Appendix 1. Analytical Methods Performed at TDI Brooks International, Inc. (TDI), Laboratory for Triazines in Water and Organic and Inorganic Compounds in Fish Tissues

Triazines in Water

Flowing water was collected according to U.S. Geological Survey (USGS) protocols (1996 [2006]). For analysis of organics in water, liquid-solid extraction (LSE) and capillary column gas chromatography/mass spectrometry (GC/MS) were used (U.S. Environmental Protection Agency [EPA] Method 525.2; EPA, 1995). Triazines, internal standards, and surrogates were extracted from a 1-liter (L) sample by passing it through a disk or cartridge containing a solid matrix with a chemically bonded C-18 organic phase (LSE). Organic compounds were extracted from the LSE by using small quantities of ethyl acetate followed by methylene chloride, with further evaporation of the solvent. Sample components were separated, measured, and identified by GC/MS (Method 525.2; EPA, 1995).

Aliphatic, Aromatic, and Chlorinated Hydrocarbons and Polybrominated Diphenyl Ethers in Tissues

Tissue Extraction

Thawed tissues having been stored at -20 degrees Celsius (°C) were homogenized by mechanical methods, and a 1-gram (g) aliquot was removed and dried at 105°C to constant weight to determine the percentage of moisture (weight/weight basis). Wet and dried samples were weighed to the nearest milligram. The remaining samples were stored in precleaned jars and frozen (-20°C) until analysis. Automated extraction apparatus (ASE 200 Accelerated Solvent Extractor, Dionex, Sunnyvale, Calif.) was used with 0.5–15 g of tissue samples having been mixed with Hydromatrix (Agilent Technologies, Santa Clara, Calif.). Using 100 percent dichloromethane inside stainless steel extraction cells at 100°C and 200 pounds per square inch (psi), the extracts were dissolved and were collected in 60-milliliter (mL) glass vials and concentrated to 1–3 mL by using an evaporative solvent reduction apparatus (water bath or TurboVap II Evaporation System, Caliper Life Sciences, Hopkinton, Mass.). Sample aliquots were weighed for determining percentage of lipids, and the remaining portions were processed through a clean-up column (activated silica gel and alumina) and high-performance liquid chromatography to minimize matrix interference. Extracts concentrated to a final volume of 0.5 mL were submitted for determination of aliphatic, aromatic, and chlorinated hydrocarbons (Lauenstein and others, 1993; EPA, 1997a, 2001a; Zuloaga and others, 2000).

Aliphatic Hydrocarbon Determination by Gas Chromatography/Flame Ionization Detection (GC/FID)

Target analytes were analyzed by using a Hewlett Packard (HP) model 5890 Series II Gas Chromatograph with a Flame Ionization Detector (Hewlett Packard Company, Palo Alto, Calif.) operated in a splitless mode. An HP-1MS capillary column (30-m × 0.25-mm inner diameter [ID] and 0.25-mm film thickness) was used to resolve peaks. The carrier gas was helium at a flow rate of 1.5 mL/minute (min). The temperature of the injection port was 300°C, and transfer line was 300°C. The initial oven temperature was 60°C, and the ramp rate was 12°C/min to a final oven temperature of 180°C. Normal alkanes with 10–34 carbons and the isoprenoids pristine and phytane were determined by using this procedure.

Aromatic Hydrocarbon Determination by Selected Ion Monitoring (SIM)–Gas Chromatography/Mass Spectrometry (GC/MS)

Quantitation of polycyclic aromatic hydrocarbons (PAHs) and their alkylated homologues was done with a HP model 5890 GC and model 5972 MS operated in SIM by using a capillary column. The GC was operated in splitless mode, and the capillary column was an HP-5 ms (60-m × 0.25-mm ID and 0.25-mm film thickness) (Agilent Technologies). The carrier gas was helium at a flow rate of 1 mL/min. The temperature of the injection port was 300°C, and transfer line was 290°C. The mass spectrometer scanned from 35 to 500 atomic mass units (amu) per second or less by utilizing 70 volts of electron energy in electron impact ionization mode. The relative peak heights of the primary mass ion compared to the confirmation or secondary mass ion fell within 30 percent of the relative intensities of these masses in a reference mass spectrum (EPA, 1997c).

Organochlorine Determination by Gas Chromatography/Electron Capture Detection (ECD)

Quantitation was performed on an HP model 5890 GC equipped with an ECD. Between 1 and 5 mL of sample was injected by using an HP model 7673A autosampler. The primary capillary column was a J&W DB-5 (30-m × 24-mm ID and 0.25-mm film thickness). The second column, a confirmation column, was a J&W DB-17HT (30-m × 0.25-mm ID and 0.15-mm film thickness). The inlet system was splitless, and the carrier gas was helium at a flow rate of 1 mL/min. For the analysis of standard halogenated hydrocarbons, the temperature of the injection port was 275°C, and the detector was 325°C. The initial oven temperature was 100°C with a hold time of 1 min. The ramp rate was 5°C/min to 140°C with a hold time of 1 min, followed by a ramp rate of 1.5°C/min to 250°C with a hold time of 1 min and a ramp rate of 10°C/min to 300°C with a final hold time of 5 min.

Additional column chromatography is required to separate polychlorinated biphenyls (PCBs) from toxaphene/pesticides and planar PCBs. An aliquot of the extract prior to clean-up with high-performance liquid chromatography was processed through a 3 percent deactivated silica gel column packed in dichloromethane, which was then flushed with pentane. The sample extract was transferred to the top of the column and flushed with 100 mL of pentane; this fraction contained PCBs and dichlorodiphenyltrichloroethanes (DDTs). This fraction was further processed by column chromatography packed with activated carbon/Celite (1:19 and 5 percent by weight) (World Minerals Inc., Santa Barbara, Calif.) and flushing with 1:4 dichloromethane/cyclohexane mixture. The instrument was operated in the splitless mode with helium as the carrier gas with a flow rate of 1 mL/min. The temperature of the injection port was 275°C, and the detector was 325°C. The initial oven temperature was 100°C, held for 1 min. The ramp rate was 10°C/min to 150°C, followed by a ramp rate of 6.0°C/min to 270°C with a hold time of 3 min.

The retention time of sample analytes fell within 15 seconds of the retention time of analytes in a calibration standard or retention index solutions. The levels of aroclor and toxaphene were determined by using retention index solutions of both complex mixtures. Aroclor were determined in a similar method to that described in U.S. Environmental Protection Agency (1997a, b).

Quality Control

Five concentrations of calibration solutions were prepared from commercially available analytes. For analytes of interest, a response factor relative to the internal standard was determined at each calibration level. All five response factors were averaged for a mean relative response factor. An Aroclor mixture consisting of Aroclor 1242, 1248, 1254, and 1260 was used as a retention time index solution for individual PCBs not found in the calibration solution. The individual PCB retention times were determined on the basis of pattern recognition. A calibration curve was established by analyzing each of five calibration standards (5, 20, 40, 80, and 200 picograms per microliter [pg/µL]) and by fitting the data to a quadratic equation. Each extraction batch per chemical group was analyzed as an analytical set including standards, samples, and some or all of the following quality control samples: method-blank, duplicate, matrix-spike, matrix-spike duplicate, and (or) blank spike, blank spike duplicate, and standard reference material.

Metals in Tissues

Tissue Extraction

Procedures took place at the Laboratory & Environmental Testing, Inc. (3501 Berrywood Dr., Columbia, Mo.). Tissue samples were homogenized after being freeze-dried, and wet and dry weights were used to determine percent moisture, as before. Tissues were digested in a closed Teflon (DuPont) vessel by using a microwave.

Quantitation of metals was done according to the methods developed and accepted by U.S. Department of the Interior and generally accord with EPA Methods 200.7 (Martin and others, 1994) and 200.8 (Creed and others, 1994). For digestion, the normal sample weight was 0.500 g dw, with 5.0 mL trace metal grade nitric acid and 1 mL of high purity hydrogen peroxide. Samples were heated to 180°C, and after cooling, they were diluted to 50.0 mL. For digestion for arsenic and selenium, magnesium nitrate dry ash was used, and the normal sample weight was 0.500 g dw, with 10 mL trace metal grade nitric acid, 2 mL of trace metal grade hydrogen chloride (HCl), and 10 mL 40 percent magnesium nitrate hydrate in a 100 mL Pyrex (Corning Incorporated) beaker. Samples were refluxed overnight at 70–80°C and then heated to dryness and placed in a muffle furnace and ramped at 1°C/min to 500°C and held for 1 h. After addition of 10 mL 50 percent HCl, samples were refluxed on a hot plate for 1 h, cooled, and diluted to 50 mL. Instrumentation for analyses included an Optima 7300 DV ICP-OES (Perkin Elmer, Waltham, Mass.) and atomic absorption by AAnalyst 600 with or without a FIA S1000 (Perkin Elmer).

For determination of methylmercury, the procedure was modified for tissue and based on EPA Method 1630 (2001b). Methylmercury was defined as all methylmercury forms and species found in the digestate. Samples were digested with methanol and potassium hydroxide and then ethylated prior to analysis with a Model III cold-vapor atomic fluorescence spectrometer (Brooks Rand Labs, Seattle, Wash.) (EPA, 2001a).

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