# DETERMINATION OF ULTRA-TRACE LEVEL POLYCHLORINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS IN FISH SAMPLES USING AN AUTOMATED CLEANUP TECHNIQUE

Marcia Ryel, Joseph Palausky, Thomas Dux, and Thomas Goodman

Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri, 64110, USA

## Introduction

Twelve fish samples were collected and analyzed for seventeen 2, 3, 7, 8-substituted polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs). The requested detection limits for tetra substituted PCDDs/PCDFs was 100 femtograms per gram (fg/g) or parts-per-quadrillion (ppq) on a wet weight basis. In order to achieve this low level, extraction, cleanup, and analysis procedures all required modification from the techniques presented in US EPA Method 8290[1] and 1613B[2].

A large mass of tissue was extracted and with this mass, it was apparent that the extracted lipids would present a challenge to meeting the desired detection limit. The traditional method for lipid removal, gel permeation chromatography, was considered but rejected because of the loss of extract in loading the column using conventional automated systems. Recent success with tissue samples using an automated cleanup technique on the Fluid Management Systems Power Prep™ system provided a workable alternative. Analysis was conducted using a low level standard at 0.25 pg/mL for tetra substituted PCDDs/PCDFs. The final procedure resulted in reporting limits of 50 fg/g for tetra PCDD/PCDF, 250 fg/g for penta-hepta PCDD/PCDF, and 500 fg/g for octa PCDD/PCDF.

## Methods and Materials

All samples were prepared and analyzed according to isotope dilution quantitation techniques presented in US EPA Method 8290 and 1613B. The 12 fish samples were thoroughly homogenized and a 50-g sub-sample was removed for extraction. Sodium sulfate was added and mixed with the samples. Three quality control samples including a method blank and duplicate laboratory control samples were prepared, each consisting of 50 grams of sodium sulfate. The fish and quality control samples were fortified with a mixture of $^{13}C_{12}$–labeled PCDD/PCDF internal quantitation standards as described in Method 1613B[1] and allowed to equilibrate for one hour. After equilibration, the samples were extracted by Soxhlet for 18 hours with a 50:50 (v/v) mixture of dichloromethane and hexane (B&J distilled in glass). The extracts were concentrated to dryness and the remaining lipids were determined gravimetrically. The lipids were dissolved in hexane and fortified with a $^{37}Cl_4$-labeled TCDD as a clean-up efficiency standard.

For cleanup, the samples were processed using the automated Fluid Management Systems (FMS) Inc. Power Prep™. Normal procedure for the isolation of PCDD/PCDF congeners uses a series of three disposable columns including a multi-layered (acid/base/neutral) silica column, an (acid/base/neutral) alumina column, and an AX-21 carbon column (all procured from FMS). For lipid removal, an additional disposable high-capacity acid-silica column was added prior to the multilayer silica column.

The lipid removal efficiency of the high capacity silica column had been previously evaluated by an experiment with corn oil. In the experiment, it was determined that the high column removes up to 99 percent of the corn oil during elution. With large mass samples, >5g of lipid mass was expected. In

order to maintain the efficiency of the high capacity column, samples with greater than 5g determined lipid were split 50:50 (v/v), processed separately, and recombined following Power Prep™ elution.

Prior to loading samples, each extract was filtered through a 0.45mm filter to prevent scratching of the internal valves or clogging of the system lines. All solvents used in FMS processing are Burdick and Jackson (distilled in glass). The extracts were loaded and eluted with hexane through the high capacity acid silica column and through the multilayer silica column onto the alumina column. After complete silica elution, the alumina column was pre-eluted with 2 percent (v:v) dichloromethane in hexane. The alumina column was then eluted to the carbon column in the forward direction with 50 percent (v:v) dichloromethane in hexane. The carbon column was then eluted in the forward direction with 50 percent (v/v) ethyl acetate in toluene and the PCDDs/PCDFs were eluted from the carbon column in the reverse direction with toluene. The extracts were concentrated by Turbo-Vap™ and fortified with two $^{13}C_{12}$ labeled recovery standards in tridecane. Samples were allowed to concentrate to a final volume of 10mL for analysis.

Sample analysis was performed using a Fisons Autospec Ultima high resolution mass spectrometer (HRMS) operated at mass resolution of >10,000. A 60-meter DB-5ms fused silica column was used with conditions specific for separating 2,3,7,8-TCDF and 2,3,7,8-TCDD from all other TCDF/TCDD isomers. A six point calibration curve was utilized with a TCDD concentration of 0.25 pg/mL as the low level standard. All US EPA Method 1613B criteria were met for initial calibration, daily calibration, and for isomer resolution.

Data reduction procedures were conducted using the Opusquan HRMS data system. Concentrations of native PCDD/PCDF analytes were calculated by the isotope dilution methodology using the $^{13}C_{12}$-labeled added to the samples prior to extraction. Therefore, the native results are recovery corrected for the labeled analog performance.

**Results and Discussion**

Table 1 presents the average recovery of IQS and cleanup standards in the 12 samples as well as the results for the quality control samples. Both accuracy as demonstrated by the average recovery and precision as expressed by relative standard deviation (RSD) were well within method objectives.

The method blank (MB) did not contain any target analytes above 1/3 the minimum level which is based on the lowest calibration standard, the final volume, and the extracted mass. A detection limit of 50 fg/g for TCDD/TCDFwas obtained which was an improvement over the objective of a 100 fg/g for TCDD/TCDF  The absence of background in the method blank is attributed to the high quality reagents and columns, the use of disposable columns, and the minimization of transfer, solvent-exchange, and concentration steps in Power Prep™ cleanup.

**Table 1.** Quality Control Results for Fish Tissue Analysis

| | 12 Samples Average | | Method Blank | LCS/OPR Analysis | | |
|---|---|---|---|---|---|---|
| Analyte | % Recovery | RSD | (pg/g) | Test (ng/mL) | Average % Recovery | %RPD |
| Native | | | | | | |
| 2,3,7,8-TCDF | — | — | U (0.0500) | 10 | 115 | 0.44 |
| 2,3,7,8-TCDD | — | — | U (0.0500) | 10 | 93.0 | 3.2 |
| 1,2,3,7,8-PeCDF | — | — | U (0.250) | 50 | 130 | 0 |
| 2,3,4,7,8-PeCDF | — | — | U (0.250) | 50 | 109 | 0.92 |
| 1,2,3,7,8-PeCDD | — | — | U (0.250) | 50 | 125 | 1.6 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 1,2,3,4,7,8-HxCDF | — | — | U (0.250) | 50 | 109 | 2.8 |
| 1,2,3,6,7,8-HxCDF | — | — | U (0.250) | 50 | 102 | 2.0 |
| 2,3,4,6,7,8-HxCDF | — | — | U (0.250) | 50 | 109 | 2.8 |
| 1,2,3,7,8,9-HxCDF | — | — | U (0.250) | 50 | 96.5 | 3.1 |
| 1,2,3,4,7,8-HxCDD | — | — | U (0.250) | 50 | 115 | 5.2 |
| 1,2,3,6,7,8-HxCDD | — | — | U (0.250) | 50 | 104 | 1.0 |
| 1,2,3,7,8,9-HxCDD | — | — | U (0.250) | 50 | 102 | 2.0 |
| 1,2,3,4,6,7,8-HpCDF | — | — | U (0.250) | 50 | 94.0 | 2.1 |
| 1,2,3,4,7,8,9-HpCDF | — | — | U (0.250) | 50 | 111 | 3.6 |
| 1,2,3,4,6,7,8-HpCDD | — | — | U (0.250) | 50 | 119 | 2.5 |
| OCDF | — | — | U (0.500) | 100 | 108 | 3.7 |
| OCDD | — | — | U (0.500) | 100 | 113 | 3.5 |
| **IQS** | | | | | | |
| 13C-2,3,7,8-TCDF | 86.6 | 14 | 85.0 | 100 | 101 | 5.4 |
| 13C-2,3,7,8-TCDD | 77.9 | 14 | 76.4 | 100 | 89.1 | 3.4 |
| 13C-1,2,3,7,8-PeCDF | 94.6 | 14 | 92.6 | 100 | 106 | 5.6 |
| 13C-2,3,4,7,8-PeCDF | 108 | 14 | 107 | 100 | 125 | 6.0 |
| 13C-1,2,3,7,8-PeCDD | 78.2 | 14 | 76.0 | 100 | 86.0 | 5.2 |
| 13C-1,2,3,4,7,8-HxCDF | 77.3 | 13 | 77.5 | 100 | 95.3 | 8.9 |
| 13C-1,2,3,6,7,8-HxCDF | 86.5 | 13 | 88.0 | 100 | 107 | 9.8 |
| 13C-2,3,4,6,7,8-HxCDF | 78.6 | 15 | 79.3 | 100 | 96.0 | 9.9 |
| 13C-1,2,3,7,8,9-HxCDF | 88.6 | 14 | 86.6 | 100 | 104 | 10 |
| 13C-1,2,3,4,7,8-HxCDD | 65.2 | 14 | 65.5 | 100 | 77.1 | 7.1 |
| 13C-1,2,3,6,7,8-HxCDD | 85.5 | 14 | 86.0 | 100 | 105 | 11 |
| 13C-1,2,3,4,6,7,8-HpCDF | 94.2 | 14 | 95.7 | 100 | 113 | 9.7 |
| 13C-1,2,3,4,7,8,9-HpCDF | 80.4 | 14 | 81.2 | 100 | 97.2 | 7.7 |
| 13C-1,2,3,4,6,7,8-HpCDD | 80.4 | 14 | 80.6 | 100 | 95.8 | 8.3 |
| 13C-OCDD | 64.3 | 28 | 71.5 | 100 | 86.8 | 8.3 |
| **Cleanup** | | | | | | |
| 37Cl-2,3,7,8-TCDD | 84.7 | 14 | 83.7 | 100 | 99.4 | 3.0 |

U - Undetected at reported detection limit based on lowest calibration standard, final volume, and mass extracted.

Native analyte results are presented in Figure 1. Toxic equivalency quotients (TEQs) were calculated using I-TEF values[3] based on non-detected analytes at a concentration of 0 (zero) and at the minimum level (ML).

It is observed that for samples with higher lipid content, the TEQ is largely driven by the detected analytes. For samples with lower lipid content, however, the TEQ can be significantly different depending on the value substituted for non-detected analytes. Regardless of the handling of non-detects, using the described procedure demonstrates the ability to achieve ppq-level sensitivity with high accuracy and precision.

**Acknowledgements**

This work was sponsored by the Maine Department of Environmental Protection.

# ANALYSIS I

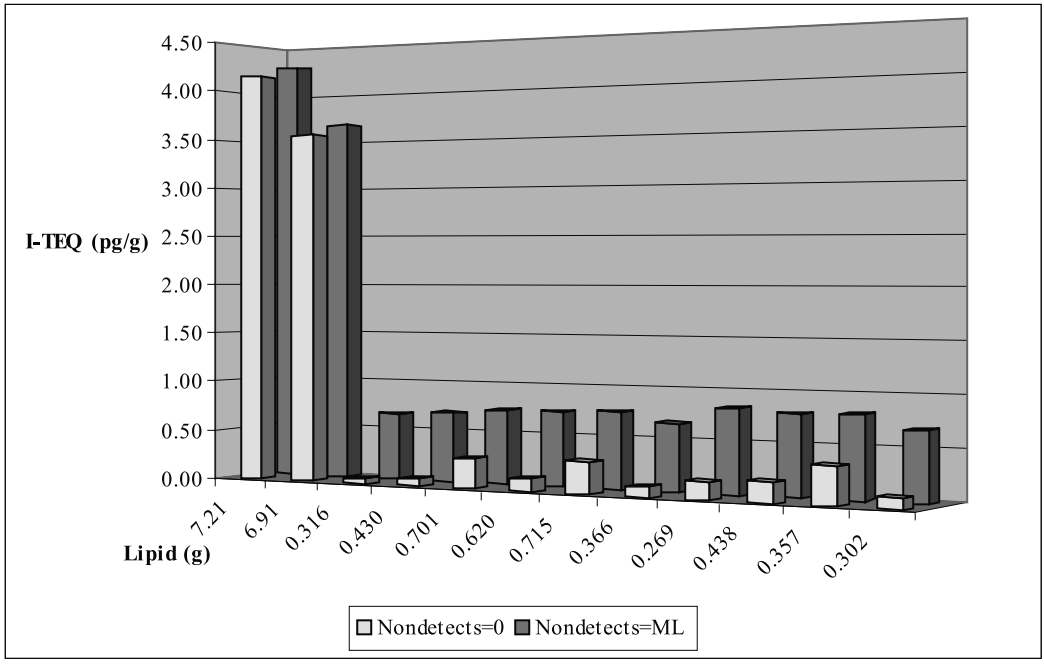


**Figure 1.** I-TEQ Based on Extraction of 50 gram Fish Tissue Samples (pg/g wet weight)

**References**

1. USEPA Method 8290, "Polychlorinated Dibenzodioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by High-Resolution Gas Chromatography / High Resolution Mass Spectrometry (HRGC/HRMS), Revision 0." September 1994.
2. USEPA Method 1613, "Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, Revision B." Oct. 1994.
3. Van den Berg et al., Toxic Equivalency Factors (TEFs) for PCBs, PCDDs, PCDFs for Humans and Wildlife, Environmental Health Perspectives 106:775-792, 1998.