



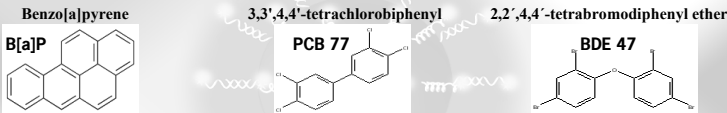
Towards a multiplex flow cytometric immunoassay for Persistent Organic Pollutants in food.



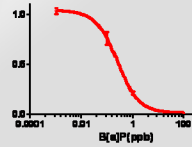
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Objectives

The development of a multiplex flow cytometric immunoassay (FCIA) to detect several Persistent Organic Pollutants (POPs) in fish, fish feed and cereal using marker compounds such as:

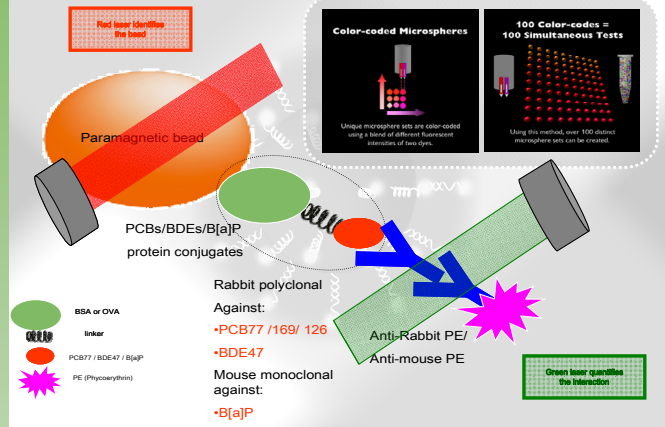


A highly sensitive FCIA to detect B[a]P with an IC₅₀ of 0.3 µg / L in buffer was developed previously*



*Meimaridou *et al.*, 2010 submitted to Analytica Chimica Acta

Assay format



FCIAs development

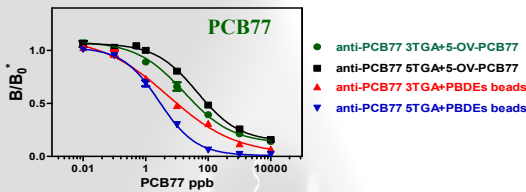


Figure 1: PCB77 calibration curves obtained in the FCIA with two polyclonal antibodies against PCB77 in combination with PCB77 & BDE47 protein conjugates coated beads

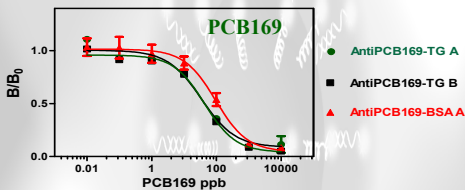


Figure 2: PCB169 calibration curves obtained in the FCIA with three polyclonal antibodies against PCB169 in combination with PCB77 protein conjugates coated beads

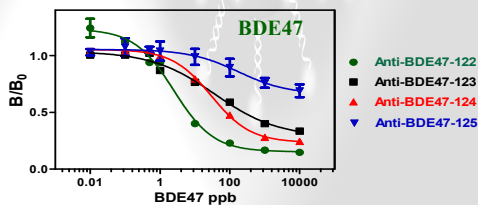


Figure 3: BDE47 calibration curves obtained in the FCIA with four polyclonal antibodies against BDE47 in combination with BDE47 protein conjugates coated beads

*B₀ is the maximum MFI of the blank measurement and B the MFI obtained with the different POPs concentrations. Each point represents the mean of three replicates ± SD

Cross-reactivities (CR%)

Table 1: Percentage of cross-reactivity (CR%) obtained with anti-PCB77 5TGA & PBDEs beads and anti-PCB77 3TGA & PCB77-5OVA beads for 11 PCBs in the present described FCIAs and/or a formerly developed ELISA

PCB	Cross-reactivity (CR) %		
	BDE47 beads	PCB77-5OVA beads	ELISA (Milan <i>et al.</i> , 2001)
PCB 77	100.0	100.0	100.0
PCB 126	48.5	7.4	6.7
PCB 169	1.1	1.4	0.4
PCB 81	n.m.	5.9	0.0
PCB 105	n.m.	3.7	0.0
PCB 156	8.0	1.6	0.0
PCB 157	19.0	0.6	0.0
PCB 123	7.5	1.0	0.0
PCB 118	2.7	0.6	0.0
PCB 114	4.8	0.6	0.0
PCB 167	1.2	0.0	0.0
PCB 189	0.5	0.0	0.0

n.m. = not measured

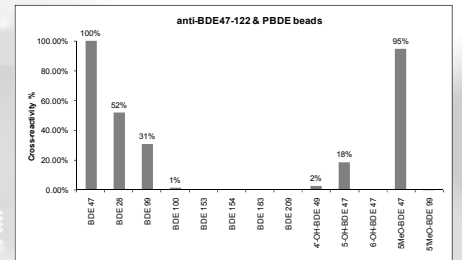


Figure 4: Percentage of cross-reactivity (CR%) obtained with anti-BDE47-122 & PBDEs beads for the 12 PBDEs in the present described FCIA.

Conclusions

- ✓ The FCIA sensitivity for PCB77 in buffer is similar to the one of the previously developed ELISA (IC₅₀ = 2 µg/L).
- ✓ The FCIA sensitivity for PCB169 is higher than the former ELISA for PCBs (Milan *et al.*, 2001).
- ✓ The FCIA sensitivity for BDE47 (IC₅₀ = 2.5 µg/L) is less than the previously developed direct inhibition ELISA (Weilin *et al.*, 2008).

Future plans

- ✓ Check the FCIA's sensitivity for PCB126.
- ✓ Optimize the FCIA for PBDEs.
- ✓ Combine all the FCIAs.
- ✓ Simplify sample preparation.
- ✓ Apply the FCIAs in food samples.