EPA |

| Guideline | Guideline Description | Test Substance | Due | Response Code | Submission Information | Current Status |
|---|---|---|---|---|---|---|
| 850.1035 | Acute tox, mysid | TPA | 12 months | 9 | 4/29/2013 - submitted Waiver - Defer once review completed on Parent study. | Waiting for response from EPA |
| 850.1075 | Acute tox, estuarine/marine fish | DCPA | 18 months | 1 | 4/29/2013 - (Marine) Developing data for marine.<br>1/29/2014 – Submitted data (MRID 49307511). | Waiting for response from EPA |
| 850.1075 | Acute tox, freshwater fish | DCPA | 18 months | 5 | 4/29/2013 - Request to upgrade MRID 41054827.<br>1/29/2014 – Submitted justification. | Waiting for response from EPA |
| 850.1075 | Acute tox, freshwater fish | DCPA | 18 months | 5 | 4/29/2013 - Request to upgrade MRID 41054826.<br>1/29/2014 – Submitted justification. | Waiting for response from EPA |
| 850.1075 | Acute tox, freshwater fish | TPA | 12 months | 4 | 4/29/2013 - (Freshwater) Promised to submit existing data.<br>1/29/2014 – Submitted data (MRID 49307518). | Waiting for response from EPA |
| 850.1075 | Acute tox, freshwater fish | TPA | 12 months | 9 | 4/29/2013 - (Freshwater) submitted Waiver - Defer once review completed on Parent study.<br>(NOTE - Did not segregate two freshwater responses on DCI response document. This should be a "9" as stated within 4/29/2013 submission.) | Waiting for response from EPA |
| 850.1075 | Acute tox, estuarine/marine fish | TPA | 12 months | 9 | 4/29/2013 - (Marine) submitted Waiver - Defer once review completed on Parent study. | Waiting for response from EPA |
| 850.1300 | Aquatic invertebrate life-cycle, freshwater | DCPA | 12 months | 1 | 4/29/2013 - Developing data.<br>1/29/2014 – Submitted data (MRID 49307510). | Waiting for response from EPA |

| Guideline | Guideline Description | Test Substance | Due | Response Code | Submission Information | Current Status |
|---|---|---|---|---|---|---|
| 850.1300 | Aquatic invertebrate life-cycle, freshwater | TPA | 12 months | 9 | 4/29/2013 - submitted Waiver - Defer once review completed on Parent study. | Waiting for response from EPA |
| 850.1350 | Aquatic invertebrate life-cycle, estuarine/marine | DCPA | 12 months | 1 | 4/29/2013 - Developing data.<br>1/29/2014 – Submitted data (MRID 49307512). | Waiting for response from EPA |
| 850.1350 | Aquatic invertebrate life-cycle, estuarine/marine | TPA | 12 months | 9 | 4/29/2013 - submitted Waiver - Defer once review completed on Parent study. | Waiting for response from EPA |
| 850.1400 | Fish early life-stage | DCPA | 12 months | 4 | 4/29/2013 – Promised to submit existing data.<br>1/29/2014 – Submitted data (MRID 49307520). | Waiting for response from EPA |
| 850.1400 | Fish early life-stage | TPA | 12 months | 9 | 4/29/2013 - submitted Waiver - Defer once review completed on Parent study. | Waiting for response from EPA |

| Guideline | Guideline Description | Test Substance | Due | Response Code | Submission Information | Current Status |
|---|---|---|---|---|---|---|
| 850.2100 | Acute avian oral, passerine species | DCPA | 12 months | 1 | 3/27/2013 – Protocol requested by Margaret Hathaway via phone conversation.<br>4/29/2013 - Developing Data, Submitted Protocol.<br>2/29/2014 - EPA asked if AMVAC would agree to use the HLS passerine acute approved protocol.<br>3/6/2014 - AMVAC agreed to use the Passerine acute protocol and requested extension request until 10/30/2014.<br>9/30/2014 – Submitted data (MRID 49477601) | Waiting for response from EPA |
| 850.2300 | Avian reproduction | DCPA | 24 months | 6 | 4/29/2013 - Cited Existing Study - MRID 47550001 and MRID 47550002. | Waiting for response from EPA |
| Terrestrial and Aquatic Nontarget Organisms Data Requirements, Environmental Fate Data Requirements | | | | | | |
| 850.1730 | Fish bioconcentration | TPA | 12 months | 9 | 4/29/2013 - submitted Waiver - based on guideline criteria. | Waiting for response from EPA |
| Toxicology Data Requirements | | | | | | |
| 870.3465 | Subchronic inhalation tox study - 28 day | DCPA | 24 months | 1 | 4/29/2013 - Promised Study, but requested 28 day study instead of 90 day.<br>8/7/2013 - Received an informal approval to complete the 28 day inhalation.<br>1/29/2014 – Submitted data (MRID 49307501). | Waiting for response from EPA |

| Guideline | Guideline Description | Test Substance | Due | Response Code | Submission Information | Current Status |
|---|---|---|---|---|---|---|
| 870.6200 | Neurotoxicity battery (acute and subchronic studies) | DCPA | 12 months | 1 | 4/29/2013 - Developing Data.<br>1/29/2014 - Two studies included in January submission (MRID 49307502, MRID 49307503). | Waiting for response from EPA |
| 870.7800 | Immunotoxicity | DCPA | 12 months | 9 | 4/29/2013 - Waiver submitted.<br>9/23/2013 - Received informal approval to waive this requirement. | Guideline Waived |
| 860.1900 | Field accumulation in rotational crops | DCPA | 36 months | 5 | 4/29/2013 - Request to upgrade current study.<br>7/30/2014 – Received EPA HED review dated 7/7/2014 stating Data remains outstanding.<br>7/31/2014 - Received EPA HED review dated 10/23/2013 stating additional data is requested.<br>9/24/2014 – In response to reviews, AMVAC submitting justification submission for 860.1900. | Waiting for response from EPA |
| ss-1066 | Chronic Sediment - Hyalella Azteca | DCPA | 24 months | 1 | 4/29/2013 - Developing Data, Submitted Protocol.<br>10/20/2014 – Received EPA review dated 3/20/2014. | 12/15/2014 – Notified EPA the Final reports are anticipated to be submitted by June 15, 2016. |

| Guideline | Guideline Description | Test Substance | Due | Response Code | Submission Information | Current Status |
|---|---|---|---|---|---|---|
| ss-1069 | Chronic Sediment - Chironomus dilutus | DCPA | 24 months | 1 | 4/29/2013 - Developing Data, Submitted Protocol.<br>10/20/2014 – Received EPA review dated 3/20/2014. | 12/15/2014 – Notified EPA the Final reports are anticipated to be submitted by June 15, 2016. |
| ss-1072 | Chronic Sediment - Leptocheirus plumulosus | DCPA | 24 months | 1 | 4/29/2013 - Developing Data, Submitted Protocol.<br>10/20/2014 – Received EPA review dated 3/20/2014. | 12/15/2014 - Notified EPA additional method development needed by the lab and proposes to update the Agency by March 31, 2015. |
| ss - 1075 | Avian inhalation toxicity | DCPA | 12 months | 9 | 4/29/2013 - Submitted Waiver - delete Air application use. | Waiting for response from EPA |
| ss - thyroid tox | Comparative thyroid study | DCPA | 24 months | 1 | 4/29/2013 - Developing Data, Submitted Protocol.<br>10/21/2014 – Received EPA review dated 11/19/2013. EPA requested revised protocol by the end of November. | 11/26/2014 – Submitted revised protocol. |