MEMORANDUM

To: RMP Technical Review Committee
Date: March 10, 2008
From: Sarah Lowe and John Ross (SFEI)
Brian Anderson (UCD-MPSL)

Re: **Selection of Sub-lethal Sediment Tests for the RMP Annual Status and Trends Cruise**

The 2008 RMP Status and Trends program (RMP S&T) will be collecting benthos, sediment toxicity and chemistry at 27 stations this summer in order to implement and evaluate the Sediment Quality Objectives (SQOs) assessment methods for bays and estuaries that have been approved by the State of California.

The RMP S&T conducts both acute and sub-lethal sediment toxicity tests as recommended by the SQO assessment methods. However, the sub-lethal bivalve development test is an elutriate test, which is not recommended by the SQO program. The TRC needs to decide if the RMP should adopt (or add) the sediment-water interface (SWI) bivalve test to comply with the SQO protocol recommendations.

To assist in this decision, we have compiled the following:

1) Background information from the SQO Staff Report;
2) A brief review of the 1999 – 2001 elutriate and SWI side-by-side test comparisons;
3) Description of a new study (by SCCWRP) to determine optimal equilibration time for homogenized sediment for the Sediment-Water Interface Exposure Method as an alternative to collecting intact sediment cores; and
4) General discussion points and suggestions for further evaluation of the SWI method.

1. Background

SQO Sediment Toxicity Test Recommendations

Section 5.5.2.2 of the Draft Staff Report (SWRCB, 2007) describes the recommended sediment toxicity tests for SQO assessments for Bays and Estuaries. That report recommends a list of specific sediment tests in order to maintain a high level of consistency and data quality around the State. Toxicity tests on sediment porewater and elutriate samples were not recommended because of potential changes in metal toxicity due to oxidation, change in sample pH, sorption of contaminants to test chambers, confounding effects of ammonia toxicity, and elimination of sediment ingestion as a route of uptake. These recommendations were based on a review of sediment toxicity test protocols provided to the SQO program by their Scientific Advisory Panel (need to confirm this with Steve Bay).

The SQO assessment protocol recommends that at least two different toxicity tests be employed: an acute toxicity test with survival endpoints and a sub-lethal test with growth or development endpoints. Although the RMP S&T program currently performs both an acute and sub-lethal sediment toxicity test, the sub-lethal bivalve development test is an elutriate test, which is not recommended by the SQO program.

RMP S&T Sediment Toxicity Side-by-Side Elutriate and SWI tests (1999-2001)
From 1999 to 2001, the RMP conducted side-by-side comparisons of the bivalve elutriate and SWI exposure systems using bay mussel *Mytilus galloprovincialis* larvae. While the results of the two methods are available through the SFEI data access tool, no 'official' summary report or statistical review was ever conducted. At the time, a general review of the data and discussions between the subcontracting laboratory, RMP staff, and the Water Board led to the decision that the two test methods essentially provided similar results (with a few exceptions). Additional consideration of the prominent roll of sediment resuspension in the relatively shallow SF Estuary (through daily tidal action) contributed to the idea that the elutriate test was probably a pretty reasonable toxicity indicator for the RMP. Because of the lack of a compelling argument to switch methods, the fact that few monitoring programs were using the SWI method, and the fact that collecting the SWI cores is more labor intensive, the TRC decided not to switch methods.

New Possible Alternative to Intact Sediment Cores for Use in the SWI Method
Studies conducted as part of the initial development of the SWI exposure system demonstrated greater toxicity in tests conducted with intact cores vs those conducted using homogenized sediments. These results indicated greater flux of metals into sediment overlying water using intact samples relative to homogenized samples. However, no experiments were conducted to evaluate the effects of sample equilibration on toxicity tests using homogenized samples. It is possible that given a sufficient equilibration period to allow re-establishment of the original (undisturbed) sample's redox characteristics, SWI tests with homogenized sediment might be comparable with intact samples.

Because of concerns over the additional labor required to collect and store intact sediment cores, SCCWRP is conducting a small-scale study to investigate using homogenized sediments as an alternative in SWI exposures. This study will evaluate results of tests conducted using intact cores vs those conducted with homogenized sediments that have been allowed to settle and equilibrate for different periods. This alternative is being evaluated for possible use by the Bight-08 monitoring effort in Southern California.

2) Review of the RMP S&T Side-by-Side Bivalve Methods Study

The RMP sampled 14 sediment stations between 1999 and 2001 for a total of 52 samples. Two 48-hour, sub-lethal bivalve development tests were run side-by-side using homogenated sediment elutriates (1:4 dilution), and intact sediment core samples (SWI).

The actual toxicity test procedures were the same for both sediment exposure methods so only the test set-up process is described below.

RMP sub-lethal sediment toxicity – 48-hour bivalve development test (elutriate):
The elutriate test uses bay mussel *Mytilus galloprovincialis* larvae with a sub-lethal endpoint of mean percent normal alive. Fifty grams of sediment is added to 200 mL of Granite Canyon seawater forming an elutriate mixture that is shaken vigorously for 10 seconds, and then allowed to settle for 24 hours (Tetra Tech 1986). The elutriate solution is then pipetted into replicate containers and inoculated with mussel embryos for a 48-hour exposure (US EPA 1995).

Alternate sub-lethal sediment toxicity – 48-hour bivalve development test (SWI):
The SWI test also uses *Mytilus galloprovincialis* larvae with a sub-lethal endpoint of mean percent normal alive. The tests are conducted with intact sediment core samples taken with minimal disturbance from the Van Veen grab sampler. Test containers consist of a polycarbonate tube with a 25-µm screened bottom placed so that the screen was within 1 cm of the surface of an intact sediment core (Anderson et al., 1996). Salinity adjusted seawater is poured into the core tube and allowed to equilibrate for 24 hours before the start of the test.

Statistical Methods
The raw elutriate and SWI replicate data for each station were compared using a one-tailed t-test with equal variances not assumed. The t-test was conducted to indicate whether differences between test methods were statistically significant ($p < 0.01$).

Results
Comparison of mean % normal alive results from the two test methods showed good agreement between 87% of the results (45 of 52 samples – nine of which showed significant toxicity in both test methods). Thirty-nine of these samples showed less than 10% difference (RPD) between the two test results.

Fifteen samples were significantly toxic and, of those, seven samples had very different results between the two test methods and disagreed on the significance level of toxicity (Table 1). It is not clear why these samples showed such different results using the two test methods but it is worth noting that these samples largely came from two stations.

Table 1. Seven of fifty-two samples (13%) sharply disagreed on the significance level of toxicity and the actual % normal alive results

		Elutriate % Normal Alive			SWI % Normal Alive		
Station Code	**Cruise**	**Toxic?**	**Mean**	**StDev**	**Toxic?**	**Mean**	**StDev**
BA10	2000-07	*	0	0		84	7
BA21	1999-02		89	7	*	55	7
BA21	2000-07	*	0	0		88	10
BA21	2001-08	*	59	13		86	8
BF21	1999-07	*	0	0		80	8
BF21	2000-07	*	0	0		84	4
BF21	2001-08	*	0	0		83	5

Mean % normal alive results were generally higher in elutriate versus SWI tests (60% of the comparisons). The elutriate test results indicated that 29% of the samples were significantly toxic while 19% of the SWI results indicated toxicity.

Significant differences were found for 27% of the t-test comparisons ($p < 0.01$, 14 of 52 comparisons); however in only 6 of these cases did sample toxicity differ for the two test methods, i.e. toxic versus non-toxic. In five instances the elutriate method was found to be both toxic and significantly different from the SWI method (non-toxic). There was one instance where the SWI result was found be significantly toxic while the elutriate result was non-toxic (BA21, 1999-02).

Conclusions

The two elutriate and SWI test methods showed good agreement in 87% of the samples with confounding results in seven samples, largely from two estuarine habitat stations located in the Lower South Bay and Suisun Bay Regions of the Estuary (BA21 and BF21). 60% of the elutriate test results showed higher % normal alive measures. However, the elutriate tests had more significantly toxic samples (29% vs. 19%) and when there was toxicity it was much more pronounced than observed in the SWI test (more 0% normal alive results). The reasons for the differences in toxic response between the two test methods and are not well understood, but it does indicate that, under some conditions, homogenization of samples may significantly influence sediment toxicity results.

Anderson et al. (2001) indicated that porewater results were sometimes more toxic than the SWI results in an embryo-larval toxicity comparison study using sea urchin larvae. The bivalve elutriate study (with a 1:4 dilution) showed similar characteristics.

3) New Small Study to Investigate Alternative Sampling Options for SWI test

In preparation for the Bight-08 monitoring in Southern California this summer, SCCWRP is conducting a small study to determine optimal equilibration time for homogenized sediment for the Sediment-Water Interface Exposure Method as an alternative to collecting in-tact sediment cores (test species will be *Mytilus galloprovincialis*). Because of the inconvenience of sampling and transferring in-tact sediment cores to the laboratories, the SCWRP study will evaluate if there is an optimal equilibration time for homogenized sediment when used in the sediment-water interface exposure system.

The optimal equilibration time will be determined when toxicity and metal flux rate values are most similar to those achieved with intact cores. The study will also evaluate if there are potential holding time effects on intact cores.

4) Discussion

Estimated Cost Increase (negligible)

1) Sampling: It will take about 15 – 25 minutes to collect an additional sediment grab at each toxicity station (adding about 9 additional hours to the cruise). This is within the RMP's logistics cost budget: the budget includes one or two

contingency days for un-expected delays or events and therefore the new sampling will be covered within the budget.

2) Analysis: The cost increase for the 48-hour bivalve development test using the SWI method (instead of the elutriate method) is ~$20/sample ($550/cruise)

Comparison to Historic RMP data

There may be a significant differences in results from the Lower South Bay and Suisun Bay/Grizzly Bay Regions (where there was most disagreement between the methods for unknown reasons (BA21 and BF21)). Previous research has demonstrated greater toxicity in SWI tests conducted using intact samples relative to homogenized samples, and chemical analyses have shown great flux to sediment overlying water in the intact samples. However, the greater toxicity observed in the sediment elutriate tests may be due to methods used to prepare the elutriate. In this case the use of a 1 to 4 ratio of sediment to seawater may result in a concentration of contaminants in the elutriate solution relative to the overlying water in the intact SWI sample. This hypothesis has not been confirmed using chemical analysis. .

Comparison to other programs

The SWI exposure system was evaluated as part of BPTCP sediment toxicity test studies conducted prior to establishment of the RMP. In addition, the SWI method was evaluated as one of several chronic sediment toxicity test protocols as part of the SQO program. These studies confirmed the utility of this test system. Other than these evaluations, there is little historical data using the SWI methods outside of the BPTCP, SQO and RMP programs.

Trade offs in sampling and transport of intact and homogenized samples

- Added sampling time
- Maintaining good core integrity as the core is collected and the bottom and top lids are secured is difficult making sample-handling an additional 'noise' factor.
- Possible loss of interstitial water through leaking from the bottom of the cores. (These issues shouldn't be an issue with an experienced crew.)

Ecological Relevance of the SWI exposure method

It is thought that the intact sediment cores are more ecologically relevant than the homogenated sediment elutriate exposure methods because of changes in oxidation and chemical constituents during mixing. The SWI intact cores may better address effects of fluxed contaminants on epibenthic species as a result (Anderson et al. 2001). However, there is a lack of literature to support these hypotheses.

Replicate, intact SWI samples (6 cores are sampled/ station) provide true field replicates, unlike homogenized sample replicates, allowing for spatial characterization of toxicity at a station.

Information Gaps

- No clear understanding of how to interpret results from the elutriate vs intact SWI methods when sediments are likely to be toxic. This would require chemical analyses of elutriates and sediment overlying water in the SWI core.
- Can homogenated sediments be used in the SWI test? This is being investigated by SCCWRP prior to the Bight '08 program. However, because of limited resources, this study is being conducted using only 4 samples, and will likely not provide sufficient information for recommendation to the SQO program. Similar studies using additional samples will likely be required to develop SWI procedures using homogenized samples so that results are comparable to intact samples.

References

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