APPENDIX C

CHEMICAL METHODOLOGIES
SEDIMENT AND TISSUE MEASUREMENTS

FIELD COLLECTIONS

Sediment samples were taken with a modified 0.1m^2 Van Veen sediment sampler, and subsamples were taken from the top 2-cm of each grab sample for testing. The samples were analyzed for grain size, dissolved sulfides, total organic carbon, priority pollutant metals, and priority pollutant organic compounds.

Grain Size

Samples of sediment were removed from the Van Veen sampler with a metal spoon, placed in plastic cups, transported to the laboratory, and refrigerated.

Dissolved Sulfide

Portions of the Van Veen sample were taken into an open-ended plastic syringe and placed in a pore water press. Pore water was squeezed into an attached plastic syringe which is then filtered through a 0.45 um filter into a polypropylene tube containing 2-3 drops of 2 N zinc acetate solution. It is filled to the brim, then capped excluding air, and refrigerated.

Total Organic Carbon (TOC)

Test portions of sediment samples were removed with a metal spoon, placed in acid-rinsed amber glass bottles, covered with Teflon lined caps, and refrigerated.

Priority Pollutant Metals

Sample portions were removed from a Van Veen Sampler with a plastic spoon, placed into acid-rinsed plastic cups, capped, and frozen. For field collection of sediment samples, see Chapter 5 (Materials and Methods).

Priority Pollutant Organic Compounds

Sample portions were taken from a Van Veen sampler with a metal spoon, placed into hexane- and acetone-rinsed glass containers and refrigerated. For field collection of tissue samples, see Chapter 7 (Materials and Methods).

LABORATORY PROCEDURES

Sediment Grain Size

Sediment grain size is used to normalize sediment chemistry data and to differentiate changes in benthic communities caused by the outfall from the effect of substrate type. Until 1996, sediment grain size was determined by the classical technique of particle sizing by sorting fractions through sieving and sedimentation. In 1996, this manual methodology was replaced by an instrumental method using the Coulter LS 230 particle size analyzer (PSA) which measures particle size by the scattering of light. Light scattering technology utilizes the relationship between particle size, wavelength, and the angle of the scattered light that is transmitted through an optical system to a series of detectors. Laser diffraction particle sizing is done by analyzing the pattern of scattered light produced when particles pass through a laser beam. The angular range is from close to 0 to 160 degrees relative to the incident light beam. PSA incorporates both laser diffraction and Polarization Intensity Differential Scattering (PIDS for the smaller particles 0.04-0.8um). The instrument calculates the results based on both the Fraunhofer and Mie theories of light scattering. It measures particle size from 0.04 um to 2000 um in a single scan using 116 size channels. A representative sample is introduced into the Fluid Module sample cell of the instrument. The sample is then dispersed in tap water and circulated through the measuring cell, and the various particle sizes are determined by detection of scattered light and displayed by volume percent in differential distribution. Coarse material (gravel >2 mm) can introduce negative bias by blocking the imaging of the smaller particles. Samples with measurable amounts of particles in the higher range must be sieved with a 2 mm (# 10) sieve prior to instrumental analysis. Specific particle size control samples are used for verification of the performance of the particle size analyzer. In 2006, Coulter LS 230 was replaced by Coulter LS 13-320. The LS 13-320 is an improvement over the LS 230 in terms of instrument performance (shorter analysis time) as well as improved software; it has more software capabilities as well as being more user-friendly (it runs on Windows XP as compared to Windows 3.1 for LS 230).

Dissolved Sulfide

Dissolved sulfide was determined colorimetrically using the methylene blue method from Standard Method 4500 D (APHA 1998). A 7.5 mL portion of sediment pore water sample was transferred to a culture tube. Amine-sulfuric acid reagent and ferric chloride solution were added and mixed. If sulfide was present, a blue color appeared in the solution. After 3-5 minutes, diammonium hydrogen phosphate was added to mask the yellow color of excess ferric chloride. Absorbance was measured after 3-15 minutes using a Shimadzu spectrophotometer at 667 nm. The results were compared with a standard solution of sodium sulfide, which was standardized with iodine and then diluted to the appropriate concentrations. The results were expressed in parts per million (ppm).

Total Organic Carbon

Total Organic Carbon was analyzed using the combustion-infrared method from Standard Method 5310 B (APHA 1998), with modifications for solid/slurry samples. An Apollo 9000 Total Organic Carbon Analyzer with a Rosemount Dohrmann Model 183 TOC Boat Sampler for solid matrices was used.

Samples were analyzed in their original slurry state. Inorganic carbon was removed by reaction with acid prior to analysis. Portions of each sample were dried at 105°C to determine the percentage dry weight. Total organic carbon was then calculated based on dry sediment weight and reported as mg/Kg.

Priority Pollutant Metals

Sediments

All priority pollutant metals (except mercury) in sediment samples were analyzed by EPA method 200.7 (EPA 1994) using a Varian Vita Pro inductively coupled plasma spectrophotometer (ICP). Prior to analysis of all metals, except mercury, the sediment samples were digested according to EPA method 3050 (EPA 1986). This digestion procedure was used to prepare sediment, sludge, and soil samples for the determinative step by method 200.7.

The sediments were pre-dried at 60^{o}C before sample preparation. Approximately 2 grams each of well- mixed sample were digested according to EPA method 3050 and diluted to a final volume of 100 mL in volumetric flasks with deionized water.

Mercury was analyzed by cold vapor technique in Standard Methods 3112 (APHA, 1998). Prior to the determinative step using a Cetac M6000 cold vapor mercury analyzer, approximately 1.0 gram of pre-dried sediment sample was digested with nitric acid and hydrochloric acid in presence of potassium permanganate. The digested sample was diluted to a final volume of 50 mL with distilled water.

Reagent blanks were run throughout the preparation and analytical procedures. Necessary corrections of the data were made using reagent blank results. The sediment results were expressed as mg/kg of dry sediment. Approximate detection limits of various metals analyzed according to the above conditions are presented in Table C-1. To check the precision and accuracy of analytical data, a QC sample, a spiked sample, a duplicate spiked sample, a NIST standard, and a reference sample were run with each batch or every ten samples analyzed, whichever was more frequent.

All reagents were trace metal grade. The water was deionized. The glassware and plastic containers were cleaned by soaking in 30 percent nitric acid solution followed by a deionized water rinse.

Tissue

Metals in tissues were analyzed essentially by the same procedures as described above for sediment metal analysis, except for a few modifications, described below.

Frozen tissue samples were thawed and, immediately before sample preparation, were homogenized thoroughly using a mortar and pestle. Approximately two grams of homogenized tissue was used for digestion by EPA Method 3050 (EPA 1986). The digested samples were diluted to 50 mL with distilled deionized water.

For mercury analysis, approximately 1gram of tissue sample was used for digestion. The rest of the digestion and analytical procedures were the same as those described for sediment analysis. Mercury was analyzed using the cold vapor technique from Standard Method 3112 (APHA 1998).

All results were expressed as μg/kg (wet weight). The approximate detection limits of metals in various tissues analyzed by the above conditions are presented in Table C-1.

Priority Pollutant Organic Compounds in Sediment and Tissue

Sediment and tissue samples were thawed, extracted with solvents, and the extracts further processed to remove interfering substances and to concentrate analytes. Base-Neutral and Acid extractable organic compounds (BNA) were analyzed with a gas chromatograph/mass spectrometer (GC/MS). Organochlorine pesticides and polychlorinated biphenyls (PCB) were analyzed using a GC equipped with electron capture detectors (ECD).

All raw data for organic compounds in sediments and tissues was reported in µg/Kg wet weight.

Sample Preparation and Extraction

Sediment and tissue sample preparation and extraction were done using SW-846 EPA Method 3545 (EPA 1986), Pressurized Fluid Extraction (PFE) procedure. A portion of sample was weighed and dried with anhydrous sodium sulfate to form a homogenized fine powder. Extraction was performed using a Dionex Accelerated Solvent Extraction (ASE) 200 model extractor. The powder was loaded into an extraction cell and extracted three times with 1:1 methylene chloride/acetone mixture for 5 minutes at 100 degrees Celsius and 1500 psi. The extract from the ASE was ready for BNA, Pesticides, and PCBs cleanup and analyses.

Base-Neutral and Acid Extractable Organics

The method for BNA in SW 846 EPA Method 8270 (EPA 1986) was followed. Prior to quantitative analysis, the ASE extract was cleaned by acid-base partition. Sample extract was adjusted to a pH > 12 to "salt" out the acids. The Base/Neutral components were extracted 3 times with methylene chloride. The organic phases were combined and concentrated to an appropriate volume. This was followed with a florisil cleanup used to further reduce matrix interferences. The aqueous phase, after the extraction at pH 12, was adjusted to pH 2 to convert salts back to acids, and then extracted with three portions of methylene chloride. The acid portion extracts were combined with the B/N portion before concentrating to 1 mL for GC/MS analysis.

An Agilent 5890 GC equipped with a 5972 MSD, an auto sampler model 7673, and a 30 m x 0.25 mm i.d. DB5-ms fused silica capillary column was used for identification and quantification by means of internal standards. It was operated in the electron-impact-ionization mode and checked with decafluorotriphenylphospine.

Organochlorine Pesticides and Polychlorinated Biphenyls (PCB)

The procedure given in SW 846 EPA Methods 8081 and 8082 (EPA 1986) were followed to determine pesticides and PCBs respectively. A florisil cleanup procedure was performed on the ASE extract before pesticides and PCBs quantification analysis. The ASE extract was solvent exchanged to hexane, concentrated, cleaned through a Florisil column, and separated into two fractions by eluting the column with 6% ether/hexane and 50% ether/hexane.

An Agilent GC equipped with dual ECD detectors and fused silica capillary columns (DB5 and DB 1701) was used for identification and confirmation. Quantification was relative to external standards.

Table C-1. Method detection limits of metals in sediments and tissues.

METALS	SEDIMENTS	FISH MUSCLE	FISH LIVER
	mg/dry kg	**µg/wet kg**	**µg/wet kg**
As	0.13	100	500
Cd	0.03	30	100
Cr	0.10	40	100
Cu	0.27	90	500
Hg*	0.7*	19	100
Pb	0.25	110	500
Ni	0.32	70	500
Ag	0.04	20	50
Zn	0.59	1140	1000

*Mercury units are µg/Kg

LITERATURE CITED

American Public Health Association. 1998. Standard Methods for the Examination of Water and Wastewater, 20th Ed. American Public Health Association, Washington, D.C., unpaged.

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USEPA 1994. Method 200.7. Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry, Revision 4.4, 1994. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Office of Research and Development, Cincinnati, Ohio.