ERDC TN-DOER-R5
September 2004

Analysis of Uncertainty in Estimating Dioxin Bioaccumulation Potential in Sediment-Exposed Benthos

PURPOSE: This technical note demonstrates two methods for analyzing uncertainty in estimating theoretical bioaccumulation potential (TBP) in benthic organisms residing in or on sediments contaminated with polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDD/F). An additional question addressed is whether congener-specific biota/sediment accumulation factors (BSAFs) in the TBP calculation provide a more certain estimate than simply using a generalized BSAF representing all congeners.

BACKGROUND: Regulatory decisions regarding the suitability of dredged sediment for disposal in open waters of the United States are made using a tiered approach, as described in the implementation manuals for the Federal Clean Water Act, Section 404 and Section 103 of the Marine Protection, Research, and Sanctuaries Act (U.S. Environmental Protection Agency/U.S. Army Corps of Engineers (USEPA/USACE) 1991, 1998). TBP is a simple equilibrium partitioning-based model recommended in both manuals for estimating levels of neutral organic chemicals that could result in the tissues of organisms exposed to sediments. The model is used in a screening mode to indicate whether the sediments in question are either so clean or so contaminated that a decision regarding disposal can be made at that point, or to indicate the necessity of definitive (and more costly) bioaccumulation testing.

The model delivers a point estimate, expressed as a probable organism tissue concentration of a chemical of interest, given a known concentration of the chemical in sediment and the organic carbon content of the sediment. TBP incorporates a partition coefficient, BSAF, which is the ratio of lipid-normalized concentration of a chemical in an organism to organic carbon-normalized concentration of the chemical in sediment to which the organism is exposed. BSAFs for a given chemical can vary over orders of magnitude in different species and sediments. The BSAF value used in the model, the sediment chemical concentration, the sediment organic carbon content, and the lipid content of the target organism all contribute uncertainty to the TBP model. Without an indication of the range of uncertainty in the calculated TBP, the model has very limited predictive capability. For that reason studies were sponsored by the USACE Dredging Operations and Environmental Research (DOER) Program to evaluate and apply methods for uncertainty analysis to the calculation of TBP.

INTRODUCTION: In a previous study, two methods of uncertainty analysis were compared: (1) a computational method using root-sums-of-squares (RSS) to combine random and systematic error, and (2) a simulation method that uses bootstrap resampling of replicated model input parameters to calculate statistical uncertainty parameters. These were used to test the degree of correspondence between TBP estimations made from sediment chemistry data and the actual tissue concentrations of polynuclear aromatic hydrocarbons (PAH) in organisms exposed to the sediments (McFarland and Clarke 1999, Clarke and McFarland 2000). The uncertainty

methods are explained in the references cited, and results obtained with each method are reported.[1] The RSS computational method is relatively simple and easily incorporated into a spreadsheet. This method combines systematic method error[2] and propagated measurement error (random and systematic) to calculate total error (TE). The range of uncertainty is the value of TBP ± TE. For PAHs, TBP uncertainty by RSS was less than a factor of two in most cases, and was always less than a factor of five. The bootstrap method is computer intensive but is more precise, and provides nonparametric statistical uncertainty measures such as standard errors and confidence intervals. Tests of significance can also be performed. Using bootstrap statistics on the same PAH data set, TBP uncertainty was always less than a factor of two. These methods are similarly applied to calculate TBP uncertainty for PCDD/Fs in the present study.

METHODS:

Experimental Design. Dioxin and dibenzofuran concentration data of coastal shelf sediments, organic carbon content of the sediments, lipid content of the biota collected with the sediments, and BSAFs calculated from data contained in the USACE ERDC BSAF database[3] provided input parameters for the TBP model:

$$TBP = BSAF*(Cs/fOC)*fL \quad (1)$$

where

Cs = concentration in sediments, pg g^{-1}, dry weight
fOC = decimal fraction sediment organic carbon, dry weight
fL = decimal fraction organism lipid, wet weight
BSAF = biota/sediment accumulation factor (unitless)
TBP = theoretical bioaccumulation potential, pg g^{-1}, wet weight

Uncertainty was computed for TBP using both the RSS and bootstrap methods. Tissue concentrations of PCDD/Fs measured in the biota collected with the sediments were then compared with the predicted concentrations (TBP) and their ranges of uncertainty.

Field Collections. Sediments and resident organisms were collected using a 0.1-m^2 Smith-McIntyre grab sampler in the New York Bight Apex, at an area surrounding 40°20.48' North, 73°52.34' West during 20-24 August, 1991. Sediment samples were emptied into plastic sorting trays prepared by acid washing, hexane rinsing, and rinsing with site water prior to use. A 0.5-L aliquot of sediment was removed from each grab sample and composited. At the end of each sampling day, the composited sediments were homogenized, large organisms were removed by hand, and five 1-L replicate samples were placed in acid-cleaned, hexane-rinsed glass jars with teflon lids. Care was taken to ensure that no head space remained in the containers. The samples were stored under refrigeration (4°C) until the end of the field sampling effort, then shipped in

[1]McFarland and Clarke (1999) is available in pdf format on the USACE Engineer Research and Development Center website: *http://www.wes.army.mil/el/dots/eedptn.html* . Readers are referred here for explanations of TBP, BSAFs, and calculation of RSS uncertainty.

[2]Systematic method error is the error inherent in the model itself, were all input data measured with perfect accuracy. It is estimated by comparison of model predictions to actual bioaccumulation.

[3]The Engineer Research and Development Center Biota/Sediment Accumulation Factor (BSAF) and Lipid Database can be found at: *http://www.wes.army.mil/el/dots/database.html*

coolers via overnight air freight to the U.S. Army Engineer Research and Development Center (ERDC), Waterways Experiment Station (WES) for physical and chemical characterization.

The remainder of each sediment sample was sorted in the field to collect organisms. This was accomplished by washing the sediment through 0.5-mm mesh sorting screens with collection site seawater to separate the organisms from the sediment. No single grab contained sufficient organisms of any taxonomic group to be treated as a sample for chemical analysis. Therefore, organisms were collected and pooled by taxa in plastic sorting trays. The taxonomic identification of the benthos collected and wet weight in grams of each are given in Table 1. The collected organisms were maintained alive in fresh collection site seawater until the end of each day, when a decision was made as to which taxonomic groups had sufficient biomass for chemical determination. These organisms were then placed together in plastic Whirl-Pac® bags and frozen on board the vessel. All molluscs were frozen in their shells with the exception of *Mercenaria mercenaria*, which were shucked on board the vessel due to their large size. Insufficient Crustacea were found to constitute a pooled sample, and those that were found were not analyzed. Five pooled samples resulted: (1) Mollusca (bivalves), (2) Lumbrineridae, (3) *Nephtys sp.* (Polychaeta), (4) Misc. Polychaetes, and (5) *Cerebratulus lacteus* (Nemertea). Samples were stored in shipboard freezers at < 0°C, until shipped to WES by overnight air freight for subsampling and preparation for chemical analysis.

Table 1
Benthic Organisms and Weights (g) of Pooled Samples Collected at the New York Bight Apex Site

Polychaetes		**Crustaceans**	
Nephtys sp. (incisa or picta)[1]	119	Isopods	5
Unknown worm parts	105	Assorted	8
Pherusa sp. (affinis)	24		
Lumbrineridae	393	**Molluscs**[2]	
Glycera sp.	48	*Nucula sp.*	94
Diopatra cuprea	12	Unsorted molluscs (primarily *Nucula* and shell hash)	459
Orbiniidae	21	*Astarte sp.*	198
Opheliidae	2	*Nassarius trivittatus*	4
Sigalion arenicola	6	*Ensis correctus*	44
		Yoldia limatula	6
Nemerteans		*Spisula solidissima*	62
Cerebratulus lacteus	82	*Mercenaria sp. (mercenaria)*	60

[1]Species names in parentheses are tentative identifications.
[2]Mollusc weights include shells.

Chemical Analysis. All PCDD/F tissue and sediments were analyzed at the Battelle Columbus Dioxin Laboratory, Ohio. Samples were analyzed following a revised version of Method 8290 for dioxin/furan analysis (USEPA 1996). Following extraction, the seventeen 2,3,7,8-chlorine-substituted PCDD/F congeners and homologue groups were analyzed using combined capillary column gas chromotography/high resolution mass spectrometry (GC/MS).

Lipids were determined gravimetrically on a 100-µL aliquot of a dichloromethane extract removed prior to clean-up steps for GC/MS analyses, and blown to dryness under N_2. The residue remaining after drying was weighed in a tared pan on a Cahn C31 electronic microbalance. Lipids were reported as a percentage of the sample wet weight. Although only a

few (2 to 4) replicates could be analyzed due to insufficient quantity of material, the results for most species were consistent among the replicate samples.

Total organic carbon (TOC) in sediment samples was analyzed using EPA method 415.1 (USEPA 1983). The organic carbon in the sample was converted to carbon dioxide by wet chemical oxidation and the CO_2 evolved was measured by infrared on an Oceanographic International Model 700™ TOC analyzer. Sediment samples were prepared externally using the ampule method to convert TOC to CO_2.

BSAF Derivation. Seven published studies were found in the ERDC BSAF and Lipid Database reporting BSAFs of benthically coupled organisms for PCDD/F congeners or homologues (Table 2). Most of the reports were of field studies and most were freshwater regimes. The freshwater organisms included four species of fish, three species of mussel, and one each of insect, crayfish, and shrimp. Of the saltwater organisms, two species were clam and two were crab. Most of the tissues reported were whole body, but two studies reported BSAFs calculated for ovary and liver in fish and hepatopancreas in crabs. No data were excluded from the compiled dataset. In all, 97 usable BSAF values were found in the database. In addition to these, 52 BSAF values were added to the dataset from an unpublished report in which the oligochaete, *Lumbriculus variegatus*, was exposed in the laboratory for 28 days to sediments from three sites in Lake Ontario.[1] Most of the BSAF information included sample size (*n*) and an estimate of variability (usually standard error of the mean). Fractional *n*'s sometimes resulted for the Lake Ontario BSAFs when *n* was estimated as the mean of differing sample sizes for the four BSAF input parameters.

Table 2
Organisms Included in the ERDC-WES BSAF Database for which PCDD/F BSAFs Were Pooled to Calculate BSAFs and Statistics of Table 3

Species	Type of Organism	Fresh or Salt	Type of Study	Tissue	Reference
Macoma nasuta	Clam	S	Lab	Whole Body	Pruell et al. (1993)
Corbicula japonica	Clam	S	Field	Whole Body	Kang et al. (2002)
Callinectes sapidus	Crab	S	Field	Hepatopan.	Schell, Campbell and Lowe (1993)
Cancer magister	Crab	S	Field	Hepatopan.	Yunker and Cretney (2000)
Orconectes virilis	Crayfish	F	Field	Whole Body	Currie et al. (2000)
Ameiurus melas	Fish	F	Field	Ovary	Schell, Campbell and Lowe (1993)
Cyprinus carpio	Fish	F	Lab	Whole Body	Kuehl et al. (1987)
Catostomus commersoni	Fish	F	Field	Whole Body	Currie et al. (2000)
Ictalurus nebulosus	Fish	F	Field	Liver	Schell, Campbell and Lowe (1993)
Hexagenia sp.	Insect	F	Field	Whole Body	Currie et al. (2000)
Dreissena polymorpha	Mussel	F	Field	Whole Body	Marvin et al. (2002)
Dreissena bugensis	Mussel	F	Field	Whole Body	Marvin et al. (2002)
Pyganodon grandis	Mussel	F	Field	Whole Body	Currie et al. (2000)
Lumbriculus variegatus	Oligochaete	F	Lab	Whole Body	Pickard (2002)[1]
Palaemonetes pugio	Shrimp	F	Lab	Whole Body	Pruell et al. (1993)

[1]Unpublished data.

[1] Scott W. Pickard, CELRB-TD-OT, USACE District, Buffalo, 1776 Niagara Street, Buffalo, NY, 14207, USA, 716-879-4404.

Mean BSAFs for each of the seventeen 2,3,7,8-Cl-substituted PCDD/F congeners and for homologue groups are reported with relevant statistics in Table 3. The BSAF distributions were skewed for many of the congeners and homologue groups (Figure 1). Total error for each of the mean BSAFs was estimated as follows:

1. Calculate mean square error (MSE) as an estimate of the "within treatment" variance based on individual sample variances s_i^2, where

$$\text{MSE} = \Sigma [\ s_i^2\ (n_i - 1)\ ] / \Sigma\ (n_i - 1) \quad (2)$$

2. Calculate overall variance for each congener or homologue group as an estimate of the "between treatment" variance, using all of the BSAFs for each chemical.

3. Overall $N = \Sigma\ n_i$ for each chemical[1] (3)

4. Total Error = $[\ (\text{MSE} + \text{Overall Variance}) / \text{Overall } N\ ]^{1/2}$ (4)

The total error for each mean BSAF was then used as a standard error to derive 95-percent confidence intervals (Table 3):

$$\text{CI} = \text{mean BSAF} \pm t_{0.95,\text{overall N-1}} * \text{total error} \quad (5)$$

Table 3
Dioxin and Dibenzofuran BSAFs and Summary Statistics for Benthically Coupled Biota Using Studies Cited in Table 2

PCDD/F	Mean BSAF	Overall N[1]	Total Error	t	Lower 95% CL	Upper 95% CL
2,3,7,8-TCDD	0.433	85.5	0.297	1.663	-0.061	0.927
1,2,3,7,8-PeCDD	1.128	91.5	0.272	1.662	0.675	1.581
1,2,3,4,7,8-HxCDD	1.283	8.5	0.328	1.876	0.668	1.898
1,2,3,6,7,8-HxCDD	0.211	9.5	0.063	1.845	0.095	0.326
1,2,3,7,8,9-HxCDD	0.514	111.5	0.063	1.659	0.410	0.619
1,2,3,4,6,7,8-HpCDD	0.050	11.5	0.019	1.804	0.016	0.084
OCDD	0.039	10.5	0.021	1.822	0.002	0.077
2,3,7,8-TCDF	0.899	257.5	0.475	1.651	0.115	1.683
1,2,3,7,8-PeCDF	1.093	66.5	0.352	1.668	0.507	1.680
2,3,4,7,8-PeCDF	0.550	68.5	0.259	1.668	0.118	0.982
1,2,3,4,7,8-HxCDF	0.288	39.5	0.285	1.685	-0.192	0.768
1,2,3,6,7,8-HxCDF	1.105	114.5	0.159	1.658	0.842	1.367
1,2,3,7,8,9-HxCDF	0.623	8.5	0.157	1.876	0.328	0.917
2,3,4,6,7,8-HxCDF	0.463	9.5	0.086	1.845	0.304	0.622
1,2,3,4,6,7,8-HpCDF	0.032	9.5	0.009	1.845	0.015	0.048
1,2,3,4,7,8,9-HpCDF	0.252	8.5	0.043	1.876	0.171	0.333
OCDF	0.033	10.5	0.008	1.822	0.019	0.047
Total TCDD	0.255	2	0.005	6.314	0.223	0.287
Total PeCDD	0.039	2	0.019	6.314	-0.081	0.159
Total HxCDD	0.038	2	0.013	6.314	-0.041	0.116
Total HpCDD	0.019	2	0.011	6.314	-0.051	0.089
Total TCDF	0.187	7	0.058	1.943	0.074	0.299
Total PeCDF	0.151	5	0.064	2.132	0.014	0.289
Total HxCDF	0.079	2	0.051	6.314	-0.243	0.401
Total HpCDF	0.029	2	0.021	6.314	-0.102	0.160
ALL	0.573	945.5	0.139	1.646	0.344	0.802

[1]Overall N was estimated as the mean of differing sample sizes for the four BSAF input parameters (Cs, TOC, Ct, lipid)

[1] When n was not given for an individual BSAF, a default minimum sample size was used: $n = 2$ if a standard error was given, or $n = 1$ if no standard error was given. This results in conservative confidence intervals.

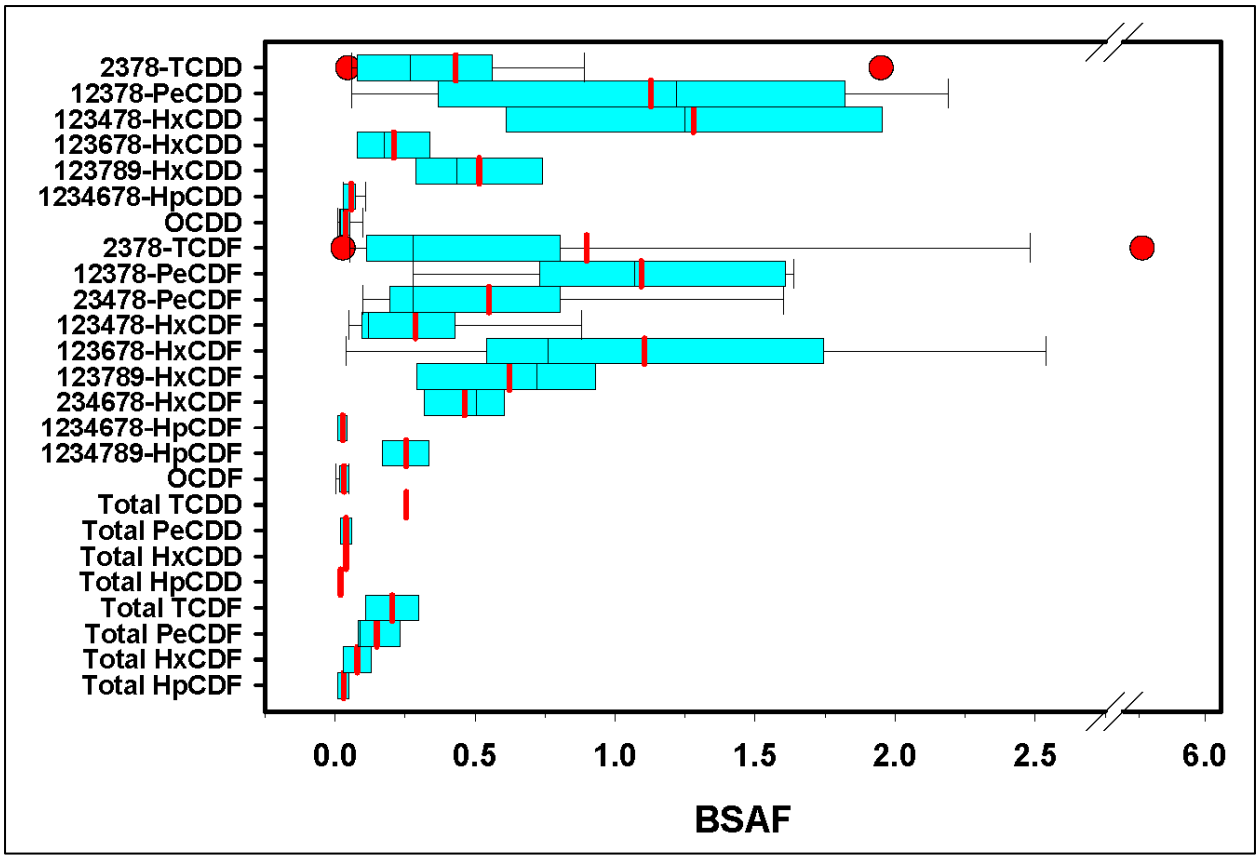

Figure 1. Boxplot of PCDD/F BSAFs calculated from data of studies cited in Table 2. Boxes are medians, 25th and 75th percentiles. Whiskers are 10th and 90th percentiles. Balls are 5th and 95th percentiles. Red (bolded) vertical lines are means

UNCERTAINTY ANALYSES: Estimates of uncertainty for TBP were determined using RSS and bootstrap methods. The latter was also used to conduct nonparametric tests of significance comparing TBP with measured mean tissue concentrations Ct. Calculations were performed using individual mean BSAFs for each PCDD/F congener or homologue, and also an overall mean BSAF for all PCDD/Fs.

Root Sum of Squares (RSS) Uncertainty. The first step in estimating the bioaccumulation potential of a chemical in a sediment and the uncertainty surrounding the estimate is to calculate TBP using Equation 1 with a BSAF for the chemical, relevant sediment concentration, organic carbon content, and lipid content of a target organism, as in the data of Tables 3 and 4. For example, substituting the data for 2,3,7,8-TCDD:

$$\text{2,3,7,8-TCDD TBP} = 0.433 * (0.474/0.0050365) * 0.0331176 = 1.350 \quad (6)$$

Table 4
TBP and RSS Calculation Data and Comparison of TBP with Measured Tissue Concentration

PCDD/F	Cs[1]			TBP[2] pg g^{-1} w.w.	Ct[3] mean	Relative % difference[4]
	mean	n	SE			
2,3,7,8-TCDD	0.474	4	0.091	1.350	1.774	-23.9
1,2,3,6,7,8-HxCDD	0.598	4	0.092	0.828	1.051	-21.2
1,2,3,7,8,9-HxCDD	0.608	4	0.133	2.057	1.047	96.4
1,2,3,4,6,7,8-HpCDD	4.390	4	1.750	1.438	3.323	-56.7
OCDD	46.086	4	18.359	11.964	25.639	-53.3
2,3,7,8-TCDF	0.718	4	0.250	4.241	3.163	34.1
1,2,3,4,7,8-HxCDF	0.458	4	0.165	0.866	0.796	8.7
1,2,3,6,7,8-HxCDF	0.203	4	0.033	1.471	0.666	121.0
1,2,3,7,8,9-HxCDF	0.328	4	0.047	1.341	0.786	70.5
1,2,3,4,6,7,8-HpCDF	1.659	4	0.554	0.344	4.858	-92.9
OCDF	3.039	4	1.086	0.656	1.741	-62.3
Total TCDD	0.521	4	0.077	0.873	3.477	-74.9
Total HxCDD	2.580	4	1.074	0.636	3.617	-82.4
Total HpCDD	6.709	4	2.949	0.836	12.004	-93.0
Total TCDF	2.609	4	1.229	3.202	15.924	-79.9
Total PeCDF	1.401	4	0.783	1.394	4.789	-70.9
Total HxCDF	1.934	4	0.693	1.005	2.598	-61.3
Total HpCDF	2.341	4	0.706	0.450	4.137	-89.1

[1]Cs: Concentration in sediment, pg g^{-1} d.w.
[2]fOC: mean = 0.0050365 d.w., n = 4, SE = 0.00161952; fL: mean = 0.0331176 w.w, n = 17, SE = 0.0036175
[3]Ct: Concentration in pooled tissues, pg g^{-1}, w.w.
[4](TBP – mean Ct)*100/mean Ct.

In order to put bounds on the point estimate (TBP = 1.35 pg g^{-1}) using the RSS method, first the method error and propagated error are estimated, and then they are combined. Method error (ME) is the error inherent in the model equation itself, and would remain even if all of the input parameters could be measured with perfect accuracy. The average ME is found using a data set containing both the input parameters for calculating TBP (mean Cs, mean fOC, and mean fL) and the associated measured tissue concentration of sediment-exposed biota (mean Ct), assuming no error in the input values. The average ME is calculated by:

$$\text{avg ME} = \sum |[\ 100 - (\text{TBP} * 100)/\text{Ct}\ ]|/n \quad (7)$$

The average TBP ME using the ERDC-BSAF Database-derived PCDD/F BSAFs in Table 3 and the New York Bight pooled sediment and pooled organism data in Table 4 was found to be 62.7 percent.[1]

Using this value, the ME for each PCDD/F congener TBP estimation was calculated as:

$$ME_i = TBP_i * \text{avg ME}/100 \quad (8)$$

[1]The average percent method error was calculated excluding the data for 1,2,3,7,8,9-HxCDD and 1,2,3,4,6,7,8-HpCDF because all replicate tissue concentrations were reported as < DL for these congeners. This was an operational decision based on the consideration that in the absence of any quantifiable concentrations within a given set of replicates, no acceptable estimates could be made.

For example,

$$\text{2,3,7,8-TCDD TBP ME} = 1.350 * 62.7/100 = 0.847 \quad (9)$$

Propagated error for each TBP estimation (PE_i) is calculated for each of the TBP input parameters separately and then combined, adaptating the method of Campbell (1982) for multiple input values.

1. The mean and the lower and upper 95-percent confidence limits (CL_{Li} and CL_{Ui}) of each of the TBP input parameters are used to first calculate TBP at each limit (TBP_{Li} and TBP_{Ui}) by substituting in Equation 1 each input parameter CL_{Li} and CL_{Ui} for that parameter and the means of all other parameters. For 2,3,7,8-TCDD these are:

Parameter	Mean	SE	df	t	CL_{Li}	CL_{Ui}	TBP_{Li}	TBP_{Ui}
BSAF	0.433	0.297	84.5	1.663	-0.061	0.927	-0.191	2.891
Cs	0.474	0.091	3.0	2.353	0.261	0.687	0.744	1.957
fOC	0.005	0.002	3.0	2.353	0.001	0.009	5.550	0.769
fL	0.033	0.004	16.0	1.746	0.027	0.039	1.093	1.608

2. Propagated error for each input parameter is calculated as:

$$PE_i = ((TBP_{Ui} - TBP_{Li})^2 / 4)^{½} \quad (10)$$

3. Overall propagated error is:

$$PE = (\Sigma PE_i^2)^{½} \quad (11)$$

For the 2,3,7,8-TCDD example, the results are:

Parameter	PE_i	PE_i^2
BSAF	1.5407	2.374
Cs	0.6064	0.368
fOC	2.3909	5.716
fL	0.257	0.066
Overall Propagated Error PE		2.920

4. Total error of TBP for each PCDD/F congener or homologue group is:

$$TE = (ME^2 + PE^2)^{½} \quad (12)$$

For the 2,3,7,8-TCDD example,

$$TE = (0.847^2 + 2.920^2)^{½} = 3.040 \quad (13)$$

The range of uncertainty using the RSS method for the example is 2,3,7,8-TCDD TBP ± TE = 1.35 ± 3.04, or between < 0.0 and 4.390 pg g^{-1} (Table 5). The mean measured tissue

concentration was 1.774 pg g^{-1} (Table 4). In this case TBP underestimated the measured tissue concentration by about 24 percent (relative percent difference, Table 4), and Ct was well within the RSS range of uncertainty (Table 5).

Above formulae and additional explanation are given in McFarland and Clarke (1999) and Clarke and McFarland (2000).

Table 5
Method Error, Propagated Error, RSS Total Error, TBP Total Error Limits, Error Ratio, and Comparison with Measured, Pooled Tissue Concentrations of Dioxins and Furans at the New York Bight Apex Benthic Sampling Location

PCDD/F	ME[1]	PE[2]	TE[3]	TBP - TE	TBP + TE	TE Interval Width ÷ TBP	Ct[4] within TE Interval?
2,3,7,8-TCDD	0.847	2.920	3.040	-1.690	4.390	4.5	Within
1,2,3,6,7,8-HxCDD	0.519	1.572	1.656	-0.827	2.484	4.0	Within
1,2,3,7,8,9-HxCDD	1.290	3.837	4.048	-1.991	6.105	3.9	Within
1,2,3,4,6,7,8-HpCDD	0.902	3.059	3.189	-1.751	4.627	4.4	Within
OCDD	7.503	26.635	27.671	-15.707	39.636	4.6	Within
2,3,7,8-TCDF	2.660	9.102	9.483	-5.242	13.724	4.5	Within
1,2,3,4,7,8-HxCDF	0.543	2.237	2.302	-1.436	3.167	5.3	Within
1,2,3,6,7,8-HxCDF	0.922	2.704	2.857	-1.386	4.327	3.9	Within
1,2,3,7,8,9-HxCDF	0.841	2.512	2.649	-1.308	3.990	4.0	Within
1,2,3,4,6,7,8-HpCDF	0.216	0.692	0.725	-0.381	1.069	4.2	Above
OCDF	0.411	1.323	1.385	-0.729	2.041	4.2	Within
Total TCDD	0.547	1.588	1.680	-0.807	2.553	3.8	Above
Total HxCDD	0.399	1.861	1.904	-1.268	2.540	6.0	Above
Total HpCDD	0.524	3.527	3.565	-2.729	4.401	8.5	Above
Total TCDF	2.008	6.989	7.272	-4.070	10.474	4.5	Above
Total PeCDF	0.874	3.335	3.448	-2.054	4.841	4.9	Within
Total HxCDF	0.630	4.548	4.591	-3.587	5.596	9.1	Within
Total HpCDF	0.282	2.194	2.212	-1.762	2.662	9.8	Above
						Avg = 5.2	

[1]Method Error (avg ME = 62.7 percent).
[2]Propagated error.
[3]Total error.
[4]Ct from Table 4.

Bootstrap Uncertainty. Bootstrap estimates of TBP means and standard errors were derived using SAS procedures and programming language to generate 1,000 bootstrap resamples[1] of each TBP input parameter. Means of the resamples were inserted in Equation 1 to generate 1,000 bootstrap estimates of TBP for each PCDD/F congener or homologue group. The mean and standard deviation of this distribution were, respectively, the bootstrap mean TBP and bootstrap standard error of the mean. Lower and upper 95 percent confidence limits for the bootstrap mean TBP were defined, respectively, as the 2.5th percentile and 97.5th percentile of the bootstrap TBP distribution (Table 6). Bootstrap bias was defined as the difference between the bootstrap mean TBP and the calculated TBP, expressed as percent of the calculated TBP.

[1] Computer resources are generally no longer a limiting factor for the number of bootstrap resamples. At least 1,000 resamples are recommended.

Table 6
Bootstrap Uncertainty Analysis Using Congener- and Homologue-Specific BSAFs of Table 2

PCDD/F	Bootstrap TBP		Bootstrap Confidence Limits		Bootstrap Uncertainty Ratio	Ct Within Bootstrap Confidence Limits?
	Mean	Se	Lower (2.5)[1]	Upper (97.5)[2]		
2,3,7,8-TCDD	1.462	0.747	0.575	3.339	1.89	Within
1,2,3,6,7,8-HxCDD	0.897	0.462	0.235	2.059	2.03	Within
1,2,3,7,8,9-HxCDD	2.238	1.055	0.814	4.870	1.81	Within
1,2,3,4,6,7,8-HpCDD	1.563	0.905	0.419	3.918	2.24	Within
OCDD	12.939	7.895	2.824	33.967	2.41	Within
2,3,7,8-TCDF	4.641	2.490	1.447	10.885	2.03	Within
1,2,3,4,7,8-HxCDF	0.938	0.649	0.179	2.693	2.68	Within
1,2,3,6,7,8-HxCDF	1.604	0.820	0.513	3.604	1.93	Within
1,2,3,7,8,9-HxCDF	1.458	0.725	0.350	3.312	2.03	Within
1,2,3,4,6,7,8-HpCDF	0.373	0.207	0.094	0.865	2.07	Above
OCDF	0.709	0.366	0.219	1.628	1.99	Above
Total TCDD	0.942	0.328	0.485	1.720	1.31	Above
Total HxCDD	0.690	0.384	0.191	1.626	2.08	Above
Total HpCDD	0.904	0.626	0.164	2.384	2.46	Above
Total TCDF	3.451	2.114	0.701	8.504	2.26	Above
Total PeCDF	1.510	1.067	0.274	4.310	2.67	Above
Total HxCDF	1.083	0.744	0.191	2.866	2.47	Within
Total HpCDF	0.482	0.320	0.078	1.275	2.48	Above

[1]Lower distribution percentile.
[2]Upper distribution percentile.

Bootstrap tests of significance comparing TBP with observed Ct were conducted for each PCDD/F congener or homologue group as follows:

1. The mean Ct was subtracted from the calculated TBP to obtain a difference *D*.

2. The Ct data were randomly resampled 16,000 times to generate 16,000 bootstrap mean Ct estimates.

3. BSAF, Cs, TOC and lipid data were randomly resampled 16,000 times to generate 16,000 bootstrap mean TBP estimates.

4. Bootstrap Ct and TBP estimates from Steps 2 and 3 were combined into a single data set, randomly allocated to 16,000 pairs, and the second member of each pair subtracted from the first to obtain a distribution of 16,000 bootstrap D estimates.

5. The original *D* from Step 1 was added to the distribution of bootstrap D and the entire distribution ordered to obtain the rank of the original *D*. TBP was considered to significantly underestimate Ct if the rank of *D* was less than 400, or to significantly overestimate Ct if the rank of *D* was greater than 15,600 (two-tailed significance level of $P < 0.025$ in each tail of the distribution or overall $P < 0.05$) (Table 7).

Table 7
Bootstrap Test of Significance for the Difference Between Mean TBP and Mean Measured Concentrations of PCDD/Fs in Pooled Benthic Organisms of the New York Bight Apex

PCDD/F	TBP	Ct	D = Ct - TBP	Rank of D in Bootstrap Distribution (N = 16,000)	Bootstrap P	TBP Over- or Under-Estimates Mean Ct?
2,3,7,8-TCDD	1.350	1.774	-0.424	2746	0.3433	no
1,2,3,6,7,8-HxCDD	0.828	1.051	-0.222	3649	0.4561	no
1,2,3,7,8,9-HxCDD	2.057	1.047	1.010	14265	0.2169	no
1,2,3,4,6,7,8-HpCDD	1.438	3.323	-1.885	501	0.0626	no
OCDD	11.964	25.639	-13.675	794	0.0993	no
2,3,7,8-TCDF	4.241	3.163	1.078	13273	0.3409	no
1,2,3,4,7,8-HxCDF	0.866	0.796	0.070	9956	0.7555	no
1,2,3,6,7,8-HxCDF	1.471	0.666	0.805	14243	0.2196	no
1,2,3,7,8,9-HxCDF	1.341	0.786	0.555	13737	0.2829	no
1,2,3,4,6,7,8-HpCDF	0.344	4.858	-4.514	54	0.0068	under
OCDF	0.656	1.741	-1.085	204	0.0255	under
Total TCDD	0.873	3.477	-2.604	1	0.0001	under
Total HxCDD	0.636	3.617	-2.980	28	0.0035	under
Total HpCDD	0.836	12.004	-11.168	19	0.0024	under
Total TCDF	3.202	15.924	-12.721	6	0.0008	under
Total PeCDF	1.394	4.789	-3.395	189	0.0236	under
Total HxCDF	1.005	2.598	-1.594	489	0.0611	no
Total HpCDF	0.450	4.137	-3.687	85	0.0106	under

Sensitivity Analysis. Sensitivity analysis was conducted for each TBP input parameter by calculating a minimum and maximum TBP using the minimum and maximum observed values of that parameter and the means of the other input parameters. A sensitivity index was computed as

$$\text{Abs} [(\max \text{TBP} - \min \text{TBP}) / \text{mean TBP}] \quad (14)$$

for each parameter averaged over all PCDD/F congener and homologue groups. Larger values of the index indicated greater contribution of the associated parameter to the uncertainty in TBP.

DISCUSSION:

RSS Uncertainty. TBP was initially calculated for each PCDD/F congener and homologue group using the corresponding congener/homologue mean BSAF from Table 3. TBP generally corresponded well with measured mean tissue concentrations for the PCDD/F congeners, and less well for the homologue groups (Table 4, Figure 2). RSS uncertainty (TBP $\pm$ TE) was determined using average method error (avg ME) of 62.7 percent and Equations 8, 10, 11 and 12; the uncertainty intervals are given in Table 5 and shown graphically in Figure 2. The RSS TE intervals included mean Ct for 12 PCDD/F congeners and homologue groups, but underestimated Ct for one congener (1,2,3,4,6,7,8-HpCDF) and five homologue groups (Table 5 and Figure 2). The poorer predictive capability of the homologue-specific BSAFs appears to be related to the smaller number of observations (in most cases N = 2) as well as probable disequilibrium between organism and sediment resulting from observations made only on filter-feeding bivalves.

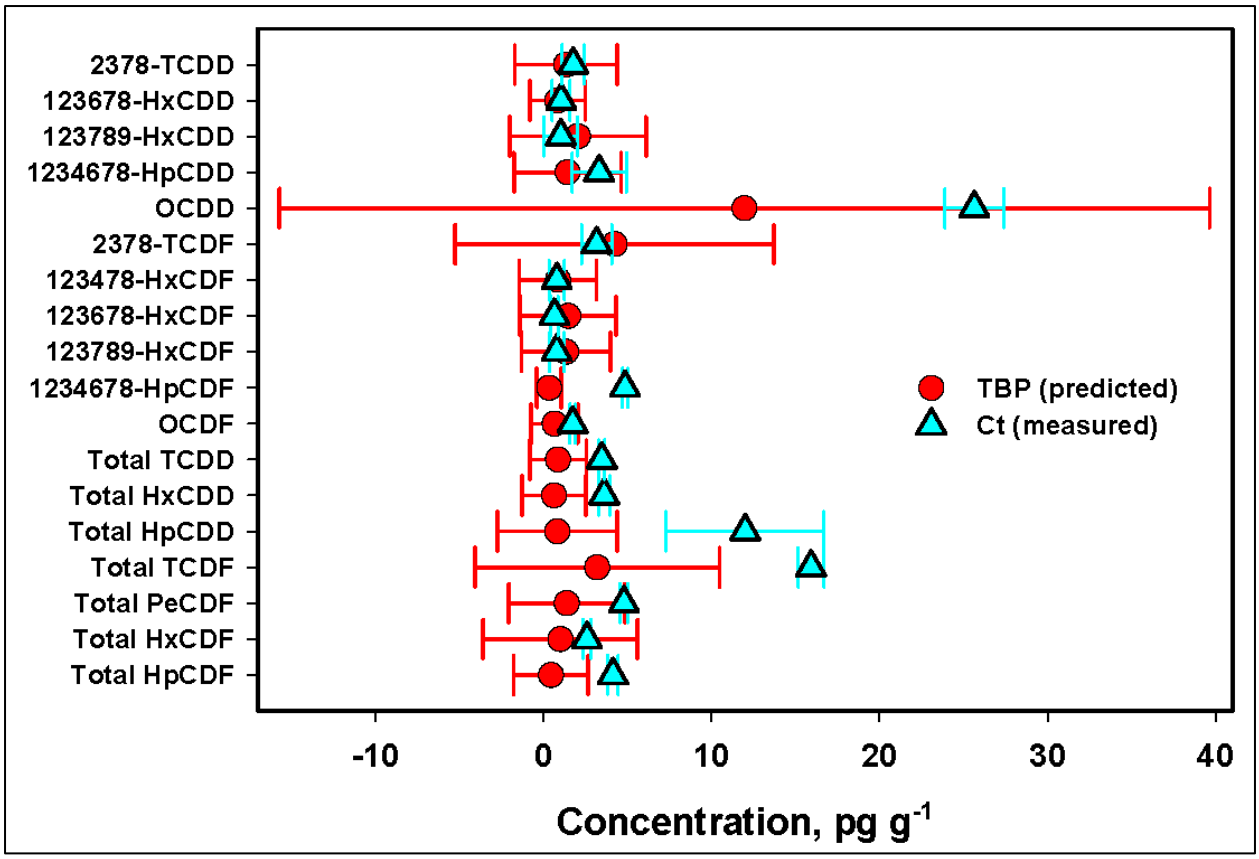

Figure 2. Mean TBP ± RSS total error estimation of bioaccumulation potential compared with measured PCDD/F concentrations (Ct ± 95 percent confidence interval) for pooled benthic organisms (Table 1) collected in the New York Bight Apex. TBPs calculated with congener- and homologue-specific BSAFs (Table 3)

RSS calculations were also performed using an overall mean BSAF = 0.573 (Table 3) for all congeners and homologue groups, to determine whether the overall BSAF could produce adequate TBP predictions. This resulted in larger average method error (157 percent) as well as larger propagated error and total error, and thus less precise estimates of TBP. Using the overall mean BSAF, TBP and its uncertainty interval were generally larger than the corresponding values calculated using congener/homologue mean BSAFs (Figure 3). The relative percent difference between TBP and mean Ct ranged from –90 percent to nearly +600 percent (Figure 3), and the broad RSS TBP uncertainty intervals always included mean Ct. On average, TBP underestimated Ct by 30 percent when congener-specific BSAFs were used, and overestimated Ct by 137 percent when the overall mean BSAF was used. Excluding homologue groups, the average relative disparity between TBP and mean Ct was +2 percent when congener-specific BSAFs were used, and +179 percent when the overall mean BSAF was used.

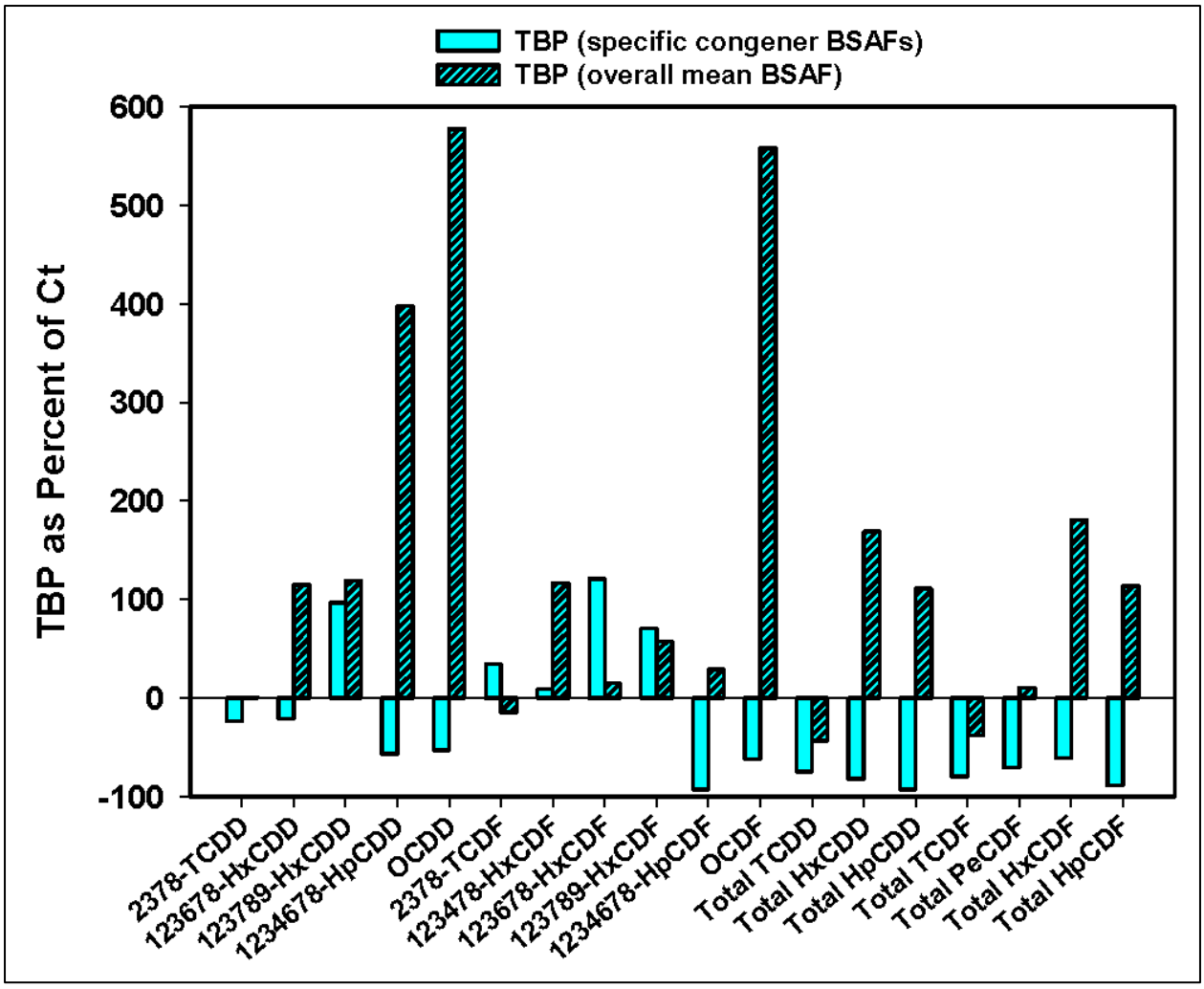

Figure 3. RSS relative percent difference between TBP and Ct using PCDD/F congener- and homologue-specific BSAFs and between TBP and Ct calculated using a single overall BSAF (formula in Table 4)

Bootstrap Uncertainty. Bootstrap calculations of TBP and uncertainty generally mirrored those of the RSS method, although with narrower uncertainty intervals (Figure 4). The bootstrap intervals are nonparametric estimates of statistical confidence intervals based solely on resampling of input parameter replicates. RSS uncertainty intervals, on the other hand, combine a method error component with incremental (propagated) error derived from parametric confidence limits for each input parameter, and thus incorporate more sources of uncertainty, leading to generally broader uncertainty intervals. Bootstrap mean TBPs determined using congener/homologue mean BSAFs (Table 6) reflected a positive bias of about 8 percent compared with the calculated TBPs. The positive bias is indicative of a skewed distribution resulting from simulations involving ratios.

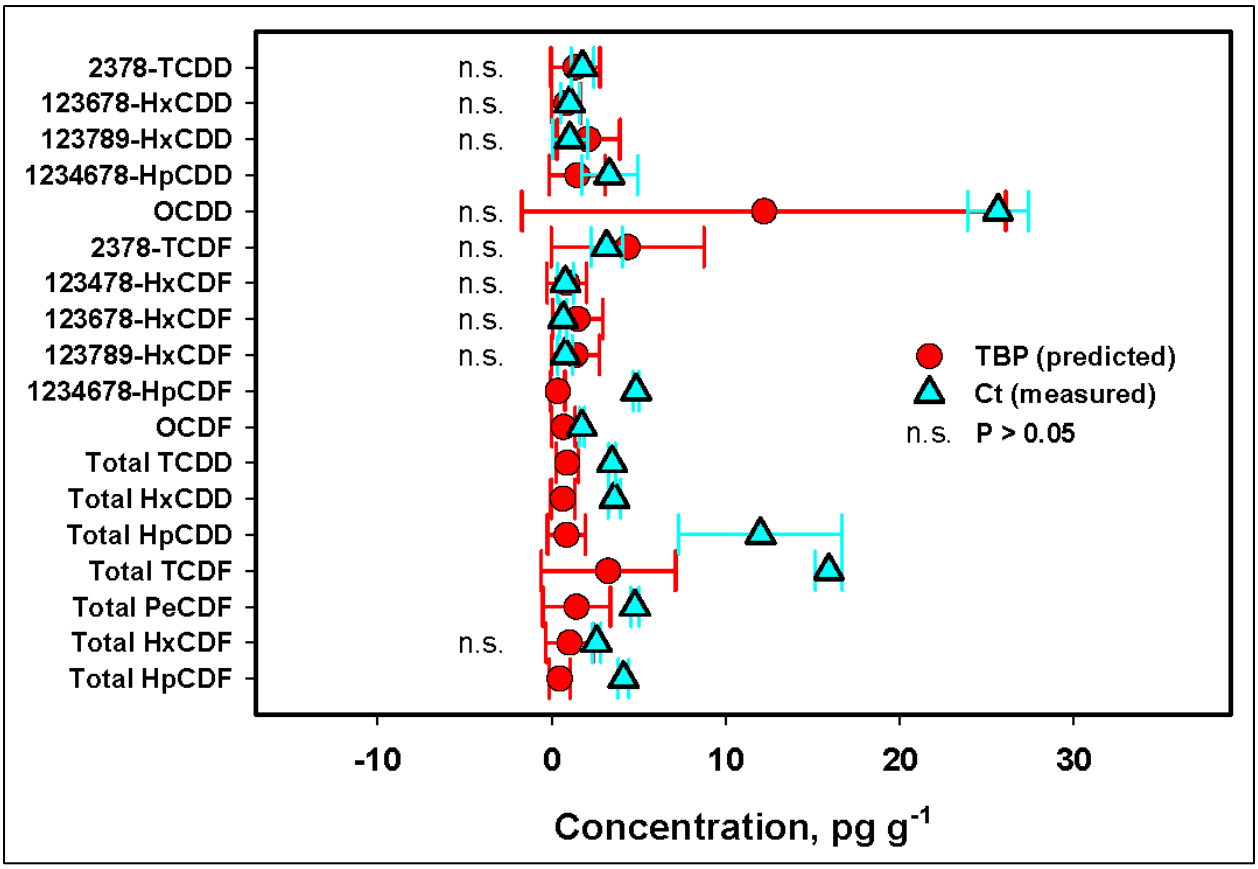

Figure 4. Bootstrap TBP ± bootstrap 95-percent confidence interval estimation of bioaccumulation potential compared with measured PCDD/F concentrations (Ct ± 95 percent confidence interval) for pooled benthic organisms (Table 1) collected in the New York Bight Apex. TBPs calculated with congener- and homologue-specific BSAFs (Table 3). Pairs marked "n.s." are not significantly different at P > 0.05 by bootstrap test of significance

The bootstrap uncertainty ratio for TBP (bootstrap confidence interval width ÷ TBP) was approximately a factor of two (Table 6). The bootstrap confidence intervals included mean Ct for the total HxCDF homologue group and for all congeners except 1,2,3,4,6,7,8-HpCDF and OCDF (Figure 4, Table 6). Tissue concentrations of these two congeners and all the other homologue groups were underestimated by bootstrap TBP.

When bootstrap simulations were performed using the overall mean BSAF, the resulting confidence intervals were broader in most cases than when congener/homologue mean BSAFs were used (Figure 5). Mean Ct fell below the lower bootstrap CL for 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD, OCDF, and total HxCDF, but was otherwise within the bootstrap confidence interval. The bootstrap relative percent difference between TBP and mean Ct was –40 percent to +200 percent when the bootstrap confidence interval included mean Ct, and +130 percent to > +600 percent when mean Ct fell below the lower bootstrap CL.

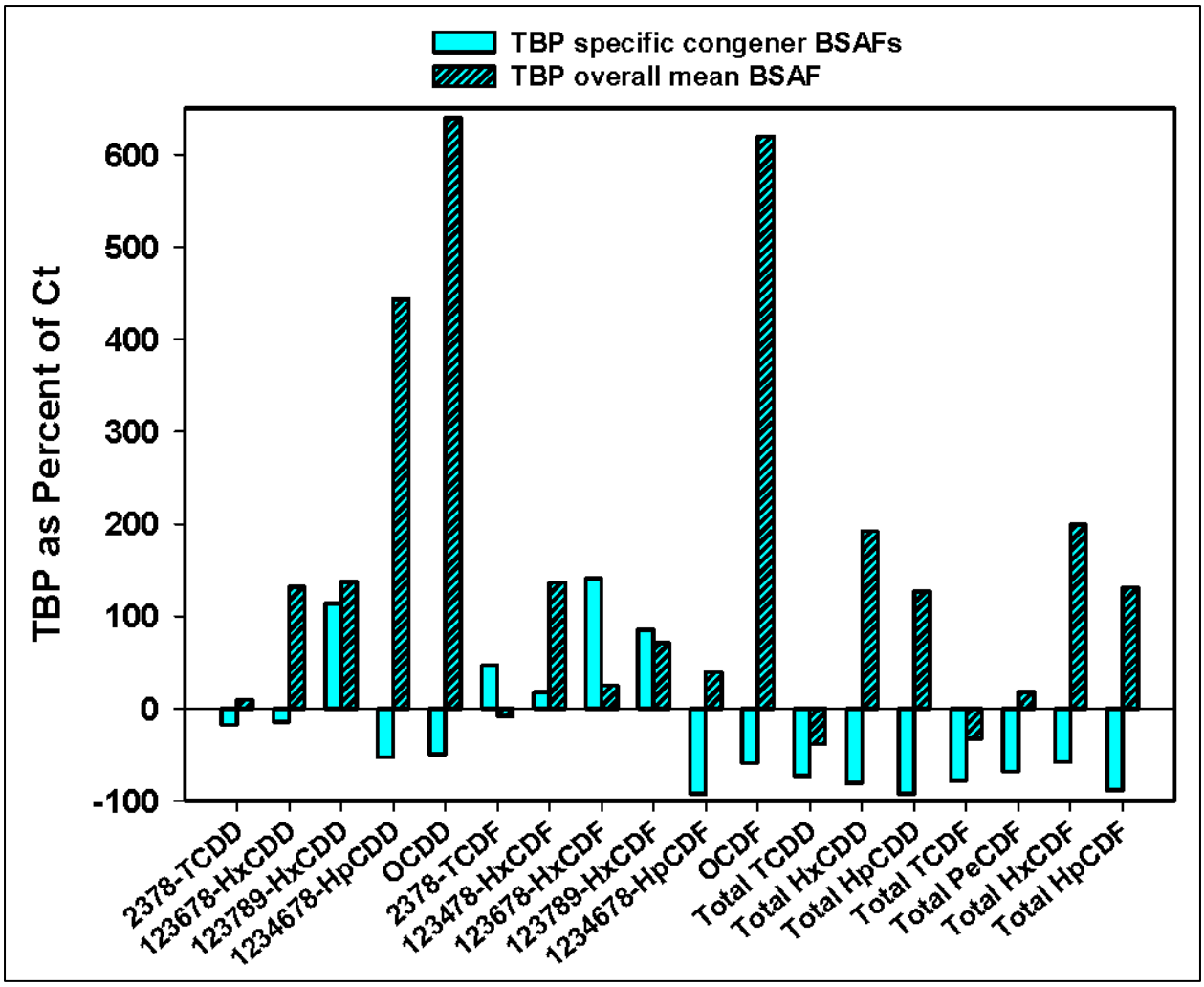

Figure 5. Bootstrap relative percent difference between TBP and Ct using PCDD/F congener- and homologue-specific BSAFs and between TBP and Ct calculated using a single overall BSAF (formula in Table 4)

The relative disparity between bootstrap TBP and mean Ct, averaged over all congeners and homologue groups, was –23 percent when congener/homologue mean BSAFs were used, and +158 percent when the overall mean BSAF was used. Excluding homologue groups, the average relative disparity between bootstrap TBP and mean Ct was +30 percent when congener-specific BSAFs were used, and +176 percent when the overall mean BSAF was used.

Bootstrap Test of Significance. TBP was compared statistically with mean Ct using bootstrap tests of significance. When TBP was calculated using congener/homologue mean BSAFs, TBP was found to significantly underestimate mean Ct for 1,2,3,4,6,7,8-HpCDF, OCDF, and all homologue groups except total HxCDF (bootstrap $P < 0.05$, Table 7). These results correspond exactly to the comparison of mean Ct with bootstrap confidence intervals in Table 6. However, when the overall mean BSAF was used to determine TBP, no significant differences between mean Ct and TBP were observed in the bootstrap tests of significance.

Sensitivity Analysis. Sensitivity indices calculated for the four TBP input parameters using the available data indicated that Cs and lipid each contributed 21 percent to TBP uncertainty, TOC contributed 26 percent, while BSAF contributed 32 percent (Table 8). BSAFs included in these analyses ranged from a minimum of 0.0033 for 1,2,3,4,6,7,8-HpCDF, to a maximum of 7.45 for 2,3,7,8-TCDF. Mean BSAFs were in the range 0.02 to 1.28.

Table 8
Sensitivity Analysis of TBP Input Parameters, Averaged Over All PCDD/F Congener/Homologue Groups

Parameter	TBP min[1]	TBP mean[2]	TBP max[3]	Sensitivity Index	Percent Contribution to TBP Uncertainty
Cs	0.762	1.942	3.793	1.404	21
TOC	4.318	1.942	1.025	1.696	26
Lipid	0.440	1.942	3.172	1.407	21
BSAF[4]	0.528	1.942	5.825	2.108	32

[1]TBP calculated using minimum value of selected parameter and means of other parameters.
[2]TBP calculated using means of all parameters.
[3]TBP calculated using maximum value of selected parameter and means of other parameters.
[4]Congener/homologue mean BSAFs.

CONCLUSIONS: This technical note illustrates two methods for estimating TBP uncertainty in evaluations involving the potential for bioaccumulation of PCDD/Fs in benthic organisms. The RSS method provides a large uncertainty interval that incorporates error due to the model (method error) and measurement error associated with each model input (propagated error). The bootstrap method constructs a statistical distribution for TBP that can then be used to determine descriptive statistics (mean, confidence interval, etc.) and conduct tests of significance. The RSS method produced an uncertainty interval with a range two to five times the magnitude of TBP, while the bootstrap uncertainty interval range was about twice the magnitude of TBP. Both types of uncertainty interval were similar in identifying the PCDD/F congeners or homologue groups for which TBP underestimated observed bioaccumulation.

Using individual congener or homologue group mean BSAFs in calculating TBP was preferable to using an overall mean BSAF from all PCDD/Fs. The overall BSAF resulted in TBPs that significantly overestimated observed bioaccumulation of some PCDD/F congeners using the bootstrap method, or in uncertainty intervals that were too broad to identify significant differences between predicted and observed tissue concentrations using the RSS method.

Whereas the bootstrap method requires SAS programs or specialized software such as Resampling Stats (*www.resample.com*), the RSS method can be implemented using a calculator or spreadsheet software and the step-by-step procedure outlined above. TBP and its uncertainty interval, generated using either of these methods, have been shown to provide reasonably accurate estimates of actual PCDD/F congener bioaccumulation in sediment-exposed benthos, when congener-specific BSAFs are used in the calculations.

TBP is implemented in Tier II of the four-tiered dredged material evaluation process (USEPA/USACE 1991, 1998). TBP can be useful in identifying sediments for which expensive Tier III or Tier IV evaluations are unnecessary, either because the sediment is acceptably clean for unrestricted open-water disposal (as evidenced by the TBP upper uncertainty limit), or

because the sediment is sufficiently contaminated (as evidenced by the TBP lower uncertainty limit) that unrestricted open-water disposal most likely would be prohibited. These decisions can be accomplished by comparing TBP uncertainty limits with appropriate tissue-based standards derived from residue-effects information for receptors of concern (Bridges et al. 1996, Bridges and Lutz 1999).

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This technical note should be cited as follows:

> Clarke, J. U., McFarland, V. A., Lutz, C. H., Jones, R. P., and Pickard, S. W. (2004). "Analysis of uncertainty in estimating dioxin bioaccumulation potential in sediment-exposed benthos," *DOER Technical Notes Collection* (ERDC TN-DOER-R5), U.S. Army Engineer Research and Development Center, Vicksburg, MS. *www.wes.army.mil/el/doer*.

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