10 TIER 3 BIOLOGICAL TESTING: BIOACCUMULATION

Bioaccumulation is the accumulation of contaminants in the tissues of organisms through any route, including respiration, ingestion, or direct contact with contaminated water, sediment, or dredged material. Tier 3 bioaccumulation testing of dredged material is required when results of sediment chemical analysis for bioaccumulative chemicals of concern (BCOCs) indicate the potential for unacceptable adverse environmental or human health effects. The tissue residues derived from bioaccumulation tests are compared to the DMMP's target tissue levels (TTLs) and reference values to assess the potential for both human- and ecological-health related effects. Important elements of this testing process are described below.

10.1 BIOACCUMULATIVE CONTAMINANTS OF CONCERN (BCOCS) AND TRIGGERS FOR BIOACCUMULATION TESTING

In 2003, the DMMP adopted a revised list of Bioaccumulative Contaminants of Concern (see DMMP, 2003c Issue Paper and DMMP, 2007 Technical Basis document) using a systematic approach that considered multiple lines of evidence for determining the bioaccumulative risk posed by a particular chemical. Revising the DMMP's BCOC list involved creation of four separate BCOC lists. **List 1** (**Table 10-1**) is the primary list of bioaccumulative contaminants of concern. Analysis for these 17 chemicals in sediments (and potentially in tissues) is required to determine dredged material suitability. Analysis of TBT (marine projects) and dioxins/furans (freshwater and marine) is required on a case-by-case basis. **Lists 2 and 3** define chemicals of potential concern for bioaccumulative effects but for which definitive data are still lacking – analysis of these chemicals is not routinely required. **List 4** chemicals are those which the DMMP does not consider to be bioaccumulative.

When measured sediment concentrations of the List 1 contaminants exceed the bioaccumulation trigger (BT) values presented in **Table 8-2**

Table 8-2, bioaccumulation testing must be performed before suitability of the test sediment for open-water disposal can be determined. The BT is set at a sediment concentration that constitutes a "reason to believe" that the chemical would accumulate in the tissues of target organisms. As a general approach, BTs were established for human health COCs at concentrations in the upper 30th percentile of the concentrations allowable for unconfined, open-water disposal (i.e., 70 percent of the difference between the SL and ML) (EPTA, 1988). The DMMP agencies revised TBT guidance in DMMP, 1996, and established a porewater BT for this chemical. The BT for chromium was set equal to the SL in a DMMP, 2011 clarification paper. The 2003 revisions to the BCOC list did not involve revisions to existing BT values. However, interim BT values were developed for six of the new List 1 chemicals using the same algorithm used in EPTA (PSDDA, 1988). The interim BT for selenium was developed in consideration of sediment concentrations reported in the literature to be associated with adverse ecological effects from bioaccumulation.

The DMMP agencies will evaluate bioaccumulative COCs at non-dispersive and dispersive sites in Puget Sound on a case-by-case basis using best professional judgment based on the latest science, and regional background approaches being developed by Ecology in Puget Sound. Until that time, the approach and guidelines outlined in this section are those that will be used by the DMMP. However, modifications proposed by applicants, based on Regional Sediment Evaluation Team (RSET) guidelines, may be considered on a case-by-case basis.

Table 10-1. List 1 Bioaccumulative Chemicals of Concern

CHEMICAL	METHOD INFORMATION	LOG K_{ow}[1]	BT (dry wt basis[2])
METALS			
Arsenic	SW846 M.6010/6020	N/A	507.1 mg/kg
Cadmium	SW846 M.6010/6020/7131	N/A	11.3 mg/kg
Chromium	SW846 M.6010/6020	N/A	260 mg/kg
Copper	SW846 M.6010/6020	N/A	1027 mg/kg
Lead	SW846 M. 6010/6020/7421	N/A	975 mg/kg
Mercury	SW846 M.7471	N/A	1.5 mg/kg
Selenium	SW846 M. 6010/6020/7740	N/A	3 mg/kg[3]
Silver	SW846 M. 6010/6020/7761	N/A	6.1 mg/kg
Zinc	SW846 M.6010/6020	N/A	2783 ug/kg
ORGANOMETALLIC COMPOUNDS			
Tributyltin (interstitial water) (bulk sediment)	Krone/Unger	3.7-4.4	0.15 ug/L 73 ug/kg
ORGANICS			
Fluoranthene	SW846 M.8270	5.12	4,600 ug/kg
Pyrene	SW846 M.8270	5.11	11,980 ug/kg
CHLORINATED HYDROCARBONS			
Hexachlorobenzene (HCB)	SW846 M.8081	5.89	168 ug/kg
PHENOLS			
Pentachlorophenol	SW846 M.8270	5.09	504 ug/kg
PESTICIDES/PCBs			
Total DDT (sum of 4,4'-DDD, 4,4'-DDE and 4,4'-DDT)	SW846 M.8081	(5.7 - 6.0)[4]	50 ug/kg
Chlordane[5]	SW846 M.8081	6.32	37 ug/kg
Dioxins/Furans	EPA 1613	5.5-13.9	10 ng/kg[6]
Total Aroclor PCBs	SW846 M.8081/2	(3.6-11)[7]	38 mg/kg OC

[1] Octanol/Water Partitioning Coefficients (log K_{OW}) for organic chemicals of concern for bioaccumulation in Puget Sound.
[2] Except where noted otherwise
[3] Based on review of sediment effect values from the literature and best professional judgment.
[4] Range of individual chemicals making up the total.
[5] Chlordane includes cis-Chlordane, trans-Chlordane, cis-Nonachlor, trans-Nonachlor, and oxychlordane. Components of chlordane were clarified at the 2007 SMARM.
[6] The BT for Puget Sound established with implementation of new interim dioxin guidelines in 2010.
[7] Range of individual congeners making up the total.

10.2 BIOACCUMULATION TEST SPECIES SELECTION

Selection of appropriate species is an important consideration for Tier 3 bioaccumulation tests. Studies have shown that the time required for any given species to achieve a steady-state tissue concentration of a chemical of concern may vary (see **Table 10-2**), or are not well known (Windom and Kendall, 1979; Rubenstein, Lores, and Gregory, 1983). As such, for a given chemical triggering a Tier 3 bioaccumulation test, the applicant should consider selecting species that will assimilate the target chemical near its steady-state concentration (if known)

within the exposure period or consider extending the exposure period. The Inland Testing Manual requires bioaccumulation testing with species from two different trophic niches, including: 1) a suspension-feeding/filter-feeding organism and 2) a burrowing deposit-feeding organism. In the northwest, the Tier 3 marine bioaccumulation test is usually conducted with both an adult bivalve (*Macoma nasuta*) and an adult polychaete (*Nephtys caecoides*). For recommended freshwater species, consult the RSET SEF.

10.3 BIOACCUMULATION TEST PROTOCOL

The standard Tier 3 bioaccumulation test utilizes the EPA protocol (Lee *et al*. 1989) and a 28-day exposure period, after which a chemical analysis is conducted of the tissues to determine the concentration of bioaccumulative chemicals of concern identified in the sediments. Protocols for tissue digestion and chemical analysis will follow the PSEP-recommended procedures for metals and organic chemicals.

For many chemicals in **Table 10-1**, it was originally assumed that the standard 28-day exposure would be sufficient for a steady-state tissue concentration to be reached. After examining the observed steady state exposures depicted in **Table 10-2**, the DMMP agencies deemed it unlikely that steady state will have been reached after 28 days for select chemicals. Therefore the DMMP agencies increased the exposure time from 28 to 45 days for the BCOCs with regularly occurring BT exceedances during DMMP project testing to better approximate steady-state conditions. A 45-day exposure should be used for PCBs, TBT, DDT, Hg, and fluoranthene during required bioaccumulation testing. For the remaining BCOCs, which have not had BT exceedances to date, if BT exceedances are observed the DMMP agencies would evaluate the need to extend the exposure period beyond the 28-day exposure period before bioaccumulation testing is initiated. Given the holding time limitations (8 weeks) and the large volume of sediment required, it has always been necessary to resample project sediments in order conduct bioaccumulation testing for all previous bioaccumulation testing conducted. Under these circumstances, it is necessary to also reanalyze the newly-collected sediment for the chemicals of concern that originally triggered the requirement for bioaccumulation testing. If the chemical concentration(s) found in the bioaccumulation test sediment are less than that measured in the original sediment analyzed, the DMMP will require that the measured tissue concentrations of that chemical be mathematically adjusted. The resulting adjusted tissue concentration reflects the bioaccumulation of a given chemical that would have been expected from exposure to the original sediment sample.

Recent bioaccumulation protocol updates:

- Use a 45-day exposure time when conducting bioaccumulation testing for specific chemicals of concern for bioaccumulation (PCBs, TBT, DDT, Hg, Fluoranthene) to ensure steady-state chemical concentrations in the tissues of the test species (*Macoma nasuta* and *Nephtys caecoides*). Increasing the exposure to 45 days will require once weekly supplemental additions of 175-mL of test or control/reference sediment to each replicate10-gallon aquarium/test chamber.
- Wet-weight biomass (of a subset of 10 individual organisms/replicates) should be measured at the beginning and end of the bioaccumulation exposure period for test, control and reference samples. This estimate of net individual growth during the exposure period will be used as an additional metric to evaluate the health of the test

animals, and to build a database that may support establishing a benthic effects-based target-tissue level.

- Each DMMU undergoing bioaccumulation testing is compared to the TTL interpretation guidelines for a specific BCOC. For test sediment tissues quantitated greater or equal to the TTL no further action is required, as the DMMU fails DMMP interpretative guidelines. DMMU quantitated less than the TTL are subjected to a one-tailed one-sample t-test to determine whether the test tissues are significantly less than the TTL.
- Use an alpha level of 0.1 (rather than 0.05) when making statistical comparisons between tissue concentrations in test and reference samples to reflect higher likelihood for within-sample variability, and to increase the power of the test to discriminate between reference and test tissue concentrations. Note that an alpha level of 0.05 should be used when making comparisons between test tissues and target tissue levels (TTLs).

To conserve laboratory space and reduce the volume of sediment required, applicants may expose *Macoma nasuta* and *Nephtys caecoides* together in the same test chambers. The total sediment requirement for co-testing is 30 liters. A considerable volume of sediment is required for testing each single test species (**Table 10-3**), and co-testing of two species in single aquaria substantially reduces the volume of sediment required for bioaccumulation testing.

Table 10-2. Percent of Steady-State Tissue Residues of Selected Metals and Neutral Organics from 10 and 28 day Exposures to Bedded Sediment

COMPOUND	% OF STEADY STATE[1,2] TISSUE RESIDUE		SPECIES	EST. BY	REFERENCES[3]
	10-DAY	28-DAY			
METALS					
Copper	75	100	*Macoma nasuta*	G[5]	Lee (unpublished)
Lead	81	100	*Macoma nasuta*	G	Lee (unpublished)
Cadmium	17	50	*Callianassa australiensis*	G	Ahsanulla et al., 1984
Mercury	ND[4]	ND[4]	*Neanthes succinea*	G	Kendall, 1978
PCBs					
Aroclor 1242	18	87	*Nereis virens*	G	Langston, 1978
Aroclor 1254	12	82	*Macoma balthica*	G	Langston, 1978
Aroclor 1254	25	56	*Nereis virens*	K[6]	McLeese et al., 1980
Aroclor 1260	53	100	*Macoma balthica*	G	Langston, 1978
Total PCBs	21	54	*Nereis virens*	G	Pruell et al., 1986
Total PCBs	48	80	*Macoma nasuta*	G	Pruell et al., 1986
Total PCBs	23	71	*Macoma nasuta*	G	Boese (unpublished)
PAHs					
Benzo(a)pyrene	43	75	*Macoma inquinata*	G	Augenfield et al., 1982
Benzo(b,k)fluoranthene	71	100	*Macoma nasuta*	G	Lee (unpublished)
Chrysene	43	87	*Macoma inquinata*	G	Augenfield et al., 1982
Fluoranthene	100	100	*Macoma nasuta*	G	Lee (unpublished)
Phenanthrene	100	100	*Macoma inquinata*	G	Augenfield et al., 1981
Phenanthrene	100	100	*Macoma nasuta*	G	Lee (unpublished)
Pyrene	84	97	*Macoma nasuta*	G	Lee (unpublished)
TCDD/TCDF					

COMPOUND	% OF STEADY STATE[1,2] TISSUE RESIDUE		SPECIES	EST. BY	REFERENCES[3]
2,3,7,8-TCDD	6	22	*Nereis virens*	G	Pruell et al., 1990
2,3,7,8-TCDD	63	100	*Macoma nasuta*	G	Pruell et al., 1990
2,3,7,8-TCDF	43	62	*Nereis virens*	G	Pruell et al., 1990
2,3,7,8-TCDF	92	100	*Macoma nasuta*	G	Pruell et al., 1990
MISCELLANEOUS					
4,4-DDE	20	50	*Macoma nasuta*	G	Lee (unpublished)
2,4-DDD	31	56	*Macoma nasuta*	G	Lee (unpublished)
4,4-DDD	32	60	*Macoma nasuta*	G	Lee (unpublished)
4,4-DDT	17	10	*Macoma nasuta*	G	Lee (unpublished)

[1] This table is modified from Inland Testing Manual (Table C), using data updated from Boese and Lee (1992).
[2] Steady-state values are estimates, as steady-state is not rigorously documented in these studies.
[3] See Boese and Lee (1992) for complete citations.
[4] ND = Not Determined. Observed AFs (accumulation factors) for field tissue levels compared with sediment levels (normalized to dry weight) averaged 4 for this species, but ranged from 1.3 to 45 among other benthic macroinvertebrate species. Laboratory 28-day exposures to bedded sediment indicated uptake fit a linear regression model over the exposure period and experimental conditions and did not approach a steady-state condition. Tissue levels observed (*N. succinea)* at 28 days amounted to only 2.5 % of the total sediment-bound Hg potentially available.
[5] G = Steady-state residue estimated by visual inspection of graphs of tissue residue versus time.
[6] K = Steady-state residue estimated from a 1st-order kinetic uptake model.

Table 10-3. Species-specific sediment volume requirements for MARINE bioaccumulation testing

SPECIES	MINIMUM SEDIMENT REQUIREMENT
Macoma nasuta	250-400 ml per beaker x 10 beakers per replicate x 5 replicates = **12.5-20 liters**
Nereis virens	200 ml per worm x 20 worms per replicate x 5 replicates = **20 liters**
Arenicola marina OR *Abarenicola spp.*	500 ml per beaker x 4 beakers per replicate x 5 replicates = **10 liters**
Co-testing: Macoma/Nephtys	4 liters per replicate x 5 replicates = **30* liters**

Highlighted: This alternative has become the generally accepted protocol for bioaccumulation testing within DMMP.
* Recent testing experience from one testing laboratory has recommended increasing the volume collected from both test and reference sediment locations from 20 to 30 liters.

10.4 BIOACCUMULATION TEST INTERPRETATION

The DMMP's numerical test interpretation guidelines, or target tissue levels (TTLs), were derived from human-health risk assessments, Food and Drug Administration (FDA) action levels, or (in the case of TBT) ecological effects. Tissue residues from bioaccumulation testing are compared to the TTLs to assess whether there has been unacceptably high bioaccumulation in benthic organisms resulting from exposure to the test sediments.

10.4.1 Human Health Effects

Most of the TTLs were developed during the PSDDA study for deep-water disposal sites, using consumption rates of bottom fish by recreational anglers, the home range of bottom fish and the size of the Elliott Bay disposal site (EPTA, 1988). For those chemicals with FDA action levels lower than the risk-based concentrations, the FDA action levels were adopted. The TTL for total PCBs was revised in 1999 based on an updated human-health risk assessment that considered subsistence seafood ingestion rates of Native American and Asian/Pacific Islander groups (DMMP, 1999). **Table 10-4** shows the current TTLs used by the DMMP for suitability determinations. DMMUs are compared to the values in this table using the approach described below.

The DMMP updated dioxin guidelines in December 2010, and these guidelines will be used in a case-by-case decision-making approach that is consistent with the narrative human health standard in the SMS rule. A project-specific case-by-case evaluation would be necessary to allow consideration of the disposal of material with dioxin levels higher than 10 pptr-TEQ. Evaluation of material with dioxin concentrations greater than 10 pptr-TEQ may require bioaccumulation testing.

A target tissue level (TTL) to be used in the bioaccumulation evaluation has not been determined for dioxins at this time. In the absence of a TTL, the dredging proponent who selects the option of bioaccumulation testing will be required to include exposure of test organisms to a suitable reference sediment as part of the bioaccumulation test. Concentrations in the project test-sediment tissue would be compared against concentrations in the reference-sediment tissue to determine the bioavailability of sediment dioxin and, thereby, the suitability of dredged material for open water disposal. Over time, a tissue database will be developed, which may allow for the adjustment of this protocol. The explicit interpretative framework for evaluating the dioxin bioaccumulation testing results would need to be developed by the DMMP before testing is initiated.

Generally, interpretation of bioaccumulation test results requires a statistical comparison of the mean tissue concentration of contaminants in animals exposed to dredged material to the TTL. The statistic employed is the one-tailed one-sample t-test (alpha level of 0.05):

$$t = \frac{\bar{x} - TTL}{\sqrt{\frac{s^2}{n}}}$$

where " $\bar{x}$ ", "s^2", and "n" refer to the mean, variance, and number of replicates associated with a contaminant's tissue concentrations from bioaccumulation testing of the proposed dredged material. For undetected chemicals, a concentration equal to one-half the detection limit will be used in the statistical analysis.

Use of the one-sample t-test is necessary to allow experimental results for bioaccumulation testing to be compared to the TTLs, which are constants. A one-*tailed* t-test is appropriate since there is concern only if bioaccumulation from the dredged sediment is not significantly less than the TTL. The null hypothesis in this case is that the tissue concentration is greater than or equal to the TTL.

If the mean tissue concentration of one or more contaminants of concern is greater than or equal to the TTL, then no statistical testing is required. The conclusion is that the dredged material is not acceptable for open-water disposal. If the mean tissue concentration of a chemical of concern is less than the applicable TTL, a one-tailed one-sample t-test is conducted and the dredged material is considered acceptable for open-water disposal if the null hypothesis is rejected.

The RSET SEF has developed TTLs for BCOCs that are protective of human health, which may be considered by DMMP on a case-by-case basis.

10.4.2 Ecological Effects

It should be noted that subsistence human exposures usually drive the lowest TTLs for highly bioaccumulative COCs. The results of a Tier 3 bioaccumulation test will be compared directly with reference results (or ecological TTLs if these are available) for statistical significance. Significant bioaccumulation of chemicals of concern in test species relative to reference areas may demonstrate the potential for food-web effects. For undetected chemicals, a concentration equal to one-half the detection limit will be used in the statistical analysis. If the results of a statistical comparison show that the tissue concentration of the chemical(s) of concern in test sediments is statistically higher (one-tailed t-test, alpha level of 0.1) than the reference sediment, the dredged material will be evaluated further to determine the potential ecological significance of the measured tissue residues.

The four factors summarized below will be reviewed as part of the suitability determination process when bioaccumulation of contaminants in dredged material tests shows significantly higher accumulation of one or more chemicals of concern. In reviewing these factors, the best available regional guidance will be used to assess the relative importance of each factor to the regulatory decision.

1. How many contaminants demonstrate bioaccumulation from dredged material relative to reference sediments?
2. What is the magnitude of the bioaccumulation from dredged material compared to reference sediments?
3. What is the toxicological importance of the contaminants (e.g., do they biomagnify or have effects at low concentrations?). In assessing the toxicological importance, ecologically-based TTLs may be set on a project-specific basis by the regulatory agencies based on a review of the current residue-effects literature. A statistical comparison will be made to ecologically-based TTLs using the one-sample t-test described under human-health effects.

   One exception to the project-specific nature of ecologically-based TTLs is the TTL for TBT (**Table 10-4**), which was adopted from a CERCLA risk assessment (EPA, 1999) for Harbor Island/Elliott Bay that used a weight-of-evidence approach. The TBT TTL represents a residue that is associated with reduced growth in a number of invertebrate species including polychaetes and crustaceans and is, therefore, broadly applicable.
4. What is the magnitude by which contaminants found to bioaccumulate in laboratory test tissues exceed the tissue burdens of comparable species found at or in the vicinity of the disposal site?

If results of the bioaccumulation test in Tier 3 are found to be equivocal, or there is a concern that steady-state body burdens in test organisms were not achieved and/or cannot be estimated,

further testing may be required in Tier 4 before a regulatory decision can be made on the suitability of the dredged material for unconfined open-water disposal.

Table 10-4. Target Tissue Concentration Values for Chemicals of Concern

CHEMICAL	TTL mg/kg ww
Arsenic	10.1
Cadmium	TBD
Chlordane [1]	0.3 *
Chromium	TBD
Copper	TBD
Dioxins/Furans	TBD
Fluoranthene	8400
Hexachlorobenzene	180
Lead	TBD
Mercury	1.0 *
Pentachlorophenol	900
Pyrene	TBD
Selenium	TBD
Silver	200
TBT	0.6 [2]
Total Aroclor PCBs	0.75 [3]
Total DDT [4]	5.0*
Zinc	TBD

Legend:
ww = wet weight; dw = dry weight;
*FDA Action Level
TBD = to be determined on a project-specific basis.
[1] Chlordane includes the chlordane isomers and metabolites cis-Chlordane, trans-Chlordane, cis-Nonachlor, trans-Nonachlor, and oxychlordane
[2] The target tissue level for TBT was derived from a CERCLA risk assessment and is based on site-specific considerations of ecological risk for benthos found in the Harbor Island/Elliott Bay area, but the DMMP concluded it is appropriate for use at other DMMP disposal sites.
[3]The target tissue level for PCBs is based on site-specific considerations of subsistence human exposure in Elliott Bay and may not be appropriate for all disposal sites.
[4] Total DDT is determined by summing the p,p'- isomers of DDT and its metabolites (DDD and DDE).