Publication No. 02 03 087

Arsenic Speciation in Puget Sound Fish and Crab

Quality Assurance Project Plan

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January 6, 2002
[November 16, 2001 – draft]

Washington State Department of Ecology
Environmental Assessment Program
Olympia, Washington

1998 303(d) listings addressed by this project: Sinclair Inlet (Segment WA-15-0040), Port Orchard (WA-15-0030), and Port Washington Narrows (Segment WA-15-0050) - arsenic in edible fish tissue.

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Background and Problem Statement

Sinclair Inlet, Port Orchard, and Port Washington Narrows have been listed by the state of Washington under Section 303(d) of the Clean Water Act for non-attainment of the EPA human health criteria for arsenic. The listings are based on arsenic concentrations measured in various flatfish species collected in 1989 (Cubbage, 1992).

In the Cubbage study, five composite samples consisting of pooled muscle tissue from English sole, sand sole, C-O sole, rock sole, and flathead sole* had total recoverable arsenic concentrations ranging from 3.7 to 20.2 mg/Kg (wet weight basis). The composites were formed using tissue samples from mixed species, as encountered at each sampling site; four to five individuals per composite (Figure 1).

Ecology's 303(d) listing criterion for arsenic in edible fish and shellfish tissue is 0.0062 mg/Kg, calculated as the product of EPA's bioconcentration factor (44 L/Kg) and water column criterion (0.14 ug/L) (National Toxics Rule, 40 CFR Part 131; Ecology Water Quality Program Policy 1-11). Although this criterion is for inorganic arsenic, it has been the practice of the Ecology Water Quality Program (WQ) to list waterbodies based on total recoverable arsenic data. For 303(d) listings based on toxics in edible tissue, Ecology requires a minimum of at least two single fish samples or one composite formed from at least three individual organisms.

Other Washington State 303(d) listings for arsenic in tissue include: Eagle Harbor (shellfish), Dyes Inlet (shellfish), Port Washington Narrows (shellfish), and the lower Columbia River (fish). The Environmental Assessment Program (EAP) plans to do sampling related to the shellfish listings in the spring of 2002. EAP (Richard Jack) and WQ (Cheryl Niemi) are currently working on a project to evaluate the human health criteria for arsenic in freshwater. In addition to the Columbia River fish tissue listing, there are a number of other freshwater listings for arsenic in the water column, for which EAP has been doing ambient monitoring of total recoverable arsenic.

In discussing the Sinclair Inlet/Port Orchard/Port Washington Narrows fish tissue data, Cubbage noted the following:

"Crecelius et al. (1989) examined fish from 13 bays in Puget Sound and found little variation between sites in the concentrations of metals. The one exception in that study was arsenic. Higher concentrations of arsenic were found in sites near an historic source of arsenic, the ASARCO smelter in Tacoma. If the samples collected near this source at Point Defiance are excluded from consideration, the arsenic concentrations in the present study average nearly twice the concentrations in other areas of Puget Sound. Note that earlier studies in Sinclair Inlet found arsenic in fish muscle at approximately the same concentrations as the reference area in Discovery Bay (here Cubbage is referring to

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**Parophrys vetulus, Psettichthys melanostictus, Pleuronichthys coenosus, Lepidopsetta bilineata, Hippoglossoides elassodon*

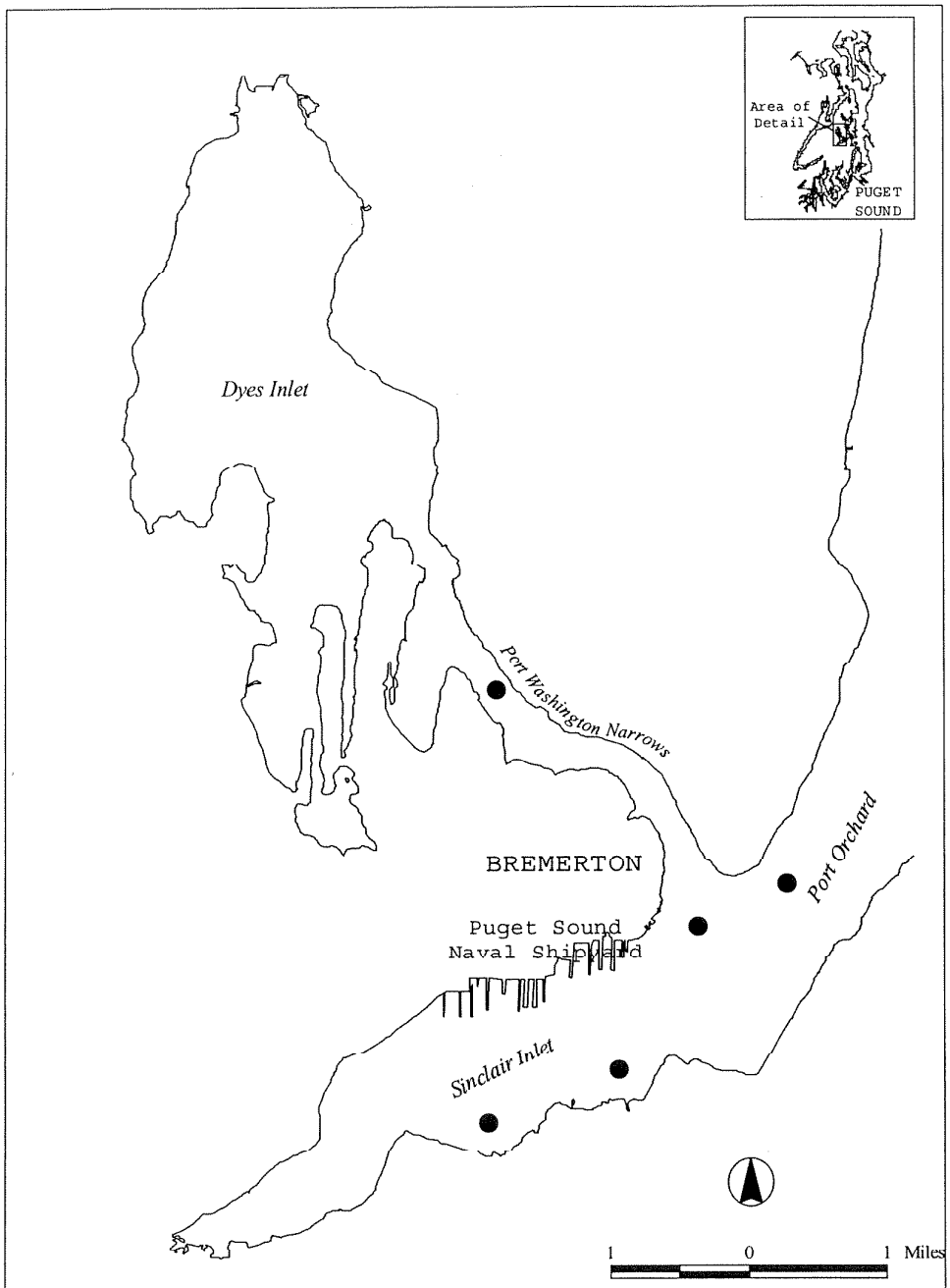

Figure 1. Location of Cubbage (1992) Fish Samples Analyzed for Total Recoverable Arsenic

Gahler et al. 1982). Again, these higher concentrations found in this study are without clear explanation, however, because of limitations in the comparability of the data, they cannot denote a temporal trend."

In fish and shellfish tissue, arsenic is primarily in organic form as arsenobetaine (Ballin et al., 1994). The toxic species of primary concern are inorganic arsenic, dimethylarsinic acid (DMA), and monomethylarsonic acid (MMA), which are minor constituents. Information on the relative amounts of these forms in Puget Sound fish and shellfish is limited to the data summarized by Lorenzana (2001) in Table 1. The general picture emerging from the available local and national data is that marine shellfish have the highest, but also most variable, concentrations of inorganic arsenic, followed by marine fish and freshwater fish, in that order (Donahue and Abernathy, 1999). Puget Sound marine organisms may commonly exceed the arsenic criterion that Ecology uses for 303(d) listing. (DMA and MMA are not taken into consideration for 303(d)). Dietary exposure to arsenic via seafood and other food products is currently an issue of concern and research.

Table 1. Arsenic Speciation Data for Puget Sound Fish and Shellfish Composite Samples (mg/Kg, wet) [median value as summarized by Lorenzana, 2001]

Species	Location	N =	Total Arsenic	Inorganic Arsenic	DMA
Sand dab	Commencement Bay	18	4.5	0.01	1.1
Rock sole	Commencement Bay	2	17	0.05	4.2
Red rock crab (cooked)	Commencement Bay	4	3.6	0.03	0.33
Littleneck clam	Marrowstone Island	12	2.2	0.02	0.45
Cockle clam	Marrowstone Island	9	1.1	0.02	0.13
Oyster	Marrowstone Island	1	2.1	0.01	0.20
Littleneck clam (cooked)	Sequim Bay	3	6.9	0.02	0.11

Analyzed by Battelle Marine Sciences Laboratory, Sequim, Washington; EPA Method 1632, Rev. A

Project Description

The primary goal of this project is to provide WQ with data showing what concentrations of total and inorganic arsenic occur in the edible tissues of Puget Sound fish and crab, and how these concentrations vary between locations and species. This information will help WQ determine the appropriateness of listing Sinclair Inlet, Port Orchard, Port Washington Narrows, or other parts of Puget Sound for arsenic in fish and crab tissue. The data will also be useful in the event a TDML is done.

A secondary goal is to provide arsenic speciation data in support of an ongoing study by EPA Region 10 to characterize the human health risk posed by arsenic in Puget Sound fish, shellfish, and seaweed.

The project will be conducted by EAP and will rely on samples previously collected by the Washington State Department of Fish and Wildlife (WDFW) for the Puget Sound Ambient Monitoring Program (PSAMP).

Project objectives are as follows:

- Obtain accurate and representative data on the concentrations of total arsenic, inorganic arsenic, MMA, and DMA in muscle tissue of individual English sole collected from Sinclair Inlet, Commencement Bay, Elliot Bay, and a Hood Canal reference area.
- Conduct a similar analysis on composite muscle tissue samples from several other fish species and Dungeness crab collected at various locations throughout Puget Sound.
- Analyze the data for differences between sampling sites and species.
- Assess the frequency with which inorganic arsenic exceeds the 303(d) listing criterion.
- Make recommendations on retaining or removing the 303(d) arsenic listings for Sinclair Inlet, Port Orchard, and Port Washington Narrows.
- Make recommendations on the appropriateness of 303(d) listing other Puget Sound waterbodies for arsenic.
- Provide this information to WQ, EPA Region 10, affected Ecology regional offices, and other interested parties.

Organization and Schedule

EAP Project Lead – Art Johnson (360/407-6766)
WDFW Contacts – Sandie O'neill (360/902-2843), Jim West (360/902-2842)
EPA Region 10 Contact – Roseanne Lorenzana (206/553-8002)
EAP Toxics Studies Unit Supervisor – Dale Norton (360/407-6765)
Manchester Environmental Laboratory Director – Stuart Magoon (360/871-8813)
Manchester Laboratory QA & Sample Management – Karin Feddersen (360/871-8829)
Manchester Laboratory Data Reviewer – to be determined
Battelle Marine Sciences Laboratory – Eric Crecelius (360/681-3604)
Ecology Quality Assurance Officer – Cliff Kirchmer (360/407-6455)
EIM Data Entry – Morgan Roose (360/407-6458)

December 7, 2001	Submit first sample set to contractor via Manchester Laboratory
January 24, 2002	Laboratory analyses completed and data reported to Manchester
February 11, 2002	Manchester data review to project lead; data provided to EPA
February 2002	Submit second sample set to contractor via Manchester Laboratory
April 2002	Laboratory analyses completed and data reported to Manchester
May 2002	Manchester data review to project lead; data provided to EPA
July 2002	Draft project report completed
August 2002	Final project report completed
October 2002	Data entered into EIM database

Data Quality Objectives

Table 2 shows project targets for accuracy, precision, and bias, and the reporting limits required.

Table 2. Measurement Quality Objectives

Parameter	Accuracy (% deviation from true value)	Precision (RSD)	Bias (% of true value)	Reporting Limit (mg/Kg wet)
Tot. Rec. Arsenic	30%	10%	10%	0.1
Inorganic Arsenic	40%	15%	10%	0.005
MMA	40%	15%	10%	0.01
DMA	40%	15%	10%	0.01

The reporting limits are the lowest currently achievable. The limit for inorganic arsenic is at the 303(d) listing criterion. These reporting limits should be sufficient to consistently quantify the analytes of interest, based on the available data.

Sampling Design

The samples proposed for analysis are shown in Table 3. The species, number of samples, and locations were dictated by the tissues available from recent PSAMP fish collections and available funding. WDFW has no PSAMP samples from Port Washington Narrows.

December 2001 Sample Set – These samples were selected to provide a survey of arsenic levels in harvested Puget Sound fish and crab. The species to be analyzed--English sole, Quillback rockfish, Dungeness crab, Coho salmon, and Pacific herring--represent a range of feeding types, habitats, and life histories. The Coho samples are from returning wild adults.

Table 3. PSAMP Fish Samples for Arsenic Speciation

Location	Date	Species	N=	Comment
		Samples to be Submitted December 2001		
Hood Canal	2000	English sole	1	Composite of 15
Commencement Bay	2000	English sole	1	Composite of 15
Sinclair Inlet	2000	English sole	1	Composite of 15
Elliot Bay	2000	English sole	1	Composite of 15
Strait of Juan de Fuca	2000	Quillback rockfish	1	Composite of 8
Foulweather Bluff	1997	Quillback rockfish	1	Composite of 12
Elliot Bay	2001	Quillback rockfish	1	Composite of 12
Hood Canal	2001	Dungeness crab	1	Composite of 11
Commencement Bay	2001	Dungeness crab	1	Composite of 15
Port Gardner	2001	Dungeness crab	1	Composite of 15
Skagit River	2000	Coho salmon	1	Composite of 12
Duwamish River	2000	Coho salmon	1	Composite of 12
Nisqually River	2000	Coho salmon	1	Composite of 12
Cherry Point	2001	Pacific herring	1	Composite of 15
Port Orchard	2001	Pacific herring	1	Composite of 15
Squaxin Pass	2001	Pacific herring	1	Composite of 15
		Total November samples =	16	
		Samples to be Submitted February 2002		
Hood Canal	2001	English sole	15	Individual fish
Commencement Bay	2001	English sole	15	Individual fish
Sinclair Inlet	2001	English sole	15	Individual fish
Elliot Bay	2001	English sole	15	Individual fish
		Total February samples =	60	
Total lab cost + QC samples, @ $275*/sample =			$23,100	

*including 25% surcharge from Manchester

Three to four locations were selected for each species to give good spatial coverage over Puget Sound and include both urban/industrial and reference sites (Figure 2). Based on the arsenic and other chemical data that has been collected by WDFW for PSAMP (presented at Puget Sound Research conferences), Hood Canal and eastern Juan de Fuca Strait were selected as reference sites. It is unclear at present if any of the Coho and herring sites represent reference conditions.

Each sample will consist of a composite of from 8 to 15 individual organisms, in accordance with the tissues available through WDFW. To the extent possible, the fish and crab in each composite will be of similar size and age (age has been determined by WDFW). Approximately equal numbers of male and females will be included in each composite, except all crab samples are males, as per harvest rules. The WDFW fish muscle samples are skin-off fillets.

February 2002 Sample Set – The purpose of these samples is to determine if there are significant differences in arsenic levels among fish from four sampling sites in Puget Sound – Sinclair Inlet, Commencement Bay, Elliot Bay, and Hood Canal. Commencement Bay has the highest water column and sediment concentrations of arsenic reported in Puget Sound (Crecelius et al., 1998; Johnson and Summers, 1999; SEDQUAL database). Elliot Bay and Commencement Bay are, in general, the most chemically contaminated embayments in the Sound.

Because the 303(d) listings in question are based on flatfish, English sole will be analyzed. Factors that make this species a potentially good indicator of local conditions are its restricted home range and benthic, as opposed to pelagic, feeding habit.

Fifteen individual sole will be analyzed from each sampling site. To the extent possible, the size, age, and number of males and females will be similar among sites. The ages of the English sole collected in 2001 are currently being determined by WDFW; therefore, submittal of these samples is being delayed until February.

A sample size of at least 15 was recommended by WDFW, based on the variability seen in their past PSAMP fish tissue data (composite sampling) and best professional judgment. Based on the range of inorganic arsenic concentrations reported for Commencement Bay flatfish by Lorenzana (2001), a crude estimate of the variance is 0.50. For individual samples taken from a population with a variance of 0.50, a sample size of 15 has a 95% chance of the relative error of the estimated mean being less than 25% (Gilbert, 1987).

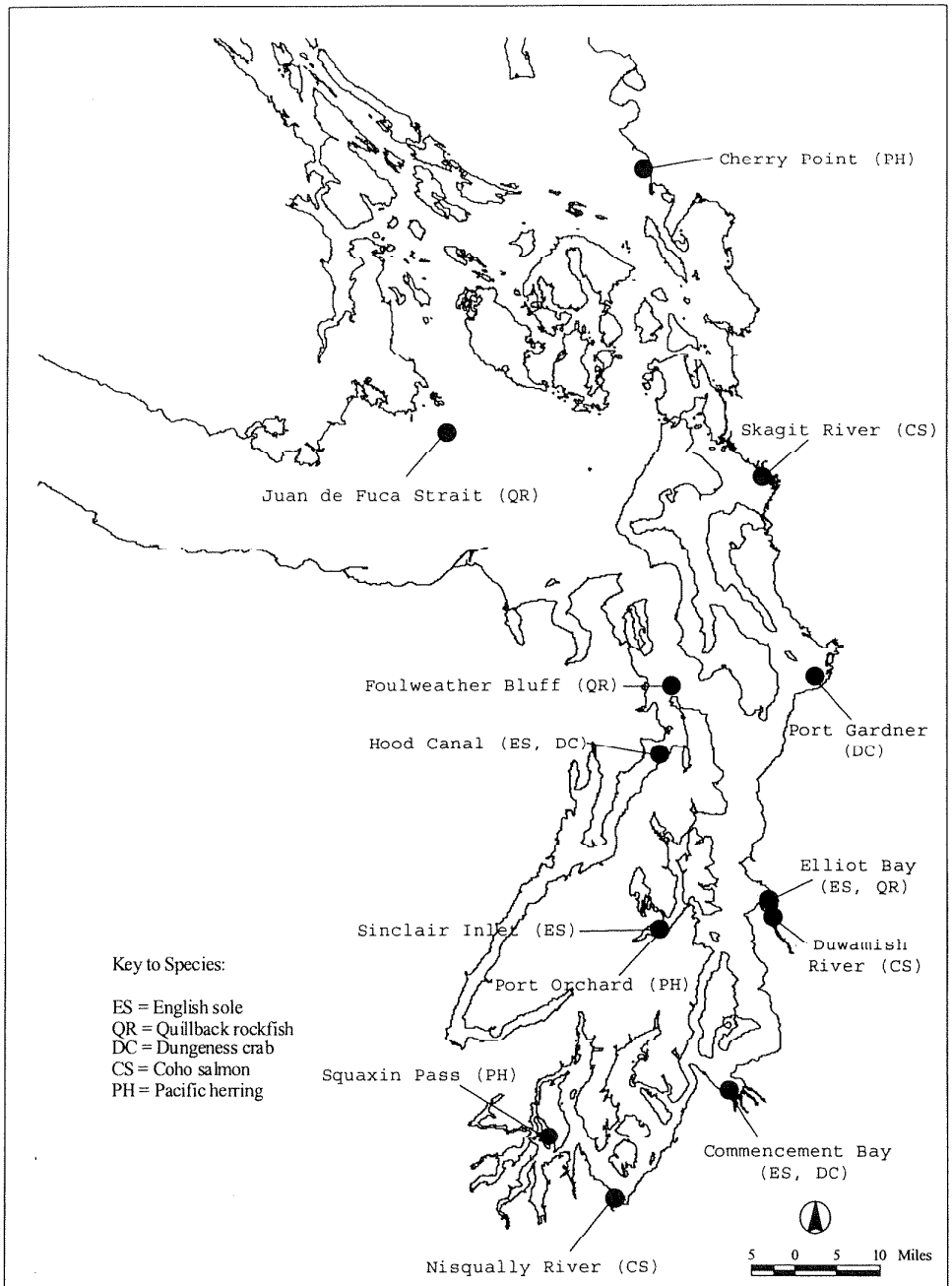

Figure 2. WDFW PSAMP Sampling Stations and Species Selected for Arsenic Speciation Analyses

Tissue Preparation

The samples are currently frozen at WDFW, some as individual samples, others already composited. The tissue was prepared by WDFW following PSAMP protocols (PSWQAT, 1997). Samples needing to be composited will be homogenized at Ecology HQ following routine EAP methods (Johnson, 1999 a,b), placed in 8-oz glass jars with Teflon lid liners cleaned to EPA specifications (EPA, 1990), and re-frozen pending analysis.

The recommended holding time for analyzing arsenic in tissue samples used in Puget Sound studies is two years (PSWQAT, 1997). No holding times have been established for inorganic arsenic, MMA, or DMA. In the opinion of Dr. Eric Crecelius, Battelle Marine Research Laboratory, who developed the arsenic speciation method being used for this project, holding time is not an issue for any form of arsenic in frozen samples (11/20/01 email).

Chemical Analysis

Table 4 shows the number of tissue samples to be analyzed, expected range of results, and the laboratory procedures to be used. The samples will be analyzed by Battelle Marine Sciences Laboratory, a contractor selected by Manchester.

Table 4. Laboratory Procedures

Analyte	Sample Matrix	Number of Samples[a]	Expected Range of Results	Sample Prep	Analytical Method
Tot. Rec. Arsenic	tissue	16 / 60	0.5 - 20 mg/Kg	NaOH	EPA 200.8[b]
Inorganic Arsenic	tissue	16 / 60	0.001 - 0.10 mg/Kg	NaOH	EPA 1632 Rev. A
MMA	tissue	16 / 60	<0.1 mg/Kg	NaOH	EPA 1632 Rev. A
DMA	tissue	16 / 60	0.1 - 5 mg/Kg	NaOH	EPA 1632 Rev. A

[a]December / February sample sets, excluding laboratory QC samples

[b]or equivalent method

Total recoverable arsenic will be analyzed by ICP/MS using EPA Method 200.8 or equivalent.

Arsenic speciation will be done by EPA Method 1632, Revision A: *Chemical Speciation of Arsenic in Water and Tissue by Hydride Generation Quartz Furnace Atomic Absorption Spectrometry*. This method is recommended by the EPA Office of Water for determining arsenic species in tissue samples. It is currently being used for the EPA National Study of Chemical Residues in Lake Fish Tissue (which includes local samples collected by EAP) and for the previously mentioned human health study by EPA Region 10.

After the analyses are completed, the contract laboratory will return excess sample to Manchester where it will be saved frozen for 60 days.

Quality Control

Table 5 shows the quality control (QC) samples to be analyzed for this project.

Table 5. Minimum Quality Control Procedures for Each Sample Submittal

Parameter	Method Blank	Replicate Analyses	Stand. Ref. Material	Matrix Spike	Matrix Spike Duplicate
Tot. Rec. Arsenic	1/20 samples	2	2	1/20 samples	1/20 samples
Inorganic Arsenic	1/20 samples	2	- -	1/20 samples	1/20 samples
MMA	1/20 samples	2	- -	1/20 samples	1/20 samples
DMA	1/20 samples	2	- -	1/20 samples	1/20 samples

Field QC

No field QC samples will be analyzed.

Laboratory QC

Laboratory QC samples will include method blanks, matrix spikes & matrix spike duplicates, analytical replicates, and a standard reference material. These samples will be analyzed at the frequency indicated in Table 5.

The procedures and criteria for analyzing blanks and matrix spikes are described in the methods. One sample from each sample set will be analyzed in triplicate; these samples to be identified by the project lead. A standard reference material, NRCC DORM-2 (dogfish muscle), will be analyzed in duplicate with each sample set. The certified

concentration of total recoverable arsenic in this material is 17.7 +/- 2.1 mg/Kg. No SRMs are available for inorganic arsenic, MMA, or DMA.

Data Review, Verification, and Validation

Manchester will conduct a review of the contract laboratory's data and case narratives. Manchester will verify that methods and protocols specified in the QAPP were followed; that all calibrations, checks on quality control, and intermediate calculations were performed for all samples; and that the data are consistent, correct, and complete, with no errors or omissions. Evaluation criteria will include the acceptability of instrument calibration, procedural blanks, spike sample analyses, precision data, laboratory control sample analyses, and appropriateness of data qualifiers assigned. Manchester will prepare a written report on the results of their data review.

The project lead will review the contract laboratory's data package and Manchester's data validation report. The project lead will check these data and reports for completeness and reasonableness. Based on these assessments, the data will be either accepted, accepted with appropriate qualifications, or rejected and re-analysis considered.

Data Quality Assessment

Once the data have been reviewed, verified, and validated, the project lead will make a determination if the data can be used to make the determinations and decisions for which the project was conducted. Results from analyzing the laboratory QC samples will be used to judge if the MQOs have been met. The SRM will indicate directly if the accuracy target for total recoverable arsenic has been met.

If the results are satisfactory, analysis of variance, or other appropriate test, will be used to test for among-site comparisons of English sole. Results from the composite samples will be compared to identify potentially significant differences between sites and species. The exceedance frequency of the 303(d) listing criterion for arsenic will be determined for each sampling site and species, taking the precision and bias of the data into account.

Reports

On or before July 2002, the project lead will prepare a draft report on results of the study and provide it to WQ for review. The report will include:

- Maps of the study area showing sampling sites.
- Descriptions of field and laboratory methods.
- Sample information (lengths, weights, sex, age).

- Discussion of data quality and the significance of any problems encountered in the analyses.
- Summary tables of the chemical data.
- Observations on significant or potentially significant findings with respect to species and site differences.
- Summary and review of other data on arsenic speciation in fish and shellfish tissue.
- Recommendations for 303(d) listing or de-listing, and other recommendations as appropriate.
- Complete data set and data reviews as an appendix to the report.

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