

APPENDIX H-5B

**Clam (*Pitar morrhuana*) Analytical Results:
Final Report**

Prepared for

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US ARMY CORPS
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New England District

Contract No. DACW33-01-D-0004

Delivery Order No. 13

August, 2002

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**Long Island Sound
Disposal Site Study**

Final Report

for

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Long Island Sound Disposal Site Study**

Submitted to

**Department of the Army
U.S. Army Corps of Engineers
North Atlantic Division
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1.0 INTRODUCTION

The following validated data report is for Delivery Order 13, *Long Island Sound Disposal Site Study*. This report includes pesticide/PCB, PAH and bis(2-ethylhexyl)phthalate, butyltins, metals, dioxin/furan, dioxin-like PCB congeners, radionuclide, percent dry weight and lipid results for the clam samples analyzed in support of the Benthic Chemistry Testing (Task 3).

1.1 Background

To support the production of the Long Island Sound Environmental Impact Statement (LIS EIS), benthic organisms were collected to assess the bioaccumulation of sediment contaminant in organisms that are exposed to the sediments of the disposal sites versus non-disposal areas. Benthic organisms were collected (including the clam *Pitar morrhuana*) in July and August, 2000 from two dredged material disposal sites (CLIS and NLDS) (see Figure 1). Collections were performed by ENSR of Acton, MA, and details of the benthos collection are provided in the “Field Summary Report, July/August Survey 2000” (ENSR 2000).

After collection, all samples were transferred to Woods Hole Group (WHG) in Wareham, MA. Samples were placed in sealed glass jars at WHG and were stored frozen. Sample custody was transferred to Battelle on 1/22/02. Upon receipt at Battelle, all samples were logged in, assigned new Battelle IDs and stored frozen until further processing and analysis.

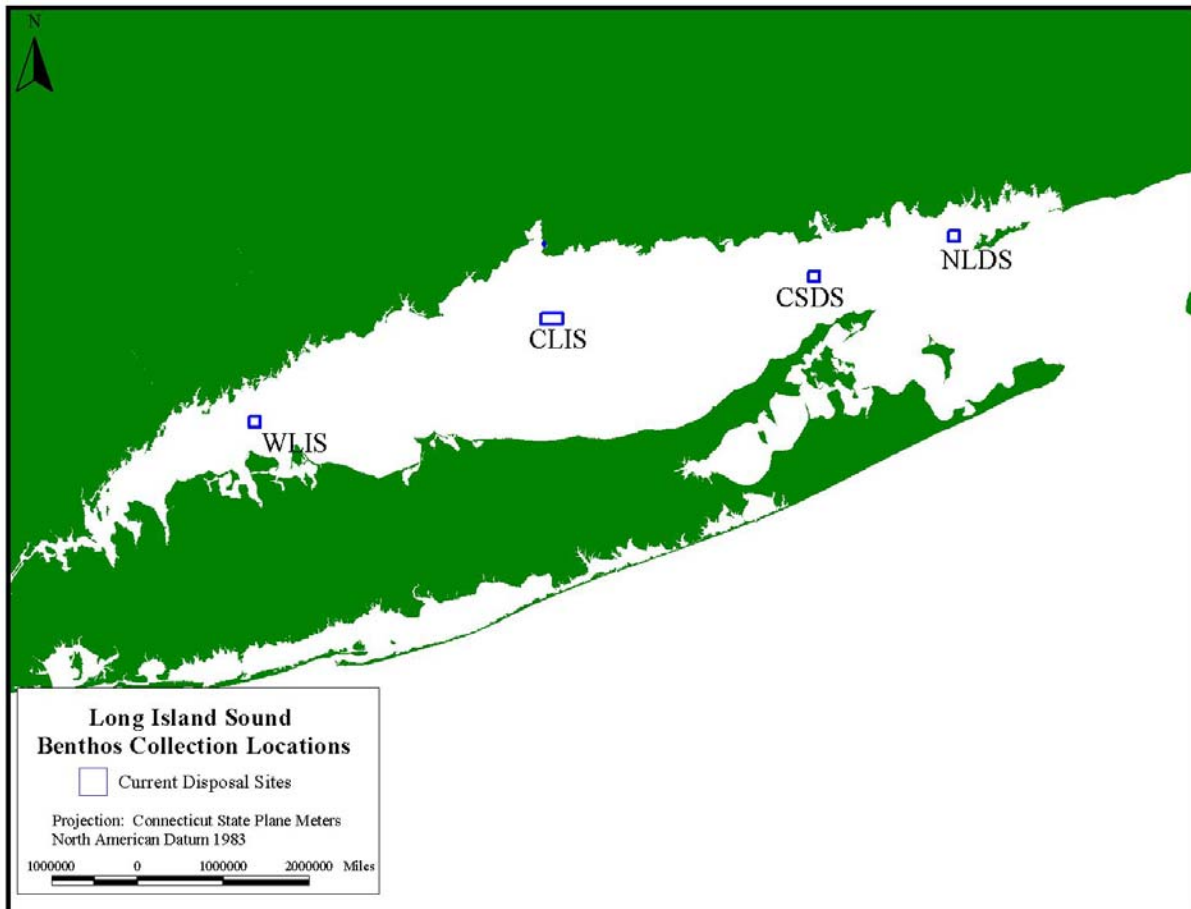


Figure 1. Benthic Sampling Locations.

1.2 Summary of Sample Processing and Analyses

All samples were received in good condition. Samples were stored frozen (at, or below -20°C) until processing. Clam samples were received in their shells. All samples were shucked. Homogenization by station was performed at Battelle, Duxbury using titanium instrumentation to allow for splits for metals analyses. Aliquots for the various analyses were removed, placed in appropriate jars, and forwarded to the specified laboratories for analyses.

Table 1 lists the laboratories that performed sample analyses. Table 2 summarizes the analytical tasks performed on each sample. All samples were not tested for all analytical parameters.

Table 1. Summary of Analytical Labs

Analysis Parameters	Laboratory	Third Party Validator
Pesticides/PCB	Battelle, Duxbury, MA	NA
PAH & Bis(2-ethylhexyl)phthalate	Battelle, Duxbury, MA	NA
Butyltins	Battelle, Duxbury, MA	NA
Metals	Battelle, Sequim, WA	NA
Dioxin/Furan, Dioxin-like PCB congeners	PSC Analytical Services, Ontario, Canada	Ecochem, Inc., Seattle WA. (1)
Radionuclide	STL St. Louis, Earth City, MO	NA
Lipids	Battelle, Duxbury, MA	NA

NA indicates Not Applicable

(1) Only one batch per matrix was sent to Ecochem for Tier III level validation.

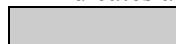
1.3 Data Verification/Validation

Laboratory data generated for this study received internal verification and validation by the Quality Assurance (QA) officers from each participating laboratory. Second-level verification of all data was performed at Battelle, Duxbury by comparing results with specific measurement performance criteria (MPCs) defined in the Quality Assurance Project Plan prepared for this study (Battelle 2002). The dioxin/furan and dioxin-like PCB congener clam data package was submitted by PSC Analytical for third party validation, which was conducted by Ecochem Inc, of Seattle, WA. The validation report is included as Attachment 8 of this report.

Table 2. Clam Samples Summary of Analyses.

Sample ID	Study Area	Station	Battelle ID	PCB Aroclor, Pest, PAH	Butyltins	Metal	Dioxin/furan and Dioxin like PCBs	Radionuclide	Lipids
LIS04CLREFC5	CLIS	REF	V1273	X	X	X	X	X	X
LIS06CLREFC2			V1293	X		X	X		
LIS06CLREFC4			V1295	X		X	X		X
LIS04CLFVPC4	CLIS	FVP	V1277	X	X	X	X	X	X
LIS06CLFVPC1			V1296	X		X	X		X
LIS06CLFPC3			V1298	X		X	X		
LIS04CLN93C2	CLIS	NHAV93	V1279	X	X	X	X	X	X
LIS06CLN93C1			V1299	X		X	X		
LIS06CLN93C2			V1300	X		X	X		X
LIS04NLLRFC2	NLDS	LRF	V1258	X	X	X	X	X	X
LIS06NLLRFC2			V1283	X		X	X		
LIS06NLLRFC5			V1286	X		X	X		
LIS04NLRLLCC1	NLDS	RLC	V1262	X		X	X	X	X
LIS04NLRLLCC2			V1263	X		X	X		X
LIS04NLRLLCC3			V1264	X		X	X		X
LIS06NLRLLCC1			V1287	X	X	X	X		X
LIS04NLSEAC1	NLDS	SEA	V1265	X		X	X	X	
LIS06NLSEAC1			V1289	X	X	X	X		X
LIS06NLSEAC2			V1290	X		X	X		X

X indicates analyses performed

 Indicates analysis not performed

2.0 METHODS

Chemical analysis of clam samples for pesticide/PCB, PAH, bis (2-ethylhexyl) phthalate, butyltin, metal, dioxin/furan and dioxin-like PCB congener, radionuclide, and lipid were conducted following methods and SOPs as described in *Quality Assurance Project Plan: Long Island Sound Study* (January, 2002). Exceptions and unusual circumstances have been documented and are noted.

2.1 Pesticide/PCB

Tissue samples were extracted for Pesticide/PCB analysis following general NS&T methodologies. *Pitar Morrhuana* (Clam) samples were homogenized using a titanium Tekmar Tissuemizer and approximately 10 grams of tissue was extracted three times with dichloromethane using maceration techniques. The combined extract was dried over anhydrous sodium sulfate, concentrated, processed through alumina cleanup column, concentrated, and qualitatively split for further purified on a HPLC cleanup column. The post-HPLC extract was concentrated, fortified with Recovery Internal Standard (RIS) and split quantitatively for Pesticide/PCB analysis and PAH analysis. Pesticide/PCB extracts were then solvent exchanged into hexane. Extracts were analyzed using gas chromatography/electron capture detection

(GC/ECD), following general NS&T methods. Sample data were quantified by the method of internal standards, using the RIS compounds. Final data tables report surrogate recovery data.

2.2 PAH and Bis(2-ethylhexyl)phthalate

Tissue samples were extracted for PAHs following general NS&T methodologies. Clam tissue samples were homogenized and approximately 10 grams of tissue was extracted three times with dichloromethane using maceration techniques. The combined extract was dried over anhydrous sodium sulfate, concentrated, split quantitatively with one portion processed through alumina cleanup column, concentrated, and further purified using a HPLC cleanup column. The post-HPLC extract was concentrated, fortified with RIS and split quantitatively for pesticide/PCB and PAH analysis. PAH extracts were analyzed using gas chromatography/mass selective detection (GC/MS) and selective ion monitoring (SIM) mode following general NS&T methods. Sample data were quantified by the method of internal standards, using the RIS compound Acenaphthene d-10. Final data tables report surrogate recovery data. Pre-HPLC extracts were analyzed separately for bis(2-ethylhexyl)phthalate due to potential loss of this compound to the HPLC column, and data were incorporated into the final reports.

2.3 Butyltins

Approximately 10 grams of clam was spiked with a Surrogate Internal Standard (SIS: tripropyltin (TPET)) to monitor laboratory efficiency and extracted with hexane and the chelating agent tropolone. Following extraction, the cationic butyltin compounds were converted to nonpolar *n*-hexyl derivatives with commercially available *n*-hexylmagnesium bromide via a Grignard reaction. The extract was cleaned up through a Silica/Florisil gel liquid chromatography column. The butyltins were collected in a conventional hexane eluate from the Silica/Florisil column. The extracts were analyzed by GC/FPD using a tin-specific photometric filter. Data are reported in units of ug/kg wet weight. This procedure utilizes the method of internal standards. The SIS is added at the beginning of the extraction procedure and carried through all steps of the method. The concentrations of target analytes in the samples are calculated relative to the SIS. The overall recovery efficiency of the method is measured by calculating the recovery of SIS relative to the recovery internal standard RIS dipropyltin (DPT), which is added just prior to GC analysis. All peaks are manually integrated due to the extreme fluctuations in baseline noise associated with this analysis.

2.4 Metals

Eleven metals were analyzed: silver (Ag), arsenic (As), beryllium (Be), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb), selenium (Se), and zinc (Zn). To prepare the tissues for analysis, they were freeze-dried then blended in a Spex mixer-mill. Sample percent moisture/dry weight was determined according to Battelle SOP MSL-C-003. Tissue samples were digested using aqua regia according to Battelle SOP MSL-I-024, *Mixed Acid Tissue Digestion*. An approximately 500-mg (dry weight) aliquot of each sample was combined with nitric and hydrochloric acids (aqua regia) in a Teflon bomb and heated in an oven at 130°C (±10°C) overnight. After heating and cooling, deionized water was added to the tissue digestate to achieve analysis volume and the digestates were submitted for analysis.

Sample digestates were analyzed for Ag, As, Be, Cd, Cr, Cu, Ni, and Se using inductively coupled plasma-mass spectrometry (ICP-MS) according to Battelle SOP MSL-I-022, *Determination of Elements in Aqueous and Digestate Samples by ICP/MS*. This procedure is based on two methods modified and adapted for analysis of solid sample digestates: EPA Method 1693, *Determination of Trace Elements in Ambient Waters by Inductively Coupled Plasma-Mass Spectrometry* and EPA Method 1640,

Determination of Trace Elements in Water by Preconcentration and Inductively Coupled Plasma-Mass Spectrometry.

The initial analysis of Cr by ICP-MS showed an over-recovery of Cr in the SRMs and QC sample results did not meet data quality objectives, most likely due to a polyatomic interference with carbon during ICP-MS analysis. Cr was reanalyzed using the method of standard addition. Digested samples of SRM TORT-2, a lobster liver matrix, were spiked with calibration standards and used to calibrate the ICP-MS. The instrument applied a correction factor to sample values, subtracting false Cr concentrations that were contributed by the matrix interference from each sample.

Sample digestates were analyzed for Pb and Zn by inductively coupled plasma-atomic emission spectrometry (ICP-AES) following SOP MSL-I-027, *Determination of Metals in Aqueous and Digestate Samples by ICP-AES*. Sample digestates were analyzed for Hg using cold-vapor atomic absorption spectroscopy (CVAA) according to Battelle SOP MSL-I-016, *Total Mercury in Tissues and Sediments by Cold Vapor Atomic Absorption*.

All results were reported in units of $\mu\text{g/g}$ on a dry-weight basis and converted to $\mu\text{g/g}$ on a wet-weight basis, calculated using the percent dry weight of each sample. The results for analysis of Pb were reported as blank corrected concentrations (see discussion in Section 4.0); results for analysis for all other metals were not blank corrected.

2.5 Dioxin/Furan and Dioxin-like PCB Congeners

Clam extracts were cleaned, and analyzed for the seventeen 2,3,7,8 – substituted PCDD/PCDF following the general procedures in EPA Method 1613, Revision B, as described in PSC Analytical Services SOP ORG-310. Clam meat samples were also extracted and analyzed for dioxin-like PCBs (also referred to as 12 WHO congeners) following the general procedures in EPA Method 1668, Revision A, as described in PSC Analytical Services SOP ORG-307. The extraction allows for both dioxins/furans and the dioxin like PCB congeners, with subsequent splitting of the extract into thirds for separate clean-up and analysis of the dioxins/furans from the dioxin-like PCB congeners and archive.

The PCDDs and PCDFs are extracted from solid samples with a solvent mixture of 50:50 hexane/dichloromethane. Following extraction, the samples are cleaned up via GPC and/or carbon (if sample necessitated) and passed through a series of columns, which remove the bulk of the organic matrix, which co-extracted with the PCDD/Fs. The resulting fraction was concentrated to 2mL for analysis. Final volume for injection was 20 μL . Qualitative/quantitative analysis for PCDD/Fs was performed using separation by high resolution capillary gas chromatography, and measured by high resolution mass spectrometry (HRMS). PCDD/Fs were identified by comparing gas chromatograph retention times and the ion abundance ratios of the m/z 's with the corresponding values obtained for standards.

The GCMS system is calibrated and the analyte concentrations are determined using an isotope dilution technique. Quantitation is based on the use of internal standards and relative response factors (RRFs).

2.6 Radionuclides

Severn Trent Laboratory analyzed a subset of clam samples for radiochemical parameters.

2.6.1 ^{60}Co and ^{137}Cs

Clam samples were prepared for ^{60}Co and ^{137}Cs following STL SOP's STL-RC-0025, STL-RC-5016, and STL-RD-0101. Clam samples were placed in either a 25 mL or 100 mL counting geometry.

2.6.2 Isotopic U

For the analysis of isotopic uranium, SOPs STL-RC-5016, STL-RC-0238, and STL-RC-0100 were followed for sample preparation and SOP STL-RD-0201 for the analysis. The appropriate aliquot of clam meat was placed in a quartz crucible and U-232 tracer, used as a yield monitor, was added to the sample. The crucible was placed on a hotplate at low heat to dry. The crucible containing the dry sample was placed in a muffle furnace and burned in a controlled fashion. The temperature was increased to 150°C and held at this temperature for 1 hour. The temperature was then ramped at the rate of 1°C/minute to 575°C. The sample was kept at this temperature for 7.5 hours. After cooling, 3M nitric acid + 1M Al(NO₃)₃ was added to the crucible and refluxed on a hotplate at low temperatures. This solution was taken through the separation procedure.

2.6.3 ⁹⁰Sr

Selected clam shell samples were analyzed for ⁹⁰Sr following SOPs STL-RC-00050, STL-RC-5016, and SL 13021. Shell samples were ground to a powder. An aliquot was placed in a crucible and the calibrated strontium carrier, used as a yield monitor, was added to the sample aliquot and the crucible placed on a hotplate at low heat to dry. The crucible containing the dry sample was placed in a muffle furnace and burned in a control fashion. The temperature was increased to 150°C and held at this temperature for 1 hour. The temperature was then ramped at the rate of 1°C/minute to 575°C. The sample was kept at this temperature for 7.5 hours. The sample residue was refluxed in nitric acid and transferred to a beaker where it was refluxed in concentrated nitric acid. Fuming nitric was added to the precipitate. Strontium and chemical separation was performed on the precipitate.

2.7 Lipids

Percent total lipids found in clam samples were determined using a method based on the original Bligh and Dyer method (Bligh and Dyer, 1959) for extracting lipids, Battelle Duxbury SOP 5-299. Modifications included using a much smaller sample aliquot (<10 grams wet) and using centrifugation rather than filtering to separate and isolate the appropriate solvent layers. Lipids are extracted using specific ratios of sample moisture: chloroform: methanol. The method is described in Battelle SOP 5-299 *Determination of Tissue Lipid Concentration Using the Modified Bligh and Dyer Method*.

3.0 RESULTS

The analytical data presented in this report are provided as Attachments to this report as follows:

- Attachment 1—Pesticide/PCB Congener Results**
- Attachment 2—PAH and Bis(2-ethylhexyl)phthalate Results**
- Attachment 3—Butyltin Results**
- Attachment 4—Metal Results**
- Attachment 5—Dioxin/Furan and Dioxin-like PCB Congener Results**
- Attachment 6—Radionuclide Results**
- Attachment 7—Lipid and Percent Dry Weight Results**
- Attachment 8—Third Party Validation Report**

Sections one through seven of this document are organized as follows:

1. A QA/QC narrative, which includes a discussion of the QC results and a description of any MPC exceedances, including the impact, if any, they may have on the overall field sample data.
2. Summary report tables for all QC samples, presented on a concentration (blanks and laboratory triplicates), recovery (LCS, MS, MSD) and/or percent difference (SRM) basis.
3. Summary report tables for all authentic samples, presented on a wet weight, concentration basis.

4.0 QC SUMMARY

All results were reviewed following an EPA Tier II-like validation. The results of all Quality Control (QC) checks and procedures were evaluated against established project specific MPCs (Battelle 2002) and used to assess and qualify samples results. Detailed QA/QC narratives are included with the analytical data in Attachments 1 through 8, as described above. Because of the intended use and interpretation of the data, some qualifiers were changed from what was originally stated in the QAPP. Specifically, a “B” qualifier now only pertains to blank concentrations that are greater than the reporting limit and a “U” qualifier is for data detected at or below the sample specific MDL or sample concentrations that are within five times detected blank concentrations. The results from the analyses of QC samples, with few exceptions, met MPCs specified in the QAPP. Exceptions are flagged appropriately throughout the data tables. Of particular note:

4.1 Pesticide/PCB

PCB 49 was under recovered in both the Matrix Spike and the Matrix Spike Duplicate. The under recovery was exclusive to PCB 49 and was due to a negative peak caused by matrix interference.

4.2 PAH and Bis(2-ethylhexyl)phthalate

A number of PAHs were detected in the blanks associated with the hepatopancreas batches. PAHs are routinely found in procedural blanks at levels above the MDL. Because sample results are being reported down to the MDL, all sample concentrations that were less than 5 times the concentration found in the associated blank were flagged with a “U”, regardless of whether the blank concentration was above the MDL or above the RL. Those values flagged with a “U” indicate that there is most likely a significant contribution from the blank. No other corrective action was taken because the blank contamination found in these samples was well below the target detection limit of 20 ppb and the source of blank contamination is variable and at the present is not controllable.

Several percent recoveries (RPDs for MS/MSDs and RSDs for triplicate analyses), were outside of the control limits, however, the contingency criteria was met for most of these exceedances (i.e. the spike concentration must be greater than 5 times the native level (for MS) or the analyte concentration must be at least three times the RL (for Triplicates)).

4.3 Butyltins

TBT was detected in the method blank at 6.45 ug/kg. While this value is below the Method RL, all sample results were less than 5 times this value and may be biased due to background contamination. All sample results are flagged with a “U” to indicate this potential bias. Low levels of TBT are often found in the method blank due to contamination both from ambient sources of TBT and from small quantities present in one of the reagents used in the derivitization step of the method.

4.4 Metals

Pb was detected in the blank at a concentration greater than five times its MDL. The presence of Pb in the blank is most likely due to laboratory contamination of one of the reagents used in sample processing. (This situation has occurred in other projects conducted in the MSL metals laboratory recently. Since the discovery of Pb contamination in method blank analyses, the contaminated reagent has been identified and eliminated.) The blank-corrected results for the Pb analysis are a more accurate representation of true Pb concentrations in the samples. The laboratory recommends using the blank-corrected Pb values in any database, reports, or data analysis. Zn was detected in the blank at a concentration of 0.054 µg/g, which is greater than the RL but less than 5 times the MDL. No Zn concentrations in samples associated with the blank were less than 5 times the blank concentration; therefore, no data were flagged or affected.

4.5 Dioxin/Furan and Dioxin-like PCB Congener

A number of dioxin and furan compounds were detected in the procedural blanks, however concentrations were all below the RL. Blank concentrations for 5 of the dioxin-like PCB congeners were found at levels above the RL. While corrective action for RL (RL=PQL) exceedences of blanks is to re-extract, this was not always an option due to limited tissue mass. This blank contamination was a result of running the procedural blanks immediately after the ongoing precision and recovery (OPR) standard. All blanks (for both dioxin/furan and dioxin-like PCB congeners) were re-analyzed with a solvent blank run prior to the procedural blanks along with two representative samples from each batch, to ensure carryover was only an issue associated with the blanks. The result for field samples remained essentially the same while blank results were much improved. Target analyte concentrations for all PCB method blank re-runs were lower, in some cases by an order of magnitude. As a result of the re-analysis, the original data reported for the procedural blanks was qualified with an “R” to indicate the data has been rejected and the new blank data has been reported. Sample data was re-evaluated against the new procedural blank data and reported in the data tables. All sample concentrations of both dioxin/furans and PCB congeners that were within 5 times the blank concentration (using the EPA Region II Tier III validation action levels) were flagged with a “U” to indicate possible bias due to the blank.

The SRM analyzed with these samples (EDF 2525) is a highly contaminated natural fish matrix with relatively low levels of dioxin/furans and dioxin-like PCBs. Because of the nature of the material (high background contamination) SRM recoveries were variable for both Dioxin/Furans and one dioxin-like PCB congener. All dioxin/furan target analytes are certified in SRM 2525, however, they are certified at relatively low levels, many below the RL, resulting in PDs greater than 30%. Only 5 of the dioxin-like PCBs are certified, one of which (PCB 169) is just above the RL. The SRM MDL for 1,2,3,4,7,8-HxDF was elevated due to interference caused by coelution with diphenyl ether. PCB 169 and 1,2,3,4,7,8,9-heptafuran in the SRM are routinely recovered above certified values. The lab had made an effort to remove the matrix interference, without success. Another SRM, EDF 2526, a fortified clean natural fish matrix, with more attainable target levels, will be run to see if any improvement can be made.

4.6 Radionuclides

All quality control samples processed with samples requiring ^{80}Co , ^{137}Cs , and Isotopic Uranium analysis met data quality objectives.

4.7 Lipids

All quality control samples processed with samples requiring lipid analysis met data quality objectives.

5.0 TIER III VALIDATION SUMMARY

The report summarizing the results of full Tier III data validation performed on the dioxin/furan and dioxin-like PCB clam results and associated quality control (QC) samples is provided in Attachment 8 of this report. The data validation is based on QC criteria documented in the above listed methods; the *Quality Assurance Project Plan: Long Island Sound Study, Task I QAPP (Final)*, Battelle, January 2002; the *U.S. EPA Region II Data Validation SOP for EPA Method 1613, Revision A*, U.S. EPA, September 1999; and the *U.S. EPA Region 10 SOP for the Validation of Method 1668, Toxic, Dioxin-like, PCB Data*, U.S. EPA, December 1995.

5.1 Correctable Deficiencies

The laboratory did not flag quality control outliers using the flags as specified in the QAPP (page 27). However, the laboratory did flag outliers and defined the flags in the case narrative. No action was taken. No other correctable deficiencies were noted.

5.2 Non-Correctable Deficiencies

Low levels of target compounds were present in all method blanks, for both the PCB congener and dioxin/furan analyses. Five PCB concentrations exceeded RLs. Corrective action specified in the QAPP was to re-extract the batch, however, due to limited sample mass, this was not possible. Concentrations of PCDD/Fs were less than the PQL, so no additional corrective action was required by the laboratory. During validation, the data were qualified as detailed in the data validation reports.

For the dioxin/furan analyses, several of the labeled compound recovery values were outside the control limits specified in Table 5.6 of the QAPP. No corrective action is required. During validation, the data were qualified as detailed in the data validation reports.

Several compound concentrations were outside the control limits for the SRM analyses associated with the PCB congener analyses. The specified corrective action is to flag the outliers. The flags were added to the EDD by the reviewer. During validation, the data were qualified as detailed in the data validation reports.

5.3 Comments

No data were rejected. Overall, the data are useable for the intended purposes.

6.0 REFERENCES

Battelle, 2002. Tasks 1 Quality Assurance Project Plan for Long Island Sound Disposal Site Study.. Prepared under contract for U.S. Army Corps of Engineers North Atlantic Division, New England. Contract No. DACW33-01-D-0004, Delivery Order No. 13. January 11, 2002.

ENSR, 2000. "Field Summary Report July/August 2000 Survey at the New London Disposal Site (NLDS) and Central Long Island Sound Disposal Site (CLIS)". Prepared for the USACE NAE under contract DACW33-96-D-004. ENSR Document No. LIS-2000-F09-BT. December 2000.