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

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OFFICE OF WATER

MEMORANDUM

SUBJECT: Technical Panel Recommendations Concerning Use of Acute Amphipod Tests in Evaluation of Dredged Material

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TO: EPA Regional Ocean Dumping Coordinators

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Over the past two years, the U.S. Army Corps of Engineers (Corps) and the Environmental Protection Agency (EPA) have been working jointly toward development and implementation of two testing manuals for evaluating dredged material proposed for disposal in aquatic environments. These documents are titled, "Evaluation of Dredged Material Proposed for Ocean Disposal - Testing Manual" and "Evaluation of Dredged Material Proposed for Discharge in Inland and Near Coastal Waters - Inland Testing Manual". The Ocean Disposal Manual was published in 1991, and the draft Inland Testing Manual was recently distributed for Corps and EPA review. Following publication of the Ocean Disposal Manual, as the Corps and EPA began to implement this revised ocean testing protocol, some laboratories experienced problems conducting amphipod bioassays and replicating laboratory test results. Some of the laboratories conducting the tests attributed these problems to ammonia and hydrogen sulfide toxicity, as well as amphipod sensitivity to grain size. In order to evaluate the use of amphipod bioassays in the dredged material regulatory programs, EPA and the Corps convened a

meeting of Experts on June 18, 1993. This memorandum transmits the findings of that meeting and subsequent discussions.

The meeting participants supported the continued use of amphipod bioassays in the dredged material regulatory programs, and recommended application of the guidance provided in this memo until EPA publishes standard sediment toxicity test protocols in 1994.

The meeting participants reviewed the results of EPA research on test protocol development, and the influences of grain size, ammonia, and hydrogen sulfide toxicity. Standard acute amphipod toxicity test method protocols to be completed by EPA this year (for five species) will include this information. Tables 1 and 2, attached to this memorandum, contain test condition acceptability ranges - based on the "best professional judgement" of the EPA researchers developing the standard protocols - for the following test organisms used to evaluate dredged material: marine and estuarine amphipods (Rhepoxynius, Ampelisca, Eohaustorius, Leptocheirus), a freshwater amphipod (Hyalella), a freshwater midge (Chironomus), and a freshwater oligochaete used in bioaccumulation tests (Lumbriculus). Test condition acceptability ranges are given for temperature, salinity, grain size, and ammonia. Hydrogen sulfide toxicity is not believed to be a problem if dissolved oxygen levels are maintained in the overlying water. At certain open-water dredged material disposal sites (e.g., dispersive situations and situations with well-oxygenated overlying water), ammonia and hydrogen sulfide may not be contaminants of concern. Whenever chemical evidence of ammonia is present at toxicologically important levels, and ammonia is not a contaminant of concern, the laboratory analyst should reduce ammonia in the sediment's interstitial water to below 20 mg/l before adding the benthic test organism. Ammonia levels in the interstitial water can be reduced by sufficiently aerating the sample at saturation and replacing two volumes of water per day. The analyst should measure interstitial ammonia each day until it reaches 20 mg/l. After placing the test organism in the sediment, the analyst should ensure that ammonia concentrations remain within an acceptable range (see Tables 1 and 2) by conducting the toxicity test with continuous flow or volume replacement not to exceed two volumes per day. Table 3 lists several peer-reviewed papers that deal with the information discussed above. A comparison of life cycle/ecological characteristics for the marine and estuarine amphipod species mentioned above is presented in Table 4.

The EPA researchers developing the standard protocols recommended that laboratories running the amphipod toxicity tests take the following steps to reduce the likelihood of obtaining invalid test results.

1) Minimize handling stress of the organisms.

2) Ship the test animals to laboratories quickly at appropriate temperatures.

3) Make certain that proper temperature and other water quality characteristics are always maintained for the test animals.

4) For marine tests, run tests within ten days of receiving test animals in the laboratory. (Tests with some species may need to be run sooner.)

5) Conduct concurrent reference toxicity tests at the start of a sediment test.

6) Feed the test animals if necessary before use.

7) Use the proper life stage of animal for the test.

8) Always run necessary controls for the tests.

9) Remember that all amphipod test species are not the same, and be aware of species specific differences in test acceptability conditions.

10) Culture Hyalella azteca at the testing laboratory.

It is recommended that test acceptability conditions (including interstitial water ammonia) be measured before initiating a test. If any test conditions lie outside of acceptability ranges, alternative test species may be chosen for use whose test acceptability conditions match the dredged material. (But for ammonia, follow the guidance in paragraph 3 of this memo.)

The panel discussed performance requirements for selecting a contractor. It was recommended that as part of the "request-for-proposal" process, contractors should be required to submit three sets of control data to show that they can successfully run the particular test. More detailed guidance is available in the draft document "QA/QC Guidance for Laboratory Dredged Material Bioassays" USACE, Waterways Experiment Station [D. Moore, T. Dillon, J. Word, J. Ward, MP XX-93 (draft may be obtained from senior author)]. EPA and the Corps will work on additional detailed guidance for QA/QC of biological tests in 1994.

EPA and the Corps recognize the need for the development of standard amphipod test protocols, and for continued training on amphipod toxicity test methods. EPA will publish and distribute standard acute toxicity test method protocols for all species listed in the attached tables in FY 94. The Corps and EPA will continue to hold training workshops on the test methods, and to develop training tools such as videos describing test method protocols. EPA and the Corps will also initiate discussions on the feasibility of developing a laboratory certification or accreditation program to support dredged material regulatory activities.

If you have additional questions concerning the amphipod bioassays described in this memo please contact the following persons. For questions concerning the freshwater test contact Dr. Gary Ankley at EPA's environmental research laboratory in Duluth, Minnesota 218-720-5603; for questions concerning the marine and estuarine amphipod tests contact Dr. Norm Rubinstein at EPA's environmental research laboratory in Narragansett, Rhode Island 401-782-3002, Dr. Rick Swartz at EPA's environmental research laboratory in Newport, Oregon 503-867-4031, or Dr. Tom Dillon at the U.S. Army Corps of Engineers Waterways Experiment Station in Vicksburg, Mississippi 601-634-3922.

Attachments

Table 1

FRESHWATER SEDIMENT TOXICITY AND BIOACCUMULATION TEST APPLICATION CONDITIONS

PARAMETER	Hyalella	Chironomus	Lumbriculus
Temperature (°C)	23	23	23
Overlying Salinity (ppt)	<15	<1	<1
Grain Size (% silt/clay)	full range	pending	full range
Total Ammonia (mg/L NH_3+NH_4)	*	*	*
Sulfides	**	**	**

*The toxicity of total ammonia to Hyalella azteca is a function of both water hardness and pH. For Lumbriculus variegatus and Chironomus tentans total ammonia toxicity increases as pH increases, with little apparent effect due to hardness. For a frame of reference, the 10-d LC50 for total ammonia in Lake Superior water (40-42 mg/L hardness) is 17.5 (14.8-20.7) mg/L at pH 7.5 for Hyalella azteca, 21.4 (19.2-23.9) mg/L at pH 7.8 for Lumbriculus variegatus, and 186 (156-222) mg/L at pH 7.7 for Chironomus tentans. A framework for deciding whether observed sediment (or elutriate) toxicity may be due to ammonia is presented in EPA/USACE (1993; Appendix F).

**Hydrogen Sulfide is not likely to be a problem in these tests if adequate dissolved oxygen levels are maintained in the overlying water.

EPA/USACE. 1993. Evaluation of dredged material proposed for discharge in inland and near coastal waters - testing manual (Inland Testing Manual). Draft Report. U.S. Environmental Protection Agency, and U.S. Army Corps of Engineers, Washington, DC.

Table 2

MARINE AND ESTUARINE AMPHIPOD TOXICITY TEST APPLICATION CONDITIONS

PARAMETER	Rhepoxynius	Ampelisca	Eohaustorius	Leptocheirus
Temperature (°C)	15	20	15	25
Overlying Salinity (ppt)	>25	>20	2-34	2-32
Grain Size (% silt/clay)	<90	>10	full range	full range
Ammonia (total mg/L, pH 7.7)*	<30	<30	<60	<60
Ammonia (UI[1] mg/L, pH 7.7)*	<0.4	<0.4	<0.8	<0.8
Sulfides	**	**	**	**

*A framework for deciding whether observed sediment (or elutriate) toxicity may be due to ammonia is presented in EPA/USACE (1993; Appendix F). This document should be consulted if ammonia is suspected to be a contaminant of concern.

**Hydrogen Sulfide is not likely to be a problem in these tests if adequate oxygen levels are maintained in the overlying water.

[1]Unionized

EPA/USACE. 1993. Evaluation of dredged material proposed for discharge in inland and near coastal waters - testing manual (Inland Testing Manual). Draft Report. U.S. Environmental Protection Agency, and U.S. Army Corps of Engineers, Washington, DC.

Table 3

American Society for Testing and Materials. E1367-92. Guide for conducting 10-day static sediment toxicity tests with marine and estuarine amphipods. In: Annual Book of ASTM Standards, Water and Environmental Technology, Vol. 13.04, Philadelphia, PA, 1992.

American Society for Testing and Materials. E1383-93. Standard guide for conducting sediment toxicity tests with freshwater invertebrates. ASTM 1993 Annual Book of Standards Vol. 11.04, Philadelphia, PA, 1993.

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Table 3, Continued

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Table 4

COMPARISON OF FOUR MARINE AND ESTUARINE AMPHIPOD SPECIES FOR ACUTE TESTS

Characteristic	Rhepoxynius	Ampelisca	Eohaustorius	Leptocheirus
Substrate Relation	Free burrowing	Tube dwelling, closed	Free burrowing	Tube dwelling, open
Zoogeography	Pacific	Atlantic-Gulf San Francisco Bay	Pacific	Atlantic
Habitat	Polyhaline	Poly-upper mesohaline	Oligo-mesohaline	Oligo-meshohaline
Life Cycle	Annual	30-40 days	Annual	30-40 days
Availability	Field	Field-culture	Field	Field-Culture
Response Data Base	Extensive	Extensive	Low to moderate	Low to moderate
Ecological Importance	High	High	High	High