CONfIDENCE: Contaminants in food and feed: Inexpensive detection for control of exposure

WP1a

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

Anastasia Meimaridou *[a], Dimitrios Mintzas [a], Milan Franek [b], Weilin L. Shelver [c], Willem Haasnoot [a], Michel W.F.Nielen [a,d]

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:

Benzo[a]pyrene
B[a]P

3,3',4,4'-tetrachlorobiphenyl
PCB 77

2,2´,4,4´-tetrabromodiphenyl ether
BDE 47
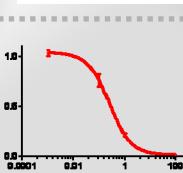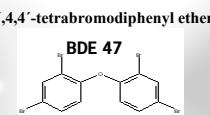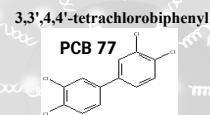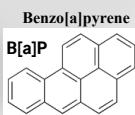
A highly sensitive FCIA to detect B[a]P with an IC_{50} of 0.3 µg / L in buffer was developed previously*

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

Assay format

Paramagnetic bead

PCBs/BDEs/B[a]P protein conjugates

Rabbit polyclonal Against:
• PCB77 /169/ 126
• BDE47

Mouse monoclonal against:
• B[a]P

Anti-Rabbit PE/ Anti-mouse PE

BSA or OVA linker
PCB77 / BDE47 / B[a]P
PE (Phycoerythrin)

FCIAs development

PCB77
- anti-PCB77 3TGA+5-OV-PCB77
- anti-PCB77 5TGA+5-OV-PCB77
- anti-PCB77 3TGA+PBDEs beads
- anti-PCB77 5TGA+PBDEs beads
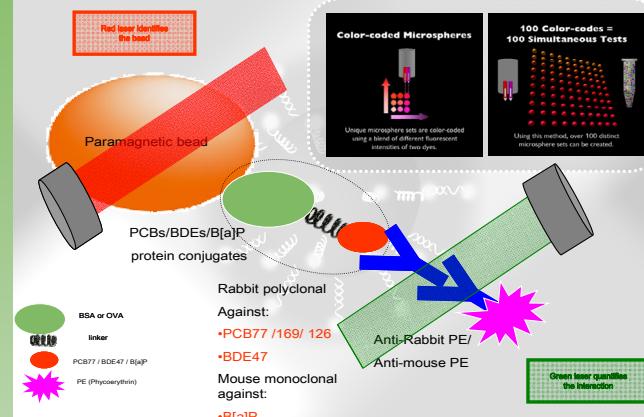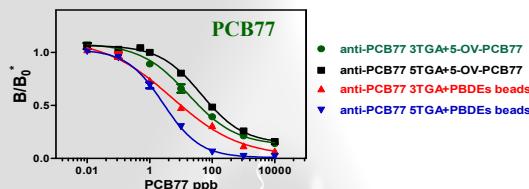
Figure 1: **PCB77** calibration curves obtained in the FCIA with two polyclonal antibodies against PCB77 in combination with PCB77& BDE47 protein conjugates coated beads


PCB169
- AntiPCB169-TG A
- AntiPCB169-TG B
- AntiPCB169-BSA A
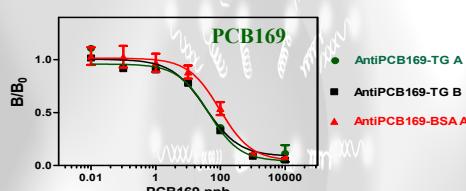
Figure 2: **PCB169** calibration curves obtained in the FCIA with three polyclonal antibodies against PCB169 in combination with PCB77 protein conjugates coated beads


BDE47
- Anti-BDE47-122
- Anti-BDE47-123
- Anti-BDE47-124
- Anti-BDE47-125
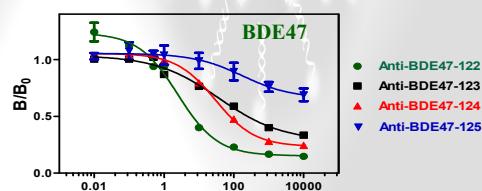
Figure 3: **BDE47** calibration curves obtained in the FCIA with four polyclonal antibodies against BDE47 in combination with BDE47 protein conjugates coated beads

*B_0 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

	Cross-reactivity (CR) %		
	BDE47 beads	PCB77 -5OVA beads	ELISA (Milan et al.,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	
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	
PCB 118	2.7	0.6	
PCB 114	4.8	0.6	
PCB 167	1.2	0.0	
PCB 189	0.5	0.0	0.0

n.m. =not measured


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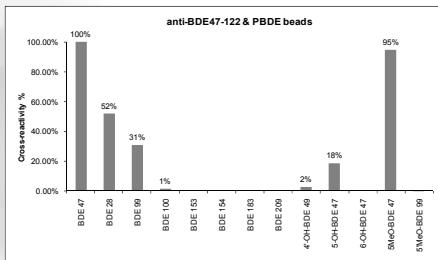
Figure 4: Percentage of cross-reactivity (CR%) obtained with anti-BDE47-122 & PBDEs beads for the 12 PBDEs in the present described FCIA.

Conclusions

✓ The FCIA sensitivity for PCB77 in buffer is similar to the one of the previously developed ELISA (IC_{50} =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 (IC_{50} = 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.

a RIKILT - Institute of Food Safety, Wageningen UR, P.O. Box 230, 6700 AE Wageningen, the Netherlands Fax: +31 317 417717. E-mail: anastasia.meimaridou@wur.nl
b USDA-ARS Biosciences Research Laboratory, P.O. Box 5674, Fargo, ND 58105, USA
c Department of Biotechnology, Veterinary Research Institute, Hudcova 70, 621 32 Brno, Czech Republic
d Laboratory of Organic Chemistry, Wageningen University, Dreijenplein 8, 6703 HB Wageningen, the Netherlands