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Interlaboratory Study on Quantitative Methods of Analysis of C_{10}−C_{13} Polychloro-*n*-alkanes

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Seven laboratories participated in an international interlaboratory comparison exercise to compare the quantitative methods used for measuring C_{10}−C_{13} polychloro-*n*-alkanes (PCAs). Participants were supplied with two solutions (PCA-1, PCA-70) containing PCAs of "known" but unstated concentrations, and two real world samples (fish extracts FE1 and FE2) each consisting of a cleaned up extract (lipid-free) of a fish tissue known to contain PCAs. A well-characterized commercial formulation, PCA-60, of stated concentration was also supplied and was used as the external standard for the exercise. Participants having other commercially available C_{10}−C_{13} PCA mixtures were encouraged to use them as external standards for the study, and the choice of the quantitative method employed was left to participants, though all were based on high-resolution gas chromatography with detection by electron capture negative ion mass spectrometry (plus electron capture detection in one case). The results of the study met with mixed success. For measurements on the PCA-1 sample, whose composition and gas chromatographic profile were quite different from the PCA-60 sample, the determined concentration was 99.3 ± 19.5 ng/μL (mean ± the standard deviation of the laboratory means); the *true* concentration of this mixture was 74 ng/μL. For the PCA-70 sample, which has a composition and gas chromatographic profile similar to that of PCA-60, the result was 297 ± 132 ng/μL, compared to the true concentration of 118 ng/μL. It is still unclear why the larger discrepancy arises for the latter sample; this observation implies that different commercial formulations used as standards would provide quite different estimates of PCA concentrations. The interlaboratory precision for measurements on the FE1 sample (coefficient of variation (CV) of 27%) was better than that for the FE2 sample (CV of 47%). An explanation for the larger variation is that some of the quantitative procedures used in measuring PCA levels in the FE2 sample did not take into account the effects of coeluting interferences, which are observed at nominal mass spectral resolution, thus making some of the values too high.

Polychlorinated *n*-alkanes (PCAs) are a class of industrially prepared mixtures of the general formula $C_nH_{2n+2-z}Cl_z$ having carbon chain lengths from C_{10} to C_{30} and chlorine content from 30 to 70% by mass.[1−3] Also known industrially as chlorinated paraffins (CPs), they are formed by direct chlorination of *n*-alkane feedstocks with molecular chlorine under forcing conditions. These reactions, which have low positional selectivity,[4−6] yield complex formulations consisting of mixtures of optical isomers and congeners.[7] Based on the principal *n*-alkane feedstocks, which are derived from petroleum fractions, commercial PCA mixtures fall into three different categories: C_{10}−C_{13} (short), C_{14}−C_{17} (medium), and C_{20}−C_{30} (long). These mixtures are further subcategorized into their weight content of chlorine: 40−50%, 50−60%, and 60−70%.[2]

PCAs are generally used where the demand for chemical stability is high;[8] their more common applications include use as high-temperature lubricants in metal-working machinery and as flame retardant plasticizers, while their more limited applications include use as adhesives, paints, rubber, and sealants.[3]

In the United States, C_{10}−C_{13} PCAs have been placed on the Environmental Protection Agency (EPA) Toxic Release Inventory (TRI) and in Canada are classified as Priority Toxic Substances under the Canadian Environmental Protection Act (CEPA). Presently, PCAs represent the largest group of chlorinated hydrocarbons produced in Western Europe and in North America.[9] Global consumption estimates for 1993 were reported to be 300 kt/yr.[2]

Much attention has been given to the C_{10}−C_{13} PCAs, which have the greatest potential for environmental release,[2] exhibit the highest toxicity of PCA products, and because of their environ-

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(1) Serrone, D. M.; Birtley, R. D. N.; Weigand, W.; Millischer, R. *Food Chem. Toxicol.* **1987**, *25*, 553−562.
(2) Environment Canada, Priority Substances Program, CEPA Assessment Report, Chlorinated Paraffins. Commercial Chemicals Branch, Hull, Quebec, 1993.
(3) Zitko, V. In *Handbook of Environmental Chemistry*; Springer-Verlag: New York, 1980; Vol. 3A, pp 149−156.
(4) Colebourne, N.; Stern, E. S. *J. Chem. Soc.* **1965**, 3599−3605.
(5) Fredricks, P. S.; Tedder, J. M. *J. Chem. Soc.* **1960**, 144−150.
(6) Chambers, G.; Ubbelohde, A. R. *J. Chem. Soc.* **1955**, 285−295.
(7) Mukherjee, A. B. *The Use of Chlorinated Paraffins and their Possible Effects in the Environment*; National Board of Waters and the Environment: Helsinki, Finland, 1990; Series A 66.
(8) Svanberg, O.; Linden, E. *Ambio* **1979**, *8*, 206−209.
(9) Swedish National Chemicals Inspectorate. KEMI Report No.1, 1991.

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mental mobility could have adverse effects on terrestrial and aquatic organisms and on humans.[10]

To date, there has been limited information on the levels and fate of PCAs in the environment. This arises both from the difficulty associated with quantifying PCAs because of the complexity inherent to commercial formulations and from the limited knowledge of their physical−chemical properties.[10] These mixtures, which may contain thousands of positional isomers,[11] generally elute by gas or liquid chromatography over a wide retention time range,[10] and components are not resolved to baseline, even with high-resolution columns.[12] To further complicate their analyses, these compounds can undergo selective environmental and metabolic transformations resulting in analyte elution signals that differ from commercial formulations, which are used as external standards.[10] In addition, commercial mixtures are known to contain additives; this makes quantitative measurements tenuous at best.[10]

In an attempt to increase our understanding of problems in the analysis of PCAs, an international interlaboratory study has been conducted to compare existing and newer methods for their quantitative analysis. Because there are no certified or standard reference materials containing PCAs of known content for use as check samples, comparing the results from this exercise should be treated with some caution. Despite these limitations, an attempt was still made to assess the interlaboratory precision and *accuracy* of the analytical methodologies used for quantifying these compounds.

EXPERIMENTAL SECTION

Materials. Two short-chain (C_{10}−C_{13}) commercial PCA products, one of 60% chlorine by mass (PCA-60) and the other of 70% chlorine by mass (PCA-70) were graciously provided by the manufacturers (Dover Chemical Corp., Dover, OH, and Occidental Chemical Corp., Niagara Falls, NY, respectively). 1,5,9-Decatriene and aldrin were purchased from Aldrich Chemical Corp., Oakville, ON, Canada.

Sample Preparation for Interlaboratory Study. Seven laboratories, including our own, participated in the study. Each laboratory received a total of five solutions that were prepared at the Freshwater Institute (FWI). Four of the five solutions were stored in vials sealed with Teflon-lined caps and containing glass inserts inscribed with a volume marker and were weighed at FWI prior to shipping. Participants were asked to check the weights of each vial and also the volume marker to ensure that no volume losses occurred during transportation. The fifth solution was prepared and stored in a sealed glass ampule.

The primary standard solution used in this study (vial A) was prepared in isooctane by carefully weighing a known amount of the PCA-60 commercial mixture to give a final solution concentration of 88 ng/μL. Participants were encouraged to use other commercially available C_{10}−C_{13} PCA mixtures as external standards but to at least use the supplied standard for the quantitative measurements. The general appearance of the high-resolution gas chromatogram of the PCA-60 mixture has been previously reported.[11]

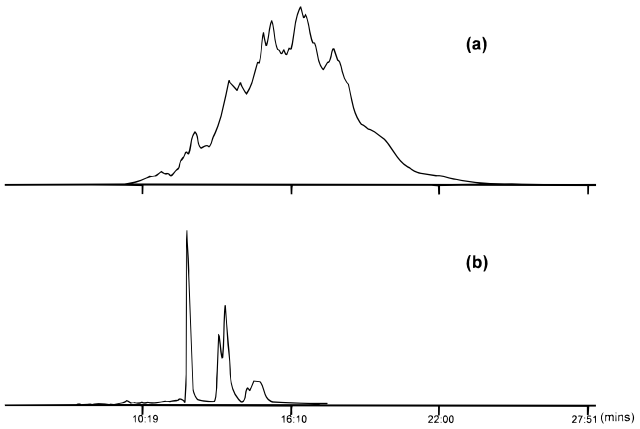

Figure 1. HRGC/ECNI-MS total ion chromatogram (*m*/*z* 65−600) of (a) PCA-70 (C_{10}−C_{13}, 70% Cl by mass) and (b) PCA-1 at an ion source temperature of 120 °C, with argon as the moderating gas, and 1 s/decade scan rate.

A second solution (vial B), containing 1 mL of an unstated amount of the PCA-70 commercial mixture, was prepared by carefully weighing a known amount of the mixture into isooctane. Participants were asked to quantify this solution versus the primary standard solution (vial A) or by using other commercially available C_{10}−C_{13} PCA mixtures. The high-resolution gas chromatography/electron capture negative ion mass spectrometry (HRGC/ECNI-MS) total ion chromatogram (TIC) of the PCA-70 mixture, determined at FWI, is shown in Figure 1a. Its general appearance is typical of chromatograms reported previously.[12−16]

The third solution (vial C), PCA-1, was prepared by purifying the products derived from the chlorination of 1,5,9-decatriene, as previously described.[11] Each vial was filled with 1 mL of this solution. Participants were asked to quantify this solution versus the primary standard solution (vial A) or versus other commercially available C_{10}−C_{13} PCA mixtures. The HRGC/ECNI-MS TIC of the PCA-1 mixture, determined at FWI, is shown in Figure 1b.

A lipid-free extracted fish tissue sample, FE1, known to contain PCAs, but of unstated concentration, was supplied in vial D. The fish used in this study was a yellow perch (~300 g) that had been netted at the mouth of the Detroit River at Lake Erie in August 1995. The extraction and isolation of PCAs from fish tissue also has been described.[11] Nonane was added to the extract to a final volume of 550 μL. Vials containing volume indicators and 50 μL of this solution, each corresponding to the extract from 2.7 g of tissue, were sealed with Teflon-lined caps. The formula group

(10) Tomy, G. T.; Fisk, A. T.; Westmore, J. B.; Muir, D. C. G. *Rev. Environ. Contam. Toxicol.* **1998**, 158, 53−128.
(11) Tomy, G. T.; Stern, G. A.; Muir, D. C. G.; Fisk, A. T.; Cymbalisty, C. D.; Westmore, J. B. *Anal. Chem.* **1997**, *69*, 2762−2771.
(12) Tomy, G. T.; Muir, D. C. G.; Westmore, J. B.; Stern, G. A. Proceedings of the 41st Annual Conference on Mass Spectrometry and Allied Topics, San Francisco, CA, May 30−June 4, 1993.
(13) Muller, M. D.; Schmid, P. P. *J. High Resolut. Chromatogr. Chromatogr. Commun.* **1984**, *7*, 33−37.
(14) Schmid, P. P.; Muller, M. D. *J. Assoc. Off. Anal. Chem.* **1985**, 68, 427−430.
(15) Murray, T. M.; Frankenberry, D. H.; Steele, D. H.; Heath, R. G. Chlorinated Paraffins: A report on the findings from two field studies, Sugar creek, Ohio and Tinkers Creek, Ohio, Vol. 1, Technical Report, U. S. Environmental Protection Agency, EPA/560/5−87/012, 1988.
(16) Metcalfe-Smith, J. L.; Maguire, R. J.; Batchelor, S. P.; Bennie, D. T. *Occurrence of Chlorinated Paraffins in the St. Lawrence River near a Manufacturing Plant in Cornwall, Ontario*; Aquatic Ecosystem Protection Branch, National Water Research Institute, Department of the Environ.: Burlington, ON, Canada. 1995.

Table 1. GC Parameters Used by Participating Laboratories

lab no.	column phase	column dimension (m × mm)	injection type	injection port temp (°C)	temperature program	injection mode	GC/MS interface temp (°C)	carrier gas
1	DB-5	30 × 0.25	splitless	220	50 °C; hold 1 min, 150 °C at 70 °C/min; hold 1 min, 260 °C at 7 °C/min; hold 8:18 min, 280 °C/min at 10 °C/min; hold 13 min	manual	250	He
2	CP−Sil8	50 × 0.21	splitless	250	90 °C; hold 3 min, 180 °C at 30 °C/min; hold 12 min, 280 °C at 5 °C/min; hold 40 min	autoinjector	280	He
3	(a) DB-5MS	24 × 0.32	on-column	80	80 °C; hold 3 min, 280 °C at 7 °C/min; hold 10 min	manual	280	He
3	(b) HP-5MS	20 × 0.25	on-column	80	80 °C; hold 3 min, 170 °C at 20 °C/min; hold 0 min, 280 °C at 7 °C/min; hold 15 min	manual	na[a]	H_2
4	DB-5MS	30 × 0.25	splitless	250	40 °C; hold 1 min, 150 °C at 25 °C/min; hold 1 min, 215 °C at 7 °C/min; hold 1 min, 280 °C at 5 °C; hold 13 min	manual	300	He
5	DB-5MS	30 × 0.25	on-column	60	60 °C; hold 2 min, 280 °C at 10 °C/min; hold 15 min	autoinjector	250	He
6	HP-1	25 × 0.20	splitless	260	90 °C; hold 1 min, 150 °C at 25 °C/min; hold 0 min, 300 °C at 15 °C/min; hold 5 min	autoinjector	260	He
7	DB-5MS	30 × 0.25	splitless	220	150 °C; hold 1 min, 260 °C at 7 °C/min; hold 8:18 min, 280 °C at 10 °C/min; hold 13 min	autoinjector	280	He

[a] na, not applicable.

Table 2. Mass Spectrometer Parameters Used for ECNI by Participating Laboratories

lab no.	MS make and model	MS type	electron energy (eV)	accelerating voltage (kV)	resolving power	source temp (°C)	pressure (Torr)	reagent gas
1	FM-8200	BE sector	120	3	1000	200−210	2.1×10^{-4} [a]	CH_4
2	HP-5988A	quadrupole	200	na[a]	1000	100	1[c]	CH_4
3	VG-Tritech	EBE sector	50	4	1000	100	9.7×10^{-5} [a]	CH_4
4	HP-5989A	quadrupole	300	na	1000	125	0.43[c]	CH_4
5	HP-5989A	quadrupole	230	na	1000	125	1.4[c]	CH_4
6	VG-Autospec	EBE sector	33	8	11−12000	∼160	1.5×10^{-5} [a]	CH_4
7	Kratos-Concept	EBE sector	∼180	5.3	∼12000	120	2×10^{-4} [a]	Ar

[a] Ion manifold pressure. [b] na, not applicable. [c] "Actual" pressure of ion source.

abundance profile (i.e., the relative abundance of each $C_nH_{2n+2-z}Cl_z$ formula) of this extract has been reported previously.[11]

The fifth solution (vial E), shipped in a sealed glass ampule, contained 100 μL of the same extract in nonane (corresponding to extract from 3.2 g of tissue), but aldrin (5 ng/μL) was also added, to be used as the volume corrector. This solution was supplied later to the participants to replace vial Ds shipped earlier that had been damaged in transit. Participants were asked to quantify at least one of the extracts (vial D or E) against the primary standard solution (vial A) or by using other commercially available C_{10}−C_{13} PCA mixtures.

GC Parameters. Because of the broad elution profile, unresolved even on high-resolution GC columns, characteristic of these mixtures,[10] the type of GC column and conditions used for chromatography were not anticipated to have profound effects on the final results, and thus, the choice of GC conditions employed was left to the participants. The GC parameters chosen by the various laboratories are shown in Table 1. All the columns used were nonpolar, with dimethyl- or phenylmethylpolysiloxane stationary phases. Two different columns were used by laboratory 3, one for mass spectral detection, the other for electron capture detection. Two laboratories used on-column injection while the others used the splitless mode of injection; three laboratories chose to perform their injections manually and the others used an automated injector. Solutions of analyte and standard were injected separately; each solution contained the same internal standard so that the mass spectral responses could be corrected for changes in volumes injected.

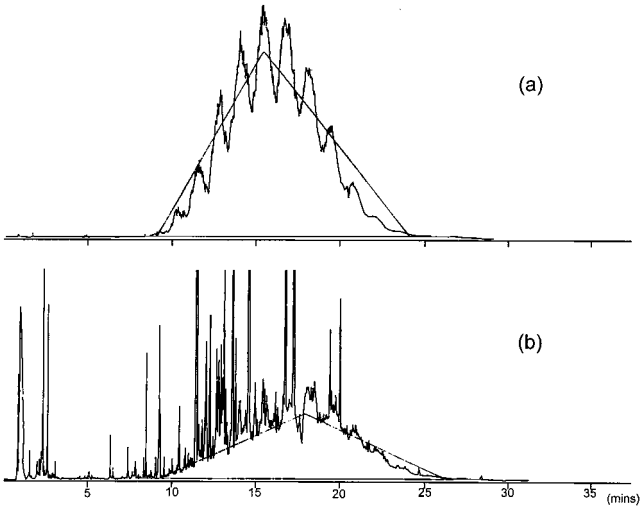

Figure 2. GC-ECD chromatograms of (a) PCA-60 and (b) FE2 samples showing the triangles used in calculating the respective areas.

Mass Spectrometer Parameters. The conditions used are shown in Table 2. Instrument types included three quadrupole systems (laboratories 2, 4, and 5) and four magnetic sector instruments (laboratories 1, 3, 6, and 7). Two laboratories (6 and 7) performed the analyses at high resolving power (RP > 10 000) while others used the MS operating at nominal RP. All laboratories performed the analyses under ECNI conditions. Six laboratories

Table 3. Methods Used by the Participating Laboratories

lab no.	method	GC volume/ MS sensitivity calibrant[a]
1	For all PCA mixtures, formula group abundance profiles were generated by SIM of the two most intense MS peaks of the $[M - Cl]^-$ isotopic group for all the formula groups described by Tomy et al.,[11] in eight retention time windows. For quantitation, similar correction factors[11] were applied to these profiles. No corrections to the integrated signals of the FE samples were made to allow for coeluting interferences that can arise at low RP.[11] Concentrations of individual PCA homologue groups were reported and single-point calibration was used to relate the response of the standard to those of the analyte solutions.	hexachlorobenzene
2	Analysis was by SIM of the two most intense peaks of the $[M - Cl]^-$ isotopic group of each molecular formula eluted. The integrated value of each signal was corrected when necessary by subtracting from it the signal arising from the ion having one fewer chlorine and two more carbon atoms (see text). The signal of interest was multiplied by the factor necessary to obtain the total signal of all peaks in the ion type. Quantitation was achieved by comparing the *doubly* adjusted areas of the external PCA-60 standard to those of the analytes (all except the PCA-1 analyte, for which the first correction was unnecessary) determined in the same manner. Because of the large number of ions, all solutions except that of PCA-1 had to be injected four times, i.e., one injection for each homologue group. For the FE samples, the responses of coeluting interferences were subtracted from the total area; no information was given on how interferences were identified. Single-point calibration was used to relate the response of the standard to those of the analytes.	tetrachloronaphthalene
3	(a) MS method: Analysis was by SIM of the two most intense peaks in the isotopic group of the $[M - Cl]^-$ ion of four abundant formula groups in the PCA-60 external standard, viz. $C_{11}H_{16}Cl_6$, $C_{11}H_{15}Cl_7$, $C_{11}H_{14}Cl_8$, and $C_{12}H_{19}Cl_7$; quantitation was achieved by comparing the integrated areas of these ion signals in the standard to those in the analyte, treated in the same manner. No corrections to the total integrated area of the FE sample signals were made to correct for coelution of other organochlorines. Multipoint calibration was used in relating the responses to those of the PCA-60 standard.	none
3	(b) ECD method: The areas of the signals for the standard and the analytes were determined by(i) a *triangulation method*, i.e., constructing a triangle by drawing its base from the start of the PCA elution to its end with its apex at the maximum signal,similar to that described by Walter and Ballschmiter[18] and illustrated in parts a and b of Figure 2 for the PCA-60 and FE2 samples, respectively, and (ii) electronic integration of the broad unresolved PCA signal. In Figure 2b, the areas of sharp GC peaks (assigned to organochlorine interferences) superimposed on the broad PCA signal were ignored.	na[b]
4	Analysis was by SIM of the same m/z values of the $[M - Cl]^-$ ions recommended by Tomy et al.[11] The signals of each MS peak were adjusted by multiplying the integrated area of the specific m/z peak by the factor necessary to give the total response for the ion type. Quantitation was achieved by comparing these adjusted values for the external PCA-60 standard to those of the analytes, determined in the same manner. Because of the large number of ions, all solutions except that of PCA-1 had to be injected twice. The efficiency of the method was improved by dividing the SIM procedure into 6−10 retention time windows. No corrections to the total integrated signals of the FE sample were made to correct for the possible presence of other organochlorines.	none
5	By scanning from 550 to 300 u, the responses of PCA-60 and another commercial formulation, Cereclor-S52 (C_{10}−C_{13}, 52% Cl by mass), were determined by integrating the area of the broad PCA signal (see Figure 2). The sharp well-resolved peaks present in the FE samples, determined not to be PCAs by examining their ECNI mass spectra, were not included in the integration. The corrected response of the analyte was then compared to the responses of both external PCA-60 and Cereclor-S52 standards determined from separate injections. Individual PCA homologue groups were not distinguished and so total PCA levels were reported. Single-point calibration was used to relate the responses of the analytes to the standards.	none
6	Formula group abundance profiles, based on the described SIM protocol[11] (i.e., by monitoring the two most intense peaks of $[M - Cl]^-$ ions), were generated. Three injections for each of the solutions had to be made owing to software limitations (generation of SIM windows was not possible for the large number of ions monitored) except for the PCA-1 sample, which has fewer ions. For quantitation, the responses of the most abundant components in the PCA-60 standard and the analyte were measured. Three GC/MS calibrants were used, a different one to elute in each injection. The measurements were free from interferences. Single-point calibration was used to relate the response of the external standard to those of analytes and concentrations of individual homologue groups were reported.	^{13}PCB congeners 118, 153, and 180
7	Formula group abundance profiles, based on the described SIM protocol[11] (i.e., by monitoring the two most intense peaks of $[M - Cl]^-$ ions), were generated. For quantitation, the responses of the most abundant components in the PCA-60 standard and the analyte were measured. The measurements were free from interferences. Single-point calibration was used to relate the response of the external standard to those of analytes and concentrations of individual homologue groups were reported.	$[^{13}C_8]$mirex

[a] When necessary, an internal calibrant of each laboratory's choosing was added to the test and standard solutions to allow for the variability of GC injection volumes and variability of mass spectral negative ion production efficiency. [b] na, not applicable.

used methane as their moderating/reagent gas, while one laboratory used argon. Because of the different pressure transducers fitted on the various machines, some laboratories reported manifold pressures (1×10^{-6}−2×10^{-4} Torr) and others the pressure in the ion source volume (0.4−1.4 Torr). The ion source temperatures ranged from 100 to 210 °C.

Methods Used by the Laboratories. The analytical methods used by the laboratories are outlined in Table 3, with the following additional comments.

Laboratory 1 performed the MS analyses at RP = 1000, owing to poor sensitivity at high RP. A possible explanation for the poor sensitivity could be the high ion source temperature (200−210 °C) used; the relative abundances of the monitored $[M - Cl]^-$ ions are known to decrease with increasing ion source temperature.[11,17]

Laboratory 2, using a quadrupole MS, made corrections for superimposed signals for PCA components giving ions of the same nominal m/z. For example, the $[M - Cl]^-$ ion of $C_{10}H_{15}Cl_7$ ($C_{10}H_{15}{}^{35}Cl_6{}^-$ monitored) was corrected for the overlap from the isobaric $C_{12}H_{20}{}^{35}Cl_2{}^{37}Cl_3{}^-$ ion from $C_{12}H_{20}Cl_6$. The value to be subtracted was estimated from another signal in the ion group.

(17) Tomy, G. T.; Tittlemeir, S. A.; Stern, G. A.; Muir, D. C. G.; Westmore, J. B. *Chemosphere* **1998**, 37, 1395−1410.

(18) Walter, B.; Ballschmiter, K. *Fresenius J. Anal. Chem.* **1991**, *340*, 246−249.

Table 4. Summary of Interlaboratory Results for PCA Analysis[a]

lab no.	PCA-1 concn (ng/μL)	PCA-1 CV	PCA-1 % error	PCA-70 concn (ng/μL)	PCA-70 CV	PCA-70 % error	FE1 concn (ng/μL)	FE1 CV	FE2 concn (ng/μL)	FE2 CV	instrumental method
1	81 ± 1	0.01	10	309 ± 12	0.04	160	nd		58 ± 6	0.1	LRMS/SIM
2	100		35	480		310	nd		42		LRMS/SIM
3(a)				78 ± 8	0.10	30			22 ± 6	0.3	LRMS/SIM
3(b)	nd			267 ± 13[b]	0.05	130	nd		36 ± 6[b]	0.2	ECD
3(c)				267 ± 16[c]	0.06	130			31 ± 5[c]	0.2	ECD
4	120 ± 20	0.17	63	310 ± 40	0.13	160	nd		80 ± 20	0.25	LRMS/SIM
5(a)	75		2	352		200	36.7		35.4		LRMS/TIC
5(b)	102[d]		38	477[d]		300	55.7[d]				LRMS/TIC
6	88.9		21	236.7		100	32.2		nd		HRMS/SIM
7	128 ± 1	0.007	74	163.6 ± 16	0.1	40	54 ± 2	0.04	24 ± 1	0.04	HRMS/SIM
mean ± sd[e]	99.3 ± 19.5			297 ± 132			44.6 ± 11.9		41 ± 19		
CV	0.20			0.44			0.27		0.47		
ADM	17.6			98.5			10.2		16.2		
true[f]	74			118							

[a] The external standard was PCA-60, except as noted. LRMS, low-resolution mass spectrometry; HRMS, high-resolution mass spectrometry; ECD, electron capture detection; SIM, selected ion monitoring; TIC, total ion current; CV, coefficient of variation; ADM, average deviation from mean; nd, not determined. [b] Triangulation method of quantitation. [c] Integrated area of PCA elution signal. [d] Based on Cereclor-S52 as the external standard. [e] Mean ± standard deviation of the laboratory means. [f] Expected result by weighing.

Laboratory 3 performed its analyses both by MS detection (SIM at RP = 1000) and by electron capture detection (ECD), employing two methods of integration of the ECD signal. For all analyses, total PCA concentrations rather than concentrations of homologue groups were reported.

Laboratory 4 performed the analyses by SIM with a quadrupole MS.

Laboratory 5 employed a quadrupole MS operating in the full-scan mode (TIC) for its analyses, and reported its results with respect to two standards.

Laboratories 6 and 7 performed the analyses by SIM at high RP (11 000−12 000).

RESULTS AND DISCUSSION

The results from this study are shown in Table 4. The accuracy and precision of the analytical methods can be evaluated, to some degree, from the measurements reported for the PCA-1 and PCA-70 mixtures because they contain PCAs of *known* concentration. The PCA-70 mixture was selected because its elution profile closely resembles that of the supplied external standard (PCA-60). In contrast, PCA-1 has a profile that is very different from that of PCA-60. Our rationale for using these two analytes was to see whether the quantitative data would become more accurate for mixtures with profiles more closely resembling that of the standard. By weighing, the concentrations of the PCA-1 and PCA-70 mixtures were 74 and 118 ng/μL, respectively. The data derived from quantitative measurements made on the FE samples would, of course, provide an estimate on the interlaboratory precision.

PCA-1 Mixture. Six laboratories were able to quantify the PCA-1 mixture; because of its choice of ions, this mixture could not be quantified by laboratory 3. The reported concentrations ranged from 75 to 128 ng/μL, with a mean of 99.3 ± 19.5 ng/μL (i.e., mean ± the standard deviation of the laboratory means). Compared to the concentration by weighing, the errors range from 2 to 74%; the corresponding coefficient of variation (CV) is 20%. Considering the number of different quantitative methods employed, the CV of 20% is acceptable.

By using LRMS, and under full-scanning conditions (m/z 300−550), laboratory 5 achieved good accuracy for their measurement made with respect to the PCA-60 standard; however, the measurement made with respect to the Cereclor-S52 standard was ∼1.4 times the true value.

The remaining laboratories (1, 2, 4, 6, 7) all monitored selected ions. Laboratory 1 also achieved good accuracy by LRMS/SIM; its value of 81 ng/μL for the PCA-1 mixture is only slightly higher (∼10%) than the *true* value. Laboratories 2 and 4, both employing LRMS/SIM quantitation methods, reported values that were 35 and 63% higher than the true value, respectively. Laboratories 6 and 7, both employing HRMS/SIM methods, reported respective levels exceeding the true value by 21 and 74%. An uncertainty in SIM is lack of knowledge of relative response factors for the monitored ions under various experimental conditions. This may be a disadvantage relative to full-scanning methods where the uncertainties in relative response factors would tend to be averaged out when many ions are monitored.

PCA-70 Mixture. All seven laboratories reported results for this solution. Compared to the weighed value of 114 ng/μL, the errors range from −30 to +310%. The mean is 297 ± 132 ng/μL, the CV is 44%, and the average deviation from the mean (ADM) is 98.5 ng/μL. The CV and ADM are, surprisingly, higher than those for measurements reported on the PCA-1 mixture. With the exception of the SIM measurement of laboratory 3, the other laboratories all exceeded the true value for this mixture.

As noted, laboratory 3 achieved good accuracy by using LRMS and by monitoring four prominent ions; however, the ECD measurements gave results about twice as high as the true value. Laboratories 1 and 4 obtained similar results, which were both ∼160% higher than the true value, while the results of laboratory 5 were ∼3 and ∼4 times the true value. The latter results are of interest because they illustrate that different commercial formulations used as standards provide quite different estimates of PCA concentrations; relative to the Cereclor-S52 standard, they are ∼40% higher than relative to the PCA-60 standard, an observation

that can be extended to their estimates for the PCA-1 and FE1 samples. Laboratories 6 and 7, relative to the PCA-60 standard, reported measurements on the PCA-70 mixture that were 100 and 40% higher than the true value, respectively.

Fish Extract Samples. Owing to the problems encountered with some samples in transit, only three laboratories were able to report results for the FE1 sample. Reported values range from 32 to 56 ng/μL, with mean = 44.6 $\pm$ 11.9 ng/μL; the CV is 27%, and ADM is 10.2 ng/μL.

Laboratory 5 submitted two results for the FE1 sample, namely, 37 ng/μL with respect to the PCA-60 standard and 56 ng/μL with respect to the Cereclor-S52 standard. The laboratory 6 result of 32 ng/μL, is ~60% of that of laboratory 7 (54 $\pm$ 2 ng/μL) although both used HRMS/SIM for quantitation.

Six laboratories submitted results for the FE2 sample, ranging from 22 to 80 ng/μL with a mean of 41 $\pm$ 19 ng/μL; the corresponding CV is 47%, and the ADM is 16.2 ng/μL.

By using their triangulation method and LRMS detection, laboratory 3 reported a value of 22 $\pm$ 6 ng/μL for the FE2 sample which agrees well with the laboratory 7 result (24 $\pm$ 1 ng/μL). By using ECD, laboratory 3 reported values of 36 $\pm$ 6 (triangulation method) and 31 $\pm$ 5 ng/μL (electronic integration), which are in good agreement with the laboratory 5 result (35.4 ng/μL). The laboratory 4 result of 80 $\pm$ 20 ng/μL for FE2 is twice as high as that of laboratory 2 (42 ng/μL) and ~1.4 times higher than that of laboratory 1 (58 $\pm$ 6 ng/μL). The slightly higher values of laboratories 1 and 4 can probably be attributed to the fact that neither laboratory took into account the effects of coeluting interferences. If these two values are treated as outliers, the CV becomes 25%, very similar to that found for the FE1 sample.

CONCLUSIONS

From this study, we can assess the variability associated with the quantitative methods used for determining C_{10}–C_{13} PCAs. Extreme results lie within a factor <2 for the PCA-1 and FE1 samples, ~4 for the FE2 sample, and ~6 for the PCA-70 sample. Some of the variability may not be a function of the instrumental method alone but is introduced when different commercial formulations are used as external standards (recall the differences between the two results reported by laboratory 5). In general, the reported values on the PCA mixtures of known concentration, i.e., PCA-1 and PCA-70, are typically higher than their respective true values. One reason that reported measurements on the FE1 sample are more consistent than those made on the FE2 sample could be that the former measurements were all made by laboratories that corrected for coeluting interferences, while the latter measurements included laboratories that chose not to do so. We recommend, therefore, that procedures that rely on low-resolution mass spectrometry should try to eliminate, or correct for, responses of other organochlorine interferences where possible.

It remains unclear why results for the PCA-70 mixture, whose GC profile and composition are similar to those of the PCA-60 external standard, are less accurate than results for the PCA-1 sample, whose GC profile and composition are quite different from the external standard. One explanation could be that the amount of additives/stabilizers used by manufacturers, which are undetectable under ECNI or ECD conditions, are quite different in the two commercial formulations, thereby making the preparation of solutions for use as standards tenuous at best. To circumvent the effects of stabilizers or other unknown additives, future external standards used for estimating PCA concentrations could take the form of purified commercial formulations or of synthetic mixtures made by chlorinating pure *n*-alkanes under free-radical conditions.

The possibility that coeluting interferences may elevate a result is minimized by using HRMS/SIM methods, if a laboratory has adequate instrumentation to do so. Uncertainties remain owing to unknown relative response factors for production of the negative ions monitored and also to lack of reliable standards. For well-behaved samples, such as PCA-1 (which contains few impurities), comparable results are obtained by LRMS/SIM, LRMS/TIC and ECD methods. For more complex samples, such as PCA-70, the ability to adequately correct for coeluting interferences is important.

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