

Relative performance of immunochemical (enzyme-linked immunosorbent assay) and gas chromatography–electron-capture detection techniques to quantify polychlorinated biphenyls in mussel tissues

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Abstract

Results from polychlorinated biphenyls (PCB) analyses of mussel tissue extracts by immunoassay (PCB RaPID Assay[®]) and conventional gas chromatography–electron-capture detection (GC–ECD) are described and compared. Mussels from natural populations with diverse concentrations of PCBs, mussel tissue fortified with technical Aroclor[®] 1254 and a certified reference material are included.

A strong correlation is reported between “total” PCBs quantified by both techniques ($r^2 = 0.95$, $n = 27$). Immunoassay results, however, exhibited lower values compared to GC–ECD, particularly when GC results are corrected for procedural recovery. A reduced antibody response, due to differences in the congener composition between the mussel extracts and Aroclor[®] 1254 (used to raise and calibrate the ELISA), provides the most likely explanation for this difference. Non-parametric statistical analyses confirmed that, although differing from Aroclor[®] 1254, PCB congener compositions in the mussel extracts most closely resemble that of Aroclor[®] 1254. At very high PCB concentrations ($>30 \mu\text{g g}^{-1}$ dry weight), however, ELISA results are statistically different ($P < 0.01$) from GC–ECD results, which is likely to be related to the solvation capacity of ELISA diluent. Similarity analysis showed high correlations between the most prominent congeners in Aroclor[®] 1254 and immunoassay results. This analysis did not, however, identify a specific chlorine substitution pattern to which the immunoassay preferentially responded.

Whilst GC–ECD affords the capability to quantify individual congeners of different reactivity and toxicity, the data reported do indicate that immunoassay offers a rapid and inexpensive alternative method for estimation of “total” PCBs at environmentally significant levels. It is, however, necessary to remove extraneous lipids to reduce matrix effects in the immunoassay. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

During the last decade, environmental immunoassays have been developed to detect selected pollutants in water and sediment/soil samples. Several commercial kits are now available for this purpose and offer rapid screening at comparatively low cost. Recently, environmental researchers have started to apply the technique to the analysis of biological media in which pollutants and their metabolites can become concentrated [1–5]. Biological monitoring data is often essential to provide a measure of exposure to biologically “available” contaminants. Methods to provide this data, however, are often complex.

Conventional biomonitoring methods, which use chromatographic techniques, are often time consuming, labour intensive, and expensive. Immunochemical methods, such as enzyme-linked immunosorbent assays (ELISAs), are easier and less expensive to use, can be very specific to the chemical or group of chemicals (e.g. PCBs), may offer improved limits of detection, and are easily adapted for use in the field.

The principles of ELISA have been previously described [6–8]. Among several ELISA formats available, the polyclonal PCB RaPID Assay[®] (Strategic Diagnostics Inc., Newtown, PA, USA) using magnetic particle-based immunoassay was selected for this study. This format has been applied to the detection of contaminants in different matrices such as water [9,10], sediment/soil [11–14], organisms [4] and food [2,3,15]. In addition, magnetic particle ELISA has shown better precision and sensitivity compared to formats where the antibody is passively adsorbed to polystyrene tubes [16,17].

Whilst primarily designed for analyses of polychlorinated biphenyls (PCBs) in water, our goal was to adapt and evaluate the effectiveness of this immunoassay method in measuring PCB levels in the biological tissues of exposed invertebrates (mussels). These evaluations included: (a) adaptation of our routine sample work-up procedure to combine with ELISA; (b) determination of matrix effects relating to co-extracted biogenic materials; (c) assessment of cross-reactivities of the antibodies with technical PCB mixtures (Aroclors); (d) assessment of reproducibility and limits of detection; (e) determination of how well immunoassay results correlate with more defini-

tive gas chromatography–electron-capture detection (GC–ECD) results.

2. Experimental

2.1. Materials

Certified solutions of PCB congeners (CLB-1) were purchased from the NRCC (Halifax, NS, Canada). Other authentic standard PCB congeners were purchased from QMx Laboratories Ltd. (Safron Walden, UK). The 55 congeners were selected for quantification based on their resolution under the selected GC conditions. Numbering of the PCB congeners followed the IUPAC system. Aroclor[®] solutions ($\sim 100 \mu\text{g ml}^{-1}$ in hexane) of selected PCB mixtures (Aroclor[®] 1242, 1248, 1254 and 1260) were purchased from Ultra Scientific (North Kingstown, RI, USA). Working solutions of individual Aroclors were prepared in hexane for GC calibration, and in methanol for ELISA calibration and cross-reactivity determinations. Internal standard (PCB 29; 2,4,5-trichlorobiphenyl) was purchased from QMx Laboratories Ltd. (Safron Walden, UK). Standard Reference Material[®] (NIST-SRM 2977—freeze-dried mussel tissue) was obtained from the National Institute of Standards & Technology (NIST, Gaithersburg, USA). Solvents of glass distilled grade were obtained from Rathburns Chemicals Ltd. (Walkerburn, UK). Solvents were batch tested for PCB contamination.

2.2. Environmental mussel samples

Mussel samples were taken from four sites in New Bedford Harbour (MA, USA) (*Geukensia demissa*) and from one site in Whitsand Bay (Cornwall, UK) (*Mytilus edulis*). Locations were selected to afford diverse levels of contamination and thus provide a robust test of the ELISA procedure. Further details about sampling sites at New Bedford Harbour (Sites 1–4) are given elsewhere [18].

2.3. Extraction of mussel tissue

Immunoassay kits for soil/sediment analyses generally recommend methanolic extraction which results

in a solution compatible with the immunosorbent assay [13,17]. Extractions involving polar solvents, however, are generally not as effective for complex matrices and very hydrophobic contaminants [19,20]. For this reason, Soxhlet extraction, which yields high recoveries (and is used in our routine sample preparation procedure for PCB analysis), was selected to provide extracts for this study. This does, however, coextract extraneous lipids which can introduce matrix effects with the ELISA. In addition, hydrophobic solvents (e.g. isooctane, hexane, etc.) inhibit the ELISA. For example, Zajicek et al. [4] have reported significant interference by isooctane on a PCB ELISA (based on antibody-coated magnetic particles) even at trace 0.1% (v/v) concentrations. Therefore, a solvent exchange procedure (hexane to methanol) is essential when using high sensitivity ELISA such as PCB RaPID Assay[®].

PCBs were analysed using a sample preparation method modified from Kannan et al. [21] and Nakata et al. [22]. Briefly, freeze-dried mussel tissue samples (~1 g), fortified samples, standard reference material and procedural blanks were spiked with an internal standard (PCB 29). The samples were Soxhlet extracted into 200 ml of hexane/dichloromethane (1:1) for 16 h. The extracts were then concentrated down to a few ml using rotary evaporation followed by pure nitrogen “blow down”. The extracts were transferred to glass columns (26 cm × 15 mm i.d.) packed with 20 g Florisil[®] and then dried using a gentle flow of nitrogen. PCBs were eluted with a mixture of 120 ml acetonitrile and 30 ml hexane-washed water. The eluants were collected in a separatory funnel containing 100 ml of hexane and 600 ml of hexane-washed water. After shaking and phase separation, the hexane layer was concentrated to exactly 4 ml. The sample extracts were split (volumetrically) for analysis of PCBs by immunoassay (25%) and chromatography (75%). Extracts for ELISA were solvent exchanged into methanol (as described by Zajicek et al. [4]). Samples for chromatography were treated with concentrated sulphuric acid and then cleaned-up and fractionated using 12 g of Florisil[®] (activated at 130 °C for 12 h). Elution was performed using 105 ml of hexane to yield the first fraction (containing the PCBs), followed by 150 ml of hexane/dichloromethane (80:20) (the second fraction containing organochlorine pesticides).

2.4. Quantification using GC–ECD

Quantification was achieved using a gas chromatograph (Hewlett-Packard 5890 series II) with a ⁶³Ni ECD and fitted with a 60 m × 0.25 mm i.d. (0.25 μm thickness) HP-5MS fused-silica capillary column (Hewlett-Packard, USA). The oven temperature was programmed from an initial temperature of 40–160 °C at the rate of 20 °C min⁻¹; 160 °C was maintained for 5 min and the temperature was then programmed to 260 °C at the rate of 2 °C min⁻¹; from 260 °C the temperature was increased at 10 °C min⁻¹ to 290 °C where the temperature was held for 10 min. Thereby, 1 μl sample volumes were automatically injected into a cold “on-column” injector. The detector temperature was maintained at 300 °C. Hydrogen (“high purity” grade) was the carrier gas at a flow rate of 1.5 ml min⁻¹ at 40 °C. Nitrogen (“ECD grade”) was used as make-up gas at a flow rate of 60 ml min⁻¹. Both H₂ and N₂ gases were further purified by moisture, hydrocarbon, and oxygen filters before use. Data were acquired and processed using Hewlett-Packard ChemStation[®] software.

Quantification of the individual 55 congeners was through external calibration using CLB-1. For the calculation of “total” PCBs (sum of 128 congeners), congeners for which authentic standards were not available were identified from relative retention times (RRTs) provided in the literature [23–25] and response factors (RFs) provided by Erickson [25] were used in quantification. Congeners with similar retention times but different chlorine substitutions were investigated by GC/MS (Hewlett-Packard Model 5890 II Plus GC and a 5972 mass selective detector (MSD) (Palo Alto, CA)). “Total” PCBs, quantified using GC–ECD, were used for comparison with the immunoassay results.

2.5. Enzyme-linked immunosorbent assay (ELISA)

Individual sample extracts in methanol (20–200 μl aliquots) were diluted (1:1000–2000) using 50% v/v methanol/buffer solution (containing stabilisers and preservatives) as required by the method. They were then analysed in triplicate for “total” PCBs together with four calibration standards of Aroclor[®] 1254 (0, 0.25, 1.0 and 5.0 ng ml⁻¹).

A polyclonal commercial ELISA, PCB RaPID Assay[®] (Strategic Diagnostics Inc., Newtown, PA,

USA), was used according to the manufacturer's instructions. Briefly, appropriate amounts of samples or standards, antibody-coated microbeads (anti-PCB antibodies immobilised onto paramagnetic particles) and enzyme conjugate (PCB-horseradish peroxidase) were mixed and incubated to allow competition for binding to the specific antibody. After washing twice with kit buffer using a magnetic rack to retain the antibodies, substrate (hydrogen peroxide) and chromogen (3,3',5,5'-tetramethylbenzidine) were added and incubated. Stop solution (2 M sulphuric acid) was added and the colour produced was measured at 450 nm using an Optimax microplate reader (Molecular Devices, Menlo Park, CA). Sample absorbance was compared to a linear regression equation using a logarithm of the concentration versus logit B/B_0 standard curve to calculate the final concentration of PCB (where B/B_0 is the absorbance observed for a sample or standard divided by the absorbance at the zero standard). Sample concentrations, expressed as "total PCBs" (Aroclor[®] 1254 "equivalents"), were calculated by multiplying results by the appropriate dilution factor.

2.6. Quality assurance

Recoveries of PCBs analysed by GC–ECD (examined in triplicate by spiking 4.0 µg of PCB standard (55 congeners) into Soxhlet extracted mussel tissue) ranged from 60 ± 9 to $104 \pm 12\%$, and averaged 91%. Detection limits for individual congeners ranged from 0.1 to 2.0 ng g⁻¹ (dry weight). Appropriate blanks were analysed and, in addition, reference material NIST-SRM 2977 was analysed simultaneously. Results for all congeners quantified by GC–ECD in the reference material were within $94 \pm 12\%$ ($n = 3$) of the mean certified values.

2.7. Safety considerations

Laboratory coats, gloves (preferably nitrile) and laboratory spectacles must be worn when manipulating samples and running the immunoassays. Analyses must be run in well ventilated areas such as a fume cupboard. Solutions within the kits contain methanol which is a volatile solvent and irritant. Special care must be taken when using the standard and enzyme conjugate solutions of the RaPID Assay[®], which

contains PCBs. Wastes generated during the analyses must be disposed of in a responsible manner.

2.8. Statistical analyses

A standard Student's *t*-test was used to examine differences between both techniques across sampling sites. Principal component analysis (PCA) and similarity analysis were performed with Primer[®] for Windows[®] (Version 5; Primer-E Ltd., Plymouth, UK).

3. Results and discussion

PCB congener patterns in contaminated environmental matrices often resemble those of the commercial/technical PCB mixtures (Aroclor[®] 1016, 1242, 1248, 1254 and 1260) or their combinations. As a result, immunoassay antibodies for PCB analysis have been raised and calibrated against technical Aroclors (e.g. Aroclor[®] 1254 [4,17]; Aroclor[®] 1248 [5]). The RaPID Assay[®] PCB ELISA was raised and calibrated against Aroclor[®] 1254. For these reasons, performance testing of the immunoassay procedure included other technical Aroclor[®] mixtures.

Some organisms, however, can accumulate a modified composition of congeners depending on the extent of environmental alterations, and the bioaccumulative and metabolic capabilities of the organisms [26]. Results for the ELISA are, therefore, compared with GC–ECD results to better understand the ELISA response to environmental PCB compositions.

3.1. Performance of ELISA

Matrix effects were carefully studied. A preparatory clean-up (see Section 2.3) was selected to remove the bulk of extraneous lipids. Ten mussel sample extracts were diluted (to cover the entire range of the standard calibration curve) and then analysed by ELISA. Results were compared to similar curves resulting from Aroclor[®] 1254 standards. A lack of parallelism between samples and standard curves can indicate matrix effects [6]. The slope of the curves (slope = 0.663 ± 0.023 , $r^2 = 0.97 \pm 0.04$, $n = 10$, not shown) was unaffected by the dilutions, providing evidence

Table 1
Specificity (cross-reactivity) of Aroclors in the PCB RaPID Assay[®]

Aroclor [®]	Chlorination (%)	MDL ^a (ng ml ⁻¹)	50% B/B ₀ ^b (ng ml ⁻¹)	Cross-reactivity
1260	60	0.08 (0.04)	2.72 (1.00)	1.07
1254	54	0.08 (0.03)	2.90 (1.07)	1.00
1248	48	0.16 (0.07)	6.90 (1.98)	0.42
1242	42	0.35 (0.07)	14.20 (2.68)	0.20

Values in parentheses are S.D. ($n = 3$).

^a MDL: method detection limit (90% B/B₀).

^b 50% B/B₀: concentration required to inhibiting one-half of the colour produced by the negative control.

that no significant matrix effects were present using the selected analytical conditions.

The method detection limit (MDL), as estimated at 90% B/B₀ for the Aroclor[®] 1254 calibration dilutions, was 0.08 ng ml⁻¹. The 50% B/B₀ (concentration required to inhibit one-half of the colour produced by the negative control) was 2.9 ng ml⁻¹ (Table 1). This sensitivity approached the estimated detection limit for individual congeners (blank + 3S.D.; [27]) analysed by the GC–ECD technique (0.05–1.01 ng ml⁻¹). The assay detection limit for mussel tissue was 0.6 µg g⁻¹ (dry weight), which is the 90% B/B₀ corrected for the dilution used. Quantification using the ELISA must be within the range of the standard curve (0.6–40 µg g⁻¹) and appropriate dilutions must be made. The sensitivity can be improved by reducing the dilution up to a limit that guarantees no matrix effect. The coefficient of variation (%CV) within the assay was less than 11 ± 4% ($n = 10$), which is similar to conventional analytical variability.

Within the analytical protocol, internal standard PCB 29 (40 ng g⁻¹ dry weight) was added to enable improved quantification by GC–ECD (to correct for recovery). Thus the compound was also present in the extracts analysed by ELISA (0.005–0.01 ng ml⁻¹). Tests to investigate the potential for this to affect the immunoassay results revealed that even ten times the amount of PCB 29 added as internal standard produced no measurable ELISA response.

Although the PCB RaPID Assay[®] was raised and calibrated against the technical Aroclor[®] 1254 mixture, compositions of PCBs in the environment can vary and reflect other Aroclors (or their combination) thus resulting in different ELISA responses. The ELISA was, therefore, tested against other commercial Aroclors (1242, 1248 and 1260). Results relative

to Aroclor[®] 1254 are given in Table 1 and demonstrate that the assay is broadly responsive to all the Aroclors tested. The ELISA response for Aroclor[®] 1242 and 1248 is smaller indicating that these congener mixtures have lower binding efficiency than those of Aroclor[®] 1254. Conversely, Aroclor[®] 1260 has a higher binding efficiency than Aroclor[®] 1254, inferring that it has a higher proportion of strongly binding congeners. These results indicate that the antibodies have increased affinity for congeners with a higher degree of chlorination. Similar relative cross-reactivities for the Aroclors have been reported by Zajicek et al. [4] and Lawruk et al. [17] using a magnetic-particle PCB ELISA and Zajicek et al. [5] using a EnviroGard[™] tube format PCB ELISA. Results were also similar to those reported by manufacturer (PCB RaPID Assay[®] manufacture's insert).

Lawruk et al. [17], using the same magnetic-particle PCB ELISA, showed that PCB antibodies are most reactive to the Aroclors that largely contain 4, 5, and 6-chlorine-substituted homologues (i.e. Aroclor[®] 1248, 1254, 1260) because Aroclor[®] 1254 (which was used as the PCB immunogen) is comprised of 94% of these homologues. It is, however, difficult to determine which specific congeners are most reactive to the antibodies. Carlson [28] suggested that a PCB immunoassay (EnviroGard[™]) had a greater specificity for congeners with 2,4,5-substitution pattern and its 2,4- and 2,5-subsets, which represent a significant portion of Aroclor[®] mixtures. In the present study, the response of the assay was within a factor of 2.5 for Aroclors 1248 and 1260 and is in agreement with the significance of 2,4-, 2,5- and 2,4,5-substitution in these congener mixtures. In general, as the percent chlorination of Aroclors increases, so does their content of congeners chlorinated in the 2-,4-,5-,2',-4',-5'-positions

and hence ELISA cross-reactivity [5]. Lawruk et al. [17] showed, however, that a coplanar PCB (PCB 126; 3,3',4,4',5-pentachlorobiphenyl) was the most reactive of a selection of congeners tested. Another pentachlorobiphenyl (PCB 114; 2,3,4,4',5-) along with selected tetra- and hexachlorobiphenyls showed much lower reactivities.

3.2. Comparison of results from ELISA and GC–ECD

In total, 27 mussel tissue samples were analysed by ELISA and GC–ECD. These included mussels from natural populations (with diverse concentrations of PCBs from 0.3–100 $\mu\text{g g}^{-1}$ dry weight; $n = 20$), tissues fortified with Aroclor[®] 1254 (0.7–40 $\mu\text{g g}^{-1}$ dry weight; $n = 3$), and replicates of a certified standard reference material (SRM 2977, $n = 4$). To enable comparison of the results obtained by both techniques, the GC results were not corrected for procedural recovery since ELISA results cannot be corrected for this factor. Losses during the analytical procedure

were typically less than 20% (based on recoveries of the internal standard). Since mussel extracts were processed using the same general analytical procedure for both GC and ELISA analyses, losses should be comparable. However, minor differences might be expected because the extracts for ELISA analyses went through an additional solvent exchange and dilution, whilst the GC extracts were further cleaned-up and fractionated using Florisil[®]. The technique for GC–ECD was improved because some degradation of chromatographic performance was observed. GC extracts were analysed prior and after the final clean-up, and no significant differences were found between both techniques (Student's t -test; $p \leq 0.05$).

Comparison of “total” PCB data for both techniques (Σ 128 congeners for the GC–ECD; Aroclor[®] 1254 “equivalents” for ELISA) shows a high correlation between the immunoassay and GC results ($r^2 = 0.95$, slope = 1.28, $n = 27$) (Fig. 1). ELISA results, however, were consistently lower than those obtained by GC by a factor of 0.83 (3–29%). A reduced antibody response, due to differences in the

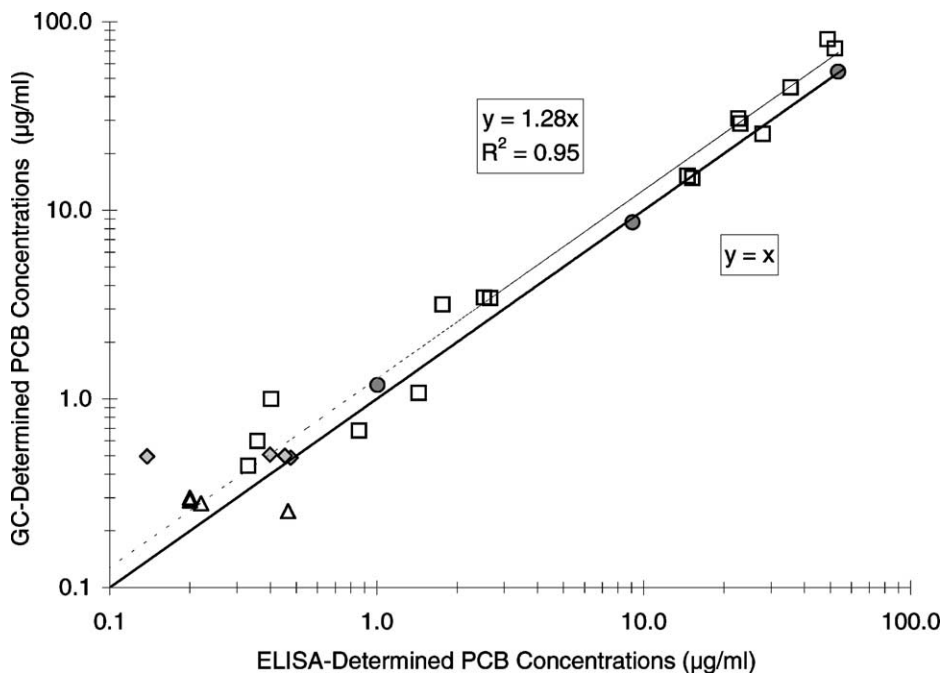


Fig. 1. Correlation between PCB concentrations in field-contaminated samples (New Bedford Harbour (\square); Whitsand Bay (\diamond)), Aroclor[®] 1254-fortified mussel tissue (\bullet) and Standard Reference Material (\triangle). GC-determined PCB concentration— Σ 128 congeners; ELISA-determined PCB concentration—Aroclor[®] 1254 “equivalents”.

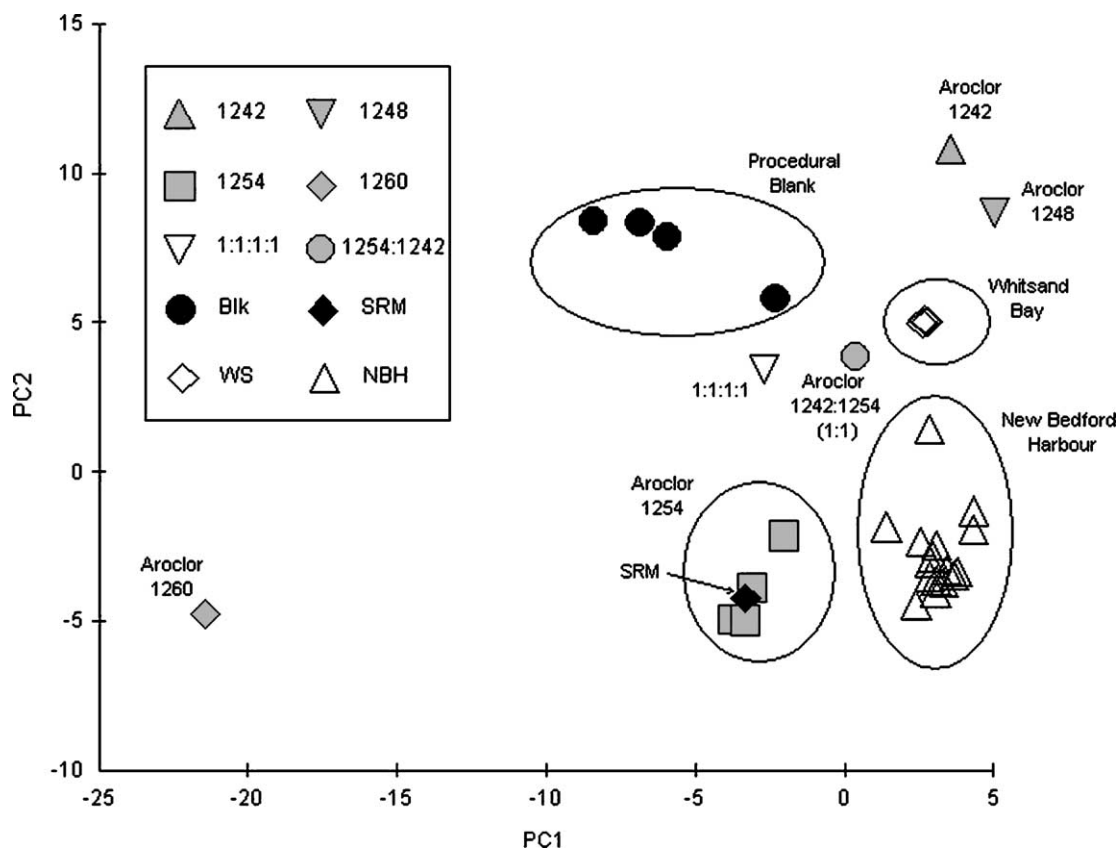


Fig. 2. PCA for Aroclor[®] mixtures and environmental samples analysed by GC–ECD. 1:1:1:1—equal parts of Aroclor[®] 1242, 1248, 1254 and 1260; 1254:1242—equal parts of Aroclor[®] 1242 and 1254; SRM—standard reference material; WS—Whitsand Bay (UK); NBH—New Bedford Harbour (USA); 1254—Aroclor[®] 1254 and Aroclor[®] 1254-fortified mussel tissue.

congener composition between the mussel extracts and Aroclor[®] 1254 (used to raise and calibrate the ELISA), provides the most likely explanation for this discrepancy.

To further investigate differences between the PCB congener compositional patterns, the GC–ECD data were subjected to PCA (PC1—29.9%; PC2—27.8%) (Fig. 2). The majority of the PCB mixtures in most mussel extracts, although differing from technical Aroclors (or their combinations), are confirmed to be most closely related to the technical Aroclor[®] 1254. Mussel samples from Whitsand Bay, however, are shown to comprise either a mixture of Aroclors or an environmentally altered technical mixture (Fig. 2). The good agreement achieved between the ELISA

and GC results is enhanced because the mussel extract PCB composition resembles that of Aroclor[®] 1254. This is supported by the fact that the samples fortified with pure Aroclor[®] 1254 exhibit ELISA/GC ratios very close to one (Fig. 1).

Our results are consistent with levels and congener patterns previously reported for New Bedford Harbour [26,29,30]. Whilst physical, chemical and metabolic processes can potentially discriminate between congeners, the GC–ECD data closely matches the patterns reported for other bivalves, sediments and water in New Bedford Harbour [26,29]. This is in agreement with negligible metabolism of PCBs by the mussels [31], although Lake et al. [26,32] do suggest enhanced accumulation of mid range congeners.

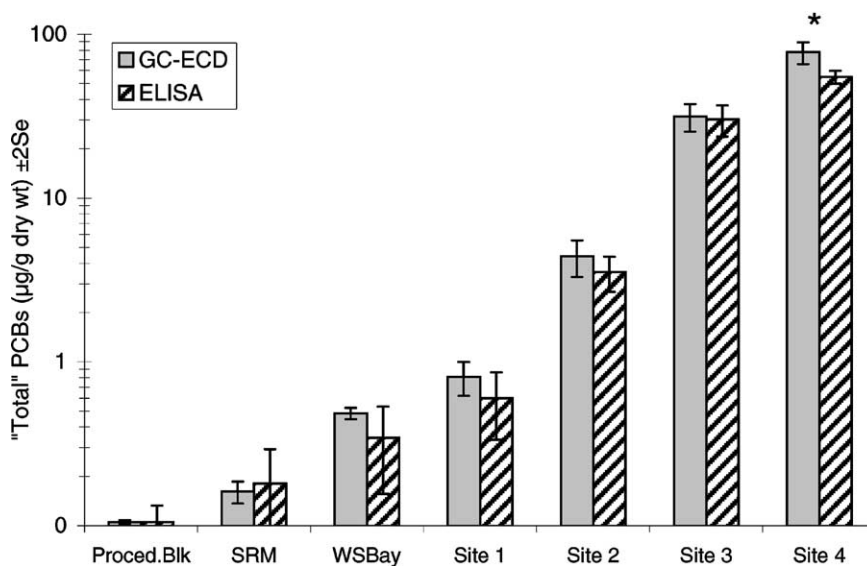


Fig. 3. Results ($\mu\text{g g}^{-1}$ dry weight) of procedural blanks, standard reference material (SRM) and environmental samples (Sites 1, 2, 3 and 4—New Bedford Harbour, USA; WSBay—Whitsand Bay, UK) analysed by ELISA and GC-ECD. Values shown are means (± 2 standard errors, $n = 4$). * — t -test $P < 0.01$. "Total" PCB—GC (Σ 128 congeners); ELISA (Aroclor[®] 1254 "equivalents").

No previous data are available for mussels from Whitsand Bay, UK.

A comparison of PCB data for the different sites and samples is presented in Fig. 3. Comparison of means (± 2 S.E.), based on four samples for each site, shows that the techniques are comparable over a wide range of concentrations. Indeed, no significant differences were detected ($P > 0.05$) with PCB concentrations up to $30 \mu\text{g g}^{-1}$. It is, however, revealed that for the samples with the highest concentrations of PCBs, whilst the trend in increased concentration is shown by both techniques, results from the two methods are statistically different ($P < 0.01$) (Fig. 3). This marginal difference probably relates to the fact that even after dilution, the measurement was made at the upper part of the calibration curve. It is notable, however, that larger differences can be observed between individual environmental samples taken from the same location [33]. Thus, the lower ELISA results could potentially relate to methodological differences, especially solvent exchange and sample dilution. It was necessary to transfer the ELISA extract from hexane to methanol. This involved taking the extract to dryness followed by redissolution of the residue

in methanol. Although this can result in losses of volatiles or problems related to dissolution of hydrophobic contaminants into a comparatively polar solvent, Zajicek et al. [4] have shown good and quantitative recoveries for this procedure with PCBs. It is also noteworthy that the ELISA diluent (50% v/v methanol/buffered solution) is polar and the solubility of hydrophobic chemicals is likely to be reduced. Li and Andren [34] measured solubilities of different PCBs (PCB 3, PCB 30, and PCB 155) in mixtures of water and methanol. Concentrations of PCBs in the extracts in the present study are generally well within the solubility limits determined by Li and Andren [34]. With the most concentrated samples (Site 4, Fig. 3), however, limits of solvation are being approached.

Finally, a second exploratory statistical procedure, similarity analysis (Primer[®]), was used to investigate the correlation between immunoassay results and individual congener distributions. It revealed the highest correlations to be between prominent congeners in the Aroclor[®] 1254 mixture and supports the conclusion that PCB ELISA results are affected by the degree of chlorination. It did not, however, identify

any specific substitution pattern to be more highly correlated.

4. Conclusions

ELISA can be used to measure “total” PCBs in hydrophobic extracts following removal of lipids and non-polar solvent, confirming recommendation of Zajicek et al. [4].

The accuracy of PCB ELISA measurements can be maximised by grouping samples with a common source of PCB contamination and by using an appropriate technical PCB mixture as the calibration standard. The reactivity of the polyclonal antibody used allows the detection of Aroclor[®] 1248, 1254 and 1260, with a good degree of agreement. In the present study, where environmental samples were contaminated with PCB patterns similar to that of Aroclor[®] 1254, consistent results are reported.

Although GC–ECD affords the capability to quantify individual congeners of differing reactivity and toxicity, the data reported indicates that ELISA analyses of mussel tissue offer a rapid general indication of the level of contamination. Even though Soxhlet extraction and partial clean-up is necessary to remove lipids, ELISA is not as time consuming or expensive as GC analyses. Because the same sample is used and extracted for both ELISA and GC–ECD analyses, initial ELISA screening can be used to identify samples appropriate for chromatography. Differences between both techniques will occur when GC results are corrected for procedural recovery (based on recoveries of the internal standards). After sample preparation, twenty quantitative ELISA analyses (in duplicate) can be obtained in <2 h. The procedure described, which involves Soxhlet extraction and partial purification, is not, however, suitable for adaptation to “on-site” monitoring of PCBs.

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