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

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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.

In addition, the DMMP agencies drafted a clarification paper for the 2013 SMARM with guidelines for addressing potential non-treatment effects from ammonia and sulfides. That paper elicited constructive comments from consultants and bioassay labs that resulted in the agencies postponing implementation of the guidelines until more work could be done. Since then, the Corps of Engineers has had additional ammonia and sulfides testing done for four federal navigation projects.

This clarification paper addresses issues raised by commenters in 2013 (Gardiner and Hester, 2013; Caldwell and Thompson, 2013) and reflects the advancement in knowledge gained through testing done by Analytical Resources and Port Gamble Environmental Sciences (now Environ) for the federal navigation projects (DOF/SEE, 2014, 2015a, 2015b; Herrera/NewFields, 2014; PGES, 2014) and research by Northwestern Aquatic Sciences (Caldwell and Irissarri, 2015). Those portions of the 2013 draft clarification paper that did not elicit comments have not been revised. This includes the 'Problem Identification', 'Literature and Data Review' and 'Derivation of Purging Triggers' sections.

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 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 non-treatment 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.

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

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

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. The following are factors which were considered in making this decision:

- 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 than those predicted from the shorter-term research tests.
- 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.
- 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.

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. 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. Unionized ammonia and hydrogen sulfide concentrations must be derived from measurements of total ammonia and sulfides using test-specific pH, temperature and salinity measurements.

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. Following are details of the recommended procedure:

1. Bulk ammonia measurements should continue to be done by the chemistry lab on composited sediment representing each DMMU. For total sulfides, rather than conducting bulk analysis on sediment from a single core prior to compositing, the DMMP agencies recommend analyzing total bulk sulfides on composited sediment. This change will provide a more realistic assessment of the concentration of total sulfides in sediment archived for bioassays. Exceptions to this revised procedure for total sulfides might need to be made for sediment testing performed for both cleanup and DMMP characterization; and for projects where wood waste in new surface material may be an issue. In those cases, total sulfides should continue to be performed on single cores.

2. While bulk measurements made by the analytical laboratory can provide an early warning of potential non-treatment effects in bioassays, these measurements are not always predictive of the ammonia and sulfide concentrations to which bioassay organisms will actually be exposed. Aqueous concentrations measured by the bioassay lab are more meaningful in this regard. For bedded sediment tests using *Neanthes*, *Eohaustorius* and *Rhepoxynius*, porewater is the medium of exposure. For the tube-building amphipod *Ampelisca*, as well as the bivalve and echinoderm species used in the larval development test, the overlying water is the medium of exposure. Therefore, for those DMMUs that will undergo bioassays, ammonia and sulfides need to be measured in the medium of exposure prior to running the bioassays.

This can be accomplished by the bioassay lab for *Neanthes*, *Eohaustorius*, *Rhepoxynius* and *Ampelisca* by setting up a single beaker for each DMMU in the manner that would be done for the amphipod and juvenile infaunal bioassays. 175 ml of sediment are placed in a beaker, with seawater added to bring the total volume up to 950 ml. The beaker is aerated and allowed to equilibrate for 24 hours. Total ammonia, total sulfides, pH, temperature and salinity are then measured in the porewater (for *Neanthes*, *Eohaustorius* and *Rhepoxynius*) and the overlying water (if *Ampelisca* is used).

For the larval test, a single beaker for each DMMU is set up as it would be for the bioassay. 18 ml of sediment are placed in a beaker along with 900 ml of seawater. The sediment is suspended by shaking vigorously for 10 seconds and then allowed to settle for 4 hours. Total ammonia, total sulfides, pH, temperature and salinity are then measured in the overlying water.

During bioassay testing, temperature and salinity are maintained within standard ranges. In contrast, pH is monitored but not adjusted. Using the temperature and salinity that will be maintained during each of the bioassays, plus the pH measured in the overlying water and porewater, calculate the unionized ammonia and hydrogen sulfide concentrations.

3. If unionized ammonia and hydrogen sulfide concentrations in the interstitial water are below the purging triggers in Table 1, or if any of the chemicals of concern exceeding SL are subject to significant loss or alteration of bioavailability during purging (to be determined in consultation with the DMMP agencies), set up the bioassays normally, without sacrificial beakers or purging. Run the ammonia reference toxicant test concurrently with a bioassay if the Ref Tox trigger is exceeded for the test organism being used.

4. If a purging trigger is exceeded for the species being used – and contaminant loss or alteration of bioavailability due to purging has been determined not to be a significant issue – prepare for purging.

Purging methods:

For sediment toxicity testing, there are a variety of approaches used by regulatory agencies, project proponents and laboratories to purge samples. Purging is most often performed either by replacing overlying water twice a day plus continuous aeration, or by aeration alone. Once the unionized ammonia and/or hydrogen sulfide concentrations are below the trigger levels in Table 1 for all test samples (labs should use the minimum purging required to bring concentrations below the NOEC), the bioassay may be initiated. Each batch of test sediments must have associated and similarly purged control and reference sediments.

For *Neanthes*, *Eohaustorius* and *Rhepoxynius*, the bioassay is set up with a sufficient number of sacrificial beakers to monitor ammonia/sulfides in interstitial water during purging. Ammonia/sulfides are also monitored in the overlying water. For *Ampelisca*, the bioassay may be set up without sacrificial beakers and ammonia/sulfides monitored in only the overlying water during purging.

For the larval test, purging via water exchanges after bioassay setup may result in loss of colloids/suspended sediments that are a critical part of the sediment evaluation. Thus, if purging is to be conducted for the larval test using water replacements, it must be conducted prior to test beaker setup. This can be accomplished by placing enough material for bioassay setup (five test beakers plus a water-quality beaker) into a single "combined" beaker and purging that beaker. Ammonia/sulfides are measured in the overlying water of the combined beaker. Purging by water replacement resulted in a loss of only 0.04% of the total wet weight of sediment used in a purging experiment performed for a federal navigation project (Herrera/Newfields, 2014). The DMMP agencies consider this a de minimis loss. At the end of the purging period, the sediment from the single combined beaker would be distributed to the individual test and water quality beakers and the larval test would commence.

If purging for the larval test is conducted by aeration alone, the test and water quality beakers are set up as they would be for the bioassay, but without the test organisms being introduced. Aeration is applied until the ammonia/sulfides concentrations in the overlying water fall below the NOEC.

Ammonia and sulfides can continue to be generated in sediment during the bioassays themselves. Therefore, if the water replacement method is used for purging, water exchanges may need to continue during the bioassay. This may be done for bedded sediment bioassays in which exposure to porewater is a critical factor. This includes the *Neanthes* test, and the amphipod test using *Eohaustorius* or *Rhepoxynius*. *Ampelisca* would only require continued water exchanges if concentrations of ammonia or sulfides in the overlying water rise above the NOEC. Continued water exchanges are not possible for the larval test. Due to the small volume of sediment used for this bioassay, aeration alone will typically be sufficient to maintain ammonia/sulfides concentrations below the NOEC.

The above describes the general approach. However, should purging be pursued for a project, there are many ways to execute the details of the purging protocol. The DMMP policy is to minimize purging to the extent practical. Purging time will be based on the range of ammonia or sulfide values measured for the test samples. Laboratories with purging experience can generally estimate, based on initial interstitial ammonia or sulfide values, the purging time required to reduce concentrations below the NOEC.

Standard Reporting of Data:

Reporting must include the following:

- All interstitial and overlying ammonia and total sulfides measurements made during pre-tests, purging and bioassay testing
- pH, temperature and salinity measurements (to be taken concurrently with all ammonia and sulfides measurements)
- calculated unionized ammonia and hydrogen sulfide concentrations for all measurements made during pre-tests, purging and bioassay testing
- dates and times of all measurements
- equations used to calculate unionized ammonia and/or hydrogen sulfide
- all ammonia Ref Tox test data
- a detailed description 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. In addition, metals bioavailability and toxicity can be influenced by purging. In order to better understand the potential for contaminant loss from the purging process due to volatilization, Seattle District conducted pre- and post-purging chemical analysis (Herrera/NewFields, 2014) for the water replacement and aeration-only methods for the larval test. There was no systematic loss of contaminants apparent in either method. While this limited testing provided evidence that contaminant loss due to volatilization may not be an issue for the purging methods described in this paper, the DMMP agencies will continue to consider the specific contaminants triggering biological testing in decisions regarding purging. If contaminants may potentially be lost or their toxicity altered 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. new surface material containing wood waste) and purging may not be allowed. Purging is also not allowed for cleanup evaluations. For projects that include both cleanup and DMMP evaluation, side-by-side testing of both purged and non-purged sediments may be required.

Applicability of these Recommendations:

The intent of these recommendations is to reduce the incidence of non-treatment effects from ammonia and sulfides in DMMP bioassays and to generate supplemental data to facilitate interpretation of bioassay results when non-treatment effects cannot be totally eliminated. While not required, the dredging proponent assumes the risk of dredged material being found unsuitable for open-water disposal if potential effects of ammonia and sulfides are not proactively addressed. Proactively addressing ammonia and sulfides requires advanced planning. Sufficient volumes of sediment must be collected for sacrificial beakers; the pretesting and purging procedures must be included in the sampling and analysis plan; and holding times must be considered. The dredging proponent will need to balance the cost of these procedures against the cost of upland disposal of dredged material that fails toxicity testing due to non-treatment effects from ammonia/sulfides.

Ammonia and sulfides are more likely to be present in deeper sediments or sediments containing a significant fraction of organic material such as wood waste. Therefore, the type of sediment being tested will need to be assessed to determine the likelihood for elevated ammonia and sulfides. Initial bulk ammonia and sulfides testing by the analytical lab will also provide valuable information in this regard.

Alternative procedures from those discussed in this paper may be proposed on a project-specific basis. Justification for the selected procedures must be clearly articulated in the sampling and analysis plan.

Coordination with the DMMP Agencies:

It is critical that close coordination with the DMMP agencies be maintained throughout the process, from development of the pre-bioassay testing procedures in the sampling and analysis

plan, to decision-making about purging and details of the purging procedure itself. All procedures must be approved by the agencies before the procedures may be performed.

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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

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