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APPLICATION OF EPA METHOD 1668A TO THE ANALYSIS OF DIOXIN-LIKE PCBs AND TOTAL PCBs IN HUMAN TISSUE AND ENVIRONMENTAL SAMPLES.

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Introduction

High resolution gas chromatography/mass spectrometric (HR GC/MS) analysis methods have evolved to meet the increasing demand for reliable congener specific determination of polychlorinated biphenyls (PCBs) in environmental samples. One method, EPA Method 1668A[(1)], permits determination of all 209 PCB congeners including full isotope dilution quantification of the twelve dioxin-like congeners assigned TEF values by the World Health Organization (WHO). We have applied this method to a variety of matrices including soil, sediment, tissue, sewage sludge, ash, human blood and human milk for the determination of all PCB congeners, PCB Homologue Totals and Total PCBs. The HR GC/MS technique is highly sensitive and selective, however, the potential for congener to congener interference exists and we have found that careful attention to the extract clean-up procedures and chromatographic resolution is required. To facilitate comparison of PCB congener data to historical PCB Aroclor equivalent results we calculated Aroclor® equivalent approximations using conversion factors determined from analysis of Aroclor® formulations.

Methods and Materials

Samples are analyzed in accordance with EPA Method 1668A.

Samples are spiked with labeled surrogate standards and extracted. An aliquot of labeled clean-up standard is spiked into the sample extract and Florisil, layered acid/base silica and alumina chromatographic column clean-up procedures are performed to isolate the PCB congeners. The extracts are reduced in volume and spiked with an aliquot of recovery (internal) standard prior to instrumental analysis. Table 1 identifies the labeled standards used.

An optional carbon chromatographic column procedure is employed to isolate the dioxin-like PCB congeners where necessary.

High resolution mass spectrometric (HRMS) analysis is performed using a Micromass Autospec Ultima, equipped with a Hewlett Packard 6890 gas chromatograph and a CTC autosampler. An Alpha data system running Micromass software is used to control the instrument and acquire data. Chromatographic separation is achieved with a Supelco SPB-Octyl column (30 m, 0.25 mm i.d. x 0.25 µm film thickness). A second chromatographic column analysis is performed on a J&W Scientific DB-1 column (30 m, 0.25 i.d. x 0.25 µm film thickness).

Table 1. Labeled compounds spiked into sample extracts

^{13}C labeled Standard	Amount added per sample
Surrogates (added prior to extraction)	
1, 3, 4, 15, 19, 37, 54, 77, 81, 104, 105, 114, 118, 123, 126 155, 156, 157, 167, 169, 188 189, 202, 205, 206, 208, 209	2000 pg each
Cleanup (added after extraction)	
28, 111, 178	2000 pg each
Recovery (added prior to instrumental analysis)	
9, 52, 101, 138, 194	2000 pg each

A six-point calibration series, each solution containing 27 native and 35 labeled congeners, encompassing a concentration range of 0.2 to 2000 pg/µL is analyzed. Included in the 27 native analytes are the 12 dioxin-like congeners and 15 windowing congeners, of the 35 labeled standards 27 are exact $^{13}C_{12}$ labeled isomers of the native analytes, three are $^{13}C_{12}$ labeled clean-up standards and five are $^{13}C_{12}$ labeled recovery (internal) standards. Relative response factors (RRF) are generated for each of the native analytes in each of the six solutions. A single-point calibration standard is used to generate RRFs and relative retention times (RRTs) for the remaining 182 congeners in accordance with the method. Calibration performance is monitored every 12 hours using the mid-level calibration standard.

PCB congener concentrations are calculated by isotope dilution/internal standard quantification techniques using Micromass' OPUSquan software.

Results and Discussion

A chromatogram of all 209 congeners is shown in Figure 1. This chromatogram illustrates the retention time overlap of the homologue groups. The HRMS analysis separates each homologue group by mass; however, there are response contributions from loss of chlorine fragment ions to the ion traces in one and two homologue groups lower.

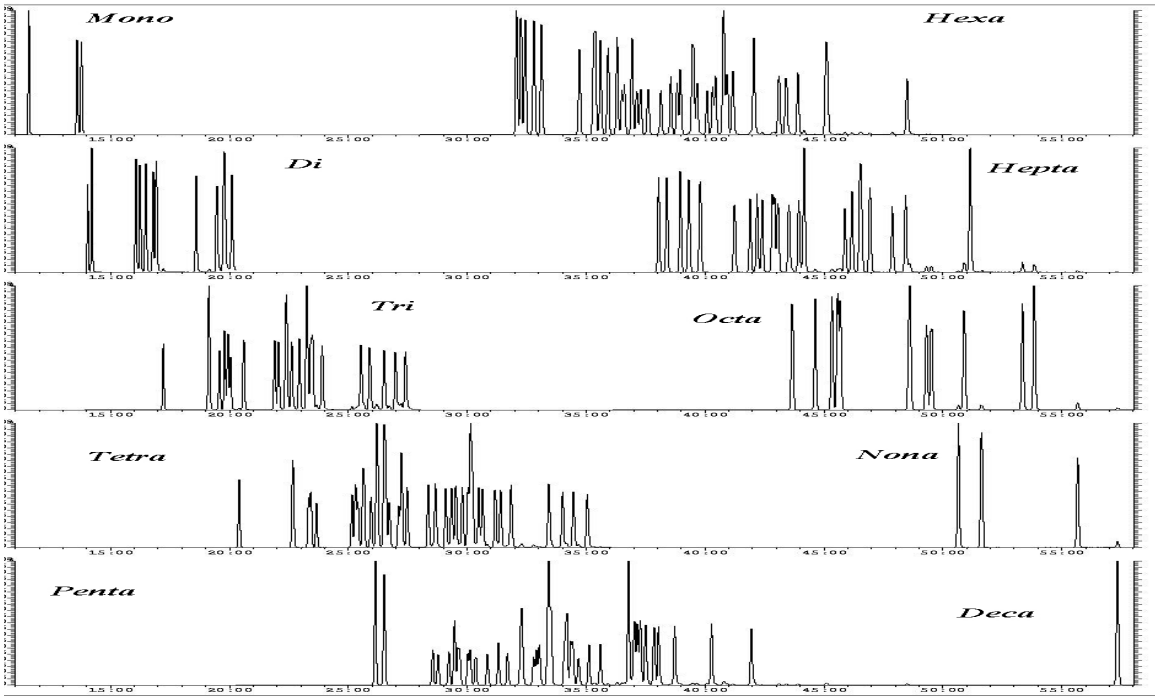

Figure 1. Elution times of PCB homologue groups on the SPB-Octyl column

The contribution could be up to 15% of the area response of the interfering higher chlorinated congener, possibly causing a high bias in the determined concentration of the target congener. This can be significant when the concentration of the target analyte is much lower than that of the interfering congener, and if the target is one of the dioxin-like PCB congeners this can substantially impact the TEQ calculated for the sample. For example, the response of the pentachlorinated congeners ($^{12}C_{12}H_5{}^{35}Cl_4{}^{37}Cl$ and $^{12}C_{12}H_5{}^{35}Cl_3{}^{37}Cl_2$) can be elevated by the response of a fragment ion from a heptachlorinated congener eluting at the same time ($^{12}C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2$ and $^{12}C_{12}H_3{}^{35}Cl_2{}^{37}Cl_3$, respectively).

Analysis on the SPB-Octyl column allows complete chromatographic resolution of the dioxin-like PCBs 77, 105, 114, 118, 156/157 (as a pair), 167, and 189 from all other congeners. The remaining dioxin-like PCBs (81, 123, 126 and 169) can be affected by closely eluting congeners or by dechlorination

interferences. The interferences cause integration difficulties by masking of the peak front, peak tail or both, as shown in Figures 2 through 5. These illustrations are arranged with the target congener in the top chromatogram, the ^{13}C isotopically labeled congener (for retention time reference) in the second chromatogram and the interfering congener in the final chromatogram(s), with the exception of PCB 123 (Figure 3) as the interfering congener is from the same homologue group. The peak areas are printed above native peaks for comparison.

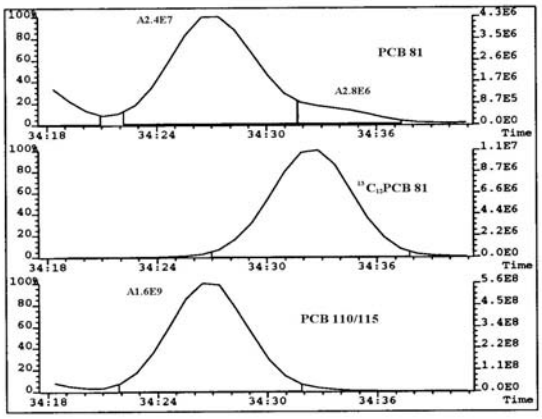

Figure 2. PCB 110/115 fragmentation interference with PCB 81

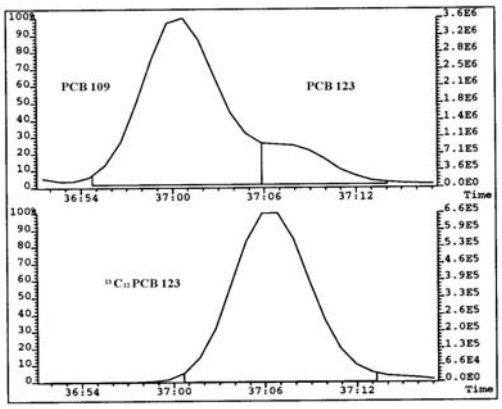

Figure 3. PCB 109 elution interference with PCB 123

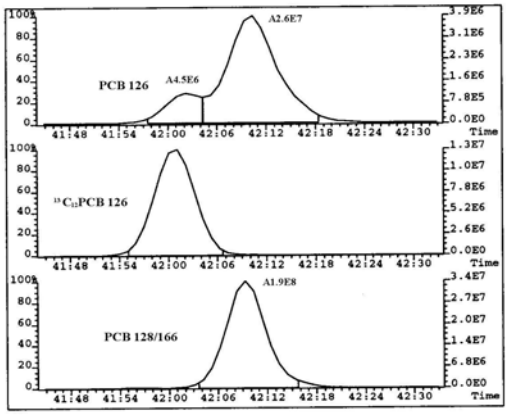

Figure 4. PCB 128/166 fragmentation interference with PCB 126

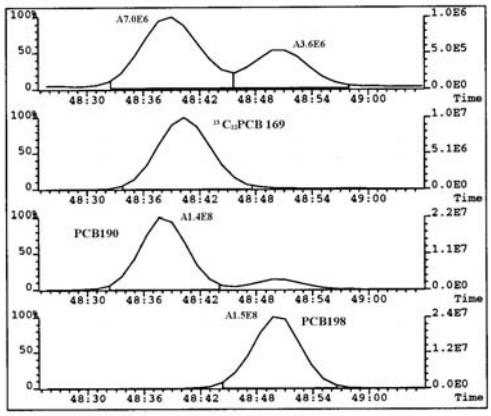

Figure 5. Masking of PCB 169 by PCB 190, 198 interferences

The effect of a fragment of PCB 110/115 (a pentachlorobiphenyl) on quantification of PCB 81 (a tetrachlorobiphenyl) is illustrated in Figure 2. A similar situation exists for quantification of PCB 126 due to interference from a fragment of a closely eluting hexachlorobiphenyl as shown in Figure 4. Figure 3 illustrates that on the SPB-octyl column PCB 123 may be difficult to quantify due to a closely eluting higher concentration congener (PCB 109) of the same degree of chlorination. The quantification of PCBs 81, 123 and 126 can, however, often be performed from the SPB-Octyl column analysis with good reliability depending on the relative concentrations of the interferences.

The interferences encompassing the retention time of PCB 169 prevent its quantification on SPB-Octyl. As shown in Figure 5 the signal from the fragments of heptachlorinated congeners 190, 198 overshadow the PCB 169 signal. Accurate quantification of PCB 169 requires analysis on a different GC column such as DB-1. Alternatively it can be reported from the SPB-Octyl as a non-detect with an elevated detection limit based on the signal of PCB 190, 198 fragments in the PCB 169 channel.

Analysis of the sample extracts using the DB-1 chromatographic column is performed to confirm the concentration of PCB 169. This column provides good resolution of PCB 169 from all other congeners. DB-1 may not be suitable for quantification of PCB 123 and 126. The co-eluting PCBs 107/109/124 affect PCB 123 on the DB-1 column in the same fashion as PCB 109 does on the SPB-Octyl column. Similarly, the dechlorination fragment of PCB 129 affects PCB 126 as the two congeners elute within one second of each other on the DB-1. PCB 81 may also be subject to interference from a fragment of PCBs 87/117/125.

Where interferences are present, definitive results for PCBs 81, 123, 126 can be obtained by analysis of the sample extract following carbon column isolation. This technique can be used to separate the 12 dioxin-like congeners from the remaining PCBs so that reliable quantification can be achieved.

The congener distributions in environmental and human tissue samples can vary considerably and the occurrences of the interferences are dependent on the particular sample congener profile. Recognition of the presence of the interfering congeners is critical for proper evaluation of data reliability and for making decisions on which combinations of SPB-Octyl, DB-1 or carbon fractionation are required.

In addition to the congener specific analysis, Homologue Total and Total PCB concentrations are determined from the individual PCB congener results. In this case the convention for reporting detection limit must be well defined. One approach is to use the highest detection limit achieved for any congener contributing to the Total and, where necessary, censor individual congener results to this detection limit.

Aroclor equivalent concentrations can be determined by multiplication of the summed concentrations of a suite of congeners characteristic of the particular Aroclor® formulation. Appropriate conversion factors are determined by congener analysis of the pure Aroclor® formulations.

Sample specific detection limits based on instrumental noise are in the range of 0.5 to 2 pg/sample. Since PCBs are ubiquitous, practical detection limits for some congeners are limited by the lab background input detectable in procedural blanks. Statistically calculated detection limits are in the range of 2 pg/sample for PCBs 77, 81, 114,123, 126,169, 10 pg/sample for PCBs 156, 157, 167, 189 and less than 50 pg/sample for all other PCB congeners.

The high sensitivity of the method is critical to reliable quantification of the dioxin-like PCBs, however, since within-sample congener concentrations can span several orders of magnitude additional analyses on diluted extracts may often be required to bring all congener responses within the calibration range. For even moderately contaminated samples some congener concentrations may routinely exceed the working concentration range of the method using standard sample sizes. In these cases analysis of smaller subsamples and/or an increase in the quantity of labeled surrogate spike with subsequent extract dilution are required to achieve isotope dilution quantification.

References:

(1) EPA Method 1668, Revision A: Chlorinated Biphenyl Congeners in Water, Soil, Sediment, and Tissue by HRGC/HRMS