# EXTENSION OF U.S. EPA METHODS 0023A/8290 TO INCLUDE $^{13}C_{12}$-LABELLED MONO-, DI-, AND TRI-CHLORINATED DIBENZO-*p*-DIOXIN AND DIBENZOFURAN STANDARDS

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**Introduction**

Concentrations of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are typically reported only in terms of the tetra- through octa-CDD/CDF isomers, either as homologue sums or as subsets thereof. This practice is common because the toxic isomers of interest are solely a subset of the tetra- through octa-CDD/CDF homologues and, hence, only the tetra- through octa-CDD/CDF $^{13}C_{12}$-labelled standards are commercially available. The mono- through tri-CDD/CDF isomers, however, are important toward understanding formation mechanisms and source/receptor relationships. Their relative abundance provides evidence for chemical formation mechanisms and equilibrium composition models. Their concentration levels, relative to the other homologues, provide additional information on the dioxin Afingerprint@ for source attribution.

Until only recently, the ability to accurately quantify the mono- through tri-CDD/CDF isomers has been relatively restricted. Isotopically labelled ($^{13}C_{12}$) standards for the mono- through tri-CDD/CDF isomers, analogous to those used to quantify tetra- through octa-CDD/CDF, are needed to ensure accurate quantitatation. Unfortunately, the commercial availability of labelled mono- through tri-CDD/CDF standards is severely limited.

Analysis for PCDD/Fs via EPA Methods 0023A/8290 utilizes the Aisotope dilution technique.@ In this method, a known amount of an isotopically labelled ($^{13}C_{12}$) homologue of each target analyte is used to quantify the target analyte. In theory and practice, the isotopically labelled homologue behaves chemically in an almost identical manner as the unlabelled or Anative@ homologue. As a result, the labelled standards can be used as both internal and surrogate standards which provide a means to internally compensate for method biases as well as assessing quantitative measurement performance.

The measurement of PCDDs/PCDFs from combustion sources by EPA Methods 0023A/8290 makes use of tetra- through octa-CDD/CDF $^{13}C_{12}$-labelled standards for several purposes. Presampling surrogate standards are spiked directly onto the sampling media (XAD-27) prior to sample collection to determine quantitative measurement performance inclusive of sampling activities and effects. The pre-extraction/internal standards, spiked after sampling but before extraction, are used to quantitate the target native compounds as well as the presampling $^{13}C_{12}$-labelled surrogates. The internals are also used to determine extraction efficiencies and losses due to cleanup procedures. If the internal standards are not all extracted or suffer partial loss during the cleanup procedure, so will the native targets. The standards= relative concentration with respect to the natives remains constant, allowing for internal adjustment of the measured

native concentration. A final, recovery standard is added to the sample after cleanup and prior to analysis to quantitate the internal standards and determine absolute internal standard recovery.

For these reasons, the U.S. EPA has had synthesized and tested mono- through tri-CDD/CDF $^{13}C_{12}$-labelled standards for their performance in EPA/NRMRL=s sampling/analytical procedures for PCDD/PCDFs, based on EPA Methods 0023A/8290. The availability of these mono- through tri-CDD/CDF $^{13}C_{12}$-labelled standards will allow a more accurate quantitative measurement of these lower-chlorinated isomers. It is emphasized, however, that the procedures and results detailed in this paper are for EPA/NRMRL=s research purposes and are not a part of the official EPA Methods 0023A/8290.

**Experimental**

Because of the need to accurately quantify native mono- through tri-CDDs/CDFs, we contracted with Cambridge Isotope Laboratories (Andover, MA, USA), to synthesize five $^{13}C_{12}$-labelled, mono-, di-, and tri-CDD/CDF (M-, Di-, Tr-CDD/F, respectively) standards to complement the three already available. The resulting eight standards and their proposed use are identified in Table 1. Ideally, use of the listed mono-, di-, and tri-CDD/CDF as internal pre-extraction (internal) standards and use of the additional 2,3-DiCDD and 2,8-DiCDF as presampling surrogates would prove sufficient. However, because of our use of commercially prepared custom mixes, we were not able to separate out the 2,3,7-TrCDD for use as an internal standard in this work.

Table 1. Previous and **Newly Synthesized** Isotopically Labelled $^{13}C_{12}$ Standards

| Presampling Standards (Surrogates) | Pre-Extraction Standards (Internals) |
|---|---|
| | **2-MCDF**<br>**2-MCDD** |
| **2,3-DiCDD**<br>2,8-DiCDF | 2,7-DiCDD<br>**2,4-DiCDF** |
| 2,3,7-TrCDD | **2,4,8-TrCDF** |

A series of tests were performed to assess the quality of mono- to tri-CDD/CDF measurements following slight modifications of procedures in EPA Methods 0023A/8290 after incorporating the new $^{13}C_{12}$-labelled mono- to tri-CDD/CDF isomers. Specifically, tests were performed with the following objectives:

1) - determine whether individual isomers within each homologue exhibit similar mass spectrometer responses (relative response factors, RRFs),
2) - determine whether individual mono- to tri-CDD/CDF isomers within each homologue exhibit similar recoveries during the sample cleanup process (e.g., 2-MCDF and 4-MCDF),
3) - determine whether our existing cleanup procedure would be suitable for inclusion of mono- to tri-CDD/CDF homologue quantitation (e.g., mono-CDF vs. tri-CDD), and
4) - determine the optimal allocation of the existing labelled standards to indicate overall measurement performance for the mono- to tri-CDD/CDF homologues.

To meet these objectives, several different sample types were prepared and examined. All available native mono- to tri-CDD/CDF isomers were analyzed by high-resolution gas chromatography/low-resolution mass spectrometry (HRGC/LRMS) to examine objectives 1 and 2. Table 2 lists the number of natives evaluated.

Table 2. Availability of Analytical Standards

| Homo-logue | Possible Isomers | | Isomers w/ Standards Available | |
|---|---|---|---|---|
| | -CDD | -CDF | -CDD | -CDF |
| Mono- | 2 | 4 | 2 | 2 |
| Di- | 10 | 16 | 4 | 3 |
| Tri- | 14 | 28 | 8 | 4 |

**Results and Discussion**

Standard solutions were analyzed directly and compared to an internal standard to determine relative response factors (RRFs). Twenty unlabelled, internal standards (the available Anatives,@ Table 2) with five $^{13}C_{12}$-labelled standards (Table1) were directly analyzed via HRGC/LRMS (three standards co-elute, and so were not tested). Table 3 (first data column) shows the standard deviations of the RRFs for the native isomers within each homologue relative to a labelled homologue-specific standard from Table 1. The relative standard deviations suggest that the GC/MS responses of individual isomers within each homologue are very similar (<16 % deviation from the average).

The second data column of Table 3 shows that quantitation of the natives is very successful, through use of the internal standards stated in Table 1. The recoveries of these natives fall within the prescribed 70-130% criterion for the presampling surrogates in EPA Methods 0023A/8290. It should be noted that the concentration step of the cleanup process should not be allowed to go to dryness. Several samples that went to dryness exhibited large loss of both mono-CDD and mono-CDF isomers and were excluded from inclusion in these tables.

These same standards were then subjected to the three-step (acid/base silica gel, alumina, activated carbon columns) chromatographic sample cleanup process and the absolute recoveries determined for each native isomer. The standard deviations in the second data column of Table 3 show that the isomers within each homologue respond similarly (< 6 % std dev) to the column cleanup procedures, satisfying objective 2. This suggests that the mono- to tri-CDD/CDF isomers respond favorably to the current cleanup procedures and that none of the native isomers are preferentially lost during cleanup.

Table 3. Isomer-Specific RRF Variation and Recovery Values for Native Isomers

| Homologue | RRF Std Dev | Avg % Recovery and Std Dev thru Cleanup |
|---|---|---|
| MCDD | 0.018 | 113.5 5.4 |
| MCDF | 0.153 | 105.4 5.4 |
| DiCDD | 0.030 | 103.3 1.7 |
| DiCDF | 0.087 | 115.7 1.9 |
| TrCDD | 0.053 | 107.4 5.7 |
| TrCDF | 0.076 | 106.3 3.5 |

Finally, actual emissions samples with XAD-27-spiked

standards were analyzed by isotope dilution to verify suitability of the mono-tri-CDD/CDF quantitative approach. The eight isotopically labelled standards were subjected to the same cleanup process to examine objectives 3 and 4. The recoveries, shown in Table 4, pass the hepta- to octa-CDD/CDF criterion of 25 - 130 % and the tetra- to hexa- CDD/CDF criterion of 40-130% except for the mono-CDD/CDF homologues. The lower recoveries on the mono- CDD/CDF indicate that they are the most sensitive indicators of sampling loss, likely due to their higher volatility. Due to the above-mentioned losses during dryness, it makes more sense to use the newly synthesized mono-CDD/CDF labelled isomers as internal standards to aid quantitation rather than as indicators of loss during sampling. The three labelled standards listed in Table 1 serve as adequate presampling standards; when more standards are available, a mono-CDD/CDF should also be used as a presampling standard.

Table 4. Absolute Recoveries of Isotopically-Labelled Mono-TriCDDs/Fs

| Congener | Average % Recovery |
|---|---|
| 2-MCDD | 38.7 7.2 |
| 2-MCDF | 28.9 3.9 |
| 2,7-DiCDD | 52.8 7.4 |
| 2,3-DiCDD | 51.9 9.4 |
| 2,4-DiCDF | 50.3 8.4 |
| 2,8-DiCDF | 51.9 6.2 |
| 2,3,7-TrCDD | 61.5 5.0 |
| 2,4,8-TrCDF | 60.9 8.2 |

**Conclusions**

The use of newly available, $^{13}C_{12}$-labelled, mono- to tri-CDD/CDF standards has been demonstrated by incorporation into existing EPA Methods 0023A/8290. Accurate quantification of mono- to tri-CDD/CDF isomers and homologues is possible. Use of the DiCDD and DiCDF as presampling surrogates is an adequate indicator of mono- to tri-CDD/CDF measurement quality. Similar recovery values indicate that homologue-specific isomers respond similarly to cleanup procedures and suggest that only a single cleanup procedure (EPA Methods 0023A/8290) is necessary.

**References**

U.S. EPA Test Method 0023A ASampling Method for Polychlorinated Dibenzo-p-Dioxins and Polychlorinated Dibenzofuran Emissions From Stationary Sources,@ in Test Methods for Evaluating Solid Waste, SW-846 (NTIS PB88-239223). Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, DC, December 1996.

U.S. EPA Test Method 8290 APolychlorinated Dibenzo-p-Dioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by High-Resolution Gas Chromatography/High Resolution Mass Spectrometry (HRGC/HRMS) Rev. 0, in Test Methods for Evaluating Solid Waste, SW-846 (NTIS PB88-239223). Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, DC, September 1994.