MEMORANDUM

To:	Technical Committee/Risk Assessment Subcommittee
cc:	Mike Johns, Nancy Musgrove, Windward Environmental LLC
From:	Lisa Saban, Windward Environmental LLC
Subject:	Fish/Decapod (Crab/Crayfish) Tissue Sampling Design for the Lower Passaic River Restoration Project
Date:	August 6, 2009

This memo summarizes sampling design elements for the collection of fish and decapod tissue in the Lower Passaic River Study Area (LPRSA) in support of the human health and ecological risk assessments (HHRA/ERA). The design addresses the two main sampling objectives related to fish and decapods outlined in the 2006 Field Sampling Plan Volume 2 (FSP2) prepared by Malcolm Pirnie et al. (Malcolm Pirnie et al. 2006) for the US Environmental Protection Agency and its Partner Agencies (USEPA /PA[1]):

- Determine if exposure to site-related contaminants in the LPRSA poses unacceptable risks to fish and decapod populations
- Determine if the consumption of fish and decapod poses unacceptable risks to human and ecological receptors

The general sampling design is summarized below. Estimates of sample sizes and analytes proposed for evaluation follow. Further details are provided in the quality assurance project plan (QAPP) that will be submitted to USEPA/PA.

General Sampling Design

- The main data type that will be collected to meet FSP2 objectives is tissue residue in target fish and decapod (crab and crayfish) receptors.
- The overall sampling design is a simple stratified random design applied to known or likely habitat areas of the LPRSA.
- Per the agreements resulting from the January 14-15, 2009 meetings between the USEPA/PA and the CPG, the general sampling design divides the LPRSA into two major zones according to surface water salinity: the estuarine zone and the freshwater zone. Consistent with the preliminary salinity reaches defined in the Problem Formulation Document (PFD) (Windward and AECOM 2009), the estuarine zone includes both the brackish and transition river segments from River Mile

[1] The Partner Agencies include the US Army Corps of Engineers (USACE), the New Jersey Department of Transportation (NJDOT), the New Jersey Department of Environmental Protection (NJDEP) and the state and federal Natural Resource Trustees (NJDEP, National Oceanic and Atmospheric Administration [NOAA], and US Fish and Wildlife Service [USFWS]).

(RM) 0 to RM 10, and the freshwater zone includes the freshwater river segment from RM 10 to RM 17.4.

- The freshwater and estuarine zones are further subdivided into reaches approximately 2 miles in length to help allocate the sampling effort throughout each zone and support the calculation of zone-wide estimates of receptor-specific mean chemical tissue concentrations.
- Target receptors from the estuarine and freshwater zones will be collected to represent species consumed by humans and key fish and decapod feeding guilds.
    - Estuarine zone target receptors are mummichog (benthic omnivore), white perch epibenthic/pelagic invertivore[2]), American eel (demersal piscivore), and blue crab (benthic omnivore).
        - Mummichog will be evaluated only in the ERA because this species is not consumed by humans; all other estuarine target species will be used in both the HHRA and ERA.
    - Target receptors for the freshwater zone are darter or killifish (benthic omnivore), channel catfish or bullhead (demersal invertivore/omnivore), largemouth bass (pelagic piscivore), and crayfish (benthic omnivore).
        - Crayfish, darter, and killifish will be evaluated only in the ERA because these species are not consumed by humans; catfish or bullhead and bass will be used in both the HHRA and ERA.
        - Estuarine blue crab will be collected in the freshwater zone, if found, and composites will be included in a study-area-wide mean to be used for both the ERA and HHRA.
- Samples will be composites of multiple fish or decapods[3] to provide sufficient tissue mass and for consistency with the previous USEPA-approved 1999 ecological sampling plan (ESP) biota sampling program (Tierra Solutions 1999) that was implemented in the lower 7 miles of the river.
    - As requested by USEPA (April 6, 2009), individual fish collected from the field of a sufficient size to meet analytical mass requirements (and quality control [QC] requirements and splits) will be analyzed as separate samples.
- Composites will be created for each target tissue type and analyzed separately. The number of individuals in a single composite will be based on analytical mass requirements and actual catch in the field.
- Target tissue types for the HHRA include fish fillet and several types of blue crab tissue samples, including combined muscle and hepatopancreas composite samples and muscle-only composite samples. Target tissue types for the ERA include whole-body fish and whole-body crab and crayfish. To meet the needs of both risk assessments with one sampling event, fish fillet will be analyzed separately from the remaining tissue (carcass) in fish receptors being analyzed for both the HHRA and ERA. Fillet chemical concentrations will be combined mathematically (proportionally

[2] Young fish (less than 2 years) are epibenthic invertivores (amphipods, insect larvae); while the older fish also prey on larger benthic organisms (e.g., mud crabs) and pelagic organisms (e.g., shrimp and sometimes smaller fish).

[3] Composite tissue sampling provides a cost-effective approach for developing an estimate of the mean concentration of chemicals of potential concern in tissue (USEPA 2002), composite samples are consistent with the HHRA data use objective of estimating mean concentrations in tissue consumed by humans over a long-term period of exposure (USEPA 1989a, 1989b, 2000), and composite samples ensure sufficient tissue mass for the program's large analytical requirements and provide comparability with the sampling that was conducted under the USEPA-approved ESP program.

to their average weights in each species[4]) with carcass chemical concentrations to compute whole-body concentrations for the ERA. A similar approach will be used to represent crab tissue residues in the ERA. Per USEPA request, a limited number of composite samples will also be collected for analysis of blue crab hepatopancreas-only tissue. Per agreement with USEPA, the purpose of these data is to qualitatively compare hepatopancreas-only tissue concentrations with muscle-only tissue concentrations in the uncertainty section of the HHRA and show the relative difference in bioaccumulation potential in the two tissue types.

- Inasmuch as it may not be possible to collect adequate tissue mass at each specified sampling location to constitute a full analytical sample, the following sampling design considerations will be implemented in coordination with USEPA during sampling to ensure that the QAPP elements are satisfied or determine whether they need to be adjusted (detailed on QAPP Worksheet 11).
    - All collection methods (e.g., baited traps, trotlines, gillnets, electrofishing) will be attempted up to five times at each target sampling location (where each method is appropriate within the LPRSA) within each 2-mile reach.
    - For all species, sampling locations may be resampled or moved to different locations within the targeted 2-mile reach based on the catch success.
    - After the five attempts have been exhausted, a chemical prioritization scheme will be employed for the analysis of the volume of tissue collected. The prioritization is presented in Worksheet No. 10 of this QAPP.
    - Some unsuccessful sampling locations may need to be relocated or abandoned or new ones added to ensure that the QAPP elements are satisfied.
- An electronic database that provides fish collection locations (coordinates and depths); trap deployment and retrieval times; and length, weight, and gender (if determinable) of each individual fish collected for analysis will be maintained.

Estimates of Sample Size

In environmental investigations, decisions about the number of samples collected are the most crucial components of the sampling design. Not only does the number of samples directly affect the cost of the investigation, but also the ability to identify patterns, answer questions, and make inferences about risks in areas of the river that are not directly assessed. The overall approach to estimating the number of composite samples to represent tissue types for target receptors in each zone relied on the following steps:

- Existing fish and crab tissue data from the ESP and Contaminant Assessment and Reduction Program (CARP[5]) datasets were evaluated for key contaminant groups (e.g., polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzofurans [PCDDs/PCDFs], mercury, polychlorinated biphenyls [PCBs], polycyclic aromatic hydrocarbons [PAHs], pesticides) to help determine statistical characteristics (variability and skewness) of tissue residues in target receptors (where data were available). Parametric and non-parametric[6] statistical methods were used to compute

[4] Whole-body chemical concentrations from fillet and carcass will be calculated on a weighted average basis according to following equation: ((fillet concentration x fillet weight) + carcass concentration x carcass weight)) / (fillet weight + carcass weight).

[5] CARP data were collected within the New York/New Jersey Harbor, including the LPRSA. Data are available at: http://www.carpweb.org/main.html. Only those data from the LPRSA were used in the sample size estimates.

[6] Non-parametric sample size calculations are based on Chebyshev's inequality and bootstrapping.

sample sizes needed to achieve different levels of precision in the estimate of the mean tissue concentration for each species (e.g., ability to estimate within 50%, 100%, or 150% of the true mean) based on the statistical characteristics of the existing data.

  - Because mummichog (and darter or killifish) data will be used for multiple purposes, the sample design also considered sample size needed to detect a relationship between sediment and tissue.

- Sample size requirements to calculate a 95% upper confidence limit on the mean (95% UCL) using ProUCL (Version 4.00.02) (USEPA 2007a) and a minimum frequency of detection of the chemicals of potential concern (COPCs) of 60% were used to adjust the sample size estimates for each species.

The proposed number of composite samples that will be collected for fish and decapod tissue are summarized in Table 1. Proposed sample sizes for fish and decapod tissue are summarized in Table 1 and are based on the agreement between CPG and USEPA as presented in the Sample Size Estimate Term Sheet (Attachment V). Additional details regarding the derivation of the sample sizes are provided in the following sections.

Table 1. Sample size proposed for fish and decapod tissue chemistry collection

Feeding Guild[a]	Target Species	Zone[b]	No. of Locations per Zone	No. of Samples per Location	No. of Samples per Zone[b]	Type of Sample	Total No. of Analytical Samples
Benthic omnivore – forage fish	mummichog	estuarine	13	3	39[c]	whole body	39
	darter or killifish species	freshwater	14	3	42[c]	whole body	42
Invertivore/ omnivore	white perch	estuarine	12	2	24[d]	skin-on fillet and carcass[e]	48
	channel catfish or bullhead	freshwater	13	2	26[d]	skinless fillet and carcass with skin[e]	52
Piscivore	American eel	estuarine	12	2	24[d]	skinless fillet and carcass with skin[e]	48
	largemouth bass	freshwater	13	2	26[d]	skin-on fillet and carcass[e]	52

Feeding Guild[a]	Target Species	Zone[b]	No. of Locations per Zone	No. of Samples per Location	No. of Samples per Zone[b]	Type of Sample	Total No. of Analytical Samples
Epibenthic omnivore	blue crab	estuarine[f]	12	field determined[g]	24[c, d, f]	muscle/ hepatopancreas combined[h]	63
			12	field determined[g]	24 [c, d, f]	carcass[h]	
			12	field determined[g]	12 [d]	muscle only[h]	
			3	field determined[g]	3	hepatopancreas only[h]	
		freshwater[f]	9	field determined[g]	17[c]	muscle/ hepatopancreas combined[h]	30
			9	field determined[g]	9	muscle only[h]	
			4	field determined[g]	4	hepatopancreas only[h]	
	crayfish	freshwater	9	3	27[c, d]	whole body	27
Total							**401**

[a] Target species are organized according feeding guilds designated for the ERA. The target demersal (bottom-dwelling) species for the HHRA are blue crab (estuarine), American eel (estuarine) and channel catfish/brown bullhead (freshwater). The target pelagic species for HHRA are white perch (estuarine) and largemouth bass (freshwater).

[b] Zones represent the estuarine (RM 0 to RM 10) and freshwater (RM 10 to RM 17.4) habitats within the LPRSA.

[c] Blue crab, crayfish, mummichog, and darter or killifish samples will be co-located with sediment samples collected as part of the benthic invertebrate QAPP in order to derive site-specific biota-sediment accumulation factors. In addition to chemical residues for these samples, lipid content for tissues and organic carbon content for sediment will be analyzed.

[d] Sample size was adjusted to address ProUCL (Version 4.00.02) requirements, assuming a minimum frequency of detection of 60%.

[e] Carcass tissue will be composed of the remaining (non-fillet) portion. Tissue type chemical concentrations will be combined mathematically (proportionally to their average weights in each species) to calculate whole-body chemical concentrations.

[f] Target sample size (n = 24) is based on blue crab collected from the estuarine zone. Additional blue crab samples may be collected from the freshwater zone if sufficient numbers of blue crabs are captured in the freshwater zone.

[g] Three crab traps will be deployed per location in both the estuarine zone and the freshwater zone. However, the number of samples collected per location will vary for all blue crab tissue sample types based on the number of crabs that are collected and on analytical tissue mass requirements.

[h] Blue crab muscle/hepatopancreas combined and muscle-only tissue samples are to satisfy HHRA data needs; carcass (i.e., non-edible soft tissue) and muscle/hepatopancreas combined tissue samples will be combined mathematically to yield all soft tissue concentrations for the ERA. Because crayfish is the target ERA species for the freshwater zone, carcass tissue samples are not required for this zone. The HHRA will use data from combined blue crab muscle/hepatopancreas samples as the basis for quantitatively evaluating the reasonable maximum exposure of individuals under current and future exposure scenarios for both cancer and non-cancer health effects following USEPA Superfund guidance, guidelines, and policies. Risks associated with the consumption of hepatopancreas-only and muscle-only tissue will be discussed qualitatively in the uncertainty section of the HHRA.

RM – river mile

Statistical Characteristics of Existing Data

Estimates of the number of composites depend on the precision needed and the anticipated variability and statistical distribution (especially skewness) of the data – higher variability and/or skewness requires larger sample sizes to achieve a given level of precision. Existing individual fish tissue data collected in the Lower Passaic River (LPR) and reported in the CARP database were initially used to provide a preliminary estimate of the variance in tissue chemical concentrations over the entire LPRSA. This variance, represented by the coefficient of variation (CV),[7] was calculated for several species (whole-body[8] mummichog, eel, and white perch) and target chemical groups (PCBs, DDTs, PAHs, individual and toxic equivalent [TEQ] sums of PCDDs/PCDFs, cadmium, and mercury). Summary statistics for these datasets are provided in Addendum 1.

Overall, the variance of the tissue-residue chemicals was relatively low – almost all CVs were below 1.5[9] (i.e., the standard deviation was less than 150% of the mean), and most were below 1.0 (Figure 1).

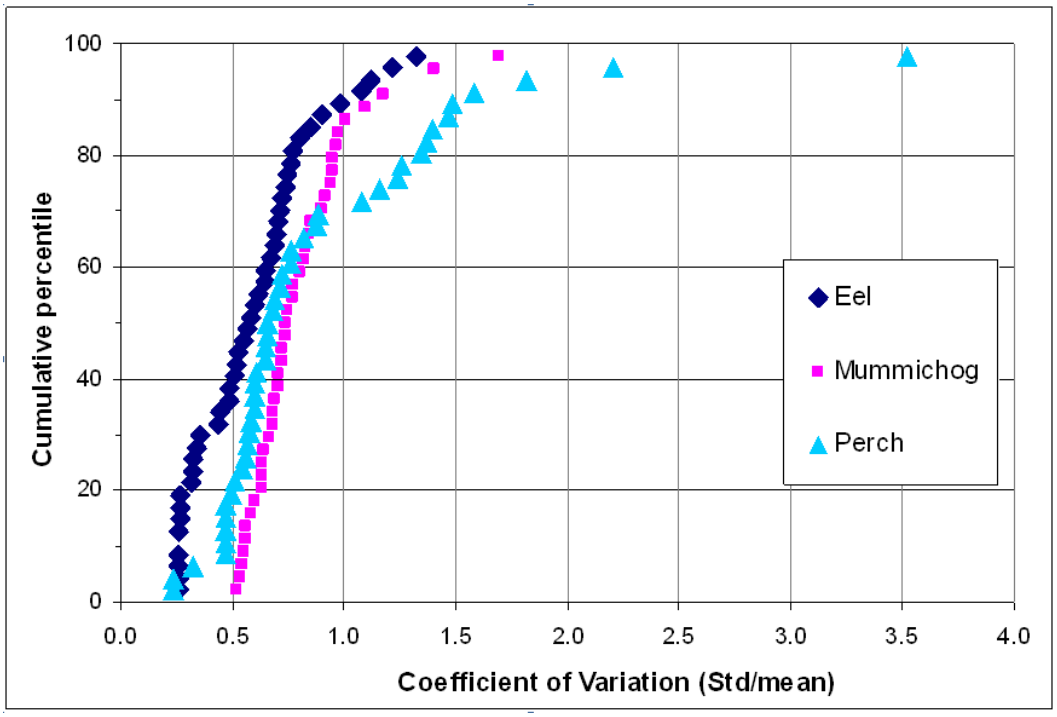

Figure 1. Cumulative frequency distribution of coefficients of variation in whole-body tissue concentrations of individual chemicals for whole-body mummichog and white perch and eel fillet

[7] The CV is a standardized estimate of the variance and is calculated by dividing the standard deviation by the mean.

[8] Eel and perch had heads and viscera removed.

[9] Chemicals with CVs greater than 1.5 in the CARP dataset included octachlorodibenzofuran and individual PAHs (benzo(g,h,i)-perylene, benzo(e)perylene, and benz(a)anthracene).

Variability in tissue data[10] collected in the lower 6 miles of the river during the 1999 to 2001 ESP program was used to refine sample size requirements because data quality for this dataset, especially the sum and total calculated chemical concentrations, was better understood. Almost all[11] of the calculated CVs from the ESP dataset (including different tissue types) were less than 1.3, and most were less than 1.0 (summary statistics for all chemicals and tissue types used in this evaluation are provided electronically as Addendum 2). A CV of 1.3 was carried forward in the sample size calculations as a reasonable estimate of variance for samples that will be collected in the entire study area.

In terms of skewness, some chemical distributions were approximately normal (e.g., total DDTs in white perch [Figure 2]), but most displayed some level of skewness (e.g., total chlordane concentrations in mummichog [Figure 3]). Shapiro Wilks test of normality found that the perch DDT data were not normally distributed ($p = 0.003$) and that the mummichog total chlordane concentrations data were also not normally distributed ($p < 0.0005$).

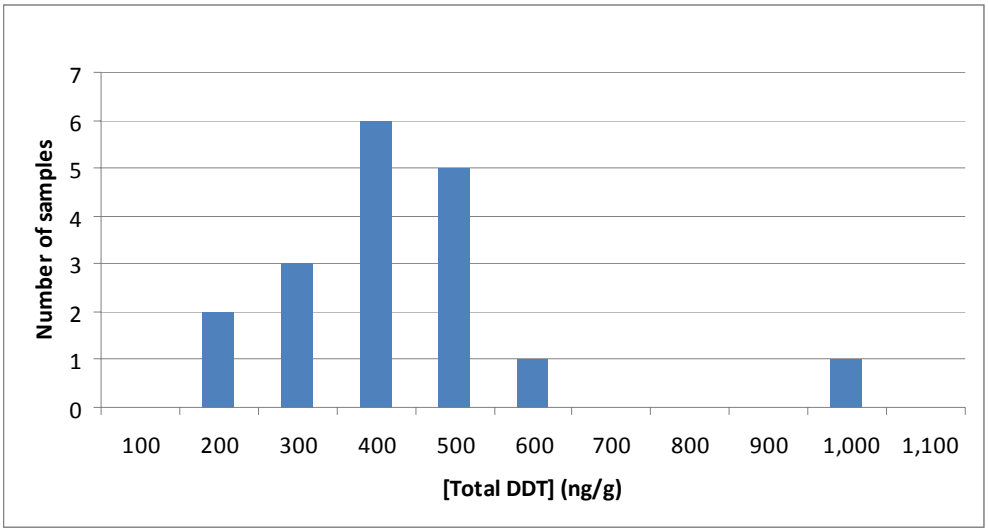

Figure 2. Example of approximate normal distribution of total DDT in whole-body white perch

[10] Tissue types included whole-body American eel, white perch, bass, and mummichog; all soft body tissue, edible muscle, and hepatopancreas for crab, and fillet for eel (skin on), white perch (skin off), and bass (skin off).

[11] A small fraction (< 1.5%) of chemicals measured in the various tissue types had CVs > 1.3.

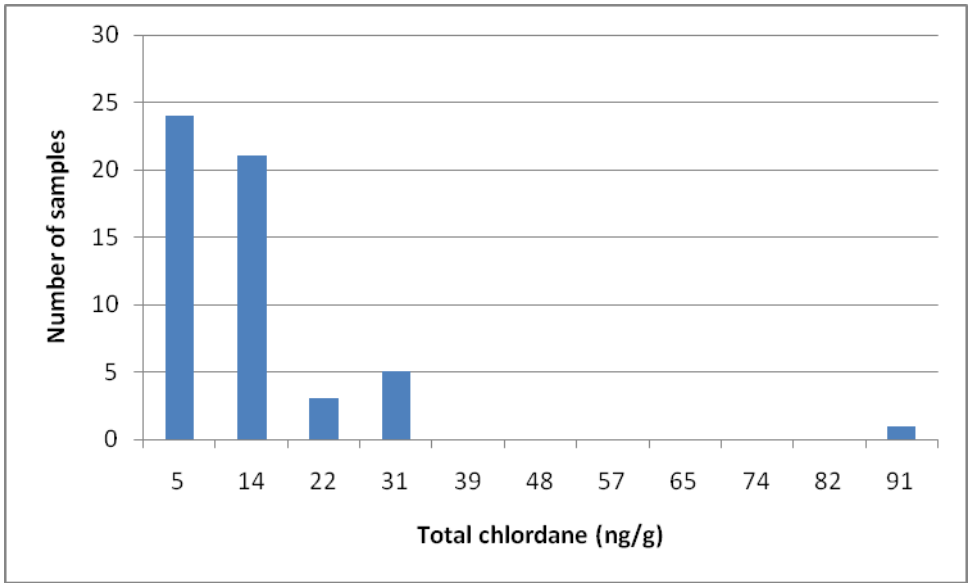

Figure 3. Example of skewed distribution of total chlordane in whole-body mummichog

USEPA (2007b) recommends using a non-parametric 95% Chebyshev confidence interval when the standard deviation of the log-transformed data is greater than 0.5 (Table 2). Although none of the data investigated had standard deviations of the log-transformed data that were greater than 1 (which would require the use of a 97.5% or 99% Chebyshev UCL), a number of chemicals had standard deviations of the log-transformed data that were greater than 0.5 (Table 3). Consequently, sample sizes that will be ample to accommodate skewness are recommended.[12]

Table 2. Summary table for the computation of a 95% UCL of the unknown mean, μ_1, based on a skewed dataset (with all positive values) without a discernable distribution, where $\hat{\sigma}$ is the standard deviation of log-transformed data

$\hat{\sigma}$	Sample Size, n	Recommendation
$\hat{\sigma} \leq 0.5$	for all *n*	95% UCL based on Student's t- or Modified-t statistic
$0.5 < \hat{\sigma} \leq 1.0$	for all *n*	95% Chebyshev (mean, stdev) UCL
$1.0 < \hat{\sigma} \leq 2.0$	$n < 50$	99% Chebyshev (mean, stdev) UCL
	$n \geq 50$	97.5% Chebyshev (mean, stdev) UCL
$2.0 < \hat{\sigma} \leq 3.0$	$n < 10$	Hall's Bootstrap UCL*
	$n \geq 10$	99% Chebyshev (mean, stdev) UCL
$3.0 < \hat{\sigma} \leq 3.5$	$n < 30$	Hall's Bootstrap UCL*
	$n \geq 30$	99% Chebyshev (mean, stdev) UCL

[12] Similar ranges of CVs, standard deviations of log-transformed data, and bootstrap results were obtained for major chemicals (e.g., total PCBs, dioxin/furans) and fish species using data from the CARP dataset.

$\hat{\sigma}$	Sample Size, n	Recommendation
$\hat{\sigma} > 3.5$	$n < 100$	Hall's Bootstrap UCL*
	$n \geq 100$	99% Chebyshev (mean, stdev) UCL

UCL – upper confidence limit on the mean

Table 3. Coefficient of variation of raw data, standard deviation of log-transformed data, and number of samples collected for major chemicals in the ESP dataset

Species	Chemical	CV Raw	Stdev Log	n	Recommended UCL Method
Mummichog (whole body)	dieldrin	0.40	0.37	54	Student's t
	total chlordane (calc'd)	1.19	0.66	54	95% Chebyshev
	sum DDE (calc'd)	0.90	0.65	54	95% Chebyshev
	total DDTs (calc'd)	0.67	0.65	54	95% Chebyshev
	total PCBs (Aroclors)	0.30	0.32	54	Student's t
	total PCDDs/PCDFs (calc'd, 1/2-DL)	1.33	0.56	54	95% Chebyshev
	mercury	0.58	0.39	54	Student's t
White perch (whole body and fillet)	total chlordane (whole body)	0.59	0.72	18	95% Chebyshev
	total chlordane (fillet)	0.72	0.73	6	95% Chebyshev
	total DDTs (calc'd) (whole body)	0.45	0.41	18	Student's t
	total DDTs (calc'd) (fillet)	0.23	0.26	6	Student's t
	total PCBs (Aroclors) (whole body)	0.41	0.49	18	Student's t
	total PCBs (Aroclors) (fillet)	0.20	0.19	6	Student's t
	total PCDDs/PCDFs (calc'd, 1/2-DL) (whole body)	0.30	0.33	18	Student's t
	mercury (whole body)	0.56	0.44	18	Student's t
Adult striped bass (whole body and fillet)	total DDTs (calc'd) (whole body)	0.45	0.65	9	95% Chebyshev
	total DDTs (calc'd) (fillet)	0.80	0.76	11	95% Chebyshev
	total chlordane (whole body)	0.76	0.92	9	95% Chebyshev
	total PCBs (Aroclors) (whole body)	0.58	0.79	9	95% Chebyshev
	total PCBs (Aroclors) (fillet)	0.48	0.45	11	Student's t
	total PCDDs/PCDFs (calc'd, 1/2-DL) (whole body)	0.35	0.58	8	95% Chebyshev
	total PCDDs/PCDFs (calc'd, 1/2-DL) (fillet)	0.72	0.85	7	95% Chebyshev
	mercury (whole body)	0.30	0.36	9	Student's t
	mercury (fillet)	0.56	0.41	10	Student's t

Species	Chemical	CV Raw	Stdev Log	n	Recommended UCL Method
American eel (whole body and fillet)	total chlordane (fillet)	0.44	0.46	7	Student's t
	total PCBs (Aroclors) (whole body)	0.76	0.96	6	95% Chebyshev
	total PCBs (Aroclors) (fillet)	0.24	0.29	7	Student's t
	total PCDDs/PCDFs (calc'd, 1/2-DL) (whole body)	0.41	0.42	6	Student's t
	total PCDDs/PCDFs (calc'd, 1/2-DL) (fillet)	0.31	0.30	7	Student's t
	mercury (whole body)	0.77	0.65	6	95% Chebyshev
	sum DDE (calc'd) (whole body)	0.59	0.68	6	95% Chebyshev
	sum DDE (calc'd) (fillet)	0.57	0.55	7	95% Chebyshev
	total DDTs (calc'd) (whole body)	0.55	0.69	6	95% Chebyshev
	total DDTs (calc'd) (fillet)	0.76	0.69	7	95% Chebyshev
Blue crab (edible muscle and all soft tissue)	total chlordane (muscle)	0.24	0.41	22	Student's t
	total chlordane (soft tissue)	0.37	0.32	20	Student's t
	total PCBs (Aroclors) (muscle)	0.39	0.38	22	Student's t
	total PCBs (Aroclors) (soft tissue)	0.50	0.58	20	95% Chebyshev
	total PCDDs/PCDFs (calc'd, 1/2-DL); (muscle)	0.27	0.29	18	Student's t
	total PCDDs/PCDFs (calc'd, 1/2-DL) (soft tissue)	0.30	0.32	16	Student's t
	total DDTs (calc'd) (muscle)	0.23	0.38	22	Student's t
	total DDTs (calc'd) (soft tissue)	0.62	0.61	20	95% Chebyshev
	sum DDTs (calc'd) (muscle)	0.26	0.50	22	Student's t
	sum DDTs (calc'd) (soft tissue)	0.75	0.67	20	95% Chebyshev
	mercury (muscle)	0.29	0.29	22	Student's t
	mercury (soft tissue)	0.33	0.33	20	Student's t

CV – coefficient of variation

DL – detection limit

ESP – ecological sampling plan

PCB – polychlorinated biphenyl

PCDD – polychlorinated dibenzo-*p*-dioxins

PCDF – polychlorinated dibenzofurans

Stdev – standard deviation

TEQ – toxic equivalent

UCL – upper confidence limit on the mean

Sample Size Calculations to Estimate a Mean

Tissue data exhibit a wide range of variability and statistical distributional characteristics, which affects sample size calculations and, in turn, the confidence with which risk conclusions can be made. To evaluate this effect on the LPR tissue sampling design, several different methods discussed in EPA guidance were applied to the sample size calculations.

The first approach (a parametric approach) assumes the data will approximate a normal distribution (standard deviation of log-transformed data < 0.50). Equation 1[13] is generalized to Equation 2 to calculate the sample size needed to estimate a 95% UCL such that the UCL would fall within a given percentage of the true mean (*p** $\overline{X}$) with ((1-*α*)*100)% confidence, assuming that the CV was less than an assumed value.

$$n = \frac{s^2}{d^2}\left(t_{1-\alpha,n-1}\right)^2$$ **Equation 1**

$$n = \frac{CV^2(t_{1-\alpha,n-1})^2}{p^2}$$ **Equation 2**

Where:

s = the standard deviation
d = the difference to be detected
n = the sample size
t = the critical value for the Student-t distribution
p = the percentage of the mean within which the UCL should fall
CV = s/ $\overline{X}$

Assuming more skewed (non-normal) populations, the Chebyshev inequality[14] equation was rearranged to compute sample size requirements for a one-sided 95% UCL (Equations 3 and 4).

$$n = \frac{s^2}{d^2}\left((1/\alpha)-1\right)$$ **Equation 3**

$$n = \frac{CV^2((1/\alpha)-1)}{p^2}$$ **Equation 4**

The sample size calculations assumed that the CV would be less than 1.3 (maximum CV from ESP data) and that the desired precision was a 95%UCL within 100% of the mean. The sample size estimates ranged from 7 samples per species per zone (assuming the data are normally distributed) (Table 4) to 32 samples per species per zone (assuming the data are not normally distributed) (Table 5). Sample size scenarios for additional levels of variance and precision are also provided in each table.

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[13] Based on Zar (1996).

[14] The two-sided Chebyshev theorem (Hogg and Craig 1978; as cited in USEPA 2007b) shown in Equation 2-44 from the ProUCL Technical Guidance (USEPA 2007b) ($P(-k\sigma_1 \le x - \mu_1 \le k\sigma_1) \ge 1-1/k^2$) leads to a two-sided UCL in Equation 2-45 (*UCL* = $\overline{X}$ +(1/α)s_x/sqrt*(n)*) and a one-sided UCL in Equation 2-46 (*UCL* = $\overline{X}$ +((1/ α)-1)s_x/sqrt*(n))*. The use of a one-sided UCL does not reduce the sample size as much for the non-parametric case as for the parametric case because it cannot be assumed that the two-sided confidence interval expressed in Equation 2-44 is symetrical. See *also* http://www.btinternet.com/~se16/hgb/cheb.htm#Graph2 *for* derivations of the one-sided UCL.

Table 4. Sample sizes needed to estimate a mean with a range of precision goals (within a given percent of the mean) for populations with a range of variance magnitudes (as represented by CV) for symmetrical populations

CV	PRECISION (percent of mean)						
	25%	50%	75%	100%	125%	150%	200%
0.5	13	5	4	3	3	3	3
0.75	27	9	5	4	4	3	3
1	46	13	7	5	4	4	3
1.1	54	15	7	5	4	3	3
1.3	76	21	10	**7**	5	5	3
1.5	99	27	13	9	6	5	4
2	176	46	22	13	9	7	5

Note: Sample sizes based on normal theory for symmetrical populations.

CV – coefficient of variation

Bold indicate sample size associated with the maximum CV from the ESP tissue dataset, given assumptions of normality.

Table 5. Sample sizes based on Chebyshev inequality for skewed populations

CV	PRECISION (percent of mean)						
	25%	50%	75%	100%	125%	150%	200%
0.5	76	19	8	5	3	3	3
0.75	171	43	19	11	7	5	3
1	304	76	34	19	12	8	5
1.1	368	92	41	23	15	10	6
1.3	514	128	57	**32**	21	14	8
1.5	684	171	76	43	27	19	11
2	1216	304	135	76	49	34	19

CV – coefficient of variation

Bold indicate sample size associated with the maximum CV from the ESP tissue dataset, given assumptions of non-normality.

Given this wide range of resulting sample sizes, a second non-parametric method was applied to the data to refine the sample size estimate. A bootstrapping technique, which repeatedly resamples the dataset, was used to estimate the sample size needed so that 95% of the bootstrap calculated means would fall within 100% of the sample mean.[15] ESP concentrations of PCDDs/PCDFs, PCB TEQ, DDT, DDE, chlordane and dieldrin in mummichog, eel, white perch, and crab tissues were used in the bootstrapping exercise. For each ESP species-chemical dataset, 1,000 bootstrap samples (iterations) of size n were selected, with replacement, from the empirical concentrations. Sample size (n) ranged from 3 to 55, so that 1,000 samples of n=3, n=4,…, n=55 were created.

[15] The percentile bootstrap method as described in ProUCL Section 2.4.9.3 (USEPA 2007b).

The mean of each bootstrap sample and the quantiles of each set of 1,000 bootstrap means were calculated. The upper 95th percentile of the 1,000 calculated bootstrap means for each set was considered to be the bootstrap estimate of 95%UCL for that sample size. The ratio of the 95th percentile bootstrap mean was divided by the mean of the 1,000 bootstrap means to determine the "precision" of the 95%UCL for that sample size (i.e., how many times greater the 95%UCL was than the mean).[16] This ratio (hereafter, "Ratio95") was then plotted against bootstrap sample size to visually display the relationship of sample size to precision (Figure 4), and the minimum bootstrap sample size with a Ratio95 less than 2.0 (95%UCL < 100% of mean bootstrap mean) was selected as the minimum sample size needed to achieve the desired precision. Figure 4 can be used to determine the sample size associated with any desired precision for any of the chemicals plotted.

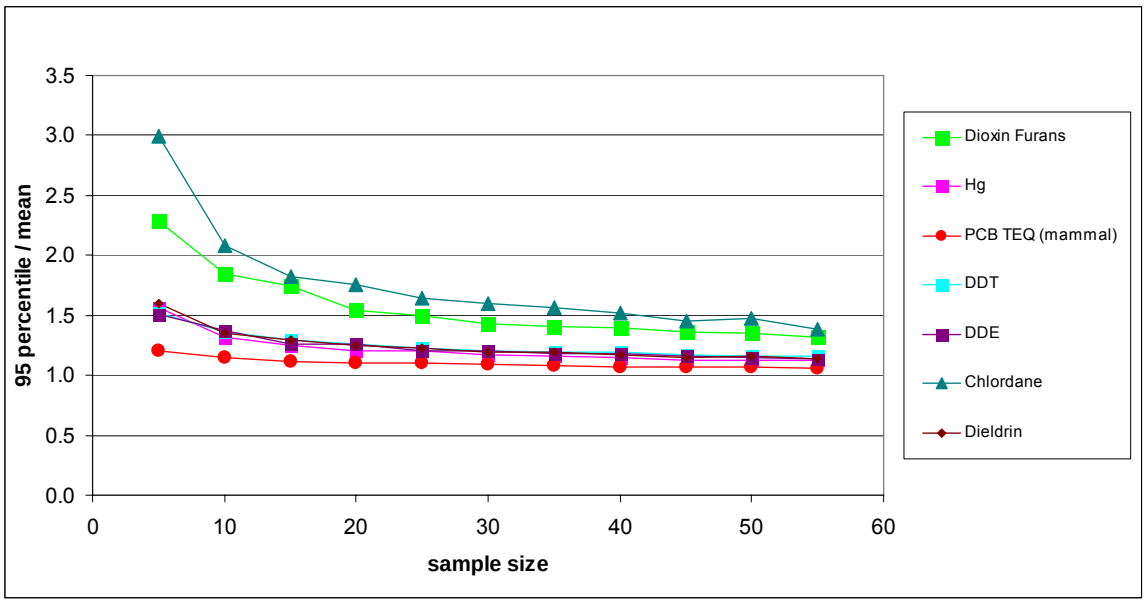

Figure 4. Relationship between bootstrap sample size and ratio of 95 percentile bootstrap mean to sample mean for mummichog whole-body residues. Results are based on 1,000 bootstrap iterations for each sample size.

According to USEPA (2007b), the percentile method can sometimes underestimate the UCL if the data are highly skewed, so sample calculations were also calculated for 97.5% confidence to cover cases with more extreme skewness. The ratio of the 97.5 percentile bootstrap mean to the mean bootstrap mean was then calculated, and an example is plotted against bootstrap sample size in Figure 5.[17]

The results of these two bootstrapping are presented in Table 6.

[16] The 95 percentile bootstrap mean was also divided by the original sample mean. Results were very similar and so only results of dividing by the mean of the bootstrap means are presented here.

[17] The bootstrap-t method, described in ProUCL Section 2.4.9.5 (USEPA 2007b), is considered more reliable than the percentile method when data are skewed and was used to check the results of the percentile method; results did not differ from those presented here.

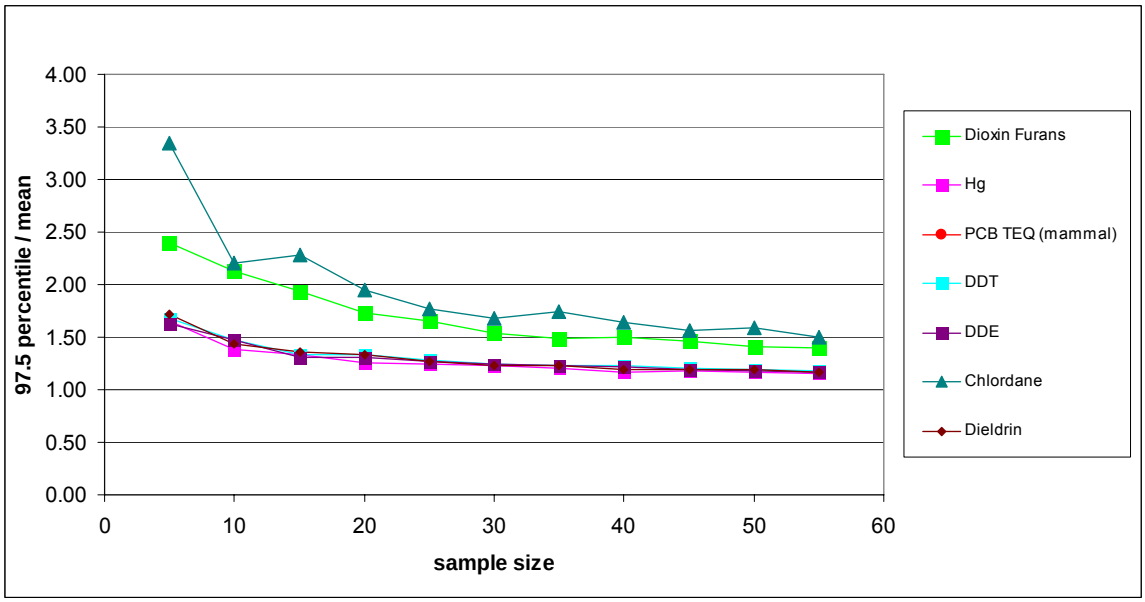

Figure 5. Relationship between bootstrap sample size and ratio of 97.5 percentile bootstrap mean to sample mean for mummichog whole-body residues. Results are based on 1,000 bootstrap iterations for each sample size.

Table 6. Sample size estimates based on bootstrapping

Species	Zone	Number of Samples Per Tissue Type Based on 95th Percentile	Number of Samples Per Tissue Type Based on 97.5 Percentile
Mummichog (whole body)	estuarine	13	20
White perch (whole body or fillet)	estuarine	< 5	<5
American eel (whole body or fillet)	estuarine	< 5	<5
Blue crab (edible muscle or all soft tissue)	estuarine or study-area wide	< 5	<5

RM – river mile

Sample Size Calculation to Characterize a Relationship Between Sediment and Mummichog Tissue

The sample size necessary to detect a linear correlation between sediment and tissue was also evaluated for mummichog (and darter or killifish, using mummichog as a surrogate). The calculation, based on Zar (1996), was used to estimate the sample size needed to detect a correlation (Pearson Correlation Coefficient, r) if the true correlation is equal to a given value. A sample size of 13 to 19 would also allow a correlation between sediment and tissue concentrations to be detected if the true correlation is greater than 0.7 ($r > 0.70$, $r^2 > 0.50$) or 0.60 ($r > 0.60$, $r^2 > 0.36$) respectively (Table 7).

Table 7. Sample size needed to detect a significant Pearson correlation coefficient (*r*) and the resulting confidence interval around *r*

R	R^2	N TO DETECT DIFFERENCE FROM *R* = 0	R LCL	R UCL
0.1	0.01	783	0.03	0.17
0.2	0.04	194	0.06	0.33
0.3	0.09	85	0.09	0.48
0.4	0.16	47	0.13	0.62
0.5	0.25	29	0.16	0.73
0.6	0.36	19	0.20	0.83
0.7	0.49	13	0.24	0.90
0.8	0.64	10	0.34	0.95
0.9	0.81	7	0.46	0.99
0.95	0.90	5	0.42	1.00
0.99	0.98	4	0.60	1.00

n = sample size needed to detect a slope different from 0, if the true R = x

r = true correlation coefficient between sediment and tissue

RLCL –lower confidence limit on r

RUCL – upper confidence limit on r

Sample Size Adjustments to Address ProUCL Requirements

ProUCL will be used to calculate the 95% UCLs for each species within a zone for use in the HHRA and ERA. ProUCL requires a minimum of six detected concentrations; otherwise, it defaults to the use of a maximum concentration. The frequency of detection in the historical ESP data was evaluated for COPCs in tissue. Typically, chemical group totals (e.g., total PCBs) included at least one detected constituent in each sample, so the frequency of detection was 100% (with the exception of total chlordanes and total endosulfans). However, individual constituents that may also be of concern in the risk assessment (e.g., individual pesticides) were less frequently detected (Addendum 2). The sample size estimates for all receptors except mummichog were increased to ensure that a sufficient number of detected values of important constituents will be available for use in ProUCL, even if the detection frequency was as low as 60%. Mummichog sample size estimates did not need to be adjusted, due to the larger sample size recommendations. Based on ProUCL minimum data requirements for calculating the 95th UCL, assuming detection frequency of 60%, a sample size of 10 samples/species would be needed. Use of the parametric sample size estimate of 7 and this same frequency of detection would require the collection and analysis of 12 samples.

Uncertainties in Sample Size Estimates

Several important issues affect the use of existing data to estimate sample sizes for the upcoming risk assessments. Historical data used to evaluate sample sizes have been collected from only the lower 6 miles of the current study area. These data are being used to represent likely conditions throughout the entire study area, but it is not known if fish tissue residues in the upper estuarine or freshwater zones will have similar magnitudes of variance. Although tissue data from the broader CARP dataset and other river systems such as the Lower Duwamish Superfund site suggest that the typical range of variability has been captured in the existing data, if tissue concentrations in the upper reaches of the LPRSA are more variable

than concentrations from lower reaches, the precision of estimates of mean risk for these areas will be lower. This issue will be addressed as an uncertainty in the risk assessments.

Sample sizes of historical data for some receptors, tissues, and chemicals from the ESP are relatively small (< 10). Although the designs used to collect these data were based on sound sampling principles, it is still possible that the data underestimate the range of variability in current concentrations. If this is the case, it is possible that the sample sizes derived from these data will not provide the precision that is needed to characterize risks. This uncertainty has been addressed, in part, by using several different methods to estimate sample size requirements, and estimates based on parametric and bootstrapping methods have been concordant.

Because of the costs associated with collecting large numbers of samples and historical difficulties in collecting adequate tissue mass for large numbers of samples, the sample sizes evaluated here strike a reasonable balance between the financial and ecological costs of removing fish from the system and the needs for adequate data to characterize risks.

Sampling Locations

Samples will be placed within each 2-mile river reach in areas of known or likely habitat based on the 2007 field reconnaissance (Windward 2009 QAPP, in preparation) and prior field sampling events (Tierra Solutions 1999). At least three target sampling locations have been identified in each reach; however, additional sampling areas may be identified in the field in order to collect sufficient numbers of fish to meet the minimum tissue mass requirements for the proposed number of samples. Target sampling areas for mummichog will be located in mudflat areas; darter/killifish target sampling areas will be located in any available habitat (mud or sandflats; vegetated shallows) in the four freshwater reaches. Actual sampling locations and sampling contingency plans will be provided in the QAPP.

Tissue Analytes

The low-resolution core (LRC) program analyte list was used as the basis for the development of the proposed analyte list for the fish and decapod tissue. Table 8 provides a summary of the chemical groups that were analyzed in the LRC sampling effort and identifies the analytical groups that are proposed for fish and decapod tissue analyses.

Table 8. Analyte groups for tissue sampling

Analyte Group	Proposed for Analysis in Fish/Decapod Tissue	Rationale for Exclusion
Metals[a]	yes	
Mercury and methylmercury	yes	
Butyltins	yes	
Semivolatile organic compounds	yes	
PAHs (including alkylated PAH)	yes (excluding alkylated compounds)	Alkylated PAH compounds are not typically evaluated in tissue samples because of the lack of adequate human or ecological toxicity values.
Volatile organic compounds	no	It is not possible to analyze VOCs in tissue samples because of volatilization during sample preparation.

Analyte Group	Proposed for Analysis in Fish/Decapod Tissue	Rationale for Exclusion
PCB – congeners	yes	209 congeners
PCB – Aroclors	yes	
PCDD and PCDF congeners	yes	
Pesticides	yes (excluding toxaphene)	Toxaphene was not detected in any of the LRC sediment samples
Herbicides	no	Herbicides were rarely detected in surface sediment samples in the LRC sampling event.

[a] Aluminum, antimony, arsenic (total and inorganic), barium, beryllium, cadmium, calcium, chromium, cobalt, copper, iron, lead, magnesium, manganese, nickel, potassium, selenium, silver, sodium, thallium, titanium, vanadium, zinc.

ERA – ecological risk assessment

HHRA – human health risk assessment

LRC – low-resolution core

PAH – polycyclic aromatic hydrocarbon

PCB – polychlorinated biphenyl

PCDD – polychlorinated dibenzo-*p*-dioxins

PCDF – polychlorinated dibenzofurans

VOC – volatile organic compound

There are 10 chemical groups that were analyzed in the sediment for the LRC study, and 6 of these (metals, mercury, SVOCs, PCBs (congeners and Aroclors), PCDDs/PCDFs, and butyltins) are also proposed for analysis in the fish and decapod tissue samples with no changes. Two chemical groups are proposed for analysis in fish tissue sampling with amended target analyte lists (PAHs and pesticides). Two chemical groups are not proposed for analysis in the fish tissue samples (VOCs and herbicides). Further rationale for the tissue analyte list is provided in the following sections for each of the chemical groups.

Polycyclic Aromatic Hydrocarbons

PAHs are present throughout the LPR and were detected in the LRC sediment samples with detection frequencies ranging from 86 to 99% in the sediment samples. PAHs have also been detected in the existing fish tissue datasets for the LPRSA (e.g., see Addendum 1). PAHs are most commonly analyzed as individual compounds. However, in the LRC study, groups of alkylated PAH compounds were also analyzed (i.e., C1-naphthalenes, C2-phenanthrenes). The analysis of alkylated PAH can provide important information regarding the source of the compounds to the sediment (i.e., pyrogenic vs. petrogenic sources), but requires modifications of existing analytical methods.

Review of the available datasets shows that there are no tissue data for groups of alkylated PAH compounds. There are data available for individual alkylated PAH (i.e., 1-methyl naphthalene). The analysis of the groups of alkylated PAH compounds is not necessary for tissue samples because of the fact that the toxicological assessments used in the ERA and HHRA do not require the analysis of alkylated PAH compounds. In addition, the fact that PAHs are readily metabolized in fish tissue renders the evaluation of PAH signatures in tissue less relevant for the purpose of assessing PAH sources. PAH compounds are proposed as analytes for the fish tissue samples. However, groups of alkylated PAH are not proposed as analytes.

Organochlorine Pesticides

Organochlorine pesticides were detected in LRC sediment samples with detection frequencies ranging from 0 to 90% in the sediment samples. The pesticide toxaphene was not detected in any of the sediment samples analyzed. In addition, toxaphene was not detected in any of the 145 tissue samples that were analyzed for toxaphene in the existing tissue dataset. Based on this evaluation, organochlorine pesticides, with the exception of toxaphene, are proposed for analysis in the fish tissue samples.

Volatile Organic Compounds

The detection frequency of individual VOCs ranged from 0 to 94% in the LRC surface sediment samples. VOCs are not analyzed in fish tissue because of the fact that these compounds are lost to volatilization in the processing of the samples. The assessment of risk due to exposure to VOCs for both human and ecological receptors is assessed based on exposure to VOC in sediment and surface water.

Herbicides

Herbicides were rarely detected in the LRC sediment samples. The detection frequencies ranged from 0.7 to 4% of the LRC sediment samples. Herbicides were detected in 6 surface sediment samples (0 to 0.5 ft) out of the 115 locations sampled. Five of these detected samples were within the LPRSA (one was above the dam). The five detects came from LPRSA samples collected at RM 4.25 (channel), RM 7 (channel), RM 7 (side channel), RM 7.85 (channel), and RM 12.56 (side channel/shoal area). Therefore, herbicides are not proposed as analytes for the fish tissue samples.

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