

## APPENDIX C

### CHEMICAL METHODOLOGIES

#### FIELD COLLECTION

Sediment samples were collected with a chain-rigged Van Veen sediment sampler from designated sampling points, and for each collection, portions for various analyses were collected from the top 2-cm of a single 0.1 m<sup>2</sup> grab sample for testing. Water and effluent samples were collected using various sampling devices. The samples were preserved following EMD Standard Operation Procedures (SOP) and later analyzed for grain size, dissolved sulfides (pore water), total organic carbon (TOC), oil and grease, cyanide, ammonia, acute toxicity, chronic toxicity, priority pollutant metals, and priority pollutant organic compounds.

#### Grain Size

Sediment samples were removed from the Van Veen grab with a metal spoon, placed in plastic cups, transported to the laboratory, and refrigerated.

#### Dissolved Sulfide

Sediment samples were taken into an open-ended plastic syringe and placed in a pore water press. Pore water for each sample was squeezed into an attached plastic syringe containing 2-3 drops of 2-N zinc acetate solution. The mixture was placed in a polypropylene tube, capped excluding air, refrigerated, and analyzed within 16 hours.

#### Total Organic Carbon

Sediment samples were collected with a metal spoon, placed in acid-rinsed amber glass bottles, covered with Teflon lined caps, and refrigerated. About 0.5 mL of H<sub>2</sub>SO<sub>4</sub> was added as preservative.

#### Oil and Gease

Samples were collected as 1-Liter grabs.

#### Toxicity

An autosampler was used to collect TIWRP effluent on a bi-hourly basis, over a 24-hour sampling period, resulting in a 24-hour composite sample. Receiving water was collected using a brass flopper bottle, which was replaced by a non-metallic General Oceanics Inc. ABS plastic GO-FLO Water Sampler, at mid-depth.

## **Priority Pollutant Metals**

Sediment samples were collected with a plastic spoon, placed into acid-rinsed plastic cups, and frozen.

## **Priority Pollutant Organic Compounds**

Sediment samples were collected with a metal spoon, placed into hexane- and acetone-rinsed glass containers and refrigerated.

## **LABORATORY PROCEDURES**

### **Grain Size**

Sediment grain size data 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, manual methodology was replaced by an instrumental method using the Coulter LS 230 Particle Size Analyzer (PSA) which measures particle size by light scattering. 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. The PSA incorporates both laser diffraction and Polarization Intensity Differential Scattering (PIDS) for the smaller particles 0.04-0.8 $\mu$ m. The instrument calculates the results based on both the Fraunhofer and Mie theories of light scattering. It measures particle size from 0.04  $\mu$ m to 2000  $\mu$ m 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 Reverse Osmosis (RO) water and circulated through the measuring cell and the various particle sizes are determined by the 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 particles in the higher range must be sieved with 2 mm (# 10) sieve prior to instrumental analysis. Specific particle size control samples are used for verification of the performance of particle size analyzer.

### **Dissolved Sulfide**

Dissolved sulfide was determined colorimetrically using the methylene blue method from Standard Method 4500 S- D. A 7.5 mL portion of sediment pore water sample was transferred to each of two matched test tubes. 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. Absorbance was measured after 3 15 minutes using a UV-Visible spectrophotometer at 664 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**

TOC was analyzed using the combustion-infrared method from Standard Method 5310 B, with modifications for solid/slurry samples. An Apollo 9000 TOC Analyzer with 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.

### **Oil and Grease**

One-Liter grab samples were extracted with three successive portions of hexane, dried over anhydrous sodium sulfate, and the oil and grease in the extract was determined gravimetrically.

### **Cyanide**

Cyanide was analyzed using the semi-automatic colorimetric measurement method from EPA Method 335.4. Cyanide, as molecular hydrogen cyanide (HCN), is released from samples containing cyanide by means of a reflux-distillation operation under acidic conditions and absorbed in a scrubber containing sodium hydroxide solution. The cyanide in the absorbing solution is then determined using the Lachat Flow Injection Analyzer (FIA).

### **Ammonia**

Ammonia values were determined following EPA method 350.1 for NH<sub>3</sub>, utilizing the Lachat Flow Injection Analyzer (FIA).

### **Acute Toxicity**

Acute toxicity was evaluated using the topsmelt, *Atherinops affinis*, following the EPA Protocol “Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms”, (EPA-821-R-02-012, 2002). Percent survival was the reported endpoint.

### **Chronic Toxicity**

Chronic toxicity was evaluated using the Red Abalone, *Haliotis rufescens*, following the EPA method described in “Short Term Methods For Estimating The Chronic Toxicity of Effluents and Receiving Waters To West Coast Marine And Estuarine Organisms”, (First Edition, August 1995, EPA-600-R-95\_136) Chapman, Denton, and Lazorchak. Endpoints were reported as Chronic Toxicity Units (TUc).

### **Priority Pollutant Metals in Sediments**

All priority pollutant metals in sediment samples were analyzed by EPA method 6010 using a Varian Vista Pro Inductively Coupled Plasma (ICP) spectrophotometer. Prior to analysis of all metals, except mercury, the sediment samples were digested according to EPA method 3050. This digestion procedure is used to prepare sediment, sludge, and soil samples for analysis.

The sediments were pre-dried at 60°C before sample preparation. Approximately 2 grams of well-mixed sample was digested according to EPA method 3050 and diluted to 100mL in volumetric flasks with laboratory water.

Mercury was analyzed by cold vapor technique in Standard Method 3112 with a Cetac M6000 mercury analyzer. Approximately 1.0 gram of pre-dried sample was digested with acids in the presence of potassium permanganate. The digested sample was diluted to 50 mL with deionized distilled water.

Reagent blanks were run throughout the preparation and analytical procedures. The sediment results were expressed as mg/kg of dry sediment. To check the precision and accuracy of analytical data, a duplicate, a spiked sample, a QC 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 analytical reagent grade. The water used in these analyses was purified by reverse osmosis and deionization. The glassware and plastic containers were cleaned by soaking in 30 percent nitric acid solution overnight, rinsed with distilled water, and rinsed again with deionized water.

### **Percent Lipid**

Tissue samples are extracted as soon as possible after removal from the parent living organisms so that there is little opportunity for changes to occur in the lipid components. When this is not feasible, the tissue is frozen as rapidly as possible in a sealed glass container at -20°C in an atmosphere of nitrogen.

A solvent extraction procedure was carried out to extract lipid from the tissue sample. The extracted lipids were purified from the non-lipid contaminants. And a gravimetric method was used to determine the total lipid content of the sample.

### **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).

Total Organic Halides (TOX) was analyzed using Mitsubishi TOX Analyzer by SW 846 Method 9020.

#### *Sample Preparation and Extraction*

Sediment and tissue sample preparation and extraction were done using the SW-846 EPA Method 3545, 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 a 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 analysis in SW 846 EPA Method 8270 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 1mL for GC/MS analysis.

A Hewlett Packard 5890 GC equipped with a 5972 MSD, a model 7673 auto sampler 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 (PCBs)*

The procedure given in SW 846 EPA Methods 8081 and 8082 was followed to determine pesticides and PCBs. The methylene chloride/acetone extract of tissue or sediment was concentrated and solvent exchanged to hexane. The extract was reduced in volume with a K/D concentrator, cleaned through a Florisil column, and separated into two fractions by eluting the column with 6% ether/hexane and 50% ether/hexane. The volume of each fraction was further reduced in a K/D and placed in a vial for pesticides and PCB determination using GC/ECD.

Analysis was performed on a Varian Model CP-3800 gas chromatograph equipped with dual ECD detectors. Two fused silica capillary columns (DB5 and DB1701) were used for identification and confirmation. Quantification was relative to external standards. Analytical results were reported in mg/Kg wet-wt.

## LITERATURE CITED

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