

**DEVELOPMENT OF A TISSUE TRIGGER LEVEL FOR BIOACCUMULATED
TRIBUTYLTIN IN MARINE BENTHIC ORGANISMS: WEST WATERWAY,
HARBOR ISLAND SUPERFUND SITE, SEATTLE, WA**

**U.S. ENVIRONMENTAL PROTECTION AGENCY
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Background

Tributyltin was identified as a contaminant of potential concern in the marine sediments of the Harbor Island Superfund site as part of EPA's remedial investigation (WESTON 1994). Currently, there are no federal or state sediment quality guidelines or standards for evaluating TBT concentrations in sediment. An interagency work group was formed to identify and evaluate various approaches to derive a sediment effects-based cleanup level for use in Puget Sound (EPA 1996). The work group evaluated available sediment and tissue data sets and concluded that bulk sediment concentrations appeared to be poor predictors of the bioavailable TBT fraction (EPA 1996). Few studies showed good correlations between laboratory bioassay or *in situ* benthic community responses and TBT concentrations in sediments. The group recommended that when TBT is a contaminant of concern in sediment, interstitial water concentrations should be measured and that sediment toxicity testing or *in situ* or laboratory bioaccumulation testing should be conducted to confirm the ecological significance of measured interstitial water concentrations.

For the West Waterway of the Harbor Island Superfund Site, a consortium of potentially responsible parties (PRPs) under an Administrative Order on Consent with EPA evaluated the bioavailability and the potential effects associated with TBT in sediments. The overall purpose of the work was to develop a site-specific, effects-based tissue trigger level that could be used in comparison with bioaccumulation test results to determine the need for cleanup of TBT-contaminated sediments. Effects considered relevant for the development of a site-specific level included mortality, reduced growth, and reproductive impairment. Some of the commonly reported sublethal effects such as bivalve shell thickening or induction of imposex¹ or intersex in gastropod snails were not

¹ Imposex is defined as the development of male sexual characteristics in females. Intersex is characterized as any disturbance of phenotypic sex determination between gonad and genital tract (see Bauer et al. 1997). Although the onset of imposex/intersex was not included as a relevant endpoint for this study, sterilization due to imposex/intersex was included as a relevant endpoint because it was considered a population level effect. Imposex and intersex have only been observed in meso- and neogastropods, which are snails with male and female sexes. The most current list of affected species (Davies 1999 pers. comm.) includes 140 species. Imposex and intersex have not been observed in other gastropods. An analysis of gastropods present in the subtidal benthic community near Harbor Island is presented in Table 2.

included in the evaluation because these endpoints are not population level effects and because of the lack of suitable habitat for the typically affected species (oysters and meso- and neogastropods). The West Waterway is a deep (-30 to -60 ft MLLW), heavily industrialized waterway within the Duwamish River estuary. Very little intertidal habitat is available because of extensive channelization and dredging of the waterway and no commercial or recreational shellfish beds occur. In addition, gastropods are typically not a large component of the Duwamish estuary benthic community and meso- and neogastropods make up only a small fraction of the total gastropod abundance (see Footnote 1).

The evaluation of TBT in West Waterway sediments was composed of two studies. First, a literature review was conducted to identify paired tissue residue and effects data for marine invertebrates and fish (ESI 1999a). The tissue residue data were used to develop site-specific, effects-based trigger concentrations (ESI 1999a). In addition, sediment samples were collected throughout the West Waterway for chemical and biological testing. TBT concentrations were measured in both bulk sediments and interstitial water (filtered and unfiltered) samples; a subset of sediment samples collected were used for bioaccumulation testing. Bioaccumulation testing was conducted to determine site-specific exposures to two marine invertebrate species (a bivalve, *Macoma nasuta* and a polychaete, *Nephtys caecoides*). Effects endpoints were not measured as part of the bioaccumulation study. Sediment toxicity bioassay tests were not conducted for this study because there are no approved toxicity bioassay protocols for test species that have demonstrated a sensitivity to TBT. Based on results of the bioaccumulation tests, tissue TBT concentrations were compared to the effects-based tissue trigger level derived from tissue residue effects data reported in scientific literature.

Development of a Tissue Trigger Level for TBT

The tissue concentrations of TBT associated with effects in marine invertebrates were reviewed for use in developing a trigger concentration. The opportunity for review was provided to natural resource trustees [Muckleshoot and Suquamish Tribes, NOAA, National Marine Fisheries Service (NMFS), Fish and Wildlife Service] and regulatory agencies (Army Corps of Engineers, Washington Departments of Ecology and Natural Resources)², and the PRPs involved in the sediment study for the West Waterway.

The PRPs used two approaches to estimate a site-specific TBT tissue trigger level from the selected data set (see ESI 1999a). Studies for which mortality was the endpoint of concern were not used. In addition, effects concentrations that were estimated from non-linear regression relationships were not included in the data set. The final data set that was used to derive the PRP's site-specific TBT tissue trigger concentration is presented

² Key participants included Karen Keeley (EPA), Erika Hoffman (EPA), Nancy Musgrove (WESTON; EPA Contractor), Jim Meador (NOAA NMFS), Peter Adolphson (Ecology), and Glen St. Amant (Muckleshoot Indian Tribe).

in Table 2-3 of ESI 1999a. In the first approach, the geometric means of paired no-effect and lowest observed effect concentrations were calculated to estimate the range of values within which the trigger level should occur. In the second approach, the 20th percentile of the effects data was calculated after excluding the effects endpoints associated with sterilization due to imposex and intersex in meso- and neogastropods. This resulted in a tissue trigger level of 5 mg TBT/kg dw.

EPA and other participants reviewed the trigger level proposed by the PRPs (ESI 1999a). In response to the limited data set available to derive a screening value (11 sublethal effects data points, 7 no-effects data points), EPA proposed a weight-of-evidence approach for development of a tissue trigger level for TBT. A number of scientifically sound methods are available for deriving sediment criteria and guidelines, but each method has its own advantages and limitations (EPA 1992).

Given the uncertainties in any one method, a tissue trigger level derived from a weight-of-evidence considers all available information. Different methods evaluated in the weight-of-evidence approach were based on sublethal tissue residue effects data for TBT reported in the scientific literature (see Table 2-2 of ESI 1999a), and included:

1. Identification of the lowest observed adverse effect level and the highest observed no-effects level reported in scientific literature for marine invertebrates
2. Calculation of selected percentiles for sublethal effects data for marine invertebrates
3. Estimation of the geometric mean of paired no-effect/low effect tissue data for marine invertebrates
4. Derivation of critical body residues
5. Estimation of a sublethal tissue residue threshold based on application of an acute-to-chronic ratio (based on water-only effects data) to tissue residue effects data for mortality.

LOEL/NOEL - Use of the lowest observed adverse effects level (LOEL) or the highest observed no-effects level (NOEL) is a method commonly applied in ecological risk-based screening assessments. Based on the literature review conducted by ESI (1999a), sublethal effects occurred over a range of tissue concentrations from 0.72 mg/kg (dry weight; dw) to 8.52 mg/kg dw TBT for invertebrates, including gastropods, bivalves, and polychaetes (see Table 2-2 in ESI 1999a). The no-effects data overlapped with the effects data, ranging from 0.3 mg/kg to 4.0 mg/kg dw TBT. A screening level based on the LOEL would be 0.72 mg/kg dw TBT, or would be 1.1 mg/kg dw TBT excluding effects data that were estimated from non-linear regression relationships (see Table 2-2 in ESI 1999a). A screening level based on the NOEL would be 4.0 mg/kg DW TBT. These

values defined the extent of the possible tissue trigger levels using a weight-of-evidence approach.

Percentiles - Another method for deriving a tissue trigger level is based on calculation of a descriptive statistic such as a mean or median for the sublethal effects data³. Given the few available data points and the non-normal statistical distribution, nonparametric methods provide a better representation of the attributes of the sublethal effects data. Percentiles were selected as the method of characterizing the data because of their ability to minimize the effect of extreme data points. Calculation of the 10th and 50th percentile was used because of the similarity to the approach used to develop informal screening guidelines in NOAA's National Status and Trends Program (Long and Morgan 1990). The median (50th percentile) value was 3.75 mg/kg dw TBT and a lower end (10th percentile) value was 1.33 mg/kg dw TBT for the sublethal effects data (see Table 1 of this report and Table 2-2 of ESI 1999a for sublethal effects data).

Geometric Mean - An examination of some of the more sensitive responses to TBT (i.e., Gibbs et al. 1998; Davies et al. 1988; Widdows and Page 1993; Moore et al. 1991; Salazar and Salazar 1998) suggests that a tissue trigger level for sublethal effects based on the geometric mean of no-effects and low effects sublethal data may range from 0.8 to 4.9 mg/kg dw TBT, with an overall geometric mean of 2.61 mg/kg dw TBT (0.52 mg/kg ww TBT). These estimates are based on a comparison of paired no-effect and lowest observed effect data from the five above-referenced studies and a subsequent calculation of the geometric mean of each paired observation and all five pairs combined. This method of using geometric means of no-effect and low effects data as an estimate of the chronic threshold is based on Stephan et al. 1985 (e.g., p. 30).

Critical Body Residue - Selected data from the literature were also used to derive a tissue trigger level based on the critical body residue (CBR) method. This method has been used in regulatory applications for the management of dredged material and setting sediment quality goals for dioxins in the Great Lakes (see EPA 1992, p. 7-1). The CBR predicts toxicity in terms of tissue concentrations based on the relationship between accumulation and response (McCarty and Mackay 1993). Several studies have found that when toxicity is expressed as a tissue residue, the variability between species, exposure periods, and exposure conditions are greatly reduced (McCarty 1991; van Wezel and Opperhuizen 1995; Meador 1997). This approach does not require that an organism be in thermodynamic equilibrium with the sediment contaminant level (see EPA 1992, p. 7-3), which tends to be strongly affected by exposure period⁴. In theory,

³ Studies for which mortality was the endpoint of concern were not used. In addition, effects concentrations that were estimated from non-linear regression relationships were not included in the data set (i.e., Oehlmann et al. 1998; Bauer et al. 1997).

⁴ Note that this is contrary to information provided by the Corps in the Environmental Effects of Dredging Technical Notes (ACOE 1992, p. 3), where normalization based on the time difference was recommended.

the narrow range exhibited in the tissue concentrations for a toxicant is a result of a specific mode of action that is applicable across species. The CBR can be determined directly or with an equation that relates an effect concentration (e.g., LC₅₀, EC₅₀, or LOEC) and a bioconcentration factor (BCF). For example, the CBR was originally developed using the equation $CBR = LC_{50} * BCF$ (McCarty and Mackay 1993). This relationship can be expanded and analyzed by regression analysis to determine the CBR for several species exposed to one toxicant. By plotting the concentration at which a response occurs (LOEC) versus the inverse of the BCF, the slope of the relationship will be the CBR ($LOEC = 1/BCF * CBR$) (Meador, in review).

A CBR for TBT was derived by Meador (in review) based on growth effects and BCFs reported in the literature for six species⁵. A regression analysis examining the relationship between the bioconcentration factor from water and reduced growth (expressed as an LOEC) resulted in an estimate of a critical body residue of 3 mg/kg dw TBT. (The slope of the regression was significant with an r² of 0.99). A recent NMFS study examining the effects of TBT on a single species, the polychaete *Armandia brevis*, showed that significant reductions in growth (25%) occurred at 2.36 mg/kg dw TBT in tissue (Meador and Rice, in prep).

As a final element in the weight-of-evidence approach, it was recommended that the acute-to-chronic ratio derived from the database used to develop ambient water quality criteria (water-only effects data) be applied to lethal effects tissue data presented in Table 3 (from Meador, in review) as an estimate of a sublethal threshold tissue residue concentration. Using an acute-to-chronic ratio of 14.7 (EPA 1997 as cited in Cardwell et al. 1999) and applying it to the mean species residue data associated with mortality (i.e., 48.4 mg/kg dw TBT) results in an estimate of a sublethal effects concentration of 3.29 mg/kg dw TBT.

⁵ Data used for the following species: trout, *Oncorhynchus mykiss* (Martin et al. 1989, Seinen et al. 1981, de Vries et al. 1991, Triesbskorn et al., 1994); polychaete, *Armandia brevis* (Meador and Rice, in prep); polychaete, *Neanthes arenaceodentata* (Moore et al. 1991), oysters, *Saccostrea commercialis* and *Crassostrea gigas* (Batley et al. 1989); and mussel, *Mytilus edulis* (Guolan and Yong 1995). None of these studies included an effect endpoint of sterilization due to imposex, but Batley et al. 1989 evaluated shell deformation. It is recognized that the CBR method uses both invertebrate and fish tissue sublethal effects data, while the other three methods in this weight-of-evidence approach use only invertebrate sublethal effects data. This is acceptable because the basis of the CBR method is that different species will respond similarly to similar tissue concentrations of a given chemical (see McCarty and Mackay 1993).

Although Guolan and Yong (1995) was rejected in ESI (1999a; see Table B-1), Meador (in review) included these data in the derivation of CBR. Guolan and Yong 1995 present tissue residues for gill plus viscera separately from muscle plus mantle. Based on their data, the concentrations for gill and viscera will be higher than the whole body concentrations, so a concentration for gill and viscera would overestimate the whole body concentrations. For the direct CBR method, these data are useful because it appears that the whole body tissue residue would be similar to a residue calculated with either group of tissues. Meador (in review) used the BCF (based on gill plus viscera) and LOEC.

A summary of the results of the weight-of-evidence approach is provided below (units are dw):

- 1.1 mg/kg TBT (LOEL based on gastropod sterilization, excluding estimated values; see Table 2-2 in ESI 1999a)
- 1.33 mg/kg TBT (10th percentile of sublethal effects; Table 1)
- 2.4 mg/kg TBT (*Armandia* study; Meador and Rice, in prep)
- 2.61 mg/kg TBT (average on overall geometric mean from paired observations)
- 3.0 mg/kg TBT (CBR from six species; Meador, in review)
- 3.29 mg/kg TBT (estimate based on acute-to-chronic ratio and lethal tissue residue)
- 3.75 mg/kg TBT (50th percentile of sublethal effects; Table 1)
- 4.0 mg/kg TBT (highest NOEL based on reduced mussel growth; see Table 2-2 in ESI 1999a).

Recommendations

Based on the weight-of-evidence approach, it is recommended that 3 mg/kg dw TBT⁶ (approximately 0.6 ppm ww TBT, assuming 20 percent moisture content) be used as the site-specific tissue trigger level for evaluating bioaccumulation data from the West Waterway. This level has been shown to be the tissue residue associated with reduced growth in a number of invertebrate species, including polychaetes and crustaceans. This level is very similar to the overall geometric mean of paired effect/no-effect data and the estimate of a sublethal effects level based on a multi-species acute-to-chronic effects ratio. In addition, a site-specific tissue trigger level based on the CBR approach tends to address a number of uncertainties associated with derivation of sediment effects criteria including the bioavailability of a chemical, its equilibrium state, the effects of confounding factors such as sediment type or organic content and mode of toxicity affecting the organism.

This proposed value should also be protective of bivalves and most gastropods for the growth and reproduction endpoints. This level may not protect the most sensitive species of meso- and neogastropods (e.g., dog whelks, periwinkles); however, meso- and neogastropods tend to be rare in Elliott Bay and the Duwamish River. As an example, within Elliott Bay gastropods make up from 2 to 5 percent of the total benthic community abundance and richness, while meso- and neogastropods make up only a small fraction of the gastropod population (see Table 2 of this report).

Advantages and Limitations

⁶ The tissue trigger level is reported in dry weight (dw) units because the dry weight basis is recommended in EPA 1993 (p. 122), and this basis reduces variability among species as water content varies.

Tissue residue-effects data and bioaccumulation data are increasingly being used in various regulatory and resource management programs (Jarvinen et al. 1998). Tissue residue-effects data have also been used in the development of water quality criteria that are protective of food chain effects in fish and wildlife (EPA 1992, p. 7-8). The Corps of Engineers has used bioaccumulation data as part of their process of determining unacceptable adverse impacts associated with potential disposal of dredged materials for over 20 years (ACOE 1999). The Oceans Testing Manual (a joint ACOE and EPA document) requires that bioaccumulation data be used within the context of whether or not an adverse impact would occur (ACOE 1999). In support, the Corps has compiled paired tissue residue and effects data for evaluation of bioaccumulation data for selected compounds in the Environmental Residue-Effects Database (ERED). Tissue residues form the basis of establishing exposure and linking it to effects in risk assessments (Field 1998). Other state and federal sediment or resource management programs (e.g., NMFS, Ecology) in Puget Sound either use or are considering use of tissue residue data as the basis for sediment management decisions.

The shift to bioaccumulation or tissue residue data to support program goals is based on some of the advantages associated with measures of chemical concentrations in receptors of concern. Tissue data integrate multiple exposure pathways (dermal contact, ingestion, respiration). In general, tissue residues provide a direct measure of exposure that is typically only predicted or assumed under other methods. Tissue data can also address food chain effects that are not represented in other current assessment methods. When the aquatic organism is used as human food, tissue residues can provide a direct link between human health and sediment criteria (EPA 1992, p. 7-5). When combined with effects measures, tissue data provide a strong link between a source of contamination and impacts.

Development of tissue residue effects thresholds is part of EPA's overall strategy for management of contaminated sediments throughout the United States (EPA 1994, p. 100). The method is most applicable to nonpolar organics or organometallics that are slowly metabolized and can cause chronic effects in exposed populations (EPA 1992, p. 7-5). For those chemicals (specifically metals) that can be sequestered by aquatic organisms in non-available forms, the linkage between tissue residues and effects data will tend to be misrepresented. Use of a tissue residue-effects approach also relies on the identification of species that are sensitive to the contaminants of concern.

Tissue residue and effects data do not directly identify to what level sediments should be remediated without additional predictions or investigations. Additional steps, such as measurement of sediment chemistry and other factors affecting partitioning or calculation and application of biota-sediment accumulation factor, may be required to determine sediment thresholds associated with significantly elevated tissue residues. In most cases, site-specific sediment-biota partitioning coefficients are unavailable. However, paired-sediment tissue data have been collected at a number of sites in Puget Sound and the types of investigations that would be required have well-established protocol.

Application and Use of the Tissue Trigger Level

The recommended tissue trigger level was designed to address the bioaccumulation and potential effects of TBT in sediments in the West Waterway of the Harbor Island Superfund site. As such, it takes into account site-specific resources and sediment conditions. It is not meant to be directly applicable to other sites making decisions about sediment remediation. Although this approach may have utility at other sites, direct measures of effects or other assessment methods may be more useful, and all reasonable approaches should be evaluated. Finally, sediment and porewater data collected to support the West Waterway sediment management decisions should not be viewed as representing threshold values that could be applied to cleanup of those media at other sites.

Future Considerations

In future studies of TBT-contaminated sediments at other sites, the following information should be considered:

- Based on results of the TBT study for Harbor Island (ESI 1999b), use a bulk sediment TBT screening value rather than porewater screening value based on the observed correlation between sediment and tissue TBT concentrations
- Consider measuring the health of test organisms during bioaccumulation testing (e.g., include an effects endpoint)
- Identify potential test species that have high Biota-Sediment Accumulation Factors for TBT
- Given the different types of habitat present at sites, consider performing field evaluations to identify the presence of imposex/intersex in intertidal snail populations or measuring TBT tissue concentrations of TBT in field collected organisms (e.g., oysters).

Other considerations may develop as additional studies on TBT are completed.

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Table 1. Percentile Calculations for TBT Sublethal Effects Tissue Data (based on data from Table 2-2 in ESI 1999)

TBT LOAEL (ug/kg ww)	Data Source	TBT LOAEL (ug/g dw)	Data Source
0.22	Oehlmann et al. 1996	1.1	Oehlmann et al. 1996
0.278	Gibbs et al. 1988	1.39	Gibbs et al. 1988
0.53	Bailey and Davies 1991	2.65	Bailey and Davies 1991
0.75	Davies et al. 1988	3.39	Bryan et al. 1987
0.94	Moore et al. 1991	3.75	Davies et al. 1988
1.09	Widdows and Page 1993	5.44	Widdows and Page 1993
1.13	Bryan et al. 1987	6.0	Salazar and Salazar 1998
1.2	Salazar and Salazar 1998	6.27	Moore et al. 1991
2.84	Bryan et al. 1987	8.52	Bryan et al. 1987
	Percentile		Percentile
0.24	5th percentile	1.22	5th percentile
0.27	10th percentile	1.33	10th percentile
0.33	15th percentile	1.64	15th percentile
0.53	25th percentile	2.65	25th percentile
0.94	50th percentile	3.75	50th percentile
1.13	75th percentile	6.00	75th percentile
1.19	85th percentile	6.22	85th percentile
1.53	90th percentile	6.72	90th percentile
2.18	95th percentile	7.62	95th percentile
9	Sample size	9	Sample size

Table 3. Whole-body Tributyltin Tissue Residues Associated with Mortality (from Meador, in review)

Species	Tissue Residue $\mu\text{g/g}$ (dw) ^c	Endpoint	Data Source
<i>Rhepoxynius abronius</i> ^a	58	LR ₅₀ ^b	Multiple studies
<i>Eohaustorius washingtonianus</i> ^a	42	LR ₅₀ ^b	Multiple studies
<i>Armandia brevis</i> ^a	65	LR ₅₀ ^b	Multiple studies
<i>Platichthys stellatus</i>	49	LR ₅₀ ^b	Meador 1997
<i>Eohaustorius estuarius</i>	59	LR ₅₀ ^b	Meador 1997
<i>Hyalella azteca</i>	74	LR ₅₀ ^b	Borgmann et al 1996
<i>Oncorhynchus mykiss</i> ^d	32	LR ₅₀ ^b	Hodson et al. 1988
<i>Scrobicularia plana</i>	67	100% mortality	Langston and Burt 1991
<i>Oncorhynchus tshawytscha</i>	37	100% mortality	Short and Thrower 1987
<i>Poecilia reticulata</i>	33	100% mortality	Tas 1993
<i>Neanthes arenaceodentata</i>	17	79% mortality	Moore et al. 1991
OVERALL MEAN	48.4		

^a Tissue residue represents species mean. Data from Meador (1993), Meador et al. (1993), Meador et al. (1997), and Meador (1997).

^b LR₅₀ is the tissue residue associated with 50 percent mortality.

^c Assumed dry weight to wet weight ratio = 0.2.

^d *Oncorhynchus mykiss* was previously *Salmo gairdneri*.

