DMMP CLARIFICATION PAPER

AMMONIA AND SULFIDE GUIDANCE RELATIVE TO NEANTHES GROWTH BIOASSAY

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INTRODUCTION

Bioassays are used in the Dredged Material Management Program (DMMP) to evaluate toxicity in sediments proposed for dredging. The DMMP has long recognized the potential effects of non-treatment variables such as ammonia and hydrogen sulfides on the bioassays used to assess the toxicity of dredged material, and implemented the requirement to monitor those parameters in dredged material as potential interfering non-treatment factors (http://www.nws.usace.army.mil/publicmenu/DOCUMENTS/bio_qa90.pdf). Aerating the beakers for the amphipod and sediment larval bioassay appears to have largely ameliorated the effects of hydrogen sulfide, but it is clear that ammonia continues to remain a potential non-treatment factor, which can potentially interfere with the test results particularly in deeply buried subsurface and/or fine grained sediments with high organic contents. In 2001 the DMMP agencies clarified that a water-only ammonia reference toxicant (LC_{50}) is strongly recommended and should be run concurrently with standard amphipod and sediment larval bioassays, when ammonia interference is expected (http://www.nws.usace.army.mil/publicmenu/DOCUMENTS/Ammonia.pdf). In 2002, the DMMP agencies further clarified ammonia issues relative to toxicity testing and potential ammonia purging for the amphipod bioassay (http://www.nws.usace.army.mil/publicmenu/DOCUMENTS/AmphAmmoniaClar20021.pdf). The purpose of this clarification paper is to further update the DMMP guidance relative to ammonia and sulfide for the 20-day *Neanthes* growth bioassay.

PROBLEM IDENTIFICATION

In 1992, the DMMP implemented the *Neanthes* 20-day bioassay following the Annual Review Meeting. In 1993 the DMMP established standard ammonia and sulfide monitoring requirements (http://www.nws.usace.army.mil/publicmenu/DOCUMENTS/ACF559.pdf) for conducting the bioassay based on studies by Dillon et. al. (1993), that showed a sharp threshold response by *Neanthes* to ammonia and hydrogen sulfide.

Regulatory Guidance History. The initial 1993 DMMP guidance (http://www.nws.usace.army.mil/publicmenu/DOCUMENTS/ACF559.pdf) for *Neanthes* stipulated that total ammonia should be <10 mg/Liter (overlying water) and measured total sulfides concentrations should be <3.0 mg/Liter (overlying water), and should be measured at the beginning and end of the 20 day test. It also strongly recommends that ammonia monitoring be conducted prior to the first and second water renewals, because the

aqueous ammonia concentrations may reach maximum values nearer the beginning of the test. This guidance was further updated in the 1995 PSEP protocol document (http://www.psat.wa.gov/Publications/protocols/protocol_pdfs/bioassay.pdf, pages 68-69), where it recommended performing additional water quality ammonia and sulfide monitoring prior to the first and second water exchange/renewals when the **unionized ammonia** concentration exceeds 0.7 mg/Liter or total sulfides exceeds 5 mg/Liter.

Ammonia Effects. An evaluation of DMMP dredged material testing data show a clear bulk ammonia and total ammonia (overlying water) correlation with *Neanthes* growth (Figures 1 and 2). Dillon et. al. (1993) found no apparent effects on juvenile worm survival and dry weight when exposed for three weeks to **10 mg/Liter** nominal total ammonia (overlying water) and **0.46 mg/Liter** unionized ammonia, respectively. However, when the concentrations were increased to $\geq$20 mg/Liter total ammonia (overlying water) or >0.68 mg/Liter unionized ammonia, they observed slightly, but not significantly diminished survival (80%) and dry weight reductions (65% - 69% of control) in juvenile worms. When the nominal total ammonia (overlying water) concentrations were further increased to 40 and 60 mg/Liter, corresponding to 1.25 and 2.02 unionized ammonia, respectively, there were no surviving worms. Literature values for fish and aquatic invertebrates, in general, highlight that **acute lethality** attributable to unionized ammonia range from about 0.5 to 2.0 mg/Liter, and **acute:chronic ratios** range from 5 to 20 (Dillon et. al. 1993).

The DMMP data were examined to evaluate the frequency of exceedances of the no effects, minor effects, and major effects threshold concentrations. The evaluation showed there were 24 out of 382 observations where overlying water total ammonia exceeded the 10 mg/liter no effects threshold of concern concentration, and 11 out of 382 observations where ammonia was equal to or exceeded the minor effects concentration of 20 mg/liter. There were only 2 out of 382 observations, where the major effects concentration of 40 mg/liter were exceeded. When bulk ammonia concentrations are plotted against total ammonia (day 0 observations) the corresponding graphic shows that bulk ammonia can also be used as an indicator of potential exceedances of the no effect, minor effect, and major effect ammonia thresholds (Figure 3, Table 1).

Total Sulfides Effects. In experiments by Dillon et al. (1993) all worms survived total hydrogen sulfide concentrationsin overlying water $\leq$3.4 mg/Liter. However, at slightly higher concentrations (5.5 mg/Liter) survival was reduced to 44%, and at higher concentrations of 15 mg/Liter, survival was 0%. DMMP program data collected from *Neanthes* testing conducted over the past 12 years indicate that hydrogen sulfide measurements were generally less than the 3.4 mg/Liter threshold, and do not appear to have been a significant test interference problem.

Reference Toxicant (LC_{50}) tests. Currently for the amphipod and larval bioassays, when threshold no-effects concentrations are exceeded for ammonia, the DMMP agencies recommend conducting a water only reference toxicant LC_{50} test. Currently, it is not required for the *Neanthes* test.

Table 1. Thresholds of Concern for *Neanthes* 20-day Chronic Test[1].

Parameter	No effects (0% mortality; no effect on growth)	Minor effects (~20% mortality; growth reduced 31-35% relative to control)	Major effects (~100% mortality, no growth)
Bulk Sediment Ammonia	≤ 115 mg/Kg	> 230 mg/Kg	> 400 mg/Kg
Total Ammonia (overlying water)	≤ 10 mg/L	> 20 mg/L	> 40 mg/L
Unionized Ammonia (overlying water)	≤ 0.46 mg/L	> 0.68 mg/L	> 1.25 mg/L
Total Sulfide (overlying water)	≤ 3.4 mg/L	≥ 5.5 mg/L	≥15 mg/L

Ammonia Purging. The DMMP agencies discourage ammonia purging of toxicity test samples on a programmatic basis. The DMMP agencies continue to be concerned that purging for ammonia may also result in a concomitant loss of sediment contaminants being evaluated in the bioassay. The DMMP agencies are unaware of studies that have definitively quantified the potential contaminant losses resulting from ammonia purging. Nevertheless, it is likely that there will be occasional sediment samples from DMMP projects where initial bulk ammonia (> 230 mg/Kilograms[2]) and/or total ammonia (overlying water) concentrations (> 20 mg/Liter) indicate that purging may be required in order to conduct a reliable toxicity test.

This paper is part of a general effort to clarify and update ammonia and sulfides guidance, and provide recommended approaches to reduce potential interference problems from these variables when conducting the *Neanthes* 20-day growth bioassay. The remainder of this clarification paper provides updated guidance relative to ammonia, when the documented ammonia concentration thresholds of concern (Table 1) are exceeded. The clarifications include:

1. Guidelines for standard reporting of ammonia data

[1] Total ammonia, unionized ammonia, and total sulfide concentrations from Dillon et.al. (1993).
[2] See Figure 1. Bulk Ammonia concentration calculated from regression equation:
x = (y – 0.1007)/0.0864, where **x** = mg/kg bulk ammonia, and **y** = mg/liter total ammonia.

2. Threshold ammonia concentrations and guidelines for conducting ammonia reference toxicant (LC_{50}) tests

3. Threshold ammonia concentrations above which DMMP agencies will consider allowing purging of samples.

4. Methods for purging ammonia from overlying sample water and guidelines for test initiation after purging (batching).

PROPOSED CLARIFICATION

1. Standard reporting of ammonia data

The DMMP agencies require that the following information be collected and reported for all test sediment where there is concern that ammonia toxicity may interfere with interpretation of test results, as well as appropriate control and reference samples, whether or not sample purging is allowed and then ultimately occurs.

- Total ammonia (interstitial water) from the original bulk sediment sample
- Total and unionized ammonia (overlying water) at the start of each toxicity test, e.g., at day 0, and again on day 3 (prior to the first seawater replacement)
- All water-only ammonia reference toxicant test data (LC_{50}, total and unionized)

2. Threshold ammonia concentrations and guidelines for conducting ammonia reference toxicant (LC_{50}) tests

The DMMP agencies implement the following guidance that the total ammonia **no effects** concentrations presented in Table 1 above be used as thresholds above which project proponents should conduct water-only ammonia reference toxicant (LC_{50}) tests (http://www.nws.usace.army.mil/publicmenu/DOCUMENTS/Ammonia.pdf). With the standard ammonia data (proposed above), synoptic ammonia LC_{50} data help the DMMP agencies and project proponents determine the potential extent of ammonia-related toxicity and reduce the need to purge sediment samples. Labs should already be experienced in running reference toxicant tests, however, project sampling and analysis plans (SAPs) should include a specific discussion including the lab's protocol for ammonia testing and calculating a LC_{50}, should the ammonia reference toxicant test be required.

3. Threshold ammonia concentrations for consideration of sample purging

The **minor effects** concentrations depicted in Table 1 are implemented as interim thresholds for consideration of test sediment purging.

Project proponents concerned that ammonia toxicity may interfere with interpretation of sediment bioassays **must measure** ammonia concentrations in bulk sediment samples prior to test set up and initiation. If the bulk ammonia, total ammonia (overlying water), or unionized ammonia concentrations approach or exceed the minor effects levels depicted in Table 1, then the proponent must immediately coordinate with the Dredged Material Management Office (DMMO), Seattle District U.S. Army Corps of Engineers and develop an acceptable plan for monitoring ammonia. The final decision whether or not to allow toxicity test sample purging prior to test initiation will be made by the DMMP agencies using best professional judgment, and in collaboration with the applicant. If purging is allowed and occurs, the project proponent would be required to collect and report the following additional information for each test sample and the associated control and reference samples.

- Total and unionized ammonia (mg/L) on each day interstitial ammonia is measured during purging

- Total and unionized ammonia (mg/L) on any additional days during the test, if proposed or required in the ammonia monitoring plan

4. Purging methods and test initiation

The DMMP agencies will use best professional judgment for those projects where purging may be indicated. Currently there are a variety of approaches used by regulatory agencies, project proponents and laboratories to purge samples, measure interstitial ammonia and initiate toxicity tests. In general, if purging is performed, overlying water is replaced 2x per day. Frequency of testing of the water in sacrificial containers may vary but generally occurs every 1-3 days, depending on the length of time purging is likely to occur. Once test sediment has reached the desired interstitial ammonia level, the test may be initiated, and each test sediment must have associated and similarly purged control and reference sediments.

The above describes the general approach. However, should purging be pursued for a project, there are many ways to vary the purging of samples and test initiation for individual samples or batches of samples. The DMMP strategy for any particular project will be to minimize purging to the extent practical and will be based on the **bulk ammonia** values that are provided up front. Below are some potential options for tailoring a project-specific purging regime.

- Set a number of days purging may occur overall

- Set a maximum number of days any sample may receive purging that is not required due to ammonia levels

- Batch groups of samples for test initiation -- this may be based on initial ammonia levels or on actual time taken to reach the desired ammonia level for testing (e.g. for a

group of 10 samples, batch and initiate the first 5 samples that reach the desired ammonia level, then wait and initiate the final 5 samples together – each group having associated purged control and reference sediments)

Laboratories with purging experience can generally estimate, based on initial bulk and total ammonia values, the purging time required to reduce interstitial ammonia levels to no-effects threshold concentrations. Once coordination with the DMMP has occurred and a test strategy has been developed, the labs can **a)** plan for procurement and acclimation of test organisms, **b)** sequence various batches for purging, and **c)** attempt to start toxicity tests, including those for samples that are not purged, at approximately the same time. No deviations from the current standard method of purging specified above are allowed at this time.

5. The DMMP agencies reaffirm the total sulfides no effects threshold of 3.4 mg/L for *Neanthes* testing

Concentrations above 5.5 mg/L are not expected, but should be reported to the DMMO immediately for consultation with the DMMP if encountered, to discuss possible remedies to ameliorate or reduce the potential effects.

REFERENCES

Barton, Justine. 2002. Ammonia and Amphipod Toxicity Testing. 2002 DMMP Clarification Paper. (http://www.nws.usace.army.mil/publicmenu/DOCUMENTS/AmphAmmoniaClar20021.pdf)

Dillon, T.M., D. W. Moore, and A. B. Gibson. 1993. Development of a chronic sublethal bioassay for evaluating contaminated sediment with the marine polychaete worm *Nereis* (*Neanthes*) *arenaceodentata*. Environmental Toxicology and Chemistry. Vol. 12: 589-605.

Fox, David. 1993. The *Neanthes* 20-day Bioassay – Requirements for Ammonia/Sulfides Monitoring and Initial Weight. 1993 PSDDA Clarification Paper (http://www.nws.usace.army.mil/publicmenu/DOCUMENTS/ACF559.pdf)

Kendall, David. 1990. Requirements for analyzing Sediment Conventionals in Reference Areas and for Water Quality in Bioassays. 1990 PSDDA Clarification Paper (http://www.nws.usace.army.mil/publicmenu/DOCUMENTS/bio_qa90.pdf)

PSEP, 1995. Recommended Guidelines for conducting laboratory bioassays on Puget Sound Sediments. Puget Sound Estuary Program July 1995 Revisions. (http://www.psat.wa.gov/Publications/protocols/protocol_pdfs/bioassay.pdf)

Warner, Lauran-Cole. 2001. Reporting Ammonia LC50 data for Larval and Amphipod Bioassays. 2001 DMMP Clarification Paper (http://www.nws.usace.army.mil/publicmenu/DOCUMENTS/Ammonia.pdf)

Figure 1. Scatter Plot of Bulk Ammonia versus Neanthes Growth

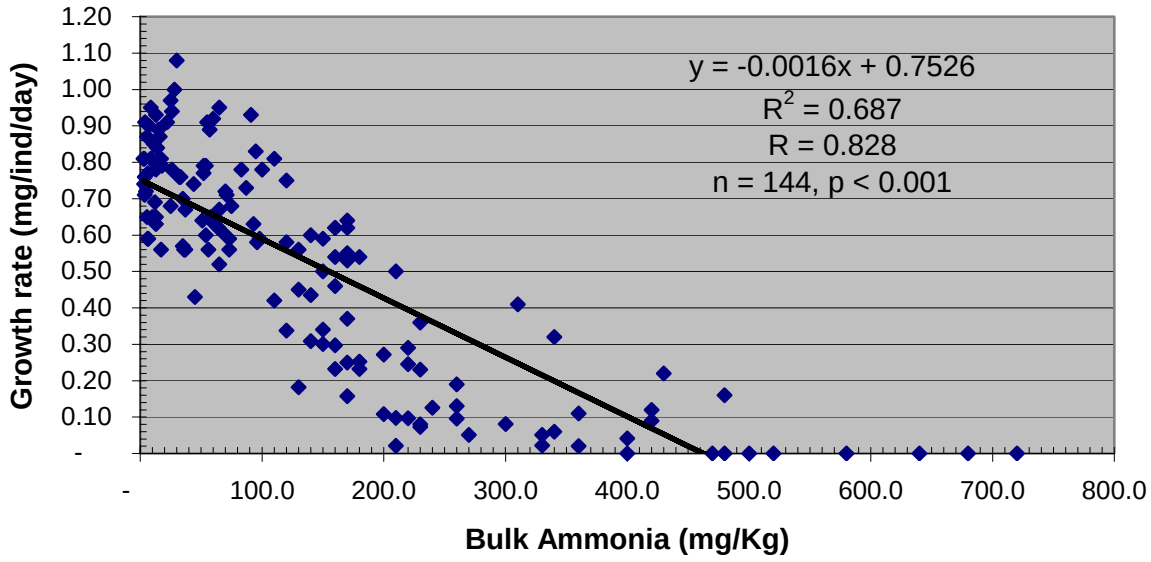
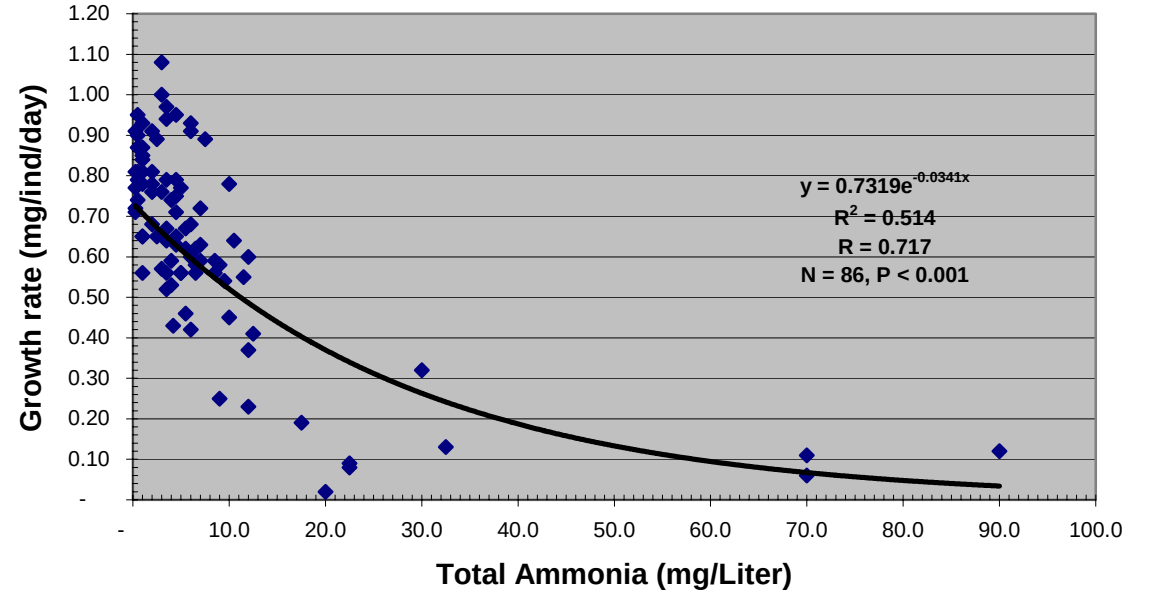


Figure 2. Scatter Plot of Total Ammonia (day 0) versus Neanthes Growth



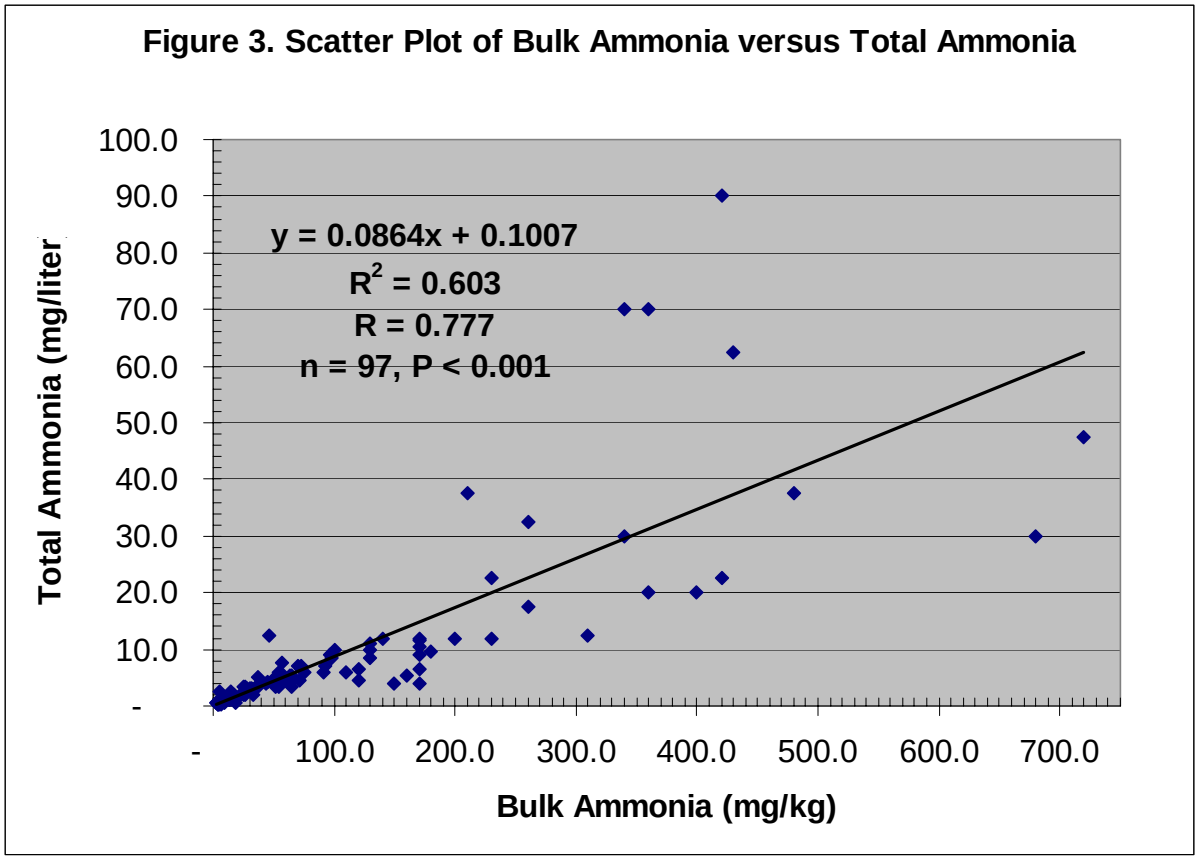


Figure 3. Scatter Plot of Bulk Ammonia versus Total Ammonia
y = 0.0864x + 0.1007
R2 = 0.603
R = 0.777
n = 97, P < 0.001
Total Ammonia (mg/liter
Bulk Ammonia (mg/kg)
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
-
-
100.0
200.0
300.0
400.0
500.0
600.0
700.0