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ANALYTICAL REFERENCE MATERIALS. PART VII.* DEVELOPMENT AND CERTIFICATION OF A SEDIMENT REFERENCE MATERIAL FOR POLYCHLORINATED BIPHENYLS ANALYSIS**

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ANALYTICAL REFERENCE MATERIALS. PART VII.*

DEVELOPMENT AND CERTIFICATION OF A SEDIMENT REFERENCE MATERIAL FOR TOTAL POLYCHLORINATED BIPHENYLS ANALYSIS**

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EXECUTIVE SUMMARY

Polychlorinated biphenyl (PCB) is one of the most frequently analyzed organic environmental pollutants. However, interlaboratory results indicated that analytical measurements for PCBs are not reliable. In order for the laboratory managers and supervisors to assess the quality of PCB data, the Quality Assurance and Methods Section has developed and certified a sediment certified reference material (CRM) for total PCB analysis. This material has already been successfully applied in several interlaboratory studies. It has also been used as in-house quality control samples or as check samples included in contracts by WQNL, WQB-OR, EPS-OR, DFO and other provincial and private laboratories.

Substances de référence pour les méthodes analytiques. Partie VII. Mise au point et homologation d'une substance de référence préparée à partir de sédiments pour l'analyse des biphényles polychlorés, H.B. Lee.

SOMMAIRE ADMINISTRATIF

Les biphényles polychlorés (BPC) sont l'un des polluants organiques les plus fréquemment analysés. Cependant, les résultats des études interlaboratoires ont démontré que les méthodes analytiques utilisées pour quantifier les BPC ne donnent pas des résultats fiables. Pour aider les directeurs et les chefs de laboratoire à évaluer la qualité des données qu'ils obtiennent pour les BPC, la Section assurance de la qualité et méthodes a mis au point et homologué une substance de référence à partir de sédiments naturels permettant de contrôler tous les aspects de l'analyse des BPC. Cette substance a déjà été utilisée dans plusieurs études interlaboratoires avec beaucoup de succès. Elle a servi à contrôler la qualité des données des laboratoires de la Division et elle a été utilisée à titre d'échantillons de vérification dans le cadre de contrats avec le Laboratoire national de la qualité de l'eau (LNQE), la Direction de la qualité des eaux (DQE) de la Région de l'Ontario, le Service de la protection de l'environnement (SPE), de la Région de l'Ontario, le ministère des Pêches et Océans ainsi que certains laboratoires du gouvernement provincial et des laboratoires privés.

ABSTRACT

A lake sediment reference material naturally contaminated with PCBs was prepared. Subsamples of this material were subjected to repetitive in-house analyses for total PCBs. Quantitative recovery of PCBs was demonstrated by performing ultrasonic and soxhlet extractions under various conditions. Cleanup of sample extracts was usually done with Florisil and the cleaned extracts were shown to be free from other major interferences, except for sulfur which was later removed by mercury. The presence of PCBs in the samples was confirmed by perchlorination of the sample extract as well as by GC-MS techniques. Most of the sample extracts were quantitated against a 1:1:1 mixture of Aroclors 1242, 1254 and 1260 on a 3% OV-1 packed column using the technique described by Webb and McCall. These results were further confirmed by quantitative GC-MS analyses of EC-1 and by inter-laboratory results provided by independent laboratories. This material shows no signs of degradation for its PCB contents over a six-year storage period at -20°C in the dark.

Key Words: PCBs, polychlorinated biphenyls, certified reference materials, quality assurance, sediment samples.

SOMMAIRE

On a préparé une substance de référence (sédiments de lac) contaminée en milieu naturel par des BPC. On a soumis des sous-échantillons de cette substance à une série d'analyses répétitives en laboratoire pour mesurer la teneur totale en BPC. On a démontré qu'il est possible d'extraire les BPC de cette substance en procédant à une extraction aux ultrasons ou au soxhlet dans diverses conditions. Les extraits ont habituellement été nettoyés avec du Florisil et se sont révélés exempts d'autres contaminants importants sauf pour le soufre qui a été éliminé à l'aide de mercure. La présence des BPC dans l'échantillon a été confirmée par la perchloration de l'extrait et aussi par la chromatographie en phase gazeuse couplée à la spectrométrie de masse. La plupart des extraits ont été quantifiés en présence d'un mélange à part égale (1:1:1) d'Arochlores 1242, 1254 et 1260 en les injectant dans une colonne remplie d'OV-1 à 3 p. 100 selon la technique décrite par Webb et McCall. Les résultats obtenus ont ensuite été confirmés par des analyses quantitatives au chromatographe en phase gazeuse et au spectromètre de masse de la substance de référence EC-1 ainsi que par les résultats interlaboratoires fournis par des laboratoires indépendants. La substance de référence ne semble avoir subi aucune dégradation quant à sa teneur en BPC après avoir été entreposée dans l'obscurité pendant six ans à -20 °C.

Mots clé : BPC, biphényles polychlorés, substance de référence homologuée, assurance de la qualité, échantillons de sédiments

INTRODUCTION

Aroclors or mixtures of polychlorinated biphenyls (PCBs) with various percentages of chlorine by weight were manufactured in the United States by Monsanto Chemical Company. Because of their general inertness, PCBs were widely used as transformer dielectric fluids, plasticizers and flame retardants, etc. in the industry. Although the production of Aroclors has been curtailed in recent years because of government regulations, disposal, dump leaching, or accidental spillage of the millions of pounds of these persistent Aroclors manufactured in the preceding years still create environmental pollution of PCBs in air, water and biota samples.

Aroclors are classified as priority pollutants by U.S. Environmental Protection Agency and environmental monitoring of such compounds was begun in the nineteen sixties because of their toxicity and persistence. Although PCB analysis is considered routine, the analytical results in many naturally-contaminated samples are often unsatisfactory as indicated by many interlaboratory round-robin studies organized by our section (1, 2) as well as by other parties (3, 4). Typically, interlaboratory relative standard deviation of PCB results in naturally contaminated sediment samples at low μg/g levels was between 25-50%. Hence, there is a need to develop a real-life reference material to monitor the performance of laboratories involved in PCB analysis.

Several years ago, our section initiated the research and development of a number of sediment certified reference materials (CRMs) for toxic organics such as PCBs (5), chlorobenzenes (6), and polynuclear aromatic hydrocarbons (7) to fulfill the requirements of our on-going quality assurance programs. Two marine sediment CRMs were recently available commercially, however, no details were given on how these CRMs were characterized. In this paper, we wish to report on our rigorous approach to analyze and certify the total PCB contents in our sediment CRM coded EC-1.

EXPERIMENTAL

Prepartion of Sediment Reference Matieral

Detailed procedures for sample collection, preliminary drying, freeze-drying, blending, bottling and homogeneity testing before and after subsampling have been described in previous publications (5, 6).

Extraction and Cleanup Sediment Samples

The soxhlet and ultrasonic extractions of PCBs in sediment samples and the partitioning and evaporation steps were identical to those employed for chlorobenzene analysis (6). Cleanup of sediment extracts was done in a 500 x 19 mm i.d. glass column filled with 20.0

g of activated Florisil and 10 mm of anhydrous sodium sulfate at the top. The PCBs in the extract were eluted by 200 mL of hexane. After addition of 3 mL of isooctane and a few boiling chips, this hexane fraction was evaporated down to ca. 3 to 5 mL using a three-stage macro Snyder column and a heating mantle. The concentrated extract was made up to 10.0 mL with isooctane. Sulfur and sulfur compounds in the cleaned-up extract were removed by vigorous agitation with mercury until the metal remained shiny.

GC-ECD Analysis

A Hewlett-Packard 5700 series gas chromatograph equipped with a Ni-63 electron-capture detector, model 7671A autosampler and a model 3390 reporting integrator was used. The analytical column was a 1.8 m x 2 mm i.d. glass column packed with 3% OV-1 on 100-120 mesh Gas Chrom Q. The temperatures were: injection port 250°C, detector 300°C, column 185°C. Flow rate 25 mL/min, carrier gas argon/methane (95+5). Aroclors 1242, 1254 and 1260 were supplied by U.S. Environmental Protection Agency. The working standard was a mixture of 1:1:1 Aroclors 1242, 1254 and 1260 in isooctane with a total concentration of 600 pg/μL. Quantitation of PCBs was done by the peak matching technique described by Webb and McCall (8).

GC-MSD Analysis

A Hewlett-Packard 5880A gas chromatograph equipped with a split/splitless injection port, Level II terminal, model 7671A autosampler, model 5970B mass selective detector (MSD), model 9816S computer, and model 9133XV 15 megabytes disc drive together with a 30 mm x 0.25 mm i.d. DB-5 fused silica capillary column which was directly interfaced with the electron impact ion source for maximum sensitivity. The GC conditions were: injection port 275°C, interface 280°C, column initial temperature 70°C (held for 1.5 min), programming rate 30°C/min (70°C to 170°C), 2.5°C/min (170°C to 260°C), oven temperature held at 260°C for 15 min. Splitless valve on for 1.5 min. Column head pressure was 4 psi. Fully automated sample injection, data acquisition, data editing and report generation was made possible with the existing "Sequencing" software and a keystroke program on the GC terminal. The detector was operated in the selected ion monitoring (SIM) mode for both confirmation and quantitation. In both cases, two characteristic ions (one for quantitation and the other for confirmation) were monitored for each PCB homologous series (Table 1). For quantitative work, the procedure described by Budde *et al.* (9, 10) was used except that the 2,2',3,4,4',5,6-heptachlorobiphenyl (congener 181) instead of 2,2',3,4',5,6,6'-heptachlorobiphenyl (congener 188) was used because of availability. Chrysene-d_{12} was used as an internal standard and the dwell time for each ion was 100 m sec.

Sediment extracts were quantitated against an isooctane mixture of nine congeners of various concentrations (Table 1). At each level of chlorination, one PCB congener in the calibration mixture was used as the concentration standard for all isomers in that group, e.g. congener 29 was used to quantitate all trichlorobiphenyls in the sample. The only exception was that decachlorobiphenyl was used as the concentration calibration standard for both nona- and deca-chlorobiphenyls in the sample. Total PCB concentration was obtained by summation of all concentrations in each homologous series. All PCB congeners were obtained from Ultra Scientific Co., Hope, RI, USA. Chrysene-d_{12} was available from Aldrich Chemical Co., Milwaukee, MI, USA.

RESULTS AND DISCUSSION

Efforts were made to ensure that the PCBs in EC-1 were quantitatively recovered. The ultrasonic and soxhlet extraction techniques which are routinely used for the extraction of organics in sediments were employed and compared. In order to have valid comparison between the extraction methods, identical cleanup (Florisil column) and quantitation (packed column Webb-McCall) procedures (see later discussions) were used in these samples.

A total of 97 PCB analyses were done on EC-1 by the ultrasonic extraction technique using a 1:1 mixture of acetone and hexane. A total of 72 analyses were also done on the same certified reference

reference material by soxhlet extraction using the 59/41 acetone/hexane mixture. All of these results were summarized in Table 2. It is obvious from these data that nearly identical results were obtained from both techniques. Since the presence of moisture has been reported to provide better recoveries of some organochlorines in soil samples (11), EC-1 samples with 0 or 30% moisture content were soxhlet extracted simultaneously for comparison. A t-test was applied to the means of these results and at 95% level of significance, no difference was found in these means indicating that a moisture content of 0 or 30% in the sediment samples had no effect on the recovery of PCBs. Another t-test was applied to sample results obtained by ultrasonic extraction under similar conditions and again no difference in PCB recovery was observed.

Since similar PCB results were obtained from samples soxhlet extracted for 3, 7, 20 and 72 hours, it implied that PCBs were exhaustively removed from this sediment after three hours of soxhlet extraction. Different solvents were also employed to see if the recovery of PCB was solvent dependent. Results indicated that both acetone and methylene chloride produced similar recoveries of PCBs as the 59/41 acetone/hexane mixture, while non-polar solvents such as hexane and benzene gave slightly lower (~ 90%) recovery.

Florisil has been used by many workers (12) to remove coextractives in sediment samples for organochlorines and PCB analyses. Although some workers have preferred to use Florisil

deactivated with a few percents of water for column cleanup, activated Florisil was used in this study because it gave better separation of some organochlorines and polynuclear aromatic hydrocarbons from PCBs.

In order to ensure the Florisil cleanup was effective, some of the samples were subjected to additional cleanup steps. In triplicate runs, the PCB fraction of the Florisil cleaned EC-1 extract was further cleaned up on a Celite and 3% deactivated silica gel column (13) and, in another case, on an activated neutral alumina column (14), according to the published methods. In both cases, no change in PCB profile and quantity could be observed in the EC-1 extracts after additional cleanup. The Florisil cleaned EC-1 extract was also subjected to ethanolic KOH treatment at 80-90°C for 30 minutes. Again, no change in the PCB components could be observed before and after the additional cleanup. These experiments indicated that Florisil cleaned EC-1 extracts were free from any major interference by other organochlorines. It should be noted that a few chlorobenzenes, p,p'-DDE and mirex present in EC-1 could not be separated from PCBs by column chromatography. Their presence, however, would not affect PCB results since their levels were relatively low as compared to PCBs.

Most of the EC-1 extracts were chromatographed on a 6-foot 3% OV-1 column operated at 185°C. The PCBs were quantitated by the established peak-matching technique developed by Webb and McCall (8). This method was used since it has been demonstrated to be better than other packed column techniques in a collaborative study (15). It is

still the official method for quantitation of total PCBs in our Water Quality laboratories and is also approved by US EPA (Method 608). Samples were quantitated against a 1:1:1 mixture of Aroclors 1242, 1254 and 1260 since preliminary runs of sample extracts indicated that the PCB components in EC-1 were very similar to the above mixture (see Fig. 1).

Extracts of EC-1 were subjected to the perchlorination procedure as described by Armour (16) using antimony pentachloride. The formation of decachlorobiphenyl in these reactions confirmed the presence of PCBs in EC-1 extracts. The perchlorination results were not used quantitatively to estimate the PCB concentration in EC-1 since the latter was a complicated mixture of several Aroclors. A concentrated extract of EC-1 containing approximately 3.0 μg/mL of total PCBs) was analyzed by a 30 m DB-5 column interfaced to a mass selective detector (MSD). Data were acquired in the selected ion monitoring (SIM) mode for the detection of the ten chlorobiphenyl homologous series, i.e. from mono- to deca- chlorobiphenyl. Two characteristic ions were used for each homologous series: one for quantitation and the other for confirmation, as shown in Table 1. Although the mono-, di-, nona- and deca- chlorobiphenyls were not present in EC-1 in detectable quantities, the presence of tri-, tetra-, penta-, hexa-, hepta- and octa- chlorobiphenyls in this reference material was confirmed by the presence of both characteristic ions at the right retention times and in the expected ratios for each of the six homologous series listed above.

EC-1 extracts were also quantitatively analyzed by GC-MSD for PCBs using a method described by Budde et al. (9, 10). Since PCB homologs have overlapping retention time windows, special precautions were taken to avoid interferences by PCB congeners containing more chlorines. Under electron ionization mode, a PCB molecule undergoes fragmentation by loss of two chlorines and to a lesser extent by loss of HCl and Cl (17, 18), thus causing interference to PCBs with one or two less chlorine atoms. In this work, the level of chlorination in each PCB peak was previously determined by a full scan run of a concentrated Aroclor mixture. The level of chlorination in a sample PCB peak was first assigned by the observed relative abundance of the two corresponding characteristic ions. This together with the information obtained in the full scan run was generally sufficient to eliminate interference generated by fragmentation ions produced by coeluting PCBs with more chlorines.

Results of the quantitative GC-MSD analysis of PCBs in EC-1 were shown in Table 3. In this case, concentration of each PCB homologous series as well as the total PCB concentration were obtained. As indicated, the GC-MS results further confirmed the GC-ECD results since the total PCB concentrations obtained by these two different quantitative methods (Tables 2 and 3) were less than 10% different from each other. The overall lower sensitivity of the MSD to most PCBs, especially the hepta- and higher chlorobiphenyls, rendered some PCB peaks at low concentrations undetected by this detector. This

could be the reason why, in the case of EC-1, the total PCB results obtained by mass selective detection were slightly lower than those by electron-capture detection.

Reference material EC-1 was used in two interlaboratory round-robin studies in two different years. In both cases, the participants were requested to analyze the material for total PCBs by using their own in-house methods and standards. The interlaboratory results (Table 4) were quite diversified because of the different extraction and cleanup methodologies as well as the calibration standards and quantitation techniques employed by various participants (1, 2). Despite all these variations, the interlaboratory medians and means of the PCB results in both studies were in excellent agreement with the in-house results as summarized in Tables 2 and 4.

The stability of PCBs in EC-1 under cold storage conditions (-20°C in the dark) was monitored twice annually. Analytical results indicated no evidence of degradation during storage over the last six years (19).

In conclusion, we have successfully prepared and certified a naturally-contaminated lake sediment reference material (EC-1) for total PCB contents on the basis of 169 in-house analyses. The reference value, 2.00 ± 0.05 μg/g (uncertainty is one standard deviation) generated by combining all GC-ECD results (Table 2) together, was further supported by GC-MS results as well as inter-laboratory results in two round-robin studies. This certified

reference material is currently being used in many of our intra-laboratory and interlaboratory quality assurance programs for PCB analysis.

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Table 1 **Ions, congeners and concentrations of calibration standards used in the capillary column GC-MSC analysis of EC-1.**

	m/z		Calibration Congener	
Homolog	Quant ion	Confirm ion	Congener no.[a]	Concentration (pg/μL)
Cl_1	188	190	1	50
Cl_2	222	224	5	50
Cl_3	256	258	29	50
Cl_4	292	290	50	100
Cl_5	326	328	87	100
Cl_6	360	362	154	100
Cl_7	394	396	181	150
Cl_8	430	432	200	150
Cl_9	464	466	209	250
Cl_{10}	498	500	209	250
chrysene-d_{12}	240	-	-	250

[a]Adopted from Ballschmiter, K. and Zell, M., Fresenius, Z. Anal. Chem., 1980, 302, 20.

Table 2 **Summary of analytical results (μg/g total PCBs) on EC-1 sediment reference material by packed column Webb-McCall quantitation method.**

Extraction	Ultrasonic	Soxhlet
No. of analyses	97	72
Range (μg/g)	1.85 - 2.15	1.88 - 2.17
Mean ± SD	2.02 ± 0.07	1.97 ± 0.08

Table 3 Composition of PCBs in EC-1 as determined by quantiative GC-MS analyses (six replicates).

Homolog	EC-1 Concentration ng/g (wt. %)
Cl_1	ND
Cl_2	ND
Cl_3	184 (9.9)
Cl_4	453 (24.3)
Cl_5	688 (36.9)
Cl_6	280 (15.0)
Cl_7	161 (8.6)
Cl_8	98 (5.3)
Cl_9	ND
Cl_{10}	ND
Total Concentration	1864

ND = none detected

Table 4 Summary of interlaboratory results for total PCBs in EC-1.

Study Number	N-27	DQC-3
No. of laboratories	15	14
No. of results uses[a]	25	12
Range of results (μg/g)	0.96-3.41	1.11-3.26
Median (μg/g)	1.96	1.75
Mean ± S.D. (μg/g)	2.05±0.61	1.98±0.69

[a]After rejection of outliers.