CLARIFICATION

ECHINODERM EMBRYO SEDIMENT BIOASSAY PROTOCOL

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INTRODUCTION

At a PSDDA sponsored bioassay workshop conducted on July 10, 1990 it was apparent that for the echinoderm embryo sediment bioassay a number of protocol issues needed clarification. The proposed clarifications ensuing from this workshop will be adopted and implemented by the PSDDA program to insure consistency in the running of this bioassay and the quality of the data for regulatory decision making. The bioassay workshop recommendations on bioassay protocol clarifications were also sent to EPA's Office of Coastal Waters for future PSEP bioassay protocol revisions.

PROBLEM IDENTIFICATION

The following protocol issues requiring better specifications are discussed as follows:

(1) **Temperature.** Due to longer development times required for the echinoderm embryo bioassay at 12 degrees Centigrade, the bioassay temperature specification should be changed to 15 degrees Centigrade. This recommendation was endorsed by the July 10, 1990 bioassay workshop participants. At 15 degrees Centigrade, most labs report that they get pluteus larval development within 48-96 hours.

(2) **Test Duration.** The workshop endorsed adopting a minimum test duration of 48 hours, with no maximum test duration specified. Experience in the PSDDA program to date has shown that some echinoderm embryo sediment bioassays may have been terminated prior to full pluteus larval development in the seawater control. To insure that the tests are not terminated prematurely, a minimum test duration specification of 48 hours is proposed for implementation.

(3) **Test Endpoint.** The workshop recommendation for test endpoint was as follows: monitor seawater control until at least 90 percent (preferably more) of the pluteus larvae are well developed with deeply invaginated preoral arms. Abnormality in the seawater control should not exceed 10 percent. No test should be terminated before 48 hours have elapsed as noted above.

(4) **Test Termination.** Carefully (gently) stir up the water in each test beaker to insure larvae are suspended in the water without disturbing the sediment; then carefully decant up to 95 percent of the water (80-95%) leaving the sediment remaining in the test beaker. The decanted water should be thoroughly mixed to insure uniform distribution of larvae prior to removing up to three 10 ml aliquots, each of which is fixed with buffered formalin. One l0 ml aliquot is then counted, while the other two are archived until counts are assured to be adequate for characterizing test replicates.

PROPOSED CLARIFICATION

The following protocol clarifications will be implemented to insure high quality data for PSDDA program regulatory decision-making.

(1) **Temperature.** The temperature specifications for the echinoderm embryo sediment bioassay should be changed to 15 +/- 1 degree, Centigrade.

(2) **Test Duration.** Tests should not be terminated prior to 48 hours, and before the pluteus larval development endpoint. Test duration normally should be 48-96 hours at 15 degrees Centigrade, but no upper limit for test duration is specified. Test duration depends on larval development.

(3) **Test Endpoint.** The test endpoint is reached when 90 percent or more of the developing larvae have reached the pluteus larval stage, where the larvae are well developed with deeply invaginated preoral arms in the seawater control. Abnormal pluteus larvae in the seawater control should not exceed 10 percent as a general rule.

(4) **Test Termination.** To terminate the test, the water in test beakers should be carefully (gently) stirred to insure that larvae are in suspension without disturbing the sediment. The water is then carefully decanted so that a maximum of 20 percent of the water remains in the beaker. The decanted water containing generally between 80 to 95 percent of the tested volume is then thoroughly mixed to insure uniform larvae distribution, and three 10 ml aliquots are removed and each fixed with buffered formalin. One 10 ml aliquot is then counted, while the other two remain archived until the counts rendered for the other replicate is assured to be adequate.