FSP Title: **Tissue POP measurement GC/MS Protocol**

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1.0 Background

Environmental pollution is one of the most serious challenges to health in the modern world. They have been found in air, groundwater, and animal fat tissues. Effective monitoring methods are indispensable for assessing environmental quality, identifying pollution sources, and implementing remedial measures.

2.0 Scope

This method is for determination of persistent organic pollutants (POP) effect like tetra- through octa-chlorinated dibenzo-p-dioxins (CDDs) and dibenzofurans (CDFs) in tissue by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS).

3.0 Materials

3.1. Equipment

- Ice
- Microcentrifuge tubes
- Vortexer
- Centrifuge
- SpeedVac
- Autosampler vials and Crimper

3.2. Reagents

- Hydrochloric acid
- Chloroform
- Hexane
- Sodium sulfate
- Methylene chloride

4.0 Procedure

This procedure assumes there is adequate amount or volume of sample available to perform all the described analysis. The minimum volume of all the study samples should be checked ahead of time. If there are samples with low volume, then the volumes described in this protocol can be adjusted as long as the ratios of the reagents are kept constant.

Important: Prepare a blank. Follow all steps for blank along with samples. All steps are performed ideally at 4 ℃.

4.1. Sample preparation

1. 500 mg liver or adipose samples were extracted using with 10 ml of 6 M hydrochloric acid and 10 mL of chloroform:hexane (1:1), then shake for 12-24 hours.
2. Take the chloroform:hexane layer and concentrate it down to about 1 mL and spike with a C13 standard.
3. The purification step was performed:
    a. Insert a glass wool plug into the tapered end of a graduated serological pipet. Pack with 1 mL of sodium sulfate, 1.5 g of Florisil topped with 1 mL of sodium sulfate and a glass wool plug.
    b. Pre-elute the activated Florisil column with 10 mL of methylene chloride followed by 10 mL of hexane:methylene chloride (98:2 v/v) and discard the solvents.
    c. When the solvent is within 1 mm of the packing, apply the sample extract (in 2 ml hexane) to the column.
    d. Elute the interfering compounds with 20 mL of hexane:methylene chloride (98:2) and discard the eluate.
    e. Elute the POP with 35 mL of methylene chloride and collect the eluate.
4. Dry down the samples using N2, and resuspend in 100 ul hexane containing 5 ug/ml internal standard.

5.0 References

5.1. Telliard, W. A. Method 1613, Revision B Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS. U.S. Environmental Protection Agency Office of Water Engineering and Analysis Division (4303) 401 M Street S.W. Washington, D.C. 20460 October **1994**.

5.2. Hui Lin, Jessica Betz, Jim Dunn, Greg Martin, David Somerville, Diana Wong, Dale Walker, and Craig Marvin. Tetra- Through Octa-Chlorinated Dioxins and Furans Analysis in Water by Isotope Dilution GC/MS/MS. Tetra- through Octa-chlorinated Dioxins and Furans Analysis in Water by Isotope Dilution GC/MS/MS (agilent.com)