

APPENDIX H-5C

**Worm (*Nephtys incisa*) Analytical Results:
Final Report**

Prepared for

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US ARMY CORPS
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Final Report

Worm (*Nephtys incisa*) Analytical Results

Long Island Sound Disposal Site Study

Final Report
for
Worm (*Nephtys incisa*) Analytical Results
Long Island Sound Disposal Site Study

Submitted to

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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 content results for the worm 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 contaminants in organisms that are exposed to the sediments of the disposal sites versus non-disposal areas. Benthic organisms (including the worm *Nephtys incisa*) were collected 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 worm 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 January 22, 2002. Upon receipt at Battelle, all samples were logged in, assigned new Battelle IDs and stored frozen until further processing and analysis.

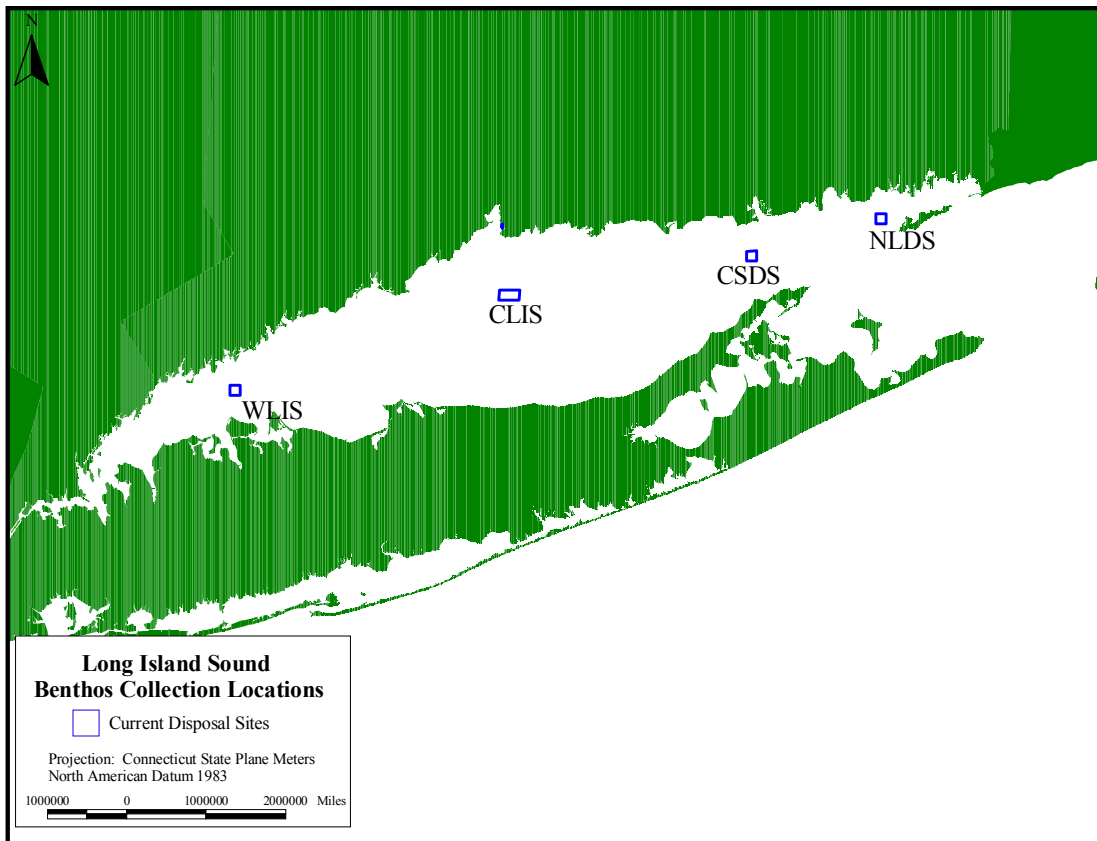


Figure 1. Benthic Sampling Locations.

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. Worm samples were homogenized 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 worm 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. Worm Sample Summary of Analyses

Sample ID	Study Area	Station	Battelle ID	PCB Aroclor, Pest, PAH	Butyltins	Metal	Dioxin/furan and Dioxin like PCBs	Radionuclide	Lipids
LIS04CLREFC2	CLIS	REF	V1270	X		X			X
LIS04CLREFC3			V1271				X		
LIS06CLREFC1			V1292	X		X	X		
LIS06CLREFC3			V1294	X	X	X	X	X (1)	X
LIS04CLFVPC1	CLIS	FVP	V1274	X		X			X
LIS04CLFVPC2			V1275	X		X			X
LIS06CLFVPC2			V1297	X	X	X	X	X	
LIS06CLN93C4	CLIS	NHAV93	V1302	X		X	X		X
LIS04CLN93C3			V1280	X		X			X
LIS06CLN93C3			V1301	X	X	X	X	X	
LIS04NLLRFC1	NLDS	LRF	V1257	X		X			X
LIS06NLLRFC3			V1284	X		X	X		X
LIS06NLLRFC4			V1285	X		X	X		X
LIS04NLSEAC4	NLDS	SEA	V1268	X		X	X	X	X
LIS06NLSEAC3			V1291	X		X	X		X
LIS06NLSEAC4			V1312	X	X	X	X		

(1) Sample V1294 was the only sample analyzed for ¹³⁷Cs and ⁶⁰Co; remaining samples analyzed for Isotopic U only.

X Indicates analyses performed

 Indicates analysis not performed

2.0 METHODS

Chemical analysis of worm 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). Due to limited tissue mass, some QA/QC samples were not analyzed for certain parameters. Detection limits for some parameters were elevated, also due to limited tissue mass. 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. Briefly, *Nephtys incisa* (worm) 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 quantitatively split for further cleanup by GPC HPLC. The post-HPLC extract was concentrated, fortified with RIS and split qualitatively for Pesticide/PCB and PAH analyses. Pesticide/PCB extracts were solvent exchanged into hexane prior to analysis. 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 Recovery Internal Standard (RIS) compounds. Sample data were reported on a wet-weight concentration basis.

2.2 PAH and Bis(2-ethylhexyl)phthalate

Tissue samples were extracted for PAHs and Bis(2-ethylhexyl)phthalate following general NS&T methodologies, as described in Section 2.1. Extracts were analyzed for PAHs following general NS&T methods. Briefly, extracts were analyzed by gas chromatography/mass spectrometry detection (GC/MS) in the selected ion monitoring (SIM) mode. Sample data were quantified by the method of internal standards, using the RIS compound Acenaphthene d-10. Sample data were reported on a wet-weight concentration basis.

Note that the 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 3 grams of worm tissue 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.

Sample data were quantified by the method of internal standards, using the SIS compounds. 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. Sample data were reported on a wet-weight concentration basis.

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, 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

Worm 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. Worm 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 were extracted from solid samples with a solvent mixture of 50:50 hexane/dichloromethane. Following extraction, the samples were cleaned up via GPC and/or carbon (if sample necessitated) and passed through a series of columns, which removed 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 were determined using an isotope dilution technique. Quantitation was based on the use of internal standards and relative response factors (RRFs). Sample data were reported on a wet-weight concentration basis.

2.6 Radionuclides

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

Severn Trent Laboratory analyzed a subset of worm samples for radiochemical parameters. Due to limited mass available, only one worm tissue sample was prepared for ^{60}Co and ^{137}Cs following STL SOP's STL-RC-0025, STL-RC-5016, and STL-RD-0101. Worm samples were placed in either a 25 mL or 100 mL counting geometry.

2.6.2 Isotopic U

Four worm samples were analyzed for isotopic uranium following, SOPs STL-RC-5016, STL-RC-0238, and STL-RC-0100 for sample preparation and SOP STL-RD-0201 for the analysis. The appropriate aliquot of worm tissue 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.

2.7 Lipids

Percent total lipids found in worm samples were determined using a method based on the original Bligh and Dyer method 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*. Sample data were reported on a wet-weight concentration basis.

3.0 RESULTS

The analytical data 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. Exceedences are flagged appropriately on the data tables. Of particular note:

4.1 Pesticide/PCB

Endosulfan II was recovered just below the lower control limit in the matrix spike. Endosulfan II is not consistently fully recovered during alumina column cleanup. No triplicate analysis was performed due to insufficient sample mass.

4.2 PAH and Bis(2-ethylhexyl)phthalate

Bis(2-ethylhexyl)phthalate was detected above the RL in the method blank. While a number of PAHs were detected in the procedural blank above the MDL, the concentrations were below the RL. 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 contribution from the blank.

Due to limited tissue mass available, no Bis(2-ethylhexyl)phthalate LCS or MS/MSD was processed with this batch. No triplicate analysis was performed due to insufficient sample mass.

4.3 Butyltins

TBT was detected in the method blank at 11.28 : g/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

Lead (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.

Cd and Zn were also present in the blank, at concentrations less than 10 times the MDL. The levels of Cd and Zn present in the blank were most likely attributable to trace levels of these metals contributed by the reagents used in sample processing. No corrective action was taken because concentrations of Cd and Zn in the samples associated with the blank were greater than 10 times their concentrations in the blank. No triplicate analysis was done due to insufficient sample mass.

4.5 Dioxin/Furan and Dioxin-like PCB Congener

A number of dioxin-like PCB congeners and dioxin and furan compounds were detected in the procedural blanks, however concentrations were all below the RL. 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 percent differences (PDs) greater than 30% from the certified value. Only five 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 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

Triplicate analysis was not able to be done due to limited sample mass, however 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 associated with the lipid determination 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 worm 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 labeled compound recovery outliers and defined the flags in the case narrative. No action was taken.

The laboratory did not flag MS/MSD, LCS, or SRM outliers. These were added to the electronic data deliverable by the reviewer. No further action was taken.

Minor calculation errors were noted for the PCB MS/MSD recovery values. The laboratory was contacted, but did not provide any corrected values. Since the errors did not significantly impact the reported results, the corrections were entered into the electronic data deliverable and no further action was taken.

The laboratory inadvertently omitted the data flags for the dioxin/furan analysis of Sample V1294. These were added to the sample summary form and to the electronic data deliverable by the reviewer. No further action was taken.

No other correctable deficiencies were noted.

5.2 Non-Correctable Deficiencies

Triplicate analyses were not performed for this matrix. Precision was evaluated using the MS/MSD results. No further action was taken.

Low levels of target compounds were present in all method blanks, for both the PCB congener and dioxin/furan analyses. However, all concentrations 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.

Most of the recovery values were less than the 80% lower control limit for the MS/MSD and LCS associated with the PCB congener analyses. These outliers were not noted in the laboratory case narrative. For MS/MSD outliers, the corrective action specified in the QAPP (Table 11.1) is to flag the outliers. This was not done. For LCS outliers, two corrective actions are listed: flag the outliers unless the majority of recovery values are outside the control limits; then, re-extract and reanalyze the associated samples. Neither corrective action was performed. The flags were added to the electronic data deliverable by the reviewer. 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. One compound concentration was outside the control limit for the SRM associated with the dioxin/furan analyses. The specified corrective action is to flag the outliers. The flags were added to the electronic data deliverable (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.