The effects of particle size and shape and animal health on toxicity test results using the amphipod *Eohaustorius estuarius*.

Estimated Cost: $30,000 from RMP (Effects of grain size in laboratory dose-response - experiments - MPSL)

$25,000 from SWRCB (Interactive effects of grain size and contaminants - SCCWRP)

Oversight Group: Exposure and Effects Work Group

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Background

The 10-day whole sediment toxicity test protocol for the amphipod *Eohaustorius estuarius* is one of the principal tests recommended for toxicity monitoring in California. Several studies have shown this species is appropriate for this application, and this is the benchmark test used in regional monitoring programs in southern California and the San Francisco Estuary. Due to concerns about limitations of methods to determine causes of persistent moderate toxicity in field sediments and the relative influence of non-contaminant factors on amphipod survival, two recent workshops sponsored by the San Francisco Estuary Institute's Regional Monitoring Program (RMP) identified specific attributes of *E. estuarius* that require additional research. Among a list of non-contaminant factors considered, the relative impacts of grain size, particle shape, and test animal condition were identified as possibly important factors affecting amphipod survival.

As part of the initial evaluation of *E. estuarius* as a test species, Dewitt et al. (1989) assessed survival of *E. estuarius* in 42 uncontaminated field sediment samples from Puget Sound, Washington and Oregon. These authors reported that "*E. estuarius* showed little sensitivity to sediments of different grain sizes: mean survival was 92.4% in sediments with $\geq$80% silt-clay content and 96.7% for coarser sediments." Environment Canada (1998) published grain size recommendations for the 10d test with *E. estuarius.* Tay et al. (unpublished study described in Environment Canada, 1998) found mean survival was 74% in mixtures with 57% clay and 99% fines. Based on these experiments, they established tolerance limits of <90% coarse grained sediment, and <70% clay. The Environment Canada (1998) 10-day guideline states that "test materials with ≥ 70% clay must not be used in a 10-day sediment toxicity test with *E. estuarius*". UC Davis conducted similar experiments using mixtures of sand and field-collected reference mud that was comprised of silt and clay. The field reference material was sieved through a 75 µm screen then mixed with sand to give sediments with 10 – 90% fines. *E. estuarius*10-day survival was ≥85% in sediments with ≤70% fines. Survival was 57% in sediment with 90% fines (Marine Pollution Studies Laboratory-Granite Canyon unpublished data). In addition to these studies, analyses of data from the RMP and elsewhere have shown that survival of *E. estuarius* in field sediments is negatively correlated with percent fine grained sediment, and with percent clay in sediment. Based on the preponderance of evidence, the effect of clay was prioritized for further study by participants of the two RMP workshops.

The toxicity workshops also identified the possible interaction of seasonal differences in amphipod health and their ability to tolerate fine-grained sediments as a high priority topic for investigation. This is based on evidence suggesting sediment toxicity in San Francisco Bay is greater in winter, and the possibility that increased winter toxicity is related to variability of the health of field collected amphipods. Seasonal changes in amphipod fitness related to nutrition, senescence, or reproductive activity have been suggested as the reason for such variations in sensitivity to San Francisco Bay sediments. The workshop participants also recommended measurement of amphipod lipid content as an indicator of animal condition. Prior studies using *E. estuarius* have shown a correspondence between tissue lipid content and changes in sensitivity to toxicants related to amphipod condition. Measurement of amphipod lipid content may provide a valuable tool for interpreting the results of future sediment toxicity surveys, but information on the seasonal changes in this parameter and its association with changes in amphipod sensitivity to stressors is needed. Combining seasonal measurements of tissue lipid with studies of the sediment particle size effects on *E. estuarius* survival will provide the information needed to evaluate the usefulness of lipid measurements in toxicity testing.

A United State Geological Survey characterization of suspended sediments in the San Francisco Estuary found that water column suspended sediments contained three clay mineral types: illite, montmorillonite (=smectite), and chlorite+kaolinite (Knebel et al., 1977). Samples were collected in the spring, summer, fall and winter seasons and covered the northern and southern reaches of the estuary. The results demonstrated that waters in the northern reaches of the estuary were dominated by chlorite+kaolinite via inputs from the Sacramento-San Joaquin river systems. Kaolinite originates from the decomposition of granite. Illite dominates the southern reach of the estuary where clay minerals are re-suspended from the estuary floor by tidal currents. Illite originates from the decomposition of micas and feldspars. Samples of the estuary sediments (bedded, not suspended sediments) showed that sediments in the northern and southern reaches were dominated by chlorite+kaolinite clays, which comprise a somewhat larger size fraction than illite clays (Knebel et al. 1977).

Part One: Establishing a Dose-Response Relationship between Sediment Clay Content and Amphipod (*Eohaustorius estuarius*) Mortality (RMP funds to MPSL-Granite Canyon $30K)

Experimental Approach

Laboratory experiments will be used to establish a dose response relationship between *E. estuarius* survival and percent chlorite+kaoliniteclay in sediment. Experiments will be conducted using a single natural reference sediments spiked with clay.

Clay-spiked sand: Reference sand will be spiked with increasing concentrations of chlorite+kaolinite clay, the dominant clay found in San Francisco Estuary sediment (Knebel et al., 1977). Clay purchased from a commercial supplier will be mixed with reference sand at ratios representative of those in the Estuary. After equilibration, 10day toxicity tests will be conducted with *E. estuarius*. One range-finder test will be conducted to establish the range of percent clay that inhibits amphipod survival. Two definitive experiments will then be conducted to confirm the dose-response relationship. Results of these experiments will be used to establish LC25 and LC50s for percent chlorite+kaolinite clay in sediment. The dose response results will also be used to examine the relationship between percent clay and amphipod survival using regression analysis to calculate of the 95% lower prediction limits of the regression of percent clay and number of survivors for *E. estuarius* (after DeWitt et al., 1989). This may be used for statistically partitioning the effects of percent clay from contamination in sediment toxicity tests with amphipods. Approximately 30 amphipods that are representative of the animals used in the test will be preserved for lipid analysis. If feasible, individuals will be measured in order to determine the variability in animal condition within each test batch. These experiments will be conducted using amphipods collected in summer-fall.

Clay-spiked reference sediment: ***Note, these experiments were originally proposed for inclusion in 2014 special study. Due to budget constraints, these experiments are now proposed for 2015 as a second phase of the laboratory grain size dosing experiments, and are not included in the 2014 SOW.***

To investigate whether clay particle shape is correlated with amphipod mortality, particle shape will be analyzed on the sand spiked with kaolin clay (described above). The original proposal described a microscopic analysis to quantify particle shape. In this analysis, particle shape characteristics will follow general methods described in Tucker (1995) using the Powers (1953) grain shape classification. These methods have been adapted by Ivano Aiello at Moss Landing Marine Laboratories to allow quantification of the relative proportion of each shape category for selected samples. In this classification, particles are categorized as either “high sphericity” or “low sphericity” and within these classifications they are further classified according to their relative angularity (e.g., highly angular to well rounded).

Ten replicate subsample smears of each clay-spiked sand or clay-spiked reference sediment from the experiments described above will be analyzed using a combination of oil-immersion and scanning electron microscopy, depending on the size distribution of the samples. The relative proportion of each particle shape category will be quantified and these values will be combined to provide a shape index value for each sample. This value will then be correlated with amphipod mortality to investigate whether there is a significant correlation between particle shape and amphipod mortality in the clay-spiked samples.

As part of the proposal review process, an alternative approach to quantifying grain size and shape was suggested based on two instruments currently in use by California State University Office of Water Programs. One instrument is a Beckman Instruments laser diffraction particle size analyzer, and the other is a dynamic flow particle imaging instrument (Sysmex FPIA3000 - Malvern Inc). The latter instrument is used for shape analysis. Laser diffraction particle size analysis will be used to quantify the size distribution of the clay-spiked sand used in this study. The most appropriate method for shape analysis is still being considered. Dr. Aiello has offered to discuss the project with CSU representatives to explore the possibilities of using their services for this study. Part of the consideration will involve cost of the analyses. In addition, it is not clear whether sonication of the samples which may be included in the particle imaging process used by the Sysmex instrument could create an artifact if there are varying degrees of clay aggregation in the clay-spiked sand. It would be preferable that whatever method is used to quantify the shape of the particles in the clay-spiked samples, the sample characterization should reflect the shape and size characteristics experienced by the amphipods during the 10d exposure. This consideration will be explored in our discussions with the CSU Office of Water representatives.

Seasonal Variation in Lipid Analysis: ***Research to measure seasonal variations in E. estuarius lipid content and examine the relationship of lipid variations to toxicity was included in previous descriptions of this special study. However, funding is not currently available to conduct this study and it is no longer proposed as part of the 2014 special study. Additional support for research related to stressor identification in San Francisco Bay has been made available by the State Water Resources Control Board, as part of an existing agreement with SCCWRP related to sediment quality objectives development. Funding from the Water Board is limited to studies of the response of E. estuarius to contaminants, and therefore cannot be used for the seasonal lipid study. The following project description (Part Two) describes proposed research to be conducted with SWRCB funding that will complement the particle size research funded by RMP.***

Part Two: Interactions between grain size and contaminant effects on amphipods (SWRCB funds to SCCWRP - $25K)

One of the hypotheses developed during the 2012 stressor identification workshop was that variations in San Francisco Bay sediment toxicity results may be due to an interaction between sediment contaminant exposure and other stressors, including particle size. San Francisco Bay sediments contain low-moderate concentrations of multiple contaminants. While no single sediment contaminant appears to be present at concentrations likely to explain the patterns of toxicity in San Francisco Bay, this toxicity may be the result of either the joint action of multiple contaminants or the interaction of contaminants and other stressors related to factors such as particle size or test organism health. This study will test this hypothesis by investigating the interaction between variations in particle size and dose of fipronil sulfone on *E. estuarius* survival. Fipronil sulfone is a toxic degradation product of fipronil, a current use pesticide of high concern for aquatic life impacts in San Francisco Bay. Results from this research are expected to provide two benefits to RMP and SWRCB: 1) provide information to develop guidance for interpreting toxicity test results in the presence of high concentrations of sediment clay, and 2) develop toxicity response thresholds for a contaminant of emerging concern that can be used for interpretation of monitoring results.

Experimental Approach

Reference sand will be spiked using with chlorite+kaolinite clay using the same methods developed in Part One of this study. A concentration series of sand spiked with clay will be prepared that brackets the LC25 for clay developed in Part One of this study. Separate portions of the clay-ammended sand will be spiked with two concentrations of fipronil sulfone, corresponding to the LC10 and LC25 (test concentrations will be based on results of previous experiments conducted for SWRCB). The resulting exposure matrix, consisting of three concentrations of fipronil sulfone (0, LC10, LC25) will be tested for toxicity to *E. estuarius* using standard exposure conditions. The results will be examined using analysis of variance to determine whether there is a significant interaction between clay content and response to fipronil sulfone. Results from this experiment may be used to supplement the recommendations developed in Part One for interpreting the results of *E. estuarius* toxicity tests in the presence of high sediment clay content.

Budget

Note: The budget reflects costs of conducting experiments in the summer season only.

Summer Testing Only			
Task*	Laboratory	Season	Cost
Effect of Sand Spiked with Clay	MPSL-Granite Canyon	1	19,460
Grain Size Analysis	Aiello – Moss Landing		2,400
Grain Shape Analysis	Aiello – Moss Landing		5,000
Reporting	MPSL-Granite Canyon		3,140
Total - RMP to MPSL-Granite			30,000
Task**	Laboratory	Season	
Interactive effects of sand-spiked clay with selected contaminants	SCCWRP		$25,000
Reporting	SCCWRP		
Total - SWRCB to SCCWRP			$25,000

* MPSL-Granite Canyon task with RMP funds; ** SCCWRP project with SWRCB funds, contractual oversight for this project will be provided by the SWRCB.

Figure 1.

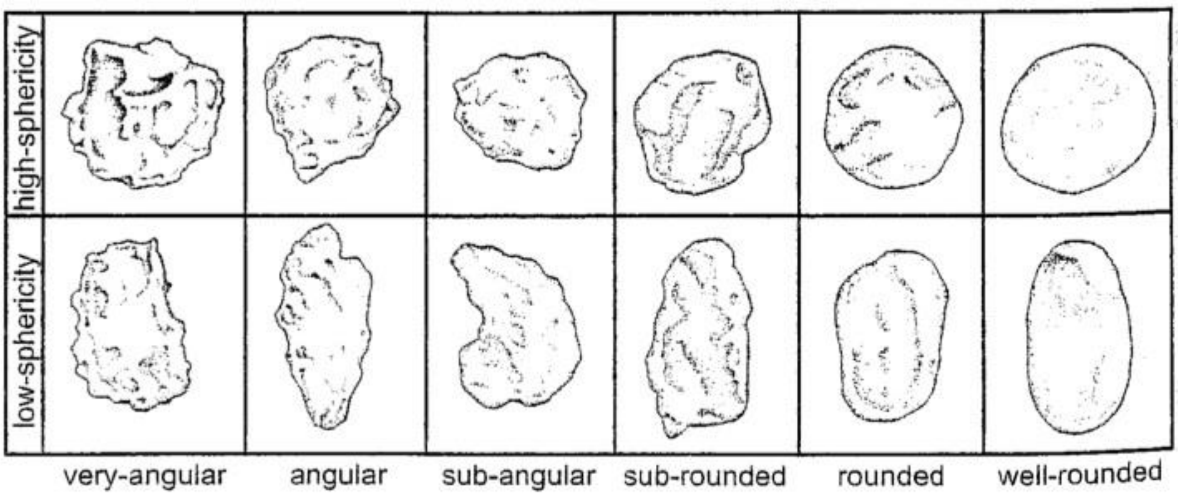

Figure 5.5 – Power's (1953) classification of grain roundness for grains displaying low sphericity and high sphericity. [From Tucker, 1995.] [Copyright © 1995 Blackwell Publishers, reproduced with permission.]

References

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