

Principle of FR analysis (I)

Environmental samples

Pim Leonards and Sicco Brandsma

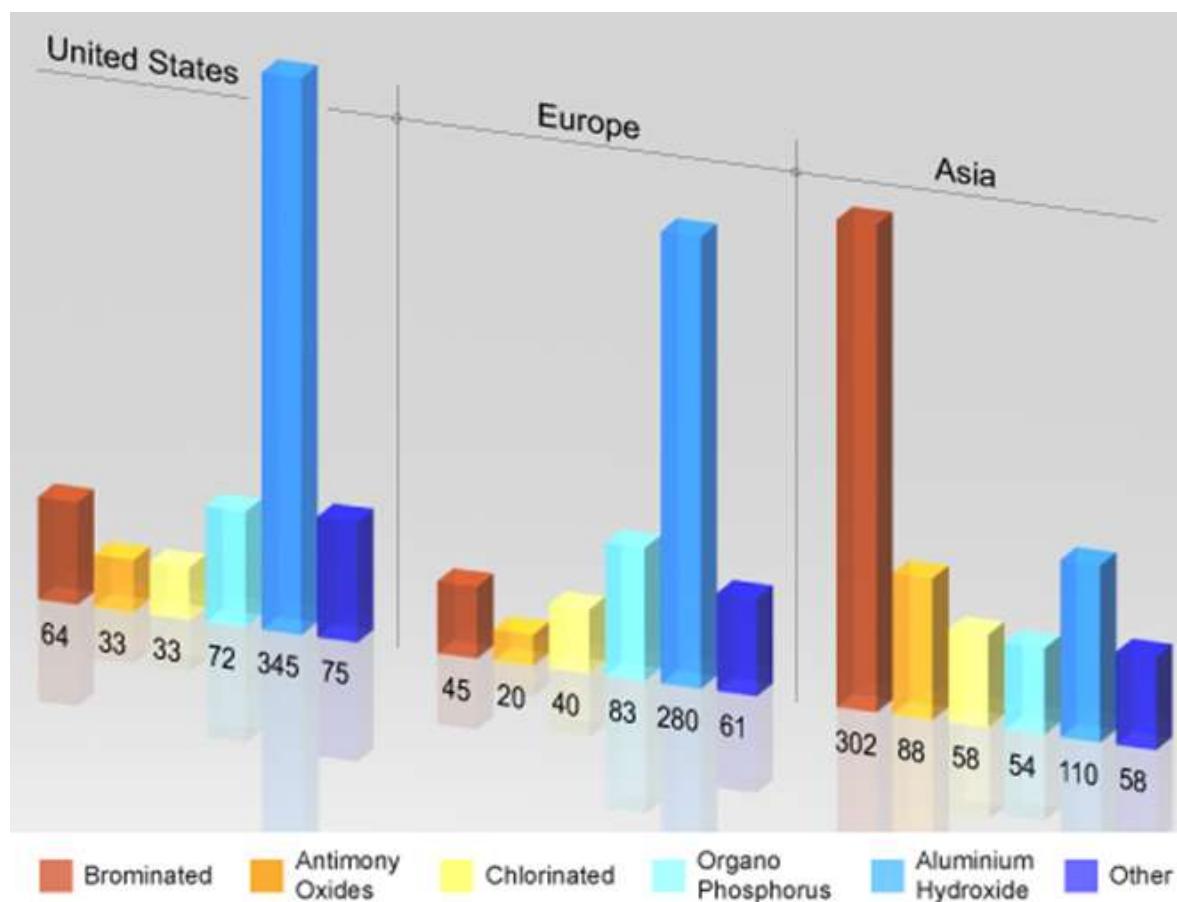
Outline

- General introduction
 - Type of flame retardants
- Sample treatment environmental samples
 - Matrix
 - Homogenisation
 - Extraction, Clean-up, derivatisation

Classes of flame retardants (FRs)

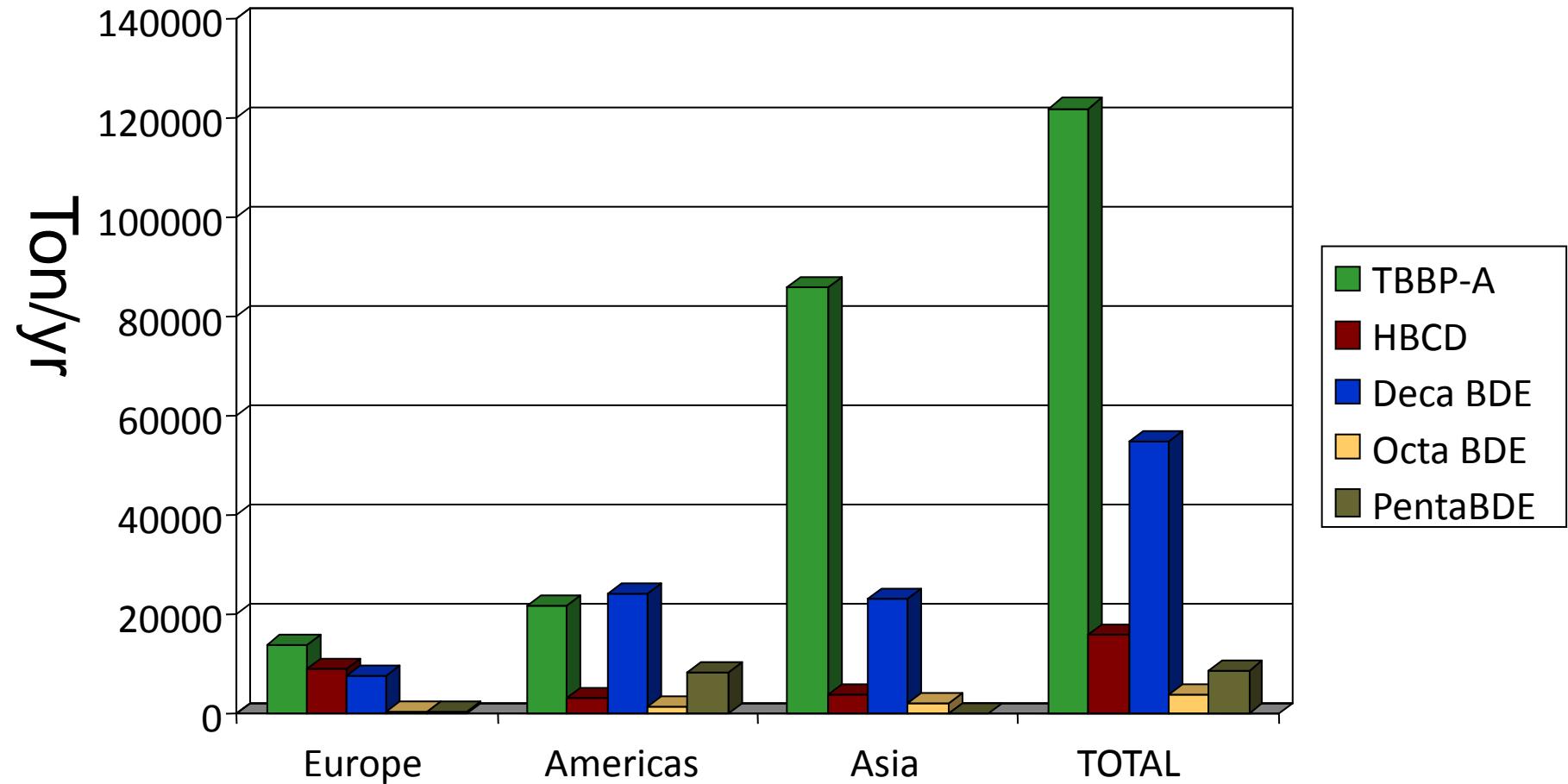
- Brominated FRs
 - polybrominated diphenyl ethers (PBDEs)
 - hexabromocyclododecanes (HBCDs)
 - tetrabromobisphenol-A (TBBP-A)
- Phosphoric FRs: - halogenated and non-halogenated
- Inorganic FRs: - antimony oxide, hydrated aluminium
- Chlorinated FRs: - polychlorinated alkanes (PCAs)

Flame Retardant Consumption 2007

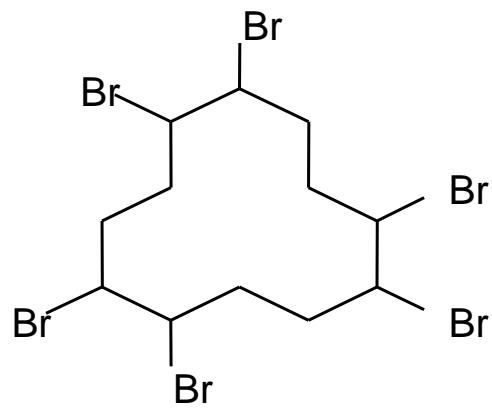
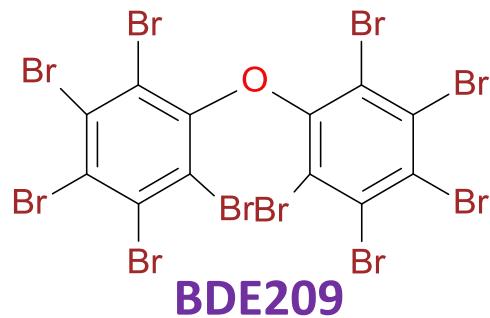
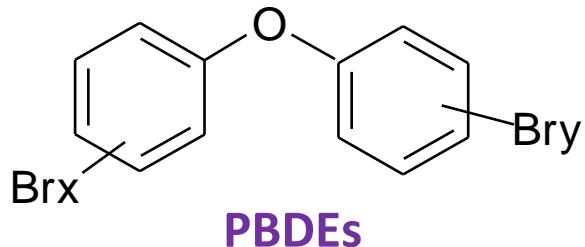


(SRI Consulting, 2008)

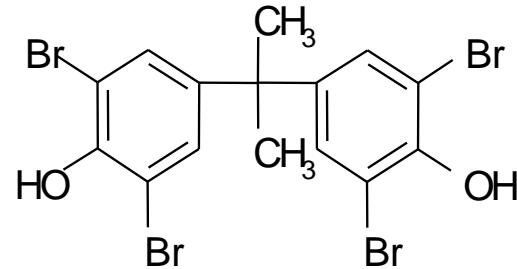
BFR production in 1999



Structures of BFRs



HBCD



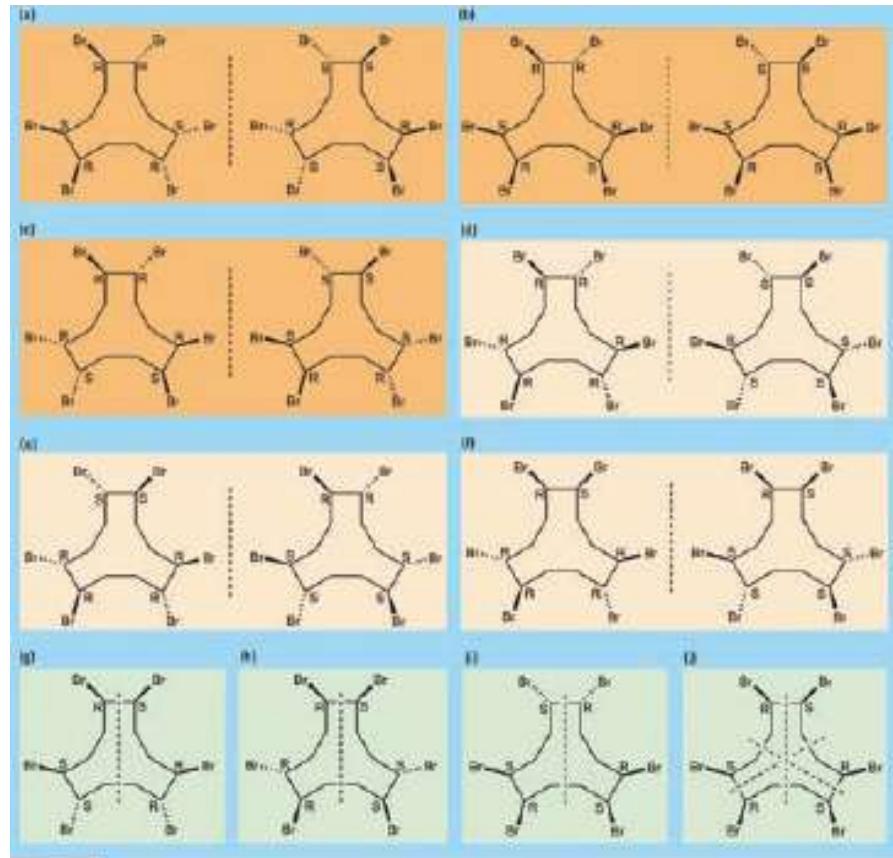
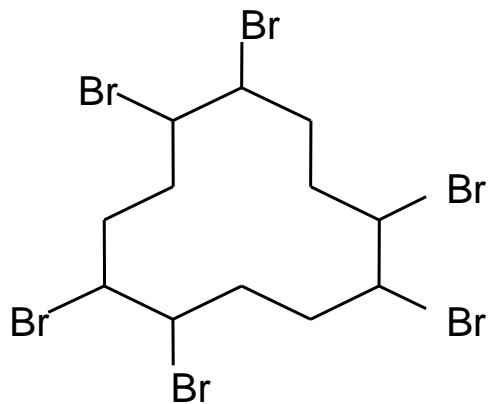
TBBP-A

Hexabromocyclododecanes (HBCDs)

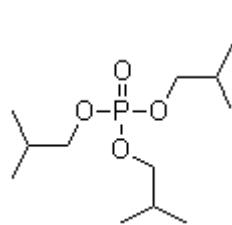
Congeners investigated in most studies:

α -, β - γ -HBCD present in the technical mixtures

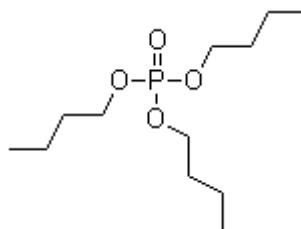
γ -HBCD dominant isomer in the technical product



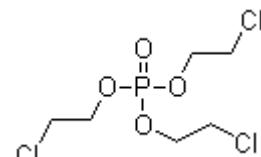
Organophosphorous flame retardants (PFRs)



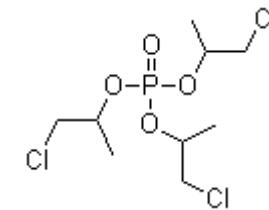
TiBP



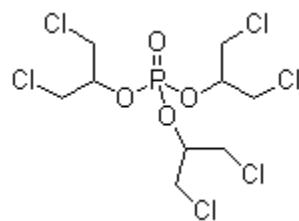
TBP



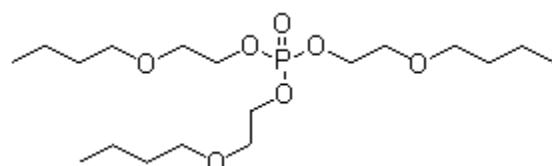
TCEP



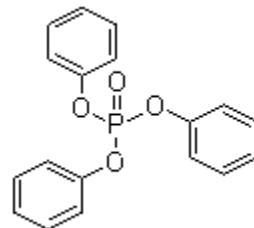
TCPP



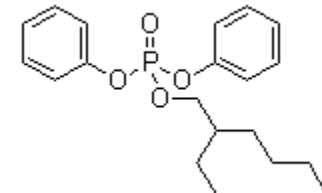
TDCPP



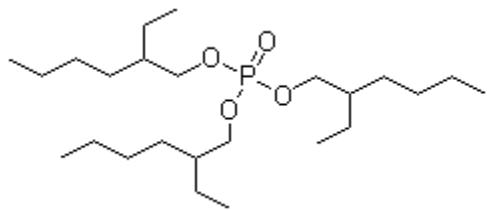
TBEP



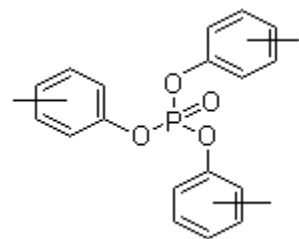
TPP



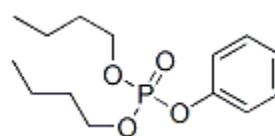
EHDP



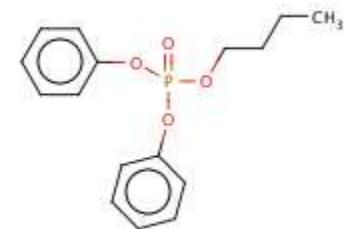
TEHP



TCP



DBPhP

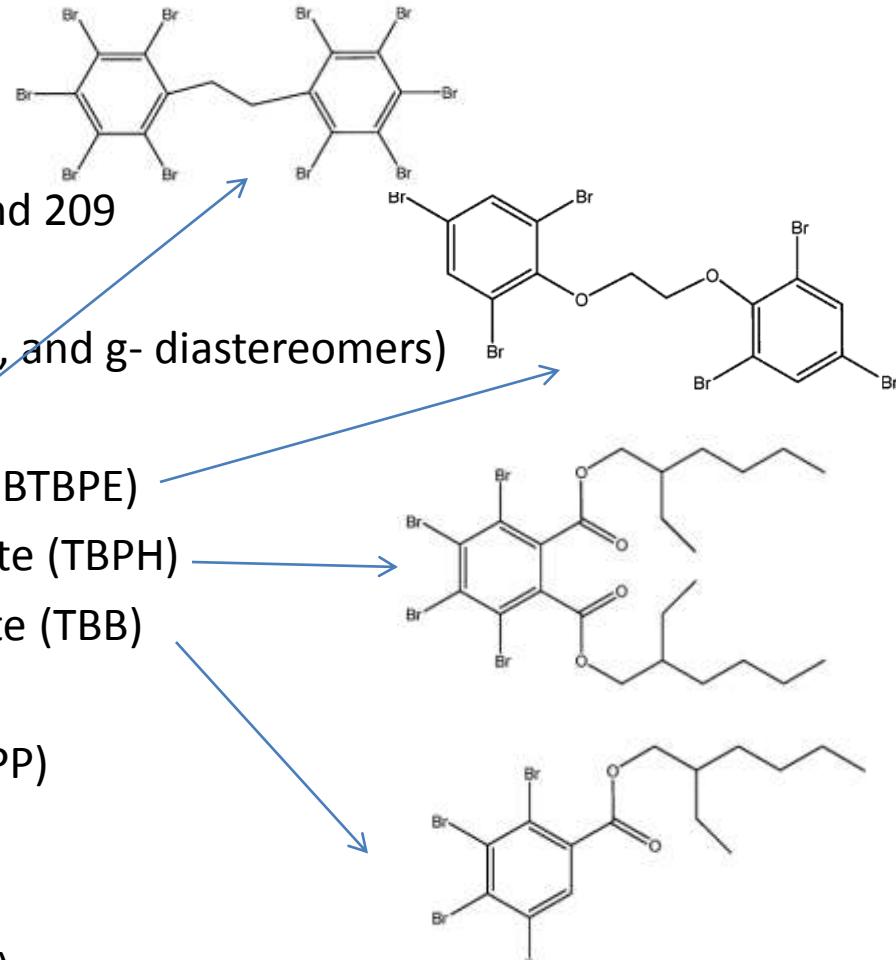


DPhBP

List of FRs monitored in INFLAME

Group 1

- PBDEs 28, 47, 99, 100, 153, 154, 183, and 209
- Tetrabromobisphenol-A (TBBP-A)
- Hexabromocyclododecane (HBCD – a, b, and g- diastereomers)
- Decabromodiphenylethane (DBDPE)
- 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE)
- 2-ethylhexyl 2,3,4,5-tetrabromophthalate (TBPH)
- 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB)
- Triphenyl phosphate (TPP)
- Tri(2,3-dichloropropyl) Phosphate (TDCPP)
- Tri(2-butoxyethyl)phosphate (TBEP)
- Tri(2-chloroethyl)phosphate (TCEP)
- Tri(monochloropropyl)phosphate (TCPP)



FR analysis

Sample treatment of
environmental samples

Why do you want to analyse the compounds?

Levels

Patterns

Toxicity

Exposure

Which FRs do you want to study?

- PBDEs
- HBCD
- TBBP-A
- TCPP
- TBB
-

Which matrix?

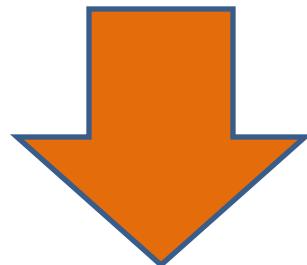
- Environment
 - Water
 - Sediment
 - Biota
 - Tissue
 - Egg
- Human (next presentation)

How to set-up a sample treatment method?

Aim Matrix

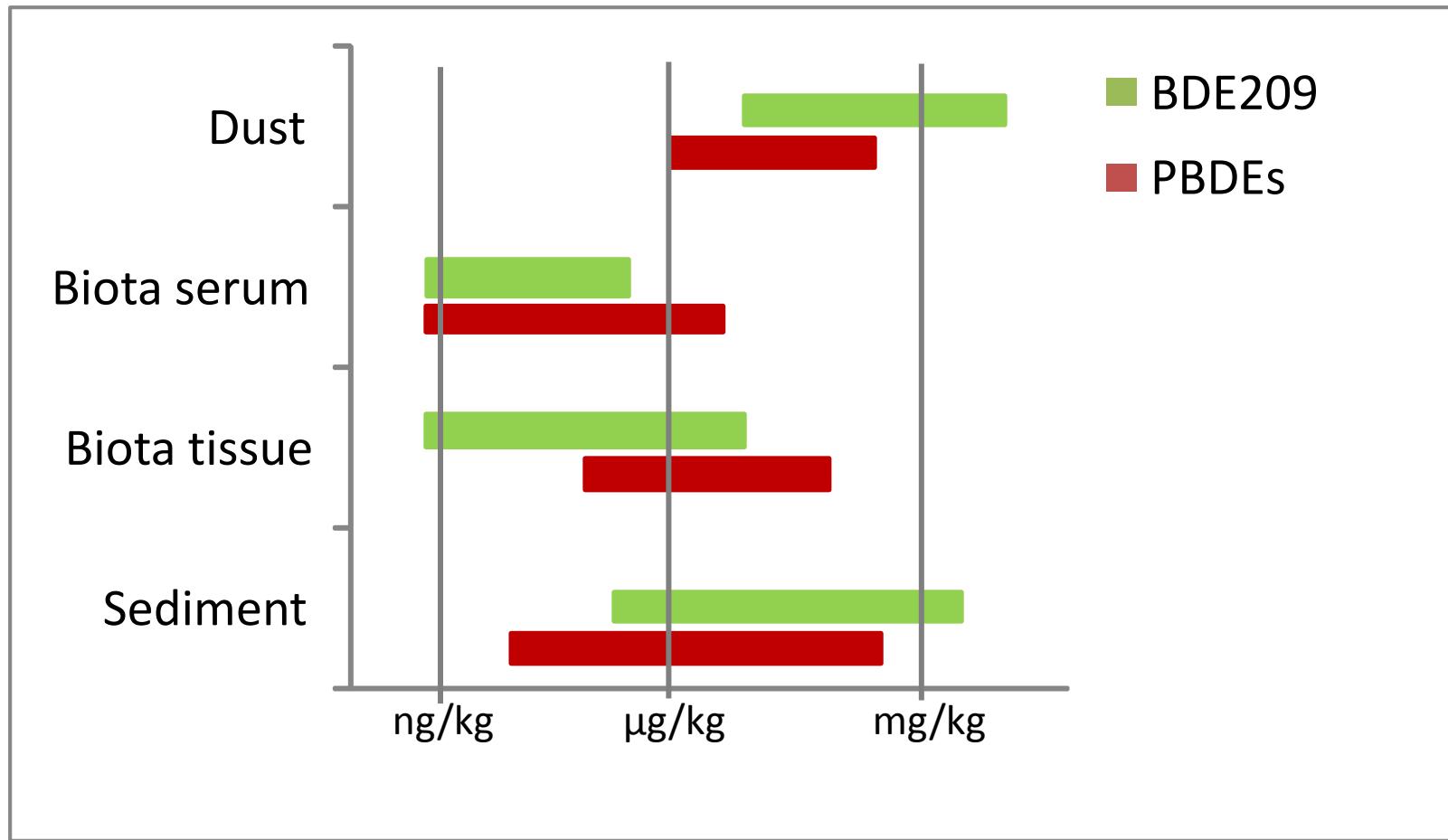


Expected level and
limit of detection

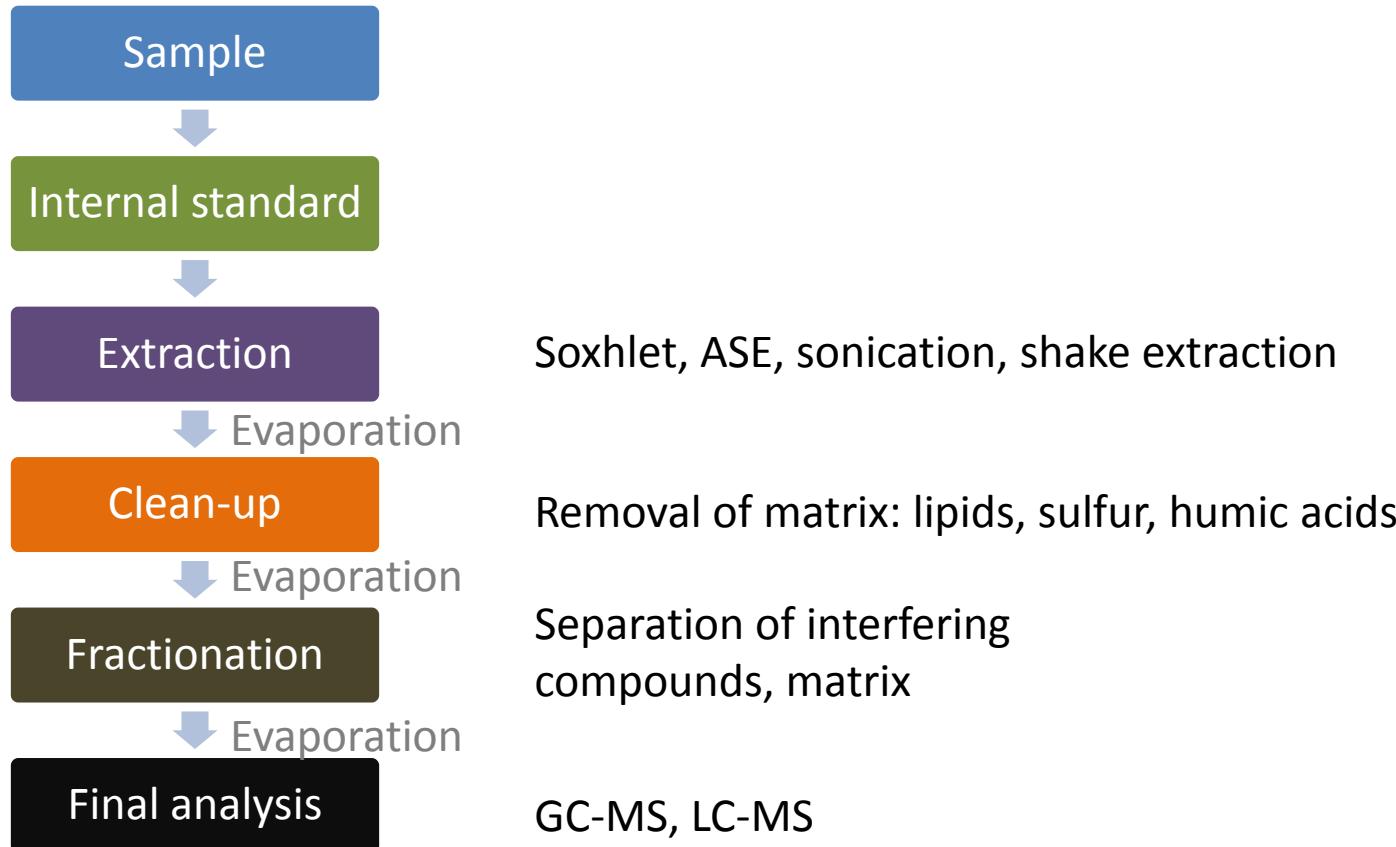


Select appropriate sample treatment
method

Schematic diagram of PBDE levels

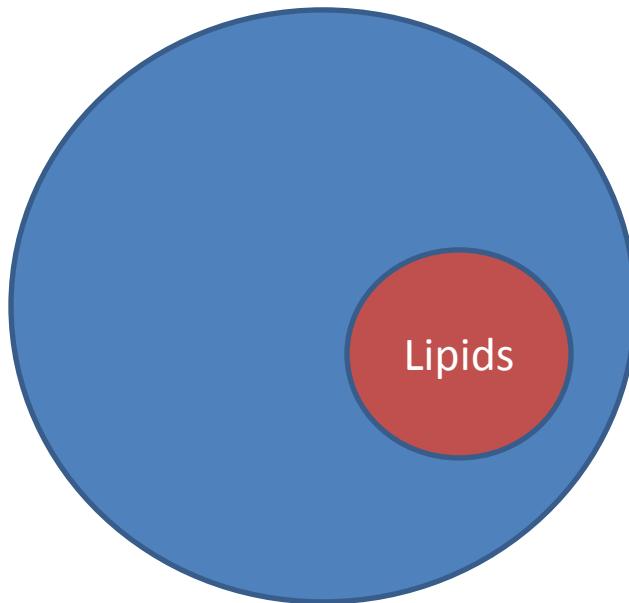


General sample treatment scheme for solid samples



Matrix vs FR

FRs are generally present at the $\mu\text{g}/\text{kg}$ or lower level!



>99% is not the compound of interest, but “matrix” e.g. lipids, proteins, sediment particles, etc.

Sample

- Grinding
- Homogenisation



- Sieving of sediments or dust
- Possibly drying
 - Freeze drying
 - Sodium sulphate
 - Hydromatrix (diatomaceous earth sorbent)



Internal standards

- Addition of internal standards
- To correct for recovery losses during sample treatment
- BFRs
 - ^{13}C -labelled standards (^{13}C -HBCD, ^{13}C -PBDEs, ^{13}C -TBBP-A)
 - F-labelled standards (F-PBDEs)
 - PBDE congeners not present in mixtures or environment (e.g. BDE58)
- Check potential overlap with native standards (retention time and mass spectrum)



Extraction

- **Soxhlet**
 - Hot organic solvent
 - Hexane:acetone
 - Toluene
 - Hexane:dichloromethane
 - Extraction time 8 hrs

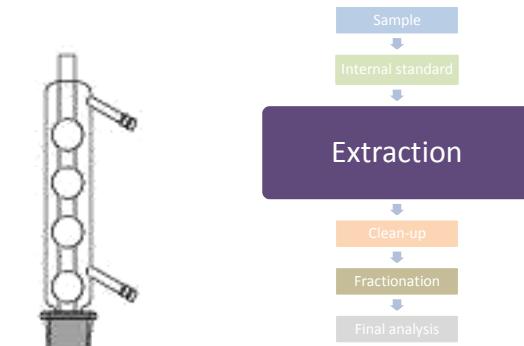
During extraction most matrix (>80%) is removed

- **Accelerated solvent extraction (ASE)**

- Hot organic solvent
- High pressure
- Extraction time 30 min
- Several cycles



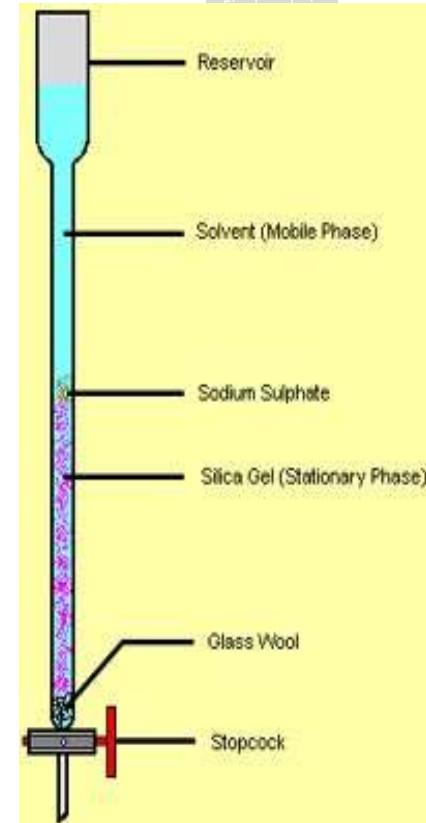
- **Shake extraction and sonication**



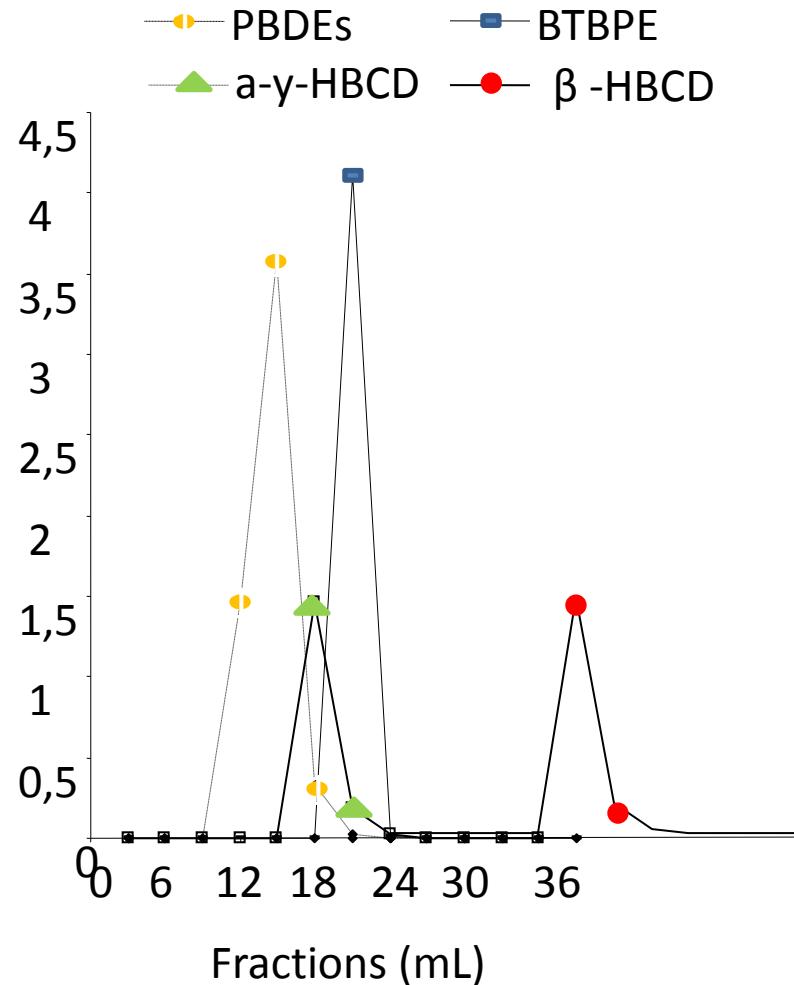
Clean-up biota and sediment



	Lipid removal	Amount solvent used	Remarks
Adsorption chromatography			
Aluminum oxide	++	10-20 ml	Sulfur not removed. Polar compounds retain
Silica gel	+	10-20 ml	Sulfur not removed
Florisil	+	10-20 ml	Sulfur not removed
Gel permeation chromatography			
	++	50-200 ml	Removal of sulfur. Effective humic acid removal
SPE	+	5 ml	Fast method
Sulphuric acid	++	10 ml	TBBP-A can breakdown
Multilayer silica columns	++	10-20 ml	KOH and H ₂ SO ₄ treated silica. Can degrade specific FRs

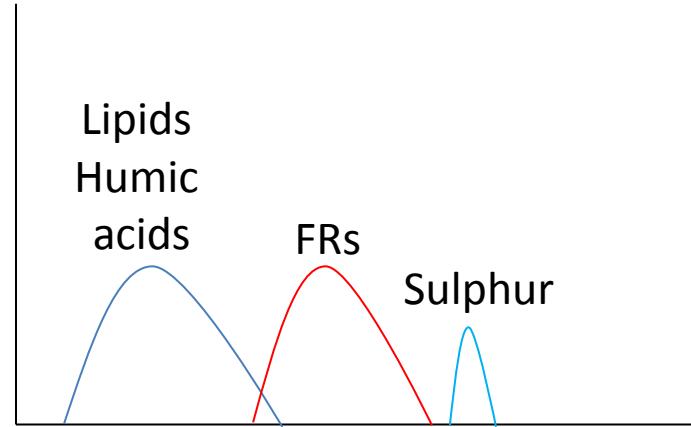


BFR elution profile silica



Sulfur removal from sediment, SPM

- Gel permeation chromatography
 - Sulfur elutes later than BFRs



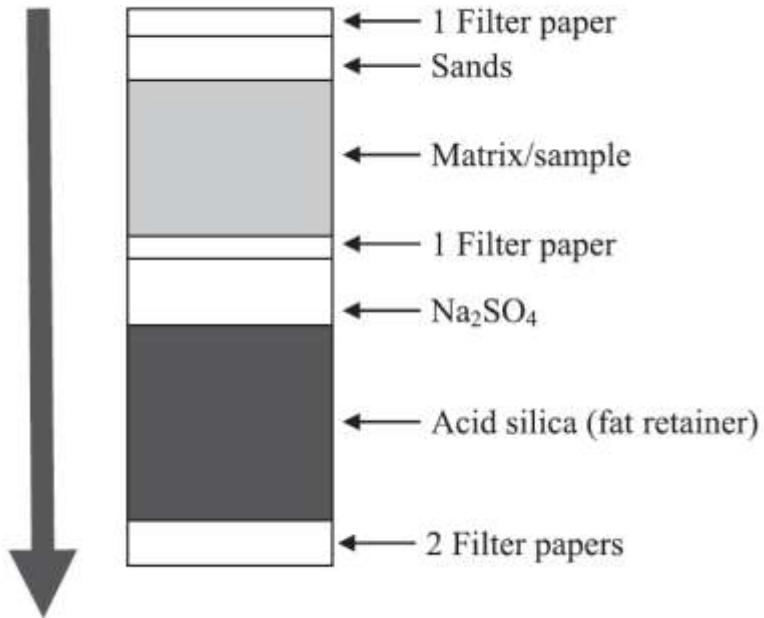
- Copper powder
- Tetrabutylammonium (TBA) sulfite reagents
 - But debromination can occur
- Silver nitrate on silica

Combined extraction and clean-up



ASE: sample+ clean-up
Method for biota or other solid samples

Flow



Recoveries (%) on different spiked level of PBDEs and PCBs in sheep liver samples.

Spiked level (ng g^{-1})	PBDE28	PBDE47	PBDE99	PBDE100	PBDE153	PBDE154	PBDE183
0.02	79	112	90	n/a	n/a	79	n/a
0.05	89	83	97	58	47	91	n/a
0.10	86	63	96	57	70	83	66
0.20	69	67	86	68	57	84	53
0.50	81	66	90	70	108	88	58
1.00	86	57	55	70	111	69	76
5.00	77	73	88	75	103	82	82
10.0	86	98	95	101	99	102	92
20.0	77	76	101	80	92	90	102
30.0	75	80	100	81	102	96	109

Zhang et al., 2011. J. Chrom A, 1218, 1203–1209

Fractionation

Fractionation or additional clean-up to:

- Remove interfering compounds
 - PBBs, PBDE-metabolites
- Remove remaining matrix
 - Lipids (cholesterol, triglycerides)



Mainly used methods:

- Silica
- Florisil
- Small columns H_2SO_4 silica
- (SPE)

Evaporation techniques



- Kuderna Danish apparatus



- Turbovap



- Rotary evaporator



- Nitrogen gas
- small volumes

Final analysis



PBDEs

- GC-MS

HBCD

- GC-MS total analysis
- LC-MS a-,b-, γ -HBCD

TBBP-A

- GC-MS derivatisation improve chromatography and limit of detection
- LC-MS analysis without derivatisation

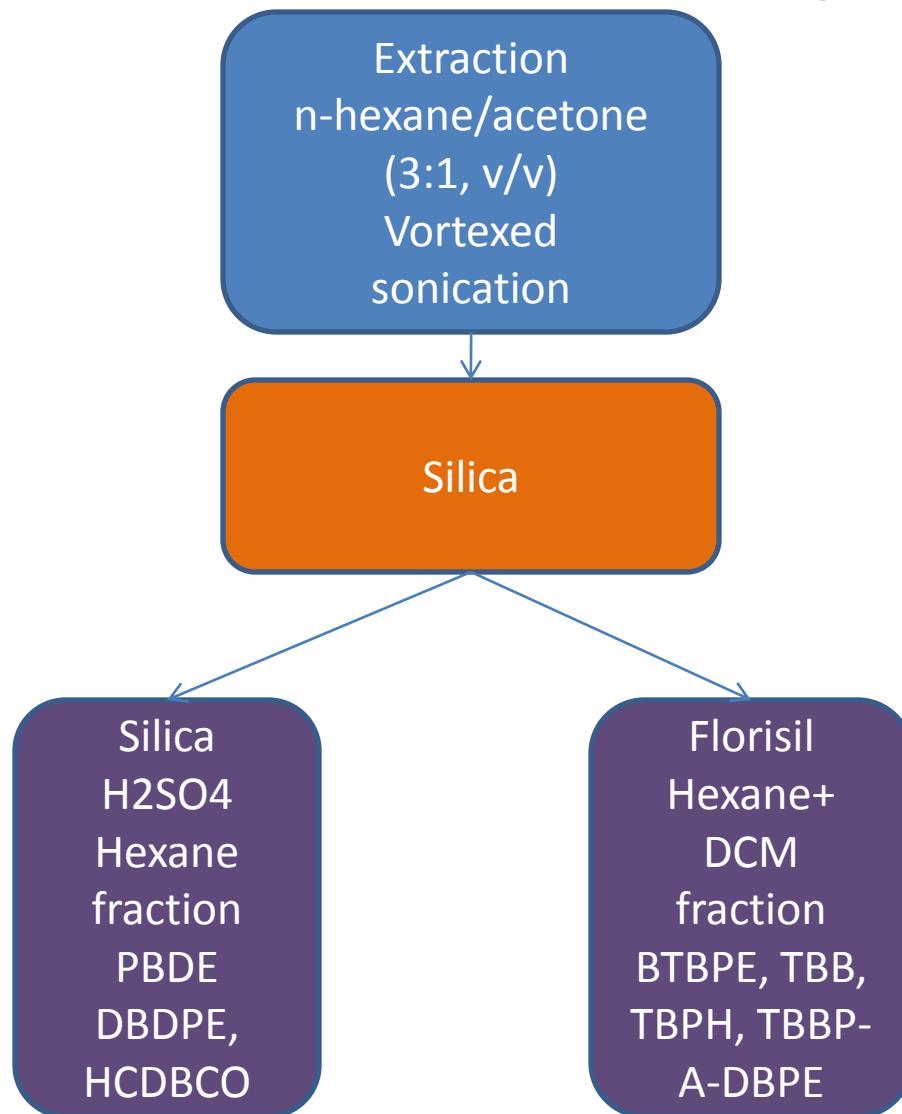
Critical factors FR analysis

- Clean glassware properly before use
- Dust
- UV-light
- β -HBCD retrains on silica gel
- Degradation possible of some BFRs by destructive clean-up methods (H_2SO_4)
- Background contamination
 - Procedural blanks are highly important!!

Recoveries

- Limit of detection
 - PBDEs: 0.001 – 0.01 ng/g wet weight
 - HBCD: 0.01 ng/g wet weight
 - TBBP-A: 0.01 ng/g wet weight
- Recoveries
 - PBDEs, HBCD, TBBP-A >70%
- QA/QC
 - Procedural blank
 - Duplicate analysis
 - Recoveries
 - Reference materials (IRM, SRM, CRM)
 - PBDEs: house dust, sediment, fish tissue, mussel tissue, whale blubber, cod, liver oil, and human serum

Example “new” FR analysis in dust

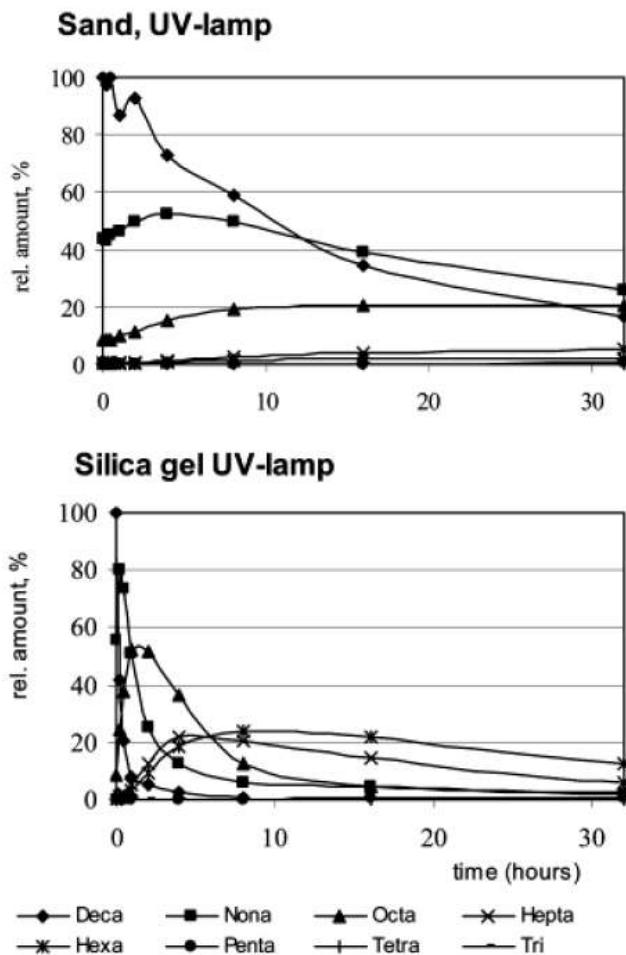


BDE209 analysis

Critical factors

- GC-MS analysis
- Internal standards
- Photo-degradation
- Solubility
- Sources of contamination

Photolytic debromination (I)



Söderström et al., Environ. Sci Technol., 2004, 38 (1), 127-132.

Photolytic debromination (II)

Half-lives (h) BDE209 on different matrices, indoors and outdoors

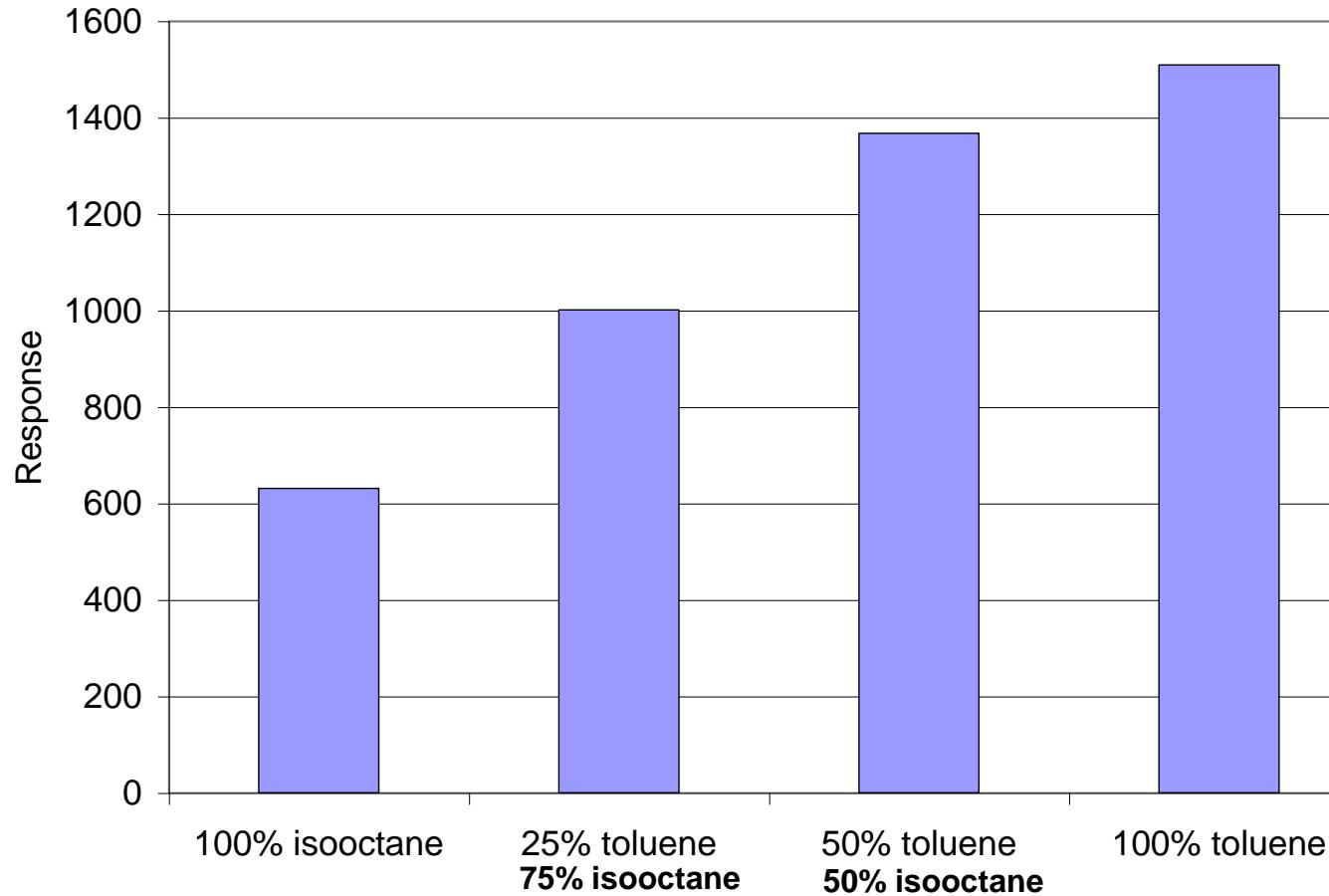
	Artificial UV-light (continuous)	Sunlight (discontinuous)	Sunlight (continuous)
Toluene	<0.25		
Silica gel	<0.25		
Sand	12	37	13
Sediment	40-60	80	30
Soil	150-200		

Söderström et al., Environ. Sci Technol., 2004, 38 (1), 127-132

Photolytic debromination (III)

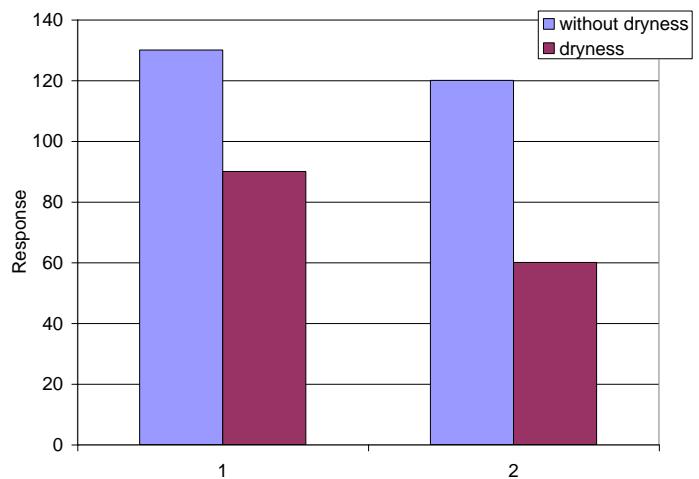
- Use of UV filters at laboratory windows and at fluorescent lightings
- Use of amber glassware or glass covered with aluminium foil

Solubility decaBDE organic solvent



Solubility decaBDE organic solvent

- Evaporation to dryness must be avoided unconditionally
- decaBDE adsorbs strongly to glassware and may not be re-dissolved completely
- Add toluene as keeper before concentrating extracts/solutions



Sources of contamination

- Laboratory infrastructure
 - Plastics, textiles, electronic equipment
- Other samples
- Reagents
- Glassware
- Atmospheric deposition
 - Dust (textile and carpet fibres, human skin, hair etc.)
- Packaging
 - EPS, PS chips, foams etc.
- GC injection system
 - rinse with toluene

Audit of glassware

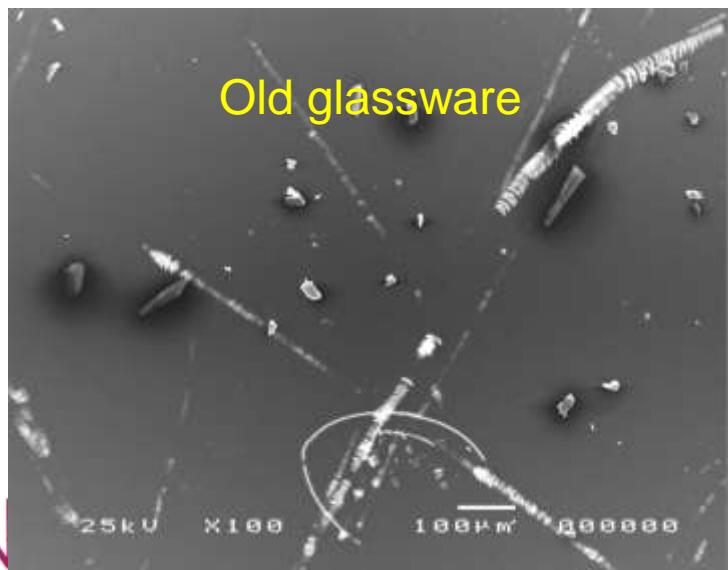
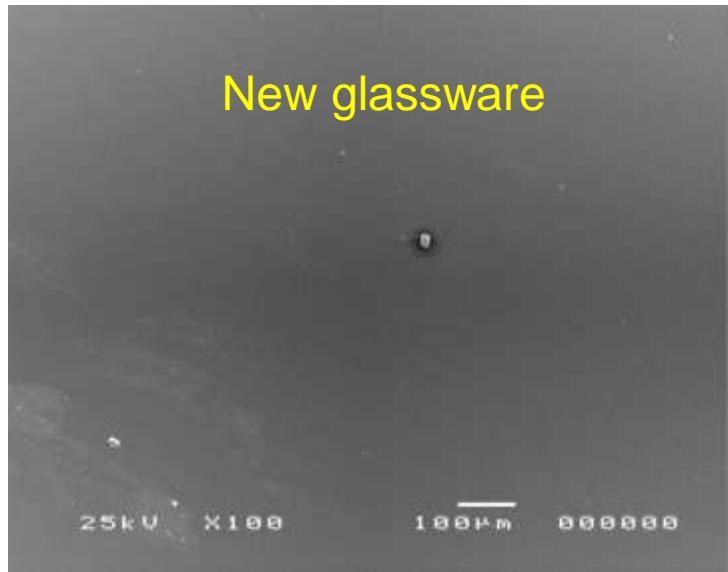
Traditional v. Modern

- Sample collection jar
- Soxhlet apparatus (4)
- Volumetric flask
- Evaporation stage (3)
- Column clean up (3)
- Evaporation stage (3)
- Fractionation stage (3)
- Evaporation stage (2)
- GC vial
- **20 pieces of glassware**
- Sample collection jar
- ASE (1)
- Volumetric flask
- Evaporation stage (3)
- GPC (1)
- Evaporation stage (3)
- Column clean up (1)
- Evaporation stage (3)
- GC vial
- **15 pieces of glassware**

(Colin Allchin, CEFAS)

Glassware as a source of contamination

(Colin Allchin, CEFAS)



Glassware issues

- Cleaning glassware is difficult
- Cleaning old, scratched glassware is even more difficult
- When “blanking” glassware think about solvents and exposure time
- Keep clean glassware clean
- Segregate glassware dependant on sample type

Dust issues

- Levels of decaBDE in dust can be very high
 - 0.1 – 10 mg/kg
- Assume 1 mg/kg in dust of laboratory
 - -> 1 pg/µg dust
 - Final volume in GC vial 1 ml
 - Will result in a concentration of 1 pg/ml in GC vial
 - If 20 pieces of glass are used, each with 1 pg decaBDE in dust -> 20 pg/ml
 - LOD 100 pg/ml

* Harrad et al Environ. Sci. Technol. 2004, 38, 2345-2350

Stapleton et al Environ. Sci. Technol. 2005, 39, 925-931

Harrad et al Environ. Sci. Technol. 2006, 40, 4633-4638

Hazrati & Harrod Environ. Sci. Technol. 2006, 40, 7584-7589

Summary guidelines

- Use ^{13}C decaBDE as internal standard
- Reduce sample exposure to glassware and reduce if possible number of pieces of glassware used
- If possible physically segregate sample by type and analysis in separate areas
- Reduce UV-light exposure (UV-filters or amber glassware)
- Reduce and avoid dust
- Use <15 m GC column
- Use short injector residence times (pulsed splitless) or on-column injection

Analysis of organophosphour flame retardants

Methodology

- Twelve aliphatic and cyclic PFRs

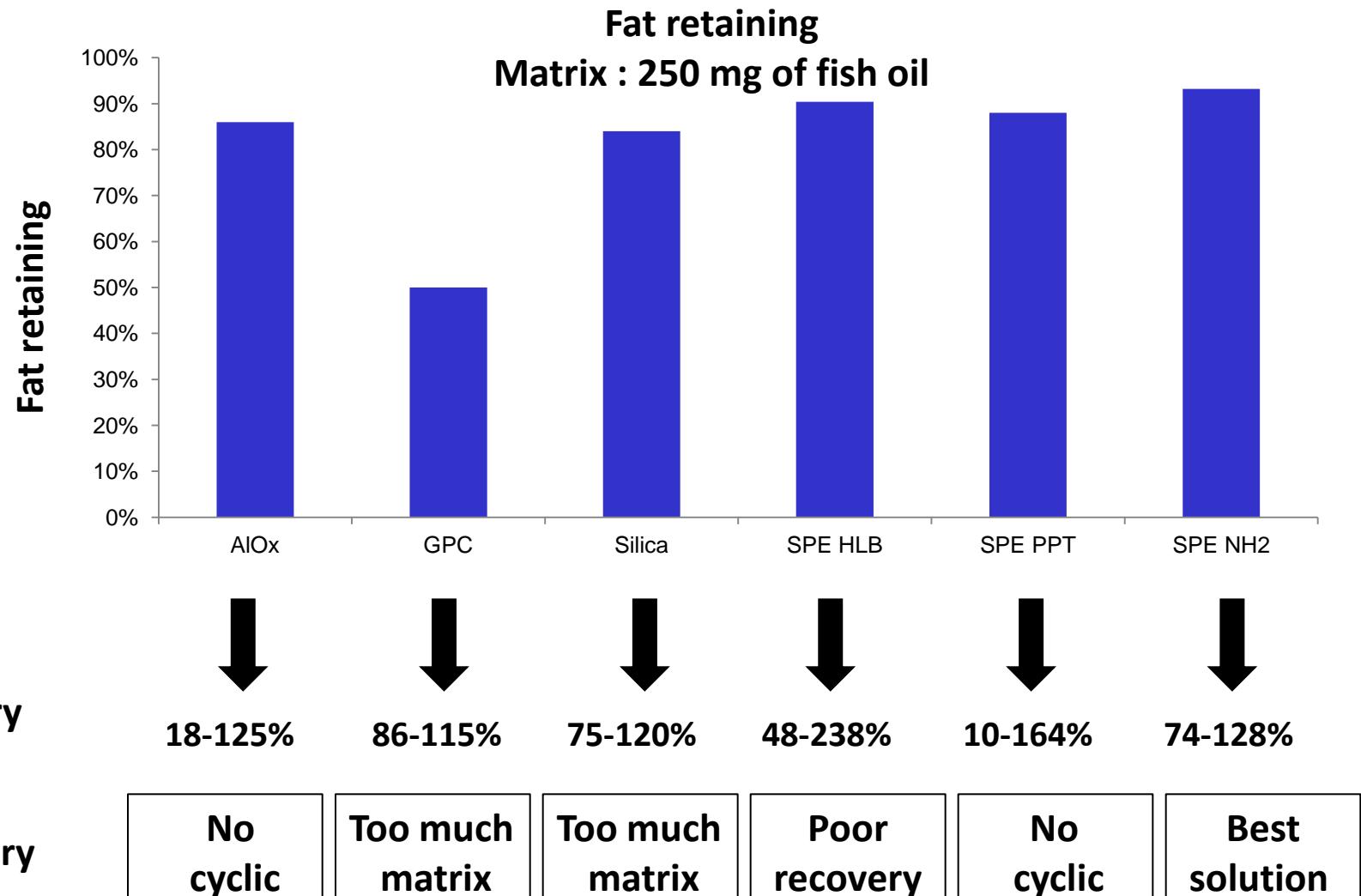
TCEP	115-96-8	tris(2-chloroethyl)phosphate	TEHP	78-42-2	tris(2-ethylhexyl)phosphate
TCPP	13674-84-5	tris(1-chloro-2-propyl)phosphate	DBPhP	2528-36-1	dibutylphenylphosphate
TDCPP	13674-87-8	tris(1,3-dichloro-2-propyl)phosphate	DPhBP	2752-95-6	butyldiphenylphosphate
TiBP	126-71-6	Tri-iso-butylphosphate	EHDP	1241-94-7	2-ethylhexyldiphenylphosphate
TBP	126-73-8	tributylphosphate	TPP	115-86-6	triphenylphosphate
TBEP	78-51-3	tris(2-butoxyethyl)phosphate	TCP	1330-78-5	tricresylphosphate (o, m, p)

- Extraction

- Accelerated Solvent Extraction (ASE)



Cleanup



Background levels

- PFRs
 - Detected in air and dust
 - Used in floor polish and as plasticizer

Mean blank	TiBP (ng)	TBP (ng)	TCPP (ng)	TBEP (ng)	TPP (ng)	EHDP (ng)
Silica/PPT blank (n=17)	5.6	12	4.4	93	0.9	0.9
SPE-NH ₂ blank (n=5)	1.7	36	3.6	7.8	1.7	0.8

Summary

- Make a plan before you start:
 - Aim
 - Compounds of interest
 - Matrix
 - Limit of detection
 - Amount of sample
 - Select methods

Guidance documents BFR analysis

- Adrian Covaci, Stuart Harrad, Mohamed A.-E. Abdallah, Nadeem Ali, Robin J. Law, Dorte Herzke, Cynthia A. de Wit. 2011. Novel brominated flame retardants: A review of their analysis, environmental fate and behaviour. *Environment International* 37 (2011) 532–556
- Boer, de J, Allchin, C, Law, R, Zeger, B, Boon JP. 2001. Method for the analysis of polybrominated diphenylethers in sediments and biota. *TrAC, Trend in Anal. Chem.*, 20, 591
- Nadeem Ali, Stuart Harrad, Dudsaddee Muenhor, Hugo Neels and Adrian Covaci. 2011. Analytical characteristics and determination of major novel brominated flame retardants (NBFRs) in indoor dust. *Anal Bioanal Chem*, 400:3073–3083
- Boer, J. de (2006). The use of GC-MS and LC-MS in the environmental monitoring of brominated flame retardants. In: M.L. Gross and R.M. Caprioli (eds.): *Encyclopedia of Mass Spectrometry*, Elsevier, Amsterdam, The Netherlands, pp.571-579.
- Covaci, A., Gerecke, A.C., Law, R.J., Voorspoels, S., Kohler, M., Heeb, N.V., Leslie, H., Allchin, C.R. & Boer, J. de (2006). Hexabromocyclododecanes (HBCDs) in the environment and humans: a review. *Environ. Sci. Technol.* 40, 3679-3688.
- Morris, S., Allchin, C., Zegers, B., Haftka, J.J.H., Boon, J.P., Belpaire, C., Leonards, P., Leeuwen, S.P.J. van & Boer, J. de (2004). Distribution and fate of HBCD and TBBPA brominated flame retardants in North Sea estuaries and aquatic food webs. *Environ. Sci. Technol.*, 38, 5497-5504.

Guidance documents BFR analysis

- Lopez, P; Brandsma, SA; Leonards, PEG, de Boer, J. 2011. Optimization and development of analytical methods for the determination of new brominated flame retardants and polybrominated diphenyl ethers in sediments and suspended particulate matter. *Anal. Bioanal. Chem.* 400 (3), 871-883.