APPENDIX VIII

PROCEDURES FOR ADDRESSING AMMONIA PRESENCE IN *MYSIDOPSIS* SEDIMENT TOXICITY TESTS (ELIZABETH SOUTHERLAND MEMO TO MARIO P. DEL VICARIO, DATED JUNE 14, 1994)

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

JUN 14 1994

MEMORANDUM

OFFICE OF WATER

SUBJECT: Recommendations for Conducting Sediment Toxicity Test with Mysidopsis bahia when Ammonia may be Present at Toxic Levels

FROM: for Elizabeth Southerland, Acting Director
Standards and Applied Science Division (4305)
Office of Science and Technology

TO: Mario P. Del Vicario, Chief
Marine and Wetlands Protection Branch
U.S. EPA Region 2

The purpose of this memorandum is to provide guidance to U.S. EPA Region 2 on conducting the mysid ten-day solid phase sediment toxicity test to evaluate dredged material for open water disposal. This guidance is provided in response to a letter mailed to Region 2 on April 22, 1994 from Monte Greges, U.S. Army Corps of Engineers, New York District, requesting guidance on running the mysid test when ammonia is present at potentially toxic concentrations.

The Office of Science and Technology held a conference call on May 16, 1994 with EPA and U.S. Army Corps of Engineers scientists and our consultants to develop an acceptable protocol for running the mysid test when ammonia may be present at toxic levels. The following protocol was recommended by conference call participants who are identified below as recipients of this memorandum.

1. The Corps of Engineers and EPA issued joint guidance on December 21, 1993 offering recommendations, based on the best available information, for reducing ammonia levels in test systems used for acute amphipod sediment bioassays. When running mysid tests, it is recommended that the procedure described in the December 21 memorandum be used with modifications pertaining specifically to Mysidopsis bahia.

2. The Corps of Engineers/EPA December 21 guidance memorandum states that 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. If chemical evidence of ammonia is present at toxicologically important levels (i.e. ammonia concentrations exceeding the species-specific acceptability ranges), and ammonia is not a contaminant of concern, the laboratory analyst running the mysid ten-day sediment toxicity test should reduce ammonia in the in the test system overlying water to the appropriate acceptable level before adding the test organism.

3. For Mysidopsis bahia, the species-specific acceptable level for unionized ammonia concentration in the test system overlying water (i.e sublethal water column concentration for a ten-day sediment test) is 0.6 mg/L in tests run at 26±1°C, 31± g/Kg salinity, and pH of 7.9-8.0 using one day old organisms . At a test pH of 7.5, the acceptable concentration of unionized ammonia is 0.3 mg/L. These acceptability levels were derived on the basis of acute toxicity tests conducted with ammonia by D.C. Miller, S. Poucher, J.A. Cardin, and D. Hansen at EPA's Environmental Research Laboratory, Narragansett, Rhode Island.

4. If unionized ammonia levels in the test system overlying water exceed the acceptability level for Mysidopsis bahia (0.6 mg/L at pH 7.9-8.0 or 0.3 mg/L at pH 7.5) the system should be flushed at a rate of two volume replacements per day until it reaches a concentration of unionized ammonia at or below the acceptability level. Overlying water should be aerated during flushing, and the analyst should measure the overlying water ammonia concentration each day until the acceptable concentration is reached. Overlying water should be sampled approximately 1 cm above the sediment surface.

5. After adding the test organisms to the system, the analyst should ensure that ammonia concentrations remain within an acceptable range by conducting the toxicity test with continuous flow or volume replacement not to exceed two volumes per day. It is recommended that overlying water concentration of ammonia be measured again at the end of the test.

6. Accurate measurement of sample pH is crucial in the calculation of the unionized ammonia fraction. EPA's Narragansett laboratory recommends the use of specific equipment and procedures for determining pH of seawater (see Attachment 1)

We are sending this memorandum concurrently to EPA Region 2 and the conference call participants who recommended guidance. We ask that conference call participants provide any comments or modifications of the recommended procedure to Tom Armitage of my staff by June 24, 1994. We will notify Region 2 if any changes in the guidance are required.

Attachment

cc: Bob Engler (COE WES)
Tom Dillon (COE WES)
David Moore (COE WES)
Monte Greges (COE NY District)
Gary Ankley (EPA ORD)
Don Miller (EPA ORD)
Norm Rubinstein (EPA ORD)
Rick Swartz (EPA ORD
Tom Chase (EPA OWOW)
Alex Lechich (EPA Region 2)
Joel O'Conner (EPA Region 2)
Dave Tomey (EPA Region 1)
John Scott (SAIC)

Use of criteria for developing water quality-based permit limits and for designing waste treatment facilities requires the selection of an appropriate wasteload allocation model. Dynamic models are preferred for the application of these criteria (U.S. EPA 1985b). Limited data or other considerations might make their use impractical, in which case one should rely on a steady-state model (U.S. EPA 1986).

IMPLEMENTATION

Water quality standards for ammonia developed from these criteria should specify use of environmental monitoring methods which are comparable to the analytical methods employed to generate the toxicity data base. Total ammonia may be measured using an automated idophenol blue method, such as described by Technicon Industrial Systems (1973) or U.S. EPA (1979) method 350.1. Un-ionized ammonia concentrations should be calculated using the dissociation model of Whitfield (1974) as programmed by Hampson (1977). This program was used to calculate most of the un-ionized values for saltwater organisms listed in Table 1 and 2 of this document. Accurate measurement of sample pH is crucial in the calculation of the un-ionized ammonia fraction. The following equipment and procedures were used by EPA in the ammonia toxicity studies to enhance the precision of pH measurements in salt water. The pH meter reported two decimal places. A Ross electrode with ceramic junction was used due to its rapid response time; an automatic temperature compensation probe provided temperature correction. Note that the responsiveness of a new electrode may be enhanced by holding it in sea water for several days prior to use. Two National Bureau of Standards buffer solutions for calibration preferred for their stability were (1) potassium

hydrogen phthalate (pH 4.00) and (2) disodium hydrogen phosphate (pH 7.4). For overnight or weekend storage, the electrode was held in salt water, leaving the fill hole open. For daily use, the outer half-cell was filled with electrolyte to the fill hole and the electrode checked for stability. The electrode pair was calibrated once daily prior to measuring pH of samples; it was never recalibrated during a series of measurements. Following calibration, the electrode was soaked in sea water, of salinity similar to the sample, for at least 15 minutes to achieve chemical equilibrium and a steady state junction potential. When measuring pH, the sample was initially gently agitated or stirred to assure good mixing at the electrode tip, but without entraining air bubbles in the sample. Stirring was stopped to read the meter. The electrode was allowed to equilibrate so the change in meter reading was less than 0.02 pH unit/minute before recording. Following each measurement, the electrode was rinsed with sea water and placed in fresh sea water for the temporary storage between measurements. Additional suggestions to improve precision of saltwater pH measurements may be found in Zirno (1975), Grasshoff (1983), and Butler et al. (1985).

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May 20, 1994

Subject: Mysid No Effect NH_3 Concentration for Lethality and pH Issues for Sediment Toxicity Test Protocols
From: Don C. Miller DCM
Research Aquatic Biologist, ERL,N
To: Tom Armitage
Office of Science and Technology (4305)

The following information is provided in response to the May 16, 1994 conference call on sediment toxicity testing where high concentrations of ammonia are present. No mysid tests are directly applicable to estimate a 10 day no lethal effect concentration for NH_3. However, data for other exposure periods are available.

1. We believe that 0.6 mg NH_3 /L in the water column should be sublethal for 10 day sediment tests with one day old *Mysidopsis bahia* at 26±1° C, 31±1 g/Kg salinity and a pH of 7.9-8.0. At a test pH of 7.5, the sublethal concentration should be approximately 0.3 mg NH_3/L.

The 0.6 mg/L value is supported by:

a. four day acute results for Test 16, per J. Cardin 8/15/86 memo, attached. Test 16 pertains to the present question as it was conducted at the above conditions. The LC50 is 1.7 mg NH_3/L. The 7% mortality observed in the 0.95 mg/L treatment probably is not significant and may be a no effect concentration for a four day test. For 10 day sediment tests, the lower treatment concentration (0.58 mg/L) may be required because the 10 day continued exposure may result in mortality at lower concentrations.

b. a 32 day chronic value, 0.232 mg NH_3 /L, which represents a lower bound no effect concentration (Miller et al., 1990, attached). This value is based on a significant effect on survival at 0.331 mg/L at the same test conditions as above This lower protection concentration reflects the greater sensitivity of mysids after maturation and young begin to develop in the brood pouch. Since eggs do not appear until day 12 to 14 (at 25°), the lower chronic value should not be applied to 10 day sediment tests, assuming one day old animals are used.

The recommended 0.3 mg NH_3 /L at pH 7.5 is supported by acute tests at pH 8.0 and 7.0 (Figure 2B, Miller, et al.). These results suggest mysid acute sensitivity to ammonia may increase as much as two-fold at pH 7.5, relative to pH 8.0, hence requiring the 50% reduction in the concentration expected to be sublethal.

2. Also important, but not specifically stated in the subject protocol, are the precautions

necessary to accurately measure pH in seawater. Accurate calculation of NH_3 concentrations in the test water requires accurate pH measurement. However, measuring pH in sea water is not straight forward, as indicated in Miller et al. (See discussion, first paragraph). Enclosed is a recommended procedure from the implementation section of the EPA saltwater criteria for ammonia. We suggest this issue be highlighted in the protocol.

3. Should additional studies be desired to better describe the NH_3 no effect concentration for mysids, we recommend: (a) flow through testing, using a pH controller, or at a minimum, 24 h monitoring of pH during day one, and (b) the tests be conducted for the range of pH conditions expected in sediment testing. The variance shown in the attached paper (Figure 2B) for static tests is due to pH drift in tests which were not monitored over night. In contrast, Figure 2A shows good agreement may be achieved with flow through tests where there was 24 h monitoring of pH during day one.

attachments: Cardin memo
Miller et al. paper
NH_3 criteria implementation

cc without attachments: N. Jaworski
G. Pesch