Multiplex Screening of Persistent Organic Pollutants in Fish using Spectrally-Encoded Microspheres

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SEVENTH FRAMEWORK PROGRAMME

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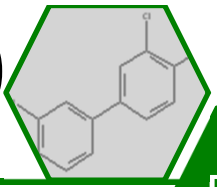
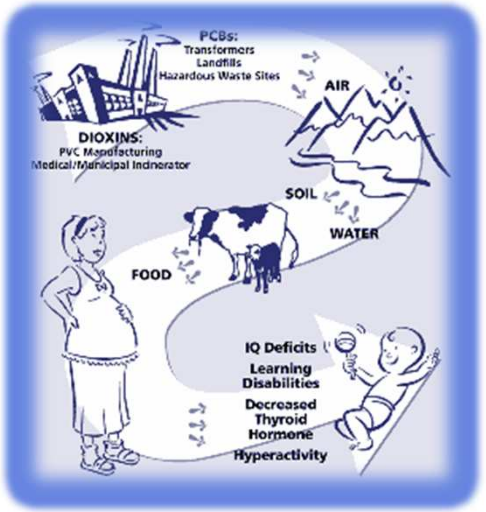
Persistent Organic Pollutants (POPs)



POPs



- ✓long-range atmospheric transport
- ✓bioaccumulation in biota
- ✓food-web biomagnifications
- ✓ adverse effects upon human & ecosystem health

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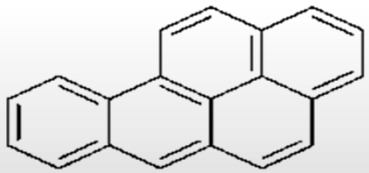
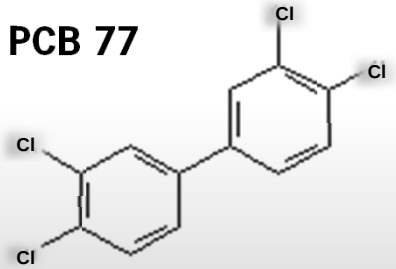
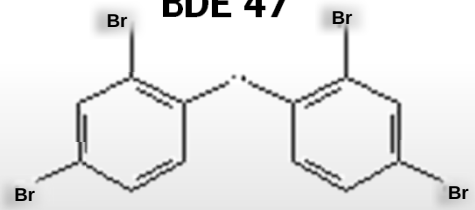
Persistent Organic Pollutants (POPs)

POPs

Polycyclic Aromatic Hydrocarbons	Polychlorinated Biphenyls	Polybrominated Biphenyls
PAHs	**PCBs**	**PBDEs**
B[a]P  Benzo[a]pyrene	PCB 77  3,3',4,4'-tetrachlorobiphenyl	BDE 47  2,2´,4,4´-tetrabromodiphenyl ether
Maximum levels (MLs) 1 ppb cereal-based food for kids 10 ppb shellfish 5 ppb smoked fish. 2 ppb edible oil ((EC)No1881/2006)	Food: 8ng WHO-PcDD/F-PCB-TEQ/kg fish meat for the sum of dioxins and DL-PCBs Fish feed: 3.25 ng WHO- PCB-TEQ/kg feedc for the sum of DL-PCBs	No limits established yet

Motivation

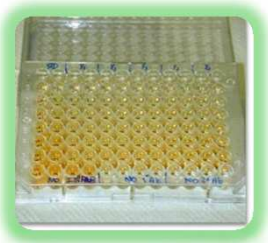
- **Persistent Organic Pollutants are known toxic, carcinogenic & mutagenic contaminants.**

- **Existing detection methods:**

  - Analytical instrumental techniques
    - **quite sensitive**
    - **rather time-consuming, expensive and laborious.**

  - Bioanalytical assays (EROD or CALUX)
    - **less time-consuming and expensive**
    - **special lab facilities for the cell culture**

  - Immunoassays
    - **Limited multiplex possibilities**



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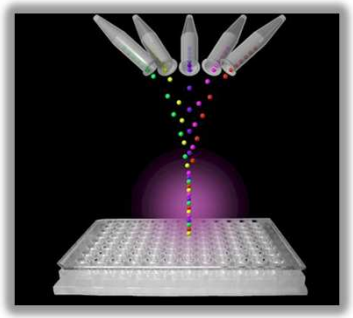
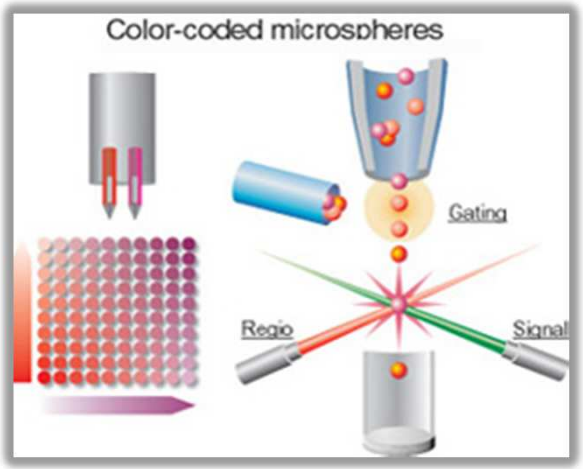
Motivation

- **A good alternative can be Flow Cytometry (FC) in combination with the xMAP technology (Luminex).**

Main advantage is the detection of multiple analytes in parallel.



http://www.labodia.com/



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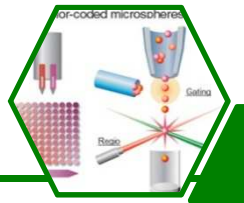
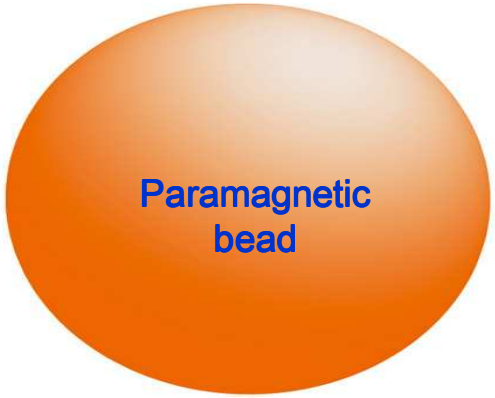
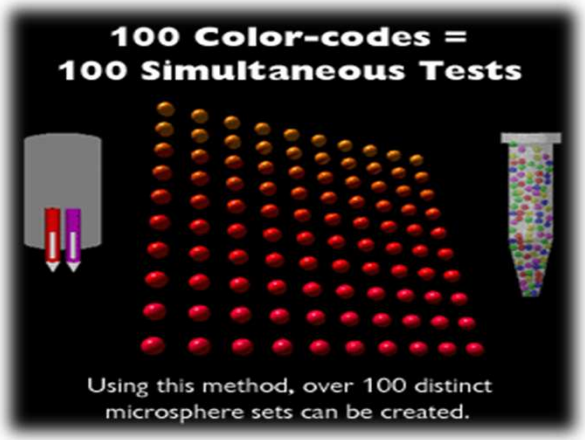
Flow cytometric color encoded microbead technology



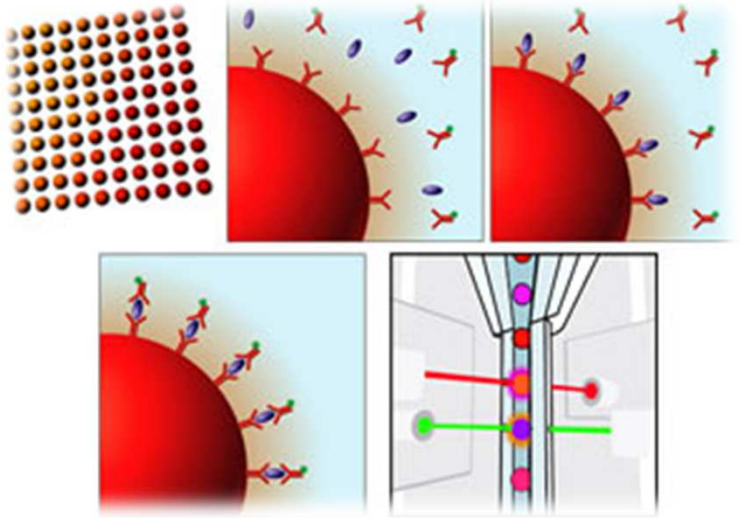
Color coded bead flow cytometry



The microsphere is a ~ 6,2 µm polystyrene paramagnetic bead with two fluorescent dyes incorporated into different ratios and with carboxyl groups on the surface.



http://www.luminexcorp.com/



www.upci.upmc.edu

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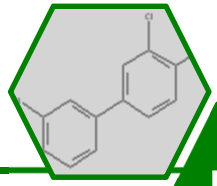
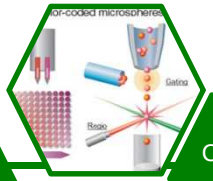
Multiplex flow cytometric color encoded Immunoassay-FCIA

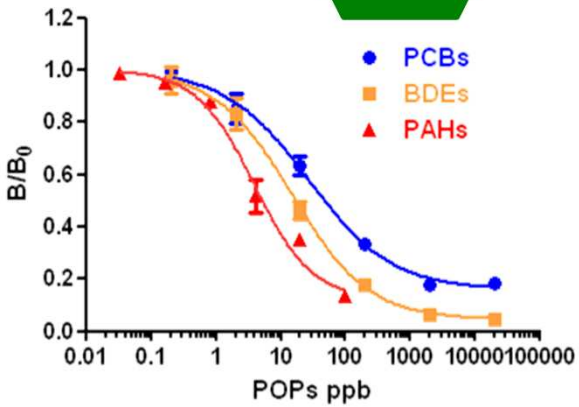


POPs



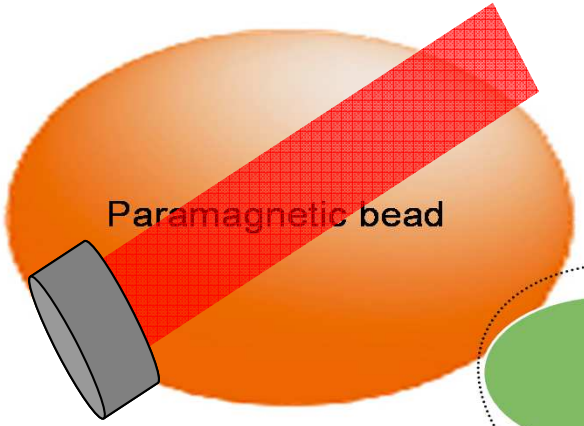
Color coded bead flow cytometry

Red laser identifies the bead

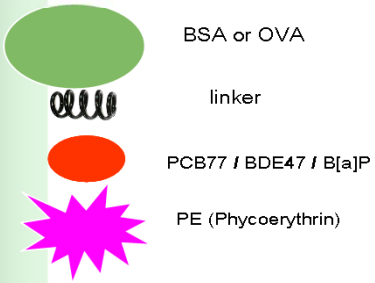


B0= [B[a]P] = 0

B = [B[a]P] = different



PCBs/BDEs/B[a]P protein conjugates

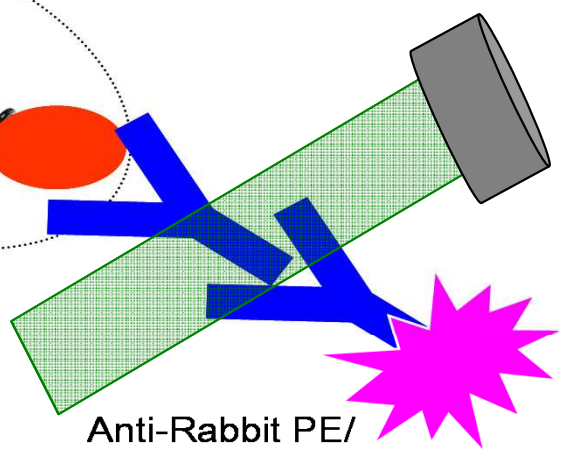


Rabbit polyclonal Against:

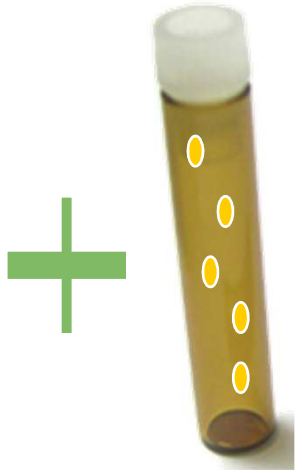
- PCB77 /169/ 126
- BDE47

Mouse monoclonal against:

- B[a]P



Anti-Rabbit PE/ Anti-mouse PE



Green laser quantifies the interaction

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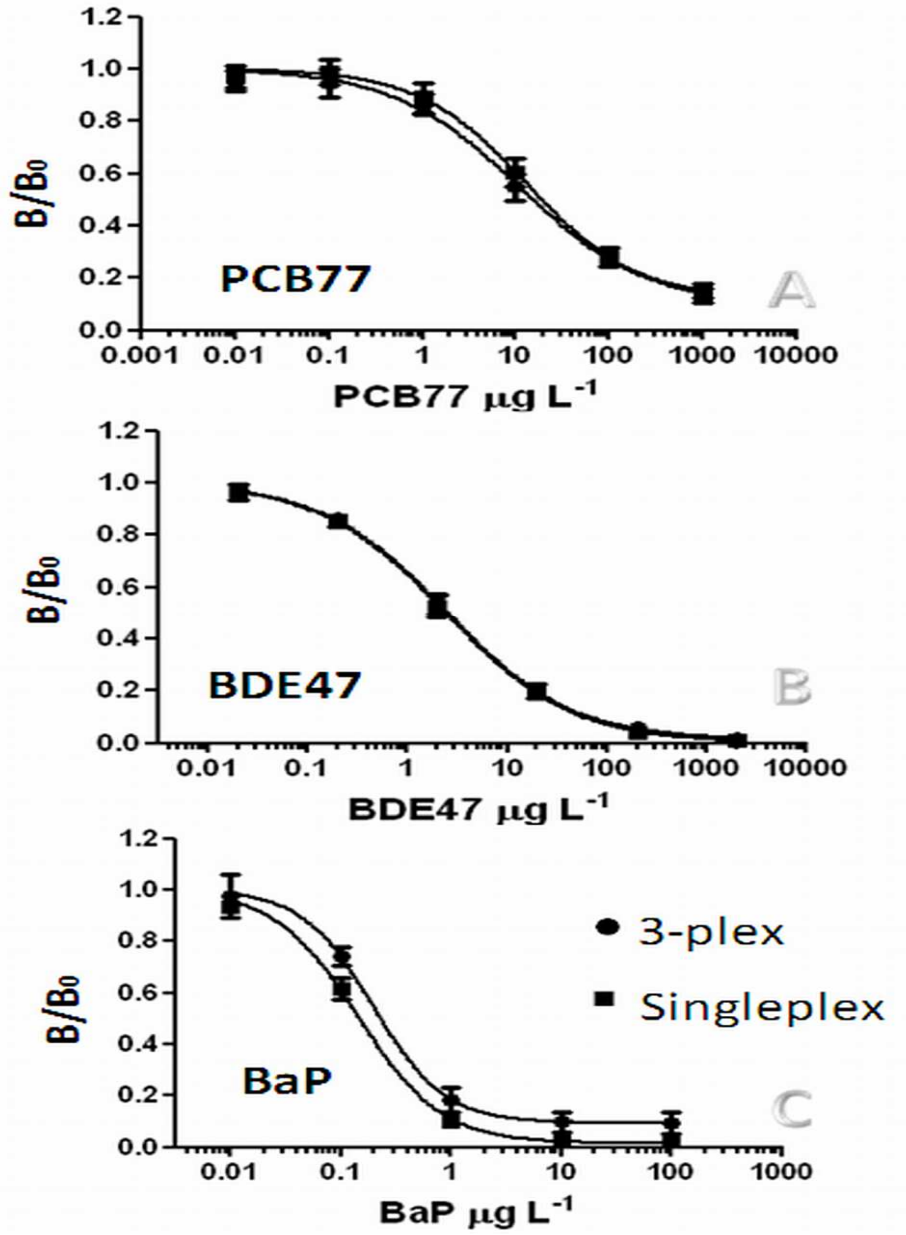
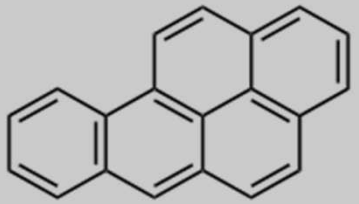
Multiplex FCIAs for PCBs, BDEs & PAHs



Multiplex FCIAs for PCBs, BDEs & PAHs -SELECTIVITY

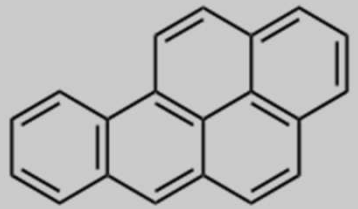
Polycyclic Aromatic Hydrocarbons (PAHs)



A1

Compound	Abbr.	Cas-number	Cross-reactivity %		
			3-plex FCIA	Single-plex FCIA	ELISA [2]
Benzo[a]pyrene	BaP	50-32-8	100	100	100
Chrysene	CHR	218-01-9	88±13	53±0.2	77
Benz[a]anthracene	BaA	56-66-3	25±5	7±0.1	13
Benzo[b]fluoranthene	BbF	205-99-2	3±0.5	8±0.9	24
Benzo[k]fluoranthene	BkF	207-08-9	3±0.7	4±0.8	5
Indenol[1,2,3-cd]pyrene	IP	193-39-5	8±1	25±0.5	45
Benzo[ghi]perylene	BghiP	191-24-2	4±1	0	1
Dibenzo[a,h]anthracene	DBahA	53-70-3	0	0±0.3	nm
Dibenzo[a,e]pyrene	DBaeP	192-65-4	0	0	nm
Dibenzo[a,h]-pyrene	DBahP	189-64-0	1±0.2	0±0.1	nm
Dibenzo[a,l]pyrene	DBalP	191-30-0	0	0±0.1	nm
Dibenzo[a,i]pyrene	DBaiP	189-55-9	5±0.5	1±0.1	nm
Benzo[j]fluoranthene	BjF	205-82-3	57±10	146±0.03	nm

Polycyclic Aromatic Hydrocarbons (PAHs)

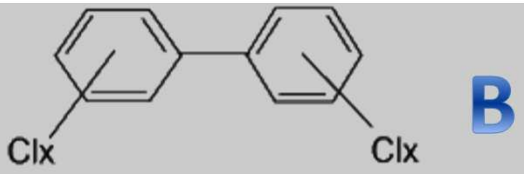


A2

Compound	Abbr.	Cas-number	Cross-reactivity %		
			3-plex FCIA	Single-plex FCIA	ELISA [2]
Cyclopenta[c,d]pyrene	CCP	27208-37-3	40±0.5	1±0.5	0
Anthracene	An	120-12-7	0	3±0.5	1
Acenaphthylene	ACL	208-96-8	0	10±1	1
Acenaphthene	AC	83-32-9	0	25±3	0
Pyrene	PY	1718-52-1	0	0.1	18
Fluorene	FL	86-73-7	0	0	0
Phenanthrene	PHE	85-01-8	0	0	1
Benzo[c]fluorene	BcFL	205-12-9	20±1	9±2	nm
Naphthalene	nA	91-20-3	0	0	0
Fluoranthene	FA	206-44-0	0	0	0
5-methylchrysene	MCH	3697-24-3	24±5	11±1	n.m.
OH-chrysene	6-OH-CHR	37515-51-8	7±2	2±0.2	n.m.
1-OHpyrene	1-OH-PYR	5315-79-7	0	2±0.1	n.m.

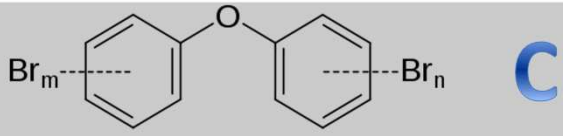
Multiplex FCIAs for PCBs, BDEs & PAHs -SELECTIVITY

Polychrorinated Biphenyls (PCBs)



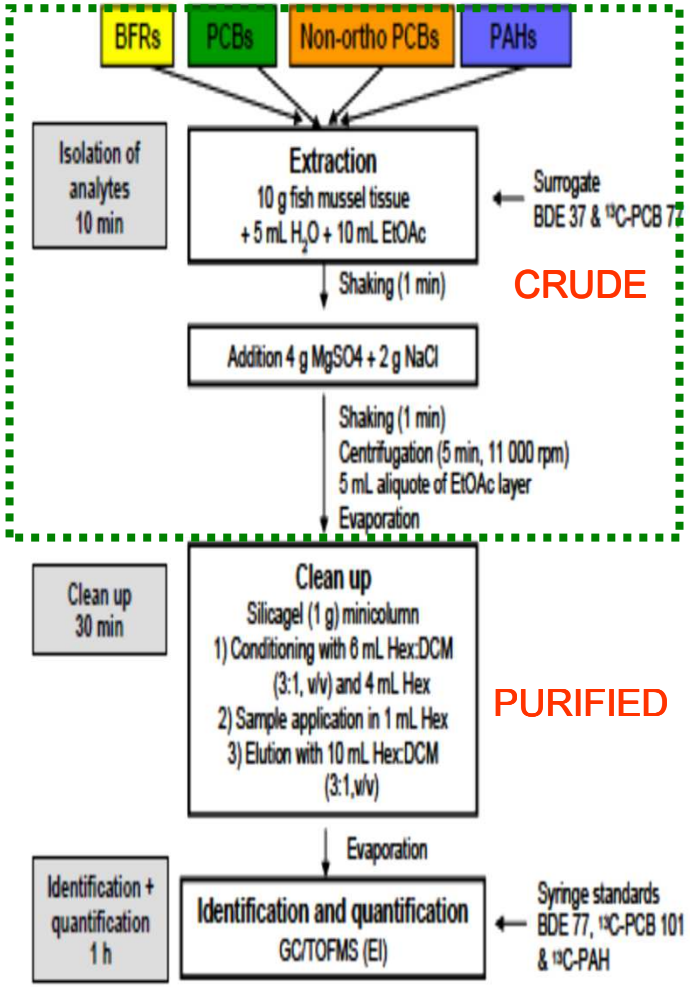
Compounds	Abbr.	Cas-number	Cross-reactivities %		
			3-plex FCIA	Single-plex FCIA	ELISA[3]
1,1'-Biphenyl, 3,3',4,4'-tetrachloro-	PCB 77	32598-13-3	100	100	100
1,1'-Biphenyl, 3,4,4',5-tetrachloro-	PCB 81	70362-50-4	2±0.35	6±3	0
1,1'-Biphenyl, 2,3,3',4,4'-pentachloro-	PCB 105	32598-14-4	12±7	4±2	0
1,1'-Biphenyl, 2,3,4,4',5-pentachloro-	PCB 114	74472-37-0	0	1±0.2	n.m.
1,1'-Biphenyl, 3,3',4,4',5-pentachloro-	PCB126	57465-28-8	23±7	7±3	7
1,1'-Biphenyl, 3,3',4,4',5,5'-hexachloro-	PCB 169	32774-16-6	9±2	1±0.5	0
1,1'-Biphenyl, 2,3',4,4',5-pentachloro-	PCB 118	31508-00-6	0	1±0.1	n.m.
1,1'-Biphenyl, 2,3',4,4',5'-pentachloro-	PCB 123	65510-44-3	1±0.1	1±0.2	n.m.
1,1'-Biphenyl, 2,3,3',4,4',5-hexachloro-	PCB 156	38380-08-4	10±3	2±0.3	n.m.
1,1'-Biphenyl, 2,3,3',4,4',5'-hexachloro-	PCB 157	69782-90-7	0	1±0.4	0
1,1'-Biphenyl, 2,3',4,4',5,5'-hexachloro-	PCB 167	52663-72-6	0	1±0.1	n.m,
1,1'-Biphenyl, 2,3,3',4,4',5,5'-heptachloro-	PCB 189	39635-31-9	0	1±0.2	0

Polybrominated Diphenyls (BDEs)



Compounds	Abbr.	Cas-number	Cross-reactivity %		
			3-plex FCIA	Single-plex FCIA	ELISA[4]
Benzene, 2,4-dibromo-1-(2,4-dibromophenoxy)	BDE 47	5436-43-1	100	100	100
Benzene, 2,4-dibromo-1-(4-bromophenoxy)-	BDE 28	41318-75-6	31±5	52±0.5	15
Benzene, 1,2,4-tribromo-5-(2,4-dibromophenoxy)-	BDE 99	60348-60-9	48±11	31±0.5	90
Benzene, 1,3,5-tribromo-2-(2,4-dibromophenoxy)-	BDE 100	189084-64-8	8±0.5	1±0.1	3
1,1'-Biphenyl, 2,2',4,4',5,5'-hexabromo-	BDE 153	59080-40-9	1±0.2	1±0.1	2
Benzene, 1,3,5-tribromo-2-(2,4,5-tribromophenoxy)-	BDE 154	207122-15-4	<0.1	0	0
Benzene, 1,2,3,5-tetrabromo-4-(2,4,5-tribromophenoxy)-	BDE 183	207122-16-5	<0.1	0	0
Benzene, 1,1'-oxybis[2,3,4,5,6-pentabromo	BDE 209	1163-19-5	<0.1	0	0
Phenol, 2,5-dibromo-4-(2,4-dibromophenoxy)-	4'-OH-BDE 49	602326-23-8	1±0.2	2±0.3	4
Phenol, 2,4-dibromo-5-(2,4-dibromophenoxy)-	5-OH-BDE 47	602326-30-7	3±0.7	18±1	9
Phenol, 3,5-dibromo-2-(2,4-dibromophenoxy)	6-OH-BDE47	79755-43-4	<1	0	1
5-Hydroxy-2,2',4,4'-tetrabromodiphenylether	5-MeO-BDE-047	602326-30-7	180±14	95±2	168
5'-Methoxy-2,2',4,4',5-pentabromodiphenylether	5'-MeO-BDE-099	n/A	2±0.7	0	1

Multiplex FCIAs for PCBs, BDEs & PAHs–Sample preparation



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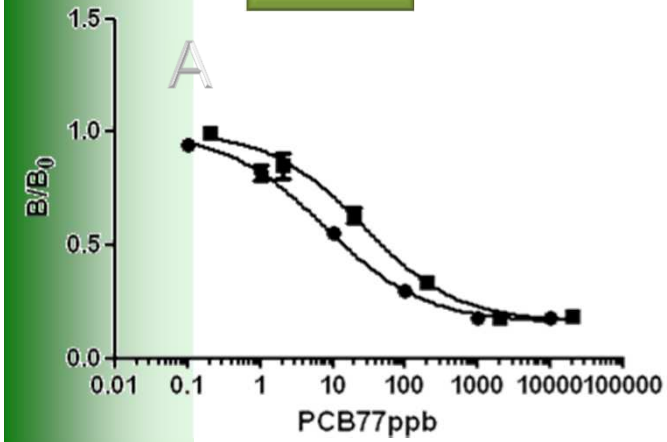
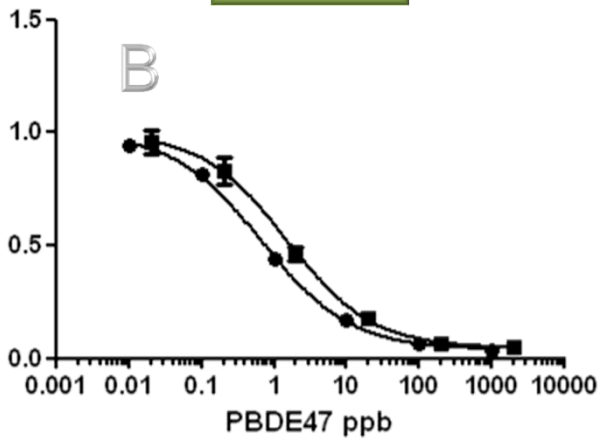
Multiplex FCIAs for PCBs, BDEs & PAHs –Fish extracts

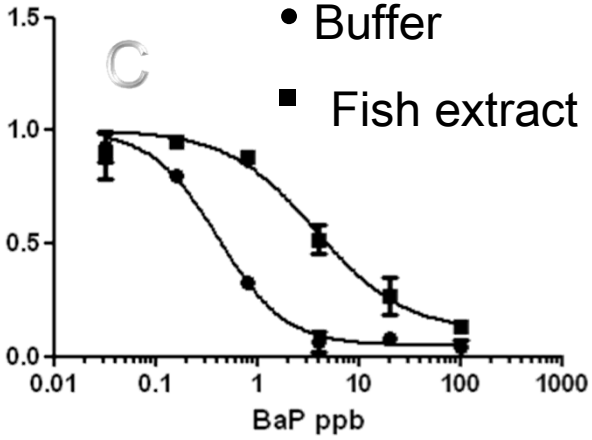
PCB77



PBDE47



BaP



- Buffer
- Fish extract

Target POPs	Matrix	Goodness of the 4P R^2 [a]	Curve steepness ($mlng^{-1}$) [b]	IC_{50} ppb in the 3plex FCIA [c]	IC_{50} ppb in ELISAs
PCB77 (A)	Buffer	0.9968	-0.8	20±2	2-15 [24]
	Fish extract	0.9927	-0.6	55±5	Not measured
BDE47 (B)	Buffer	0.9992	-0.7	2±0.1	0.135 [30]
	Fish extract	0.9902	-0.7	2±0.4	Not measured
BaP (C)	Buffer	0.9857	-1.3	0.4±0.1	0.3 [22]
	Fish extract	0.9435	-1	4±0.5	Not measured

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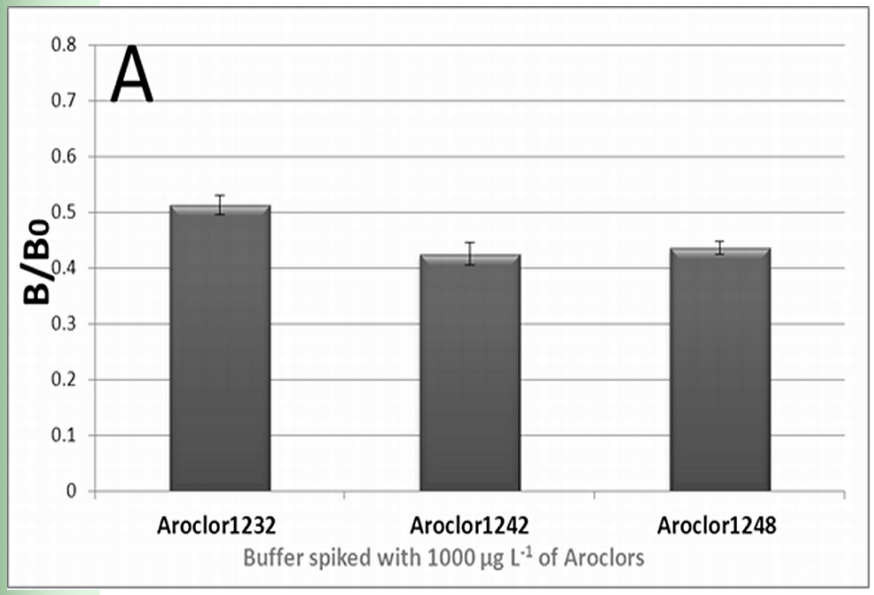
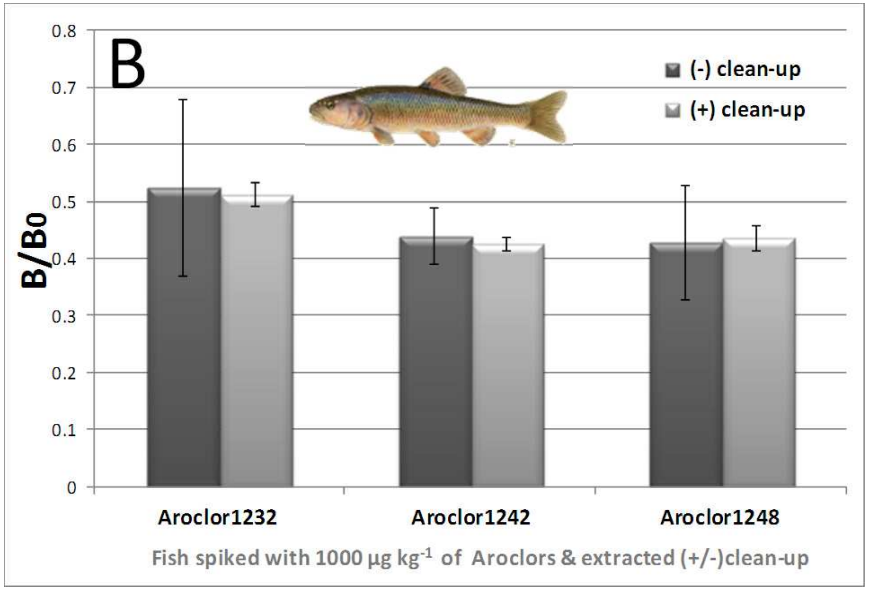
Multiplex FCIAs for PCBs, BDEs & PAHs in fish matrix

Fishes	Target POPs measured	Fat content %	µgkg-1 as measured in GC-MS	Clean-up	% of inhibition of maximum response in 3plexFCIA
Smoked trout	BaP	10	0.06	-	0
Smoked trout	BaP	11	1	-	80±2
Smoked trout	BaP	14	5	-	80±5
Smoked trout	BaP	13	14.7	-	80±3
Trout	PCBs/BDEs	2	n.d.	+	0±0.1
Chub	BDE47	1.5	0.43	+	45±2
Chub	BDE47	2	4.93	+	56±5
Chub	BDE47	2	9	+	50±4
Chub	PCB77	1.5	1.95	-	22±2

Multiplex FCIAs for Aroclors in buffer vs fish matrix





Conclusions

- ✓ 3-plex FCIA can detect BDEs, PCBs and PAHs in fish by combining 3 different immunoassays.
- ✓ 3-plex FCIA after further validation, can be a valuable screening tool for POPs in fish and other food and environmental samples prior to GC-MS.
- ✓ 3-plex FCIA meets the regulatory requirements of the EU and US food safety authorities for PCBs and PAHs.

Thank you very much for your attention

USDA

Weilin Shelver

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