SHORT COMMUNICATION

Biological monitoring of polychlorinated biphenyls in plasma: a comparison of enzyme-linked immunosorbent assay and gas chromatography detection methods

P. Griffin, K. Jones and J. Cocker

Polychlorinated biphenyls (PCBs) are ubiquitous and persistent environmental compounds. Exposure of workers handling these materials can be assessed by biological monitoring. We have compared the concentration of PCBs in the plasma of exposed workers as measured by gas chromatography with electron capture detection (mean = 40.9 ng ml^{-1}, range = 6.7–120.3 ng ml^{-1}) and enzyme-linked immunosorbent assay (ELISA) (mean = 47.1 ng ml^{-1}, range = 6.8–186.2 ng ml^{-1}). There was a good overall correlation between the two methods (n = 28, r = 0.92). We conclude that an ELISA is a useful screening tool for biological monitoring purposes where there is not immediate access to standard analytical equipment or where a very high throughput of samples is required.

Keywords: *polychlorinated biphenyls, ELISA, gas chromatography.*

Introduction

Polychlorinated biphenyls (PCBs) are ubiquitous and persistent environmental compounds. They consist of a biphenyl ring structure with differing degrees of chlorine substitution giving a potential of 209 congeners. PCBs were produced from 1929 to the 1970s and were used in electrical capacitors and transformers as well as a variety of other industrial uses (Menzer 1991). Workers decommissioning machinery containing PCBs may still be exposed to these compounds. PCBs are lipophilic in nature and are usually measured in serum or plasma samples for the purposes of biological monitoring. High resolution gas chromatography with electron capture detection (GC/ECD) or gas chromatography/mass spectrometry (GC/MS) provide the best sensitivity and specificity for trace analysis of PCBs (Krupcik *et al.* 1992, 1993). PCBs used commercially were produced as mixtures of congeners characterized by the percentage of overall chlorine substitution of the biphenyl ring. Therefore, Aroclor 1260 had 60% chlorine substitution, Aroclor 1242 had 42% substitution and so on. Due to differences in biotransformation and elimination from the body of the various congeners, the pattern of congeners measured in body fluids differ from the pattern of congeners in the PCB material involved in the original exposure (Cairns and Siegmund 1981) and it has been shown that an analysis of the congeners present in serum can be used to identify the source of exposure (Luatomo 1988). Analytical standards made up of specific congeners are used to set environmental regulatory exposure limits in several European countries (DeBoer and Doa 1991). The GC/ECD used to perform congener specific analysis is expensive to purchase and requires highly skilled technicians for its maintenance and operation. One way of reducing the overall cost of monitoring is to perform a simple screening test to identify if PCBs are present. Immunoassays can be used for this purpose. The equipment costs for immunoassays are much less than, for example, a GC/ECD and immunoassay equipment does not require such highly skilled staff for its operation. Several methods have been published describing the measurement of PCBs in environmental samples by immunoassay and show a good correlation with standard analytical techniques (Johnson and Van Emon, 1996, Lawruk *et al.* 1996). A radioimmunoassay has been developed which detected Aroclor 1260 in spiked blood at levels from 2 to 16 ppb (Newsome and Shields 1981). As yet no comparison has been made between the PCB concentrations in plasma of workers occupationally exposed to these materials as measured by immunoassay and a traditional analytical technique such as GC/ECD.

In this communication we compare the concentration of PCB in plasma of occupationally-exposed workers as measured by GC/ECD and a modification of a commercially available enzyme-linked immunosorbent assay (ELISA) for measuring PCBs in environmental samples.

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METHODS

Samples

Plasma samples were collected from workers occupationally exposed to PCBs during decontamination of machinery. Samples were analysed by GC/ECD and those which had detectable amounts of PCB (n = 29) were chosen for further analysis by ELISA.

GC/ECD

A calibration curve (100, 80, 60, 40, 20, 10 and 0 ng ml^{-1}) was prepared in horse plasma of a mixture of PCB congeners 18, 28, 44, 52, 66, 74, 101, 118, 126, 138, 153, 170, 180, 194 (Promochem). Congener 209 (40 ng) was added as an internal standard. One ml of each standard and sample were aliquoted separately into screw-capped glass tubes (Fisher Scientific). Each sample was processed in duplicate. Methanol (400 μl) was added to each tube which was then vortexed for 10 s. Hexane (4 ml) was then added, the tube capped and agitated on a rack rotator for 20 min. The tubes were then spun at 1600 × g for 15 min and 3 ml of the organic layer was transferred to a new glass tube. The hexane was removed by evaporation under nitrogen at 30 °C. At the end of this process the samples were reconstituted in 100 μl of hexane and analysed by GC/ECD (Hewlett Packard). Using helium as a carrier gas a 1 μl split injection was made into a BP5 fused silica column (50 m × 0.33 mm i.d., 0.25 μm film) (Scientific glass engineering).

Run conditions:

Injector temperature = 250°C.

Column temperature = 60 °C, rising by 5 °C min^{-1} to 280 °C and held for 21 min. Detector temperature = 300 °C.

The congeners eluted in the order 18, 28, 52, 44, 66, 74, 101, 118, 153, 138, 126, 180, 170, 194 and 209. The results were expressed as a cumulative PCB concentration.

ELISA

A calibration curve was prepared by spiking horse plasma with Aroclor 1248, in hexane, to give concentrations of 200, 100, 50, 20, 10, 5 and 0 ng ml^{-1}. The standards and plasma samples from workers were processed as described for the GC/ECD method except that samples were not processed in duplicate. After removal of hexane by evaporation the residue was redissolved in 100 μl of methanol and mixed with 900 μl of assay buffer (phosphate buffered saline (PBS), (pH 7.4) + 0.1% TWEEN 20 + 1% bovine serum albumin). The samples were then analysed using a commercially available immunoassay kit for measuring PCBs (Millipore). Briefly, an aliquot (150 μl) of each sample was pipetted, in triplicate, into the wells of an ELISA plate which had been precoated with anti-PCB. The horseradish peroxidase–PCB conjugate supplied by the manufacturer was then added and incubated for 1 h on an orbital shaker. The plate was washed five times with PBS + 0.1% TWEEN 20. The enzyme substrate solution supplied by the manufacturer (100 μl) was then added to each well and the plate incubated for another 30 min. The stop solution supplied by the manufacturer (100 μl) was then added to each well and the optical density, at a wavelength of 450 nm with correction at 650 nm, of the solution in each well measured on an ELISA plate reader. The optical density readings for each sample were averaged and the value converted to a percentage of the optical density reading of a sample without any inhibitor.

Statistical analysis

Statistical analysis was carried out on the C-Stat computer package (Cherwell Scientific).

Results

The ELISA was linear over a concentration range of 5–200 ng ml^{-1} with the bottom of the standard curve being the lower detection limit. By contrast the lower detection limit of the GC/ECD method was 1 ng ml^{-1} for each congener. The plasma samples had a mean concentration of 40.9 ng ml^{-1} (range = 6.7–120.3 ng ml) as measured by GC/ECD compared with a mean concentration of 47.1 ng ml^{-1} (range = 6.8–186.2 ng ml^{-1}) when measured by ELISA. A regression analysis carried out on the results measured in the plasma samples by each method is shown graphically in Figure 1. The correlation between the two sets of results was good (n = 28, r = 0.92) with a tendency for the ELISA to give higher results (slope = 1.13). Reference to Figure 1 shows the variation between the two sets of results. One sample has not been included in this analysis where there was no PCB as measured by the ELISA and 92 ng ml^{-1} was measured by GC/ECD. Because the plasma samples were analysed in duplicate for the GC but not for the ELISA it was assumed that the ELISA gave a false negative result. The intra-assay variation for the ELISA method was 11.9% whilst that for the GC/ECD ranged from 6.4% for congener 194 to 10.1% for congener 28.

Where the ELISA gave higher results than the GC method, no noticeable difference was seen in the congener distribution from those samples which gave similar values.

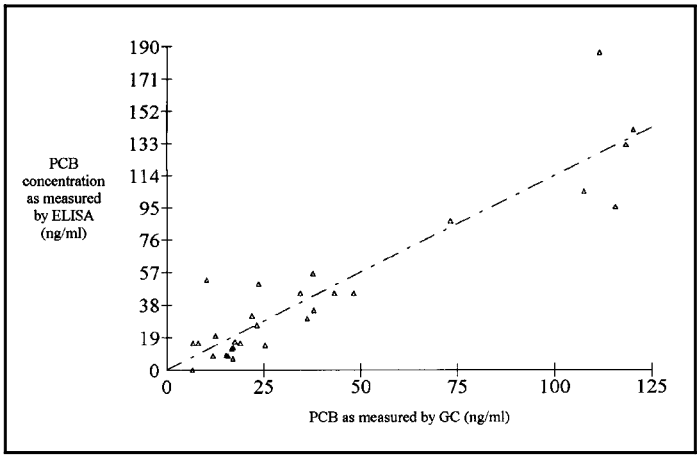

Figure 1. A comparison of PCB concentrations in plasma as measured by GC/ECD and ELISA.

Conclusion

We found a good correlation between the two methods used to measured PCBs in plasma. In a previous study Newsome and Shields (1981) recovered Aroclor 1260 from spiked blood by solvent extraction and measured the recovered PCB by radioimmunoassay and GC/ECD; both methods used Aroclor 1260 as a standard. They found a good correlation between the two methods (r^2 = 0.99). Our results are comparable with this.

The ELISA used in our study utilized Aroclor 1248 as a standard. The antibody has minimal cross-reactivity with other chlorohydrocarbons but cross-reacts with Aroclors 1016, 1242, 1254 and 1260 with a response within two-fold of Aroclor 1248 (Millipore literature). Therefore the results obtained should be regarded as Aroclor equivalents. The GC/ECD measures individually each of a range of congeners on the basis of the congeners found in populations exposed to Aroclor mixtures with differing degrees of chlorine substitution (Fait *et al.* 1989, Luatomo *et al.* 1991), the biotransformation and elimination of congeners (Cairns and Siegmund 1981, Luatomo 1988) and those used to set environmental regulatory exposure limits in several European countries (DeBoer and Doa 1991). The cross-reactivity of the anti-PCB antibody for different Aroclor mixes explains the close correlation between the GC/ECD and ELISA assays and means that the ELISA can be used as a screening procedure to detect sera which contain PCBs. A similar cross-reactivity has been noted for other anti-PCB antibodies (Johnson and Van Emon 1996, Lawruk *et al.* 1996).

One sample was excluded from the statistical analysis of the data. The value obtained using the ELISA may have been a false negative result. Such potential anomalies could be avoided by assaying two aliquots of plasma and thereby detecting experimental anomalies. This option was curtailed in this study by sample availability.

The ELISA gives a sensitive measure of PCB exposure but does not give congener specific information. Such data are useful for toxicological purposes and therefore limit the use of ELISAs to that of a screen for positive samples which can then be analysed further by traditional analytical methods. We envisage that ELISAs would be cost e

where there is not immediate access to standard analytical equipment or where a very high throughput of samples is required.

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References

CAIRNS, T. AND SIEGMUND, E. G. (1981) PCBs: Regulatory history and analytical problems. *Analytical Chemistry*, **53 (11),** 1183–1193.

DEBOER, J. AND DOA, Q. T. (1991) Analysis of seven chlorinated biphenyl congeners by MDGC. *Journal of High Resolution Chromatography*, **14,** 593–596.

FAIT, A., GROSSMAN, E., SELF, S., JEFFRIES, J., PELLIZZARI, E. AND EMETT, E. (1989) Polychlorinated biphenyl congeners in adipose tissue lipid and serum of past & present transformer repair workers and a comparison group. *Fundamental and Applied Toxicology*, **12,** 42–55.

JOHNSON, J. AND VAN EMON, J. (1996) Quantitative enzyme-linked immunosorbent assay for determination of polychlorinated biphenyls in environmental soil and sediment samples. *Analytical Chemistry*, **68,** 162–169.

KRUPCIK, J., KOCAN, A., LECLERP, P. AND BALLSCHMITTER, K. (1992) The use of reference standards for quantitative trace analysis of PCBs by HRGC. Analysis of technical PCB formulations by HRGC-FID. *Chromatographia*, **33,** 514–520.

KRUPCIK, J., KOCAN, A., LECLERP, P. AND BALLSCHMITTER, K. (1993) Reference standards for quantitative trace analysis of PCBs by HRGC. Technical PCB formulations for the calibration of ECD and MSD responses. *Chromatographia*, **35,** 411–418.

LAWRUK, T., LACHMAN, C., JOURDAN, S., FLEEKER, J., HAYES, M. AND RUBIO, F. (1996) Quantitative determination of PCBs in soil and water by a magnetic particle based immunoassay. *Environmental Science and Technology*, **30,** 695–700.

LUATOMO, M. (1988) Isomer specific biological monitoring of polychlorinated biphenyls. *Scandinavian Journal of Work Environment and Health*, **14 (1),** 60–62.

LUATOMO, M., JARVISALO, J. AND AITIO, A. (1991) Assessment of exposure to polychlorinated biphenyls: analysis of selected isomers in blood and adipose tissue. *Experimental Research*, **54,** 121–134.

MENZER, R. E. (1991) Water and soil pollutants. In *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 4th edn, M. O. Amdur, J. Doull and C. D. Klassen, eds (Pergamon Press, Oxford), pp. 872–902.

NEWSOME, W. H. AND SHIELDS, J. B. (1981) Radioimmunoassay of PCBs in milk and blood. *International Journal of Environmental Analytical Chemistry*, **10,** 295–304.

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