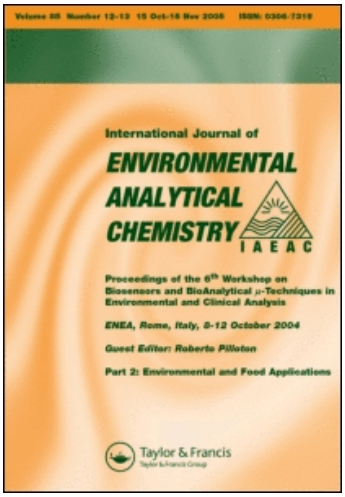

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Quantification of PCBs in Environmental Samples: Comparison of Results Obtained with Different Analytical Instruments (GC—ECD, GC—MS) and Standards

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QUANTIFICATION OF PCBs IN ENVIRONMENTAL SAMPLES: COMPARISON OF RESULTS OBTAINED WITH DIFFERENT ANALYTICAL INSTRUMENTS (GC–ECD, GC–MS) AND STANDARDS

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Polychlorinated biphenyls have been determined in sediments, macroalgae, mussels and worms sampled in the lagoon of Venice, Italy. Sample preparation included hexane extraction, clean-up with sulphuric acid, Florisil and copper and separation from pesticides by column chromatography with silica gel. Analyses were performed using methylsilicone and phenyl-methylsilicone capillary columns. A GC–ECD and a computer-assisted GC–MS performing selected ion monitoring (SIM) were employed. Quantification was based on standards prepared by combining Aroclor formulations and on mixtures prepared with selected biphenyls of different degree of chlorination (7 PCB mix).

Recovery tests conducted with an internal standard added to the samples before extraction yielded values between 79 and 102%. The precisions obtained on quadruplicate samples by using the combination HRGC–LRMS with Aroclor standards and with the 7 PCB mix were 18% and 15%, respectively. Imprecision was mainly due to uncertainties in measuring areas of low-intensity SIM peaks. With HRGC–ECD precisions were better than 7%. A systematic overestimation was observed with HRGC–ECD, probably due to inclusion of interfering compounds.

KEY WORDS: Polychlorinated biphenyl(s), PCB(s), sediment(s), mussel(s), worm(s), algae.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are chemically stable industrial compounds, which derive from the substitution of 1 to 10 chlorine atoms in the biphenyl structure. Two hundred and nine isomers and congeners are possible. Commercial formulations (e.g. Aroclor manufactured by Monsanto, USA; Clophen by Bayer, Germany; Phenoclor by Caffaro, Italy, etc.) contain complex mixtures of isomers, each identified by the global chlorine percentage (e.g. Aroclor 1242 contains 42% chlorine). These chemicals are widely used as dielectric and heat transfer fluids. Formerly they were also employed as plasticizers, wax extenders and flame retardants;[1] these dispersive uses have been prohibited by law in most countries.

PCB residues have been detected in almost all parts of the global ecosystem:

river, lakes, seas, atmosphere, fish, game and human tissues, blood, breast milk.[2–5] The toxicity of PCBs is reported to be related to the number of chlorine substituents and their position in the phenyl ring. Among the biological effects of PCBs, damage to liver, reproductive problems and immunotoxic responses are reported.[6] The water solubility of PCBs is relatively low and decrease as the number of chlorine substituents increase.[7] In water systems PCBs preferentially accumulate in suspended particulate matter, in sediments and biota.[8–12]

PCBs in environmental and biological specimens, after a sample preparation that includes solvent extraction and a number of clean-up steps, are determined by gas chromatography with electron capture detection (GC–ECD) and gas chromatography–mass spectrometry (GC–MS). For quantitation, three types of standards are generally used:

- commercial formulations such as Aroclor 1242 or 1254 or a combination of these;
- a mixture of single isomers, one for each chlorination degree;
- a mixture of selected isomers, chosen on the basis of persistence and toxicity.

The choice of the standard is based on a number of factors including the type of sample, the availability of reference compounds and, finally, the objective of the determination. The standard composed of selected isomers is difficult to provide and very expensive, but it is indispensable when specimens from higher organisms have to be analysed (i.e. organisms that are provided with an enzymatic system that selectively degrades PCBs. In other samples, such as sediments, algae and molluscs, the pattern of the commercial formulations is expected to be essentially maintained and the use of commercial formulations as standards is usually adequate.

In this work we analysed a number of samples of macroalgae, sediments, worms and mussels from the lagoon of Venice both with high-resolution GC–ECD and high-resolution GC–MS. As standards, both commercial formulations (Aroclor) and a mixture of single isomers representing diverse degrees of chlorination were used. The precision and accuracy attained with the two instrumental combinations and the two types of standards are discussed in order to assess the best compromise between ease of use, analysis time and significance of the measurements.

MATERIALS AND METHODS

The analysed samples were collected at different locations in the lagoon and were: three surface sediments (2 cm upper layer) taken in different locations; each sample was composed by mixing five subsamples; a number of macroalgae fronds (*Ulva rigida*, C. Ag.) divided into three samples of different age; many individual worms (*Nereis diversicolor*, O. F. Müller) forming one sample; many individual mussels (*Mitilus galloprovincialis*, Lam.) forming one sample. All samples were frozen, lyophylized and carefully homogenized.

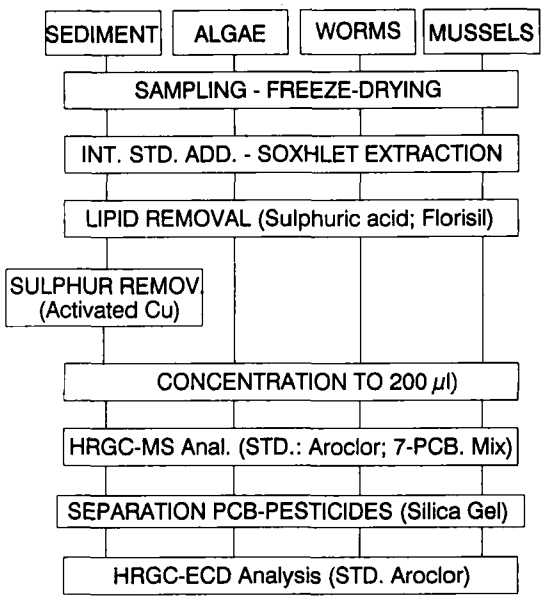

Figure 1 Scheme of the analytical procedure.

The following procedure outlined in Figure 1, was adopted. After internal standard addition (decachlorobiphenyl), ca. 2 g of samples were Soxhlet-extracted with *n*-hexane for 24 h. Lipids were then removed by treating the hexane extract, rotary evaporated to ca. 10 ml, with 1 ml aliquots of concentrated sulphuric acid. The procedure was repeated until the acid layer remained colourless. The hexane extract was then passed through a 1 cm i.d. chromatographic column filled with 1 cm of sodium sulphate on top and 1 cm activated Florisil. Sixty ml of *n*-hexane completed the elution. For removing the sulphur present in sediments, the packing of the column was modified, by adding a 2 cm layer of copper powder, activated with HCl, underneath the sodium sulphate and the Florisil layers.

Prior to analysis with GC–ECD, the PCBs were separated from interfering pesticide residues (mainly DDT, DDD, DDE, HCH), by column chromatography using silica gel (Riedel-De Haën Kieselgel S, 0.063–0.2 mm, activated at 220 °C overnight and deactivated with 1% water). The PCBs were eluted with *n*-hexane (55 ml for the silica gel batch we used); the pesticides were recovered with 70 ml of benzene.

Capillary columns, 0.2 mm i.d., 25 m long, purchased from Hewlett-Packard, coated with methylsilicone and 5% phenyl-methylsilicone were used. Analyses were run on a Hewlett-Packard (HP) 5840 A gas chromatograph equipped with an ECD and a GC–MS system composed of a gas chromatograph HP 5840 A and a 5985 B quadrupole mass spectrometer assisted by a HP 1000 computer and a HP 7920 data system.

Quantification of chromatograms run on the GC–ECD was performed by comparison with Aroclor formulations. A mixture of Aroclors 1242 and 1254 (1:1) was used for algae, mussels and worms; a mixture of Aroclors 1254 and 1260 (1:1) was employed for sediments. Twelve prominent peaks were selected and their areas were summed and compared with the sum of the corresponding ones in the samples.

Quantification with GC–MS was performed by using both Aroclor combination as with GC–ECD and a mixture containing 7 PCB isomers, one for each degree of chlorination (7 PCB mix), viz. 2-chlorobiphenyl, 2,2′-dichlorobiphenyl, 2,4,5-trichlorobiphenyl, 2,4,2′,4′-tetrachlorobiphenyl, 2,4,5,2′,3′-pentachlorobiphenyl, 2,3,4,2′,3′,4′-hexachlorobiphenyl and 2,3,4,5,6,2′,5′-heptachlorobiphenyl. Initially, also an octachlorobiphenyl was included, but it was never used for quantification as no corresponding significant peaks were found in real samples. GC–MS was operated in the selected ion monitoring mode. Ions were selected after injecting a concentrated solution containing the 7 PCBs and recording the total ion chromatogram. Two ions from the molecular ion chlorine cluster were chosen for each degree of chlorination: Cl: 186, 188 amu; Cl_2: 222, 224 amu; Cl_3: 256, 258 amu; Cl_4: 290, 292 amu; Cl_5: 324, 326 amu; Cl_6: 358, 360 amu; Cl_7: 392, 394 amu.

Identification of peaks in the samples was confirmed by checking the retention times and the ratios of intensities of ions belonging to the same cluster. Quantification was based on a single ion per degree of chlorination. The chromatograms of the standard and the samples were subdivided into seven intervals (Figure 2). and the areas of peaks were summed and the ratios calculated between those in the standard and in the sample. When using the Aroclor standards, a number of peaks per degree of chlorination were summed; when using the 7 PCB mix, only one compound per degree of chlorination was used. In the first case, the ratio (amount/area) used for quantification was based on the average ratio of all the monitored peaks in the interval. In the second case, the ratio (amount/area) of one peak per degree of chlorination was employed.

In samples, an amount of PCB per degree of chlorination was determined.

RESULTS AND DISCUSSION

Prior to instrumental analysis all samples were processed using the same procedure that included solvent extraction, clean-up to remove interferences such as lipids and sulphur and separation from pesticides. This procedure was checked with intercalibration exercises,[13] and was refined from time to time.[14,15]

Recovery tests were based on decachlorobiphenyl added as internal standard prior to analysis. The choice of this compound can be questioned, as it can theoretically be present in the samples. Preliminary analyses of samples with GC–ECD eexcluded the presence of detectable peaks at retention times as high as that of DCB. Recoveries ranged from 79% to 102%. Assuming that loss was the same for the internal standard and for PCBs, PCB concentrations were corrected accordingly.

A large part of the significance of an analytical datum is determined by the

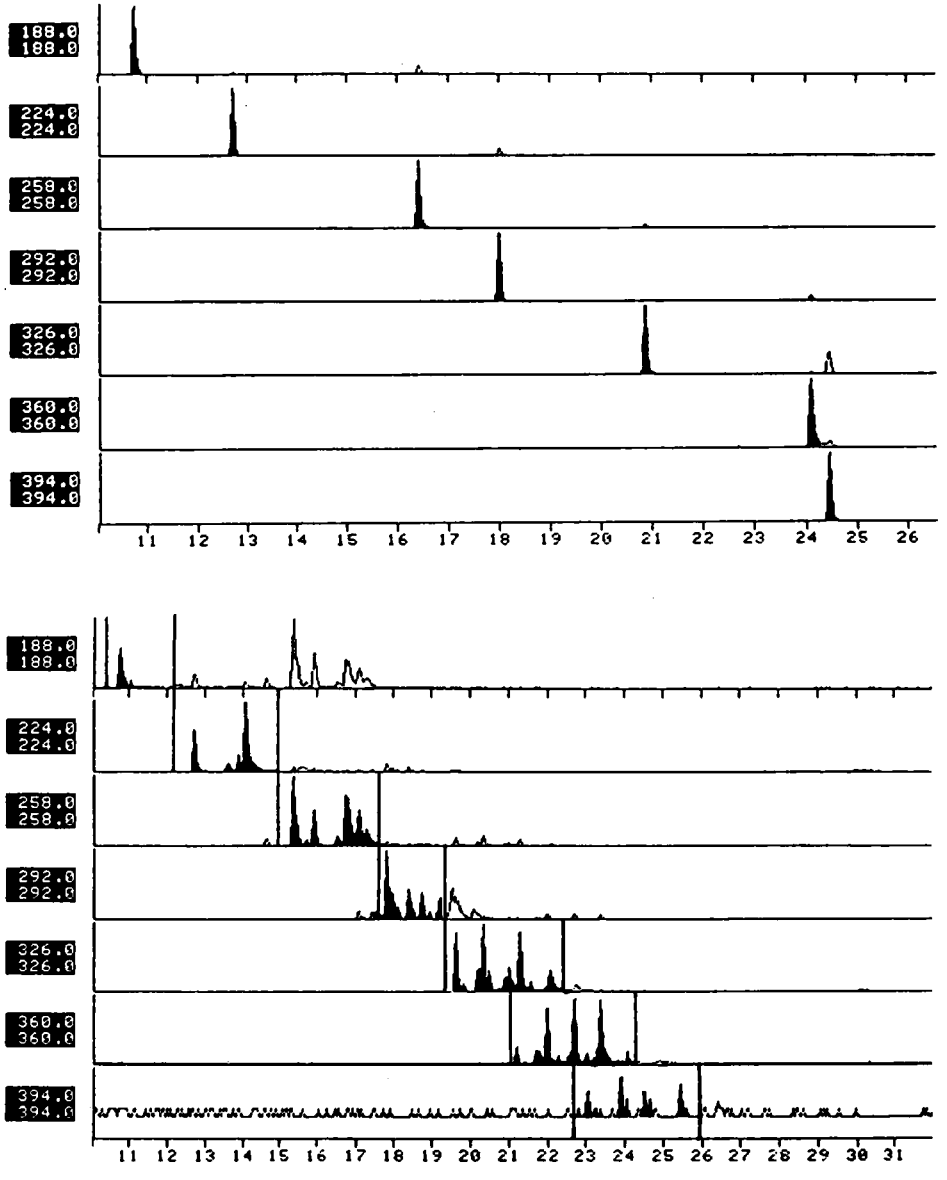

Figure 2 Quantification of PCBs with GC–MS in the SIM mode. Top, 7 PCB mix standard; bottom, Aroclor 1242 and 1254 (1:1) standard.

standard used for quantification. We have primarily evaluated the correspondence between sample patterns and combinations of Aroclor formulations (Figure 3).

A combination of Aroclors 1242 and 1254 (1:1) was found appropriate for samples of algae, worms, mussels; for sediments a mixture of Aroclors 1254 and 1260 (1:1) was judged more proper. The PCB degradation that takes place in the

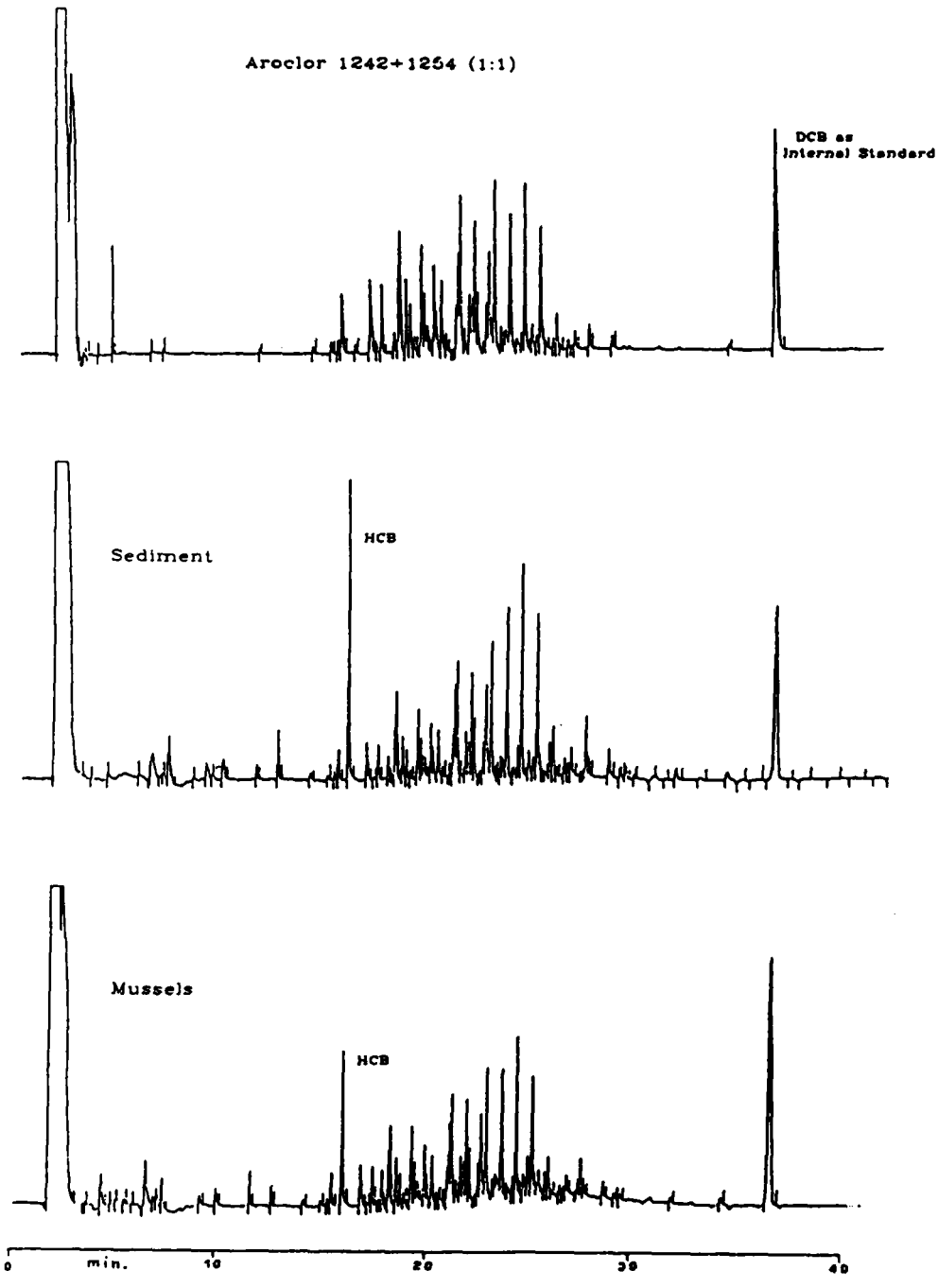

Figure 3 GC–ECD chromatograms showing the standard Aroclor 1242 and 1254, and samples of sediment and mussels.

sediment (deposited or resuspended), in algae and benthic organisms do not alter significantly the original composition of the mixture they came in contact with. For the comparison, two types of Aroclor mixtures and a composition containing seven PCB, one for each degree of chlorination were therefore used.

The standard based on Aroclor formulations can be used both for quantifying samples analysed by GC–MS and GC–ECD. The 7 PCB mix standard can only be used with GC–MS, as only by monitoring selected ions it is possible to assign the chlorination degree to the PCB represented by a peak. Quantifications made with 7 PCB mix enable us to evaluate the relative weight of any degree of chlorination in the overall mixture of the sample. By using Aroclor mixtures a preliminary qualitative evaluation is made by comparing the sample with different Aroclor formulations of increasing chlorination degree.

As regards the ease of execution, quantification with GC–MS using Aroclor formulations is more laborious than with the 7 PCB mix, but not to such an extent as to render this quantification tedious.

The comparison among quantification methodologies is therefore the comparison between: (i) two types of standards applied with the same analytical technique (GC–MS, the combination of Aroclor and the 7 PCB mix); and (ii) two analytical techniques (GC–MS, GC–ECD), when using the same Aroclor combinations.

MS in the SIM mode is much more selective than the ECD detection. Only the diagnostic ions of a substance are monitored, and the identification of a peak, primarily picked by the relative retention time, is confirmed by the ion ratios, that must be the same in the sample and in the standard within a predetermined precision (usually 20%). Analyses carried out with ECD, even though preceded by several steps to minimise interferences, can—as identification is only based on retention times—always be affected by over-evaluation due to the inclusion of extraneous substances. In the case of PCBs, residues of pesticides or other chlorinated hydrocarbons are also detected. As an example, in the chromatogram of Figure 3 at 16.25 min a very neat peak is present. It was identified as hexachlorobenzene (HCB). In the lagoon of Venice, HCB is a pollutant of industrial origin (industrial district of Porto Marghera[16]).

In Figure 4 the results obtained with GC–MS (Aroclors), GC–MS (7 PCB mix) and GC–ECD (Aroclors) are compared.

Area measurements obtained by GC–ECD were much more precise than with GC–MS. In this case areas were measured with both standards in the reconstructed chromatogram, by selecting one ion for each degree of chlorination. Areas have been measured manually by integrating the digitised areas, peak by peak, or automatically by using a computer program, supplied by the instrument manufacturer, that identifies peaks on the basis of the retention time and validates identification by comparing peak ratios. After a number of runs, the second method has been abandoned as, due to their low intensities, peaks were frequently mismatched. As no intervention was possible during program execution, the loss of time during procedure repetitions rendered the manual integration more profitable. With both integration procedures, peaks in the first half of the chromatogram and ions in the middle range of masses showed precisions around 10–12%, whereas with low mass peaks the precision was less good (12–15%) and even worse with

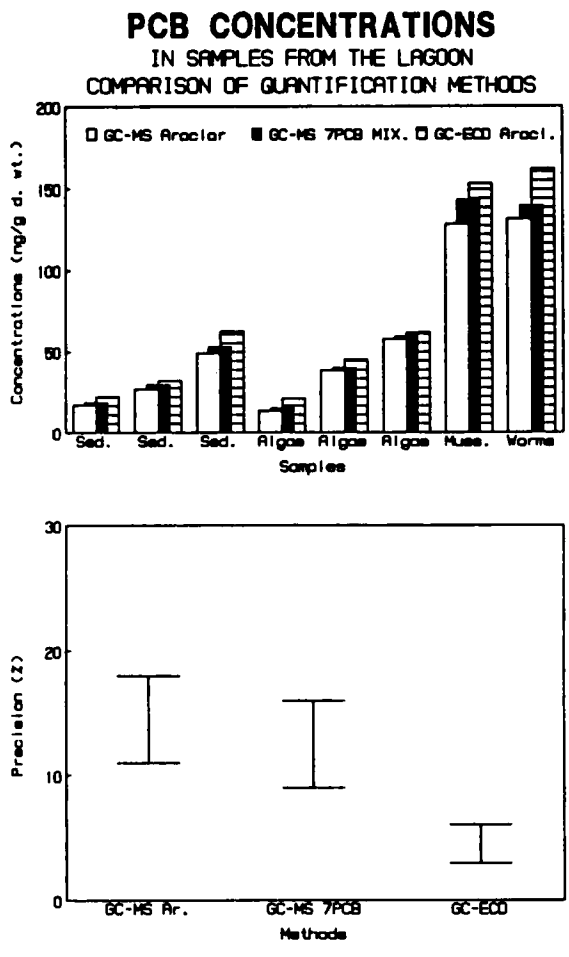

Figure 4 Comparison of results obtained with the two instrumentations (GC–ECD and GC–MS) and the two standards (Aroclor, 7 PCB mix).

high mass peaks (ca. 18%) ($n=4$ in all cases). With the 7 PCB mix a small gain in precision was observed compared to the Aroclor combinations.

With GC–ECD, precisions ranged between 3% and 7%. Differences were rather related to peak resolution than to uncertainties in area measurements. In addition, the same precision was observed over the whole chromatogram. Even when considering the best conditions with the GC–MS system and the worst ones with GC–ECD, the latter turned out to be more precise. The explanation probably resides in two facts, one related to the area measurements, as already discussed, and one to the different sensitivity of the two analytical techniques. The GC–ECD system was more sensitive than the GC–MS–SIM by a factor from 10 to 20. In fact, as the number of chlorines in the biphenyl structure increases, the sensitivity of GC–ECD also increases, whereas that of GC–MS decreases. In sediment

chromatograms, for some peaks of very low intensities imprecision with GC–MS reached 18%.

The results obtained with GC–ECD were systematically higher than those with GC–MS both using the Aroclor combinations as standard or the 7 PCB mix. The quantification with the 7 PCB mix led to results systematically higher than those with the Aroclors. However, if one takes the precision into considerationn, it is clear that, due to the high precision of GC–ECD, the difference between GC–ECD and GC–MS is significant and deserves interpretation; the one between the results obtained with the two standards using the (same) GC–MS system are not. As regards the former aspect, due to the lack of selectivity of GC–ECD, some interfering substances are included in the quantification that are easily discriminated by GC–MS in the SIM mode. From this point of view, the GC–MS data are more accurate by about 10%.

CONCLUSIONS

The most important advantage in performing PCB analyses with GC–MS, resides in the high selectivity that efficiently differentiates different chlorination degrees and discriminates interfering substances. By adequately preparing the sample in terms of amount extracted, final volume of the extract and by fine-tuning the instrument in order to move the maximum sensitivity toward higher masses, it is possible to obtain precisions around 13%. This requires good practice and skill and is time-consuming. This procedure is mandatory when, for a limited set of samples, very accurate measurements are needed.

If we consider that analyses with GC–ECD are much easier to perform and cheaper, that sensitivity and precision are very good over a wide range of concentrations, when numerous samples have to be analysed to trace time trends or distributions, analyses with GC–ECD are dependable and preferable.

References

1. O. Hutzinger, S. Safe and V. Zitko, *The Chemistry of PCBs* (CRC Press, Cleveland, OH, 1974), Chaps. 1–2, pp. 1–39.
2. R. W. Risebrough, P. Rieche, S. G. Herman, D. B. Peakall and M. N. Kirven, *Nature* **1098** (1968).
3. F. Cordle, P. Corneliussen, C. Jelinek, G. Hackley, R. Hehman, J. McLaughlin, R. Rhoden and R. Shapiro, *Environ. Health Perspect* **157** (1978).
4. M. V. Holdrinet, H. E. Braun, R. Frank, G. J. Stopps, M. S. Smout and J. W. McWade, *Can. J. Pub. Health* **68,** 74 (1977).
5. S. Safe, *Toxicol. Environ. Chem.* **5,** 153 (1982).
6. S. Safe, S. Bandiera, T. Sawyer, L. Robertson, L. Safe, A. Parkinsons, P. E. Thomas, D. E. Ryan, L. M. Reik, W. Levin, M. A. Denomme and T. Fujita, *Environ. Health Perspect.* **60,** 47 (1985).
7. A. L. Alford-Stevens, *Environ. Sci. Technol.* **20** (12), 1194 (1986).
8. S. F. J. Chou and R. A. Griffin, *PCBs and the Environment* (J. S. Waid, ed.), CRC Press, Boca Raton, FL, Vol. 1, Chap. 5, pp. 101 (1986).
9. G. R. Shaw and D. W. Connell, *PCBs and the Environment* (J. S. Waid, ed.), CRC Press, Boca Raton, FL, Vol. 1, Chap. 6, pp. 121 (1986).

10. J. C. Duinker, *Neth. J. Sea Res.* **20,** 229 (1986).
11. R. F. Bopp, H. J. Simpson, C. R. Olsen and N. Kostyk, *Environ. Sci. Technol.* **15,** 210 (1981).
12. P. Hagel and L. G. M. Th. Tuinstra, *Bull. Environ. Contam. Toxicol.* **19,** 671 (1978).
13. R. Donazzolo, L. Menegazzo Vitturi, A. A. Orio and B. Pavoni, *Environ. Technol. Lett.* **4,** 451 (1983).
14. B. Pavoni, A. Sfriso and A. Marcomini, *Mar. Chem.* **21,** 25 (1987).
15. S. Raccanelli, B. Pavoni, A. Marcomini and A. A. Orio, *Sci. Tot. Environ.* **79,** 111 (1989).
16. V. U. Fossato, E. S. Van Vleet and F. Dolci, *Archivio di Oceanografia e Limnologia* **21** (2), 151 (1988).