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Comparison of an enzyme-linked immunosorbent assay (ELISA) to gas chromatography (GC) – measurement of polychlorinated biphenyls (PCBs) in selected US fish extracts

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Abstract

The analysis of PCBs in fish tissues by immunoassay methods was evaluated using fish collected from a US monitoring program, the National Contaminant Biomonitoring Program of the US Department of Interior, Fish and Wildlife Service. Selected composite whole fish samples, which represented widely varying concentrations and sources of PCBs, were extracted and subjected to congener PCB analysis by gas chromatography (GC) and total PCB analysis using an ELISA (ePCBs) calibrated against technical Aroclor 1248. PCB congener patterns in these fishes were different from the patterns found in commercial Aroclors or their combinations as demonstrated by principal component analysis of normalized GC congener data. The sum of the PCB congeners measured by GC (total-PCBs) ranged from 37 to 4600 ng/g (wet weight). Concentrations of PCBs as determined by the ELISA method were positively correlated with total-PCBs and the ePCBs/total-PCBs ratios for individual samples ranged from 1 to 6. Ratios of ePCBs/total-PCBs for dilutions of Aroclors 1242, 1254, and 1260 and for matrix spikes range from 0.6 for 1242 to 2.5 for 1254 and 1260. These results suggest that higher chlorinated PCB congeners have higher affinity for the anti-PCB antibodies. Partial least squares with latent variable analysis of GC and ELISA data of selected Aroclors and fish samples also support the conclusion that ELISA derived PCB concentrations are dependent on the degree of chlorination. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: ELISA; Immunoassay; PCB congeners; NCBP fish; Principal component analysis

1. Introduction

Polychlorinated biphenyls (PCBs) are common hydrophobic contaminants that continue to be of environmental concern due to their chronic toxicity to fish, wildlife, and humans. Their physical/chemical proper-

ties, toxicities, distributions, and analysis have been the subject of several books and recent reviews (Erickson, 1986; Safe, 1990; Ahlborg et al., 1994). Cost and time limitations of instrumental methods make detailed studies of contaminated sites difficult (Harrison and Melnychuk, 1995; Lesnik, 1994). Enzyme-linked immunosorbent assay (ELISA) kits currently provide an alternative means for estimation of total PCB contamination (ePCBs) in soil samples (Harrison and Melnychuk, 1995; Lawruk et al., 1996), water samples (Lawruk et al., 1996; Harrison and DeBaise, 1994), and

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to a lesser extent for biological matrices, such as fish tissue (Richter et al., 1994). Although ELISA has been used successfully in simple field methods for the determination of PCBs in water (Lawruk et al., 1996; Harrison and DeBaise, 1994) and soils (Harrison and Melnychuk, 1995; Lawruk et al., 1996), a logical extension of the ELISA method is to adapt it for determination of PCB concentrations in fish tissue extracts. Others have developed sensitive and selective ELISAs for hydrophobic contaminants, such as, chlorinated cyclodienes (Stanker et al., 1991), polychlorinated dibenzo-p-dioxins (Sherry et al., 1989), and permethrin (Stanker et al., 1989) in biological tissue extracts, but in each case the success of the method was dependent on extensive sample preparation prior to the ELISA measurements. In all instances, the ELISA determinative steps retained their inherent benefits of simplicity, low cost, and speed. However the method, from extraction to analysis, was still relatively complex. We chose to evaluate the use of a commercial ELISA as a means to screen extracts from our classical sample preparation procedure for PCBs (Steingraeber et al., 1994). Extending an ELISA methodology to PCB determination in tissue extracts could provide considerable cost and time savings.

The specific objectives of this research were to: (a) determine any bias in the ELISA associated with our sample preparation methods, (b) determine the range of ELISA responses for PCB contaminated fish extracts related to the responses of selected technical PCB mixtures (Aroclor[®]s), (c) use principal component analysis (PCA) to statistically relate patterns of individual congeners of PCBs in fish to those found in the Aroclors, and (d) use partial least squares (PLS) with latent variables analysis to model the relative contributions of individual PCB congeners to the measured ELISA responses of Aroclors and fish extracts.

2. Experimental

2.1. Materials and samples

Aroclors. Stock solutions (mg/ml) of selected technical PCB mixtures (Aroclor[®]s: 1242, 1248, 1254, and 1260) were prepared in methanol and in iso-octane from neat materials received from the manufacturer (Monsanto Corporation, St. Louis, MO). Working dilutions of Aroclor 1248 ranging from 0.099 to 2.00 µg/ml were prepared in methanol for ELISA calibration. Similar dilutions of the other Aroclors were also prepared in methanol for determination of cross-reactivities relative to Aroclor 1248. The concentrations of total PCBs in the methanol stock solutions were verified by GC analysis of their iso-octane dilutions. Working dilutions of individual Aroclor stocks were prepared in iso-octane for GC

calibration as individual Aroclors and as an equal-part mixture of all four Aroclors used to measure 105 individual PCB congeners.

PCB congeners. The PCB congeners were numbered according to the convention of Ballschmiter and Zell (1980) and as subsequently revised by Ballschmiter et al. (1992). A stock solution of 2,4,6-trichlorobiphenyl (PCB 030, Ultra Scientific) was prepared in iso-octane as the surrogate congener used for GC determinations. A stock solution of ¹⁴C-2,2',4,4',5,5'-hexachlorobiphenyl (¹⁴C-PCB 153) in iso-octane was obtained from Pathfinders Laboratories, St. Louis, MO. For working dilutions of the PCB congeners we either directly diluted microliter volumes of the iso-octane stock solutions into methanol, or we exchanged the solvent from iso-octane to methanol in a manner similar to that of Zajicek et al. (1996). We used GC analyses to verify that PCB 030 was quantitatively recovered from the solvent exchange procedure, and liquid scintillation counting (LSC; Model LS3801, Beckman Instruments) was used to verify the recovery of ¹⁴C-PCB 153 through the solvent exchange procedure.

NCBP fish sample extracts. Whole body extracts were prepared from a subset (28 of 117 monitoring stations) of the US Department of Interior, Fish and Wildlife Services's National Contaminant Biomonitoring Program (NCBP) (Schmitt et al., 1990) fish collected in 1988 (Fig 1 and Table 1). A variety of quality control samples were also prepared including procedural blanks, matrix blanks prepared from hatchery fish raised at the US Geological Survey's Columbia Environmental Research Center, and matrix blanks each fortified with about 160 ng of one of the individual technical Aroclors/g (wet weight). Briefly, measured amounts of fish tissue samples (typically 30 g) were mixed with sodium sulfate (120 g), placed in 4 cm i.d. glass columns, and extracted with 300 ml of methylene chloride (Steingraeber et al., 1994; Zajicek et al., 1996). Columns containing fish tissue mixed with sodium sulfate were fortified with the surrogate congener, PCB isomer 030 (500 ng), and matrix spikes also received 5.0 µg of technical Aroclors (/spike) prior to the addition of the extraction solvent. Crude methylene chloride extracts were concentrated to 10 ml and cleaned up by two-stage reactive chromatography using multi-layer columns. The first column (2 mm i.d.) containing 6 cm (~19 ml) of coarse 30% (w/w) sulfuric acid-silica gel (SA-SG, 60–100 mesh), 10 cm (~31 ml) of standard 40% (w/w) SA-SG (70–230 mesh), and 10 cm (~31 ml) of potassium hydroxide-impregnated silica gel (KS-SG) was eluted with 150 ml of methylene chloride. After concentrating the eluates to 3 ml, the extracts were further purified by passing through a second column (1 cm i.d.) containing from top to bottom 1 cm (~0.8 ml) 40% (w/w) SA-SG and 5.0 g of silica gel. The PCBs were eluted from these absorbents with 60-ml portions of 0.5% benzene/99.5% hexane (v/v). Most pesticides and other potential analytical interferants were removed by

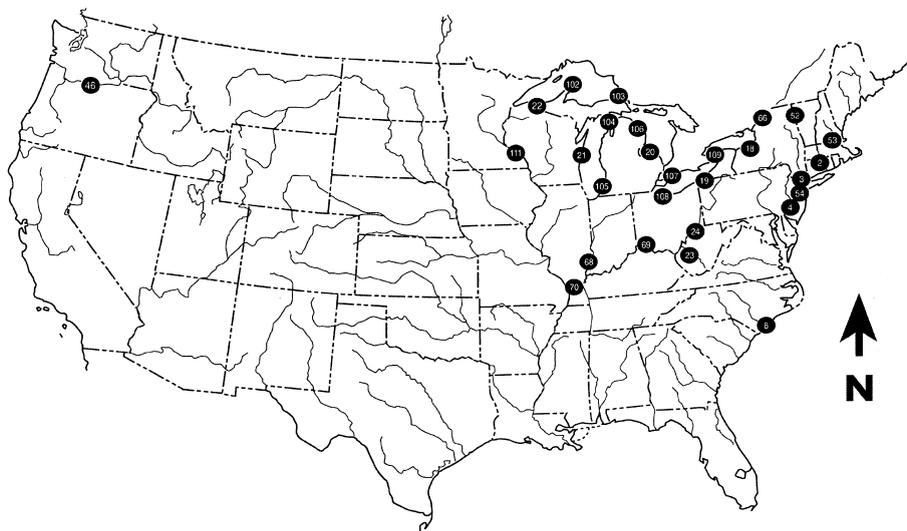


Fig. 1. National Contaminant Biomonitoring Program Stations (numbered circles) selected for detailed analysis of PCB congener concentrations and ELISA analysis of total-PCBs.

the adsorbents. Clean sample extracts, concentrated and solvent exchanged to isooctane, were either analyzed by GC, or aliquots (20–200 μ l) were transferred and the solvent exchanged to methanol as described above for the PCB congeners (Zajicek et al., 1996). These extracts were diluted with methanol as required for the ELISA measurements. The extract of common carp (*Cyprinus carpio*) collected from station 69 on the Ohio River at Cincinnati, OH was analyzed by GC, only (Table 1). Extracts of fish from the remaining 27 stations (Table 1) were selected for PCB analysis using both the GC and the ELISA methods.

Measurements and data analysis. Clean fish extracts were analyzed for 105 PCB congeners by GC using a 57 m \times 0.25 mm (id) capillary column with a DB-5 bonded phase (J&W Scientific) and hydrogen as carrier gas (Steingraeber et al., 1994), while commercial ELISA kit reagents were used to measure total PCB concentrations, [ePCBs] (Millipore Corporation, 1993). The PCB competitive ELISA kit (EnviroGard™ Test Kit PCB, 1 ppm) reagents, in a coated-tube format and configured with Aroclor 1248 calibration standards, were supplied by Millipore and used according to the manufacturers recommendations for methanolic solutions (Millipore Corporation, 1993). For our studies we chose to use the ELISA kits as routinely configured by the manufacturer. Briefly, individual sample extracts in methanol (25- μ l aliquots) were analyzed together with five calibration standards: 0, 0.1, 0.25, 0.5, and 1.0 μ g/ml in batches of 20 tubes. Dilutions of samples or calibration standards were incubated in polystyrene assay tubes containing immobilized polyclonal anti-PCB antibodies at the bottom ends. The sample matrix was washed

away, leaving only the PCBs bound to the antibodies. Then, a solution of a PCB enzyme conjugate, a horseradish peroxidase labeled PCB (PCB-HRP), was added to the tubes and the PCB-HRP allowed to bind to free anti-PCB sites on the immobilized antibodies. Unbound PCB-HRP was washed away to leave an amount of HRP enzyme that was inversely proportional to the PCB concentration in the samples or standards. Finally, a colorless substrate and chromogen were incubated with the bound enzyme to produce a blue color that was inversely proportional to the concentration of PCBs. The stop solution was added to change the color to a stable yellow. ELISA absorbances at 450 nm were measured with a Differential Photometer (Millipore Corporation), absorbance data (B) were related to that of the zero standard (B_0), and ePCBs concentrations were calculated from the semi-log relation: $B/B_0 = \text{Slope}^x \text{Log}[e\text{PCBs}] + \text{Intercept}$ using Microsoft™ Excel Software. PCA was used to compare PCB congener distributions in fish samples to the PCB congener distributions of four reference Aroclors, and PLS analysis was used to model relative PCB congener contributions to the ELISA results (Wold et al., 1984). For these statistical analyses we used Soft Independent Modeling of Class Analogy (SIMCA-S for Windows, version-6.0) pattern recognition software (Umetri AB, Umeå, Sweden).

3. Results and discussion

PCB congener patterns in contaminated soils often resemble those of the commercial PCB mixtures, and as

Table 1
Total PCB concentrations in selected NCBP fish samples collected in 1988 as determined by gas chromatography

Station # ^a	Species code ^b	River or Lake	Locations	Total-PCB ^c (µg/g)
2	CHC	Connecticut R.	Windsor Locks, CT	1170
3	WSU	Hudson R.	Poughkeepsie, NY	2670
4	CHC	Delaware R.	Trenton, NJ	1170
8	CHC	Cape Fear R.	Elizabethtown, NC	480
18	WSU	L. Ontario	Port Ontario, NY	1290
19	WSU	L. Erie	Erie, PA	440
20	C	Saginaw Bay	Bay Port, MI	740
21	LT	L. Michigan	Sheboygan, WI	2260
22	LT	L. Superior	Bayfield, WI	300
23	CHC	Kanawha R.	Winfield, WV	2120
24	C	Ohio R.	Marietta, OH	3580
46	BRB	Columbia R.	Cascade Lock, OR	40
52	WSU	L. Champlain	Burlington, VT	70
52	NP	L. Champlain	Burlington, VT	170
53	WSU	Merrimack R.	Lowell, MA	610
54	WSU	Raritan R.	Highland Park, NJ	1460
66	WSU	St. Lawrence R.	Massena, NY	210
68	C	Wabash R.	New Harmony, IN	620
69	C	Ohio, R.	Cincinnati, OH	2550
70	C	Ohio R.	Metropolis, IL	1340
70	CHC	Ohio R.	Metropolis, IL	1550
102	LT	L. Superior	Keeweenaw, MI	3700
103	LT	L. Superior	Whitefish Point, MI	550
104	LT	L. Michigan	Beaver Island, MI	1100
105	LT	L. Michigan	Saugatuck, MI	2170
106	LT	L. Huron	Alpena, MI	1720
107	C	L. St. Clair	Mt Clemons, MI	3940
108	C	L. Erie	Port Clinton, OH	620
109	LT	L. Ontario	Roosevelt Beach, NY	4620
111	C	Mississippi R.	Lake City, MN	1970

^a Stations as designated by the NCBP.

^b CHC, channel catfish (*Ictalurus punctatus*); WSU, white sucker (*Catostomus commersoni*); C, common carp (*Cyprinus carpio*); LT, lake trout (*Salvelinus namaycush*); BRB, brown bullhead (*Ictalurus nebulosus*); NP, northern pike (*Esox lucius*).

^c The sum of the individual PCB congener concentrations determined by the GC method described below.

a result, ELISA determinations of PCBs in soils have been calibrated against technical Aroclor[®] 1242, 1248, 1254, and 1260 standards (Harrison and Melnychuk, 1995; Lawruk et al., 1996). However, Schwartz et al. showed that PCB congener distributions in some fish and turtles did not resemble those of the commercial PCB mixtures (Schwartz et al., 1987). It was their suggestion that the observed differences in PCB congener distributions were due to environmental and metabolic alterations in the compositions of the congeners. Therefore, we chose to measure the relative cross-reactivities of four commonly used technical Aroclors and two individual PCB congeners to understand better the relationships between the PCB ELISA response and the congener compositions of environmental PCB mixtures.

PCB congener 030 cross-reactivity. Comparison of ELISA with GC results required evaluation of the ELISA response of the GC surrogate, PCB 030. Analysis of PCB congener 030 (dilutions) prepared in methanol demonstrated that cross-reactivity relative to

Aroclor 1248 was 24% and that the 50% *B/B*₀ concentration was 0.97 µg/ml. Therefore, the amount of PCB 030 added to samples produced no measurable ELISA response, since the dilutions for ELISA contained ≤ 0.1 µg/ml of PCB 030. Initially we had prepared methanolic dilutions of congener 030 by direct dilution of microliter volumes of an isooctane stock solution. As a control, we also added 1 µl of isooctane to a replicate 200 µl aliquot of the 0.5 µg/ml Aroclor 1248 standard to check for any bias that isooctane might contribute to the ELISA (Zajicek et al., 1996). However, the results of this experiment were inconclusive because the 0.5% (v/v) isooctane in the methanolic Aroclor 1248 standard negatively biased the PCB ELISA result by 78% (Zajicek, unpublished data). This effect of isooctane was further studied and is described below.

Isooctane effect on ELISA response. Methanolic solutions of ¹⁴C-PCB 153 (168 ng/ml), containing 0.1–1% (v/v) isooctane, were used to examine the negative bias of isooctane on the PCB ELISA. These results indicated

that 1% isooctane in methanol inhibited the binding of ^{14}C -PCB 153 by as much as 96% (Fig. 2). In other words, the ELISA absorbance measured for the 168 ng/ml PCB 153 standard (1% isooctane) was 96% higher than that of the equivalent standard without isooctane. The measured absorbance was nearly equivalent to that of the zero calibrator. Parallel measurements by liquid scintillation counting (LSC) independently verified that the ^{14}C -PCB 153, unbound to antibody, was indeed free in the sample solution and not bound to the upper, antibody-free walls of the assay tubes. Apparently, isooctane was in some way interfering with the binding reaction between ^{14}C -PCB 153 and the anti-PCB antibodies. We did not attempt to further characterize the physical/chemical nature of the interaction(s) between isooctane, the anti-PCB antibodies, and ^{14}C -PCB 153. Such interaction(s) may involve direct binding of isooctane molecules to the anti-PCB binding site. However, it is much more likely that small amounts of isooctane act in a non-specific way(s) to disrupt (or modify) critical hydrophobic interactions within the immunoassay environment. Stevens (1991) has provided an excellent summary of the physical chemistry of protein interactions with solid phases and with other proteins. Such interactions provide the energy of binding for adsorption of hydrophobic portions of proteins to hydrophobic polystyrene tube walls (Tanford, 1979). They provide the energy to stabilize protein structure through associations of hydrophobic regions within antibody molecules (Miller et al., 1987; Kellis et al., 1988) and for the interactions between hydrophobic surfaces on protein subunits (Janin et al., 1988). For small hydrophobic molecules, such as PCBs, the actual high avidity binding to the antibody active site may be driven by the energy from hydrophobic interactions. Also, at low concentrations, isooctane can form hydrophobic micro-droplets in the typical aqueous-methanolic binding medium that act to selectively partition small hydrophobic molecules, such as ^{14}C -PCB 153 out of the aqueous medium

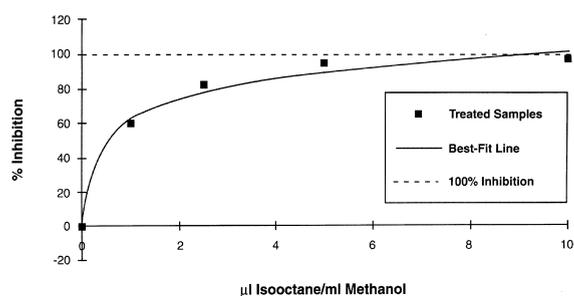


Fig. 2. Inhibition of the ELISA response toward PCB 153 by increases in concentration of isooctane in methanolic samples. Inhibition of ELISA response corresponds to increased absorbance at 450 nm. PCB 153 concentration was held constant at 168 ng/ml.

(Zajicek et al., 1996). Regardless of the actual physical chemistry involved, our results indicate that small percentages of isooctane in methanolic samples result in a relatively large negative ELISA bias. Even as little as 1 μl of isooctane dissolved in 1000 μl of methanol (0.1% (v/v) isooctane) is sufficient to inhibit the ELISA response by as much as 50%. Subsequent to our work, Millipore changed the assay procedure to one in which the sample and PCB-HRP were incubated together (Millipore Corporation, 1995). Both sensitivity and interferences were reduced by using this latter procedure for routine soil analysis (Harrison, unpublished data). However, this new procedure has not been tested for the isooctane solvent interferences shown above. Previously, Zajicek et al. (1996) reported a similar interference in a PCB ELISA based on antibody-coated magnetic particles; an immunoassay in which the sample and enzyme-labeled PCB are incubated together. Taken together, these findings indicate that it is critical when using high sensitivity PCB ELISA analyses to design solvent exchange procedures that avoid retention of traces of hydrophobic solvent (e.g., isooctane), while simultaneously maximizing the recovery of the analyte.

ELISA responses of fish extracts. Having established that the cross reactivity of PCB 030 was trivial and after removing isooctane from aliquots of sample extracts, we prepared methanolic dilutions of fish extracts from 27 NCBP stations and selected quality control samples. The results of PCB ELISA analyses of the quality control samples including: procedural blanks, matrix blanks, and extracts of fish tissue fortified with technical Aroclors indicated that relative cross reactivity ratios (apparent [ePCB] relative to Aroclor 1248) of the matrix spike samples were (Fig. 3): 1242 (0.5), 1248 (1.0), 1254 (2.5) and 1260 (2.5). The PCB ELISA was calibrated using Aroclor 1248 standards, therefore a sample containing 0.16 $\mu\text{g}/\text{ml}$ of Aroclor 1260 appeared to have 0.40 $\mu\text{g}/\text{ml}$ of ePCBs based on calibration against Aroclor 1248 (Fig. 3). These results are similar to those reported by Harrison et al. (1991) and Carlson (1995) for the relative cross-reactivities of these Aroclors with the Millipore EnviroGard ELISA. In general, as the percent chlorination of Aroclors increases, so does their content of congeners chlorinated in the 2-, 4-, 5-, 2', 4', and 5'-positions (Brinkman and de Kok, 1980; Mullin et al., 1984) and the ELISA cross-reactivity. (Carlson, 1995) tested selected PCB congeners, and his conclusion was that chlorination at the 2,5 and 2',5'-positions of the biphenyl molecule increases cross-reactivity (or binding to the anti-PCB antisera) in the EnviroGard PCB ELISA. Conversely, Carlson also found that lack of chlorination at the ortho-biphenyl positions or the 5, 5' positions resulted in decreased cross-reactivity (and antibody binding) in the ELISA (refer also to Richter et al., 1994 for selected Aroclor and PCB congener cross-reactivity data). These conclusions were also consistent

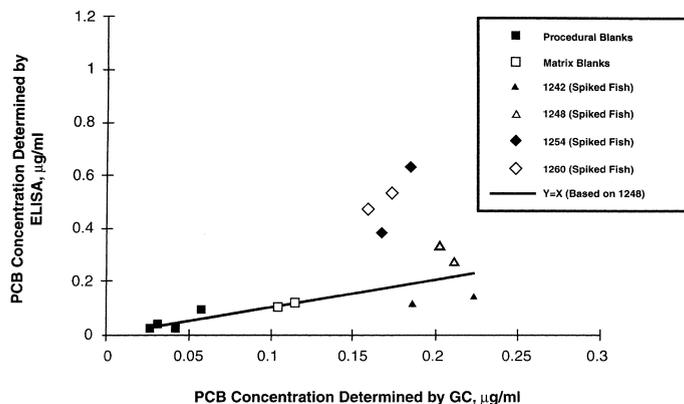


Fig. 3. Comparison of PCB concentrations determined by ELISA and GC for quality control samples. $Y = X$ line is included to show where samples containing only the mixture of PCB congeners found in Aroclor 1248, the PCB mixture used for ELISA calibration, would appear.

with the expected binding specifying of the polyclonal anti-PCB antibody mixture, based on the design of the PCB-hapten and the resulting PCB-immunogen (Carlson, 1995). Our ELISA results are consistent with the fact that technical Aroclors with 54–60% chlorination have relatively high concentrations of those congeners that have been shown to most strongly bind to the EnviroGard PCB ELISA antibodies.

Measured concentrations of ePCBs ($\mu\text{g/ml}$) were positively correlated to GC-determined concentrations of total PCBs ($\mu\text{g/ml}$) for NCBP fish extracts (Fig. 4). The relationship was defined as $[\text{ePCBs}] = -0.084 + 3.99[\text{total-PCBs}]$ with an $R^2 = 0.75$. Thus, the characteristic ELISA response of the fish samples was about 4-fold that of a comparable concentration of Aroclor 1248. The ratios of ELISA-determined to GC-determined PCB concentrations ($[\text{ePCBs}]/[\text{total-PCBs}]$) for these fish extracts ranged from less than 1 to about 6 (Fig. 4). As stated earlier this ratio was 1.0 for Aroclor

1248, 0.5 for Aroclor 1242, and 2.5 for both Aroclor 1254 and 1260 when the ELISA was calibrated with Aroclor 1248. Therefore, it seemed likely that the patterns of the PCB congeners of these fish samples were different from those of the technical Aroclors tested. It may have been that the PCB residue mixtures in some samples were selectively enriched in those congeners that bound most strongly to the ELISA's anti-PCB antibody. For these reasons, we compared the congener patterns of PCBs among fish samples and Aroclor standards by PCA.

PCA of PCB congener patterns. PCB congener patterns were analyzed by principal component analysis (PCA). Concentration-normalized PCB congener data was used to create variable loading terms (β) and principal component terms (τ), from which a three-dimensional sample similarity plot was prepared (t_1 vs. t_2 and t_3). Schwartz et al. (1987) previously used PCA modeling of PCB congener patterns determined by GC to show that PCB congener patterns in selected fish and turtle samples from a freshwater tidal marsh on the Pennsylvania shore of L. Erie (Tinicum Marsh) were different from those of technical Aroclors or their combinations. The results of our application of PCA modeling to the PCB congener patterns in the NCBP fish extracts showed that the Aroclors (and clean fish tissue spiked with them) defined the four vertices of a pyramidal volume in three-dimensional space that included Aroclors and their combinations (Fig. 5). NCBP fish samples were not modeled within the Aroclor volume, but instead extended outward in n -space from the triangular face (hashed surface) defined by Aroclors 1248, 1254, and 1260 showing that their PCB congener patterns were much different from those of commercial Aroclors, and most different from those of Aroclor 1242. These pattern recognition results provided a statistically rigorous comparison to those of Schmitt et al. (1990,

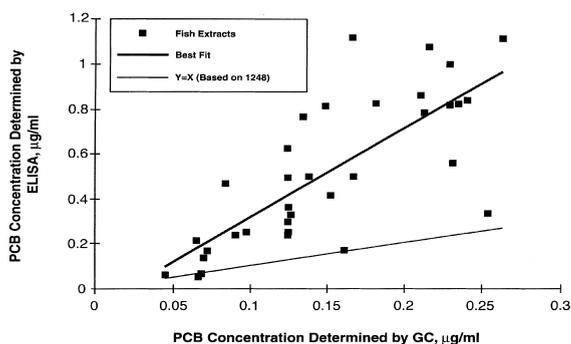


Fig. 4. Comparison of PCB concentrations determined by ELISA and GC for dilutions of fish extracts. Samples whose PCB mixtures have cross-reactivities greater than 1.0 relative to Aroclor 1248 appear above the $Y = X$ line.

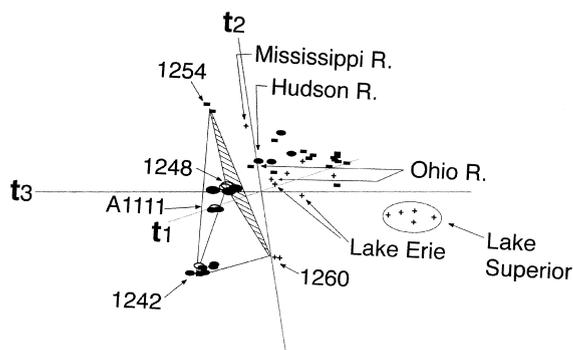


Fig. 5. 3-D PCA sample-similarity Score Plot of PCB congener patterns of 1988 NCBP fish samples and Aroclors. In this projection, the scores of the first (t_1), second (t_2), and third (t_3) principal components are combined to generate a three-dimensional image of the similarities among Aroclors and fish sample patterns. The largest points are closest to the observer.

1985) for PCB analyses of earlier fish samples from these same NCBP sites. Schmitt et al.'s PCB mixture characterizations and measurements were based on calculations that used a series of simultaneous linear equations to systematically account for relative PCB congener (GC peak) contributions in technical Aroclor mixtures. PCB congeners uniquely attributable to Aroclor 1242 were not detected in fish from these studies, while a majority of the PCB residues were characterized as mixtures of Aroclor 1254, 1260, and to a lesser extent 1248. These earlier calculations assumed that the PCB residues extracted from NCBP fish could be described as mixtures of Aroclors, but this hypothesis could not be statistically tested by their methods.

The reasons why the congener patterns of PCBs in these fishes were different from the technical Aroclor standards tested are not completely known. However, it is known that the fish collected for this study came from different locations, and thus represent different sources of PCBs. Additionally, the physical/chemical properties of the various PCB congeners impart differing environmental transport and transformation properties to these congeners (Mackay et al., 1992). The other key factors contributing to the patterns of PCB congeners observed in fishes are the differential rates of uptake and elimination. It is interesting to note that the congener patterns of PCBs in fish exposed to differing sources, in widely differing environments, and among different species had mainly the same pattern of PCB congeners. The only largely differing group from this broad statement is that composed of the lake trout from Lake Superior. (Fig. 5). The PCBs found in Lake Superior are thought to be primarily a result of atmospheric loading to the lake, with a smaller percentage of direct input as compared to the lower Great Lakes (Eisenreich et al., 1997). The sorting of PCB congeners, which occurs

through differential atmospheric transport rates, may be an important factor why the patterns of PCB congeners in these fish are separated from the other fish patterns in this study.

PCB congener contributions to measured ELISA response. Partial least squares (PLS) modeling with latent variables analysis was used to estimate the relative contributions of individual PCB congeners to the observed ELISA responses. PLS analysis finds the relationship between the block of Y dependent variables and a block of X independent or predictor variables expressed as: $Y = f(X) + E$. For cases with a single dependent variable the analysis is similar to multiple linear regression without the restriction of linearity. For our analysis the Y data matrix was composed of a single normalized ELISA response for each sample, the ePCB/total-PCB concentration ratio. The X data matrix was composed of the samples' (Aroclors and fish) corresponding GC derived PCB congener data. The PCB congener data had been first normalized to fractional composition to remove the concentration differences among the congeners of each sample, then normalized to unit variance. The PLS analyses of PCB data from Aroclor standards, clean fish tissue fortified with Aroclors, and the corresponding ELISA responses were used to cross-validate two principal components.

Together, these two principal components accounted for 87% of the X -space variability and 92% of the Y -space variability. A score plot showed that there was a linear relationship ($R^2 = 0.92$) among these Aroclor samples, generally increasing with percent chlorination (Fig. 6). The relative importance of individual PCB congeners to the Aroclor–ELISA PLS model was examined by plotting the combined-variable loading

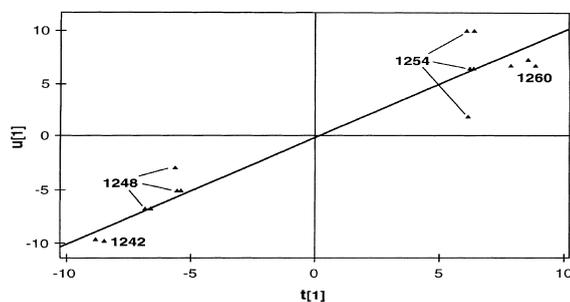


Fig. 6. PLS Score Plot for technical Aroclors, fish fortified with Aroclors, and ELISA responses for these samples. In PLS modeling, one space is used to geometrically represent the X -data matrix and a second space for the Y -data matrix. PLS then develops lines, planes or hyper-planes in the two spaces to (a) well fit the data in the two spaces, and (b) have the planes in the projection planes – the X -scores (t) and the Y -scores (u) – well correlated. This plot shows the samples (PCB congener data from Aroclors and Aroclor spikes) in the projected $X(T)$ -space and the responses (ELISA/GC ratios).

factors or variable weights ($w^*c[n]$ s for the combined X - and Y -space variables). The variable loadings were negatively correlated for Cl_2 - and Cl_3 -PCB congeners and Cl_5 - and Cl_6 -PCB congeners (lower left and upper right quadrants of Fig. 7). In contrast, variable loadings were positively correlated for Cl_4 -PCB congeners and Cl_7 - to Cl_9 -PCB congeners (upper left and lower right quadrants of Fig. 7). Beyond these general tendencies related to the degree of chlorination, we were unable to identify any consistent PCB congener substitution patterns that were most important to determining the measured ELISA responses. PLS modeling of the entire fish sample data set failed to provide any reduction in the dimensionality of the data spaces. Although, the dimensionality of a data space that included only the lake trout and channel catfish samples was reduced by PLS modeling (two principal components identified), the percentage of the X - and Y -data space variabilities included in the model was only about 50%, and these results are not presented. Another predictive approach would be to measure the responses for a representative subset of the individual compounds (e.g., PCB congeners), and then use PLS modeling to identify a predictive relationship for the entire set of chemically related compounds, similar to what Tysklind et al. (1994) did for polychlorinated dibenzofuran and dibenzodioxin compounds. Whether using Tysklind et al.'s approach or the one we have described, PLS modeling of individual congener contributions to the ELISA response of mixtures should be more certain for immunoassays based on monoclonal antibodies that are often more selective than those based on polyclonal antibodies. Finally, Carlson

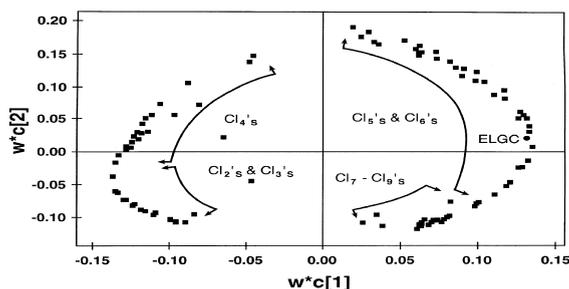


Fig. 7. PCB congener contributions (combined-loadings, w^*c 's) to the ELISA response model of Aroclors and Aroclor-spiked fish. This plot displays the correlations between the X and the Y variables. The w 's and c 's are the weights that combine the x and y variables to form the scores t and u . These weights are selected so as to maximize the correlation between T and U , thereby indirectly Y . X variables with large w^*c 's (positive or negative) are highly correlated with $U(Y)$ or ELISA responses. Arc's with radial arrows at the ends show the locations of PCB congener homologs designated as Cl_n : where n is the number of chlorines in the PCB homolog series. PCB congeners that are radially closer to the w^*c origin (0,0) belong to the homolog grouping defined by the nearest arc.

and Carlson (1998) recently reported the antibody (binding) affinity values of all 209 PCB congeners using a new monoclonal antibody-based, coplanar congener EIA (Enzyme ImmunoAssay). Data such as these will be useful in further demonstrating the predictive power of the above described PLS modeling approaches.

4. Summary and recommendations

We estimate that ELISA analysis of methanolic dilutions of samples prepared using traditional sample preparation techniques for gas chromatographic or multiple analysis will be negatively biased if isooctane is present at greater than 0.01–0.03 (v/v)%. This estimate is based on an extrapolation of our isooctane interference data shown in Fig. 2. This may be a general effect of hydrophobic solvents and co-extracted lipids on the ELISA of hydrophobic analytes (Zajicek et al., 1996). Prior to ELISA analysis of samples, the isooctane should be exchanged to methanol, or the sample extract in isooctane should be diluted about 10,000-fold or greater into methanol. This should adequately reduce the proportion of isooctane in the assayed dilution.

The ELISA response of PCB mixtures of technical Aroclors, PCB residue mixtures in fish extracts, and individual PCB congeners is generally dependent on their degree of chlorination. PCB mixtures in fish extracts are statistically different from those of technical Aroclors. The range of ELISA responses (cross-reactivities) for NCBP fish extracts encompasses that of the technical Aroclors and extends up to about twice that of the most reactive, Aroclor 1254. The highest ELISA cross-reactivity observed for these fish extracts is similar to that of 2,2',4,4',5,5'-hexachlorobiphenyl (5- to 10-fold, our data); a close structural analog of the immunogen used to generate the polyclonal antibodies that form the basis of this PCB ELISA.

From the ELISA cross-reactivities we measured in fish tissue extracts from 27 sites across the US, the range of cross-reactivity ratios relative to that of Aroclor 1248 was between about 0.5- to 6.5-fold. At the same time the relative cross reactivity ratios of the technical Aroclors tested ranged from about 0.5- fold for 1242 to about 2.5- to 3-fold for Aroclor 1254. When we examined the relationship between the ELISA [ePCBs]s and the GC [total-PCBs]s we found that the [ePCBs] = $-0.084 + 3.99^*[\text{total-PCBs}]$ with an $R^2 = 0.75$.

Based on these observations, we recommend that future screening analyses of unknown fish tissue extracts, using these PCB ELISA kit reagents, should be calibrated with methanolic dilutions of technical Aroclor 1254. In this way the cross reactivity of the calibration standards will more closely represent the mean cross reactivity of the PCB mixtures of US NCBP fish tissue

extracts, and the true [total-PCBs] would likely range from 0.5 times to 3 times the corresponding [ePCBs].

When fish samples are influenced by the same source of PCB contamination the ELISA response should be calibrated by comparison to a limited number of reference GC determinations (5–10) for split sample extracts. The results of the split sample analyses can be used to determine the cross reactivity (or systematic bias) of the ELISA for the PCB residues across the sample matrix(s) and location(s). Using this approach, the accuracy of the ELISA determinations should be similar to that of the GC determinations once the [ePCBs] have been corrected for any documented systematic bias.

PCB ELISA measurements provide a simple, inexpensive, and rapid approach to screening environmental sample extracts as long as calibration and hydrophobic solvent effects are accounted for.

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