DMMP CLARIFICATION PAPER
SMS TECHNICAL INFORMATION MEMORANDUM

CLARIFICATIONS TO THE DMMP BIOACCUMULATION PROTOCOL

Note: This erratum adds additional discussion on Statistics and alpha level, and corrects alpha level for test tissue concentration comparison with reference sediment

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INTRODUCTION

The Dredged Material Management Program (DMMP) requires bioaccumulation testing when a bioaccumulation chemical trigger level has been exceeded in a project test sediment. Similarly, the Sediment Management Standards (SMS) program may require bioaccumulation testing on a site-specific, "as needed" basis. The current EPA protocol guidance (Lee et.al., 1993; ASTM, 1995) stipulates a test exposure of 28 days, followed by tissue analysis for chemicals of human health and ecological effects concern. The DMMP protocol guidance was previously modified in 1994 (http://www.nws.usace.army.mil/publicmenu/DOCUMENTS/dmmo/acum_94.pdf) and in 1996 (http://www.nws.usace.army.mil/publicmenu/DOCUMENTS/dmmo/acum_96.pdf) to ensure compliance with the National EPA/Corps guidance (EPA/COE, 1991; EPA/Corps, 1994) and to further refine the DMMP bioaccumulation guidance on species selection, sediment volume collection and testing requirements (Battelle, 1992). The DMMP program is now working on revising the guidelines for bioaccumulative-chemicals-of-concern (BCOC) and the framework for accomplishing this work was presented at the 1998 SMARM (http://www.nws.usace.army.mil/publicmenu/DOCUMENTS/dmmo/BCOC_IP.98.htm; http://www.nws.usace.army.mil/publicmenu/DOCUMENTS/dmmo/BCOC_TS.981.pdf). In September 1999, the DMMP convened a bioaccumulation workgroup to begin the process of addressing bioaccumation guideline revision issues (e.g., revising BCOC list, PCB congener and TBT analysis, target tissue level (TTL)/bioaccumulation trigger (BT) development).

PROBLEM IDENTIFICATION

The purpose of this clarification paper is to document interim changes made by the DMMP to the bioaccumulation protocol. These changes are recommended for interim implementation in the DMMP and SMS programs pending review and final recommendations to be made by the bioaccumulation workgroup.

Exposure duration. The DMMP and SMS are concerned that the 28 day bioaccumulation test exposures may not be sufficient to achieve a tissue concentration at or near equilibrium (steady-state) with the water/sediment exposure environment for some important bioaccumulative chemicals of concern (e.g., mercury, fluoranthene, PCBs, tributyltin, DDT, etc.). Over the past three years, the DMMP have extended the

exposure period from 28 to 45 days to provide a better approximation of steady-state tissue concentrations for dredging projects conducting bioaccumulation testing. Due to the increased exposure time, it is necessary to supplement the nutritional needs of the test organisms as well as maintain exposure concentrations in the test chambers. This is accomplished by once weekly additions of 175-mL of test sediment to each of the 10-gallon aquaria test chambers.

Assessing health of test animals. In order to assess the general health and well being of the test organisms during the exposure period, the current protocol requires monitoring survival, moisture, and lipid content in the test organisms. In a recent dredging project, an additional metric, net change in body weight (growth) during the exposure period (e.g., wet-weight biomass), was assessed. The rationale for measuring tissue weight changes in test animals during a bioaccumulation test was: 1) to confirm that the test animals are in good health and capable of accumulating biologically available chemicals; 2) to develop an effects endpoint to pair with the tissue chemistry for use in risk-assessment (consistent with U.S. EPA's emphasis on tissue-residue effects); and 3) to establish a more sensitive effects endpoint (relative to survival) to determine the acceptability of test results. Wet-weight biomass data were used along with percent moisture and percent lipids in a preponderance-of-evidence approach to evaluate animal health at the end of the test in conjunction with the observed tissue accumulations of chemicals-of-concern.

Statistics and alpha level. The DMMP agencies compare the tissue chemistry data from each dredged material management unit (DMMU) to both tissue data from reference-sediment exposure and chemical-specific interpretation guidelines (i.e. Target Tissue Levels - TTLs). A one-tailed t-test is used for comparison to reference, while a one-tailed one-sample t-test is used for comparison to TTLs. In the past, the DMMP has used an alpha level of 0.05 for all statistical comparisons.

1. For comparisons to reference data, the null hypothesis (Ho) is defined as the test tissue concentration being less than or equal to the reference tissue concentration. The alpha level (the probability of making a Type I error) is defined as the probability of rejecting the null hypothesis when, in fact, the test tissue concentration really *is* less than or equal to the reference tissue concentration. However, results of recent DMMP bioaccumulation testing have shown relatively high within-sample replicate variability for some contaminants (e.g., PCB, TBT). Use of a 0.05 alpha level with these samples resulted in a marked reduction in the power to discriminate between contaminant tissue concentrations in reference and test sediment exposures. When a 0.1 alpha level was used (allowing for a higher probability of making a Type 1 error) the power of the test to discriminate between reference and test tissue concentrations was increased. Given the definition of Ho used for comparison to reference data, increasing the allowable alpha level to 0.1 results in an increase in the environmental protectiveness of the comparison between test tissues and reference tissues. In other words, improving the power to discriminate between test and reference results in a higher likelihood

of making a Type I error (concluding that contaminant concentrations in test tissue are higher than reference when they really aren't).

2. For comparisons to interpretation guidelines (e.g. TTLs), Ho is defined as the test tissue concentration being greater than or equal to the TTL. Thus, the Type I error is the probability of rejecting Ho (i.e. concluding that the test tissue concentration is statistically below the TTL), when in fact it really is greater than or equal to the reference concentration. For this type of comparison, the DMMP agencies are maintaining use of the lower 0.05 alpha level as appropriately conservative to minimize the likelihood that dredged material disposed at the sites could result in bioaccumulation exceeding TTLs.

3. Use an alpha level of 0.1 when making statistical comparisons between chemical concentrations in test and reference tissue to reflect higher within sample variability, and to increase the power of the test to discriminate between reference and test tissue concentrations. Comparisons between test tissue and interpretive guidelines should continue to use an alpha level of 0.05.

PROPOSED CLARIFICATION

The DMMP and SMS programs propose to make the following changes to the existing bioaccumulation protocol on an interim basis. These changes will remain in effect until the bioaccumulation workgroup formally reviews the technical/regulatory protocol guidance and makes recommendations to the DMMP and SMS for potential future protocol/regulatory changes.

1. Use a 45-day exposure time when conducting bioaccumulation testing for specific chemicals of concern for bioaccumulation (PCBs, TBT, DDT) to ensure steady-state chemical concentrations in the tissues of the test species (*Macoma nasuta* and *Nephtys caecoides*). Increasing the exposure to 45 days will require once weekly supplemental additions of 175-mL of test or control/reference sediment to each replicate10-gallon aquaria/test chamber.

2. Wet-weight biomass (of a subset of 10 individual organisms/replicate) should be measured at the beginning and end of the bioaccumulation exposure period for test, control and reference samples. This estimate of net individual growth during the exposure period, will be used as an additional metric to evaluate the health of the test animals, and to build a database that supports establishing an effects-based target-tissue level (TTL).

3. Use an alpha level of 0.1 when making statistical comparisons between chemical concentrations in test and reference tissue to reflect higher within sample variability, and to increase the power of the test to discriminate between reference and test tissue concentrations. Comparisons between test tissue and interpretive guidelines should continue to use an alpha level of 0.05.

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