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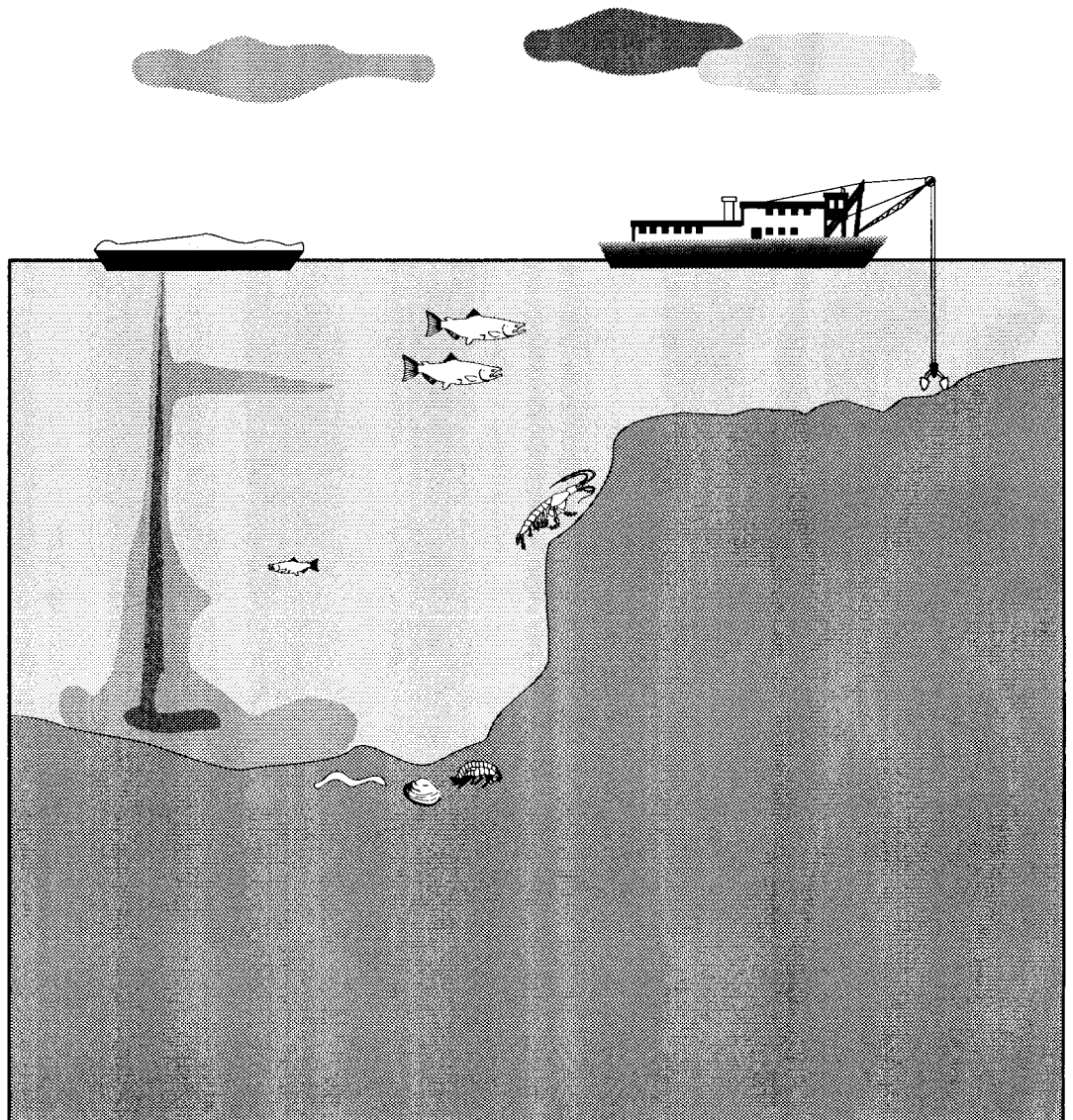EPA-823-B-98-004
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Evaluation of Dredged Material Proposed For Discharge in Waters of the U.S. - Testing Manual

Inland Testing Manual

EVALUATION OF DREDGED MATERIAL
PROPOSED FOR DISCHARGE IN WATERS OF THE U.S. - TESTING MANUAL

(INLAND TESTING MANUAL)

Prepared by

ENVIRONMENTAL PROTECTION AGENCY
Office of Water
Office of Science and Technology
Washington, D.C.

and

DEPARTMENT OF THE ARMY
United States Army Corps of Engineers
Operations, Construction, and Readiness Division
Washington, D.C.

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APPENDIX F
METHODOLOGIES FOR IDENTIFYING AMMONIA AS A TOXICANT IN DREDGED-MATERIAL TOXICITY TESTS

APPENDIX F

Ammonia Toxicity: General Overview

Ammonia is a relatively toxic compound which, in sediments, is generated from the microbial degradation of nitrogenous organic material such as amino acids (Santschi et al., 1990). Resulting interstitial (pore) water concentrations of ammonia in otherwise uncontaminated sediments can be as high as 50 mg/L (Murray et al., 1978; Kristensen and Blackburn, 1987), while ammonia concentrations in pore water from contaminated sediments may range from 50 to greater than 200 mg/L (Ankley et al., 1990; Schubauer-Berigan and Ankley, 1991). Hence, exposure of epibenthic/benthic test species to ammonia in solid phase tests can be significant. Moreover, because ammonia is released from sediments relatively readily during resuspension events (Blom et al., 1976), high concentrations can also occur in test elutriates. Both marine and freshwater studies suggest that ammonia can be responsible for toxicity observed in some laboratory sediment toxicity tests (Jones and Lee, 1988; Ankley et al., 1990).

Because ammonia is not extremely persistent, its toxicity may not be of as much concern as that from, for instance, metals or pesticides. For this reason, there has been a tendency in some situations to use open-water disposal for dredged material whose toxicity is suspected to be due to ammonia. Unfortunately it has previously been difficult, if not impossible, to validly link sediment or elutriate toxicity to ammonia when multiple sediment contaminants are present (Ankley et al., 1992), in particular because ammonia concentrations can be exceptionally high in sediments which are also toxic due to other, persistent contaminants such as inorganic and/or organic chemicals (Schubauer-Berigan and Ankley, 1991). However, recent technical developments have resulted in a logical conceptual framework, specifically a simple risk assessment, for deciding whether observed sediment (or elutriate) toxicity may be due to ammonia. Briefly, data are collected on the toxicity of ammonia to the test species of concern (effects assessment), and concentrations of ammonia are measured in appropriate test fractions (elutriate, overlying water, pore water) during the toxicity test (exposure assessment). If concentrations of ammonia in the test are large enough to result in toxicity to the test species of concern (risk characterization), a simple set of toxicity identification evaluation (TIE) procedures is next used to confirm that toxicity is indeed due to ammonia and not to other contaminants in the sediment (Ankley et al., 1992). TIE methods consist of physical/chemical sample manipulations conducted concurrently with toxicity testing in order to directly characterize and identify contaminants responsible for toxicity in complex mixtures. Further information on how this approach could be used, and important technical considerations relative to this assessment are described below.

Specific Considerations for Assessing Ammonia Toxicity in Dredged Material

The first step in assessing the potential for ammonia toxicity in a sediment test is to routinely measure ammonia concentrations in test fractions of concern, at a minimum, when starting and ending the test. Due to the influence of pH on ammonia toxicity to some species, it is essential that pH also be measured and recorded simultaneously. For elutriate tests, ammonia measurements can be made on whole elutriate. For solid phase tests, ammonia should be measured both in overlying water and in pore water, the potential routes of ammonia exposure for epibenthic and benthic species. In tests where periodic renewal of overlying water is utilized, ammonia may not be present at toxicologically significant concentrations in the overlying water (Ankley et al., 1993); nonetheless, it would still be prudent to measure ammonia in the overlying water. Regardless of whether overlying water renewal is used in a sediment test, pore water ammonia concentrations should be determined. Pore water for ammonia measurements can be isolated using any of a variety of techniques (e.g., low-speed centrifugation, squeezing, peepers, etc.). Unlike other pore water contaminants of concern (e.g., metals, nonionic organics), it does not appear that the method used to isolate pore water greatly affects observed ammonia concentrations (EPA, 1991a). Upon isolation of the appropriate test fraction(s), ammonia can be measured using any accepted technique; specific ion electrodes are rapid, simple and often used for ammonia determinations at concentrations ≥ 1 mg/L (EPA, 1979).

The next step is to compare exposure data (i.e., ammonia measurements) to toxicity data. The basis of this comparison most generally will be to ammonia toxicity data generated in water-only toxicity tests. For the elutriate tests the comparison can be made directly while, for solid phase tests, the water-only toxicity data are compared to overlying water and/or pore water ammonia concentrations. To assess the potential for ammonia toxicity in a test with a given species, it is essential that comparisons be made to toxicity data generated with that same species in tests conducted under conditions reasonably similar to the sediment test. The tendency to attempt to extrapolate toxicity data for one species to another species should be avoided. Such an approach may be appropriate for some types of risk analyses; however, for the approach described here, this type of extrapolation likely would result in erroneous conclusions. Similarly, comparisons within a species should be made only between tests which were conducted under a relatively similar set of conditions. For example, it would be inappropriate to compare toxicity data and ammonia concentrations from a short-term sediment test to water-only chronic toxicity data for that same species. In addition to test length, pH is of primary concern while hardness, salinity and temperature are of somewhat lesser concern. All of these factors can markedly influence ammonia toxicity, and must be accounted for to enable among test comparability.

Although there is a good deal of data on the toxicity of ammonia to various aquatic species (EPA, 1985), much of this information was generated using pelagic species (e.g., cladocerans, fishes), which precludes comparison to sediment exposures with commonly tested benthic species (e.g., amphipods). [Although it should be noted that these data would be useful for extrapolation to elutriate tests which commonly utilize pelagic species].

Water-only toxicity data are available for some epibenthic/benthic species of concern, however these water-only tests were often conducted under conditions quite different from those commonly used in sediment tests, which greatly limits any extrapolation to sediment tests. However, efforts are now underway to generate useful data. For example, toxicity data now exist for ammonia at four different pHs (ca., 6.5, 7.2, 7.8, 8.6) for *Hyalella azteca*, *Chironomus tentans* and *Lumbriculus variegatus* (EPA, 1991a; G. Ankley, unpublished data). Ammonia toxicity data have also been developed for the commonly tested marine amphipods *Rhepoxynius abronius*, *Eohaustorius estuarius*, *Ampelisca abdita* and *Grandidierella japonica* in four-day water-only exposures at a pH of 8.0 (Kohn et al., 1993); and, ammonia toxicity data have been generated for the polychaete *Nereis (Neanthes) arenaceodentata* (Dillon et al., 1993).

Although toxicity data exist for several pelagic and some benthic species of concern, it may be necessary for laboratories conducting dredged material tests with a particular species, under a given set of test conditions, to develop ammonia toxicity data relevant to their species/test conditions. This likely would be a wise investment of resources, in particular for those laboratories conducting large numbers of tests with dredged material.

In this regard, a major caution must be noted concerning pH in ammonia tests. Ammonia acts as a basic compound in water. The un-ionized form (NH_3) predominates at pH values greater than 9.3, while the ionized form (NH_4^+) is most abundant at pH values less than 9.3. Through the pH range of 6 to 8.3 (which is typically encountered in freshwater and marine sediment tests), the percentage of un-ionized ammonia changes approximately 250-fold. Based on models developed primarily with fish, it has been common to express ammonia toxicity data on an un-ionized (i.e., NH_3) rather than a total (i.e., NH_3 plus NH_4^+) basis. This implicitly suggests that ionized ammonia is not of great toxicological significance. While this appears to be true for fish (EPA, 1985), it does not appear to be the case for some invertebrates. For example, *H. azteca* displays the same sensitivity to total ammonia (NH_3 plus NH_4^+) over a pH range of approximately 6.0 to 8.5, suggesting that this amphipod is very sensitive to ammonium ion (EPA, 1991a; G. Ankely, unpublished data). Hence, extrapolation of ammonia toxicity data collected at only one pH value, based on un-ionized ammonia concentrations, would result in inaccurate predictions of potential toxicity of ammonia to at least this amphipod. Other invertebrates may exhibit a similar lack of predictability relative to pH/ammonia interactions. Unfortunately, relatively few ammonia toxicity tests with invertebrates have been conducted at multiple pHs; thus, it is difficult to broadly predict responses to ammonia at different pH values. To make accurate predictions of potential ammonia toxicity for a particular test species, it is important to obtain (or generate) ammonia toxicity data within the pH range in which extrapolations are made.

If measurements of ammonia in elutriate tests, or overlying and/or pore water in solid phase tests are determined to be of possible toxicological significance, it is essential that the role of ammonia in causing toxicity be confirmed. It is important to avoid the tendency to assume that if a dredged material test exhibits

toxicity and ammonia is present, that ammonia is the sole (or even major) cause of the olconcentrations of other contaminants may be present simultaneously with ammonia. In such cases the assumption that only ammonia was causing toxicity could lead to disposal decisions (i.e., open-water) that may result in serious long-term impacts to benthic communities.

Relatively simple TIE manipulations as generally described by Ankley et al. (1992), and specifically in a series of guidance manuals (EPA, 1988; 1989a; 1989b; 1991a; 1991b) may be used to determine whether (or not) ammonia is responsible for the observed toxicity. To date, these TIE methods have only been used with freshwater sediments. However, in many instances similar approaches can be used with marine sediments; also, EPA currently is developing standardized TIE methods for marine sediments.

Current sediment TIE methods are only for elutriates or pore waters and for short-term ($\leq$ 96 hour) tests (EPA, 1991a). This is not a problem if TIE procedures are to be used with toxic elutriates, because elutriate tests also generally consist only of short-term ($\leq$ 96 hour) exposures. However, solid phase tests with dredged material are generally 10 days in length. Although using pore water as a surrogate test fraction for TIE work with solid phase exposures could mean that toxicity might not be expressed in the shorter-term pore water exposures, this may not be a significant problem in the case of ammonia. In water-only exposures with three different benthic invertebrates (*H. azteca, C. tentans, L. variegatus*), the majority of toxicity due to ammonia was observed within 4 days in a 10 day test (G. Ankley, unpublished data).

There is one other important consideration relative to the use of pore water as a surrogate test fraction for solid phase sediments. Because the toxicity of ammonia to some organisms can be pH-dependent, it is imperative that pH in pore water tests mimic the pH in the initial solid phase tests. This is particularly important with freshwater sediments, because pH can drift upwards by as much as one unit over the course of a 96-hour test (Ankley et al., 1991). Methods which have proven useful for controlling pH in pore water tests include: (a) use of acids/bases in chambers with minimal head-space, (b) use of organic buffers, and (c) use of varying amounts of CO_2 in head-space overlying the pore water (EPA, 1991a; 1991b).

Most aquatic species that can be tested successfully in a water-only exposure can be utilized for TIE work. For example, cladocerans (*Ceriodaphnia dubia, Daphnia magna, Daphnia pulex*), fish (*Pimephales promelas, Oryzias latipes, Oncorhynchus mykiss*), amphipods (*H. azteca*), oligochaetes (*L. variegatus*), and chironomids (*C. tentans*) all have been used for freshwater TIE studies. The best choice of a TIE organism is, of course, the same species that was sensitive to the original elutriate or solid phase sediment of interest. For example, if toxicity was observed in solid phase sediment tests with *H. azteca*, that species would be the best choice for pore water TIE work. Of course, there are instances in which this may not be possible; for example, the test species of concern may be of limited availability. In this case, it may be possible to use surrogate species for the TIE, provided there is adequate knowledge of the sensitivity of the surrogate species to ammonia, relative to the original test

species of concern. If a surrogate species is used, upon conclusion of the TIE it is important to perform limited testing to confirm that the same compound(s) which was toxic to that species was responsible for toxicity to the original test species.

As discussed above, the toxicity of ammonia to many species can be highly pH-dependent. If the test species of concern is more sensitive to un-ionized that ionized ammonia, samples will be more toxic at high pH values than at low pHs. [Note that this again demonstrates the need for data concerning pH/ammonia interactions for specific test organisms]. If the test species exhibits this pH-dependency with regard to ammonia toxicity, the graduated pH test can be an extremely powerful tool for implicating ammonia as a suspect toxicant. The graduated pH test is conducted at a series of physiologically tolerable pHs (generally ranging from 6.0 to 8.5); if sample toxicity is greater at higher pH values, this suggests that ammonia is responsible for at least some of the observed toxicity. A number of other TIE techniques also exist for implicating ammonia. These include evaluation of relative species sensitivity (e.g., fish are generally more sensitive than cladocerans), removal of ammonia from the test samples with cation exchange resins (e.g., zeolite) and/or extended air-stripping at elevated pH values (e.g., >10) prior to toxicity testing, correlation of toxicity with measured ammonia concentrations and toxicity tests at different pH values with equitoxic concentrations of ammonia (EPA, 1989a; Ankley et al., 1990). Another useful method for confirming that ammonia is responsible for toxicity is ammonia removal followed by spiking to restore the original ambient concentrations of ammonia. The spiked sample is then tested for toxicity; if ammonia is the causative toxicant, observed toxicity theoretically should be the same as that observed in the original sample. It is desirable to conduct as many of these confirmation tests as possible because no single test is specific for ammonia, e.g., zeolite will remove cationic metals, as well as ammonia, from test samples. Failure of one or more of the tests to confirm ammonia as responsible for toxicity would indicate that other contaminants were contributing to sample toxicity.

Summary

In order to identify elutriate or solid phase dredged material toxicity due to ammonia, it is essential to make routine measurements of ammonia on appropriate test fractions. These measurements then are compared to water-only toxicity data for the same species used in the dredged material test. The water-only toxicity data should be generated under conditions (e.g., pH, test length) reasonably similar to those in the test with the dredged material. If ammonia concentrations are too low to have potentially caused the observed toxicity in the dredged material sample, other contaminants are responsible for the toxicity. If ammonia concentrations are high enough to have caused the observed toxicity, TIE procedures should be used to confirm this suspicion. When there is no TIE confirmation that ammonia is responsible for sediment toxicity, it must be assumed that persistent contaminants other than ammonia are causing toxicity.

References Cited

Ankley, G.T., A. Katko and J.W. Arthur. 1990. Identification of ammonia as an important sediment-associated toxicant in the lower Fox River and Green Bay, Wisconsin. Environ. Toxicol. Chem 9:313-322.

Ankley, G.T., M.K. Schubauer-Berigan and J. R. Dierkes. 1991. Predicting the toxicity of bulk sediments to aquatic organisms with aqueous test fractions: pore water versus elutriate. Environ. Toxicol. Chem. 10:1359-1366.

Ankley, G.T., M.K. Schubauer-Berigan and R.A. Hoke. 1992. Use of toxicity identification evaluation techniques to identify dredged material disposal options: A proposed approach. Environ. Manage. 16:1-6.

Ankley, G.T., D.A. Benoit, R.A. Hoke, E.N. Leonard, C.W. West, G.L. Phipps, V.R. Mattson and L.A. Anderson. 1993. Development and evaluation of test methods for benthic invertebrates and sediments: Effects of flow rate and feeding on water quality and exposure conditions. Arch. Environ. Contam. Toxicol. 25:12-19.

Blom, B.E., T.F. Jenkins, D.C. Leggett and R.P. Murrmann. 1976. Effect of sediment organic matter on migration of various chemical constituents during disposal of dredged material. Report No. D-76-7. U.S. Army Corps of Engineers Waterways Experiment Station, Vicksburg, MS.

Dillon, T.M., D.W. Moore and A.B. Gibson. 1993. Development of a chronic sublethal sediment bioassay with the marine polychaete *Nereis (Neanthes) arenaceodentata*. Environ. Toxicol. Chem. 12:589-605.

EPA. 1979. *Methods for Chemical Analysis of Water and Wastes*. EPA/600/4-79-020. U.S. Environmental Protection Agency, Cincinnati, OH.

EPA. 1985. *Ambient Water Quality Criteria for Ammonia - 1984*. EPA 440/5-85-001. U.S. Environmental Protection Agency, Office of Water Regulations and Standards, Washington, DC.

EPA. 1988. *Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures*. EPA/600/3-88-034. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN.

EPA. 1989a. *Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures.* EPA/600/3-88-035. U.S. Environmental Protection Agency, Environmental Research Laboratory Duluth, MN.

EPA. 1989b. *Methods for Aquatic Toxicity Identification Evaluations: Phase III Toxicity Confirmation Procedures.* EPA/600/3-88-036. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN.

EPA. 1991a. *Sediment Toxicity Identification Evaluation: Phase I (Characterization), Phase II (Identification) and Phase III (Confirmation) Modifications of Effluent Procedures.* EPA/600/6-91-007. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN.

EPA. 1991b. *Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures.* 2nd Ed. EPA/600/6-91/003. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN.

Jones, R.A. and G.F. Lee. 1988. Toxicity of U.S. waterway sediments with particular reference to the New York Harbor area. Pp. 403-417. In: J.J. Lichtenberg, F.A. Winter, C.I. Weber and L. Fradkin (Eds). *Chemical and Biological Characterization of Sludges, Sediments, Dredged Soils and Drilling Muds.* ASTM STP 976. American Society for Testing and Materials. Philadelphia, PA.

Kohn, N.P., J.Q. Ward, D.K. Nigogi, L.T. Ross, T. Dillon and D.W. Moore. 1993. Acute toxicity of ammonia to four species of marine amphipod. Submitted Manuscript.

Kristensen, E. and T.H. Blackburn. 1987. The fate of organic carbon and nitrogen in experimental marine sediment systems: Influence of bioturbation and anoxia. J. Mar. Res. 45:231-257.

Murray, J.W., V. Grundmanis and W.M. Smethie, Jr. 1978. Interstitial water chemistry in the sediments of Saanich Inlet. Geochim. Cosmochim. Acta. 42:1011-1026.

Santschi, P., P. Hohener, G. Benoit and M.B. Brink. 1990. Chemical processes at the sediment-water interface. Mar. Chem. 30:269-315.

Schubauer-Berigan, M.K. and G.T. Ankley. 1991. The contribution of ammonia, metals and nonpolar organic compounds to the toxicity of sediment interstitial water from an Illinois river tributary. Environ. Toxicol. Chem. 10:925-939.