Analysis of Persistent and Bioaccumulative Environmental Organic Pollutants in Human Placenta: NCS Project 2-18

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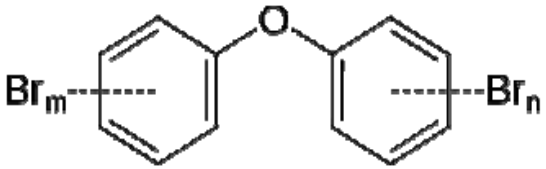
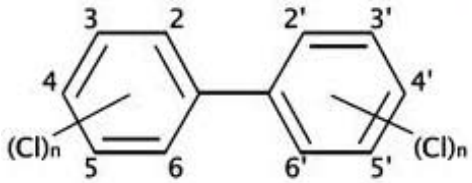
Toxic Chemicals

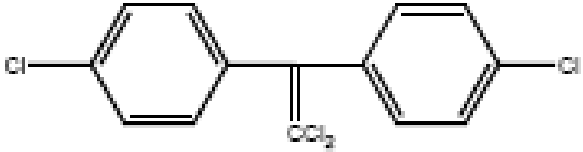
- Polybrominated diphenyl ethers (**PBDEs**) are flame retardants which were, required by law in the U.S., added to various consumer products for the protection of people from incidental fire.
- Polychlorinated biphenyls (**PCBs**) were used widely in industries during the 1950s to 1970s. Their production and uses were banned around1979 worldwide.
- DDT was a “magic” pesticide used in the 1940-1970s. The major metabolite of DDT is **DDE**.
- These chemicals are <u>persistent</u>. They do not degrade and disappear easily in nature.
- These chemicals are <u>bioaccumulative</u> thus commonly found in human and biota.



PBDEs



PCBs



DDE

Both PBDE and PCB “families” have 209 “members” called congeners, differing by the number and positions of the bromine or chlorine atoms in the molecule.

Effect of Prenatal Exposure to PCBs

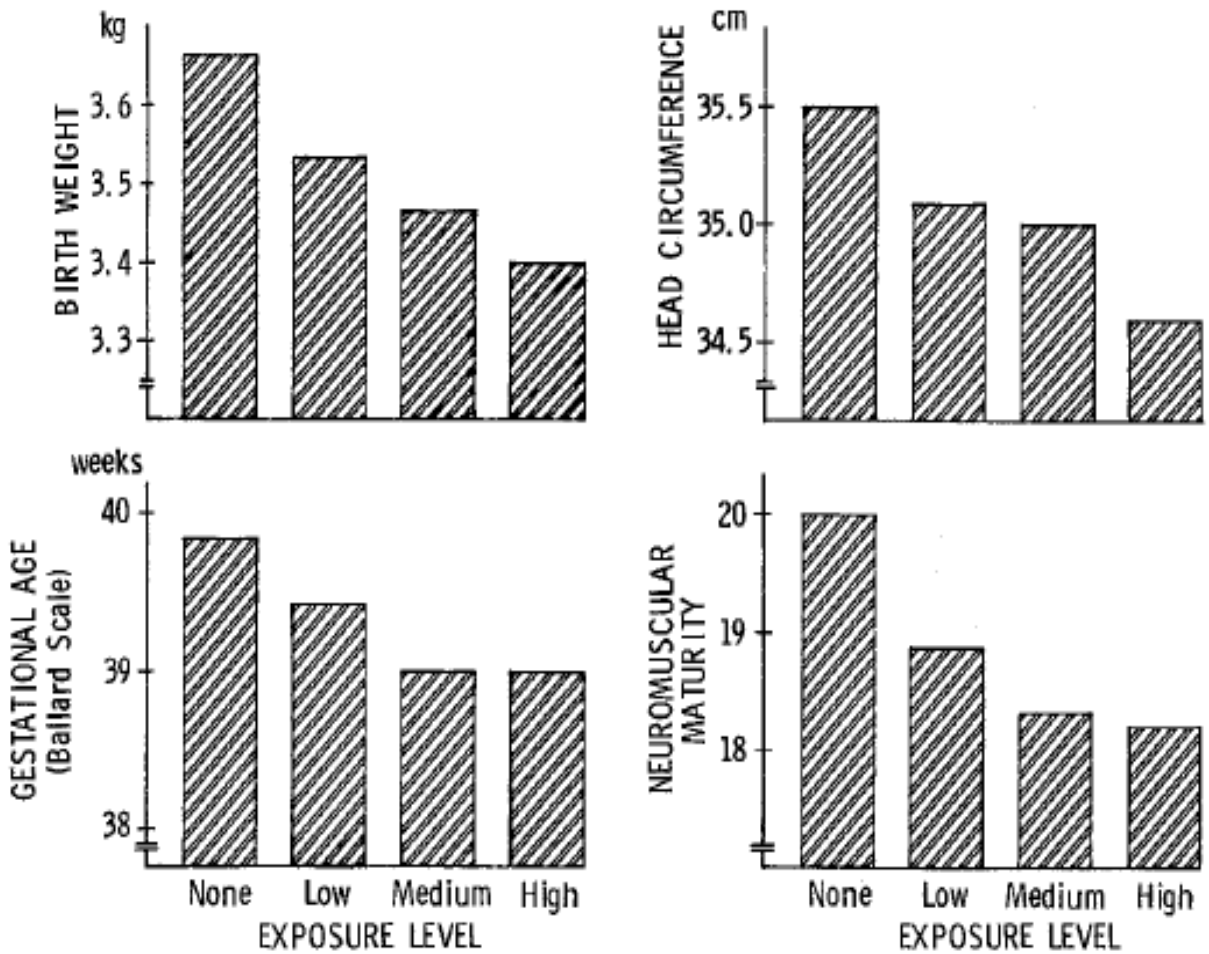
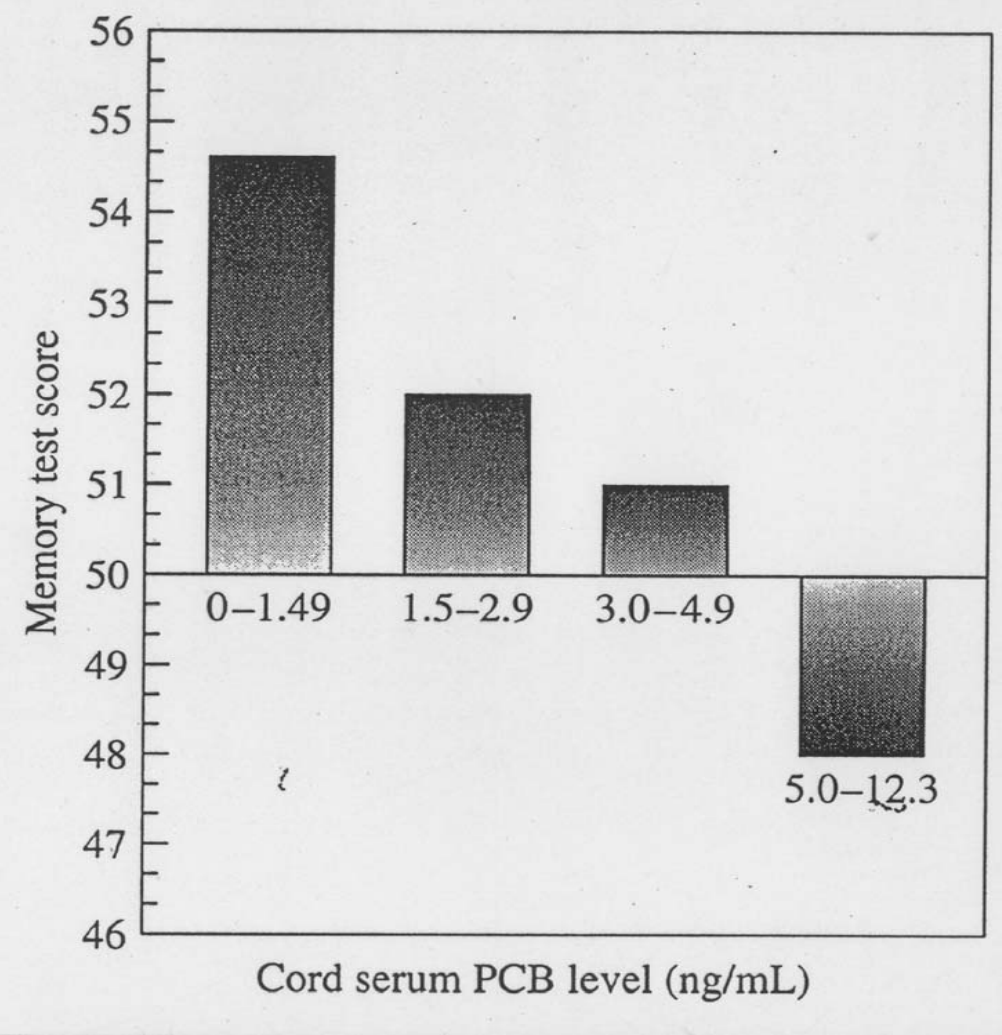
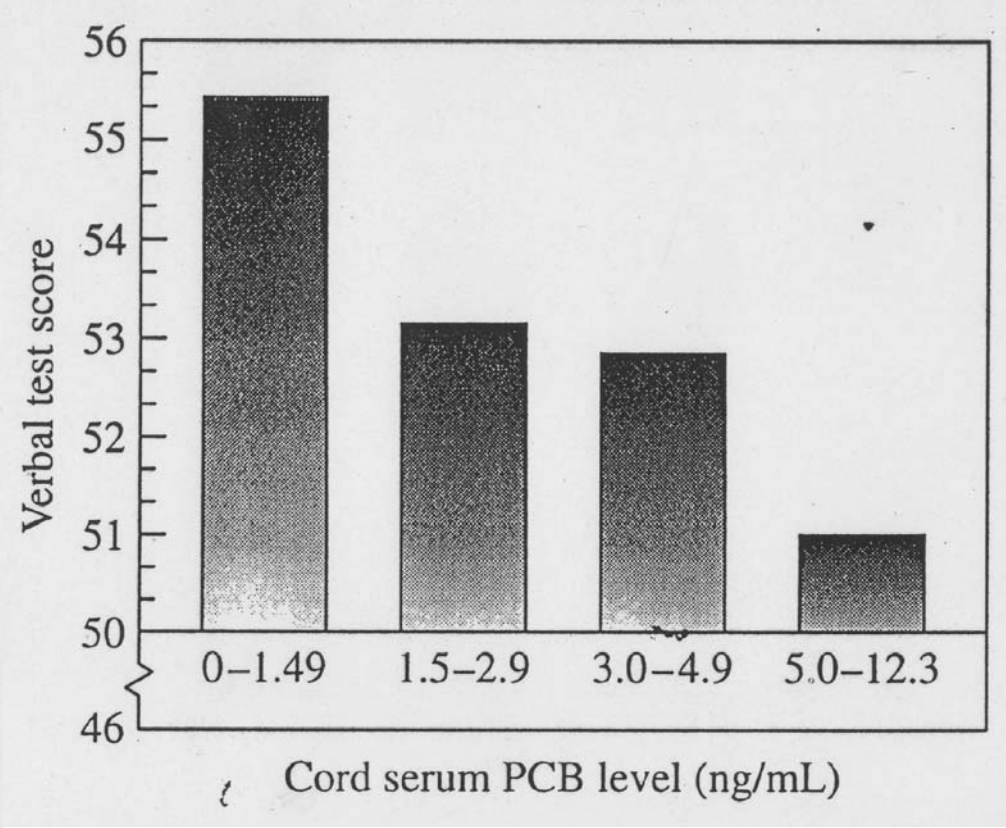


Figure. Dose-response relationships for birth weight, gestational age (Ballard examination), head circumference, and neuromuscular maturity by overall contaminated fish consumption. Group means were derived from analyses of covariance, in which they were adjusted for the effects of maternal prepregnancy weight, type of delivery, and consumption of alcohol and caffeine prior to and during pregnancy and cold remedies during pregnancy. Exposed infants were divided into three approximately equal groups: low exposure 2.0 to 3.4 kg/yr, medium 3.5 to 6.5 kg/yr, high 6.6 to 41.7 kg/yr.

Fein et al., Aug. 1984. *J. Pediatrics.* 316-320

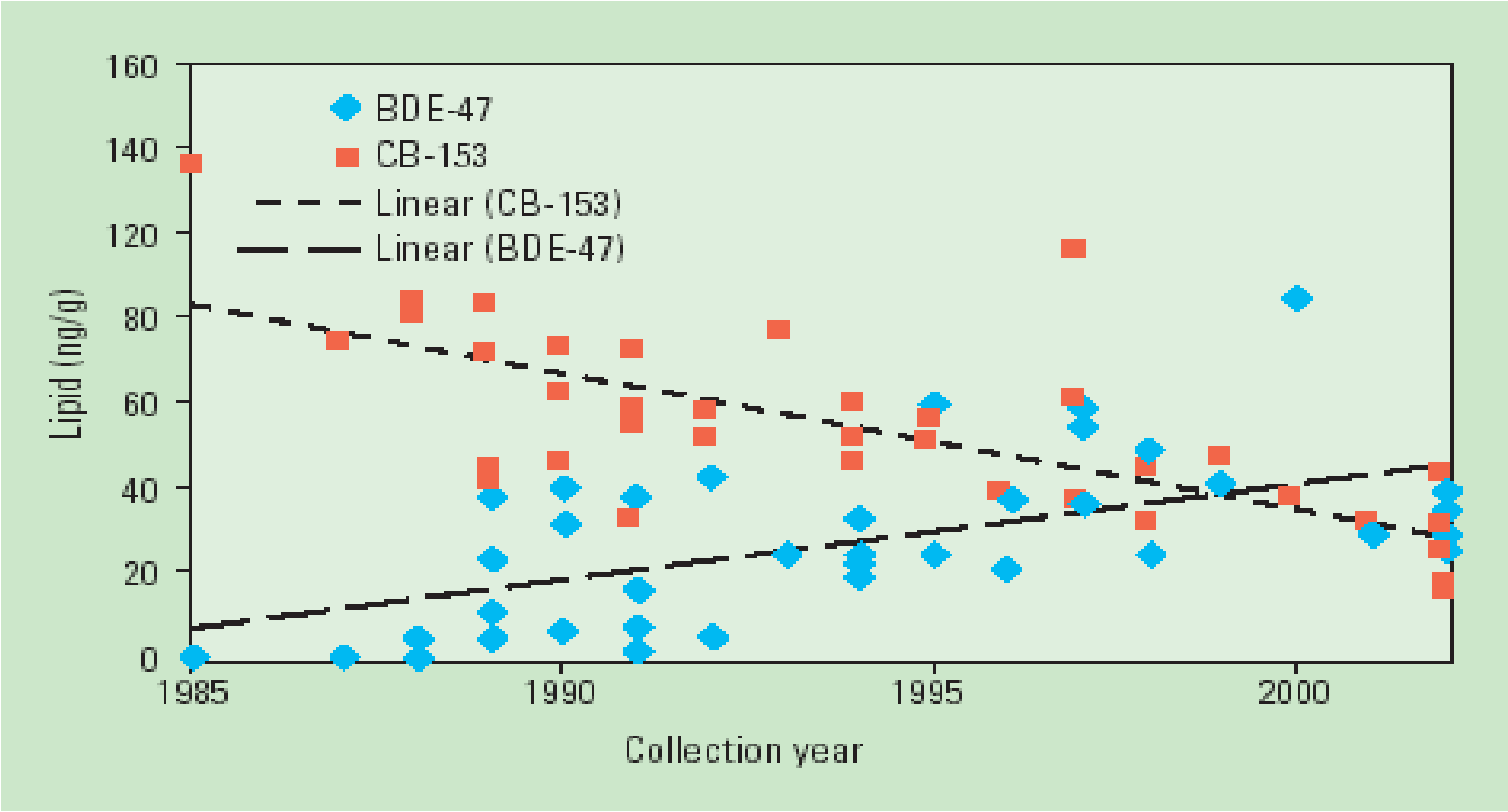
McCarthy test scores of the 1990 Lake Michigan case study of 4-year-old children.





Source: Redrawn from Jacobson et al., 1990. *J. Pediatrics* 116(1): 38-44

PBDE is up, while PCB is down



This slide, which was created for *ES&T* by Andreas Sjödin of the U.S. Centers for Disease Control, shows the levels of the most bioaccumulative PBDE congener, BDE-47, and the most bioaccumulative PCB congener, CB-153, in U.S. human blood samples.

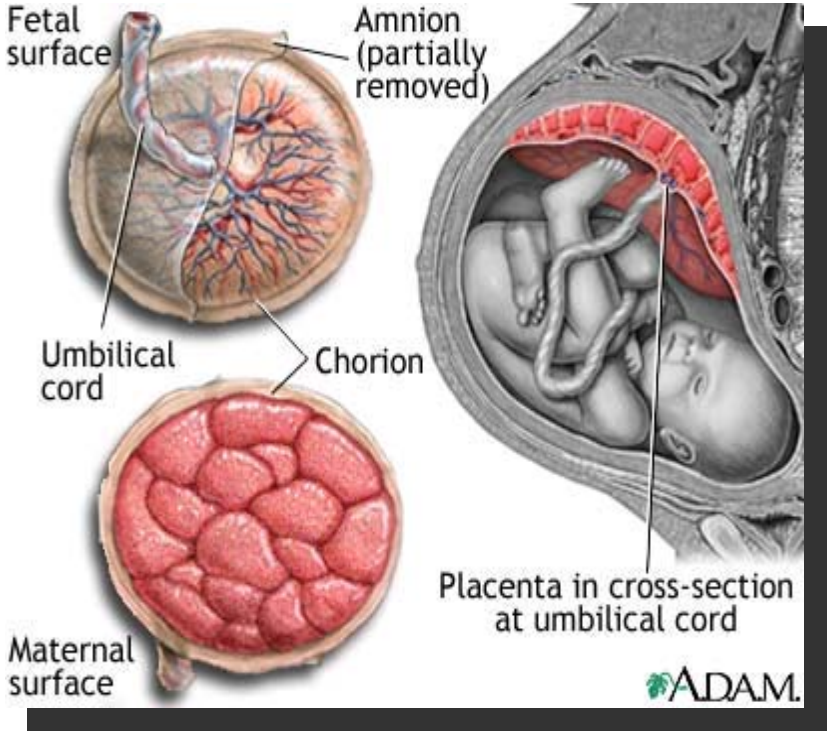
Anonymous. 2003. U.S. PBDE milestones. *Environ. Sci. Technol.*, **2003**, *37* (21), p 384A

Why Placenta ?

- In the assessments of pre- and neo-natal human exposure to toxic chemicals, most studies use maternal and cord blood.
- The advantages of using placenta include:
  - Easier sample collection
    - How many placentas are thrown away everyday?
    - Non-invasive, no afterwards health concerns.
  - Sufficient amount of each sample for multiple chemical analyses, allowing the studies on chemical synergy.
  - Placenta may better reflect the exposure during the entire pregnancy for hydrophobic compounds.
  - Placenta is the matrix to study the science (mechanisms and kinetics) of cross-placental transfer of toxic (and nutrient) chemicals.

Challenges of using placenta

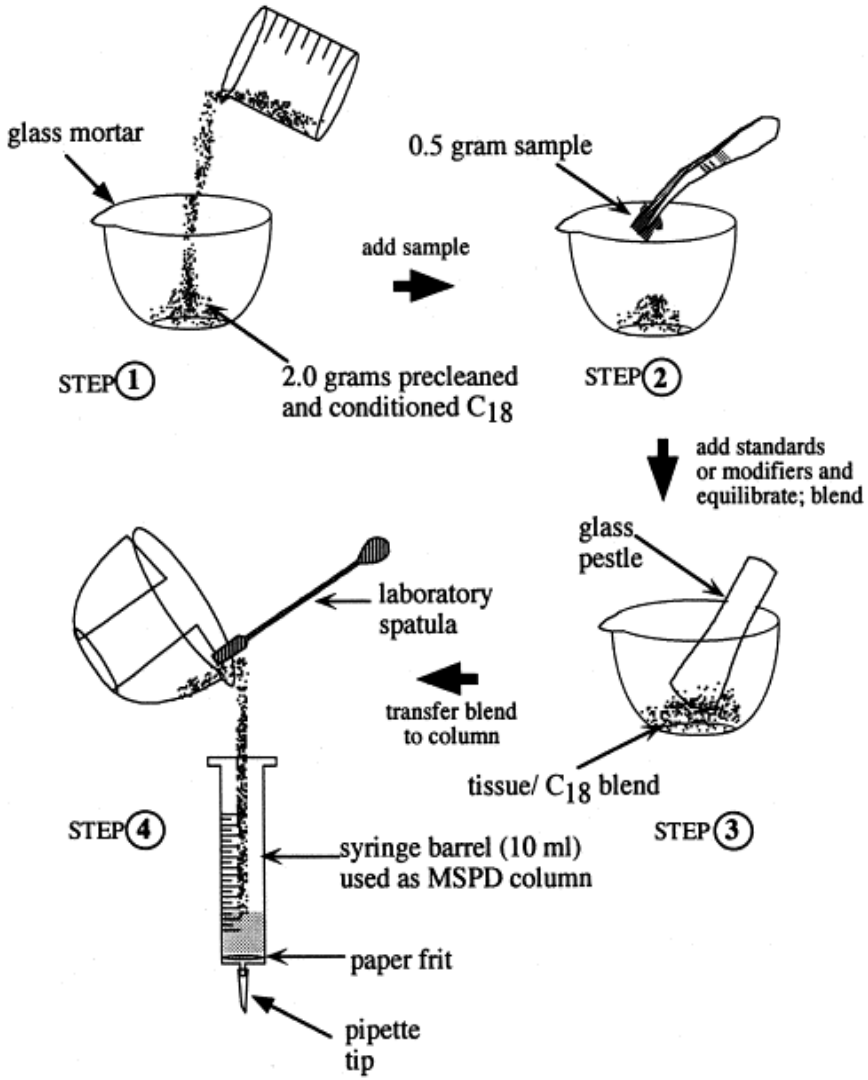
- Heterogeneity of the placenta
  - difficulty in defining “sample”
  - “whole placenta” approach
- High variation in lipid content and difficulties in determination
- Due to the low concentrations of the toxics,
  - sufficient sample mass is needed
  - easily contaminated and influenced by background



- No standard reference materials for quality control
- Limited number of studies for comparison

Matrix Solid-Phase Dispersion (MSPD)

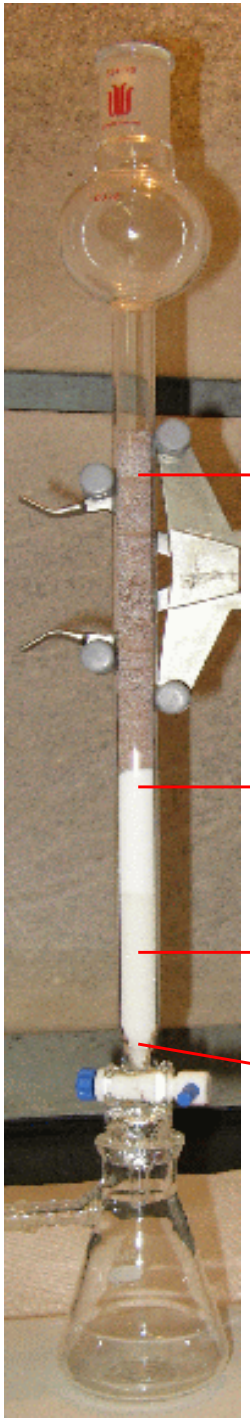
- For solid and semisolid samples, the traditional Soxhlet extraction is exhaustive, nonselective, and solvent and time consuming
- MSPD procedure was developed in the late 1980s for the extraction of drugs from biological samples.
- MSPD application in environmental analysis started in the mid 1990s.
- The advantages of MSPD include its simplicity, efficacy, low costs, and the possibility of simultaneous extraction and cleanup.



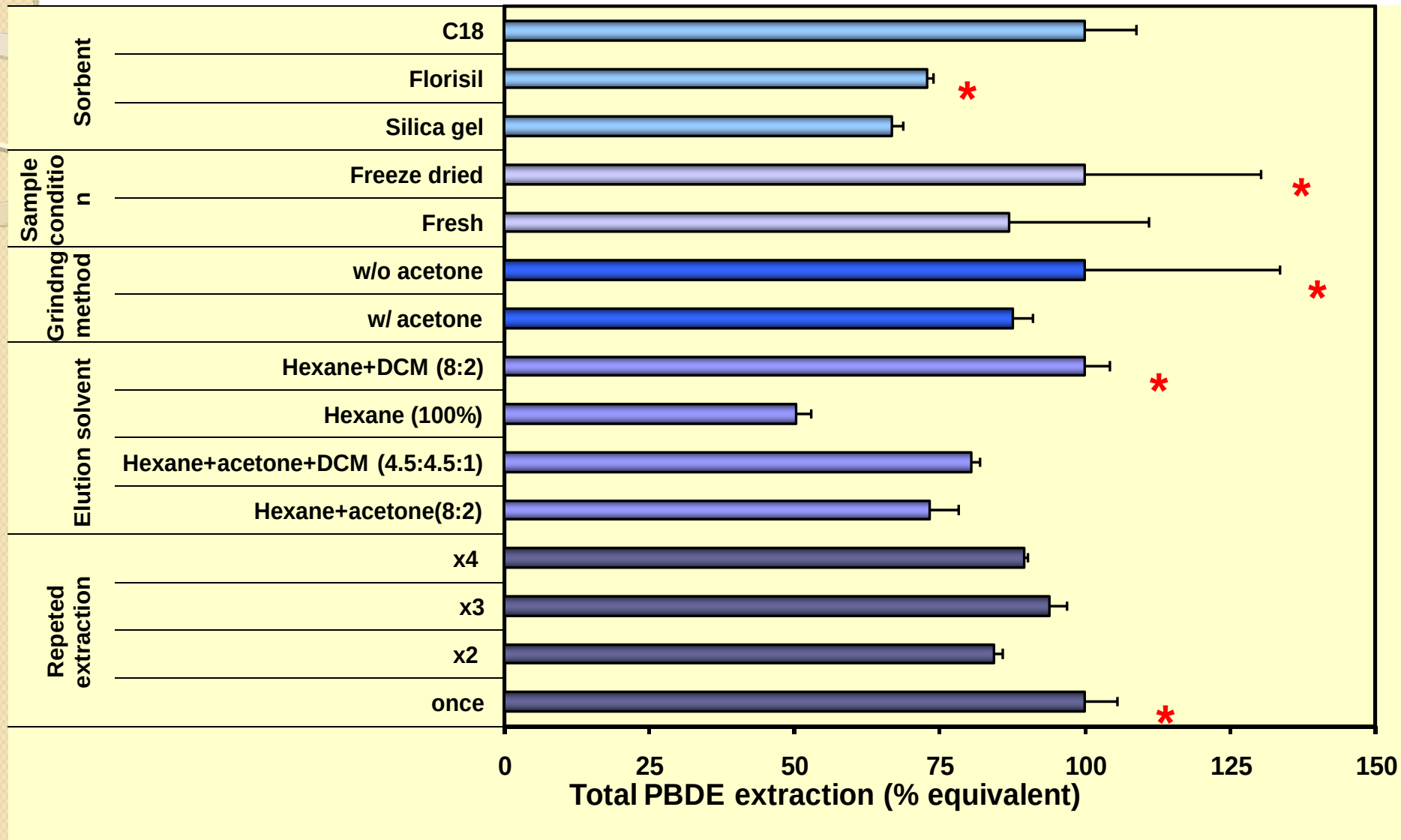
Source: Barker, S.A. *J. Chromatogr. A* **2000**, 885, 115-127

Extraction and Cleanup

- MSPD procedure
  - Grinding with glass mortar-pestle for ca 5 min, with sample: sorbent ratio of 1:2
  - A glass column (13 mm id and 30 cm length) was packed
  - Elution – using 100 mL solvent under gravitational flow.
- Cleanup
  - GPC removal of lipid
  - Further Silica gel cleanup



MSPD Optimization



Relative extraction efficiency of total PBDEs under different MSPD conditions. The error bar represents one standard deviation of the duplicated analyses. The condition with an asterisk was chosen for the optimized MSPD procedure.

Instrumental Analysis

- GC/MS
  - 120 μL injection using a PTV inlet
  - Restek Rtx1614 capillary column (15 m × 0.25 mm i.d. × 0.1 μm film)
  - ECNI MS with SIM

- GC/MS/MS
  - 120 μL injection using a multi-mode inlet
  - Restek Rtx1614 capillary column (30m × 0.25 mm i.d. × 0.1 μm film)
  - EI MRM (NCI MRM is being developed)

Quality Control

- Analytical accuracy was monitored by recovery of surrogate (^{13}C-labeled BDE118L).
- Analytical precision was determined from the relative standard deviation (RSD) of replicate analyses.
- Instrument blanks were run to ensure the GC/MS is free of any contamination.
- Procedure blanks are analyzed with each batch. Reported results are after subtractions of blank levels.
- Method detection limits (MDLs) were determined according to standard procedures.
- Method validation included the use of matrix spikes and standard reference material (SRM).

Method Validation with Spikes

Table 1. PBDE recovery from the spiked placenta samples (ng/g dw)*

Congener	**Spike level**	**Sp1**	**Sp2**	**Sp3**	**Un-spiked**	**Mean Recovery**(%)	**RSD**(%)
BDE28+33	1.32	1.22	1.16	1.22	0.0025	91± 0.03	2.71
BDE47	1.52	1.55	1.47	1.57	0.0775	95 ± 0.06	3.62
BDE66	1.43	1.52	1.31	1.42	0.0000	99 ± 0.1	7.35
BDE100	1.60	1.57	1.43	1.52	0.0150	94 ± 0.07	4.71
BDE99	1.55	1.74	1.47	1.57	0.0325	100 ± 0.1	48.58
BDE118L	1.39	1.72	1.36	1.51	0.0000	110 ± 0.18	11.59
BDE85	1.44	1.79	1.47	1.71	0.0150	114 ± 0.1	69.93
BDE154	1.57	1.72	1.43	1.55	0.0075	99 ± 0.15	9.37
BDE153	1.54	1.78	1.51	1.62	0.0350	104 ± 0.14	8.31
BDE183	1.58	1.87	1.69	1.81	0.0100	113 ± 0.09	5.10
BDE209	1.53	1.98	2.06	1.84	0.4775	97 ± 0.11	5.64

* Recovery = (concentration in spiked sample - concentration in unspiked sample) / spike level x 100%

Method Validation using SRM

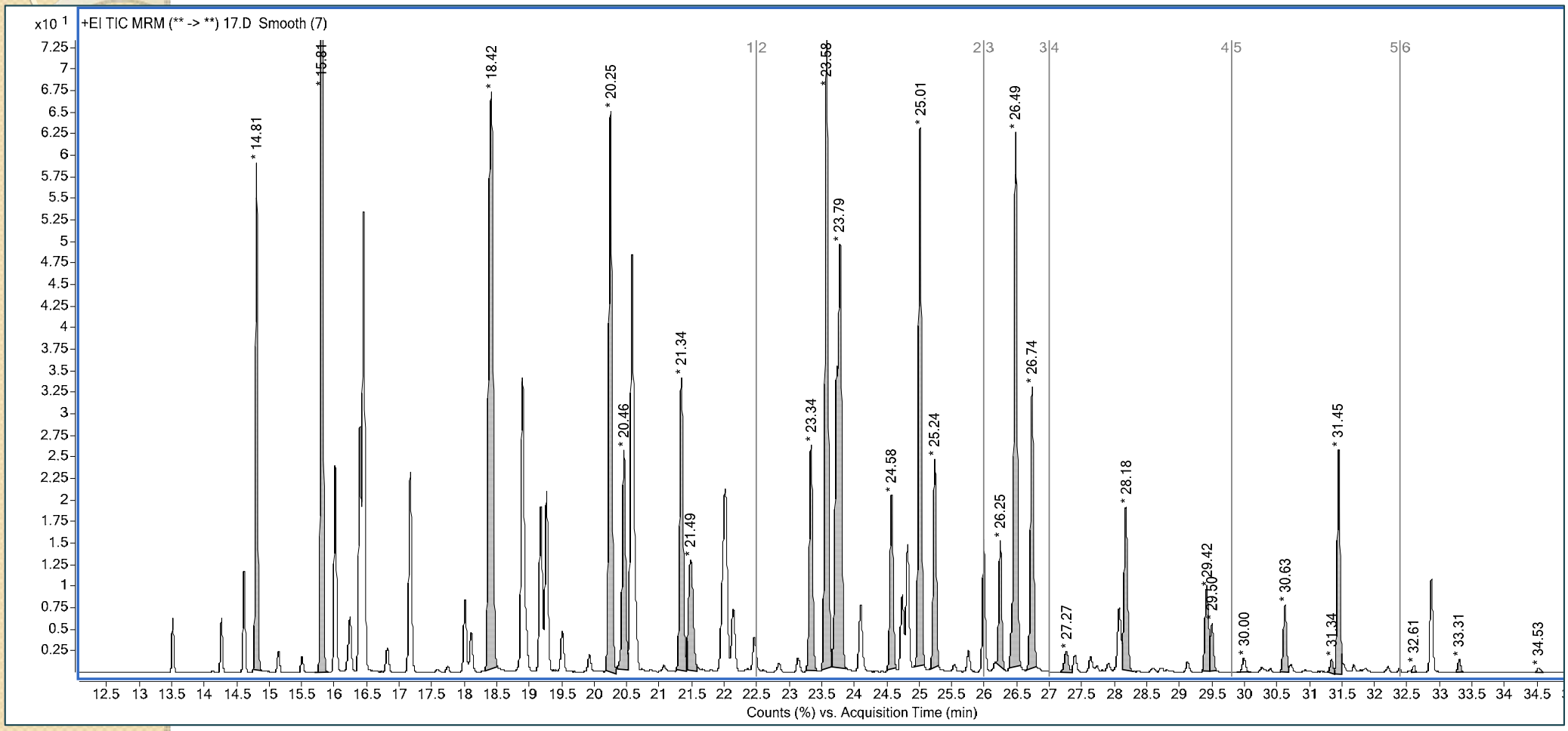
Table 2. Analytical results of SRM 1947 using optimized MSPD extraction (ug/kg ww)

PBDE	(A)	(B)	Mean	RSD (%)	Certified*	Recovery (%)
BDE28	1.26	0.94	1.10 ± 0.23	20.8	2.26 ± 0.46	49
BDE47	70.17	67.38	68.77 ± 1.98	2.9	73.30 ± 2.9	94
BDE66	1.84	1.46	1.65 ± 0.27	16.4	1.85 ± 0.13	89
BDE 100	14.82	15.50	15.16 ± 0.48	3.2	17.10 ± 0.6	89
BDE99	23.15	20.91	22.03 ± 1.58	7.2	19.20 ± 0.8	11 5
BDE154	6.49	6.66	6.57 ± 0.12	1.8	6.88 ± 0.52	96
BDE 153	4.16	4.27	4.22 ± 0.07	1.7	3.83 ± 0.04	11 0

* The value for BDE28 is a reference value.

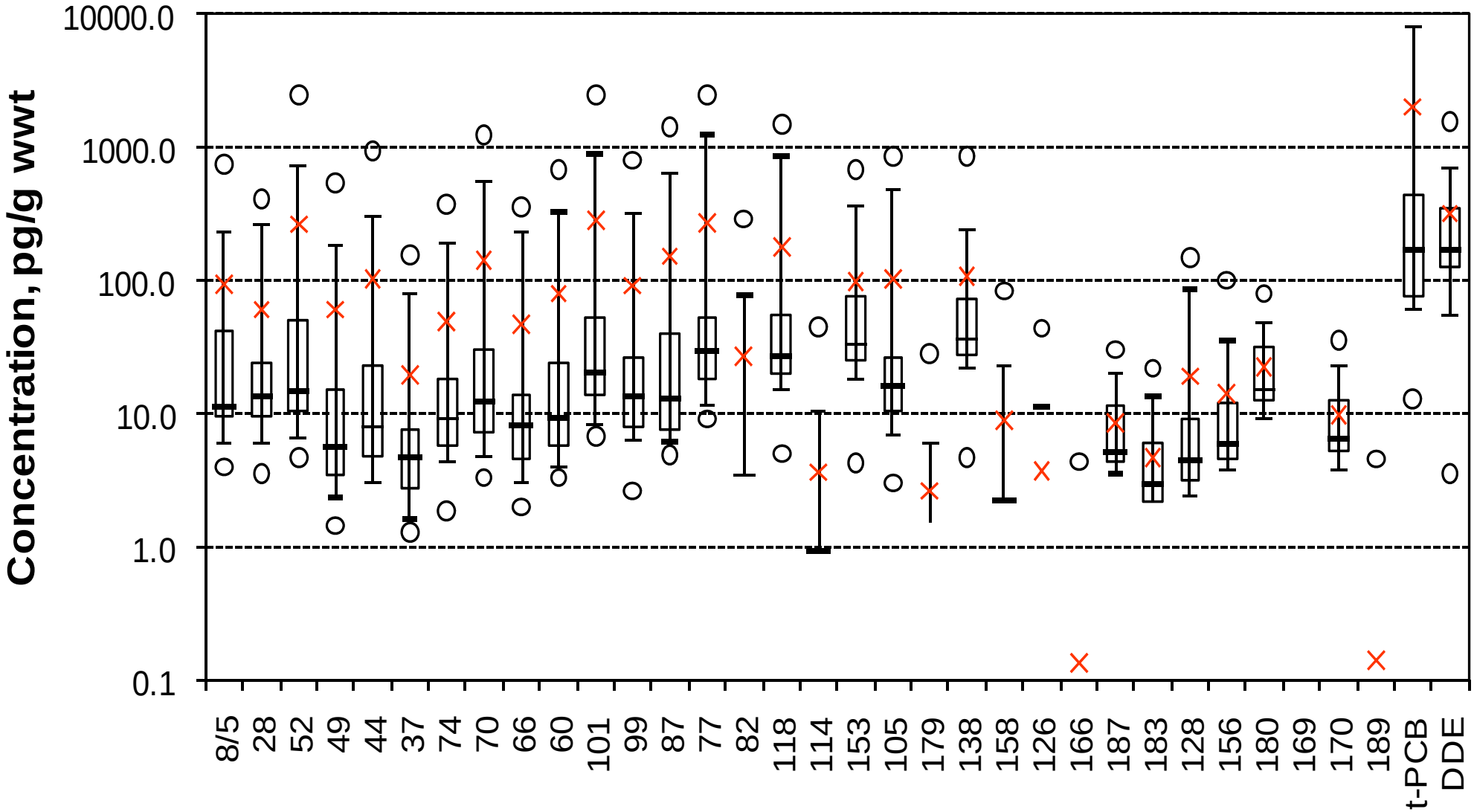
Standard reference material (SRM) 1947 is fish tissue from the Great Lakes

GC/MSMS Chromatogram for PCBs



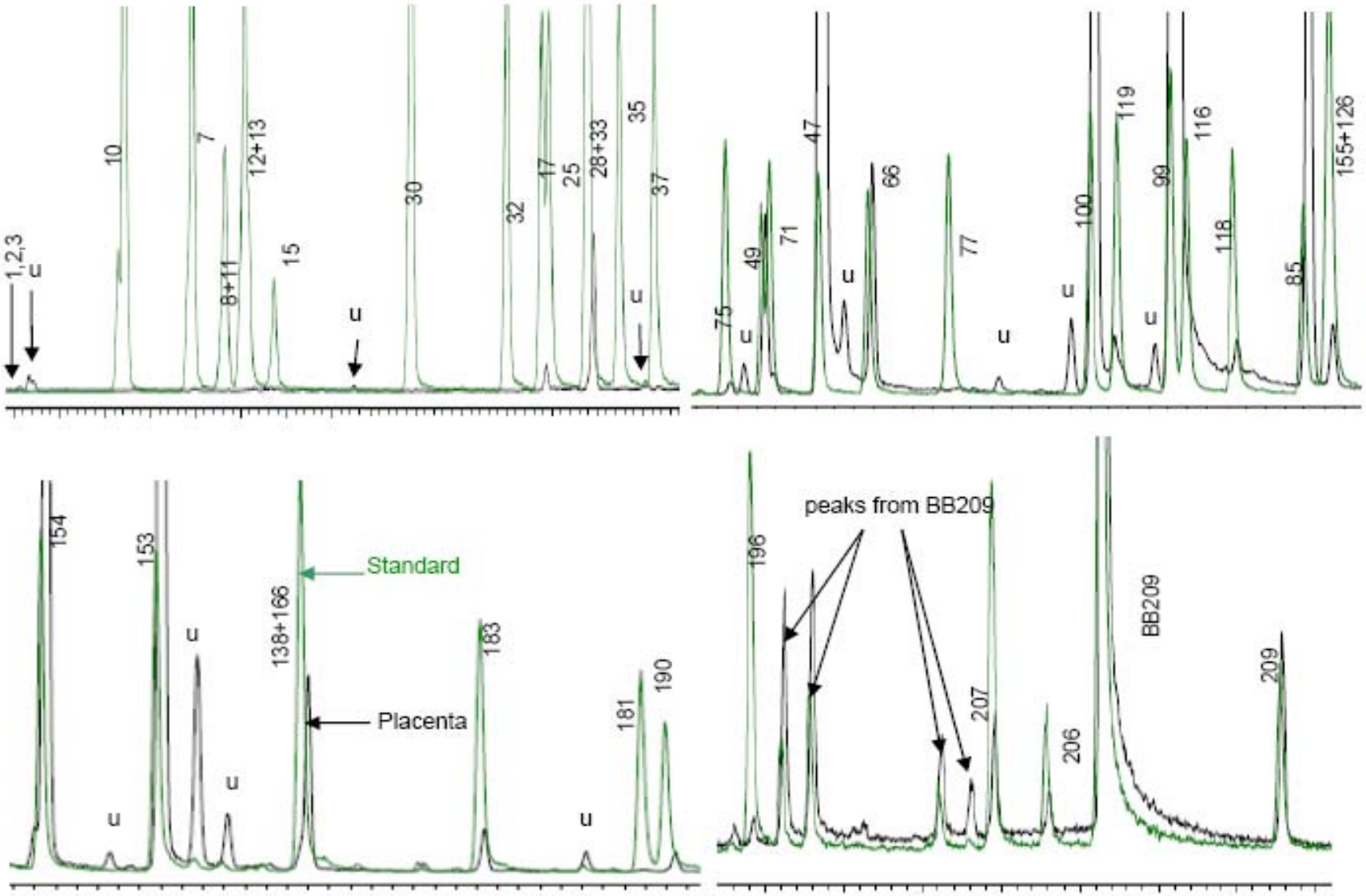
Agilent 7890/7000 GC/MSMS, electron impact (EI) ionization with multiple reaction monitoring (MRM) acquisition

PCBs and DDT in Placenta Tissue



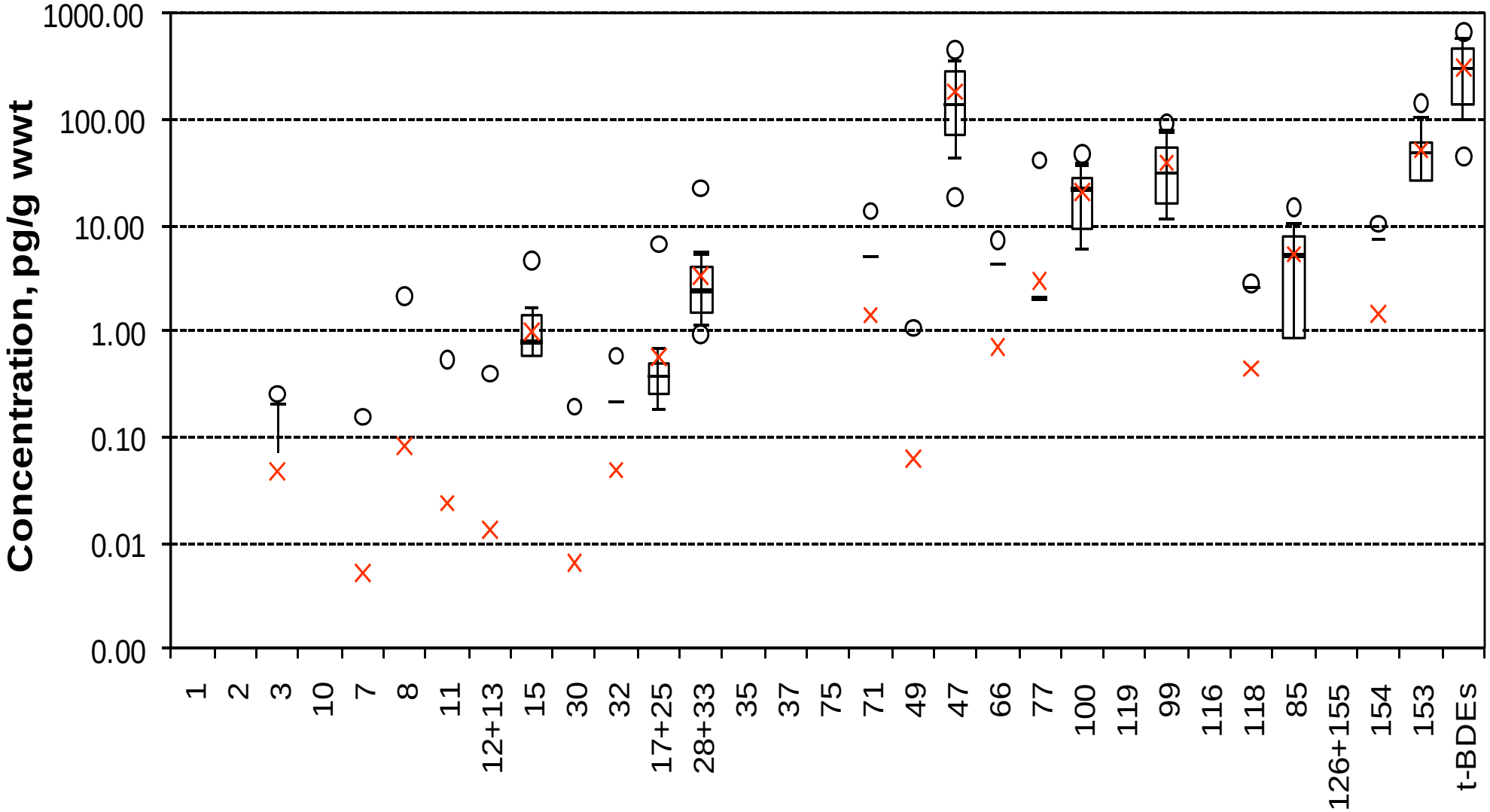
* Placenta samples (N = 33) were collected 2010-2011 in Chicago

PBDEs in Placenta



Agilent 6890/5973 GC/MS, negative chemical ionization with selected ion monitoring acquisition.

PBDEs in Placenta Tissue



* Placenta samples (N = 30) were collected 2010-2011 in Chicago

Comparison among countries

	Country	Year	N	# BDEs	£ 7Br BDE (ng/g lipid)	decaBDE (ng/g lipid)
Main et al.	Denmark	2007	129	14	0.61–3.31	NA
Main et al.	Finland	2007	56	14	0.35–9.89	NA
Gomara et al.	Spain	2007	30	9 or 15	0.14-2.3	0.05-8.4
Takasuga et al.	Japan	2006	10	9	0.234	0.25-0.56
Strandman et al.	Finland	2000	11	4	1.0 – 4.4	NA
Hirai et al.	Japan	2001	4	6	1.0±0.3	NA
Doucet et al.	Canada	1998-2006	60	7	<100 - > 10,000*	NA
This lab	U.S.A.	2008-9	5	43	380 - 2370	12-29
This lab #	U.S.A.	2010-11	34	43	45 - 670	NA

* Estimated from graph
# preliminary data

Preliminary Result Summary

- DDE was the most abundant single compound found with 100% detection rate, and averaged at 323 (range10 -1500) pg/g wet weight (wwt).
- The sum of 34 PCB congeners has a median of 378 pg/g wwt and an average of 2,360 pg/g wwt. The most abundant congener were CBs 138, 77, 153, 118, and 101 (> 20 pg/g wwt). The data for all congeners are highly right-screwed, with the averages being 1.5 to 18 (average 6.6) times higher than the medians.
- The results for PBDEs are preliminary, due to the on-going GCMSMS optimization. For 34 mono- to hepta-BDEs, the sum Σ_{34}BDE in placenta has a median of 301 pg/g wwt and an average of 310 pg/g wwt. BDE47 had the highest concentration followed by BDEs 99, 28+33, 100 and 153. These congeners were detected in almost all the samples. No correlation was found between PCBs and PBDEs. Levels of PBDEs in North America are much higher than the reported literature values from other continents.

Near-Future Plan

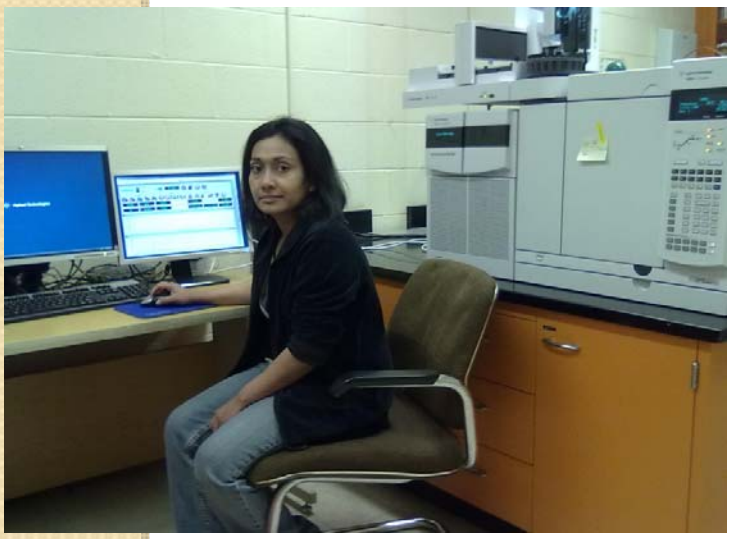
- The operating conditions of the newly purchased GC-TQMS is being further optimized, especially in negative chemical ionization (NCI), in order to improve analytical sensitivity, which becomes critical when the amount of available placenta tissue is limited.
- At present, NCS P2-18 samples from “Collection of Nine” are being analyzed for PCBs, DDE and PBDEs with the GC-TQMS.
- The improved and optimized instrument conditions will be used in the future to analyze samples collected in the NCS P2-18 pilot study and the main study.

Long Term Future Plan and Ideas

- **Within our laboratory:**
  - Include more persistent and bioaccumulative organic pollutant chemicals in the analyte list.
  - Develop/optimize laboratory procedures for the analysis of the metabolites of PBDEs, PCBs and others in human placental tissue and bloods.
- **Collaborate with other researchers:**
  - Mechanisms of cross placenta transfer
  - Chemical synergy among organics, metal, drugs, …,
  - Epidemiological investigation - health statistical inferences and correlations with various biological and sociological parameters
- **Laboratory capacity building:**
  - Dedicated instrument for higher throughput.
  - Dedicated personnel

Acknowledgements

- We thank UIC Medical Center and all placenta donors.
- We thank Dr. Richard Miller for his wonderful management of P-18. We thank all P-18 fellow researchers for their support and patience.
- Funding was from National Institute of Environmental Health Sciences (NIEHS) (Grant Number R03ES015608) and the National Children's Study (NCS) through the Greater Chicago Study Center at Northwestern University. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIEHS, NIH, or NCS.



NIEHS

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Emerging HOPs being analyzed in this lab

PCBs	>140 congeners
PBDEs	180 congeners
Mirex	Dechlorane
syn-DP	Dechlorane plus (syn)
anti-DP	Dechlorane plus (anti)
Dec602	Dechlorane 602
Dec603	Dechlorane 603
Dec604	Dechlorane 604
Kepone	Chlordecone
DBDPE	Decabromodiphenylethane
BB153	2,2,4,4,5,5-hexabromobiphenyl
BTBPE	1,2-bis-(2,4,6-tribromophenoxy) ethane
TBECH	1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane
HCBDCO	Hexachlorocyclopenta-dienyl-dibromocyclooctane
t-HBCD	Hexanbromocyclododecane

Various pesticides

TBB	1,3,5-Tribromobenzene
ATE	allyl 2,4,6-tribromophenylether
pTBX	2,3,5,6-tetrabromo-p-xylene
TBOCT	Tetrabromo-o-chlorotoluene
PBT	Pentabromotoluene
PBEB	Pentabromoethyl benzene
HBB	Hexabromobenzene
PBBB	pentabromobenzyl bromide
PBBA	Pentabromobenzyl acrylate

Many others are “under construction” of methods.