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DMMP Clarification Paper
Modifications to Ammonia and Sulfide Triggers for Purging and Reference Toxicant Testing

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INTRODUCTION

The potential for ammonia and sulfides to complicate bioassay evaluations of dredged material has been addressed in the following DMMP clarification papers:

- DMMP (1993) The *Neanthes* 20-day Bioassay – Requirements for Ammonia/Sulfides Monitoring and Initial Weight,
- DMMP (2001) Reporting Ammonia LC_{50} data for Larval and Amphipod Bioassays,
- DMMP (2002) Ammonia and Amphipod Toxicity Testing, and
- DMMP (2004) Ammonia and Sulfide Guidance Relative to *Neanthes* Growth Bioassay.

PROBLEM IDENTIFICATION

Ammonia and sulfides are potential non-treatment factors that may affect the results of bioassays. Despite the numerous clarification papers addressing these chemicals, there remain data gaps and inconsistencies in the existing guidance that limit the DMMP agencies' ability to adequately interpret the effects of these non-treatment factors or prevent them altogether. Existing deficiencies in the DMMP guidance can be categorized as follows:

Ammonia:

Threshold concentrations that would trigger purging and/or reference toxicant (Ref Tox) testing have been established for the amphipod and *Neanthes* bioassays, but not for the larval test.

Hydrogen Sulfide:

Threshold concentrations that would trigger purging[1] have been established for the *Neanthes* bioassay, but not for the amphipod and larval bioassays.

Predicting Non-treatment Effects:

The DMMP agencies currently rely on the concentration of sulfides and ammonia in bulk sediment samples to predict potential problems in the bioassays due to these chemicals. There are two flaws in this approach. First, the bulk sediment tested for sulfides and ammonia may not be representative of the sediment that will eventually be used for bioassays, due to differences in holding times and conditions. Second, with the exception of ammonia for *Neanthes*, there are no established triggers based on bulk

[1] Ref Tox testing for hydrogen sulfide is not practical due to difficulties in maintaining stable concentrations of this volatile compound during the test.

sediment concentrations. The other established triggers are based on water measurements; comparisons can only be made after ammonia and sulfide measurements are taken at the beginning of the bioassays themselves, at which point it is typically too late to initiate a purging procedure and prevent nontreatment effects from occurring.

Effects Level of Purging Triggers:

There is a discrepancy in the effects levels currently used to trigger purging. For the amphipod bioassay, the purging trigger is set at the no-effects level, while for *Neanthes* it is set at the minor-effects level. If purging is not conducted until the minor-effects level is reached, non-treatment effects can be expected to occur for concentrations above the no-effects level but below the purging trigger. For example, the ammonia trigger for purging in the *Neanthes* test is set at a concentration that could be expected to result in mortality of 20% and a growth reduction of 31-35% relative to the controls (DMMP, 2004). While within-batch Ref Tox tests can provide evidence of toxicity due to ammonia, the length of the Ref Tox test is much shorter than that of the amphipod and *Neanthes* bioassays. Therefore, quantifying the contribution of ammonia to toxicity in these bioassays based on the results of Ref Tox tests is extremely difficult. With respect to sulfides, it is not practical to even run Ref Tox tests, so setting the purging trigger at the minor-effects level is even more problematic.

Interstitial Measurements:

The DMMP water quality monitoring requirements include testing of interstitial samples for ammonia and sulfides in the *Neanthes* and amphipod biosssays. Interstitial testing requires the use of sacrificial beakers because the sediment in the beaker must be "sacrificed" in order to extract the porewater. This is not a problem unless purging is required. In order to monitor interstitial ammonia and sulfides concentrations during purging, a series of sacrificial beakers must be set up so that porewater can be extracted and measured every 1 to 3 days. The additional sediment needed for sacrificial beakers must be collected during project sampling, which means that additional cores may need to be taken to provide adequate volume.

LITERATURE AND DATA REVIEW

In order to evaluate the validity of existing triggers and establish new triggers where missing, ammonia and sulfide toxicity data for standard test organisms were collected from published studies, poster presentations at various toxicological meetings, and reference toxicity studies from laboratories. Data were expressed as endpoints including No Observable Effect Concentrations (NOECs), Lowest Observable Effect Concentrations (LOECs), and the concentration at which 50% of the population was impacted - exhibited as either abnormal development (effective concentration or EC_{50}) or mortality (lethal concentration or LC_{50}). All collected data are presented in Appendix A for ammonia and Appendix B for sulfides.

Aside from the *Neanthes* bioassay, for which there is a single definitive study evaluating the sensitivity of this species to ammonia and sulfide (Dillon, 1993; DMMP, 2004), there was a great

deal of variation in the number of studies, endpoints, and concentrations reported in the literature for the various test species. In some cases, variability in the NOECs and LC/EC_{50}s was high and resulted in overlap in these values within the same species.

DERIVATION OF PURGING TRIGGERS

The DMMP agencies entered into extensive discussions with regard to the effects level and measurement basis to be used in the derivation of purging triggers. After careful deliberation, the agencies elected to set triggers for unionized ammonia and hydrogen sulfide at the lowest NOEC, based on the concentrations of the primary toxic constituents in the overlying water. The following are factors which were considered in making this decision:

- While use of the lowest NOEC to trigger purging could result in this procedure being performed for ammonia/sulfide concentrations that are nontoxic in some cases, allowing non-treatment effects to occur by setting the purging trigger at higher concentrations could result in bioassay data that are rejected for use or difficult to interpret.
- NOEC and LC_{50}/EC_{50} values from various studies sometimes overlap each other within the same species. Only by adopting the lowest NOEC can non-treatment effects be reliably negated.
- The NOEC, LOEC and LC_{50}/EC_{50} values for *Neanthes* and the amphipod species are based on exposures of shorter duration than those used in the DMMP bioassays. Setting the purging triggers at the LOEC or LC_{50}/EC_{50} would likely result in effects levels in the longer-term DMMP tests even higher those predicted from the shorter-term research tests.
- Sulfides and ammonia are often higher in interstitial water as compared to overlying water, and interstitial water represents the most significant exposure medium for non-tube dwelling species (*Eohaustorius*, *Rhepoxynius*). Ammonia and sulfides should therefore be reduced to levels well below toxic levels in the overlying water, to increase the chances that interstitial water concentrations of these compounds will be below thresholds of toxicity.
- Most of the ammonia and sulfide toxicity data compiled in the evaluation were expressed in terms of unionized ammonia and hydrogen sulfide as these represent the predominant toxic forms of these two chemicals.
- It is unknown whether the porewater extraction procedure can alter the concentrations measured, particularly for hydrogen sulfide.
- Collecting adequate sediment volume for sacrificial beakers during purging has been problematic for some projects in the past and can mean collecting additional cores to cover testing contingencies.

PROPOSED CLARIFICATION

Unionized Ammonia and Hydrogen Sulfide Triggers:

The DMMP agencies propose using the lowest available NOEC as a trigger for purging bioassay containers prior to testing. Further, it is proposed that triggers be established for only the most toxic constituents - namely unionized ammonia and hydrogen sulfide - rather than for total

ammonia and total sulfides. For ammonia, half the NOEC is proposed as a trigger for Ref Tox testing. To avoid the need for sacrificial beakers, it is proposed that measurements be required in the overlying water only, rather than both overlying and interstitial water. The new and revised trigger concentrations are presented in Table 1.

Table 1. Ref Tox and Purging Triggers for the various bioassays.

Trigger	Bedded sediment tests				Larval tests	
	Neanthes	*Ampelisca*	*Eohaustorius*	*Rhepoxynius*	Bivalve	Echinoderm
Unionized Ammonia (mg/L) Ref Tox	0.23	0.118	0.4	0.2	0.02	0.007
Unionized Ammonia (mg/L) Purge	0.46	0.236	0.8	0.4	0.04	0.014
Hydrogen Sulfide (mg/L) Purge	3.4	0.0094	0.122	0.099	0.0025	0.01

The proposed triggers are expressed in terms of unionized ammonia and hydrogen sulfide. However, bioassay labs typically are only able to measure total ammonia and total sulfides. Unionized ammonia and hydrogen sulfide concentrations must be derived from measurements of total ammonia and sulfides using test-specific pH, temperature and salinity measurements.

The triggers apply to measurements in overlying water. However, project proponents may also opt to measure interstitial water concentrations in addition to concentrations in the overlying water. If project proponents wish to do so, they should consult with the DMMP agencies prior to initiation of the tests.

Determining the Need for Purging or Ref Tox Testing:

The DMMP agencies recommend determining the need for purging or Ref Tox testing PRIOR to the commencement of actual bioassay testing. This could be done for all test sediments or for only those test sediments that exceed DMMP screening levels. Test beakers are set up and measurements of ammonia/sulfide from the overlying water used to calculate the concentrations of unionized ammonia and hydrogen sulfide. These calculated concentrations are then compared to the trigger values presented in Table 1 to determine the need for purging or Ref Tox testing. Following are details of this procedure:

1. For each test sediment, set up two beakers as they would for the bioassays, but without the bioassay organisms:
    a. One for the larval test
    b. One for the amphipod/*Neanthes* test (larval tests have a different sediment-to-water ratio than the bedded sediment tests).

2. After the standard settling/equilibration time (the next day for the amphipod/*Neanthes* beakers and 4 hours after mixing for the larval test), measure total ammonia, total sulfides, pH, salinity and temperature in the overlying water.
3. Calculate the hydrogen sulfide concentration from the total sulfides and pH measurements and compare to the triggers in Table 1.
4. Calculate the unionized ammonia concentrations from the total ammonia, pH, temperature and salinity and compare to the triggers in Table 1.
5. Consult with the DMMP agencies regarding the need for purging.
6. If a decision is made to purge, work with the DMMP agencies to establish the details of the purging procedure.

Case-by-case Determination to Allow Purging:

The purging process may cause loss of more volatile/less hydrophobic COCs (Ferretti, 2000; Burgess et al 2003) while less volatile compounds with a higher log K_{ow} are expected to remain associated with particles and dissolved organic matter. The DMMP will consider the specific contaminants triggering biological testing in decisions regarding purging. If contaminants may potentially be lost while purging for ammonia or sulfides, then purging may be disallowed or restricted in duration. Also, in some cases, ammonia or sulfides themselves may be contaminants of concern (e.g. wood waste) and purging may not be allowed.

***Purging methods*:**

For bedded sediment toxicity testing, there are a variety of approaches used by regulatory agencies, project proponents and laboratories to purge samples. In general, if purging is performed, overlying water is replaced twice a day. Once all test sediments have reached the desired hydrogen sulfide or unionized ammonia level (labs should use the minimum purging required for overlying water to be at or below the NOEC), the bioassay may be initiated. Each batch of test sediments must have associated and similarly purged control and reference sediments.

For larval tests, purging via water changes may result in loss of colloids/suspended sediments that are a critical part of the sediment evaluation. Thus, purging for larval tests must use aeration only. The DMMP agencies recognize that this may not reduce ammonia concentrations below the NOEC, in which case the ammonia Ref Tox data will be critical in gauging the magnitude of the nontreatment effect from ammonia. Fortunately, the length of the larval Ref Tox test is the same as the test period for the bioassay itself, thereby facilitating the use of the Ref Tox results in decision-making.

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 range of ammonia or sulfide values measured for the test samples. 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 or sulfide levels.
- Batch groups of samples for test initiation -- this may be based on initial ammonia or sulfide levels or on actual time taken to reach the desired ammonia or sulfide level for testing (e.g. for a group of 10 samples, batch and initiate the first 5 samples that reach the desired ammonia or sulfide 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 ammonia or sulfide values, the purging time required to reduce concentrations to no-effects threshold concentrations.
- Run a purging pretest, prior to actual purging for bioassays, to determine the efficacy of purging and estimate the time required for purging.

Standard Reporting of Data:

Reporting must include the following:

- All pre-test ammonia and total sulfides measurements
- pH, temperature and salinity measurements (to be taken concurrently with the ammonia and sulfides measurements)
- calculated unionized ammonia and hydrogen sulfide concentrations
- dates and times of all measurements
- description/equations of how unionized ammonia and/or hydrogen sulfide were calculated
- all water-only ammonia Ref Tox test data (LC_{50}/EC_{50} data, total ammonia, and unionized ammonia)
- Purging procedure and all ammonia and/or sulfide measurements made during purging.
- Day 0 and end of test ammonia and sulfide measurements in all bioassays.

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Kendall, D. and Barton, J. (2004) Ammonia and Sulfide Guidance Relative to *Neanthes* Growth Bioassay. DMMP Clarification Paper.

Appendix A. Ammonia Literature Summary

Literature values for Effects of Ammonia on Bivalve Larval tests (all NH3 mg/L)(all water-only exposures)

Reference	NOEC	EC50	Species	
PSDDA Refinements (1993)	0.1	0.13	*C. gigas*	
Geffard et al. (2002)		0.019	*C. gigas*	This is an EC20 value from unpublished study by author referenced in 2002 paper (and converted from umole to unionized by McDonald (2005)
McDonald (2005)		0.036	*M. gallo*	Ref tox EC20 from same study = 0.028 mg/L NH3; no NOEC given for ref tox.
Phillips et al.(2005)	0.09	0.12	*M. gallo*	Also gives LOEC = 0.152 mg/L
Tang et al. (1997)	0.097	0.231	*M. gallo*	From 1997 SETAC poster abstract PMP107
Nicely (2000)	0.09		*M. gallo*	From SETAC Presentation/Poster Referenced in Phillips et al 2003.
NewFields ref tox data (2013)	0.04	0.063	*M. gallo*	Summary of LC50 data compiled by lab. Provided by Bill Gardiner 3/21/13
Greenstein et al. (1996)	0.06	0.096	*S. purpuratus*	pH 7.7 data from Table 2
Bay et al. (1993)	0.057		*S. purpuratus*	Referenced in Phillips (2005) summary table - Primary ref in book not available to DMMP
Tang et al. (1997)	0.012		*S. purpuratus*	From 1997 SETAC poster abstract PMP107
PSDDA Refinements (1993)	0.04		*S. purpuratus*	
NewFields ref tox data (2013)	0.062	0.07	*S. purpuratus*	Summary of LC50 data compiled by lab. Provided by Bill Gardiner 3/21/13
NewFields ref tox data (2013)	0.023	0.032	*Dendraster*	Summary of LC50 data compiled by lab. Provided by Bill Gardiner 3/21/13
PSDDA Refinements (1993)	0.014	0.03	*Dendraster*	Report recommends NOEC as warning level indicating that additional ammonia monitoring during test is required. EC20 value.

Literature values for Effects of Ammonia for Ampelisca tests (all mg/L)

	NOEC		LC50			
Reference	TAN	NH3	TAN	NH3	Species	
Kohn et al. (1994)	14.6	0.236	49.8	0.83	*Ampelisca*	(Seawater-only exposures)
Burgess et al. (2003)			132	0.76	*Ampelisca*	interstitial water (Sediment exposure)
Burgess et al. (2003)			78	1.54	*Ampelisca*	Overlying water (Sed exposure)
SAIC (1992)			31	1.24	*Ampelisca*	Overlying water (spiked water - sed exposure)
SAIC (1992)			28	0.21	*Ampelisca*	Pore water (spiked water - sed exposure)
SAIC (1992)			28	1.09	*Ampelisca*	Overlying water (spiked sed exposure)
SAIC (1992)			66.5	0.95	*Ampelisca*	Porewater (spiked sed exposure)
MEC (1992)			48.7	0.74	*Ampelisca*	extracted porewater
Kohn et al. (1994)	67.1	1.298	125.5	2.49	*Eohaustorius*	Seawater-only exposures
Kohn (1994)	36.3	0.677	79	1.6	*Rhepoxinius*	Seawater-only exposures

Literature values for Effects of Ammonia for Neanthes tests (all mg/L)

	NOEC		"Adverse effects" (LC/EC20)	
Reference	TAN	NH3	TAN	NH3
Dillon et al. (1993)	10	0.461	20	0.68

Lowest NOEC highlighted in red

Appendix A References

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Appendix B: Sulfide Literature summary

Literature values for Effects of Sulfides on Larval tests (H_2S, ug/L)

Reference	Dose range	NOEC	LOEC	EC50/LC50	Species
Knezovich et al. (1996)	1 to 64	5.0	9.0	10.0	*Mytilus*
Westin (2006)	2.1 to 13.3	2.5	6.3	7.0	*Mytilus* , static renewal average of two test
Knezovich et al. (1996)	1 to 64	10.0	13.0	19.0	*Strongylocentrotus*

Literature values for Effects of Sulfides on Amphipods (H_2S, ug/L)

Reference	Dose range	NOEC	LOEC	EC50/LC50	Species
Knezovich et al. (1996)	32 to 250	99	147	160	*Rhepoxynius*
Knezovich et al. (1996)	35 to 435	122	192	332	*Eohaustorius*
Westin (2006)*	1.4 to 66.4	9.4	22	40.2	*Ampelseca*

Literature values for Effects of Sulfides on *Neanthes* (H_2S, ug/L)

Reference	Dose range	NOEC	LOEC	EC50/LC50
Westin (2006)	0.5 to 123	no effects at any dose		
Dillon et al. (1993)	1400 to 15,000	3400	5500	close to 5500

Notes:
Knezovich et al., 1996 conducted sealed, flow-through, 48-h, water only exposures
Westin 2006 conducted continuous flow, 96-hr exposures for amphipods and *Neanthes* , and 48-h exposures for larval species.
Dillon et al., 1993 conducted static 96-hr, water only exposures

* *Ampelesca* tests repeated in this series. Test 1 data shown; test 2 had LOEC = LC50 which was >55.7 ug/L H2S; this test had poor control survival and high variability

proposed guidance for purging highlighted in blue.

Appendix B References

Dillon TM; Moore DW; Gibson AB. 1993. Development of a chronic sublethal bioassay for evaluating contaminated sediment with the marine polychaete worm *Nereis* (*Neanthes*) *arenaceodentata*. Environ.Toxicol. Chem. 12:589-605.

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