Dear Ms. Houk,

I managed to review, as requested, chapters 6, 7 and 8 of the EPA guidance document on Sediment TIE.

First of all, please express my compliments to Kay Ho and Rob Burgess and their coworkers for this excellent piece of work.

This also sets the tone for my review: I have no major comments, only minor comments/suggestions for further improvement of a document that is already of impressive quality. Hereby I followed, as also requested, the review questions provided by you. Please find my suggestions below.

Overall questions:
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1) Concepts and assumptions OK?

a. "Index response" (section 6.2.2., p15): for me this concept is still a bit vague. Maybe it can be explained a little bit better: what does it mean, what is its value? Maybe an example could be given?

b. "Coconut charcoal" (section 7.2.5.1, p42): I do not agree to the assumption that a decreased biomass due to the addition of charcoal indicates toxicity. I would rather like to put forward the hypothesis that charcoal sorbs nutrients. Thus reduced nutrient bioavailability correlates to biomass reduction. This is just a physical effect. Hence, in my opinion, it has nothing to do with toxicity. In fact, my hypothesis is supported by field observations. Dr. Richard G. Luthy (Stanford University) conducted/conducts field tests at a tidal mudflat adjacent to a former shipyard in San Francisco in which activated carbon is mixed into the upper sediment layer to stabilize PCBs (see a.o.: http://www.hsrc-ssw.org/bratislava/presentations/luthy/luthy-abstract.html). I asked him at this NATO sediment workshop in Bratislava (see web-link) if he found as 'side-effect' of this in-situ stabilization of PCBs in sediment a reduced biomass due to binding of nutrients to carbon. Indeed he did and he agreed that it may be caused by reduced nutrient availability.

c. Maybe the same argumentation holds true for Ambersorb resin (p47, figure 7-7, and text just below that figure)?

2) Sound conceptual, scientific basis for manipulations?

a. Yes basis is OK, no further comments.

3) Methods & logic clearly explained and scientifically OK?

a. Yes OK. The only suggestions I could think of making here is that there is considerable repetition/duplication in explaining the TIE manipulations if you read Ch. 7 (whole sediment) and Ch. 8 (IW) after each other. However, I see the logic to do it in this way. Most users will probably only read the guidance in either Ch. 7 or Ch. 8 as they probably will already have made up their mind if they want to go for whole sediment or IW TIE. So probably best to leave it as it is. However, if you want to change this (but not really necessary), you could think of moving 'generic descriptions/guidance' (i.e. what works for whole sediment as well as IW) to an Annex and then only describe in Ch. 7 and 8 what is really specific to/for the matrix tested.

Specific questions/remarks to single chapters:

Ch. 6) Designing TIE:

p13. Top page "water only": water, especially IW, will contain Suspended Particulate Matter (SPM) so it can not be considered as water only testing, unless you only use filtered water

p13. Bottom page, last bullet "naturally anoxic": IW is not completely anoxic, in fact in the field there is a sharp, almost sigmoid decrease in oxygen over a few mm's (from sediment surface to deeper in sediment layer). Furthermore, several benthic species borrow tunnels in the sediment (e.g. midge larvae and oligochaetes) and hence create a layer with a relatively higher oxygen content surrounding them.

p15. Top page "…collection, manipulation and testing COULD affect …": I think it should state WILL affect

p16. Top page "weight of evidence": does WoE not intrinsically conflict with the whole idea of TIE? TIE aims for identifying actual causes of toxicity rather then building WoE. However, I agree that for true causality one should follow al the way up till phase III TIE, where often WoE based on Phase I and II maybe enough for decision making.

p17. Middle of page "*Corophium volutar*": should be *Corophium volutator*.

p18. Table 6-2: I miss the references (e.g. below the table) to the cited methods (column 4 in Table)

p19. Table 6-2: I myself conducted several Whole sediment TIEs with *C. volutator*. Kay Ho pre-reviewed some of that work done. However, I still have to finalize that ET&C manuscript.

p19. Table 6-2: quite some TIE work (whole sediment as well as IW) done with Microtox (*Vibrio fischeri*), a.o. by myself

p20. Middle of page: *Ulva* may remove ammonia from the test matrix, but zeolite does not: it sorbs the ammonia, but it will stay in the matrix (sorbed to zeolite)

Ch. 7) Phase I Whole sediments:

p21. Just below middle of page "… clean water for renewal": why clean? Is it also not an option to use the in-situ sampled water for renewal?

p22. Top page "… lower than those expected at equilibrium, ": can you also indicate why this is the case?

p22. Top page "…type of exposure the test organism has MAY affect …": should state WILL affect

p23. Section 7.1.2.: addition of a picture/graphical representation of the test system would be nice

p24. Middle of page "YCT" is this readily available? (maybe yes in US, but maybe not in EU)

p26. Middle of page "non-ionic organics": I have also good experience in using Tenax as sorbens in whole sediment TIE (*C. volutator* and Microtox) with oil contaminated sediment (field and spiked sediment) . Kay Ho pre-reviewed some of that work done. However, I still have to finalize that ET&C manuscript.

p27. Section 7.2.1. ".. diluting the sediment before phase I ..": can you address in a few words how you do that

p29. Middle of the page "…large dynamic range": that is?

p45. Fig. 7-5: Y-axis: explain what you mean by performance, i.e. what is the endpoint?

p49. Last paragraph: could you discuss a little bit why the sand dilution blank rarely shows a substantive change in toxicity?

Ch. 8) Phase I IW:

p50. Middle of page "*Ulva* addition": should it not state "*Ulva* or Zeolite addition"? In fact probably yes as you also do that in Table 8-1 (p53).

p51. Last paragraph before section 8.1: LC50 is a lethality endpoint indeed, but lethality as endpoint is not only and always LC50. Maybe it would be valuable to discuss here when and where it is recommendable to use LC50 (so test a dilution series) in comparison to maybe an increased No. of replicates of only one test concentration.

p52. Section 8.1.1. there are also IW TIE experiences with Microtox and even Algae species

p58. Middle of the page "..at 10,000 x g": should it not be 10,000 r.p.m.?

p63. Top page "…than pH 6 (Table 8-3)": I guess it should refer to Table 8-4?

p63. Relative sensitivity …: what doe you mean by that? Are LC50s indicated in the table?

p64. Table 8-4: I miss the units to the LC50 (?/L)

p65. Equation: some work needs still to be done to bring it to 'equilibrium'

p66. Bottom page "Safety note": a bit strange to come at once with such a note. Then you could do it at more places: working with (extremely) contaminated sediments, dangerous chemicals etc.

p68. Top of page: be consistent in either using HCl and NaOH (preferred) or hydrochloric acid and sodium hydroxide (at several places in this and the next section)