San Francisco Estuary Institute
7770 Pardee Lane, 2nd Floor • Oakland, CA 94621-1424
Office (510) 746-SFEI (7334) • Fax (510) 746-7300

RMP Sediment Toxicity Stressor Identification Workshop

Meeting Summary Notes

April 7th, 2010
San Francisco Estuary Institute
First Floor Conference Room
7770 Pardee Lane, Oakland
9:00 am-3:30 pm

Meeting Participants:

Name	Affiliation
Michael Kellogg	CCSF-OBL
Patrick Conroy	CCSF-OBL
Rebeccah Schermesser	EOA
Kay Ho	EPA Naragansett
Rob Burgess	EPA Naragansett
Steve Clark	Pacific EcoRisk

Name	Affiliation
Karen Taberski	RWQCB
Keith Maruya	SCCWRP
Steve Bay	SCCWRP
Aroon Melwani	SFEI
Sarah Lowe	SFEI
Chris Beegan	SWRCB
Stephanie Fong	SWRCB

Name	Affiliation
Chris Vulpe	UCB
Brian Anderson	UCD-MPSL
Bryn Phillips	UCD-MPSL
Kelly Smalling	USGS
Rachel Allen	SFEI
Jay Davis	SFEI
Darrin Greenstein	SCCWRP

Power Point presentations were given in the morning and then there was a group discussion in the afternoon.

Introductions, Review of Agenda, and Workshop Goals. Brian Anderson

Background:

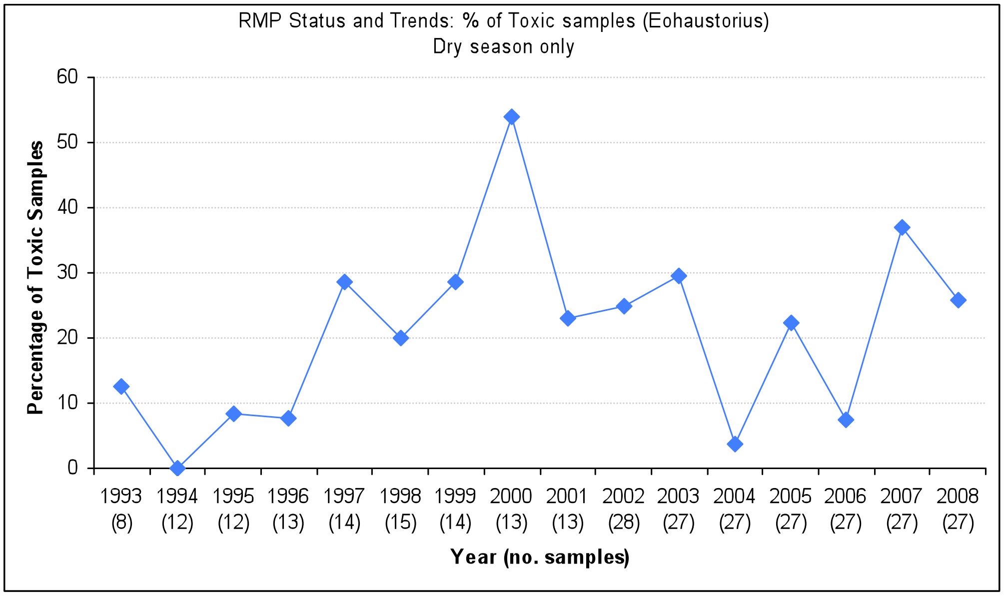
"What's killing amphipods in lab tox tests?"*Eohaustorius estuarius* (EOHA) is the 'benchmark' species for state Sediment Quality Objectives (SQO)
Previous data shows consistent moderate toxicity to this species in SF-Bay.

Why is the RMP using this species?

Rigorous comparison with other toxicity test species was conducted early in the Bay Protection and Toxic Cleanup Program and the early RMP. EOHA was the best performing laboratory test species. The RMP also uses the *Mytilus* bivalve larvae development test as a second test species. The two tests complement each other in sensitivity to contaminants and as a chronic and acute toxicity test. Both of these RMP test species were also endorsed by the recently approved SQO assessment methods by the Southern California Coastal Water Research Project (SCCWRP) who performed their own independent evaluation of laboratory test species to recommend in an SQO assessment.

Core issue:

There has been persistent moderate toxicity in SF-Bay since 1993. About 22% of the samples in the dry season have been toxic to EOHA since 2002. Need to develop tools to investigate the causes of toxicity in support of environmental management decisions. If we don't know what is causing the toxicity we can't help managers address source reduction.



Workshop goals:

- 1st of 2 workshops (next one will be at the end of this year)
- Focus future research efforts on the most important chemicals and stressors of amphipod toxicity based on the groups general expertise. Focus first on SF-Bay and then statewide.
- What toxicity identification evaluation (TIE) tools work well, which ones are deficient
- What data/information is missing in the TIE toolbox but would be useful
- Begin to outline potential studies, approaches needed to address tool development
- Agenda for workshop 2

Overview of Sediment Toxicity Issues in San Francisco Bay: Causes of Toxicity, Recent TIE Results and Research Planned for 2010. Bryn Phillips

Bryn provided an overview of Sediment Toxicity issues in SF-Bay and the TIE work conducted on solid phase and sediment elutriate samples by UCD-MPSL in recent years. The goal was to present enough of the analytical details of the recent work on whole sediment and interstitial water (IW) TIEs to have the group understand the current progress on phase II TIE methods. Another goal was to discuss ways of improving some of the analytical techniques being developed to further tease-out organic contaminants that may be causing toxicity.

Background:

Amphipod toxicity is generally higher in the winter

Often see persistent amphipod toxicity in SF-Bay

Lots of samples are moderately toxic: 50-70% survival

Infrequently see severe amphipod toxicity: 20-35% (percent survival)

Correlation studies indicate:

Contaminant mixtures have been identified as important

Relation with grain size, some metals and organics (but we know the amphipods are not sensitive to metals at most local environmental levels)

Non-contaminant factors may play a role, e.g. % clay

Q: A question from the audience asked “What is the reference site sediment used with this species?”
A: Bryn said the “control” sediment used is “home sediment” collected where the animals are collected (Oregon). No ref site from SF-Bay is used for routine testing, though some ref sites have been identified in previous studies

History of Sediment TIEs in SF-Bay

Whole sediment TIEs on samples from Redwood Creek and Grizzly Bay

1996-2004 elutriate TIEs for RMP S&T

Bivalves show divalent cations are causes of toxicity in Grizzly Bay Interstitial water TIEs have been conducted with sea urchin larvae using samples from 2 stations (very sensitive)

Mission Creek TIE Study Goal: to further develop sediment TIE methods using EOHA.

Collected sediment in winter with the goal of finding two toxic samples. Toxicity defined as amphipod survival <50% for sample to be acceptable for TIE

Hard to find very toxic sample (targeted 14 samples with only one site sufficiently toxic to warrant using in the TIE development (Mission Creek which had 48% survival).

Tested both sediment and interstitial water

Previous research has shown it is easier to manipulate interstitial water, for example, to assess toxicity of a dilution series, and this provides additional lines of evidence for resolving causes of toxicity

TIE results indicated that

- metals were not an issue (EOHA are generally not sensitive to metals at regional concentrations)
- ammonia was not an issue in whole sediment TIE, but contributed to the toxicity of the interstitial water
- organic compounds were characterized as the likely cause but additional steps were required to further separate organic groups to focus on specific organic chemical (This is the goal of a phase II TIE)
- Analysis of Amberlite eluate after exposure to sediment was used qualitatively assess organics removed from the whole sediment sample

TIE summary:

NH3 was not a factor in whole sediment but contributed to the toxicity of the interstitial water
Reduced toxicity with addition of Amberlite and Powdered Coconut Charcoal (PCC)
Analyze Amberlite eluate afterwards - toxicity and chemistry
Because the whole sediment results pointed to organics, the interstitial water TIEs focused on organics

The results of the interstitial water TIEs showed that there were inconsistencies in removal of toxicity using solid-phase extraction columns, and in eluting chemicals from the column.

Chemistry highlights:

Individual chemicals were all below know toxicity threshold values
Many chemicals were present in a complex mixture
A Sediment Quality Guideline Quotient value (SQGQ) was calculated based on methods of Fairey et al. (2001). SQGQ = 21.3, the SQGQ was 6.9 without chlordane
Some evidence of PAH toxicity - Sum PAHs exceeded LC50 for toxicity to the amphipod *R. abronius*

Have some LC50s for the amphipod EOHA, but threshold of effects information is generally lacking for estuarine taxa.

The current RMP TIE project (Causes of Tox 2009-2010) is currently developing three LC50s for EOHA (trans chlordane, cyfluthrin, pyrene).

Q: Because the test species is a wild organism, are there issues with genetic diversity?

A: We run reference toxicant tests using cadmium to see how healthy each test batch is compared to other batches over time. We see a consistent response to cadmium based on LC50 values. Also, the tests species for most of the US come from one/two sites (one supplier in Oregon).

The final part of the presentation by Bryn Phillips focused on the current TIE project for FY2009-2010. This includes development of LC50 values, and further development of whole sediment and IW TIE methods. The TIE will be conducted on the spiked sediments used in the development of LC50 values. These will include incorporation of Solid-Phase Micro-extraction (SPME) to determine how the TIE treatments influence bioavailability of the chemicals.

Application of Multiple Approaches for Stressor Investigation in Southern California Sediments. Steve Bay

Steve summarized 3 years of a SCCWRP study at Ballona Creek in Southern California (listed for toxicity and contaminants - TEs and organics). This project is working on multiple study tasks to better understand the spatial and temporal extent of contamination in the creek and to further investigate tools for understanding the potential causes of toxicity. Tasks include:

- TIE development: They have been using pyrethroid TIE methods with success but teasing out pyrethroid signatures from OC-pesticides remains problematic.
- Interpreting TIE and chemistry/ toxicity results includes comparison to thresholds of effects (LC50s, ERMs, and SQGs) and evaluating toxic units.
- Statistical correlations
- Developing methods for extracting and understanding the bio-available fraction of sediment contaminants using passive porewater samplers and SPME.

Steve stressed that we need to continue to develop documented TIE methods for investigating causes of toxicity from emerging contaminants (as well as the legacy contaminants of concern), and provide guidance for using and interpreting the results.

Analytical Challenges Associated with Identifying Chemicals Responsible for Sediment Toxicity. Kelly Smalling & Keith Maruya

Kelly's presentation discussed the fact that ambient samples contain mixtures of unmeasured compounds that may be contributing to toxicity, but for which little is known. It is difficult to sort out which compounds are a priority for TIE studies (i.e., those chemicals that are likely toxic to lab organisms, like amphipods). We need to prioritize key 'emerging contaminants of concern' from a toxicity point of view, for both developing analytical chemistry methods to measure them and/or developing thresholds of effects (LC50s). Kelly provided an approach to prioritize:

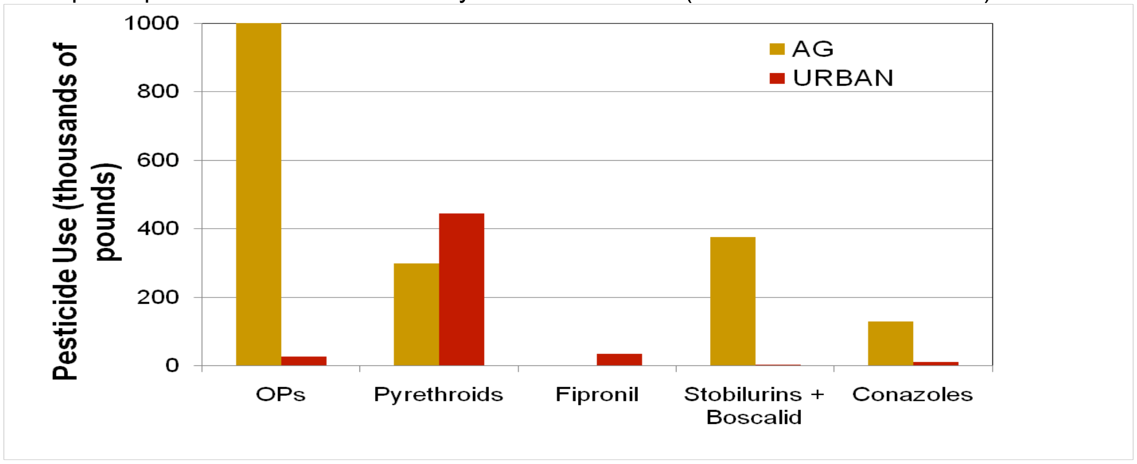
How to prioritize analyte lists for chemistry and/or effects threshold development?

Use DPR pesticide database (department of pesticide regulation – lists pounds of chemicals applied by county and land-use)

Look at occurrence in sediment/water

Need to develop toxicity thresholds – LC50 or EC50 values for newer non-standard compounds. Kelly highlighted specific concern about fungicides that are applied in high amounts based on the DPR database and also measured in ambient samples. She also was concerned about non-standard herbicides.

Example of pesticide use in all of CA by land-use in 2008 (based on DPR database):



Kelly also discussed specific issues with pyrethroid analyses: High variability in pyrethroid results. USGS was part of an inter-calibration study with several labs which resulted in usually high variability in results. This was thought to be because there are no standard methods for the analyses of pyrethroids. Also there are no certified standards to use to evaluate accuracy.

Summary of issues with pyrethroids include:

> Need robust low level detection methods (low ng/g or ng/L) to measure ambient samples at concentrations that are potentially toxic.
>
> Need to standardize methods. How do we standardize?
>
> > Need a good reference sediment that contains pyrethroids, fungicides, etc for a larger inter-lab calibration study. This would allow one to evaluate the difference between methods and develop reliable standard methods.

Keith presented findings on a method to extract the bioavailable fraction of organic compounds using solid-phase microextraction (SPME) fibers in both laboratory and environmental settings. The devices extract key contaminants of concern at levels comparable to bivalve bioaccumulation in controlled tests.

(UCD-MPSL will be using the SPME devices in the RMP 2010 study to further evaluate their utility in extracting the bioavailable fraction of organic compounds. If these devices work consistently for several organic contaminant groups, they may become useful tools for the sediment TIE toolbox).

The group identified several groups that are working on the broader issue of emerging contaminants and thought that coordination with them may be useful in developing a prioritized list of contaminants to focus on in future toxic effects studies. These groups include: The Pyrethroid Working Group, EPA, RMP’s Emerging Contaminants Workgroup, and other chemists.

Non-Anthropogenic Chemicals and Non-Contaminant Stressors and Their Role in Amphipod Mortality. Brian Anderson

Brian addressed non-anthropogenic stressors by listing many potential stressors and discussing what we know about their effects on toxicity and how they are (or are not) controlled for in laboratory tests:

- Unionized Ammonia – Y* (Aeration/pH/zeolite)
- Hydrogen Sulfide – Y (Aeration/pH)
- Other Toxins
  - e.g., Phytotoxins?
  - Hg, Mn?

  - Non-Traditional Contaminants
    - Anions – Y (SPE)
    - Polar Organics – ? (LC-MS)
    - Oxidants – Y (Na2S2O3)

- Test Organism Health
  - Salinity Effects/Acclimation – Y
  - Seasonal Health – Y (Control Chart data indicates no obvious effect)

- Grain Size
  - Percent Fines - ?
  - Percent Clay -? Particle Shape -?

*Y = can be controlled for in a lab-test, or there are TIE methods to identify them

The second part of this presentation focused on the possible effects of grain size on EOHA survival. Evidence suggests that clay size or shape could inhibit amphipod survival. This is based on graphical analysis of percent clay versus amphipod survival using uncontaminated samples from RMP monitoring. It is also based on un-published laboratory test results at MPSL

A question from the audience asked about the effect of 'black carbon' in environmental sediment samples… it tends to tightly bind organics making them not bioavailable? A question was also asked about the effect of carbon on amphipod survival. It's not clear whether carbon has this effect, since carbon and grain size parameters (clay, percent fines) all co-vary.

The group thought that the list of alternate stressors made sense, did not have others to add, and agreed that they all should be addressed (ruled out) when interpreting TIE results. Kay ho suggested Hg was not a likely cause of acute toxicity to amphipods.

Phytotoxins and grain size effects on amphipod survival are not well understood and the group thought that they merit further study (either by bringing in other experts who have been working on these issues (in the case of the phytotoxins) or by developing new research ideas (in the case of assessing the effects of clay on EOHA)). Kay Ho also suggested one way to determine if a particular sample's "toxicity" was due to chemicals vs. grain size would be to test the sample side-by-side using EOHA and *Ampelisca*. Since Ampelisca prefers fine-grained sediments, this would provide additional information on whether chemicals were the cause of toxicity. EPA Narragansett conducts *Ampelisca* exposures using minimal sediment overlying water in the test beakers. This results in a greater exposure of the amphipod to sediment interstitial water, and likely reduces their ability to isolate them from chemicals by building a tube. Previous research has suggested this behavioral characteristic might be one reason *Ampelisca* are perceived to be "less sensitive' than EOHA to contaminated field sediments.

Genomic Tools for Identifying Chemicals Affecting *Eohaustorius estuarius.* Chris Vulpe

Chris presented their fairly well-developed genomic tool, which to date has emphasized daphnids, and discussed progress towards developing a gene microarray for EOHA. They are almost done with developing the gene microarray and the next step will be to begin diagnostic tests with single contaminant dosed samples (from LC50 studies – cyfluthrin, chlordane (cis- and trans), pyrene, some specific metals, and CHCs) and ambient samples that were toxic to EOHA (from SoCal & SFBay). These results will be used to sort out gene expression 'signatures' related to a toxic effect. The daphnid studies suggests that UC Berkeley will be able to identify specific 'signatures' related to specific contaminant groups that have similar modes of biochemical action.

However Kay Ho mentioned that the EPA spent over two years working on a similar study with another estuarine amphipod, *Ampelisca abdita*, and they were not able to have such clear stressor ID results. They also used similar methods and dilutions as the UCB study. Chris said he knows the people conducting that study and would followup with them to determine why there has been a delay in providing results. For now they are not far enough along in the EOHA genomic tool development study to know if it will be able to indentify effects from specific contaminants or contaminant groups.

Afternoon Group Discussion Chairmen: Brian Anderson/ Steve Bay

The morning presentations were designed to familiarize the workshop participants with regional and state-wide work on stressor identification in recent years and to highlight areas where additional research is needed. To help focus the discussion, the afternoon discussion was divided into three subject areas:

1. Likely stressors causing amphipod mortality in SF Bay and beyond (chemical and non-chemical stressors)
2. List of TIE tools and other methods to address these stressors
3. List of information or procedural deficiencies which should be addressed to improve outcomes.

<u>1. Brian presented a "straw man" list of stressors of concern and the group discussed them.</u>

Stressors	**Group comments**
NH3	Have adequate tools to address ammonia effects in tox tests.
H2S	Have adequate tools to address sulfide effects in tox tests.
Grain Size/Clay (shape)	Worth pursuing for effects on EOHA. Could use a side-by-side with a species that is not sensitive to GS effects (e.g. *Ampelisca*). Possibly consult with a sedimentologist, or geologist familiar with quantification of particle shape and sizes to determine if a laboratory experiments could be designed to address effects of clay on EOHA. This should be done through the TWG process.
Physical toxicants (oils/smothering)	Yet unaddressed but may be a factor in some SF-Bay sediment.
Unknowns: e.g., phytotoxins	Need to talk to experts to sort out if this may be a factor in SF-Bay and CA in general. K. Taberski had ideas on who to consult
Metals	The group agreed that metals are not likely an issue with EOHA. Bivalve embryos tend to be more sensitive to these compounds.
Cations	Adequate TIE methods exist to address these
Anions	Adequate TIE methods exist to address these
Organics	

Pesticides	The group thought that pesticides would be the most important organic contaminant group to evaluate and prioritize for analytical chemistry, further TIE methods development, and LC50 development. Emphasis on emerging contaminants should focus on newly introduced pesticides
OCs	Need to evaluate further
OPs (Chlorpyrifos)	Need to evaluate further but less of an issue these days.
Pyrethroids	Important emerging pesticides – have some LC50s, need more for more test species, and need to develop standard methods and reference material for detection low-level concentrations in ambient samples.
Other	Expand to include new contaminants such as fipronil and triclosan
Fungicides	Fungicides and herbicides should be considered in the prioritization effort. But will need to really consider not only use but potential to be toxic in sediment toxicity tests.
Herbicides	As above
PAHs	Specific PAHs and PAH mixtures remain a concern in sediment toxicity tests. This is still a priority.
PCBs	Less of a concern in toxicity testing as it is unlikely to be toxic to sediment tox test organisms in acute exposures.
PCPs (personal care products)	Worth adding to the list – and to prioritize similar to fungicides/herbicides
PBDEs	Less of a concern in toxicity testing as it is unlikely to be toxic to sediment tox test organisms in acute exposures.
Mixtures	Not discussed in detail but the group agreed additive, synergistic, and removal effects are still a concern.
Organics	e.g. PBO with pyrethroids, combinations of PAHs,
Metals	
Metals/Organics	
Non-Contaminants w/ Chemicals	e.g. TOC (how TOC reduces bioavailability of some organic contaminants).
Ammonia with all	

Other comments by the group on addressing prioritization of stressors:

The group identified groups that are working on the broader issue of emerging contaminants and thought that coordination with them may be useful in developing a prioritized list of contaminants to focus on in future toxic effects studies. These groups and tools include: The Pyrethroid Working Group, EPA, RMP's Emerging Contaminants Workgroup, and other chemists. There are also QSAR tools that can be used to evaluate potential for toxicity (likely fate based on chemical characteristics): U.S. EPA QSAR provides chemical Kows and modes of action (and other information) - http://cfint.rtpnc.epa.gov/aster/
The California State Department of Toxic Substance Control (DTSC) is another resource/group that could help identifying emerging contaminants of concern.

University of Miami and Woods Hole might have additional analytical capability that could help identify unknown peaks from chromatograms. Daniel Oros' work with unknown peaks should also be re-examined.

Dave Crane might be able to help with microcystin analysis.

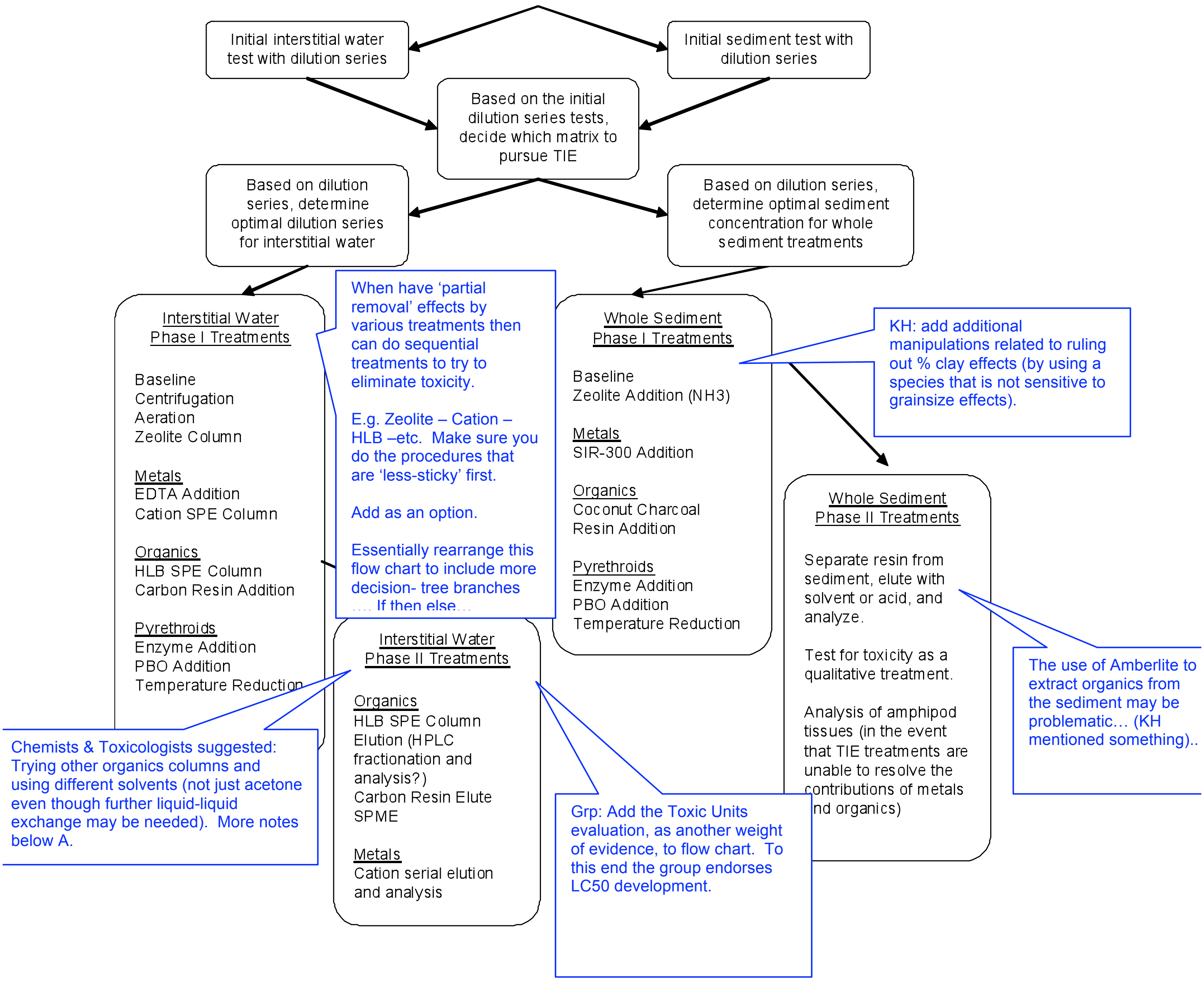
There was a suggestion that highly water soluble compounds that have low log Kow (e.g., Log Kow< 2) would not likely partition to sediments and therefore be a lower priority.

The group agreed that the issue of unmeasured contaminants is an ongoing concern. One approach to addressing the possibility that unknown or unidentified chemicals are contributing to sample toxicity would be through more thorough evaluation of chromatograms for non-targeted compounds. This could be conducted as part of the TIE process in close consultation between toxicologists and chemists.

On the topic of mixtures, it was mentioned that the genomics tools may be useful in evaluating mixtures.

2. A 'generic' flowchart of solid phase (whole sediment) and elutriate (interstitial water) TIE manipulations as described by Bryn, in the Mission Creek case study, served as a straw man template for the group to comment on current TIEs tools and methods, and make suggestions for improvement.

In general the group liked the flowchart and thought it would be really useful as a decision tool. They made some suggestions on adding a few more alternate steps based on initial sample manipulations.



More Notes related to TIE methods in flow-chart:

A. On Solid-phase extraction columns for non-polar organics:
    a. The HLB SPE Column is a balanced hydrophobic/hydrophilic column and may not strongly bind to hydrophobic contaminants (resulting in break through). The C-18 column may have stronger hydrophobic binding capabilities – will then need to address liquid-liquid extractions. …

B. Workshop participants agreed that it was important to recognize how well the TIE methods work to rule out groups of potential stressors and not to just focus on their limitations. Chris Beegan from the State Water Board endorsed that by explaining that having these tools can save millions of $$ in unnecessary TMDL listings. He really encouraged moving forward with this important tool development and endorsed that fact that these tools are robust at ruling out some potential stressors. From Aroon's notes "CB – TIEs are great tools and help to put the sediment chemistry data in context. For example, ruling out metals in TIEs is informative. Currently, TMDLs based on ERLs / ERMs that often points to metals being a problem, which would be wrong. Keep pushing forward…"

** A New 'draft' flow chart that includes many of these suggestions was developed by Bryn Phillips (UCD-MPSL) since the workshop and is available on the workshop website as a separate file. **

Workshop Summary and Action Items Group

Group discussion about:

- Prioritization of future research needs
  For next workshop will present some of the findings of the RMP 2010 Causes of Toxicity whole sediment and interstitial water TIE work and the molecular TIE (genomics) study. Also would like to invite others who have more expertise on ammonia, phytotoxins, and grain size effects. There was also some debate on the merits of including an expert on Quantitative Structure Activity Relationships (QSARs) to help develop a method to prioritize chemicals likely to be contributing to amphipod mortality.

  ACTION: Brian will develop the preliminary agenda and the group can help complete topics and invitees.

- Brief evaluation of workshop format.
  No real discussion on this but general nod of heads that this was a productive first workshop.

  ACTION: Sarah will send out a survey and compile it for the group

- Additional areas of expertise needed for next workshop
  Group suggested including other geologists, toxicologists, chemists
  SB- suggested Dave Mount and an expert on geological interactions with biota (physical toxicants).
  KT – said she will identify someone who is working on phytoplankton through the POD workgroup.

  ACTION: Brian and Sarah will follow up with workshop participant to develop a list of new invitees.

- Deliverable TASKS to complete between now and next workshop:
    a. Sarah to work with group to develop a compilation of LC50s, expanding the UC-Davis list with information provided by the group.
    b. UC Davis will redesign their TIE flowchart and circulate it for comments and refinement. The idea would be to include a 'decision tree' as general guidance of what the options are as one works through a solid-phase and elutriate TIE manipulations. (e.g. what do you do if you have grain size concerns, partial removal on multiple treatments)