

Tim Thompson Comments

1. General comment - in the opening paragraph it would be good to add a statement what conditions this procedure is applicable, and that all applicants must get approval from the DMMP agencies to use this procedure. For example, "The use of the resuspension termination procedure may be used with concurrence of the DMMP agencies when the sediments to be tested contain a high (DEFINE) fraction of wood waste or other natural organic substances that have been shown (REFERENCE) to entrain, but not impair, larval development"

Response: It is difficult to point to any one metric (e.g., % fines) that is predictive of when entrapment is going to be an issue. A few lines of evidence may be required to determine when to employ resuspension, including % fines, settling observed in wet-sieving performed on board when sediment samples are first acquired, knowledge of the site and former activities that may have contributed flocculent material, etc. The determination of larval test method will be made in coordination with the DMMP during the project planning phase.

2. General comment - Somewhere in the presentation of this I believe it would be appropriate for Ecology and the DMMP agencies to state that they do not believe that entrainment from natural organic matter to be a deleterious effect. Others might disagree with you (strongly!), but you should state that as a matter of policy you have made this determination.

Response: The entrainment referred to in this method occurs in the larval test is an artifact of the method and is defined as burial or "entrapment" by fine material settling on top of development larvae preventing them from rising into the water column. For the purposes of this method, we have replaced the word "entrainment" with the word "entrapment" to avoid confusion with zooplankton or larvae being entrained by suspended material and dragged down through the water column to the bottom.

The subsequent question would be does this type of burial and entrapment occur in dredging or site-related activities. In the case of a dredging and dredged material disposal, you do have energetic mixing of water and sediment. In such cases, "entrapment" is possible if you have larvae happen to be on the bottom during disposal. However, this is a narrow window of time (8-12 hours post fertilization) and is unlikely to be a significant effect at disposal sites. For *in-situ* evaluations (contaminated sites), there aren't naturally occurring conditions that are similar to the type of complete mixing of sediment in the water column that occurs in the PSEP test.

3. General comment - in all cases I would recommend that the standard PSEP protocol be run in tandem with the resuspension protocol for all test and control sediments.

Response: If there is *a priori* knowledge that burial may occur, resuspension may be selected as a substitute for the standard method. There may be times where standard PSEP tests have been run and there appears to be the potential for burial. In such cases, the resuspension method is used subsequent to the standard PSEP method (e.g. Port Gamble).

4. Prodissoconch I and the termination stage. The protocol discusses the PDC I for bivalves will occur at 48 - 60 hours, but practically at the test temperature for every test I have even been involved in it is pretty close to 48. I would suggest you edit the protocol to address only the 48 hour termination.

Response: It was not our intent to modify the guidance (PSEP or ASTM) regarding test duration. While it is true that the test often terminates at 48 hours, there are times that it requires additional time. Per current guidance, the test will be conducted for 48-60 hours.

5. Would also suggest you not have a variable in time before termination to resuspend. Chose 6, or 8, so that all your bioassays results are consistent. BTW, you should be able to defend why 6 or 8, and not 2 or 4.

Response: The intent was to accommodate different testing schedules of different labs/experimenters. The goal of 6 to 8 hours was to allow sufficient time for sediment to settle such that enumeration at the end of the test is not hampered. If the nature of the sediment is such that sediment remains in the water column following the 4-hour settling period at the beginning of the test one would expect that at the end of the test as well. In an effort to standardize the time, the guidance will be rewritten as "at approximately 42 hours".

6. Conflicting information is presented in the procedure:

a. 95% seawater control have reached the PDC I state (48-60 hours)

b. 6 - 8 hours prior to the 48 hour endpoint should that be PDC 1?

c. "At 38 - 42 hours" shouldn't that be 40 - 42 hours prior if it is 6 to 8 hours prior to the 48 hour termination.

d. The confusion occurs in that there is no practical way to assess if you are 6 hours from the PDC 1 stage. You have to make a decision on experience (and faith!) and simply mix at 42 hours and then if/when the test runs longer than 48.

Response: The modification indicating that at a set time (the sediments are resuspended regardless of whether the test will terminate at 48 hours or longer) should address this question of whether larvae are at PDC I prior to 48 hours (addressing a, b, d). Comment c is a remnant of the previous version.

I think my point d is worth considering; just simply mix at the 42 hour point, and then terminate at 48, or when the 95% PDC is achieved. There is really not another practicable way to set a remixing point.

Response: We will revise the 42 hour time point as "approximately 42 hours" to allow for some wiggle room for labs.

Comments from Linda Nemeth

B) Larval:

1. One potential concern is possibly that you now have a completely different method for resuspension. Do you have any data doing it this way with only 6-8 hours of settling flocculent samples? Will this work? Our concern is that it may not be long enough to settle enough so that larvae can be enumerated.

Response: Yes, there have been many test treatments that have been evaluated using this method. Six to eight hours is generally sufficient settling time for visualization. It can increase the amount of material in the test vials. The reason for the range (as opposed to an absolute value) is to allow for additional settling time. However, this has resulted in comments regarding a variable time window. The labs currently have various options to assist with visualization in the larval tests.

2. It would be good to be clear when this endpoint is going to be used. I think I have heard that it is no longer going to be used for high fines, but that may not be true. If you are revising this attachment, are you revising the SMARM clarification paper text to specify when it is to be used if that has changed? Since this attachment would likely be used without the document it is attached to (trust me) you may want to specify right in this attachment that the Neanthes dry and ash free weights are always to be used and the larval resuspension to be used ... (when?) .

Response: It is difficult to point to any one metric (e.g., % fines) that is predictive of when entrapment is going to be an issue. A few lines of evidence may be required to determine when to employ resuspension, including % fines, settling observed in wet-sieving performed on board when sediment samples are first acquired, knowledge of the site and former activities that may have contributed flocculent material, etc. The determination of larval test method will be made in coordination with the DMMP during the project planning phase.

3. There is some inconsistency in describing the larval termination as it is described, as in “48-60 hours” vs. “48 hours” vs. “95% to prodissoconch I stage”, but we know what is meant. It may not be clear for new users.

Response: we will review for inconsistencies, however, both 48-60 hours and PCD I are correct definitions of test duration.

4. Saying you resuspend 6-8 hours prior to test termination puts the lab in a bit of a guessing game, but I think the 6-8 time frame would work (assuming the time for resettling actually is adequate). For the lab it would probably be best to assume 6 hours prior to 48 hours you resuspend, aim for the 6 hours, then hope your test is developed by 50 hours and that way you don't go over the 8 hours. Usually the 48 hours is enough time, but not always. I'm not saying to pick 6 or pick 8, just that it can work with that window (IF 6-8 hours is sufficient settling time for enumeration). I would definitely leave the “approximately” in the 6-8 hour time frame. For example, if a test gets started by noon then you are resuspending at 6 a.m. which is a little early for normal staff hours. If you have the 6-8 then you can come in just a little early to resuspend the test and still be within the intent of the method and the time frame specified.

Response: The method will be revised to indicate that resuspension will occur at “approximately 42 hours” to avoid confusion. We have tried to accommodate comments regarding too loose or too strict a time frame. It is not our intent to dictate a starting time, however, labs will need to schedule tests appropriately.

5. Depending on when this resuspension is required and whether some samples in a test series would require resuspension and others would not, then double sets of the reference and control samples would be needed so that those samples can be ended both ways. Just mentioning that. Or maybe one reference sediment is for the samples requiring resuspension and another is for the standard endpoint so two reference sediments would be needed for that test series. I like that you mention that you can't do both the standard and resuspension methods on the same test chambers.

Response: In general, treatments within a project should be tested following one method. However, if both methods are included in a project, a separate control and reference should be conducted concurrently with the standard PSEP test and resuspension method.