HRMS PBDE Analysis on a Thermo DFS

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Outline

- PBDE – what is it and why are we interested in it
- EPA 1614 – HRMS analysis
- Considerations for Thermo DFS / Trace GC
- Food analysis
- High volume water analysis

Polybrominated Diphenylether (PBDE)

- PBDE flame retardants are added to some plastics, electrical and electronic equipment, upholstered furniture, non-clothing textiles and foam products. Because PBDEs are added to the products rather than chemically bound into them, they can be slowly and continuously released from the products during their manufacture, while in use, or after their disposal.
- PBDEs have been found both in the environment and in humans, including in human breast milk in Canada, the United States and Europe. While the levels in humans are very low, they have been increasing with time, and are higher in North Americans than in Europeans.
- Three commercial mixtures – Penta, Octa and Deca
- A number of countries and US states have moved to ban PentaBDE and OctaBDE which they consider to be of greatest concern. The major North American manufacturer of these two PBDEs ceased their production in 2004. Because of environmental concerns, many companies have stopped using PBDEs in their products, and it is anticipated that more will follow. In Sweden, the levels of PBDEs in breast milk decreased soon after measures to reduce their use and environmental release were introduced.

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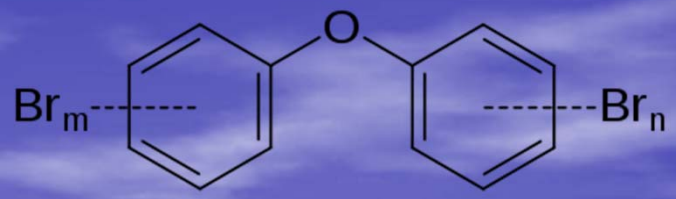
PBDE and the Stockholm Convention (POPs)

- May 2009 nine new compounds added to the POPs list including PBDE
  - Commercial Penta (PentaBDE (99, 100)and TetraBDE (47))
  - Commercial Octa (HexaBDE (153, 154) and HeptaBDE (183/175)
- Deca BDE not on the list
- All PBDE either banned or pulled off the market

Chemical Structure



- 209 possible congeners – mono thru decachlorobiphenyl
- No Toxic Equivalency Factors (TEF) yet established but thought to be in the range of PCBs

- MonoBDE 3 congeners
- DiBDE 12 congeners
- TriBDE 24 congeners
- TetraBDE 42 congeners
- PentaBDE 46 congeners
- HexaBDE 42 congeners
- HeptaBDE 24 congeners
- OctaBDE 12 congeners
- NonaBDE 3 congeners
- DecaBDE 1 congeners

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Analytical Options: Why HRMS?

- GC/ECD
  - quick and cheap – okay for dominant congeners (47, 99, 100, 153, 154, 183, 209)
  - Internal standard quantitation
  - Interferences from overlap of levels of bromination (LOB)
- GC/MSD
  - Monitor 79/81 and use like ECD
  - Run in SIM and eliminate LOB problems
  - IS or Isotope Dilution techniques (although Isotope techniques more expensive)
  - Possible to use NCI for improved detection limits
- GC/HRMS
  - Great sensitivity and selectivity (ppt and ppq)
  - Isotope dilution possible

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EPA Method 1614

- HRGC-HRMS congener specific method (August 2007)
- Uses 8 internal standards ($^{13}C_{12}$-labeled congeners)
  - Dominant congeners in commercial mixtures – 28L, 47L, 99L, 100L, 153L, 154L, 183L, 209L
  - One clean-up standard ($^{13}C_{12}$-BDE139) and two $^{13}C_{12}$-PCB recovery standards
- Five point (1-2000 ng/mL) calibration for PBDEs with labeled standard
- Single point calibration for other PBDEs (41 additional compounds)
- Two methods of quantification
  - Isotope dilution (for 8 congeners with labeled standards)
  - Internal standard – everything else
- 30 m DB-5ht column (use 15 m for BDE209)

HRMS analysis – BDE209

- 10 bromines = big molecule – m/e 957/959
- Thermal degradation at >300C
  - Byproducts NonaBDE (206, 207, 208)
  - To a lesser extent OctaBDE
- Column length critical
  - 30 m , difficult to elute in reasonable time; poor sensitivity
  - 15 m, okay for BDE209 but can you get resolution for BDE49/71
  - 6 m, good in theory but means two analysis or double column.
- Cleanliness is next to godliness!
  - BDE47 20-50 pg
  - BDE99, BDE100 5-40 pg
  - BDE209 100 pg

Thermo DFS conditions

- Thermo DFS HRMS with dual Thermo Trace GCs, splitless with surge injection
- Nine functions – one for each LOB

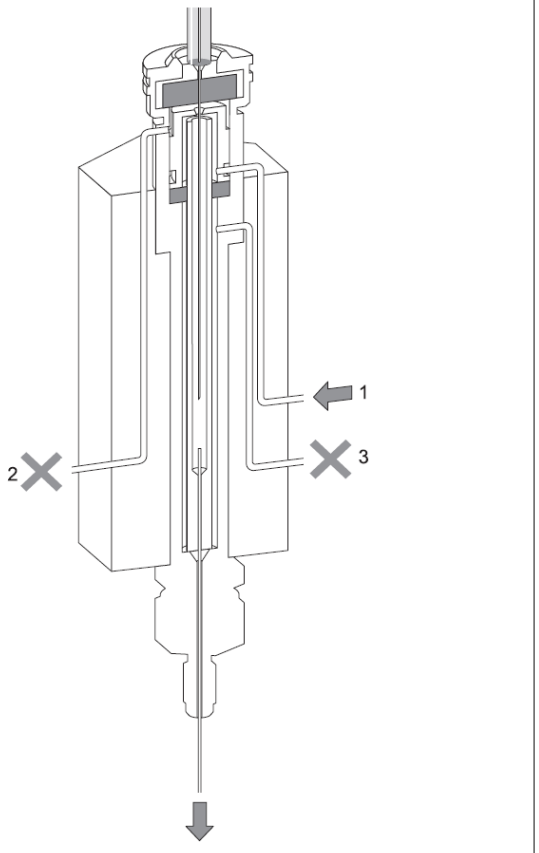
Trace GC Injection Port

Splitless injection with surge

"For narrower diameter columns (< 0.22 mm) with inherently lower flows (< 1.0 mL/min), the transfer might never be completely achieved due to back diffusion of sample vapors in the injector at a higher rate than transfer into the column.

You can counter this by using the splitless surge pressure mode. In this mode, the pressure in the injector temporarily increases during the splitless period to increase the flow into the column. You set the surge pressure, which activates during the Prep Run stage.

At the end of the splitless period, the split valve reopens and the split flow flushes the injector of any remaining sample vapors."

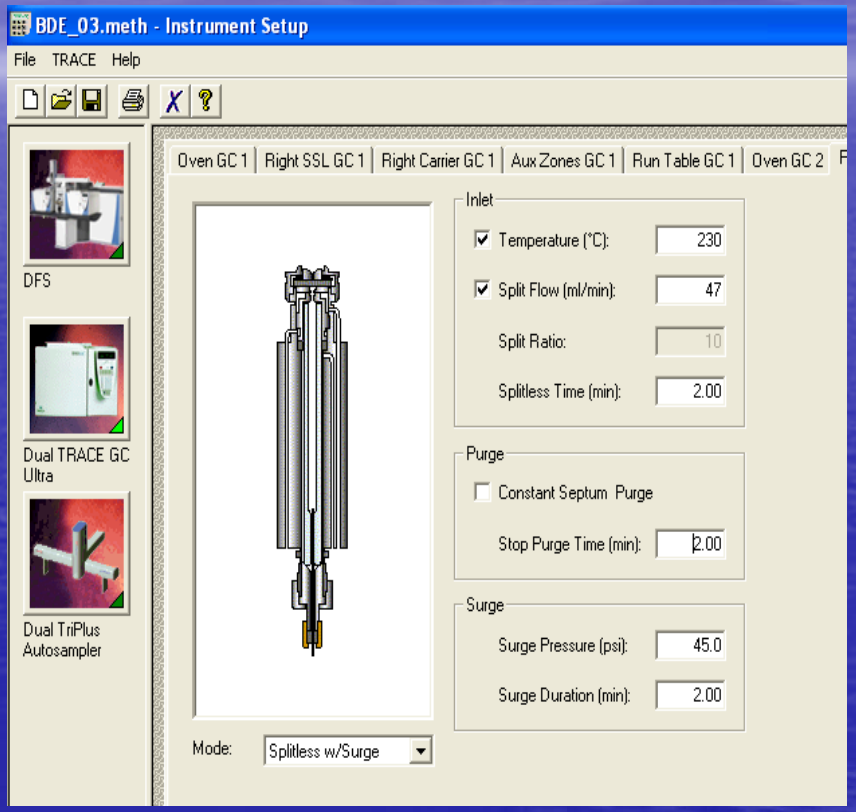


1. Carrier Gas Inlet
2. Septum Purge Line
3. Split Line

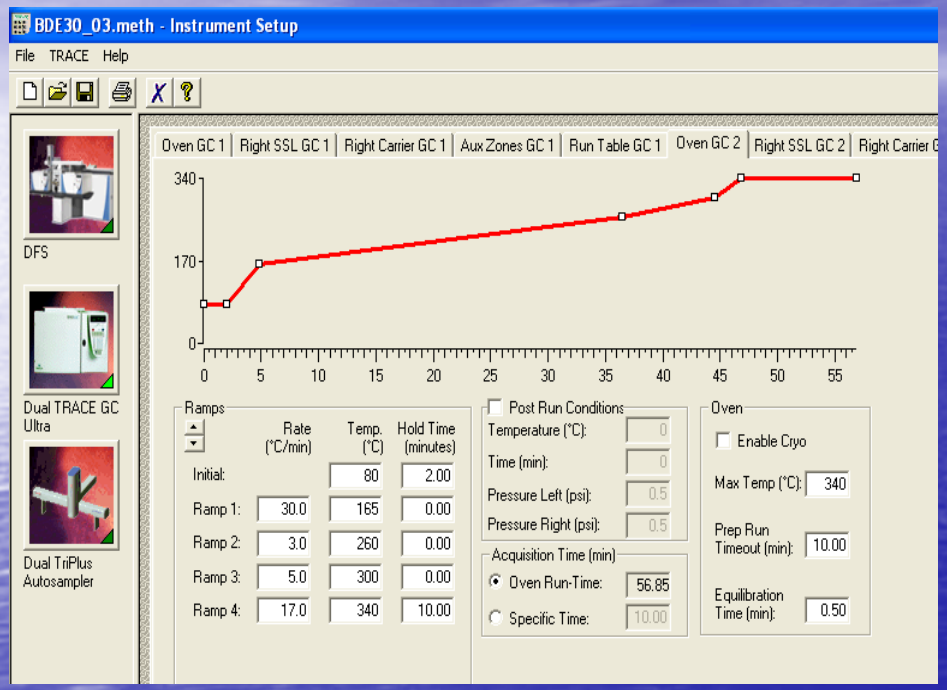
Figure 6-9. Splitless Injection Technique

Injector Parameters

- Inj temp – 230
- Splitless - 2 minutes
- Surge pressure - 45 psi for 2 minutes
- Same parameters for 15 or 30 m column



Column temperature program

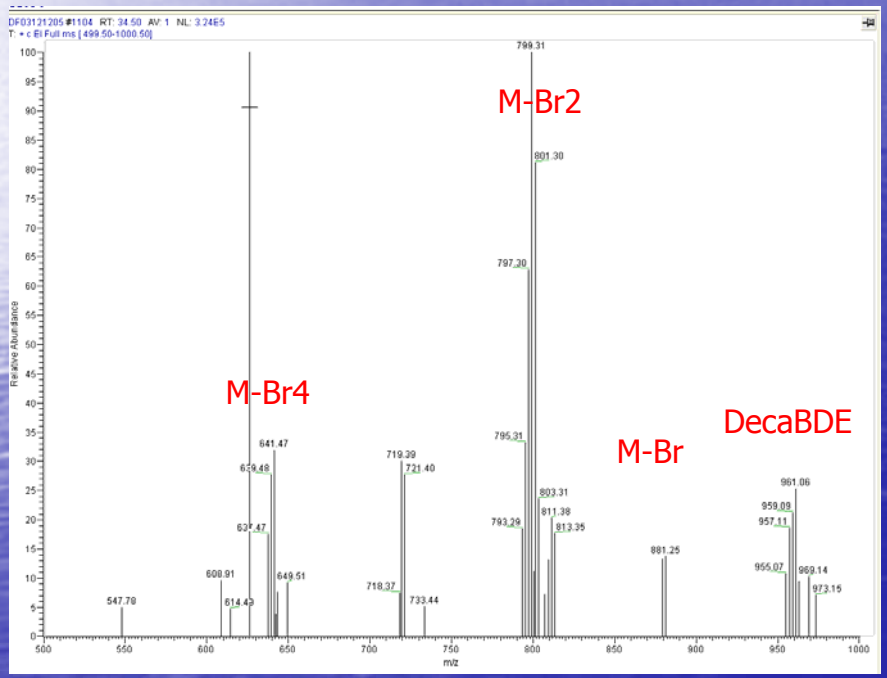


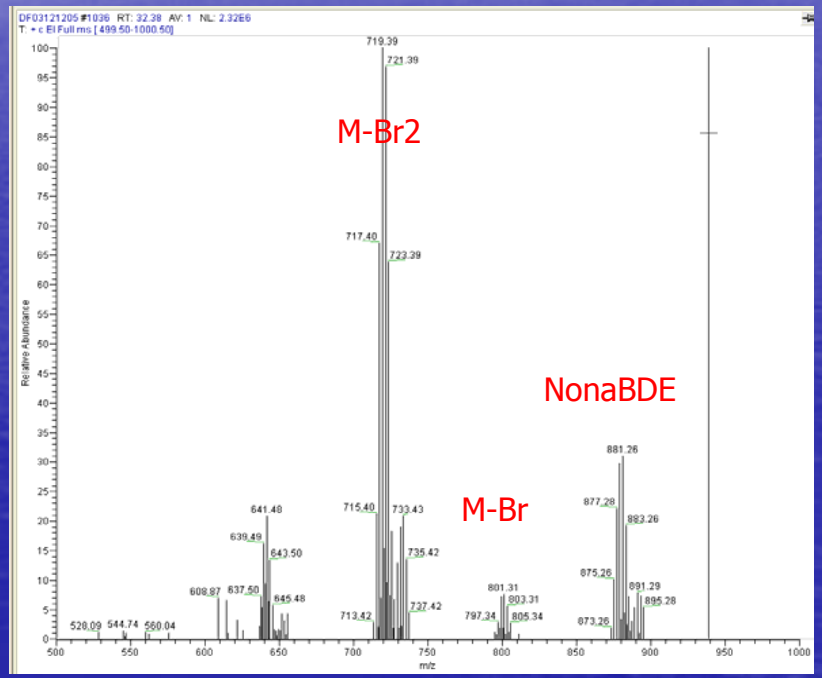
Variation depending on column length

- Initial temp 80C for 2 min
- Ramp1 – 30C/min to 165C
- Ramp2 – 3C/min to 200C (260C for 30 m)
- Ramp3 – 5C/min to 235C (or 300C for 30 m)
- Ramp4 – 8C/min to 300C
- Ramp5 – 17C/min to 340C and hold 5-10 min

BDE Mass Spectrum

Use M-Br2 for NoBDE and DeBDE





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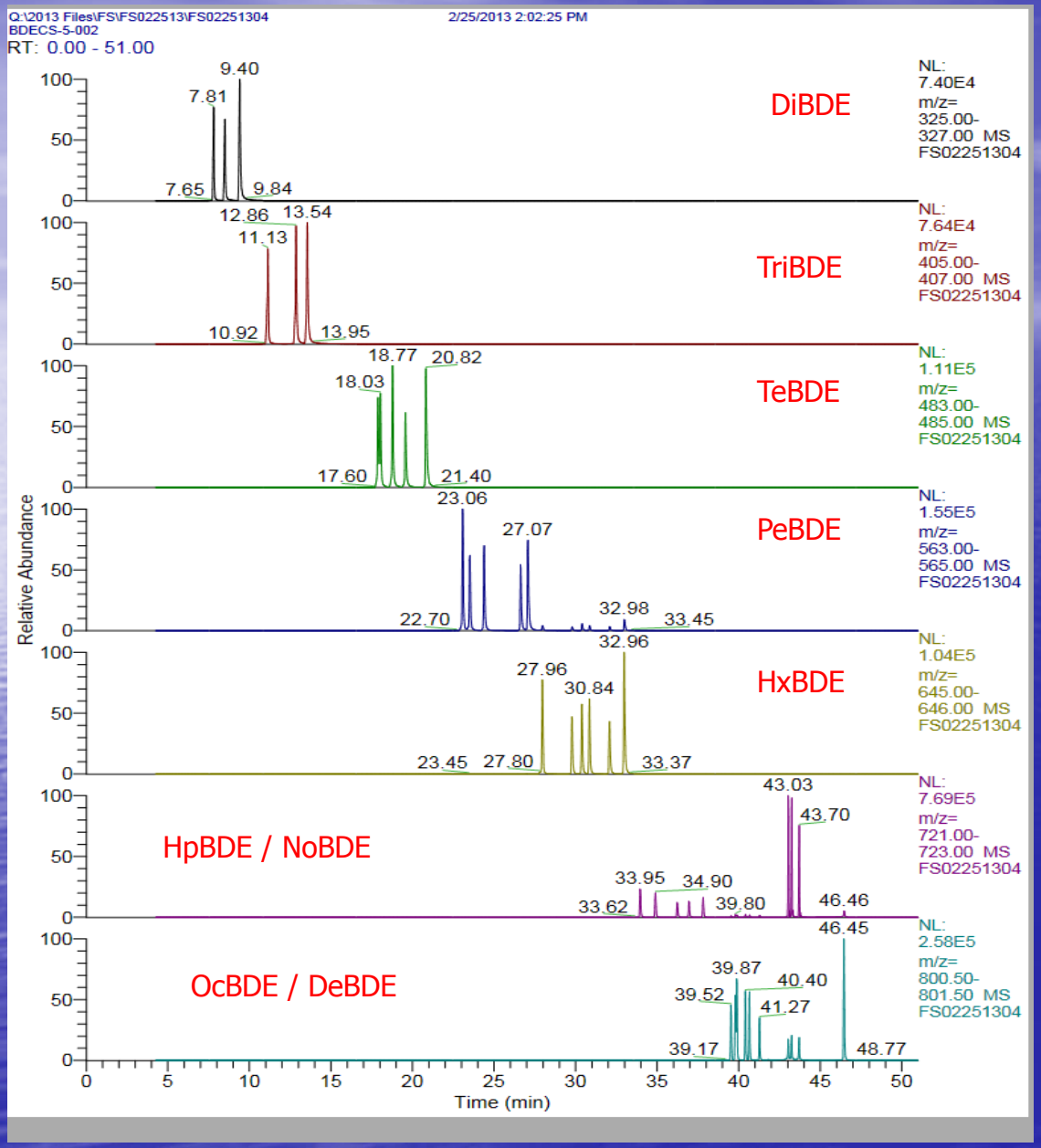

CS-5

100-1000 pg/uL

30 m column

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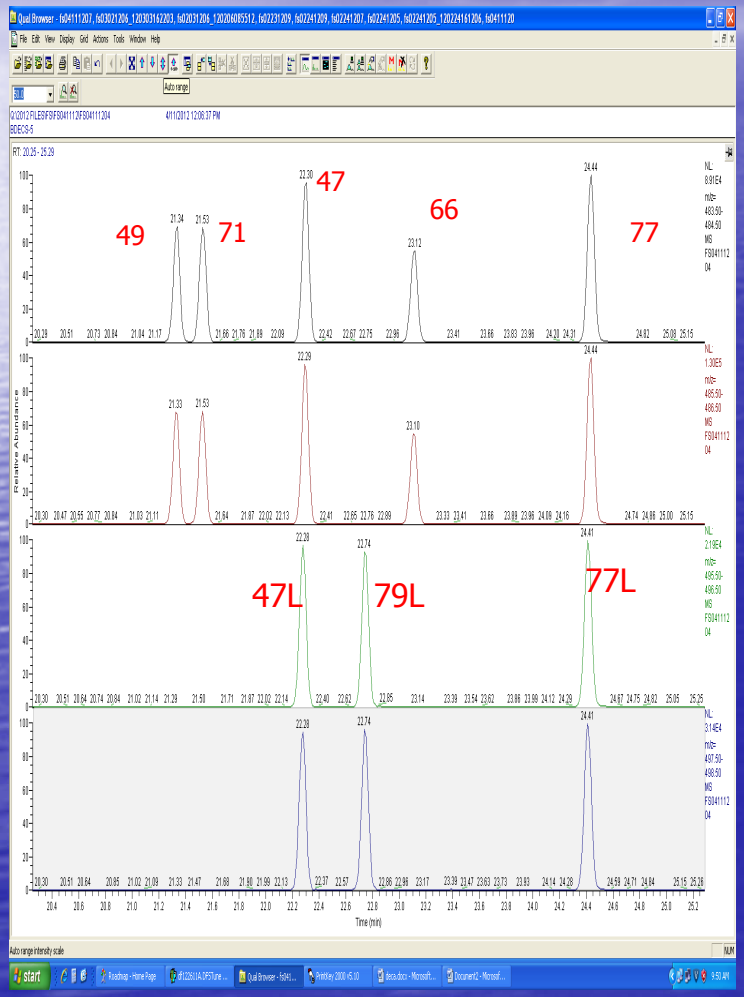
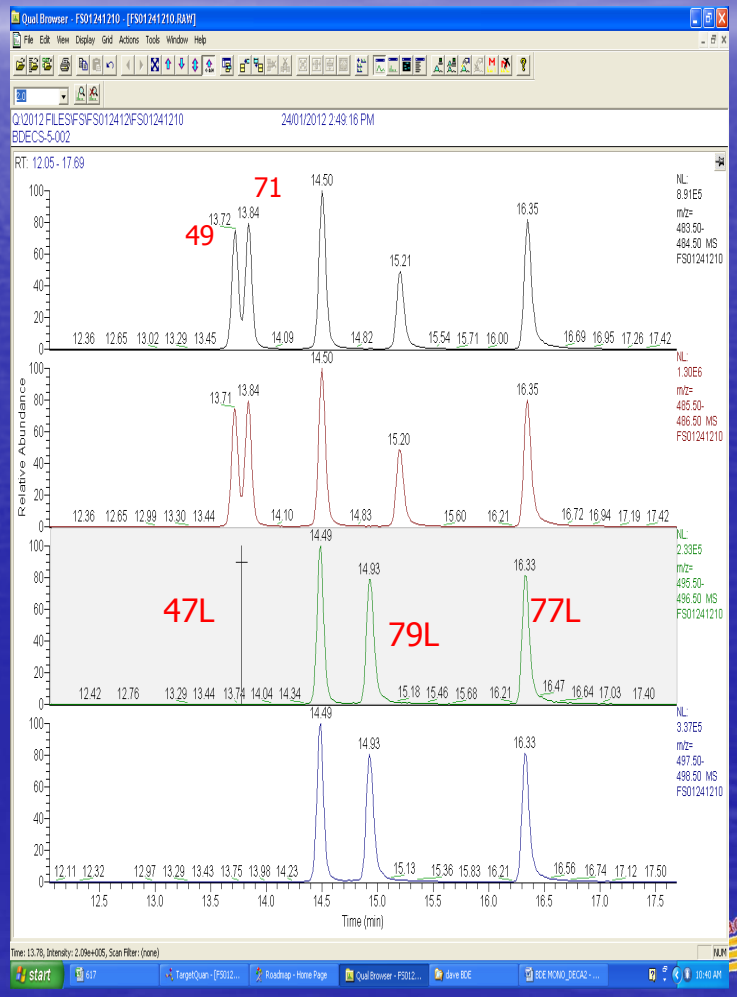
15 m or 30 m???

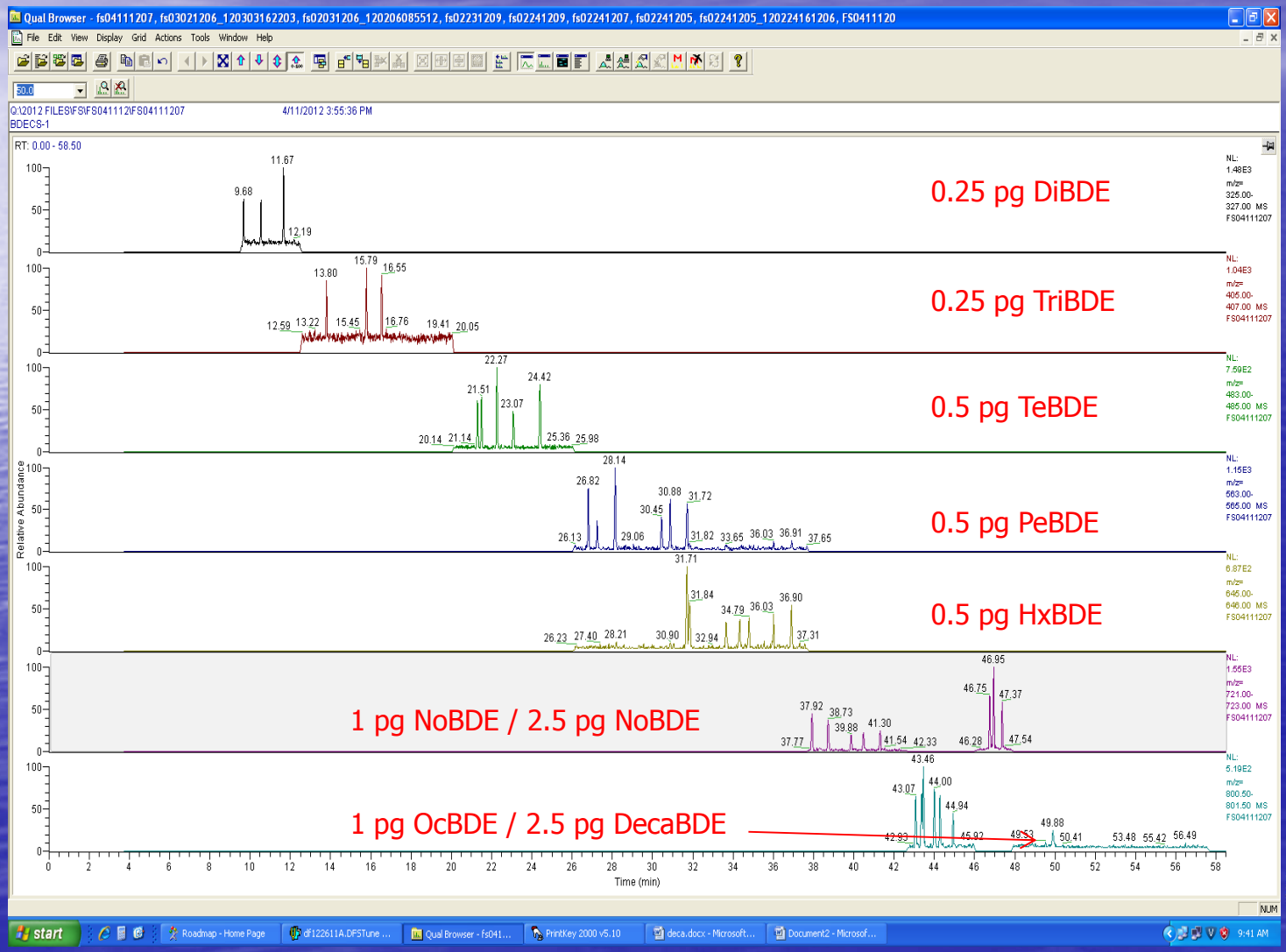
You decide!

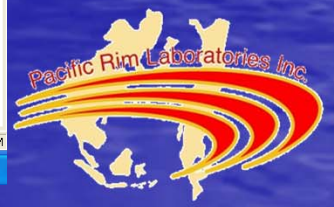
BDE49/71 resolution on 30 m and 15 m columns

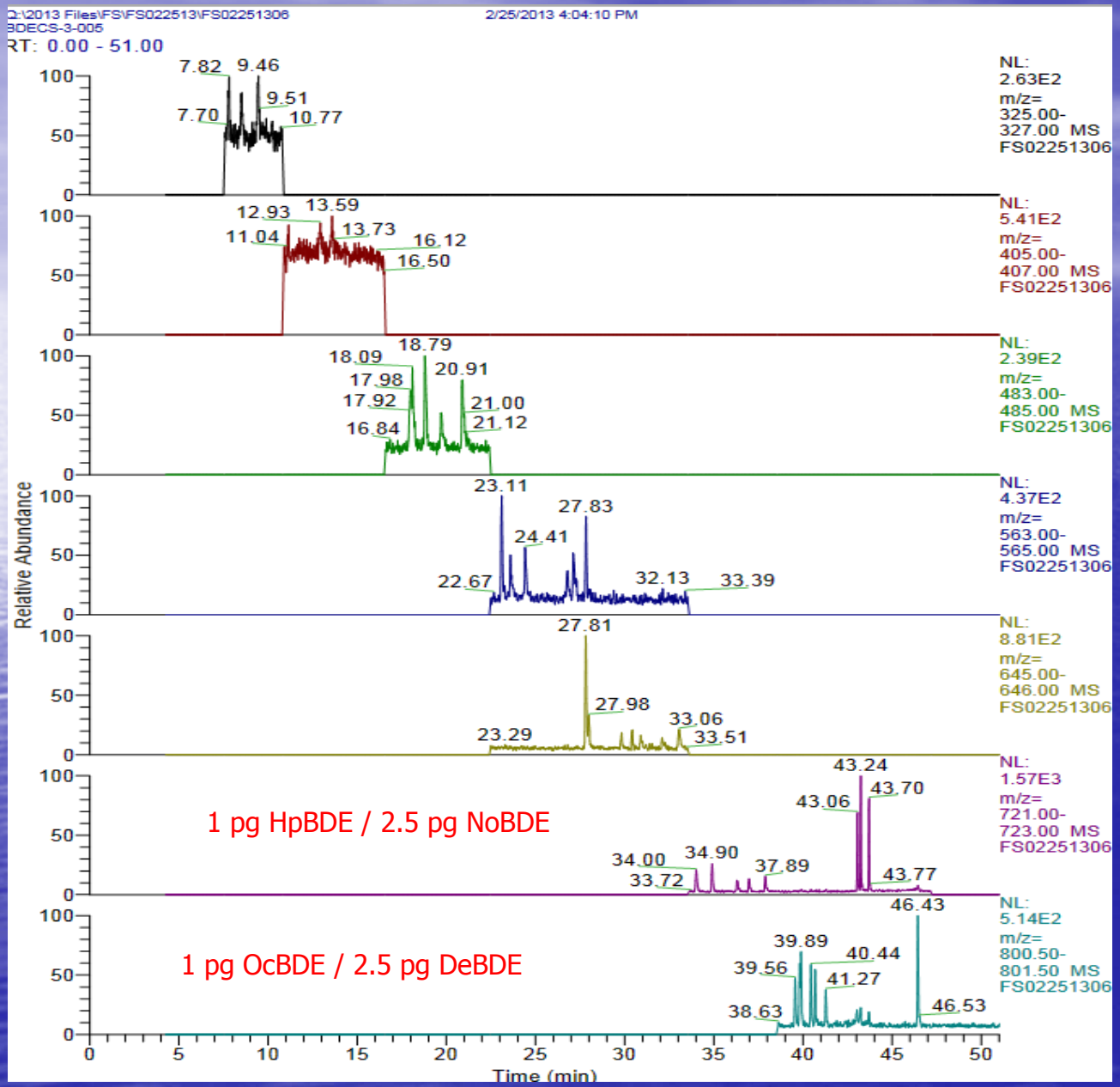




BDE CS-1 0.25-2.5 pg/µL (15 m)

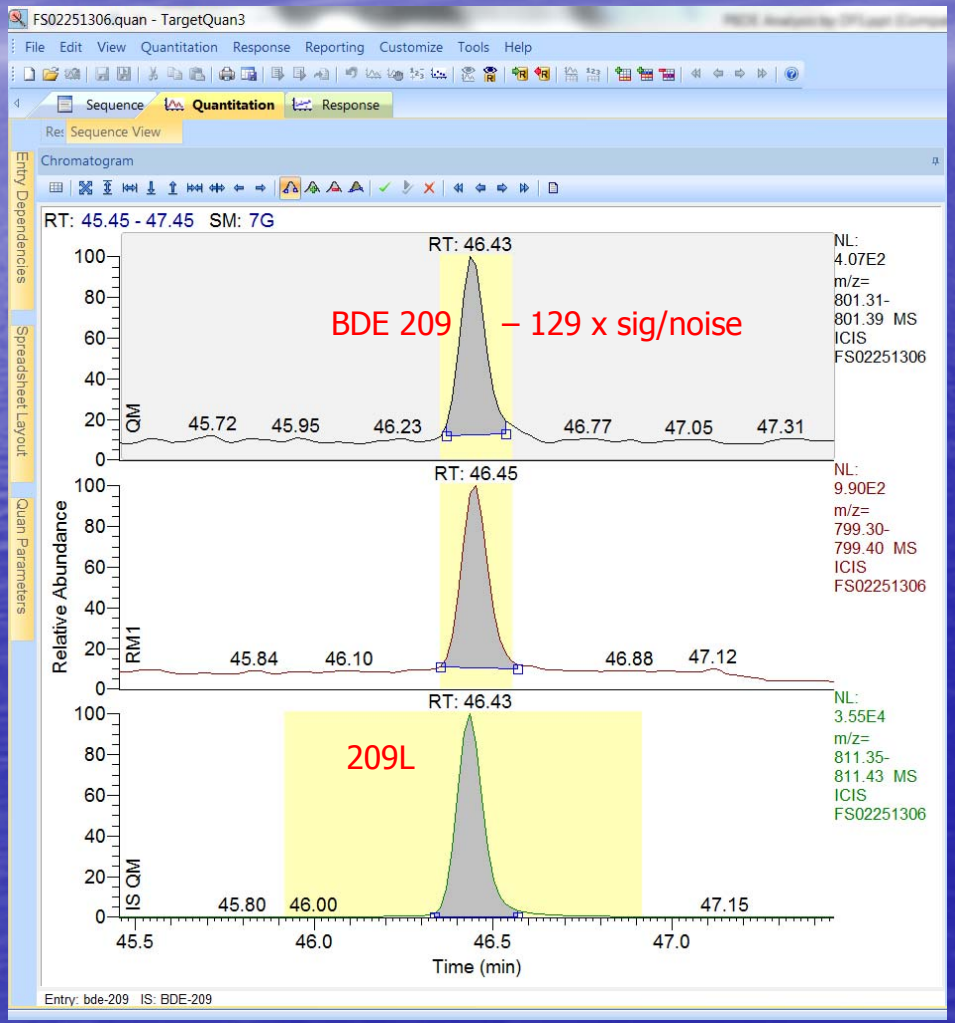






CS-1
0.25-2.5 pg/µL (30 m)

2.5 pg/uL of DecaBDE on 30 m column



Tissue Extraction

Fish, meat, cheese, nut butters, oils, butter

- 10 g of tissue is placed in a jar
- Add ^{13}C-standards
- Add 80 mL of 3N HCl and shake for 18 h
- If digested, add 9:1 DCM/acetone and shake.
- If not digested, add more acid until it is fully digested
- Extract three times with solvent mixture
- Clean-up with acid silica gel and basic alumina
- Final volume 100 uL in Toluene

Method Performance

- Six nut butter samples (10 g whole weight, 50% lipid) spiked with 0.05 – 500 ng of 34 PBDE
  - 0.01-10 ug/kg for Di-TriBDE
  - 0.02-20 ug/kg for Tetra-HexaBDE
  - 0.04-40 ug/kg for Hepta-OctaBDE
  - 0.1 – 100 ug/kg for Nona-DecaBDE
- Linear over range (r^2 – 0.98-1.00)
- Unbiased with the exception of BDE030
- Average recovery of 98%

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MDLs in various food matrices

Analyte	BDE #	MDL Oil pg/g	MDL Plant pg/g	MDL Tissue pg/g
2,4,4'-TrBDE	28	2.8	0.7	0.1
2,2',4,4'-TeBDE	47	9.2	2.3	4.9
2,3',4,4'-TeBDE	66	8.8	1	0.1
3,3',4,4'-TeBDE	77	8.9	1.8	0.1
2,2',4,4',6-PeBDE	100	4.5	1.5	4.3
2,2',4,4',5-PeBDE	99	5.1	5.4	7.4
2,2',3,4,4'-PeBDE	85	4.3	1.6	0.4
2,2',4,4',5',6-HxBDE	154	7	3.4	1
2,2',4,4',5,5'-HxBDE	153	6	3.4	0.8
2,2',3,4,4',5'-HxBDE	138	4.4	2.5	0.1
2,3,3',4,4',5,-HxBDE	156	5.6	2.2	0.1
2,2',3,4,4',6,6'-HpBDE	184	9.8	1	0.1
2,2',3,4,4',5',6-HpBDE	183	3.9	3.2	0.1
2,2',3,3',4,4',6,6'-OcBDE	197	7.2	1.5	0.1
2,2',3,3',4,4',5,6'-OcBDE	196	9.6	1.8	0.5
2,2',3,3',4,4',5,6,6'-NoBDE	207	11	4	0.7
2,2',3,3',4,4',5,5',6-NoBDE	206	17	2.2	0.5
DeBDE	209	22	3.3	23.9

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Nut Butters

BDE47 – 15 ng/kg

BDE99 – 6 ng/kg

BDE209 – 30 ng/kg

About double levels found in the blank

Whitefish (n=26) – BC River

BROMINATED DIPHENYL ETHERS		DL	Min	Max	Median
Congeners	PBDE	ng/g	ng/g	ng/g	ng/g
2,2',4-TrBDE	17	0.002	0.006	0.13	0.027
2,4,4'-TrBDE	28	0.002	0.097	1.6	0.355
2,2',4,5'-TeBDE	49	0.002	0.17	5.6	0.435
2,2',4,4'-TeBDE	47	0.002	6.5	120	32
2,3',4,4'-TeBDE	66	0.002	0.11	5	1.15
2,2',4,4',6-PeBDE	100	0.002	1.9	55	11
2,3',4,4',6-PeBDE	119	0.002	0.056	0.5	0.278
2,2',4,4',5-PeBDE	99	0.002	5.7	140	32
2,2',4,4',5',6-HxBDE	154	0.004	0.46	15	3.25
2,2',4,4',5,5'-HxBDE	153	0.004	0.66	20	4.5
2,2',3,4,4',6,6'-HpBDE	184	0.004	0.005	0.046	0.0145
2,2',3,4,4',5',6-HpBDE	183	0.004	0.005	0.22	0.066
2,2',3,3',4,4',6,6'-OcBDE	197	0.004	0.005	0.024	0.0095
2,2',3,3',4,4',5,6,6'-NoBDE	207	0.025	0.056	0.056	0.056
DeBDE	209	0.025	0.026	0.24	0.0595

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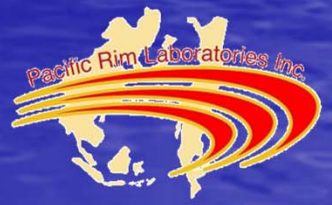
Lake Fish (24) – Western North America

			Min	Max	Median
BROMINATED DIPHENYL ETHERS		DL	Conc	Conc	Conc
Congeners	PBDE	ng/kg	ng/kg	ng/kg	ng/kg
2,4,4'-TrBDE	28	2	<	104	7.3
2,2',4,5'-TeBDE	49	2	4.6	249	38
2,2',4,4'-TeBDE	47	2	67	6041	331
2,3',4,4'-TeBDE	66	2	<	288	7.8
2,2',4,4',6-PeBDE	100	2	15	1769	72
2,2',4,4',5-PeBDE	99	2	5.2	6512	148
2,2',4,4',5',6-HxBDE	154	2	4.3	645	26
2,2',4,4',5,5'-HxBDE	153	2	5.3	314	20
2,2',3,4,4',5',6-HpBDE	183	4	<	110	2.7
2,2',3,3',4,4',6,6'-OcBDE	197	4	<	70	<
2,2',3,4,4',5,5',6-OcBDE	203	4	<	28	<
2,2',3,3',4,4',5,6'-OcBDE	196	4	<	36	<
2,2',3,3',4,5,5',6,6'-NoBDE	208	10	<	35	<
2,2',3,3',4,4',5,6,6'-NoBDE	207	10	<	109	<
2,2',3,3',4,4',5,5',6-NoBDE	206	10	<	53	<
DeBDE	209	10	<	909	15



C.L.A.M.

- The C.L.A.M. (Continuous Low-Level Aquatic Monitoring) is a submersible extraction sampler. It uses EPA-approved Solid Phase Extraction media to sequester pesticides, herbicides, PAH's, TPH, and other trace organics from water.
- Standard grab sampling only provides a few second snap shot in time, of a changing dynamic system. Automatic samplers are bulky, heavy, expensive, and only provide an intermittent sample. Passive samplers will provide a long term integrative partitioning event, but requires complicated mathematical modeling for any type of quantitative information, and is dependent on temperature, flow, and bio-fouling
- uses low flow rate extraction sampling (5-75 ml/minute), where water is drawn continuously through the extraction media first.
- provides an extraction event of up to 36 hours long, allowing capture of trace pollutants from illicit and episodic events.
- extracts the water in-situ, with the same technology the labs use on the bench, providing a pre-extracted quantitative sampling event representing up to a hundred liters of water.

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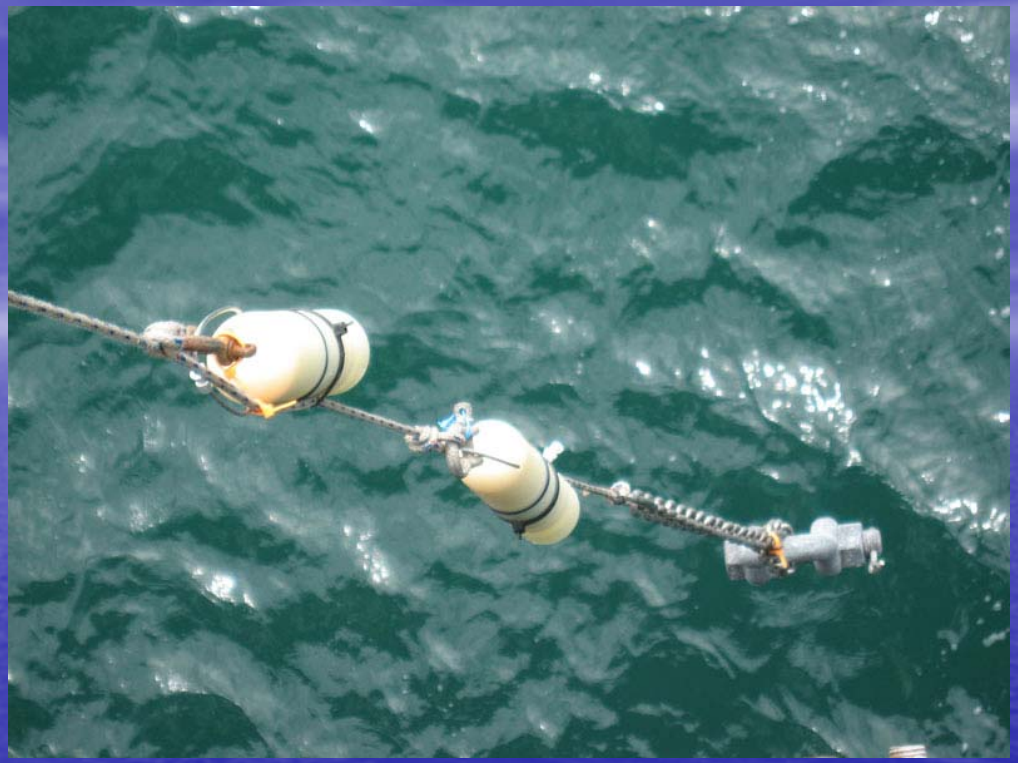
Samples

Deployed CLAM sampler for 24 h
Collected 60-90 L of water

1 L water samples collected at each site.

For more info on C.L.A.M see
www.aqualytical.com



Extraction

CLAMS eluted with 50 mL methanol

Then 2 x 25 mL of dichloromethane spiked with Internal Standards.

Mixed with 100 mL of water in separatory funnel

Gently shake to separate layers

Extract concentrated and cleaned with sulphuric acid wash, followed by acidic silica gel and basic alumina.

Concentrated to 100 uL and analyzed by GC/HRMS on Thermo DFS.

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Water samples

	Water1	Water2	Water3	Water4	Water5	Water6	Water7	Blank
	pg/L	pg/L	pg/L	pg/L	pg/L	pg/L	pg/L	pg/L
BDE028	<	<	<	<	<	<	<	<1.8
BDE047	39	41	30	136	47	137	11	<12
BDE066	<	<	<	<	<	<	<	<2.5
BDE100	<	<	<	22	<	27	<	<0.7
BDE099	35	<	<	134	17	121	<	<1.5
BDE154	12	<	<	21	<	<	<	<3.1
BDE153	<	<	<	<	<	<	<	<4.8
BDE183	<	<	<	<	<	4	<	<0.5
BDE197	<	<	<	<	<	3	<	<0.4
BDE196	<	<	<	<	<	<	<	<0.4
BDE208	<	<	<	<	<	<	<	<1.8
BDE207	<	<	<	<	<	19	<	<6.5
BDE206	<	<	<	9	<	<	<	<1.9
BDE209	146	75	723	196	45	291	89	34

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CLAM samples

	CLAM1	CLAM2	CLAM3	CLAM4	CLAM5	Blank
	pg/L	pg/L	pg/L	pg/L	pg/L	pg/L
BDE028	2	3	2	0.2	0.2	<0.03
BDE047	137	159	138	17	16	0.7
BDE066	3	3	3	0.4	0.4	<0.06
BDE100	27	29	26	2	2	0.08
BDE099	139	154	129	8	9	0.2
BDE154	12	13	11	1	1	<0.07
BDE153	12	16	13	2	0.5	<0.11
BDE183	2	2	2	1	0.1	<0.02
BDE197	2	2	2	0.9	0.5	<0.02
BDE196	1	1	1	0.1	0.1	<0.02
BDE208	12	12	10	2	0.3	0.2
BDE207	12	13	10	0.4	0.8	0.2
BDE206	16	18	12	2	0.3	<0.06
BDE209	222	267	240	11	16	2

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Thank you!