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Use of Micro Scale Solid Phase Extraction and Automated Clean Up in POPs Analysis of Milk and Serum

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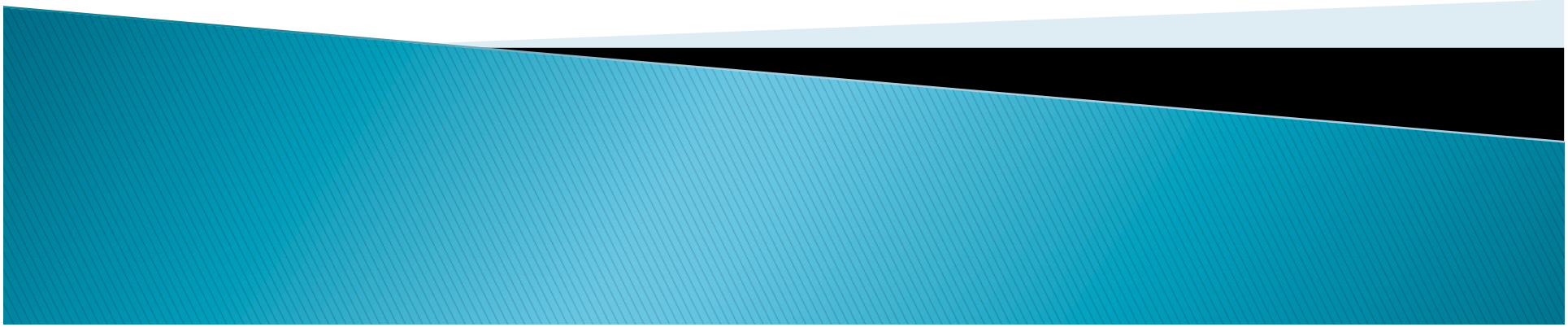

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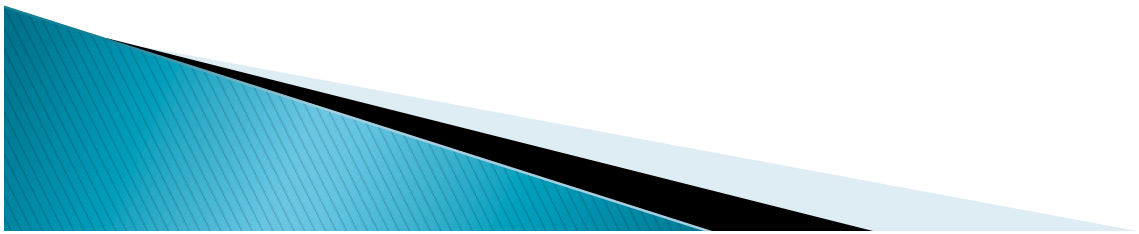
Introduction

- Stockholm Convention on Persistent Organics Pollutants 2001.
- Compounds of interest: polychlorinated dibenzo-p-dioxins (PCDDs), furans (PCDFs), biphenyls (PCBs) and poly brominated diphenyl ethers (PBDEs).
- Known toxicity.
- Strict environmental regulations in force in most countries.



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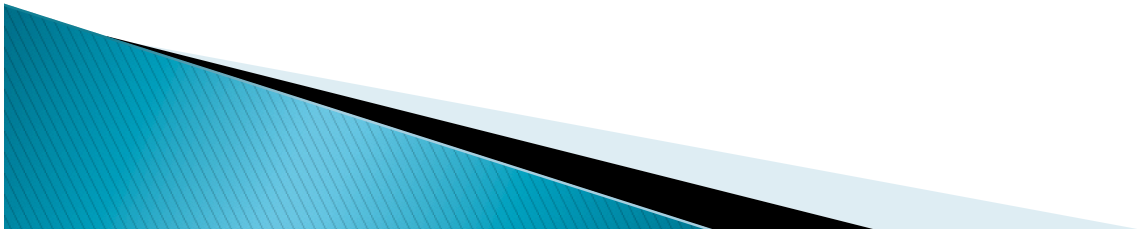
Sources

- Sources: intentional production (PCBs (phased out), PBDEs) and unwanted byproducts (PCDD/F).
- PCDD/F sources: combustion, incineration, metallurgical industry, pulp and paper bleaching/ production; low natural background (Trace Chemistries of Fire).
- Levels are dropping.
- Still at significant concentrations to pose danger.



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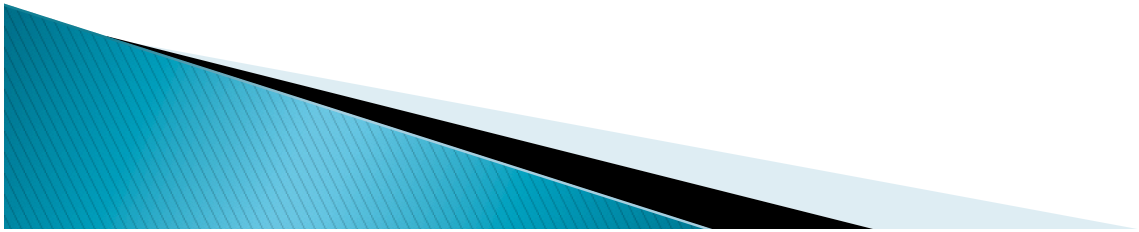
Health Effects

- Endocrine disruptors.
- Immune system.
- Nervous system.
- Reproductive functions.
- Carcinogenic.
- Chloracne.
- Main exposure (> 90%) is through dietary intake: meat, dairy, fish

Serum as matrix

- Serum is complex matrix.
- Low lipid levels (~ 600 mg/dL).
- Analytes present at low levels (fg-pg range).
- Manual methods are time-consuming; can have poor reproducibility and accuracy.
- Biomonitoring: study of POPs levels in humans to improve regulatory efforts and public health.



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First step: SPE

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Serum PCB/PBDE

- Use 2 g bovine serum with 2g water and 4 mL formic acid.
- Add 100 uL methanol and HCl, ^{13}C labeled standards, 4 g water gives 12g sample.
- Condition HLB-500 cartridge with DCM, MeOH, water, load sample.
- Dry with positive pressure (nitrogen), elute with 12 mLs DCM.
- Reduce to 3 mLs, exchange to hexane, clean up over 0.2 g silica (same system).
- Elute with 20 mL hexane, reduce to 1 mL in evaporator, then to 10 uL for analysis

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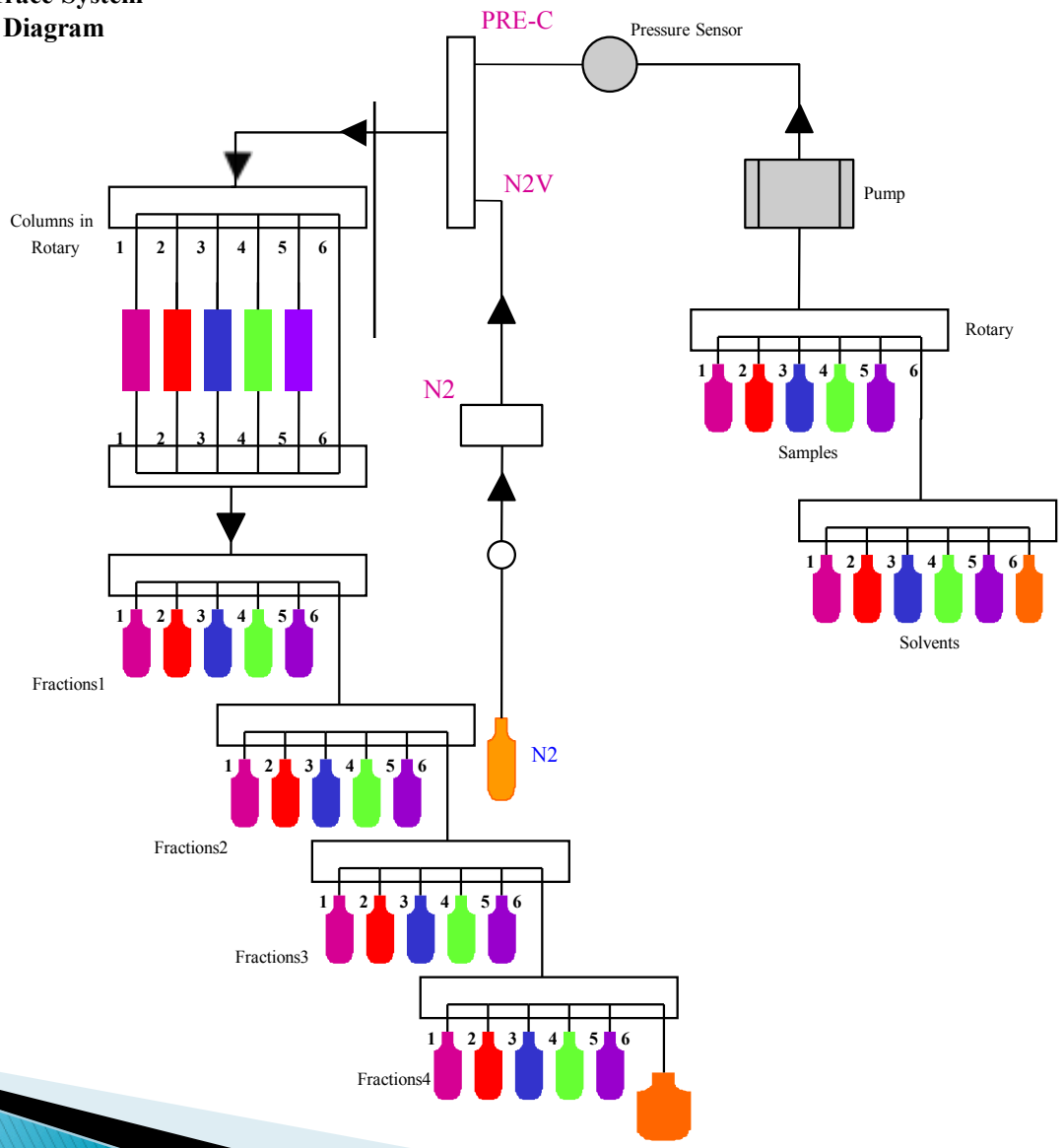
First Step: SPE

SPE-NANO Trace System
Plumbing Diagram



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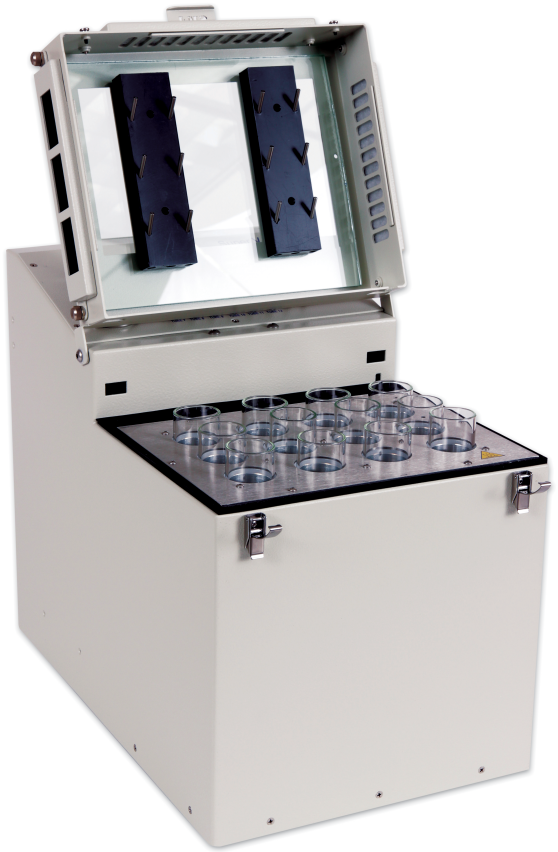
12 position evaporator 50 mLs



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SuperVap Evaporation

- System pre-heated to 45-60 ºC.
- Samples evaporated at stable T under 5-6 psi nitrogen.
- 1 mL extract vial transferred to GC vial (can have direct-to-vial feature).
- Recovery standards added (nonane/dodecane).
- Extract taken to 10 uL volume with a gentle stream of nitrogen at ambient temperature.

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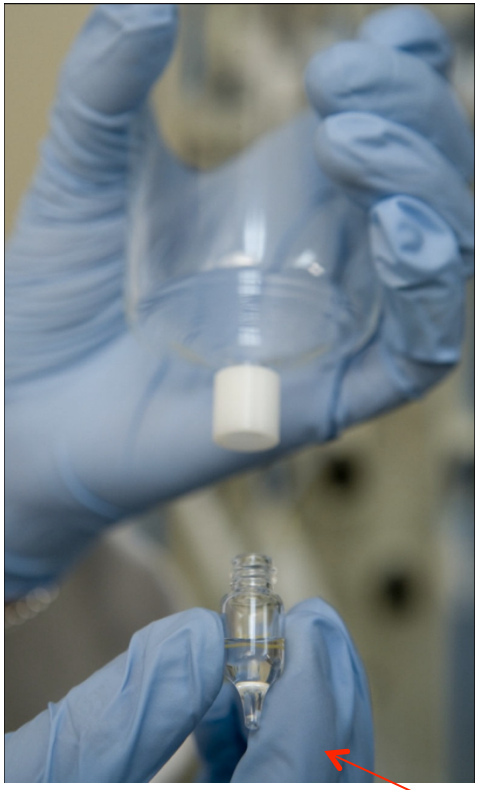
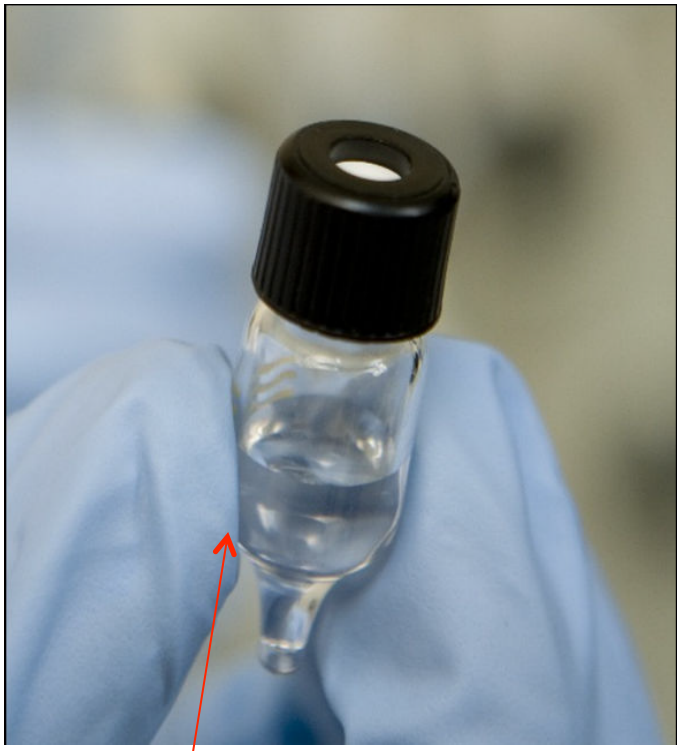
24 position vial evaporator





GC vial

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PCDD/F in Serum

- 20g bovine serum mixed with 20g water and treated with 40 mL formic acid.
- ^{13}C labeled standards added.
- 40 g water added extra to make about 120 g sample.
- Sample loaded onto C-18 cartridge (conditioned with DCM, MeOH, water); positive pressure and dried (N2).
- Cartridge eluted with 20 mLs dichloromethane.
- Volume reduction to 5 mLs, solvent-exchange with hexane.
- Then: clean up in an automated column chromatography system.

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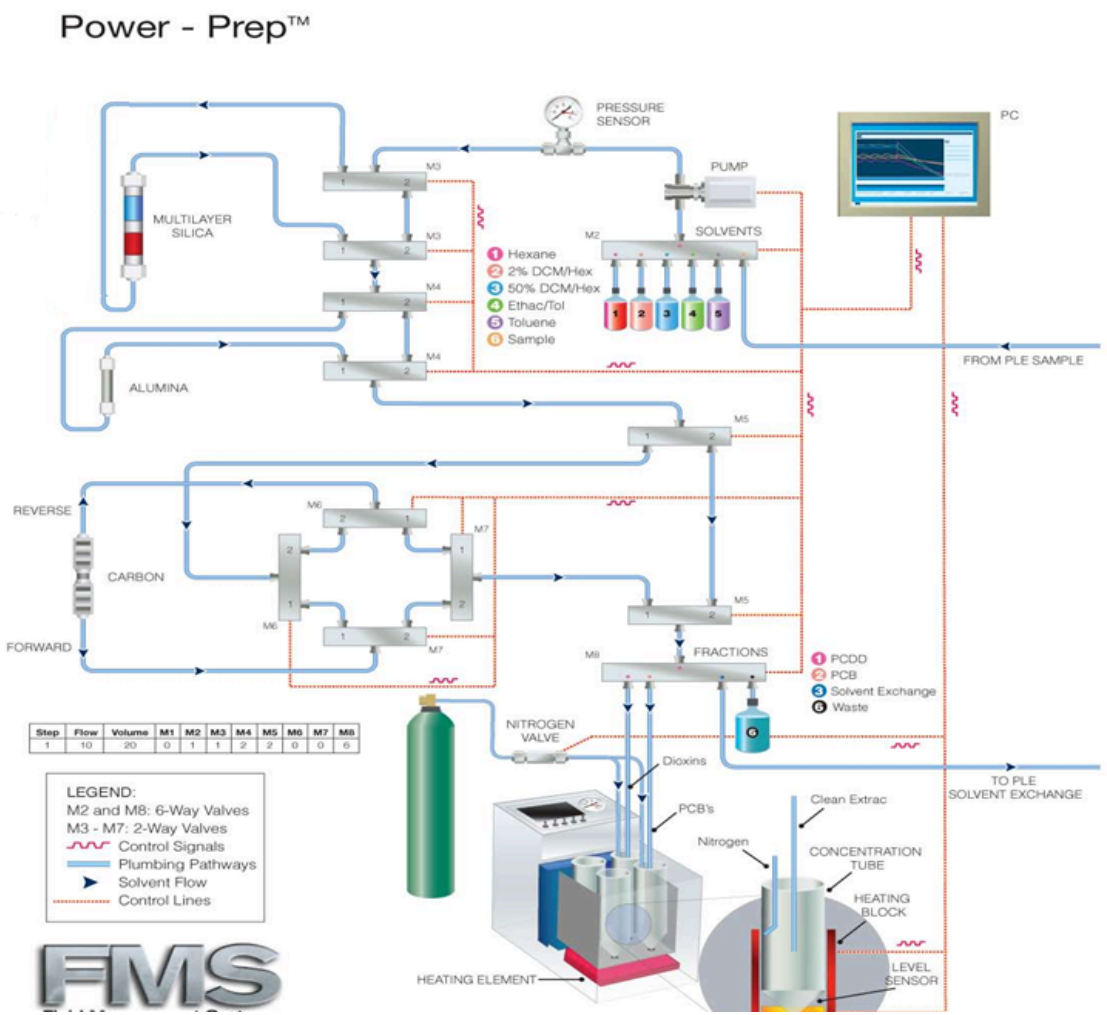
PowerPrep CleanUp System (PCDD/F)



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Clean Up (1)

- Silica - PBDE-free multilayer classical ABN silica gel column
- Alumina – PBDE-free basic alumina column
- Carbon – PBDE-free carbon/celite column

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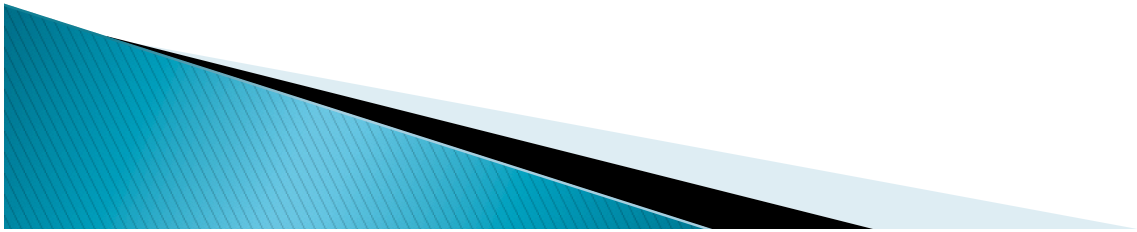
Clean Up (2)

- Condition columns 10 mLs each.
- Sample loaded onto silica column in hexane.
- Elute silica and alumina with hexane (90 mLs), then 10% DCM/hexane (70 mLs, collect PCBs if they were present).
- Elute alumina with 50 mLs DCM to get PCDD/F onto carbon.
- Back elute carbon with 35 mLs toluene, collect PCDD/F. Total volume is ~ 300 mLs of solvent.



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DFS HRGC/HRMS

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Data for serum (1)

Mean recoveries and standard deviations for 10 samples.

	Mean Rec	STDEV
2378-T4CDF	88%	7%
2378-T4CDD	89%	7%
12378-P5CDF	97%	8%
23478-P5CDF	87%	18%
12378-P5CDD	88%	19%
123478-H6CDF	93%	11%
123678-H6CDF	89%	7%
234678-H6CDF	93%	11%
123789-H6CDF	100%	14%
123478-H6CDD	97%	11%
123678-H6CDD	90%	7%
123789-H6CDD	93%	8%
1234678-H7CDF	85%	10%
1234678-H7CDD	84%	5%
OCDF	76%	6%
OCDD	74%	8%

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Data for serum (2)

Mean recoveries and standard deviations for 10 samples.

	Mean Rec	STDEV
pcb-28	72%	7%
pcb-52	72%	7%
pcb-101	73%	8%
pcb-105	93%	18%
pcb-114	94%	19%
pcb-118	84%	11%
pcb-123	90%	7%
pcb-128	88%	11%
pcb-138	85%	14%
pcb-153	82%	11%
pcb-156	98%	7%
pcb-157	96%	8%
pcb-167	91%	10%
pcb-170	90%	5%
pcb-178	77%	6%
pcb-180	98%	8%
pcb-194	75%	21%
pcb-206	75%	23%
pcb-209	64%	15%

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Data for serum (3)

Mean recoveries and standard deviations for 10 samples.

	Mean Rec	STDEV
BDE-28	80%	10%
BDE-47	83%	9%
BDE-99	76%	10%
BDE-100	78%	11%
BDE-153	83%	7%
BDE-154	73%	9%
BDE-183	77%	16%
BDE-197	48%	15%
BDE-203	64%	14%

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Milk (PCB/PBDE)

- 1 g cow milk, spiked with ^{13}C labeled standards, absorbed into 1 g Hydromatrix™ cartridge, placed into SPE.
- Dried with nitrogen (positive pressure, no solvents).
- Elution with 12 mLs DCM.
- Reduce to 3 mLs, exchange to hexane, clean up over 0.2 g silica (same system).
- Elute with 20 mL hexane, reduce to 1 mL in evaporator, then to 10 uL for analysis.

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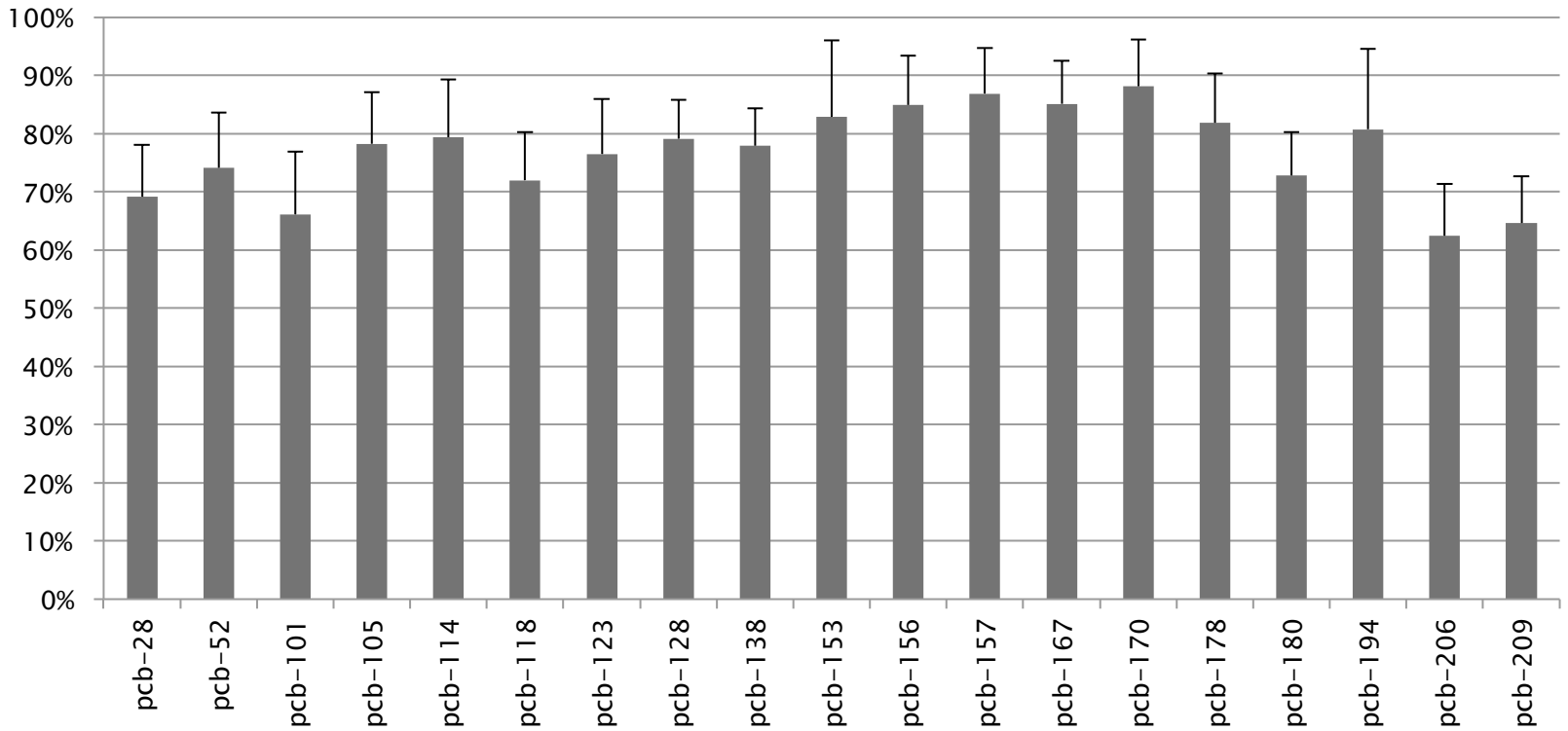
PCB recoveries (n=8)



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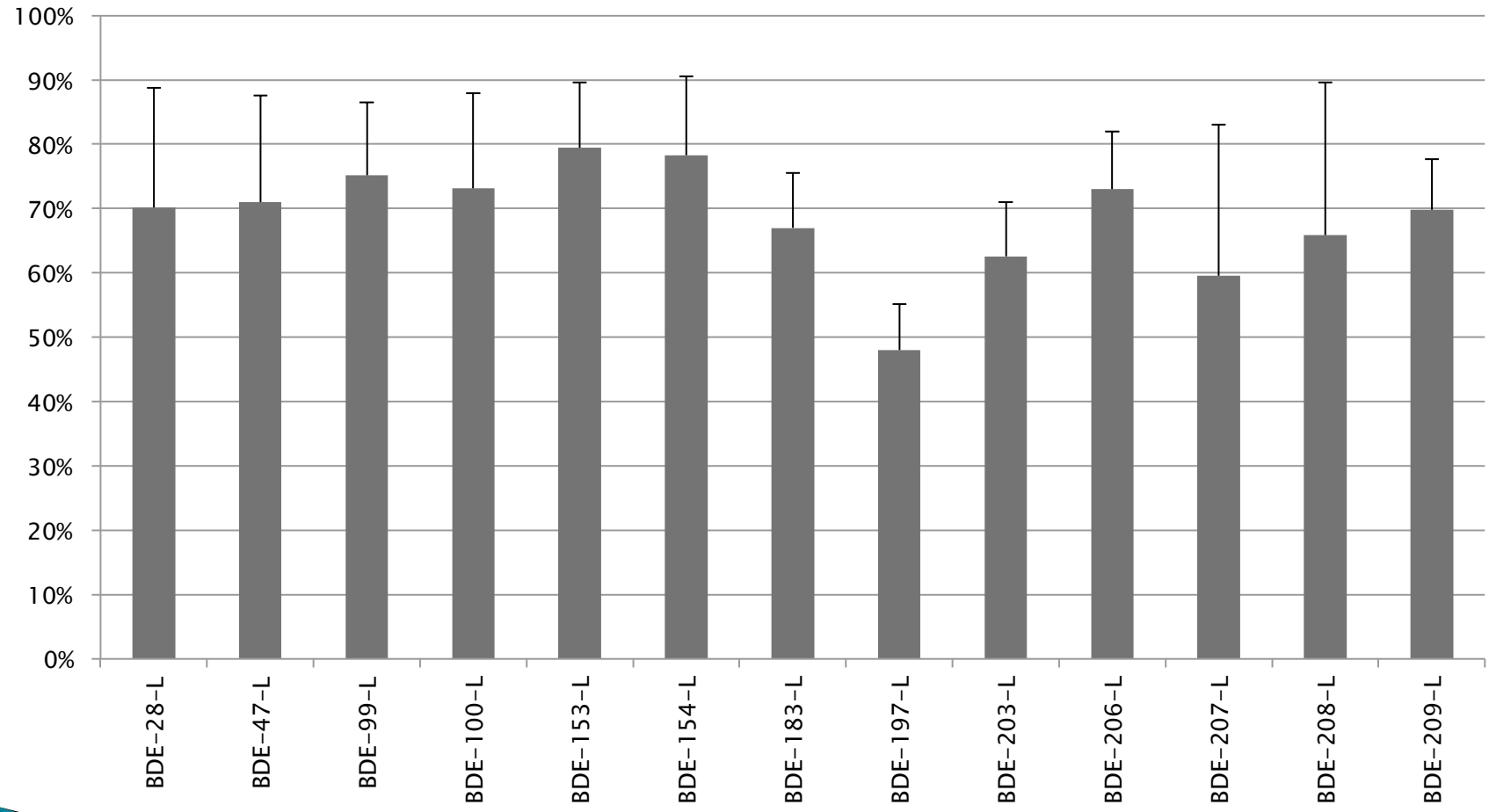
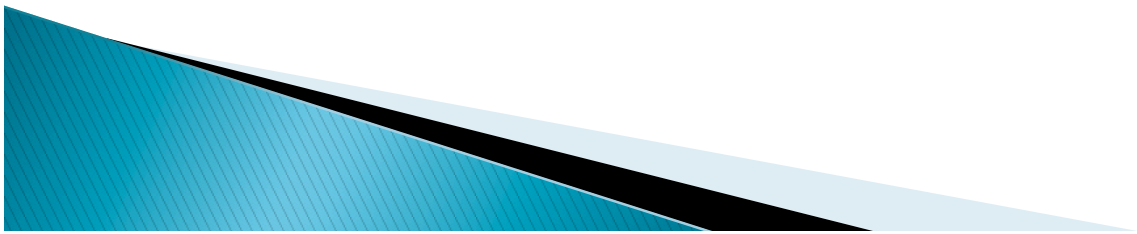
PBDEs recoveries (n=7)



Conclusions (1)

- Solid Phase Extraction of milk and serum in micro system means low solvent consumption and less background contamination.
- With PCBs and PBDEs extraction and single column clean up can be done is same system. No additional equipment needed.
- For PCDD/Fs multi column silica, alumina, and carbon clean up delivers clean samples ready for analysis now with reduced solvent use.
- Same day sample processing and analysis (HRGC/ HRMS): can be easily done in one day.

Conclusions (2)

- Excellent recoveries for POPs isolated from milk and serum.
- Ability to measure at low pg range with high res GC/MS.
- Future work: PCDD/F in milk; human milk and human serum; validation with NIST reference materials.