THE RELATIVE PERFORMANCE OF IMMUNOCHEMICAL (ELISA) AND GC-ECD TECHNIQUES TO QUANTIFY PCBs IN MUSSEL TISSUES

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Introduction

During the last decade, environmental immunoassays have been developed to detect selected pollutants in water and sediment/soil samples. 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].

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), and are easily adapted for use in the field.

The principles of enzyme-linked immunosorbent assays (ELISA) have been previously described [6-8]. Among several ELISA formats available, the polyclonal PCB RaPID Assay® using magnetic particle-based immunoassay was selected for this study.

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 biological tissues of exposed invertebrates (mussels).

Methods

Mussel samples were taken from four sites in New Bedford Harbour (Massachusetts, USA) (*Geukensia demissa*) and from one site in Whitsand Bay (Cornwall, UK) (*Mytilus edulis*).

PCBs were analysed using the sample preparation method modified from Kannan *et al.* [9] and Nakata *et al.* [10]. Briefly, freeze-dried mussel tissue samples (~1 g) were Soxhlet extracted into hexane/dichloromethane. The extract was transferred to a column packed with Florisil® (untreated) for lipid removal, and then eluted with acetonitrile/water. The eluant was collected in a separating funnel containing hexane and water. After shaking and phase separation, the hexane layer was concentrated. 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]). In the PCB RaPID Assay®, sample extracts in methanol were diluted and incubated with a PCB conjugate and solid phase polyclonal anti-PCB antiserum linked to magnetic particles. Following magnetic field separation, the proportion of enzyme-labelled conjugate was measured after the addition of the chromogenic substrate by its absorbance at 450nm. The kit was configured using Aroclor® 1254 calibration standards. Sample concentrations were calculated using a log-logit curve and

are expressed as Aroclor® 1254 "equivalents". Samples for chromatography were treated with concentrated sulphuric acid and then cleaned-up and fractionated using activated Florisil® Quantification of PCBs was performed using a GC (Hewlett Packard 6890 series) equipped with an ECD.

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, 11]; 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 [11]. Results for the ELISA are, therefore, compared with GC-ECD results to better understand the ELISA response to environmental PCB compositions.

Performance of ELISA

Matrix effects were carefully studied. A preparatory clean-up was selected to remove the bulk of extraneous lipids. Ten mussel sample extracts were diluted and then analysed by ELISA, providing evidence that no significant matrix effects were present using the selected analytical conditions.

The method detection limit (MDL), as estimated at 90% B/Bo for the Aroclor® 1254 calibration dilutions, was 0.08 ng mL^{-1}. The 50 % B/Bo (concentration required to inhibit one-half of the colour produced by the negative control) was 2.9 ng mL^{-1} (Table 1). The coefficient of variation (%CV) within the assay was less than 11 ± 4 % (n = 10), which is similar to conventional analytical variability.

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. 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 [4, 5, 11].

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

Aroclor®	% Chlorination	MDL[a] (ng mL^{-1})	50%B/Bo[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 standard deviation (n = 3)

[a] MDL – method detection limit (90 % B/Bo)

[b] 50 %B/Bo –concentration required to inhibiting one-half of the colour produced by the negative control.

Comparison of results from ELISA and GC-ECD

In total, twenty-seven samples of mussel tissue were analysed by ELISA and GC-ECD. These included mussels from natural populations, tissues fortified with Aroclor® 1254, and replicates of a certified standard reference material (SRM 2977). 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. 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®.

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,) (Fig. 1). ELISA results, however, were consistently lower than those obtained by GC by a factor of 0.83. 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 discrepancy.

To further investigate differences between the PCB congener compositional patterns, the CG-ECD data were subjected to principal component analysis (PCA). 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. The good agreement achieved between the ELISA and GC results is enhanced because the mussel extract PCB composition resembles that of Aroclor® 1254.

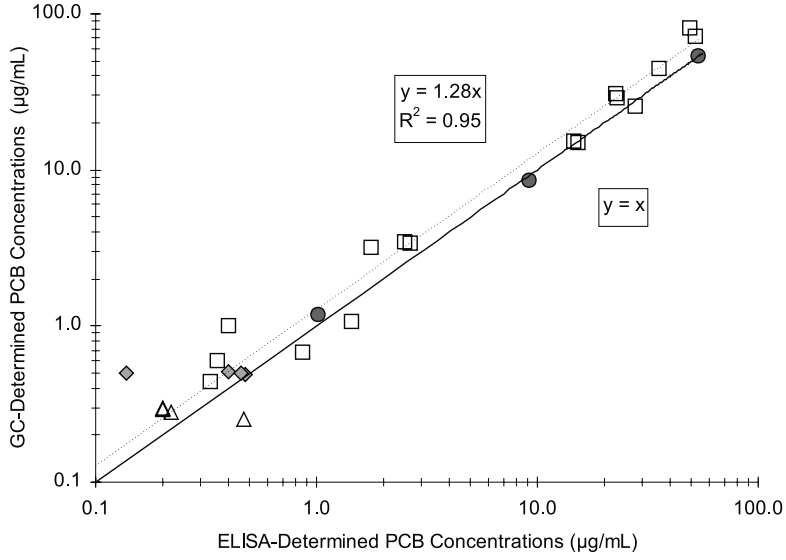

Figure 1. Correlation between PCB concentrations in field-contaminated samples (New Bedford Harbour (□); Whitsand Bay (◆)), Aroclor® 1254-fortified mussel tissue (●) and Standard Reference Material (△). GC-determined PCB concentration - Σ 128 congeners; ELISA-determined PCB concentration – Aroclor® 1254 "equivalents".

ANALYSIS II

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.

Conclusions

ELISA can be used to measure "total" PCBs in hydrophobic extracts following removal of lipids and non-polar solvent.

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).

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