Charge to the Peer Reviewers of "Sediment Toxicity Identification (TIE) Phases I, II and III Guidance Document"

This document was developed by EPA's Atlantic Ecology Division and Mid-Continent Ecology Division. Its objective is to provide guidance on the performance of sediment TIE for both interstitial water and whole sediments in marine and freshwater environments.

Background:

Sediment contamination in the United States has been amply documented and, in order to comply with the 1972 Clean Water Act, the U.S. Environmental Protection Agency must address the issue of toxic sediments. Contaminated sediments from a number of freshwater and marine sites have demonstrated acute and/or chronic toxicity to a variety of test species, as well as adverse ecological effects such as population declines and changes in community structure. However, simply knowing that a sediment is toxic has limited use. This document provides guidance on the performance of sediment Toxicity Identification and Evaluation (TIE). TIE methods allow for the identification of toxic chemicals or chemical classes causing observed toxicity. The identification of pollutants responsible for toxicity of contaminated sediments has a broad application in a number of EPA programs as the methods can be used within a total maximum daily load (TMDL) framework, to link sediment toxicity to specific dischargers, to design cost-effective remediation programs, and to identify environmentally protective options for dredged material disposal. In addition, the identification of specific problem contaminants in sediments could prove to be very useful to EPA programs involved in the development of water or sediment guidelines, and the registration of new products such as pesticides. Finally, knowledge of the causes of toxicity that influence ecological changes such as community structure would be useful in performing ecological risk assessments not only for the Agency but also for the scientific community as a whole.

Review of:

"Sediment Toxicity Identification Evaluation (TIE): Phases I, II, and III'

Edited by Kay Ho, Robert Burgess, Dave Mount, Teresa Norberg-King, and Russell Hockett

Note: This review covers Sections 6 – 10 only.

Charge:

In your review, please provide written responses to the following questions. Additional comments and recommendations for improving the document are welcome.

Overall questions:

1) Are the concepts and assumptions laid out in the document sufficiently developed and clearly articulated? If you identify deficiencies, please recommend ways to remedy them.

General Comments:
This document provides sediment TIE methods for interstitial waters and whole sediments. The document is well written (except where noted), well organized and easy to follow. The report includes very useful discussions of the implications of TIE results using the various methods, and provides illustrations from the years of EPA experience developing these procedures.

The report formatting is not consistent, I'm assuming because the various sections were written separately. In addition, data tables and figures more often than not do not include citations.

One obvious impression is that this document would be more comprehensive if it integrated some of the results and conclusions of the recent evaluation of sediment TIE methods conducted for the Water Environment Research Foundation. The WERF results are particularly relevant for Phase II solid-phase procedures. Where appropriate, I have pointed out where results of the WERF research might augment information presented in the current report. The final draft of the WERF report will then reference this EPA guidance document where appropriate. The combination of this guidance document with methods evaluated and developed for the WERF project should be very useful for those conducting sediment TIEs.

My only other general comment is that this guidance document would benefit if it also included more examples of TIE methods with west coast marine species.

Specific Comments for each section are provided below.

2) Are the scientific bases for the manipulations conceptually sound/valid?

The scientific basis for the manipulations are conceptually sound and valid.

3) Are the methods and logic clearly explained and scientifically justified? Please indicate any modifications that would improve upon the methodology.

The methods are clearly explained and scientifically justified, except where noted below.

Sections 1-5: *Introduction; Health and Safety; Quality Assurance; Equipment, Supplies and Facilities; Statistical Methods*

These sections were not reviewed.

Do these series of brief sections provide an acceptable opening to the document and provide the reader with sufficient preliminary information for understanding the material that follows? What specific additions or deletions to this section would you suggest?

Section 6: *Designing the TIE approach*

Does this section describe the differences between interstitial and whole sediment TIEs and contain logic for which approach to use, and how the approaches can be combined to help the researcher identify the cause of toxicity?

Is this section internally consistent with the other sections?

See comments below:

Page 13 (6.2.1): Note inconsistencies with formatting between this and later sections.

Table 6-1. A reference citation is needed for this and all subsequent tables that include data. If the data are un-published EPA data, this needs to be stated. Also, there is no control response provided in this table.

Page 14: I suggest that after the bullet describing differences in degree of IW exposure, the authors include an additional bullet describing logistical constraints on working with IW (e.g., problems obtaining adequate volumes). I know this is discussed elsewhere, but this is the appropriate place.

Page 15 ¶1. This section needs some references to support the points (sentences #1 and #2). Section 6.2.2, ¶2 and ¶3 : both these paragraphs have awkward wording. (e.g., ¶2 sentence #1; ¶3 sentence # 1 and #2). In fact, this whole section could benefit from editing for awkward sentences.

Page 16, ¶3. Delete this ¶, it's redundant.

Section 6.3. ¶1. Topic sentence is awkward.

Page 17, ¶2. I suggest this include the west coast amphipod Eohaustorius estuarius, this species has been used in both solid-phase and IW TIEs. The amphipod *H. azteca* has also been used in both solid-phase and IW TIEs.

The citation for this is: Anderson, BS, Hunt, JW, Phillips, BM, Tjeerdema, RS. 2006. Navigating the TMDL process: sediment toxicity. (Draft) Final Technical Report. Water Environment Research Foundation (WERF), Alexandria, VA. 74 pp, with Appendices.

Additional suggested citations for IW TIEs:

1. Science Applications International Corporation (SAIC). 2003. Guide for planning and conducting sediment pore water toxicity identification evaluations (TIE) to determine

causes of acute toxicity at navy aquatic sites. User's Guide UG-2052-ENV. Naval Facilities Engineering Service Center, Port Hueneme, CA, USA. Prepared by Science Applications International Corporation, Newport, RI, USA.

2. Norberg-King, TJ, Ausley, LW, Burton, DT, Goodfellow, WL, Miller, JL, Waller, WT (eds). 2001. Toxicity Reduction and Toxicity Identification Evaluations for Effluents, Ambient Waters, and Other Aqueous Media. SETAC Press. Pp 93-113.

Page 18, Table 6-2. Where are the footnote citations for the methods cited in this table? Also, I suggest adding plus marks for all test media and TIEs after the amphipod *Eohaustorius estuarius* (note spelling), and cite the WERF report.

Table 6-2 (cont.): After purple sea urchin (S. purpuratus). Put a plus in column for whole sediment testing and cite: Anderson, B.S., Hunt, J.W., Hester, M. and Phillips, B.M. 1996. Assessment of sediment toxicity at the sediment-water interface. In: G.K. Ostrander (ed.) *Techniques in Aquatic Toxicology*. Lewis Publishers, Ann Arbor, MI.

Also here, put a plus in column for IW TIEs and cite: Hunt, J.W., B.S. Anderson, B.M. Phillips, R. Tjeerdema, K.M. Taberski, C. J. Wilson, H. M. Puckett, M. Stephenson, R. Fairey, J. Oakden . 2001. A large-scale categorization of sites in San Francisco Bay, USA based on the sediment quality triad, toxicity identification evaluations, and gradient studies. Environ Toxicol Chem. 20: 1252-1265.

Insert *Mytilus galloprovincialis* after purple urchin. Put a plus in whole sediment testing column and Whole sediment TIEs and cite: Phillips, BM, Anderson, BS, Hunt, JW, Thompson, B, Lowe, S, Hoenicke, R, Tjeerdema, RS. 2003. Causes of sediment toxicity to *Mytilus galloprovincialis* in San Francisco Bay, California. Arch. Environ Contam. Toxicol. 45: 486-491.

Section 7: *Phase I Overview and Methods: Whole Sediments*

Does this section clearly explain the Phase I methods we have developed for whole sediments?

Is this section internally consistent with the other sections?

Are there other methods that should be referenced in this section?

See comments below:

Page 21, section 7.1. Suggest adding west coast species from Table 6-2 here, particularly mussel *M. galloprovincialis* and amphipod *E. estuarius*.

Page 22, ¶3, note that marine TIEs using *E. estuarius* are sometimes 10-d tests (static), per WERF citation.

Page 25, 7.1.3. The statement that standard (common) solid-phase tests use 8 replicates seems to apply only to freshwater tests (e.g., *H. azteca*, right?). This may be an appropriate place for me to point out that because this document was apparently written by several folks, some of whom are freshwater-centric, while others are seawater-centric, there is some disconnect between the two, especially in terms of citations and examples provided. I suggest having the marine people read the freshwater sections, and visa versa to make sure the sections are more comprehensive.

Page 26, Section 7.2. Do you want to add zero valent Mg to the list of bullets per Fig 7-1? (note the discussion of this method later on is a little tepid in terms of it's advocacy).

Page 28, ¶2. We used both formulated sediment (with peat as carbon source), and amphipod home sediment for diluting sediment in the WERF project report.

Page 29, ¶5. All examples here are for freshwater tests, suggest also using examples from saltwater tests in this discussion.

Page 31, Figure 7-2. There is no (apparent) citation for this and most of the other figures and tables showing example data in this report.

Page 41, since Ambersorb 563 and coconut charcoal were both used to some success in the previously discussed (*ad nauseum*?) WERF report, this would be a good place to cite it, once again.

Page 42, ¶2. Include a citation for the DDT example given. One other point to consider in the discussion of the utility of sediment dilution in the TIE process is that dilution may also lead researchers to overlook chemicals that are marginally toxic in 100% sample, but below toxicity thresholds in lower dilutions. This leads to emphasis on only the most toxic constituents of the sample.

Page 44, ¶3. Here's another chance for a WERF report citation. This project used coconut charcoal to varying degrees of success in freshwater and marine sediments spiked with fluoranthene, nonylphenol, and tetrachlorobenzene, as well as freshwater and marine field sediments.

Page 46, note that Ambersorb 563 (and others?) is no longer available from the manufacturer. There are alternative XAD-type resins available.

Page 48, ¶1. Cite WERF for Ambersorb 563 tests with marine and freshwater sediment spiked with fluoranthene, nonylphenol, and tetrachlorobenzene, as well as freshwater and marine field sediments.

Page 48, ¶3 (7.2.6). Rather than calling this section "Sand dilution blank", I suggest calling it "whole sediment dilution blank" and consider examples for home sediment, and formulated sediment, as well as sand.

Section 8: *Phase I Overview Methods Interstitial Waters*

> Does this section clearly explain the Phase I methods we have developed for interstitial waters?
>
> Is this section internally consistent with the other sections?
>
> Are there other methods that should be referenced in this section?

See comments below:

Page 50, Bullets: Why not include a cation SPE column in the list of IW methods? Also, why not include sequential column treatments to separate effects of cations and nonpolar organics?

Fig 8-1. I suggest that rather than limiting SPE for nonpolars to C18 column, just say SPE, since other columns have also been used successfully (e.g., OASIS HLB).

Page 52, ¶1. In WERF study we conducted IW tests with amphipods *H. azteca* (5 animals per rep) and *E. estuarius* (1 animal per rep) using small volume 20 ml glass scint vials (10ml).

¶2: Plastic containers might be appropriate for comparison to glass in situations where metals are suspected.

Page 58, ¶1. As above, suggest mentioning that other SPE columns have been used successfully in IW tests. OASIS-HLB used in WERF study.

¶4. I question the statement that elution of the SPE may not provide useful Phase I information if the column does not reduce toxicity. We found that in some cases, the column did not reduce toxicity, even in situations where solvent elutions of the column returned significant toxicity. This may be due to contaminants overwhelming the capacity of the column (breakthrough). Also, there is evidence to suggest that in IWs containing high concentrations of DOM, highly hydrophobic chemicals may largely pass through the column, giving the

impression that nonpolars are not in the sample. Experimental evidence from our lab and USGS suggests this may happen in IWs contaminated by pyrethroid pesticides.

Page 59 ¶1, delete reference to effluent testing?

Page 61, ¶1. Suggest also citing WERF report regarding for inconsistent elution of organics from OASIS-HLB column using methanol.

Page 62 . Suggest discussing utility of cation column for metals after section 8.3.4.

Page 63. Table 8-3. Some of the info here seems a little incongruous. Why not delete info for species not normally used in IW tests (fish?). Also, since this table is presented in the context of the pH test, why not include info on H2S from the Knezovitch citation and any other info on freshwater and east coast species? Include info on H2S and NH3 toxicity to H. azteca. Info on Black Rock Harbor tests is not necessary.

Section 9: *Phase II Sediment TIE Methods*

> Does this section clearly explain the Phase II methods we have developed for whole sediments and interstitial waters?
>
> Does the section explain how procedures performed in the different manipulations can be supportive of the identification of the toxicant?
>
> Is this section internally consistent with the other sections?
>
> Are there other methods that should be referenced in this section?

See Comments Below:

Page 76, section 9.2. Again, why not include the cation column in this discussion.

Page 78. Has the zero valent Mg method been published? Give the citation.

Page 82, ¶3 Section 9.2.6. Also suggest citing: Phillips et al 2003, as an example where the cation column was eluted with different concentrations of acid to separate specific metals.

Phillips, BM, Anderson, BS, Hunt, JW, Thompson, B, Lowe, S, Hoenicke, R, Tjeerdema, RS. 2003. Causes of sediment toxicity to *Mytilus galloprovincialis* in San Francisco Bay, California. Arch. Environ Contam. Toxicol. 45: 486-491.

Cation column elution with acid was also evaluated in the WERF study using both freshwater and marine IWs spiked with copper, and in ambient sample TIEs.

Page 83, section 9.2.7. Elution of cation resin with acid in solid-phase TIEs was evaluated in the WERF report. One limitation of this method discussed in this report was that using marine tests of formulated sediments spiked with high concentrations of copper, elution of the cation resin with acid showed inconsistent results in tests with the amphipod *E. estuarius*.

Page 84, final ¶. What is the date of the Heinis et al. citation?

Page 88, end of section 9.3.1. Suggest consideration of alternative Phase II solid-phase TIEs here. For example, in the WERF study, we isolated Ambersorb 563 from the sediment via sieving then eluted it using acetone. Acetone eluates were toxic to freshwater and marine amphipods. In addition, the acetone eluates were either analyzed directly using GC-MS, or subhected to HPLC fractionation and the fractions were then analyzed. The results showed that we could successfully elute nonpolar organics from Ambersorb, and measure organic chemicals in the eluate. This proved to be a useful Phase II tool, and should be discussed as an additional method to the SPMD method described here.

Note: in the first draft of the WERF report, chemical analyses of the acetone eluates and HPLC fractions often did not contain detectable concentrations of chemicals. In the interim, the chemists involved in this project used direct injection of the acetone and HPLC fractions to show that the eluates contained hydrophobic constituents, often pyrethroid pesticides. These results will be included in the final draft of the WERF report.

Page 80, Section 9.3.2. Rather than titling this section: "C18 SPE …", why not title it SPE in general, then discuss methods using different columns (e.g., C18 and OASIS-HLB)?

Page 93. end of section 9.3. Why not discuss use of IW chemistry here as an important Phase II tool?

Page 97, last ¶. As discussed above, I suggest discussing here the possibility of missing marginally toxic chemicals when you dilute samples to the point where the Phase I methods are effective at reducing toxicity. One other point that should be considered is that more research needs to be conducted to investigate the influence of longer equilibration times using coconut charcoal and carbonaceous and cation resins. It may be these treatments will work better with highly toxic sediment when longer equilibration times are employed, and this could be reduce the need for sediment dilution.

For IW tests, higher capacity SPE and cation columns might also be considered as an alternative to sediment dilution.

I suggest considering methods that combine treatments to remove metals and organics, in sequence. This could include mixtures of cation and carbonaceous resins (also with zeolite). Or, in the case of IW TIEs, sequential treatment of samples with cation SPE, then C18 or HLB SPE for nonpolar organics, to resolve toxicity due to mixtures of metals and organics.

Consider citing: Hunt JW, Anderson BS, Phillips BM. 2005. Toxicity identification evaluation of solid-phase sediment from San Francisco Bay, California, USA. Case Study 6.27. In: Norberg-King T, Ausley L, Burton D, Goodfellow W, Miller J, Waller WT (eds.) *Toxicity Reduction Evaluations and Toxicity Identification Evaluation (TIE) for Effluent, Ambient Waters and Other Aqueous Media*. SETAC Press, Pensacola, FL. Pp 319-324.

This case study describes a situation where none of the standard sediment TIE procedures were effective at removing toxicity. The only procedure that reduced toxicity of solid-phase sediment was a weak-acid-acid leach of the sediment (using 0.5 N HCl). The results implicated some acid-soluble contaminant, possibly metals.

Page 102, Section 9.9. Phase II summary.

Under this section, I would add the additional Phase II treatments discussed in the WERF report, including temperature manipulations and PBO additions for identifying toxicity due to pyrethroids (in addition to the Wheelock et al. citation for the esterase enzyme).

Section 10: *Phase III Sediment TIE Methods*

> Does this section clearly explain the Phase III methods we have developed for whole sediments and interstitial waters?
>
> Does the section explain how procedures performed in the different manipulations can be supportive of the final identification of the toxicant?
>
> Is this section internally consistent with the other sections?
>
> Are there other methods that should be referenced in this section?

See Comments Below:

Page 105. Section 10.2. Solid-phase vs IW testing.

Since both IW and solid-phase TIEs provide valuable information, I suggest emphasizing use of both procedures here in a weight-of-evidence approach.

End of Comments.

Please provide your written comments to me no later than **September 25, 2006.** Comments may be sent by regular mail to the address below, by Fax, or by e-mail to houk.virginia@epa.gov.

If you have any questions concerning the draft document or the charge, please contact me at 919.541.2815 or houk.virginia@epa.gov. We sincerely thank you for your input to our peer review process.

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