DMMP CLARIFICATION PAPER

REFERENCE AREAS FOR FRESHWATER BIOASSAYS

Prepared by Stephanie Stirling (U. S. Army Corps of Engineers) and the RSET Bioassay Subcommittee for the DMMP agencies.

Introduction/Problem identification

The DMMP program has identified a number of sites that are suitable for the collection of reference sediments for marine bioassays (PSEP1991). Suitable clean reference sites have not been identified for freshwater bioassays. The Regional Sediment Evaluation Team's Bioassay subcommittee has developed an approach for identifying suitable freshwater reference sites. This approach is explained in the attached white paper "Presentation of Process for Reference Sediment Area Identification," prepared by Dr. Taku Fuji and Tom Pinit of Kennedy/Jenks Consultants.

Proposed Clarification

Most of the DMMP agencies are represented on the RSET Bioassay Subcommittee. Because not all RSET stakeholders have been actively involved in Bioassay Subcommittee activities, the agencies are clarifying certain aspects of RSET that will apply to DMMP activities. The DMMP program will be recommending the approach outlined in the RSET white paper for the identification and selection of reference sites when needed for freshwater bioassays. However, the DMMP program has no plans to identify freshwater reference sites as they have for the marine waters; the different sediment requirements for individual watersheds and the relatively few freshwater projects that the DMMP reviews does not make it cost-effective to undertake this process at this time.

References

PSEP 1991. Reference Area Performance Standards for Puget Sound. EPA document number 910/9-91-041

DRAFT RSET WHITE PAPER – Presentation of Process for Reference Sediment Area Identification

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QUESTION/ISSUE: Is there a recommended process to follow to identify potential sediment reference areas for use in biological testing programs?

DISCUSSION: This Draft White Paper presents a methodology for identifying and selecting sediment reference areas for use in sediment bioassay and bioaccumulation testing programs. This methodology would be most useful when applied to projects in geographic areas currently lacking established, reliable reference sediment areas. However, the general concepts that are discussed in this White Paper should also be useful for assisting in the identification of reference areas for specific projects.

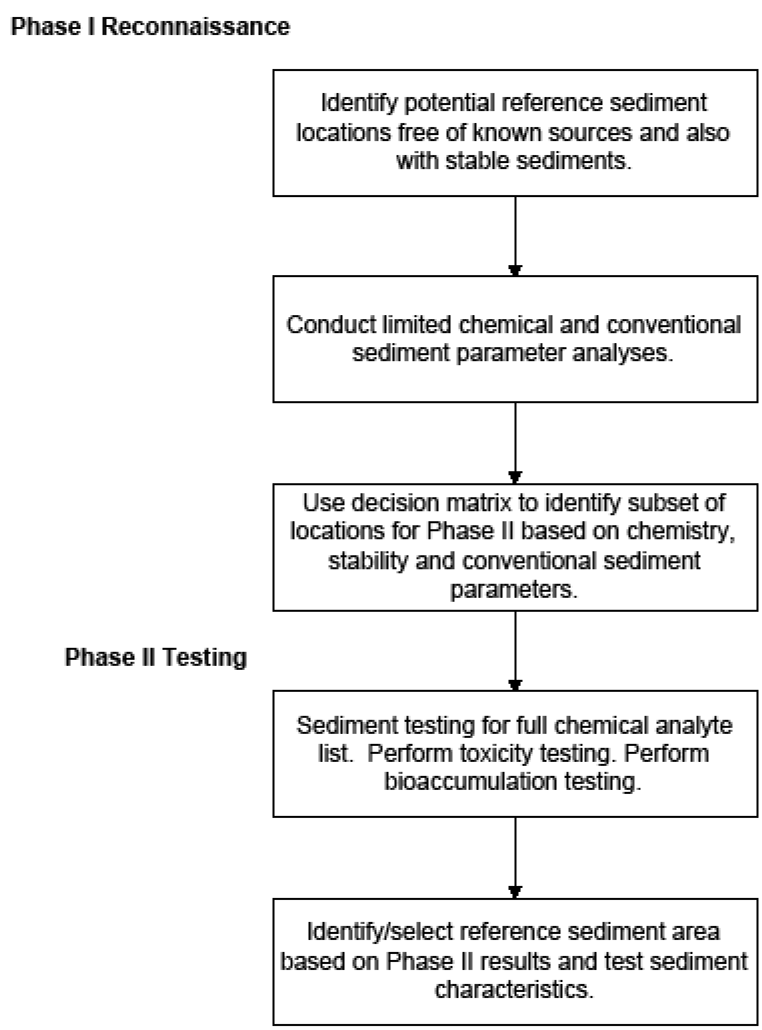
Reference sediment was previously defined in the Dredged Material Evaluation Framework (DMEF, 1998) as a "whole sediment used to assess sediment conditions that are similar as practicable to the grain size and total organic carbon (TOC) of the dredged material but is free from contamination". The Puget Sound Estuary Program (PSEP) Guidance on Sediment Bioassays (PSEP, 1995) describes the following use of reference sediments within a sediment bioassay program. "Laboratory negative control sediments generally are those from which infaunal test animals (e.g., amphipods) were collected. As such, physical and chemical sediment characteristics may be very different from those of the test sediments. Reference sediments can provide data that can be used to separate toxicant effects from unrelated effects such as those of sediment grain size (PSEP, 1995)". The reference sediment must be tested in the same batch as the test sediment and the results are used to interpret the bioassay results in accordance with established biological testing interpretive criteria (SEF, 2006).

It is important to distinguish "background sediment" from "reference sediment" for the purpose of this White Paper. While in some cases "background" areas may be the same as "reference" areas, their use within specific regulatory programs are different. Washington State has two definitions of background in their administrative code (Washington Administrative Code [WAC] 173-340-200); (1) **"Area background"** means the concentrations of hazardous substances that are consistently present in the environment in the vicinity of a site which are the result of human activities unrelated to releases from that site, and (2) **"Natural background"** means the concentration of hazardous substances consistently present in the environment that have not been influenced by localized human activities. The State of Oregon does not have rules specific to sediment background concentrations or the selection of sediment reference sites. Generally, the Department of Environmental Quality's (DEQ) approach for determining appropriate reference sites for bioassay comparison is to focus on physical similarities between the test sediment and sediment in the reference location and make sure the reference location is not contaminated. "Reference areas" as discussed in this White Paper are consistent with the WAC definition of "natural background".

The process described herein was developed to identify three potential freshwater reference sediment areas based on grain size characteristics within the Portland Harbor on the Lower Willamette River (LWR) for the U.S. Army Corps of Engineers (Hart Crowser, 2002). The reference area selection process described in this Draft White Paper is based on procedures used to select reference area locations for the Puget Sound Dredged Disposal Analysis (PSDDA) program (PTI, 1991) and the Grays Harbor and Willapa Bay, Washington Dredged Material Evaluation Procedures and Disposal Site Management Manual (Corps, 1995). The RSET Biological Testing Subcommittee has reviewed these procedures and recommends their use in identifying sediment reference areas for biological testing as discussed in the SEF.

This reference area selection process was designed to identify locations that meet specific grain size ranges and organic carbon concentrations characteristic of the test sediments. The process was designed to be conducted in two phases; a **Phase I** reconnaissance survey to evaluate potential reference areas by limited chemical and conventional sediment parameter analyses, and a **Phase II** focused evaluation of a subset of candidate reference areas by comprehensive chemical and biological testing (i.e., full suite of SEF chemicals of concern and biological testing using the amphipod *Hyalella azteca* and the midge *Chironomus tentans*). Additionally, for **Phase II**, if bioaccumulation testing is expected, a 28-day bioaccumulation test using the worm *Lumbriculus variegates* is recommended to establish baseline data. An alternative freshwater species for bioaccumulation testing that is being considered by RSET is the clam *Corbicula fluminea* and the use of this species for establishing baseline data should be discussed with the appropriate regulatory agency prior to test initiation. Sampling and testing are to be performed in a manner consistent with applicable federal and regional guidance documents (Ecology, 1995, EPA/Corps, 1998, and DMEF, 1998). The **Phase I** and **Phase II** reference sediment selection process is illustrated on the following flow chart.

Reference Sediment Selection Process Flow Chart



Phase I Reconnaissance

The objective of the **Phase I** reconnaissance survey is to screen potential locations for suitability as reference areas based on conventional sediment parameters and lack of chemical contamination. Surface (0 to 10 centimeters [cm]) sediment samples are collected that target conventional parameter ranges representative of those found in the test sediments.

Phase I involves a reconnaissance of potential reference sediment locations that are unlikely to be impacted by known contamination sources (e.g. identified clean-up sites or large municipal discharges), those believed to be hydraulically stable to permit long-term utilization (i.e., no appreciable erosion or accretion), those that exhibit preliminary grain size characteristics determined in the field by wet-sieving, those with comparable total organic carbon (TOC) content, and those with low levels of total ammonia and sulfides in sediment pore water. Ammonia and sulfides have been selected for Phase I analysis as these constituents have been identified as potentially important confounding factors for sediment bioassays. Threshold levels of concern for these constituents are available for marine/estuarine tests (Barton, 2002; Fox, 1993 and Kendall and Barton, 2004). Similar thresholds have not been established for freshwater bioassays at this time but available thresholds can be used to provide a general evaluation of pore water concentrations of these constituents.

Wet-sieving can be performed in the field using a standard No. 230 mesh, 63-um-opening sieve to obtain a rough estimate of grain size. A 50-ml aliquot of reference sediment is washed through the sieve mesh. The sediment that passes through the sieve is classified as the fine-grained (silt/clay) particle fraction, and the sediment remaining on the sieve is the coarse-grained (sand/gravel) fraction. Sediment should continue to be washed until the water passing through the sieve runs clear. The remaining coarse fraction is then emptied into a 100-ml graduated cylinder for measurement. Subtracting the remaining coarse fraction volume from the initial 50-ml volume yields the fine-grained fraction volume. Dividing the fine-grained volume by the initial 50-ml volume yields the "percent fines". Percent fines ranges can then be compared between reference and test sediments to determine similar grain sizes. Detailed information on wet-sieving protocols can be found in EPA (2000) guidance.

To assist in the identification of potential in-water areas away from known sources of contamination, there are databases available that provide a listing of identified sites in both Oregon and Washington. In Oregon, these include: Oregon Department of Environmental Quality (DEQ) Facility Profiler, the Environmental Cleanup Site Information Database (ECSI), as well as Underground Storage Tank (UST) and Leaking Underground Storage Tank (LUST) databases. In Washington, an Environmental Information Management System (EIMS) is being developed that will provide similar information in a single database. In addition, local city or county offices should be contacted to identify locations of major outfalls in the region. Based on the initial reconnaissance, a number of potential reference sediment areas are identified.

Each of the potential reference sediment areas are then subject to a limited suite of conventional parameter and chemical analyses, including laboratory grain size analysis, TOC, total ammonia and sulfides (in pore water), total petroleum hydrocarbons (NW-TPH), pesticides, and total polychlorinated biphenyls (PCBs).

Phase II Testing

The results of the **Phase I** sediment sampling are used to recommend a subset of reference areas for follow-up comprehensive chemical and biological testing (**Phase II**). To select candidate **Phase II** sampling locations, a decision matrix based on the four characteristics identified to prioritize **Phase II** sample locations is used. Ideally, candidate reference areas selected for further analysis in **Phase II** should be those shown to be substantially free of anthropogenic contaminants, those believed to be stable to permit long-term utilization (i.e., no appreciable erosion or accretion), those that matched the target grain size and organic carbon characteristics identified in the test sediments, and acceptable concentrations of ammonia and sulfide in pore water.

For the decision matrix presented below as an example, the sediment samples from each of the three target grain size ranges were evaluated with regard to each of the four characteristics and given a subjective score (based on a scale of 1 through 5, with 5 indicating the highest match with program objectives). For instance, under the analytical chemistry characteristic, all sediment samples received the highest score of 5 because none exhibited any anthropogenic contamination. For the stability characteristic, the samples collected from coves or other quiescent areas received a higher score than samples collected within or adjacent to the main channel of the river. For both the grain size and TOC characteristics, the scores were based on how closely the **Phase I** sediment samples matched the target grain size/TOC range.

Phase II testing further focused the process by selecting specific candidate areas from the potential reference area locations sampled in **Phase I**. For the Lower Willamette project, each of the candidate areas represented one grain size class, i.e. coarse, medium, fine (these grain size targets will vary with specific project objectives). Within each of the grain size classes, the candidate sediment area selected was based on limited **Phase I** analytical chemistry results (TPH, pesticides, PCBs) with non-detects or lowest detected concentrations. Given the limited chemical analyte list, the subjective scoring (1 to 5) for this criterion would be based on number of detected chemicals in reference sediments and the relative concentrations between potential reference sediments. Finally, each candidate area within a grain size class was preferentially selected based on more stable hydrologic conditions, such as back eddies, coves, or other quiescent areas off the main LWR channel.

The example decision matrix below illustrates how selection of one candidate area within each specified grain size class can be facilitated. **Phase I** potential reference locations are listed across the top of the matrix, with 4 decision criteria listed along the left-hand side of the matrix. Each of the samples is subjectively ranked on a scale of 1 to 5 per decision criterion, with 5 indicating the highest match with program objectives.

Example Decision Matrix for Fine-grained Phase I Samples

	Sample IDs		
	REF-A	**REF-B**	**REF-C**
Analytical Chemistry	**5**	**5**	**5**
Stability of Location	**3**	**4**	**5**
Grain Size Match	**4**	**5**	**3**
TOC Match	**5**	**4**	**3**
Total Score	**17**	**18**	**16**

In this example, REF-B would be the selected reference candidate area for the fine-grained size class, based on the summed rankings indicating: non-detect or lowest chemical concentrations (5); second-best sediment stability (4); best grain size match to targeted class (5); and second-best TOC concentration match to test sediments (4). This decision matrix process would be repeated for the medium-grained and coarse-grained size class samples.

Once the candidate reference areas are identified through the decision matrix process, one surface sediment sample is collected from each area. These **Phase II** sediment samples are analyzed for the full SEF Chemicals of Concern (COC) list, including:

- Metals (Sb, As, Cd, Cu, Hg, Ni, Ag, Zn)
- Polycyclic aromatic hydrocarbons (PAHs)
- Semivolatile organic compounds (SVOCs)
- Some chlorinated hydrocarbons
- Pesticides
- PCBs
- Porewater tributyltin (TBT)
- Total sulfides
- TOC
- Grain size

Sediment chemistry results are screened against appropriate screening guidelines depending on whether the reference sediments represent freshwater or estuarine/marine conditions. SEF (2006) screening levels (SLs) can be used to determine whether chemical concentrations exceed conservative levels of concern in marine/estuarine sediments. For freshwater sediments, it is envisioned that the upcoming Freshwater Sediment Quality Guidelines would be used for the purpose of a chemistry screen.

The **Phase II** candidate reference area samples are subjected to bioassay testing evaluating lethal and sublethal endpoints. Again, depending on whether this project is being conducted on freshwater or marine/estuarine sediments will determine the appropriate sets of sediment bioassays to select. For the specific example being discussed in this Draft White Paper, the following freshwater bioassays were selected; 10-day *Hyalella azteca* amphipod survival and 10-day *Chironomus tentans* midge survival and growth tests. Bioassay sediment toxicity was determined based on specified freshwater interpretive criteria in the SEF. It should be noted that specific regulatory programs may require the use of longer term freshwater bioassays (i.e., 28-day *Hyalella azteca* amphipod survival and growth test and the 20-day *Chironomus tentans* midge survival and growth test).

If bioaccumulation testing is expected, preliminary bioaccumulation testing should be conducted on the proposed reference sediments to establish baseline conditions and determine whether the reference sediments are resulting in statistically significant accumulation in test organisms. Again, as this example Lower Willamette River project was for freshwater sediments, the bioaccumulation testing program used freshwater protocols (28-day *Lumbriculus variegatus* and *Corbicula fluminea* bioaccumulation tests). As stated previously, *Lumbriculus variegates* is the standard freshwater bioaccumulation test species and the use of the bivalve, *Corbicula fluminea*, would need to be approved by the appropriate regulatory agency prior to test initiation. A paired one-tailed Student's-t test ($p < 0.05$) was conducted on Day 0 and Day 28 tissue concentrations to evaluate the statistical difference between the two concentrations, i.e. whether significant bioaccumulation had occurred over the 28-day test period. For marine/estuarine sediments, the standard marine/estuarine bioaccumulation testing protocols should be used. The Bioaccumulative Chemicals of Concern List (BCOC) list presented in the SEF should be used as the analytical program for the bioaccumulation testing.

Summary

The selection process described above allows for the systematic identification of candidate reference sediment areas for a given water body or watershed. The process is particularly useful for those projects in geographic areas currently lacking established, reliable reference areas. Final selection should target locations that match grain size and organic carbon characteristics of the test sediments, those shown to be substantially free of anthropogenic contaminants and that are as stable as possible to permit long-term utilization (i.e., no appreciable erosion or accretion).

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RECOMMENDATION: The BTS proposes including the reference sediment area selection process described in this Draft White Paper in the Revised Interim Final SEF.

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