Analytical Methods

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Mercury (Total) by EPA Method 1631B

Total mercury (Hg) concentrations were determined by EPA Method 1631, Revision B (*Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry*) and its Appendix (*Digestion Procedures for the Determination of Total Mercury in Tissue, Sludge, Sediment, and Soil*).

Analysis of total mercury in fish tissue samples by Method 1631B involved the following steps:

1) digesting 0.5 g to 1.5 g of tissue sample and refluxing with 10 mL of concentrated nitric acid,
2) diluting the digestate to volume (50 or 100 mL) with 0.02N BrCl to ensure complete oxidation of methylmercury,
3) pipetting aliquots of the diluted digestate into pre-purged $SnCl_2$-containing water,
4) purging Hg(0) from solution onto a gold trap, and
5) thermally desorbing Hg(0) from the gold trap and transferring it to a cold vapor atomic fluorescence spectrometer for quantification.

The chemical concentration in the fish tissue sample was calculated on a mass basis (i.e., relative to the mass of the sample extracted).

Arsenic by EPA Method 1632A

Total inorganic arsenic, arsenic (III), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) were directly determined by EPA Method 1632, Revision A (*Chemical Speciation of Arsenic in Water and Tissue using Hydride Generation Quartz Furnace Atomic Absorption Spectrometry*). Arsenic (V) was determined by mathematically subtracting the measured concentration of arsenic (III) from the measured concentration of total inorganic arsenic. Total arsenic (which includes organic forms such as arsenobetaine) was not measured.

Analysis of the fish tissue samples by Method 1632A involved the following steps:

1) volatilizing Arsenic (III), arsenic (V), MMA, and DMA from solution at a specific pH after reduction to the corresponding arsines with sodium borohydride,
2) sweeping the volatilized arsines onto a liquid nitrogen cooled chromatographic trap which, upon warming, allows for a separation of species based on boiling points,
3) sweeping the released arsines by helium carrier gas into a quartz cuvette burner cell, where they are decomposed to atomic arsenic, and
4) determining arsenic concentrations by atomic absorption spectroscopy.

Strictly speaking, this technique determines the valence states of arsenate (V) and arsenite (III) rather than the species of inorganic arsenic. The actual species of inorganic arsenic are assumed to be those predicted by a geochemical equilibrium model.

Polychlorinated Biphenyls (PCBs) by EPA Method 1668A

EPA Method 1668, Revision A (*Chlorinated Biphenyls Congeners in Water, Soil, Sediment, and Tissue by HRGC/HRMS*, EPA-821-R-00-002) was used to determine PCB congener concentrations in tissue samples collected during the study. There are 209 possible congeners, 12 of which have toxicological significance (i.e., the "toxic" PCBs identified by the World Health Organization). Method 1668A can unambiguously determine 126 of the 209 congeners as separate chromatographic peaks. The remaining 83 congeners do not appear as separate peaks, but elute from the gas chromatograph in groups of 2 to 6 congeners that cannot be completely resolved by the instrumentation. Ten of the 12 "toxic" congeners are resolved, and the remaining two congeners (PCB 156 and PCB 157) elute as a congener pair. Because PCB 156 and 157 have identical toxicity equivalency factors, however, it is possible to accurately calculate PCB toxic equivalence based on the 12 toxic congeners.

Analysis of the full complement of PCB congeners in fish tissue samples by Method 1668A involved the following steps:

1) drying a 10-g aliquot of a homogenized fish tissue sample with sodium sulfate,
2) spiking the dried fish sample with a labeled standard solution,
3) performing soxhlet extraction of the dried fish sample using 1:1 methylene chloride/hexane as the extraction solvent,
4) drying the extract with sodium sulfate and concentrating the extract to dryness (to measure lipid content) using Kuderna-Danish and nitrogen blow-down techniques,
5) re-dissolving the extract and removing lipids by passing the extract through a layered column of silica gel, potassium silicate, and acid silica gel,
6) completing further clean-up of the extract using back-extraction with a strong base, acid and/or sodium chloride solution, gel-permeation chromatography, silica gel, carbon, or Florisil chromatography,
7) re-concentrating the extract using Kuderna-Danish and nitrogen blow-down techniques, and
8) quantifying the target chemicals in the extract using a gas chromatograph equipped with a high-resolution mass spectrometer.

The target chemicals were identified by comparing their chromatographic retention times with those of authentic standards and by comparing the ratio of the abundance of two ions specific to each chemical with the theoretical ion abundance ratio for that chemical. Concentrations were calculated from selected ion current profile areas using either an isotope dilution or internal standard technique. The efficiency of the clean-up process was monitored by spiking the extract prior to clean-up with labeled standards (PCBs 81 and 111) and tracking the final recovery of these standards. The chemical concentration in the fish tissue sample can be calculated on a mass basis (i.e., relative to the mass of the sample extracted), or lipid basis (i.e., relative to the lipid content of the sample extracted).

Dioxins and Furans by EPA Method 1613B

The presence and concentration of seventeen 2,3,7,8-substituted chlorinated dibenzo-*p*-dioxins and dibenzofurans in each sample was determined by a slightly modified version of EPA Method 1613, Revision B (*Tetra- through Octa- Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS*, EPA-821-B-94-005). Modifications were made to the procedures in order to allow for determination of dioxins and furans at levels ten times lower than those specified in the method. Specifically, the method was modified to increase the tissue sample size used for analysis and to add a sixth calibration solution that contained all the method-specified chemicals at levels lower than the levels specified in the method to verify linearity at the lower concentrations targeted.

Determination of dioxins and furans in tissue samples by Method 1613B involved the following steps:

1) drying a 100-g aliquot of a homogenized fish tissue sample with sodium sulfate,
2) spiking the dried fish sample with a labeled standard solution,
3) preparing extracts either by soxhlet extraction using 1:1 methylene chloride/hexane as the extraction solvent or by acid digestion using hydrochloric acid and 1:1 methylene chloride/hexane,
4) drying with sodium sulfate and concentrating the extract to dryness (to measure lipid content) using Kuderna-Danish and nitrogen blow-down techniques,
5) re-dissolving the extract and removing lipids by passing the extract through a layered column of silica gel, potassium silicate, and acid silica gel,
6) completing further clean-up of the extract using back-extraction with a strong base, acid and/or sodium chloride solution, gel-permeation chromatography, silica gel, alumina, or Florisil chromatography,
7) re-concentrating the extract using Kuderna-Danish and nitrogen blow-down techniques, and
8) quantifying the target chemicals in the extract using a gas chromatograph equipped with a high-resolution mass spectrometer.

The target chemicals were identified by comparing their chromatographic retention times with those of authentic standards and by comparing the ratio of the abundance of two ions specific to each chemical with the theoretical ion abundance ratio for that chemical. Concentrations were calculated from selected ion current profile areas using either an isotope dilution or internal standard technique. The efficiency of the clean-up process was monitored by spiking the extract prior to clean-up with a labeled standard (37Cl4- labeled 2,3,7,8 TCDD) and tracking the final recovery of this standard. The chemical concentration in the fish tissue sample can be calculated on a mass basis (i.e., relative to the mass of the sample extracted), or lipid basis (i.e., relative to the lipid content of the sample extracted).

For more information on this Method, visit ***www.epa.gov/waterscience/methods/1613.pdf.***

Organochlorine Pesticides by EPA Method 1656A

Organochlorine pesticides (e.g., DDT and chlordane) and were determined by Method 1656, Revision A (*Organo-Halide Pesticides in Wastewater, Soil, Sludge, Sediment, and Tissue by GC/HSD*). The following modification was made to the procedure listed below: the tissue sample extracts were concentrated by a factor of five beyond method-specified levels before instrumental analysis. This modification ensured that all target pesticides could be quantified at levels equal to or lower than the human health screening values for recreational fishers published in Table 5-3 of EPA's *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1: Risk Assessment and Fish Consumption Limits, Third Edition* (EPA 2000d).

Analysis of the target pesticides in fish tissue samples by Method 1656A involved the following steps:

1) drying a 10-g aliquot of a homogenized fish tissue sample with sodium sulfate,
2) preparing a soxhlet extraction of the dried fish sample using 1:1 methylene chloride/hexane as the extraction solvent,
3) drying the extract with sodium sulfate and concentrating the extract to dryness (to measure lipid content) using Kuderna-Danish and nitrogen blow-down techniques,
4) re-dissolving the extract and removing lipids by gel-permeation chromatography and/or solid-phase extraction (SPE cartridges),
5) re-concentrating the extract using Kuderna-Danish and nitrogen blow-down techniques, and
6) quantifying the target chemicals in the extract using a gas chromatograph equipped with dual capillary columns and an electron capture detector.

Target chemical identification is performed by comparing the GC retention times of each chemical on two different columns with the respective retention times of an authentic standard. Quantitative analysis (e.g., determining the concentration of each chemical) was performed using an authentic standard to produce a calibration factor or calibration curve, and using the calibration data to determine the concentration of a pollutant in the extract. The chemical concentration in the fish tissue sample can be calculated on a mass basis (i.e., relative to the mass of the sample extracted), or lipid basis (i.e., relative to the lipid content of the sample extracted).

Organophosphorus Pesticides by EPA Method 1657A

EPA Method 1657, Revision A (*Organophosphorus Pesticides in Water, Soil, and Tissue by GC/FPD*) was used to determine the presence and concentration of organophosphorus pesticides (e.g., chlorpyrifos and diazinon).

Analysis of the fish tissue samples by Method 1657A involved the following steps:

1) drying a 10-g aliquot of a homogenized fish tissue sample with sodium sulfate,
2) preparing a soxhlet extraction of the dried fish sample using 1:1 methylene chloride/hexane as the extraction solvent,
3) drying the extract with sodium sulfate and concentrating the extract to dryness (to measure lipid content) using Kuderna-Danish and nitrogen blow-down techniques,
4) re-dissolving the extract and removing lipids by gel-permeation chromatography and/or solid-phase extraction (SPE cartridges),
5) re-concentrating the extract using Kuderna-Danish and nitrogen blow-down techniques, and
6) quantifying the target chemicals in the extract using a gas chromatograph equipped with dual capillary columns and a flame photometric detector.

Target chemical identification was performed by comparing the GC retention times of each chemical on two different columns with the respective retention times of an authentic standard. Quantitative analysis (e.g., determining the concentration of each chemical) was performed using an authentic standard to produce a calibration factor or calibration curve, and using the calibration data to determine the concentration of a chemical in the extract. The chemical concentration in the fish tissue sample can be calculated on a mass basis (i.e., relative to the mass of the sample extracted), or lipid basis (i.e., relative to the lipid content of the sample extracted).

Semivolatile Organic Chemicals by Method 1625C

The remainder of the target organic chemicals were analyzed by a modified version of EPA Method 1625, Revision C (*Semivolatile Organic Compounds by Isotope Dilution GC/MS*). The modifications made to this method involved fractionating the samples by gel permeation chromatography (GPC) to yield a fraction containing the phthalates and some of the lower molecular weight lipids and a lipid-free fraction containing the polar target compounds. The phthalate/lipid fraction was further cleaned using Alumina and then recombined with the lipid-free fraction so that all target chemicals could be determined in a single run.

Analysis of the target chemicals by Method 1625C involved the following steps:

1) homogenizing and spiking isotopically labeled analogs of each target chemical into the sample,
2) extracting the sample at pH 12-13 and then at pH <2 with methylene chloride using continuous extraction techniques,
3) drying each extract over sodium sulfate,
4) concentrating the extract to five mL,
5) cleaning the extract with gel permeation chromatography,
6) concentrating the extract to 0.5 mL,
7) adding internal standards to the extract, and
8) injecting a one µL aliquot of the extract into a gas chromatograph (GC).

At this point, the target chemicals are separated by GC and detected by a mass spectrometer (MS). The labeled compounds serve to correct the variability of the analytical technique. Chemical identification was performed by comparing mass spectrum and retention time to calibration standards (for most chemicals) or through reference libraries using forward search or reverse search techniques. Quantitative analysis was performed in one of the following ways using extracted ion current profile (EICP) areas:

- for compounds in which standards and labeled analogs were available, the concentration was determined using isotope dilution techniques,
- for compounds in which standards were available but labeled analogs were not, the concentration was determined using an internal standard technique, and
- for compounds in which neither standards nor known response factors were available, concentrations were determined using the sum of the EICP areas relative to the sum of the EICP areas of the internal standard.

During analysis of the samples collected in the fourth year of the study only, the laboratory employed a Florisil cleanup instead of an Alumina cleanup. This change was initiated to mitigate lipid interferences that were resulting in an excessive number of re-extractions and re-analyses in the samples collected during previous years of the study. The use of Florisil did reduce the number of re-analyses required, suggesting that this approach should be considered in any further studies.