

# Principle of FR analysis (I)

## Environmental samples

Pim Leonards and Sicco Brandsma

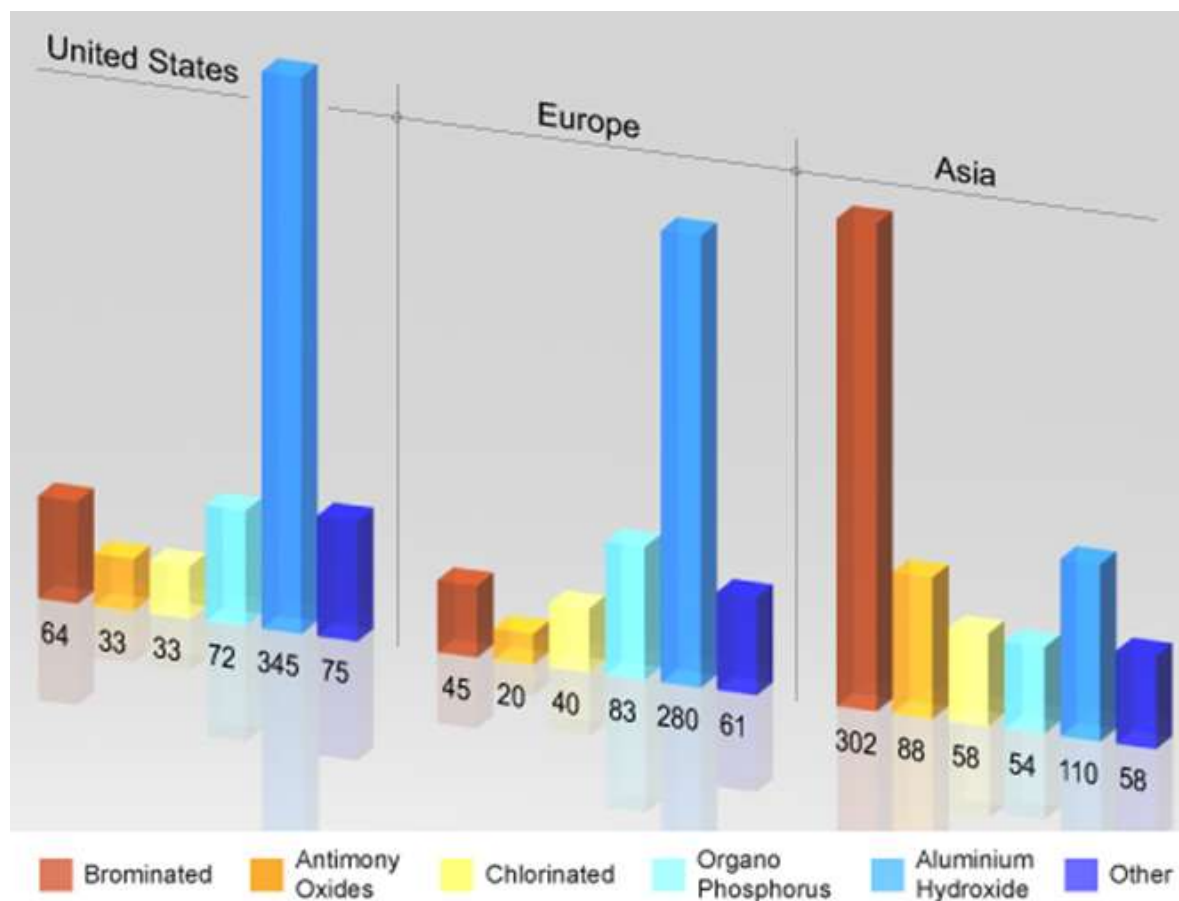
# Outline

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

# Classes of flame retardants (FRs)

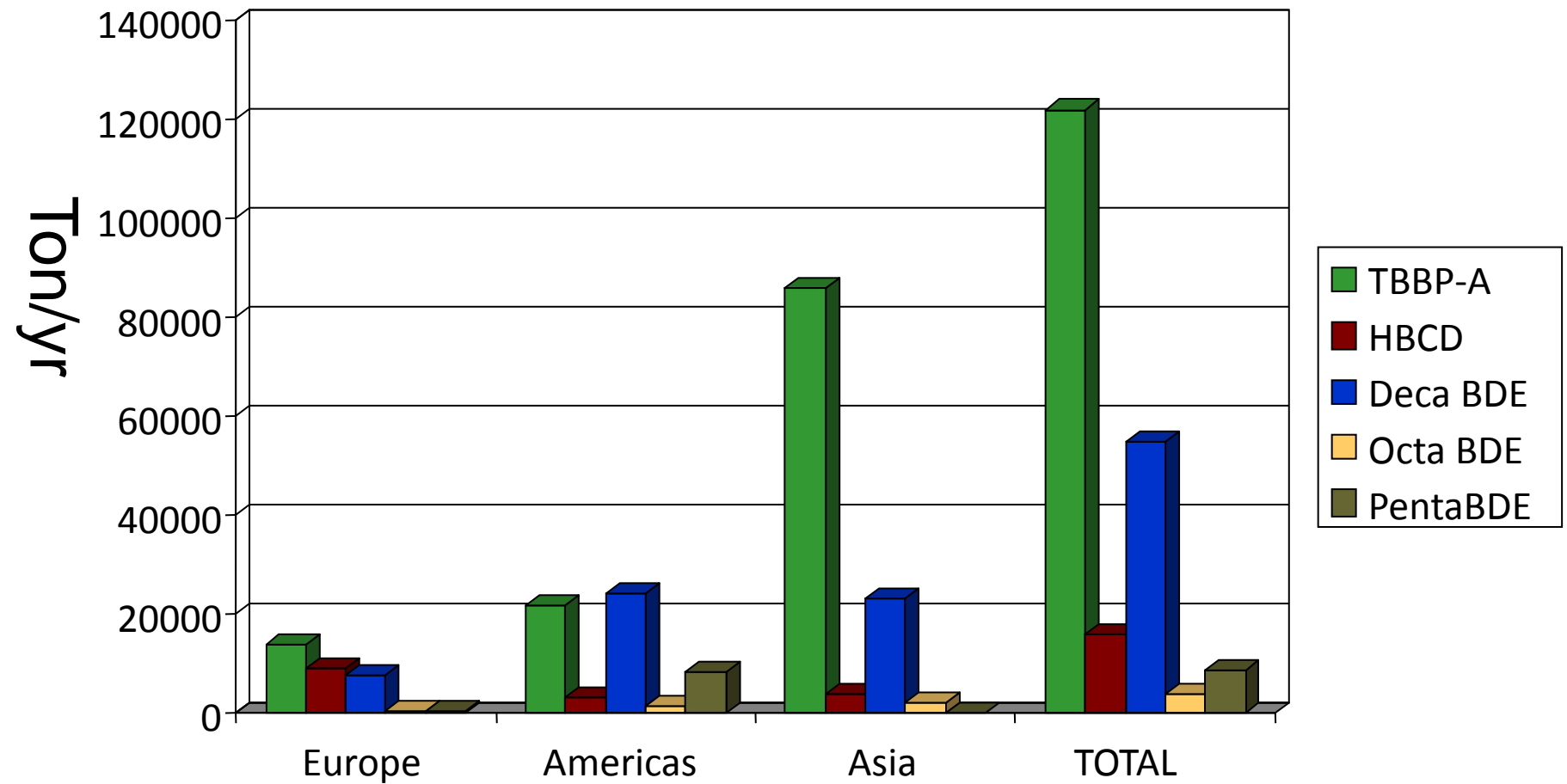
- Brominated FRs
  - polybrominated diphenyl ethers (PBDEs)
  - hexabromocyclododecanes (HBCDs)
  - tetrabromobisphenol-A (TBBP-A)
- Phosphoric FRs: - halogenated and non-halogenated
- Inorganic FRs: - antimony oxide, hydrated aluminium
- Chlorinated FRs: - polychlorinated alkanes (PCAs)

# Flame Retardant Consumption 2007

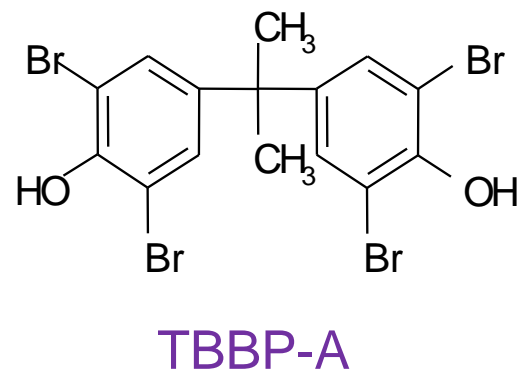
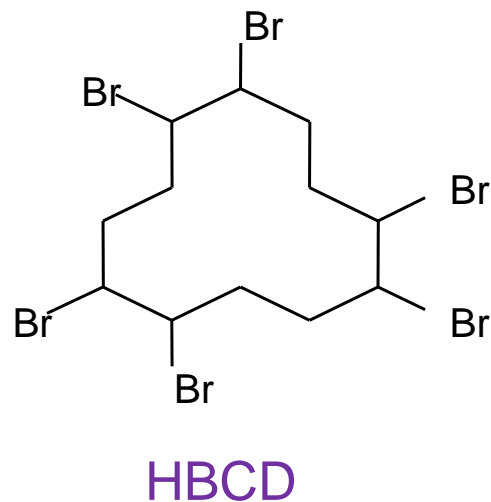
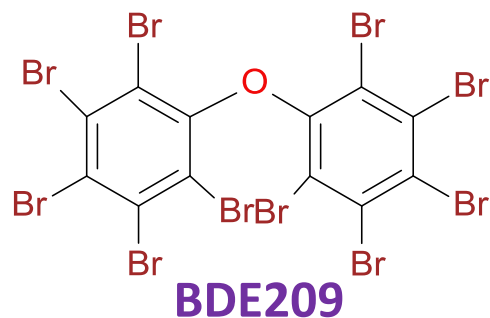
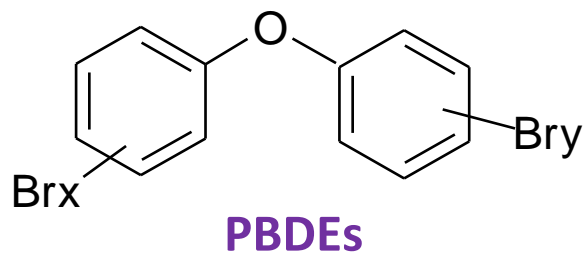


(SRI Consulting, 2008)

# BFR production in 1999



# Structures of BFRs

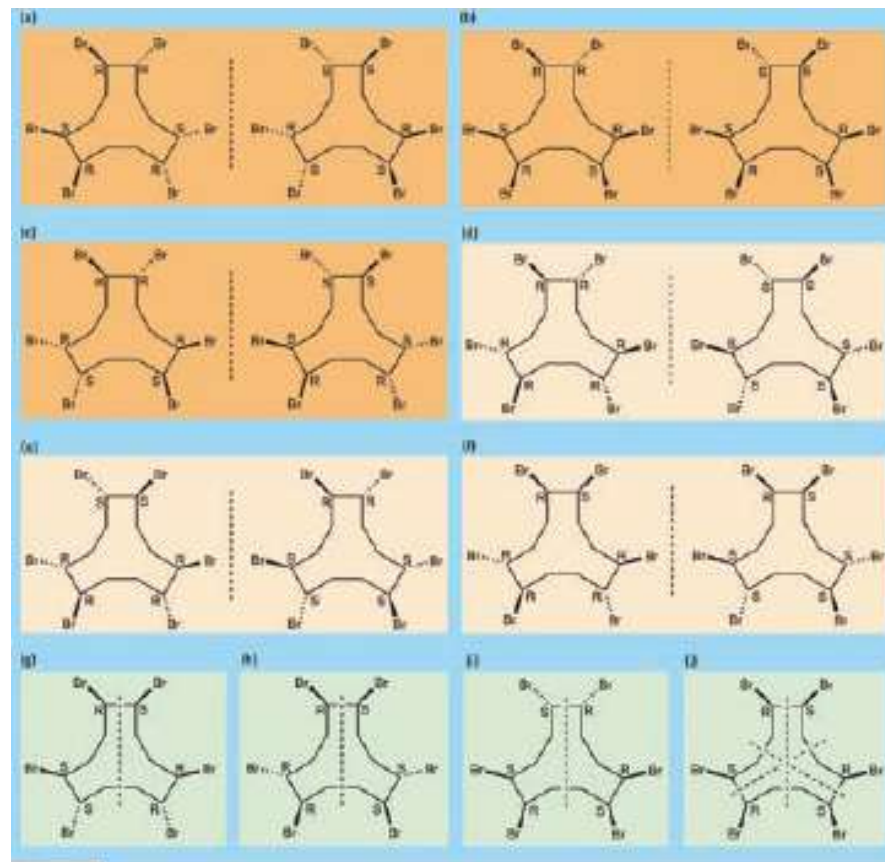
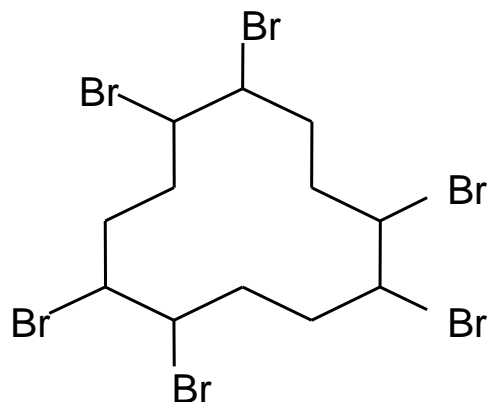


# Hexabromocyclododecanes (HBCDs)

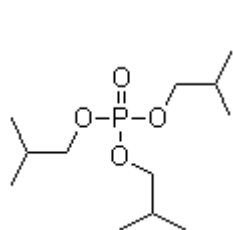
Congeners investigated in most studies:

$\alpha$ -,  $\beta$ -  $\gamma$ -HBCD present in the technical mixtures

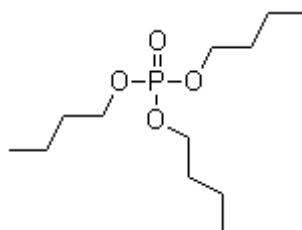
$\gamma$ -HBCD dominant isomer in the technical product



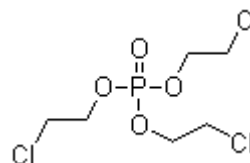
# Organophosphorous flame retardants (PFRs)



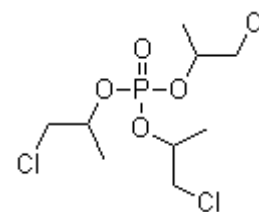
**TiBP**



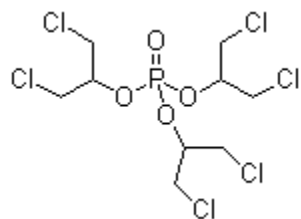
**TBP**



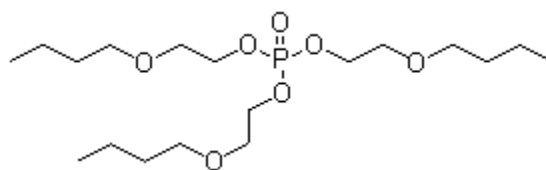
**TCEP**



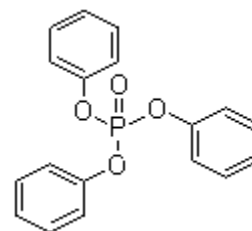
**TCPP**



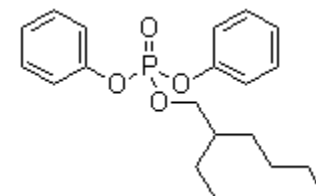
**TDCPP**



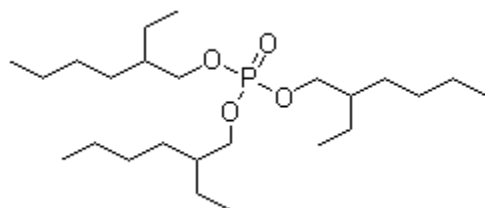
**TBEP**



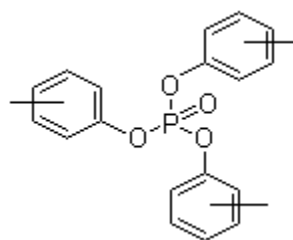
**TPP**



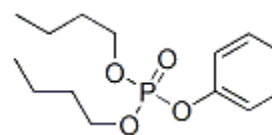
**EHDP**



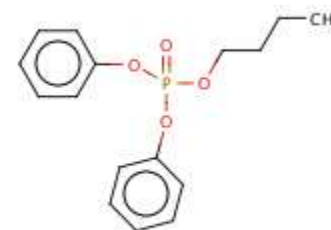
**TEHP**



**TCP**



**DBPhP**



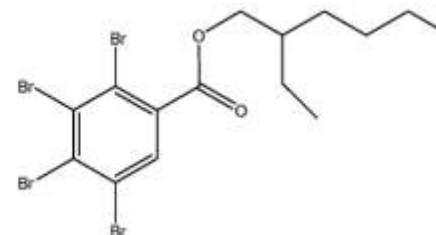
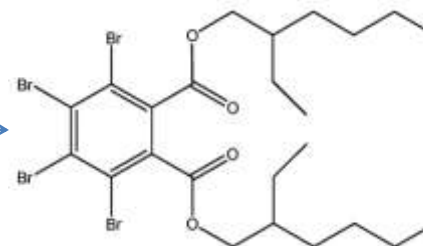
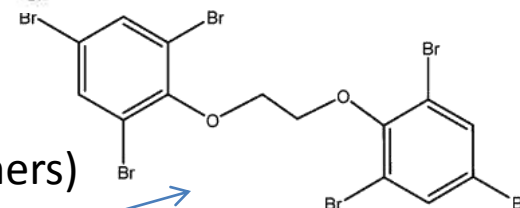
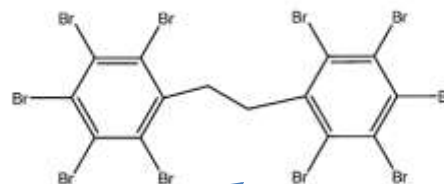
**DPhBP**



# 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

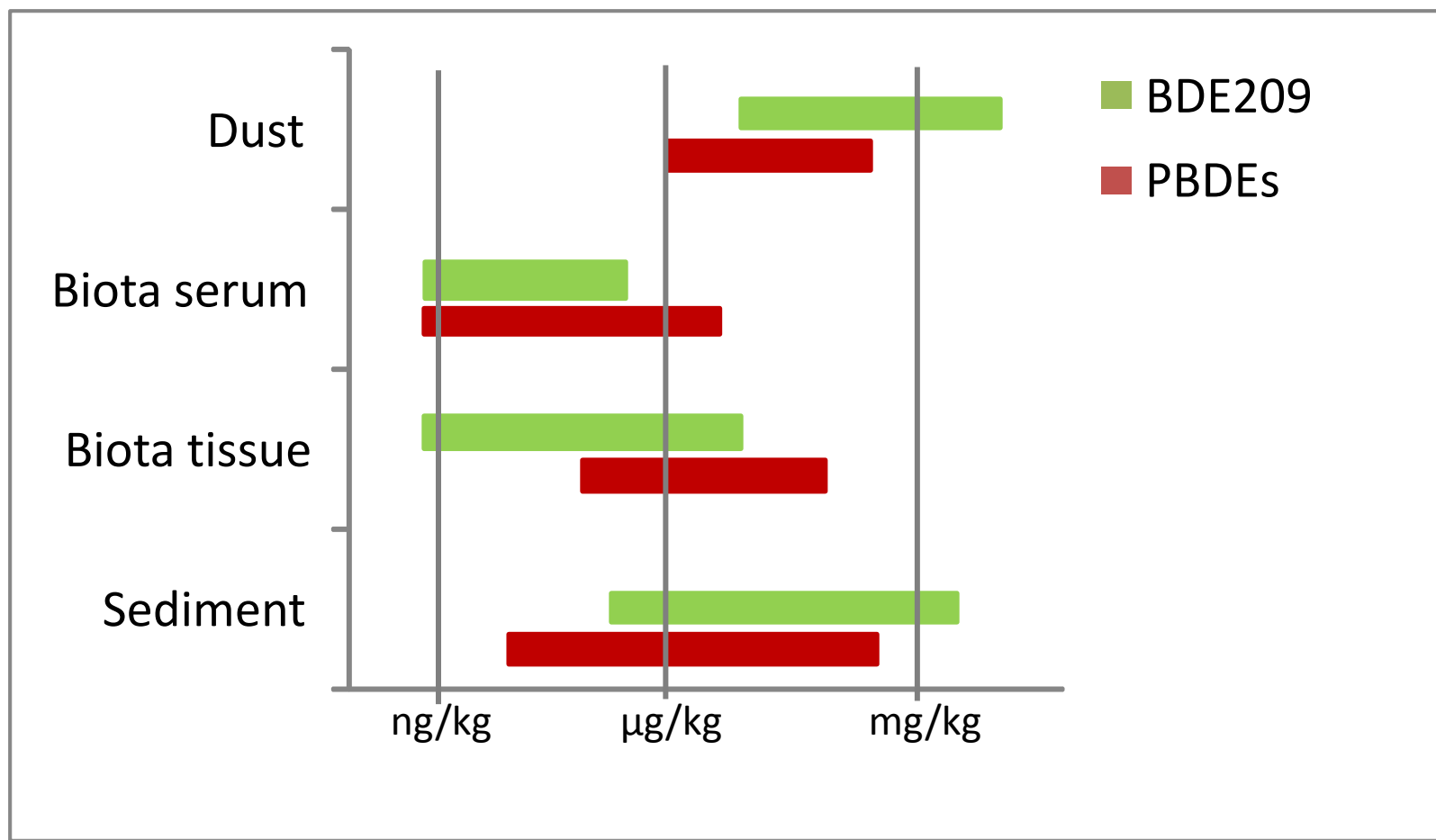


Expected level and  
limit of detection



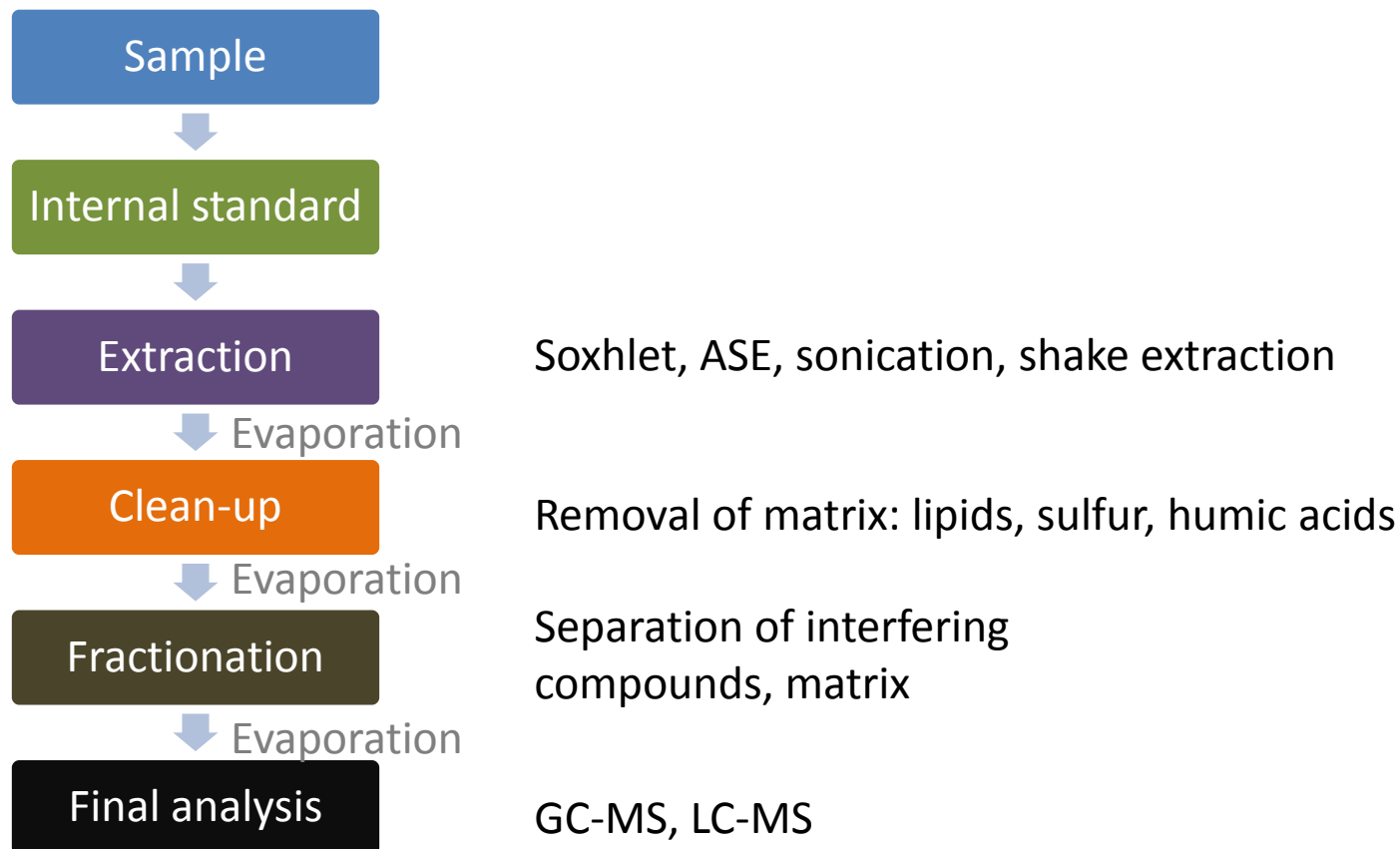
Select appropriate sample treatment  
method

# Schematic diagram of PBDE levels



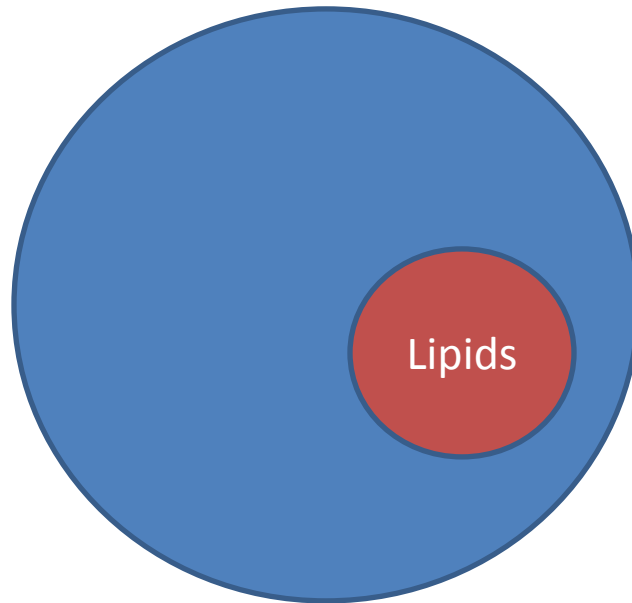


# General sample treatment scheme for solid samples



# Matrix vs FR

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



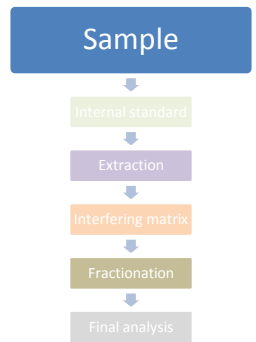
>99% is not the compound of interest, but “matrix” e.g. lipids, proteins, sediment particles, etc.

# 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
  - $^{13}\text{C}$ -labelled standards ( $^{13}\text{C}$ -HBCD,  $^{13}\text{C}$ -PBDEs,  $^{13}\text{C}$ -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

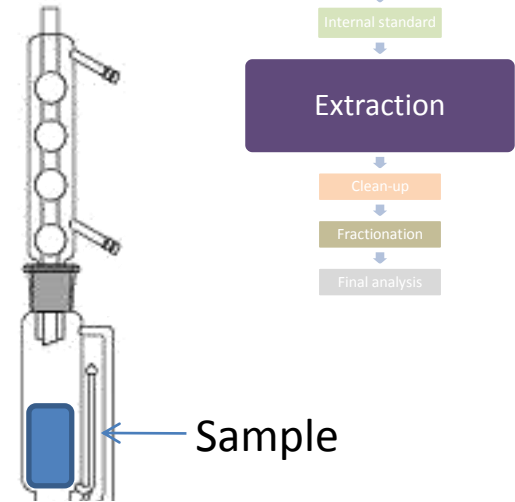
During extraction most matrix (>80%) is removed

- Accelerated solvent extraction (ASE)

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



- Shake extraction and sonication



# Clean-up biota and sediment

Sample

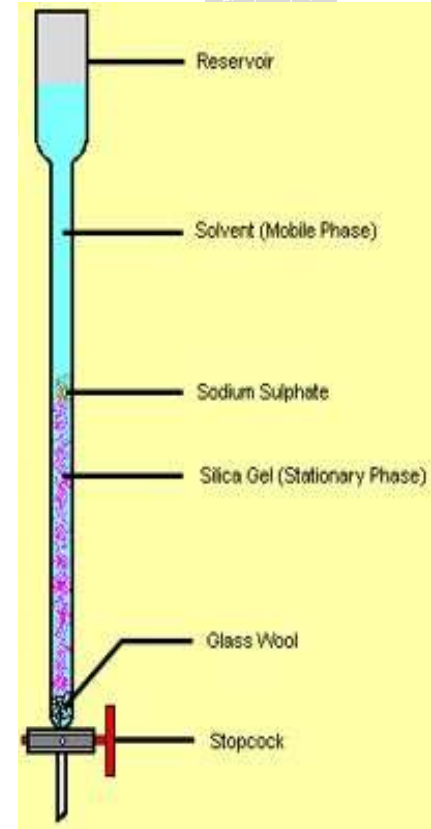
Internal standard

Extraction

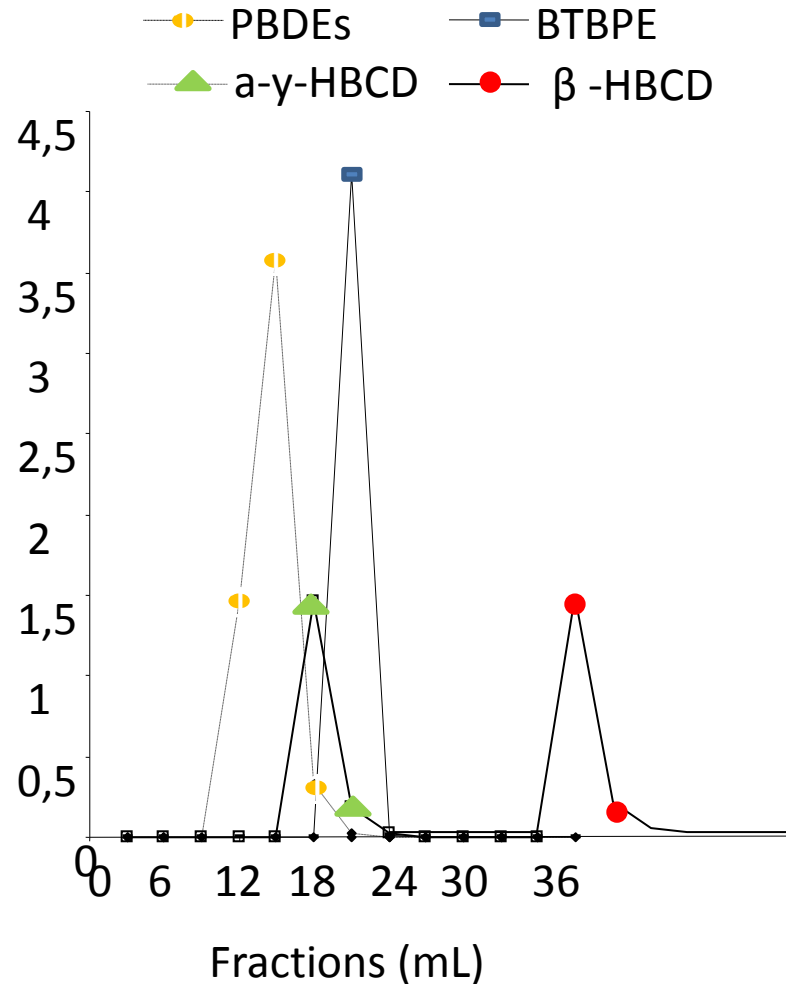
Clean-up

Fractionation

	Lipid removal	Amount solvent used	Remarks
<b>Adsorption chromatography</b>			
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
<b>Gel permeation chromatography</b>	++	50-200 ml	Removal of sulfur. Effective humic acid removal
<b>SPE</b>	+	5 ml	Fast method
<b>Sulphuric acid</b>	++	10 ml	TBBP-A can breakdown
<b>Multilayer silica columns</b>	++	10-20 ml	KOH and H <sub>2</sub> SO <sub>4</sub> treated silica. Can degrade specific FRs

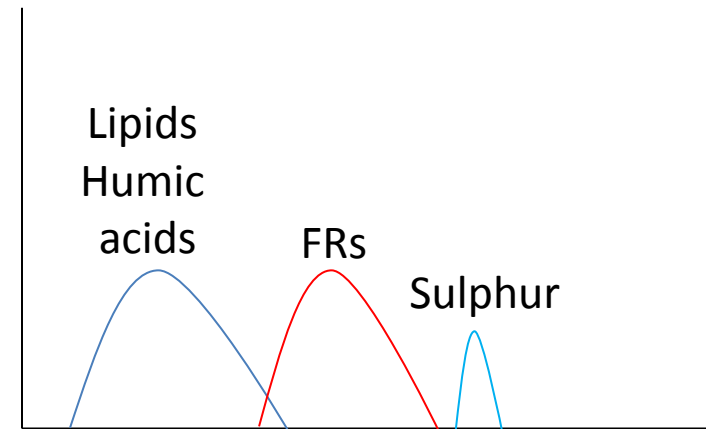


# BFR elution profile silica



# Sulfur removal from sediment, SPM

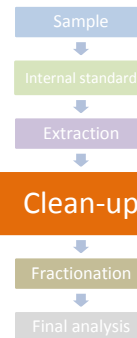
- Gel permeation chromatography
  - Sulfur elutes later than BFRs



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

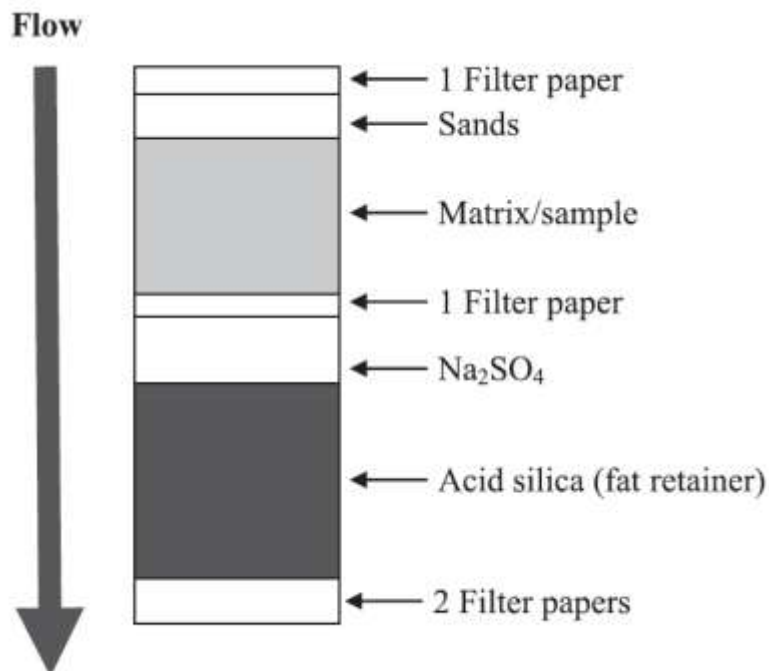


# Combined extraction and clean-up



ASE: sample+ clean-up

Method for biota or other solid samples

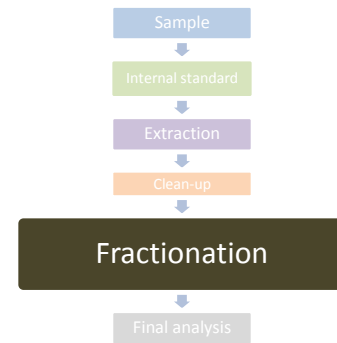


Recoveries (%) on different spiked level of PBDEs and PCBs in sheep liver samples.

Spiked level (ng g <sup>-1</sup> )	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  $\text{H}_2\text{SO}_4$  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  $\alpha$ -,  $\beta$ -,  $\gamma$ -HBCD

## TBBP-A

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

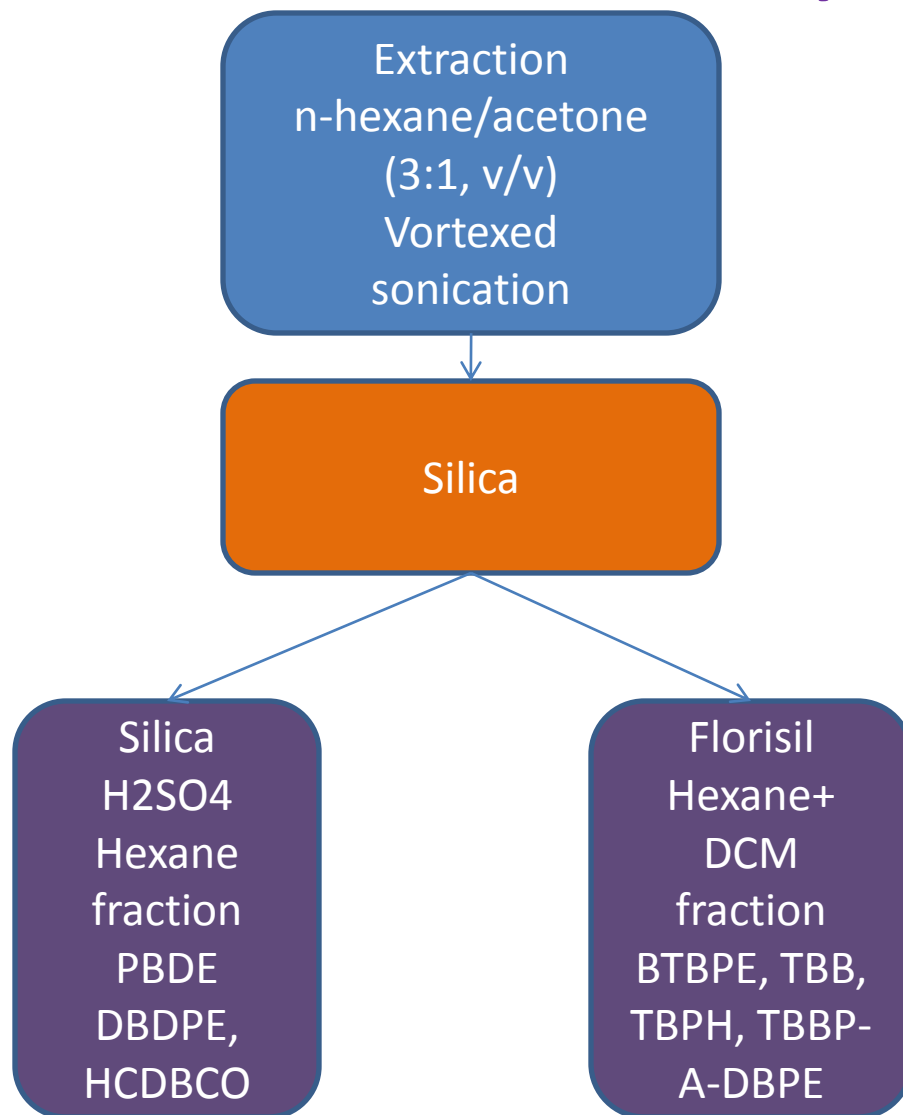
# Critical factors FR analysis

- Clean glassware properly before use
- Dust
- UV-light
- $\beta$ -HBCD retracts on silica gel
- Degradation possible of some BFRs by destructive clean-up methods ( $\text{H}_2\text{SO}_4$ )
- 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



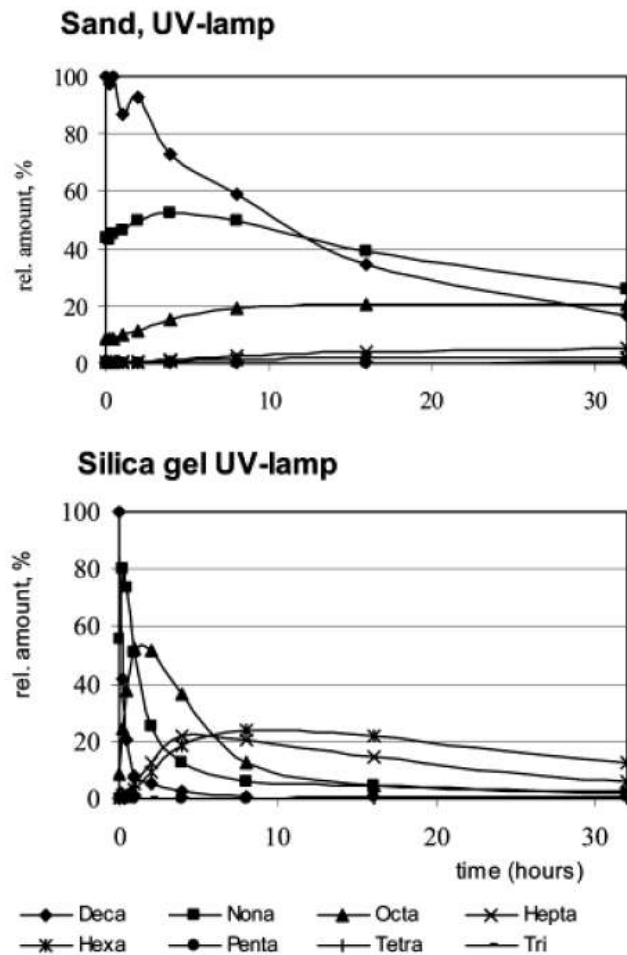
# BDE209 analysis



# 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

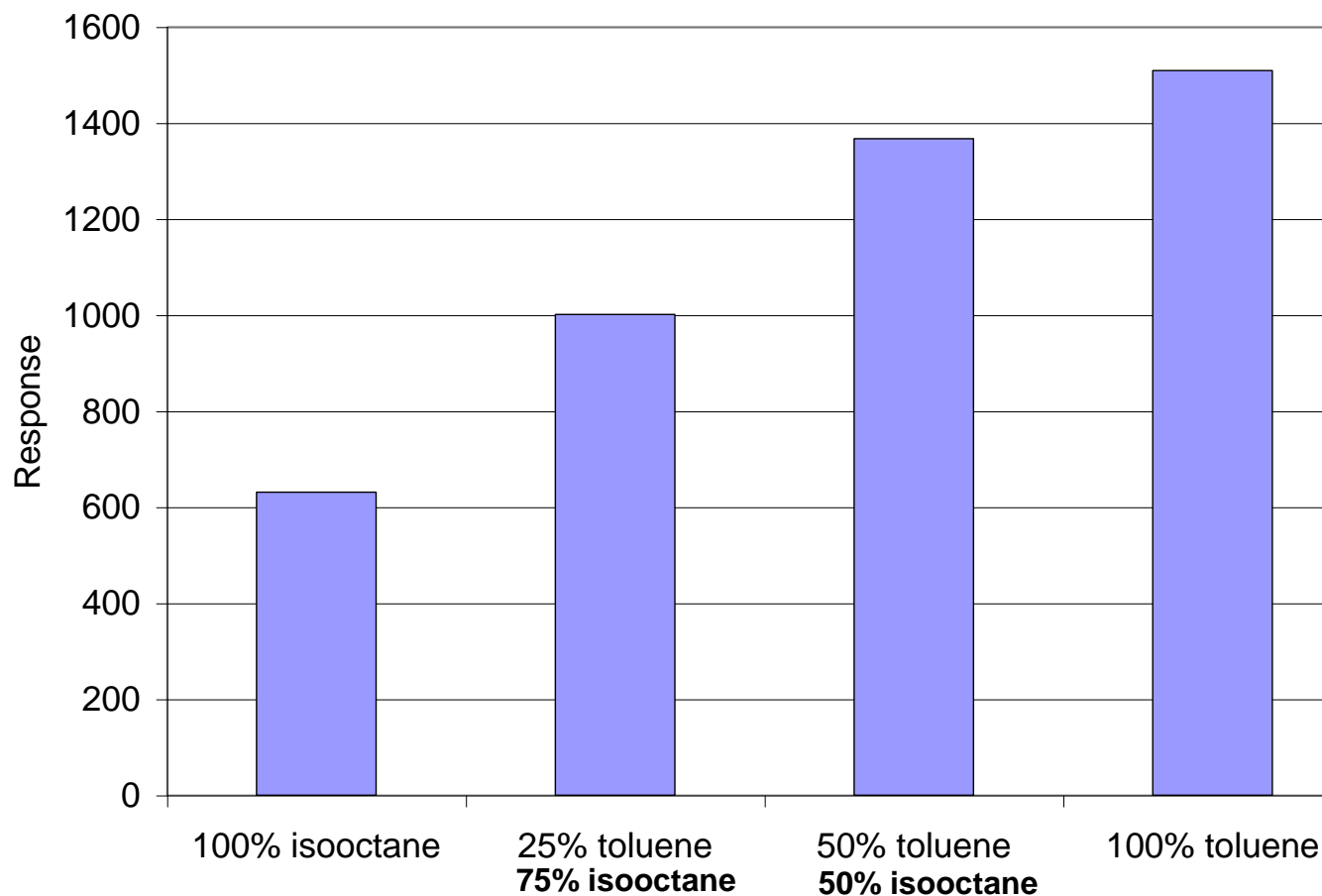
	Artificial UV-light (continuous)	Sunlight (discontinuous)	Sunlight (continuous)
Toluene	<0.25		
Silica gel	<0.25		
Sand	12	37	13
Sediment	40-60	80	30
Soil	150-200		

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

# Photolytic debromination (III)

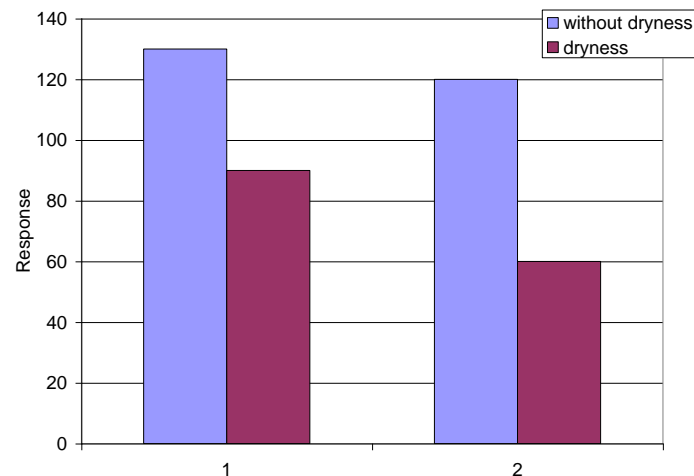
- Use of UV filters at laboratory windows and at fluorescent lightings
- Use of amber glassware or glass covered with aluminium foil

# 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

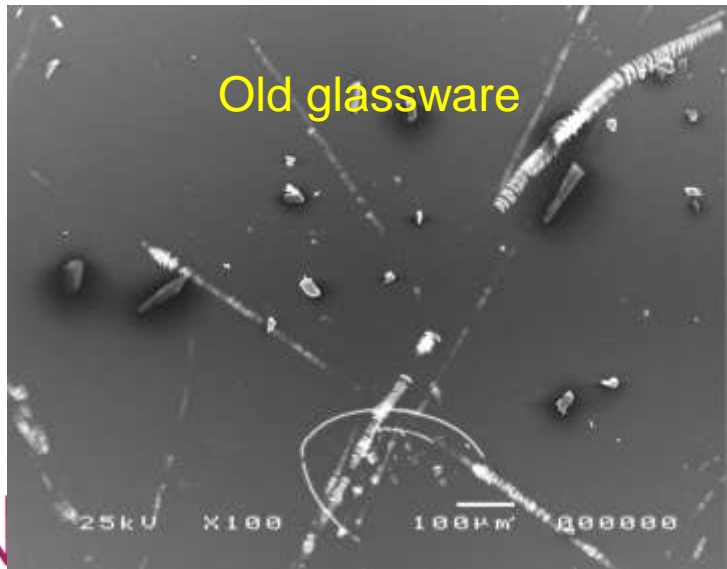
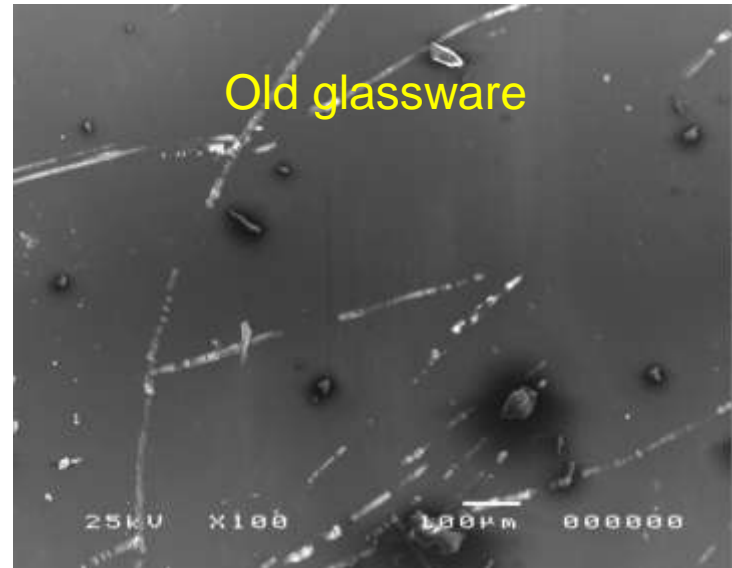
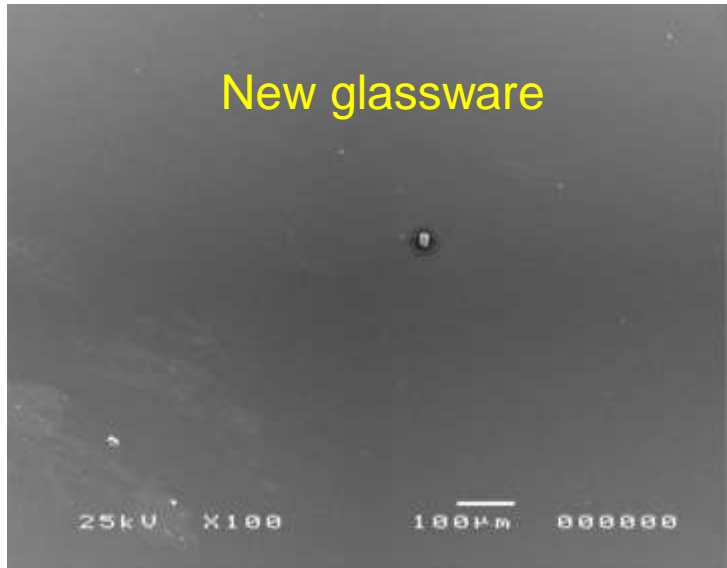
- |                                 |                                 |
|---------------------------------|---------------------------------|
| • Sample collection jar         | • Sample collection jar         |
| • Soxhlet apparatus (4)         | • ASE (1)                       |
| • Volumetric flask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

(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  $^{13}\text{C}$  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

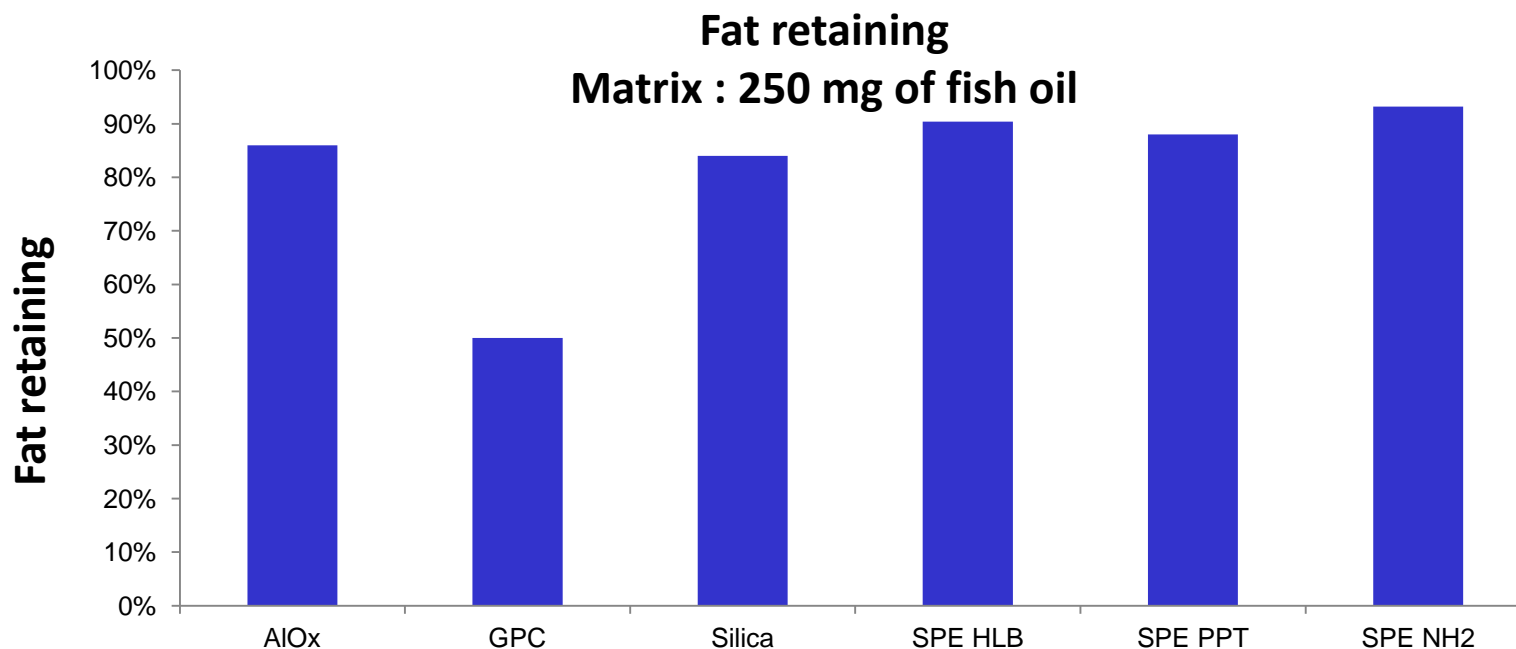
<b>TCEP</b>	115-96-8	tris(2-chloroethyl)phosphate	<b>TEHP</b>	78-42-2	tris(2-ethylhexyl)phosphate
<b>T CPP</b>	13674-84-5	tris(1-chloro-2-propyl)phosphate	<b>DBPhP</b>	2528-36-1	dibutylphenylphosphate
<b>TDCPP</b>	13674-87-8	tris(1,3-dichloro-2-propyl)phosphate	<b>DPhBP</b>	2752-95-6	butyldiphenylphosphate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

- Extraction

- Accelerated Solvent Extraction (ASE)



# Cleanup



**Recovery  
PFRs**

18-125%

86-115%

75-120%

48-238%

10-164%

74-128%

**Summary**

**No  
cyclic**

**Too much  
matrix**

**Too much  
matrix**

**Poor  
recovery**

**No  
cyclic**

**Best  
solution**

# Background levels

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

Mean blank	<b>TiBP</b> (ng)	<b>TBP</b> (ng)	<b>TCPP</b> (ng)	<b>TBEP</b> (ng)	<b>TPP</b> (ng)	<b>EHDP</b> (ng)
Silica/PPT blank (n=17)	5.6	12	4.4	93	0.9	0.9
SPE-NH2 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, Dudsadee 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 re-tardants 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.