UNIVERSITY OF SASKATCHEWAN

Synthetic and Naturally Occurring Brominated Compounds in the Marine Environment

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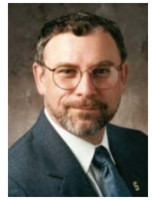
Affiliations



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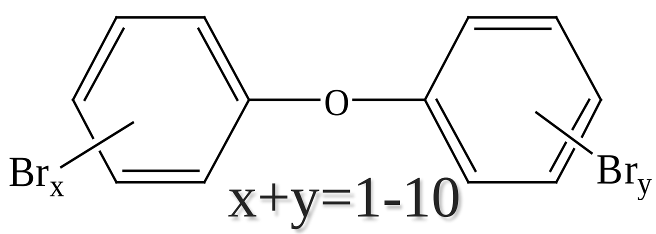
Organic Brominated Compounds

- **Halogenated organic compounds of environmental concern: fluorinated, chlorinated, and brominated**
- **Large amounts of brominated flame retardants have been produced and used in globally**
- **Many diverse types of naturally occurring organic brominated compounds have been reported, especially in the marine environment some with many bromines**
- **Relationships, contributions and potential risks of the anthropogenic and naturally occurring brominated compounds?**

Polybrominated diphenyl ethers (PBDEs)

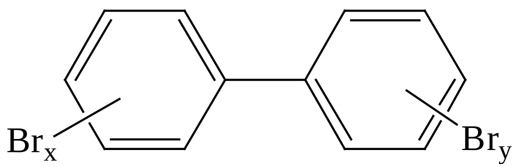
- **Synthetic flame retardants**
  - **In many products, textiles, foam for seating and electronics**
- **Ubiquitous in the environment**
- **Neurotoxins**
- **Endocrine disruption**
- **Moderately toxic at high concentrations**



E-Waste handling

Polybrominated biphenyls (PBBs)

- Synthetic flame retardants
- Became of great concern after Michigan cattle pollution accident in the U.S. in 1973
- Widely detected in the environment
- High bioaccumulation potential



$x+y=1\text{-}10$

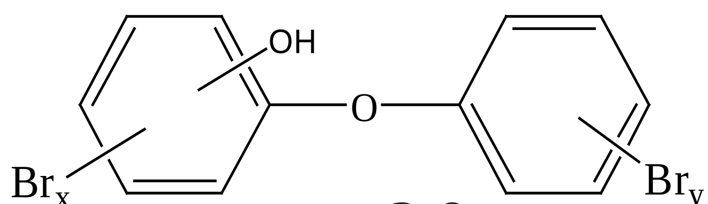
Electrical products

Hydroxylated PBDEs

- **OH-PBDEs**
  - **Natural Products**
    - ***2-OH-PBDE-47***
    - ***6-OH-PBDE-47***
  - **Bio-transformation of PBDEs**
- **Variety of Effects**
  - **Endocrine disruption**
  - **Disruption of oxidative phosphorylation**
    - ***6-OH-PBDE is acutely toxic to Zebrafish***

Marine Sponge

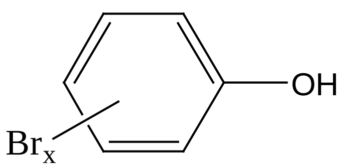
Red algae



x+y=2-9

Bromophenols (BRPs)

- **Natural compounds: a key natural flavour component of marine fish**
- **Some are used as flame retardants (2,4,6-triBRP)**
- **reported to be biotransformation products of OH-PBDEs**



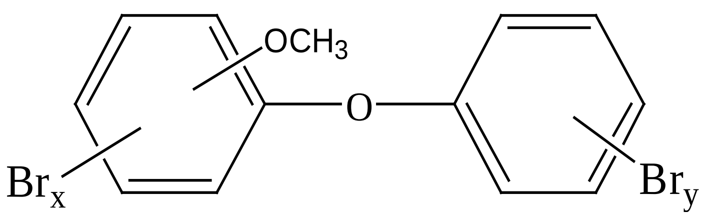
x=1-5

Marine fish

Flame Retardant

Methoxylated PBDEs

- **MeO-PBDEs**
  - **Concentrations sometimes greater than PBDE**
  - **Two abundant congeners are natural products**
    - ***2-MeO-PBDE-68***
    - ***6 MeO-PBDE-47***
  - **Suggested that they may be form from OH-PBDEs**
  - **Toxicity unknown**



x+y=2-9

Fish oil

Contribution of Synthetic and Naturally Occurring Organo-bromine Compounds to Bromine Mass

Background

- **Some well-known synthetic organo-bromines such as PBDEs have become ubiquitous environmental pollutants**
- **There are a number of unidentified organo-bromines in the environment**
- **Mass balance studies: synoptic quantification of organo-brominated compounds along with quantification of total organically bond bromine**
- **To estimate the contribution of identified and unidentified organo-brominated compounds.**

Wan Y., Jones P.D., Wiseman S., Chang H., Chorney D., Kannan K., Khim J.S., Tanabe S., Lam M.H.W., Giesy J.P., Contribution of Anthropogenic and Naturally Occurring Organobromine Compounds to Bromine Mass in Marine Organisms ***Environmental Science & Technology***, Submitted.

Sample details

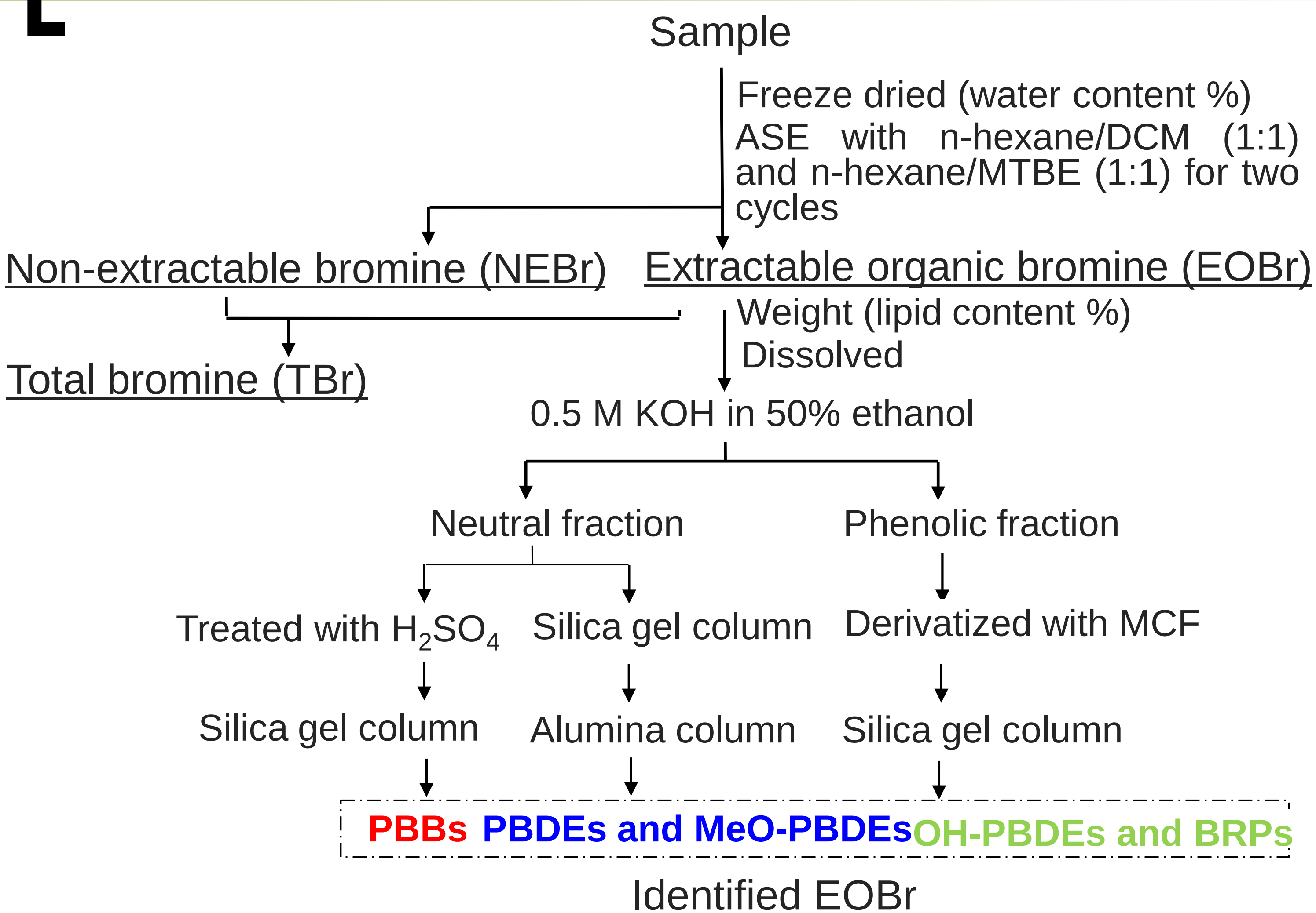
Collection Date	#	Species Name	Location
1999	10	Pacific tuna	North Pacific Ocean
1992-1996	6	Black-browed albatross	Indian Ocean, South Pacific Ocean
1994-1996	3	Grey-headed albatross	Indian Ocean
1995	1	Light-mantled sooty albatross	South Atlantic Ocean
1995-1996	2	Shy albatross	South Atlantic Ocean, Indian Ocean
1995-1996	3	Yellow-nosed albatross	Indian Ocean
1993-2002	10	Polar bear	Arctic Ocean

Liver tissues

Analytical Method

- **Target compounds**
  - **21 PBDEs, 10 PBBs, 12 MeO-PBDEs, 10 OH-PBDEs and 16 BRPs**
- **QA/AC**
  - **Recoveries for matrix spiked samples were 90-127%, 81-126%, 87-128%, 81-123% and 65-126% for PBBs, MeO-PBDEs, PBDEs, OH-PBDEs, and BRPs respectively.**
- **Derivatization**
  - **Methyl chloroformate (MCF) was used for OH-PBDE analysis**
  - **Exhibits excellent reproducibility and fewer background interferences compared to diazomethane**

Fractionation of TBr, EOBr and Identified EOBr

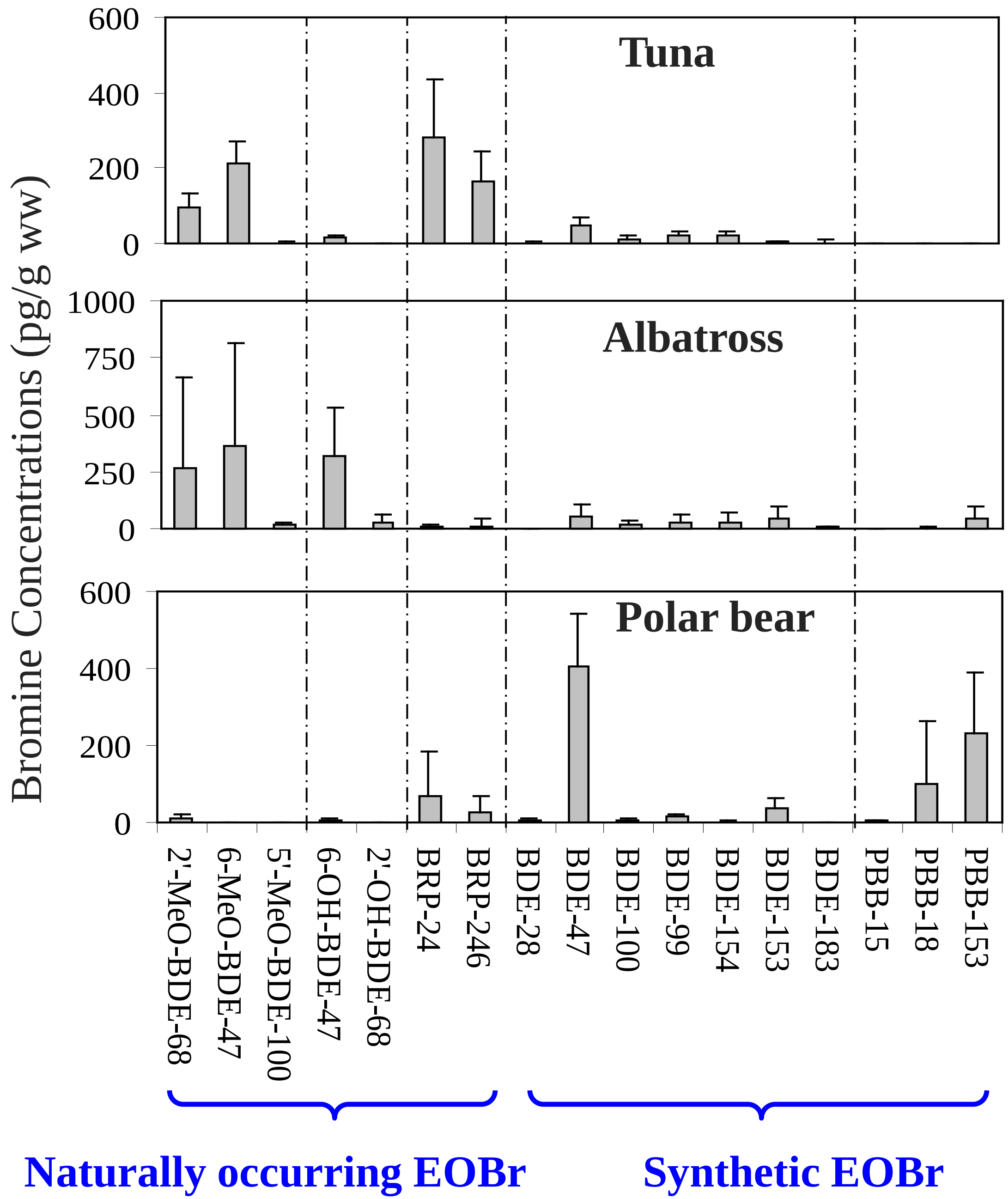


Bromine Analysis

- **INAA**
  - Samples were activated at a neutron flux of 5.0 × 10^{11} (n/cm^2)/s in the SLOWPOKE 2 nuclear reactor
  - Quantification was based on γ-peaks from 80-Br (t1/2 = 17.6 min, Eγ = 616 keV). Count duration 15 min.
- **GC-HRMS Analysis**
  - Chromatographic separation was achieved on a DB-5MS fused silica capillary (30 m length, 0.25 mm ID, 0.1 μm film thickness)
  - Resolution > 7,000
  - Injector temperature: 285 ℃; Ion source: 285 ℃.
  - Electron ionization energy: 37 eV, ion current: 750 μA.

Concentrations of Identified EOBr

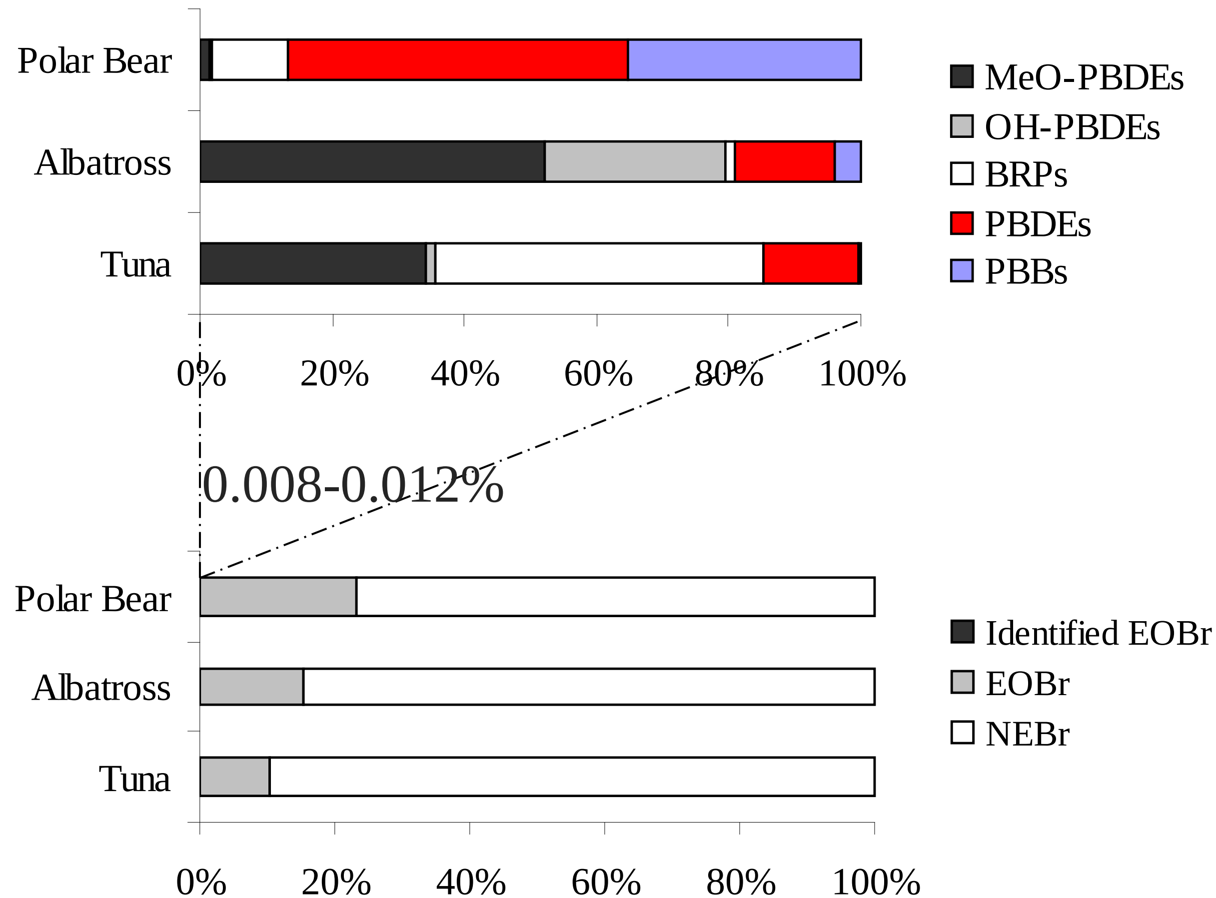
- The naturally occurring compounds were prevalent in tuna and albatross
- The identifiable extractable organic-bromine (EOBr) consisted primarily of synthetic compounds in polar bears
- Concentrations of EOBr are species- and location-specific



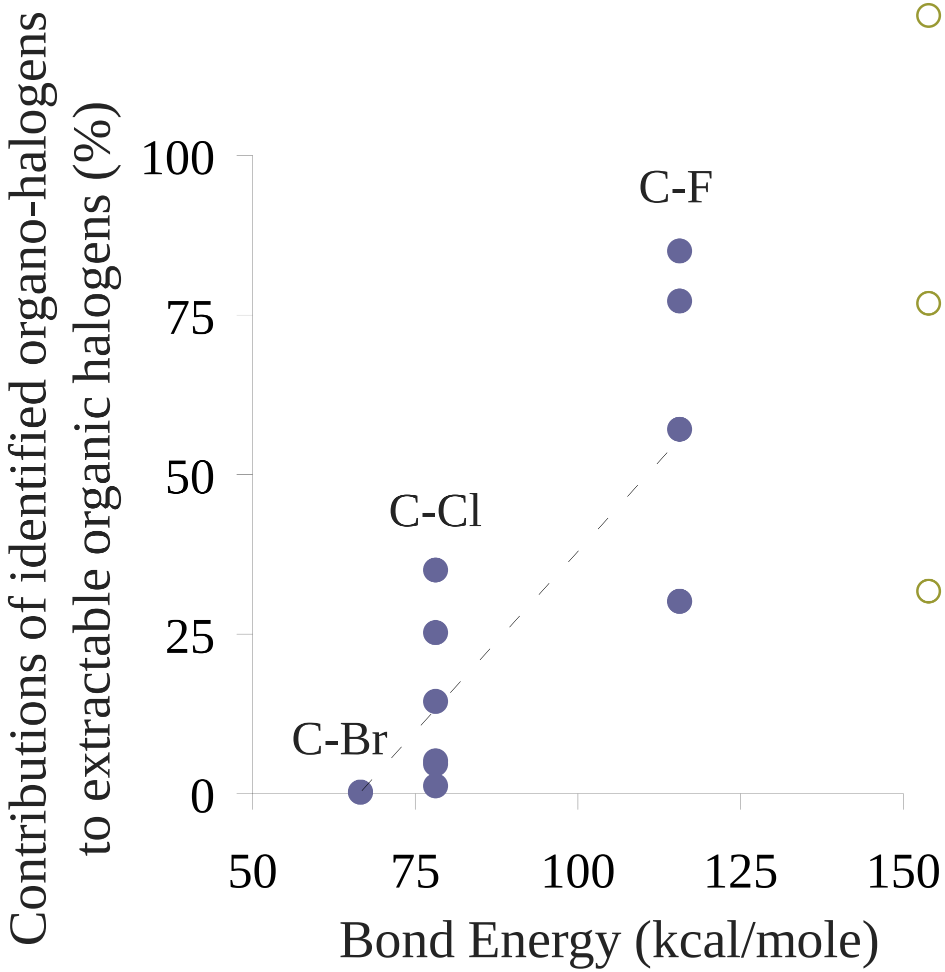
Contribution of identified EOBr, EOBr and NEBr to TBr

- **The majority of the bromine was non-extractable or inorganic (NEBr)**
- **Extractable' organic bromine (EOBr) accounted for 10 to 28% of the total bromine (TBr)**
- **0.08-0.11% and 0.008-0.012% of EOBr and TBr, respectively, could be identified**

Great diversity of naturally occurring organo-bromine compounds



Bond Energies vs Identified EOX/EOX



- More than half of the known naturally occurring organo-halogen compounds contained bromine
- As the bonds become stronger, they become less likely to be derived naturally and also less likely to be degraded natually
- Potential natural sources and impact(s) of organic brominated compounds should not be neglected: What are they? What do they do? Might they have beneficial uses?

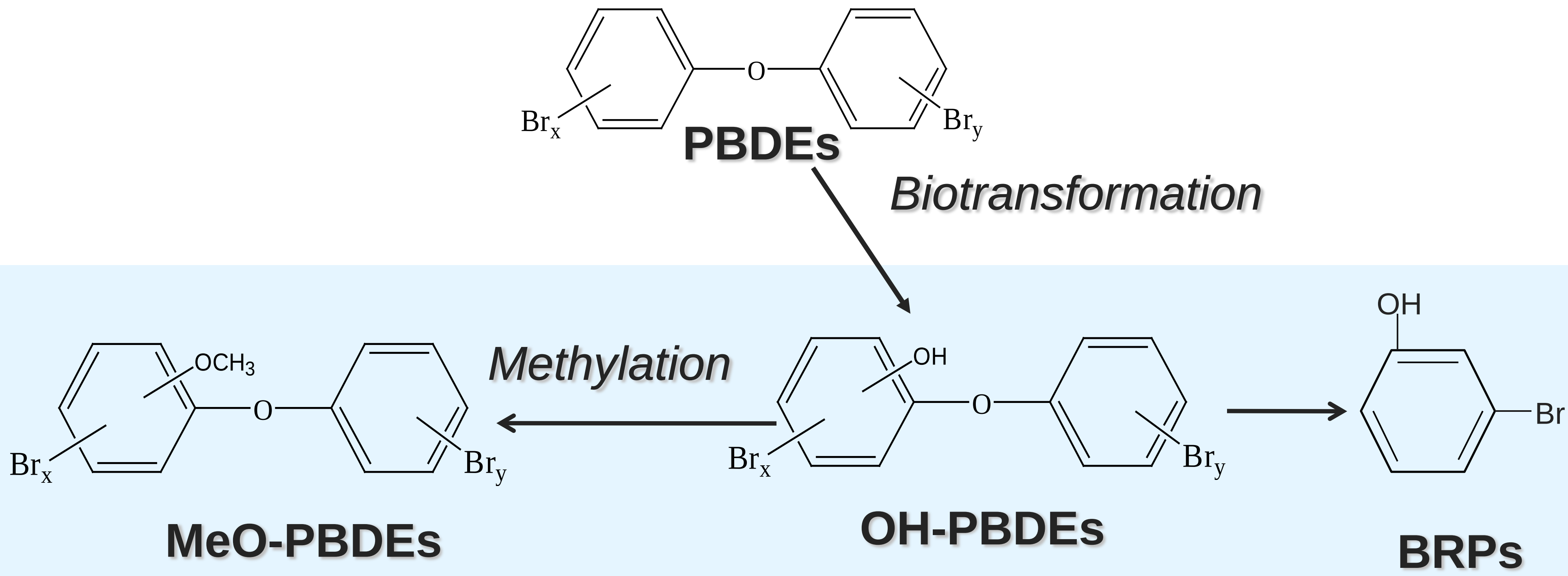
Summary

- Methods were developed for identification and quantification of TBr, EOBr and five classes of identified EOBr.
- The majority of the bromine in marine organisms was non-extractable or inorganic, with EOBr accounting for 10 to 28% of the TBr.
- Overall, 0.08-0.11% and 0.008-0.012% of EOBr and TBr, respectively, could be identified, based on prevalent classes of brominated compounds.
- The small proportion of identified EOBr was related to the great diversity of naturally occurring organo-bromine compounds

Origins and Relationships of PBDE Structurally Related Compounds

Sources and Relationships

- **Formation of OH-PBDEs is of considerable concern due to their greater toxicities relative to PBDEs and MeO-PBDEs.**
- **Conceptual model of formation of OH-BDEs and MeO-BDEs that has been proposed in the literature**



PBDEs as Precursors of OH-PBDEs ?

- **Exposure concentrations of PBDEs during *in vitro* or *in vivo* studies were large (ppm), but OH-PBDEs occurred at trace levels (<0.01-1% of PBDEs) (***Environ. Sci. Technol.* **2005**, *39*, 5342-5348; *Mol. Nutr. Food Res.* **2008**, *52*, 284-298; *Environ. Health Persp.* **2009**, *117*, 197-202.**)**
- **Relatively large concentrations of OH-PBDEs have been detected in marine organisms. (***Environ. Sci. Technol.* **2005**, *39*, 2990-2997**)**
- **These results are consistent with existence of sources of OH-BDE other than synthetic PBDEs**

What are the sources of OH-PBDEs, MeO-BDEs ?

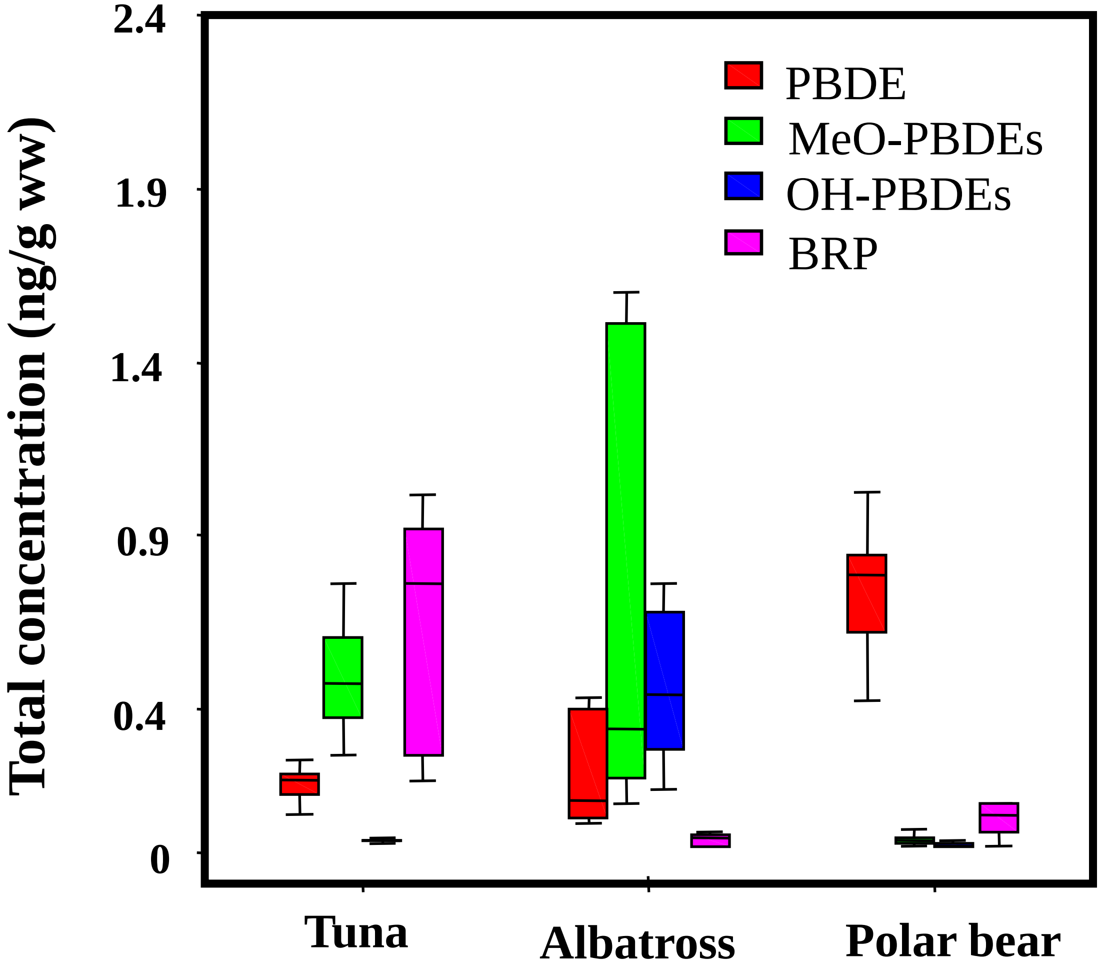
What is the relationships between PBDEs, MeO-PBDEs and OH-PBDEs?

Experimental Goals -1

- **Determine concentrations of PBDEs, MeO-PBDEs, OH-PBDEs and bromo-phenols in liver of tuna, five albatross species and polar bear collected from remote marine locations**
  - **Investigate relationships among PBDEs, MeO-PBDEs, OH-PBDEs and bromo-phenols in wildlife**

Wan, Y., S. Wiseman, H. Chang, X. Zhang, P.D. Jones, M, Hecker, K. Kannan, S. Tanabe, J. Hu, M.H.W. Lam, and J.P. Giesy. 2009. Origin of Hydroxylated Brominated Dophenyl Ethers: Natural Compounds of Man-Made Flame Retardants. *Environ. Sci. Technol.* 43:7536-7542. (DOI:10.1021/es901357u)

Concentrations of PBDEs, MeO-PBDEs, OH-PBDEs and BRPs in Marine Organisms

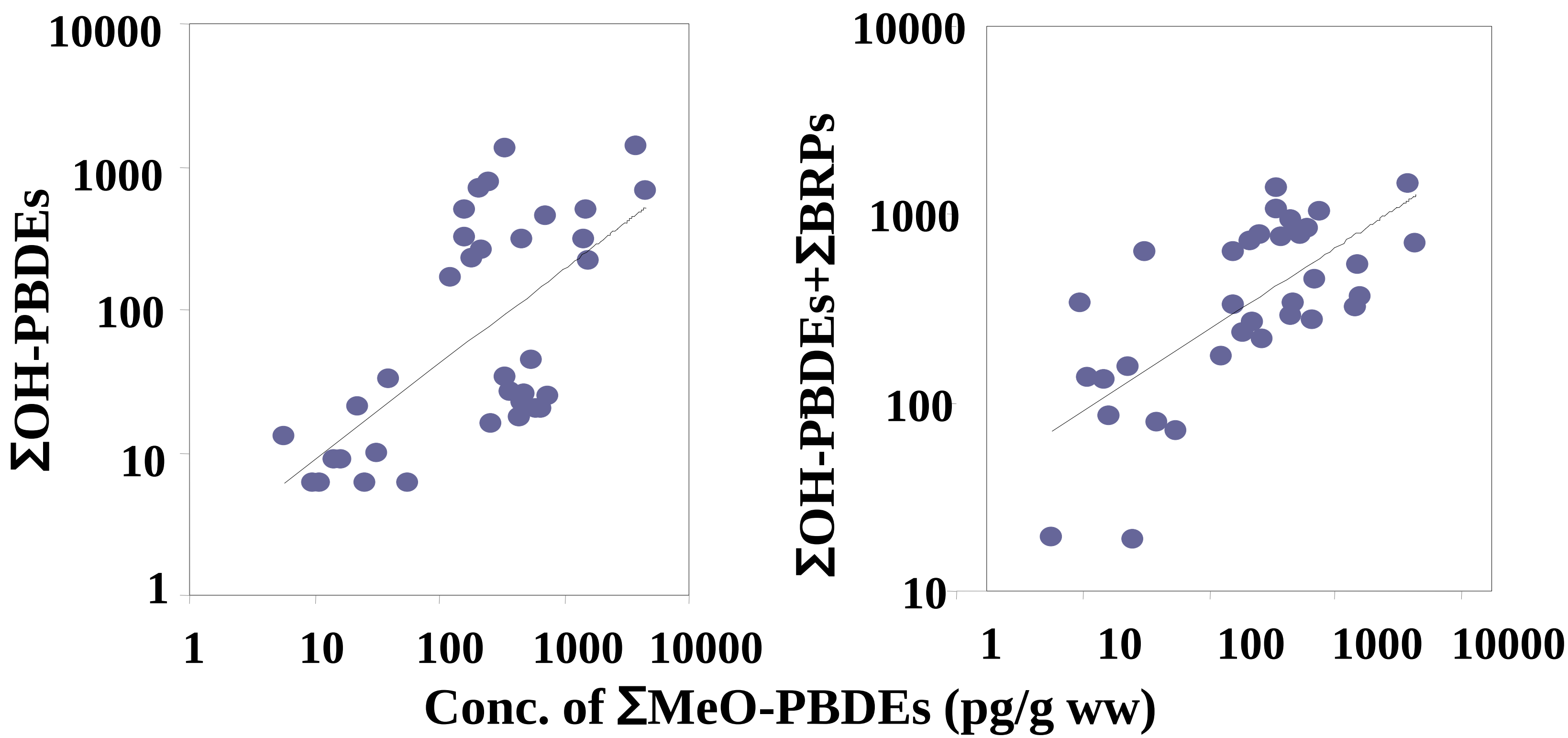


Observations

Concentration of ΣPBDEs not related to those of ΣOH-PBDEs

Possible relationships between MeO-PBDEs and OH-PBDEs

Correlations between MeO-PBDEs, OH-PBDEs and BRPs



No significant correlation between ΣPBDEs and ΣOH-PBDEs

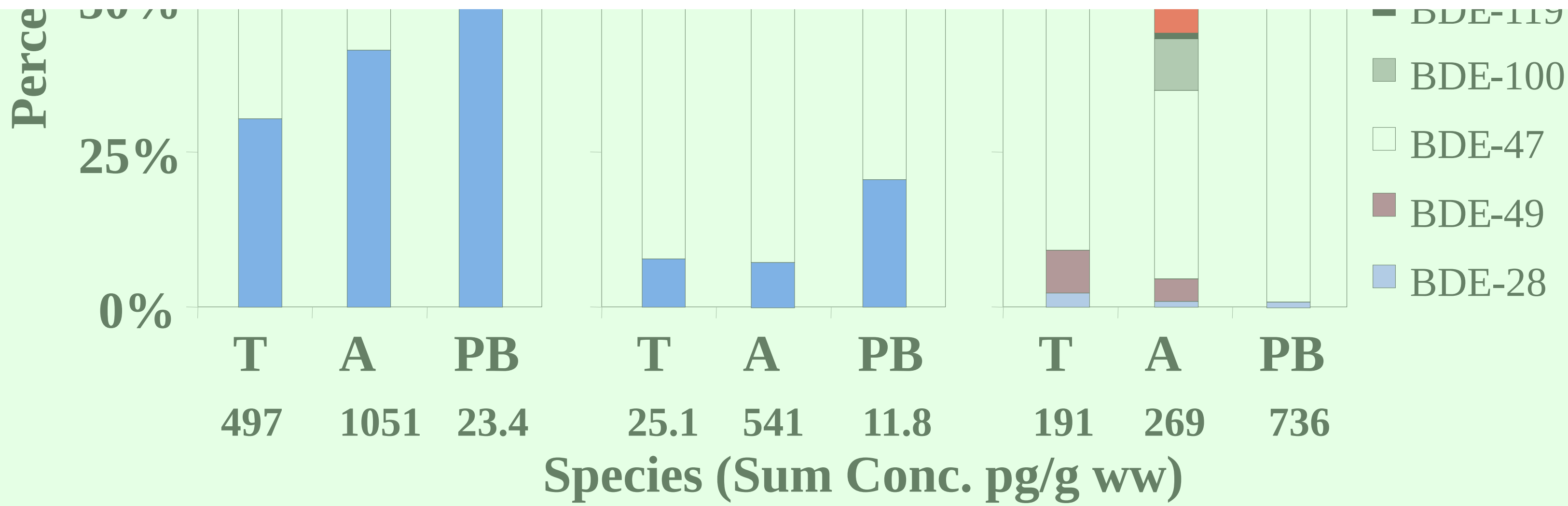
Significant correlation between ΣMeO-PBDEs and ΣOH-PBDEs

More significant correlation between ΣMeO-PBDEs and ΣOH-PBDEs+ΣBRPs

Profiles

Variations in patterns among species similar for MeO and OH-PBDEs.

Significant correlations for compounds with similar structures consistent with relationship between OH-PBDEs and MeO-PBDEs.



Experimental Goals -2

- **Investigate *in vitro* biotransformation of PBDEs, MeO-PBDEs, and OH-PBDEs in hepatic microsomes**

Wan, Y., S. Wiseman, H. Chang, X. Zhang, P.D. Jones, M, Hecker, K. Kannan, S. Tanabe, J. Hu, M.H.W. Lam, and J.P. Giesy. 2009. Origin of Hydroxylated Brominated Dophenyl Ethers: Natural Compounds of Man-Made Flame Retardants. *Environ. Sci. Technol.* 43:7536-7542. (DOI:10.1021/es901357u).

- **Investigate *in vivo* biotransformation of PBDEs, MeO-PBDEs, and OH-PBDEs in Japanese Medaka**
    - **Biotransformation of each compound**
    - **Gain insight into source(s) of each compound**

Wan Y., Liu F.Y., Wiseman S., Zhang X.W., Chang H., Hecker M., Jones P.D., Lam M.H.W., Giesy J.P., Toxic Hydroxylated PBDEs: New Evidence for Natural Origins. PNAS, Submitted

Purity Tests

		6-OH-BDE-47	6-MeO-BDE-47	BDE-47
Spiked foods	Control	N.D.	0.1	N.D.
	6-OH-BDE-47	900	0.2	1.5
	6-MeO-BDE-47	N.D.	8,000	28.3
	BDE-47	N.D.	0.2	21,000
Stock standard solutions	6-OH-BDE-47	1500,000	4.3	1,900
	6-MeO-BDE-47	N.D.	1300,000	4,800
	BDE-47	N.D.	N.D.	50,000

N.D.: not detected.

Presence of none of the contaminants in stock solutions affected conclusions drawn from the studies

In vitro metabolism of PBDEs, MeO-PBDEs and OH-PBDEs

- **Microsomes**
  - Rainbow trout, chicken, and rat
- **Exposed groups**
  - BDE-99
  - PBDE mix: ***BDE-28, BDE-49, BDE-47, BDE-66, BDE-100, BDE-119, BDE-99, BDE-85, BDE-154, BDE-153, and BDE-183***
  - 6-MeO-BDE-47
  - MeO-PBDE mix: ***2'-MeO-BDE-68, 6-MeO-BDE-47, 5-MeO-BDE-47, 4'-MeO-BDE-49, 5'-MeO-BDE-100, 4'-MeO-BDE-103, 4'-MeO-BDE-99, and 4'-MeO-BDE-101***
  - 6-OH-BDE-47
  - OH-PBDE mix: ***OH-BDE-47, 4'-OH-BDE-49, 6-OH-BDE-90 and 2-OH-BDE123***

Microsomal Incubations

Liver tissue

↓ Homogenized with cold phosphate buffer

Centrifuged for 15 min at 9000 g

↓ Take the supernatant

Centrifuged for 60 min at 100,000 g

↓ Dissolve in phosphate buffer

Microsomes

↓

Incubation with target compounds

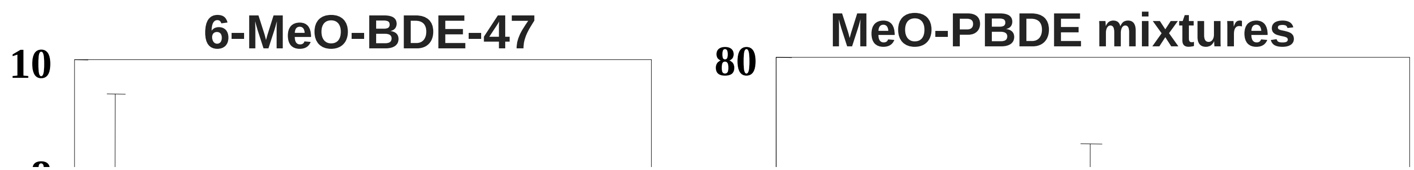
Chicken

Rat

Rainbow trout

Percentage of OH-PBDEs and BRPs in PBDE and MeO-PBDE exposed microsomes

- OH-PBDEs and BRPs not detected in PBDE exposures



Observations

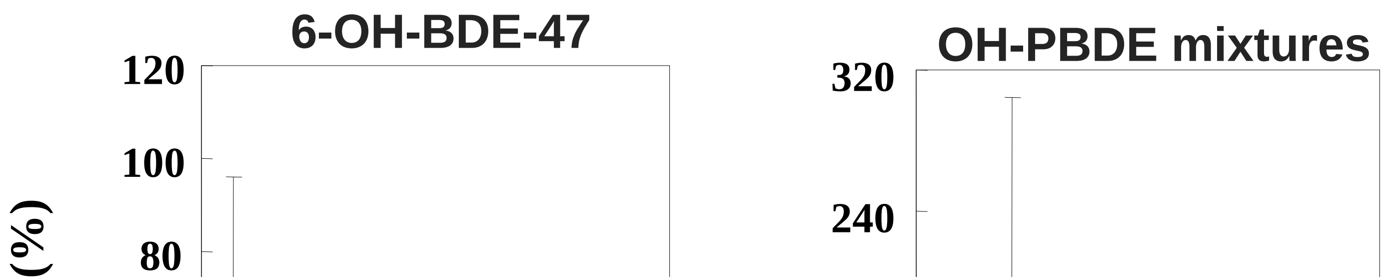
No OH-BDEs formed from PBDE!

Significant amounts of 6-OH-BDE-47 were generated from 6-MeO-BDE-47, and more OH-PBDE congeners were detected when additional MeO-PBDE congeners were incubated with microsomes.

Demonstrates biotransformation of MeO-PBDEs to OH-PBDEs at environmentally relevant concentrations.

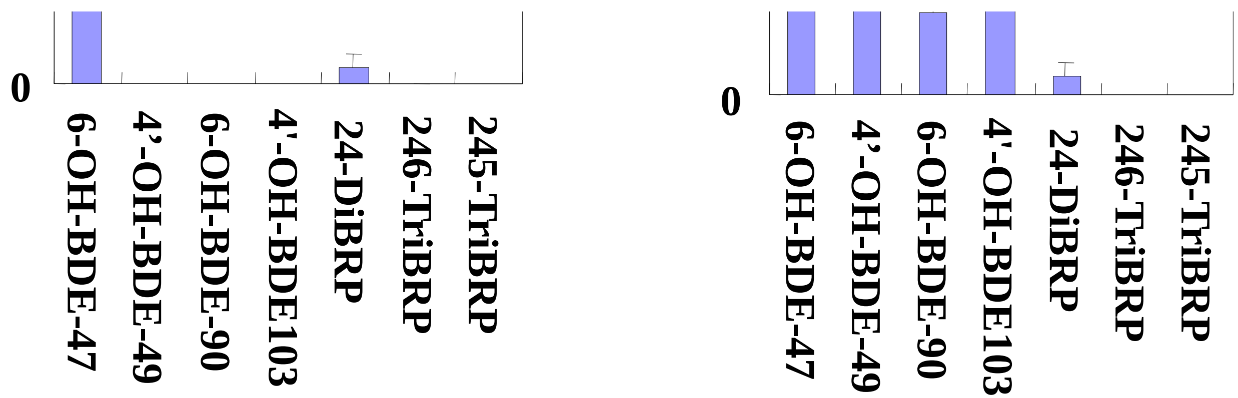
Percentage of OH-PBDE and BRP in OH-PBDEs exposed microsomes

- MeO-PBDEs were not detected in OH-PBDE exposed microsomes



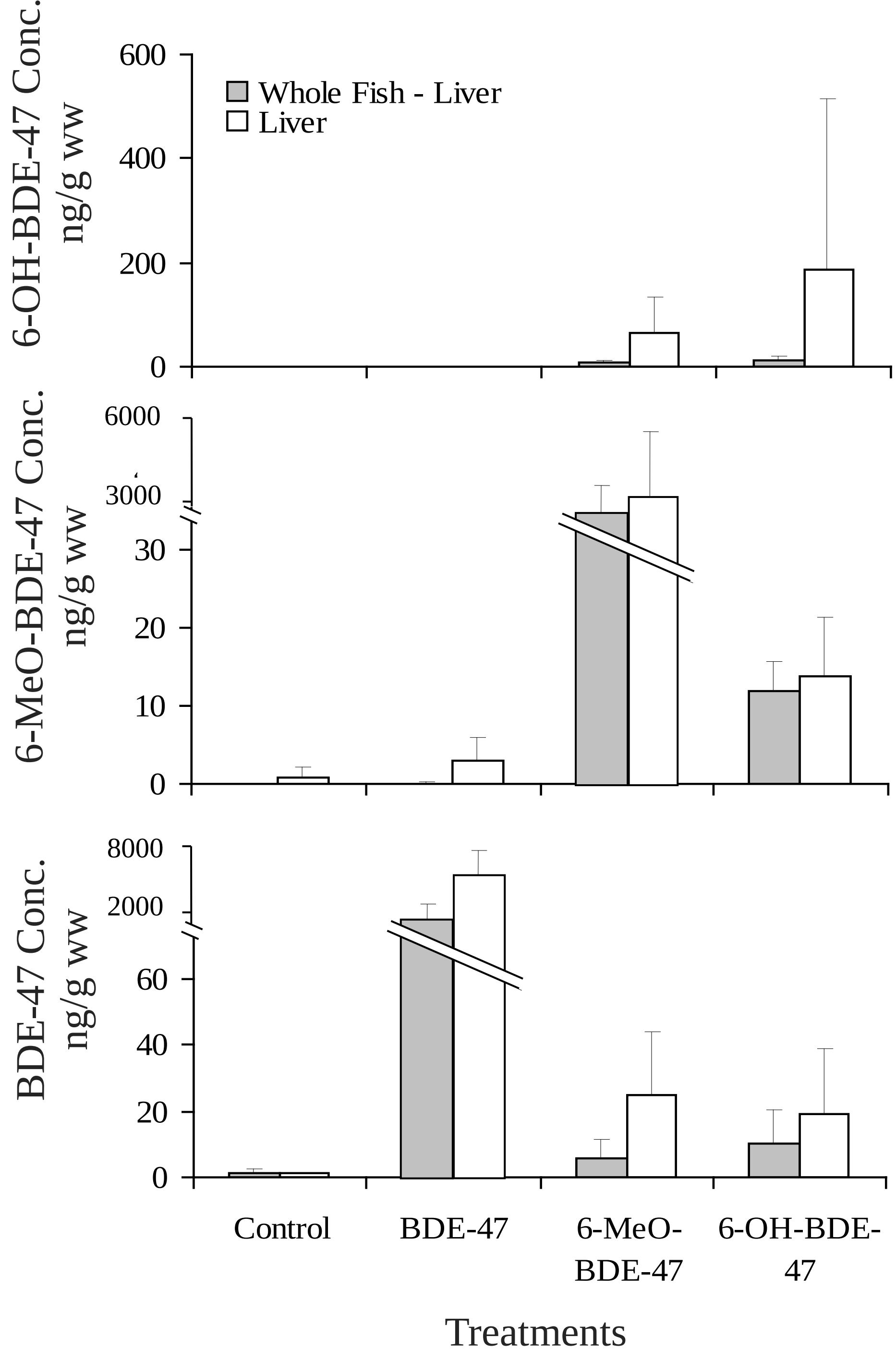
2,4-DiBRP was the major BRP congener of OH-PBDE metabolism

Concentrations of 4'-OH-BDE-49 were greater than the original exposure concentrations, suggesting the debromination of OH-PentaBDE congeners



In vivo biostransformtion of PBDEs, MeO-PBDEs and OH-PBDEs in Japanese Medaka

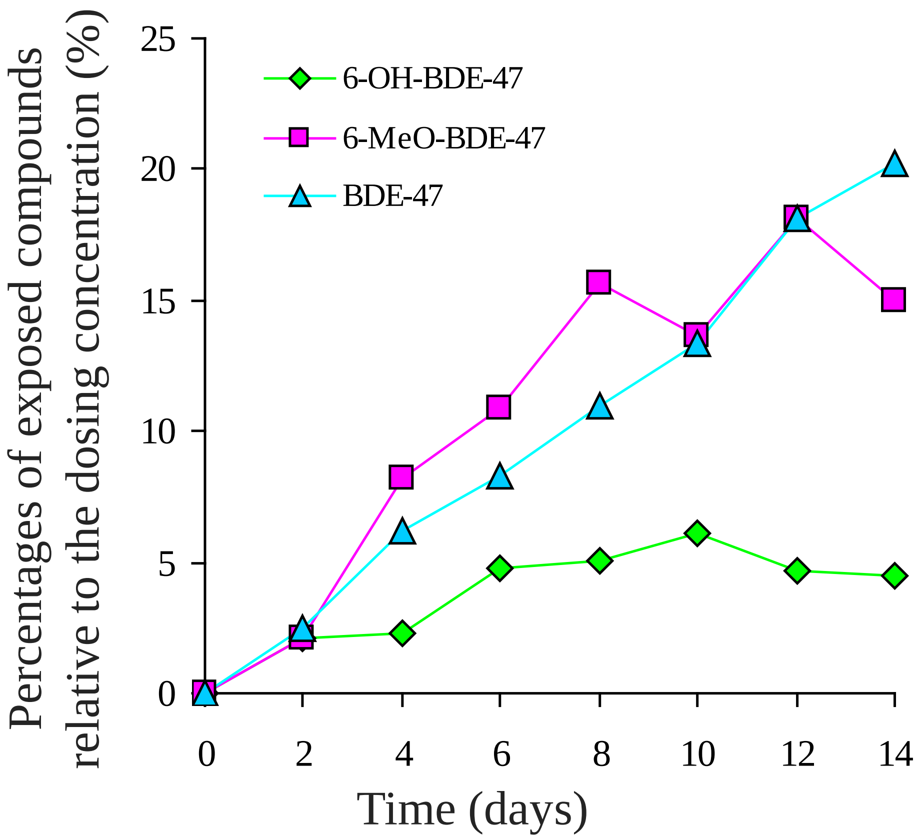
- **Fish**
  - **Freshwater Japanese Medaka (***Oryzias latipes***)**
- **Exposed groups**
  - **Control**
  - **BDE-47:**
  - **6-MeO-BDE-47**
  - **6-OH-BDE-47**
- **Exposure duration**
  - **Exposure via food for 2 weeks**



Concentrations of Target Compounds in Exposed Medaka

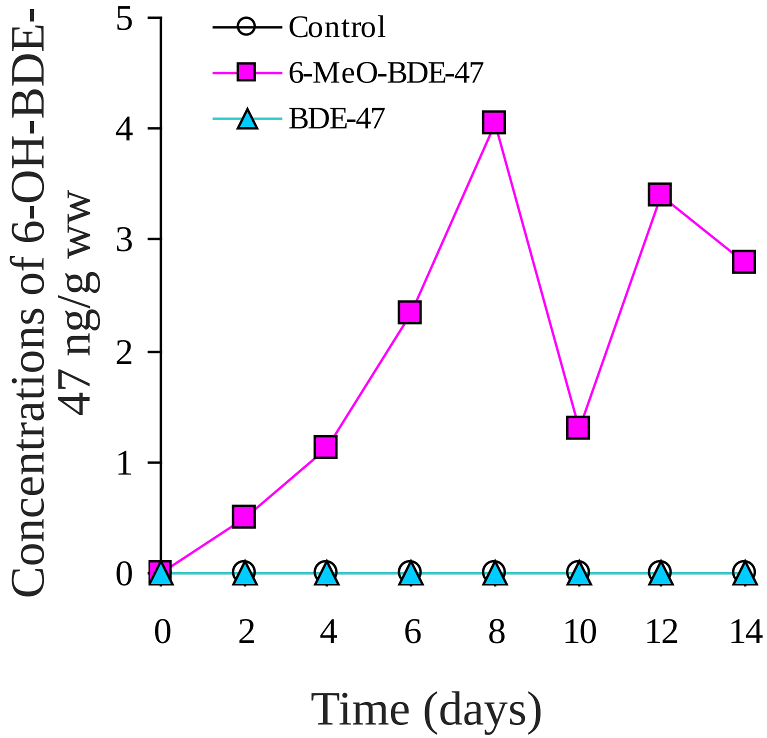
- Significant concentrations of 6-OH-BDE-47 were detected in medaka exposed to 6-MeO-BDE-47, but not BDE-47
- 6-MeO-BDE-47 was formed from 6-OH-BDE-47 in medaka
- BDE-47 observed in medaka exposed to 6-MeO-BDE-47 and 6-OH-BDE-47 is likely due to BDE-47 impurities in the stock standard solutions.

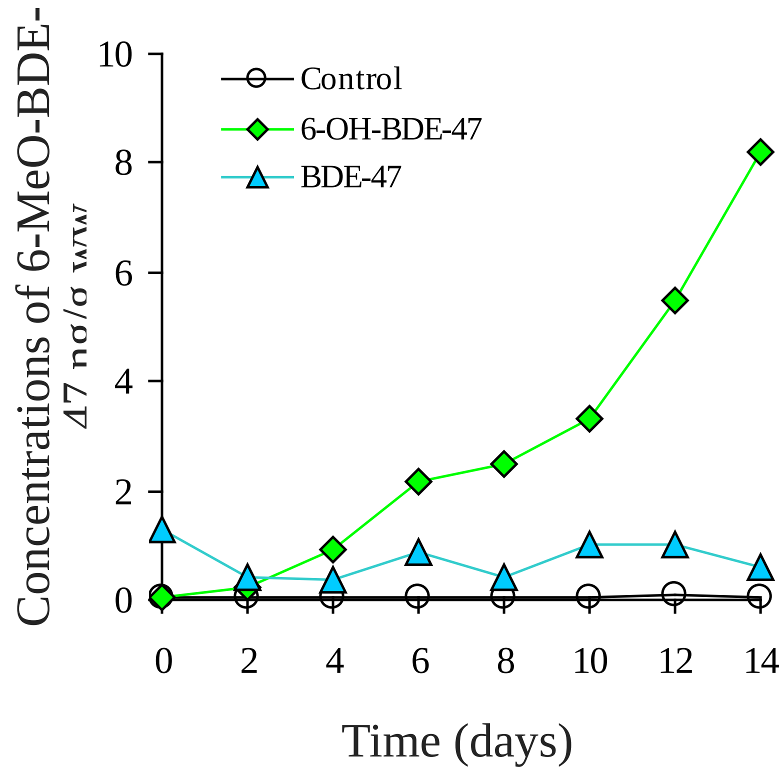
Accumulation in Eggs



- **Significant assimilation efficiencies were observed for 6-MeO-BDE-47 and BDE-47 compared to 6-OH-BDE-47 as indicated by the steep slopes for accumulation.**
- **Depuration rate of BDE-47 is likely less than that of 6-MeO-BDE-47 based on the slow assimilation rate and large concentration ratios between fish and their diet.**

Biotransformation Products in Eggs

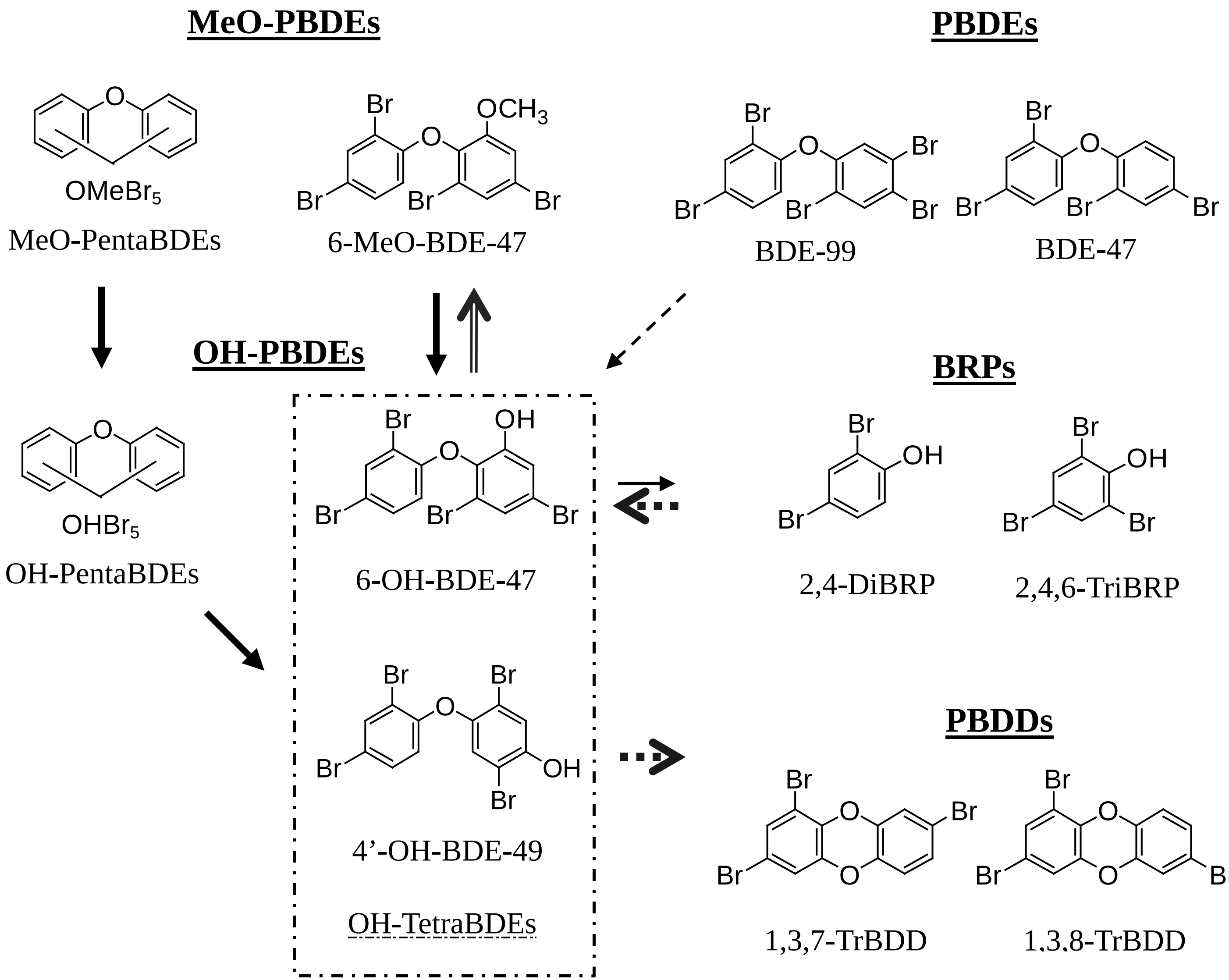




Direct *in vivo* evidence of biotransformation of 6-MeO-BDE-47 to 6-OH-BDE-47

Biotransformation of 6-OH-BDE-47 to 6-MeO-BDE-47 did not occur in hepatic microsomal fraction

Proposed metabolic relationships among brominated compounds



Summary

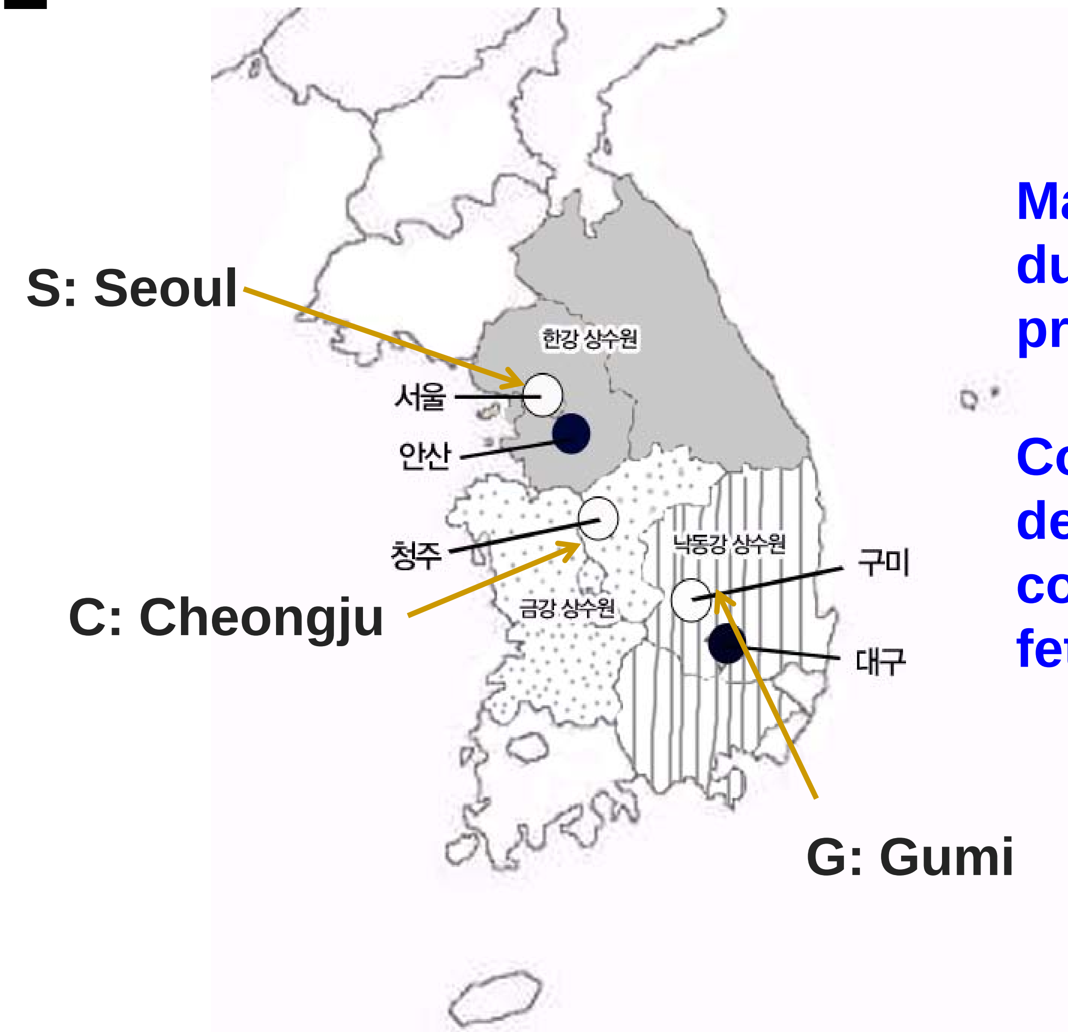
- Hydroxylation of synthetic PBDEs to OH-PBDEs was negligible
- Biotransformation of 6-OH-BDE-47 to 6-MeO-BDE-47 did not occur in the hepatic microsomal fraction
- Significant production of OH-PBDEs from biotransformation MeO-PBDEs
- MeO-PentaBDE congeners could be an important contributor of *para*-substituted OH-PBDEs
- Human exposure to MeO-PBDEs that occur naturally in marine organisms should be considered

Toxic Hydroxylated Polybrominated Diphenyl Ethers in Pregnant Women and Their Matching Fetuses

Background

- OH-PBDEs have various biological effects including disruption of thyroid hormone homeostasis, disruption of sex hormone steroidogenesis, and neurotoxicity.
- MeO-PBDEs, as a precursor of OH-PBDEs, generally accumulated to large concentrations in marine organisms.
- Pregnant women might take nutritional supplements, such as fish oil which can contain very great concentrations of MeO-PBDEs
- People living close to the ocean may hade greater concentrations OH-PBDEs, and their fetuses may be at risks due to exposure to these compounds ?

Area of populations



Maternal blood was drawn during the third trimester of pregnancy

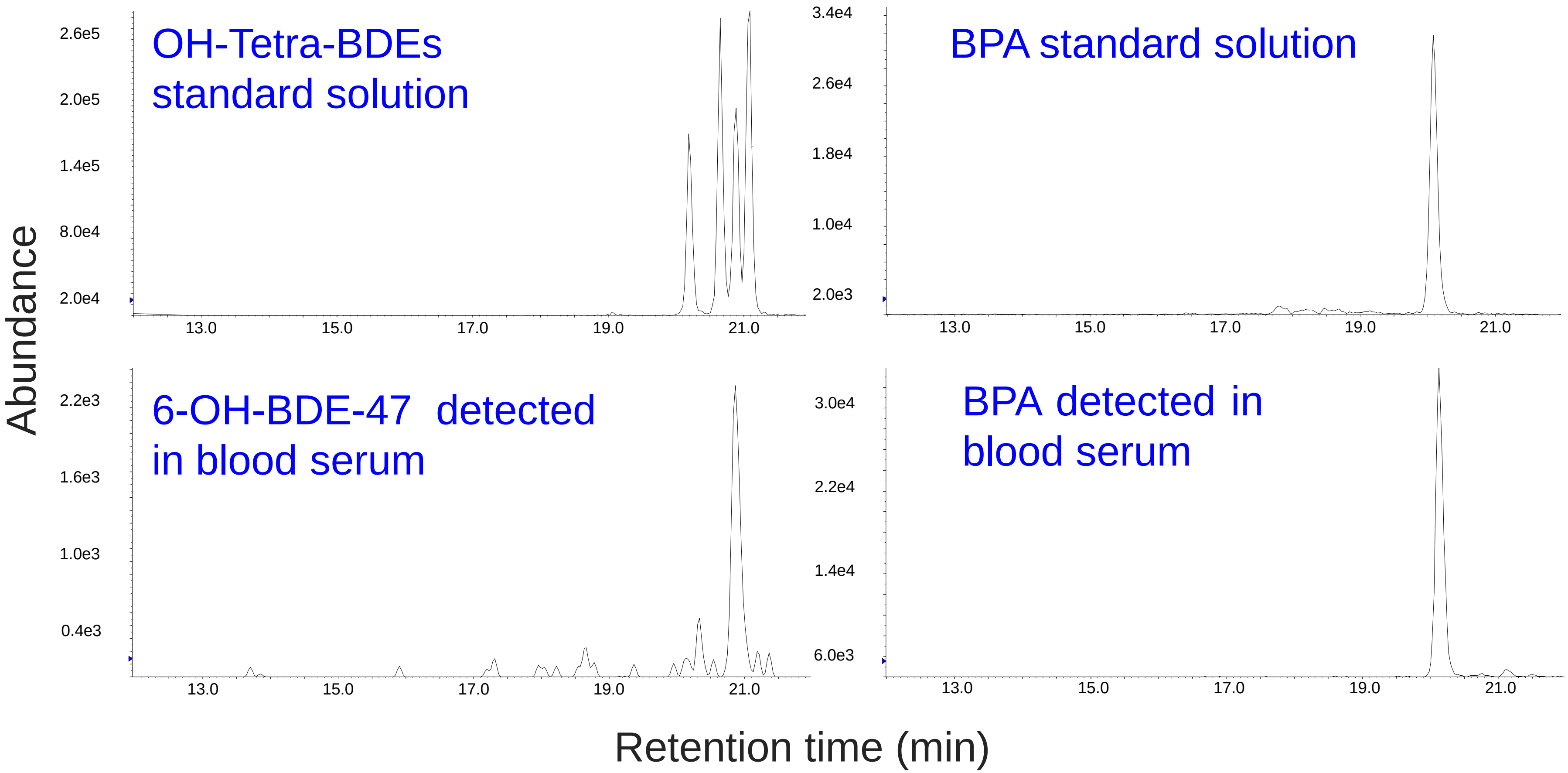
Cord blood was drawn at delivery from the umbilical cord vein of the matching fetuses

Characteristics of mothers and infants

Variable	N	Range	Mean	SD	Median
Pregnant women (n=26)					
Age (year)	26	22-39	31	4.7	31
Pre-pregnancy weight (kg)	24	45.0-80.0	55.8	9.8	50.5
Height (cm)	24	148.0-171.0	161.0	5.1	161.0
BMI (kg/m^2)	24	17.4-31.0	21.6	4.2	20.0
Parity	24	1-3	2	0.7	1
Gestational age at delivery (weeks)	24	36-41	39	1.3	39
Gestational age at blood sampling (wk)	21	20-40	36	5.1	37
Infants (n=28)*					
Sex	28	Male:13, Female:15			
Birth weight (kg)	26	2.22-4.10	3.11	0.46	3.15

* Including 3 twins. One cord blood sample was missing from one of one twin.

LC-MS/MS chromatographic profiles of OH-Tetra-BDEs and BPA



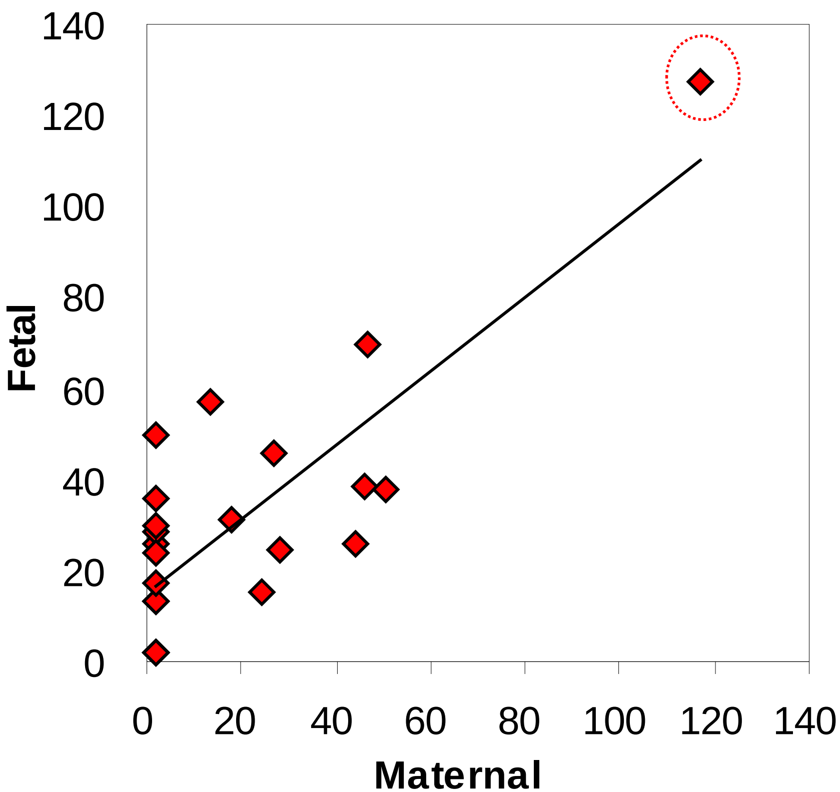
Concentrations of 6-OH-BDE47 in people worldwide

	6-OH-BDE-47					Region	Ref
	n	n>LOD	Mean ± SD	Range	Median		
	25	20	30.2±27.1	<4-127	26	2008-2009, Korea	This study
Fetal	16	16	44.6[a]	-	4.5	2003-2004, USA	Qiu et al. 2009
	6	4	1.4±2.0	<0.6-5.2	0.6	2005-2006, Japan	Kawashiro et al. 2008
	4	4	7.4±4.1	4.1-12.9	6.3	2002, Nicaragua	Athanasiadou et al. 2008
Children	10	10	8.9±8.7	1.7-25.7	6.8	2002, Nicaragua	Athanasiadou et al. 2008

OH-PBDEs in Korean pregnant women originating primarily from natural sources (marine food)

Pregnant South Korean women are exposed to relatively great concentrations of OH-PBDEs compared with people in other geographical regions

Placental transfer of 6-OH-BDE-47



Y=15.02 + 0.81 X (r=0.625, *p*=0.001).
when the circled outlier was removed,
Y=17.94 + 0.55 X (r=0.567, *p*=0.005).

The placental transfer ratio between fetal and maternal serum (F/M ratio) was 1.4±1.1 for 6-OH-BDE-47

The ratios were greater than that of BPA (<1)

Due to high affinities to TTR?

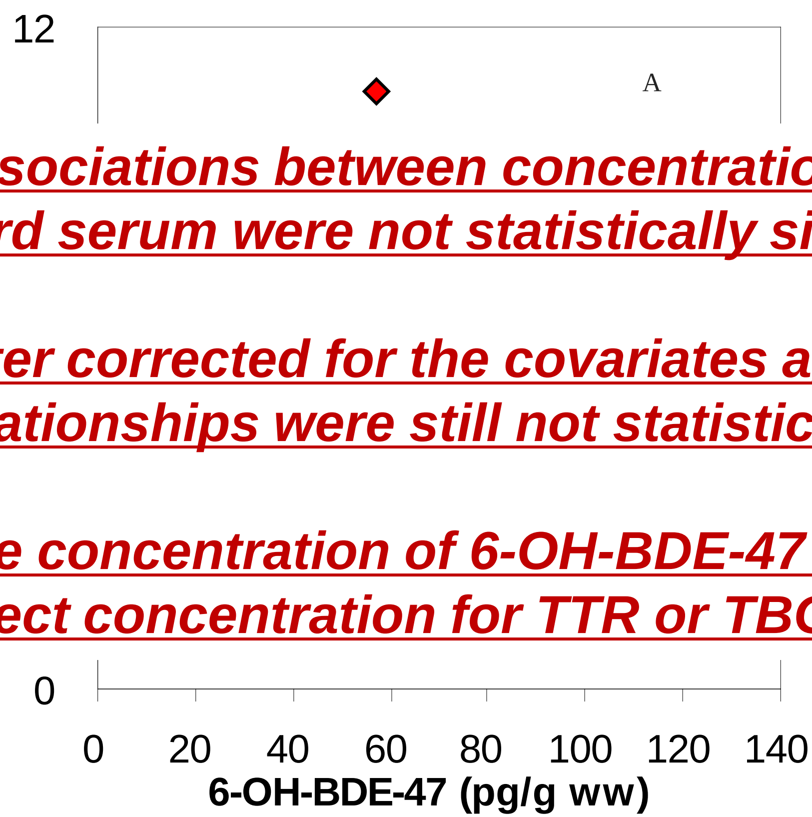
The ratios were greater than that of OH-PCBs (Netherlands: 0.6-0.7, and Japan: 0.1-0.9).

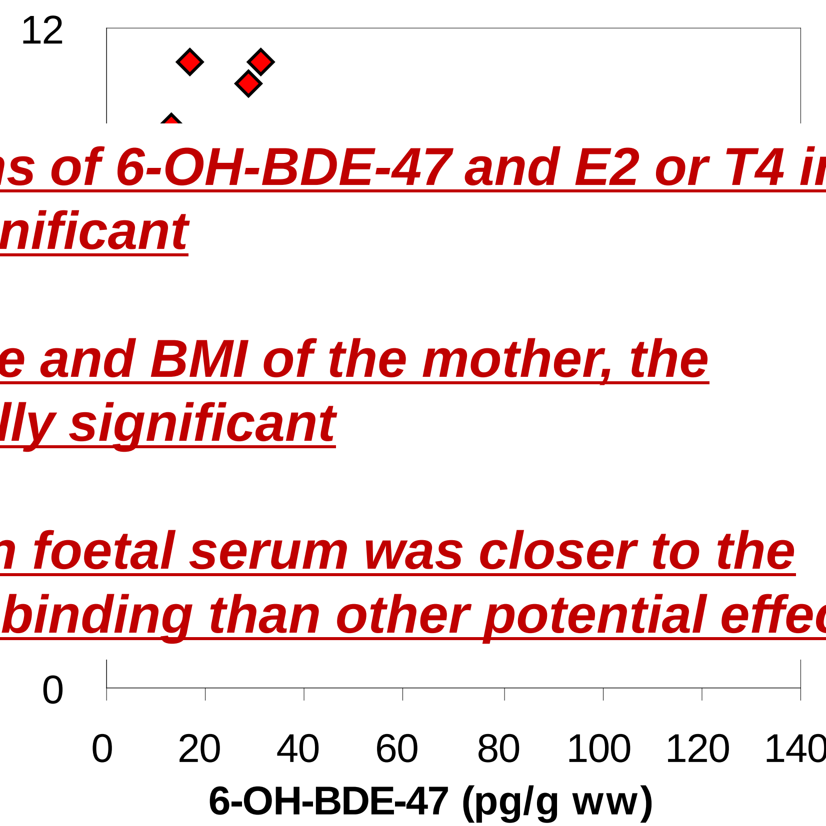
Due to high affinities to TBG?

Potential effects

- The mean concentration of 6-OH-BDE-47 detected in fetal serum was 30.2 ± 27.1 pg/g ww, or 0.06 nM, while the maximum detected concentration was 127 pg/g ww or 0.25 nM.
- The median inhibitory concentrations (IC50s) of r 6-OH-BDE-47 were 22.3-107.8 nM for TTR, and 100-867 nM for TBG in *in vitro* studies of human cells.
- Concentrations of OH-PBDEs of 100-1000 nM cause estrogenic activities, concentrations of 1000-5000 nM can cause neurotoxic effects, and concentrations of 5000-10000 nM can inhibit human placental aromatase activity.
- Thyroid and estrogen homone effects??

6-OH-BDE-47 vs E2 and T4 in Fetal Serum





Associations between concentrations of 6-OH-BDE-47 and E2 or T4 in cord serum were not statistically significant

After corrected for the covariates age and BMI of the mother, the relationships were still not statistically significant

The concentration of 6-OH-BDE-47 in foetal serum was closer to the effect concentration for TTR or TBG binding than other potential effects

Summary

- Only 6-OH-BDE-47, a naturally occurring OH-PBDE, was detected, and the exposure was related to diets of Korean women
- The placental transfer ratio between foetal and maternal blood serum for 6-OH-BDE-47 (F/M ratio: 1.4 ± 1.1).
- The F/M ratio of 6-OH-BDE-47 was different than those of BPA and OH-PCBs, possibly due to large affinities to T4 transport proteins.
- A major effect of OH-PBDE exposure might be a decrease in serum T4 concentrations.
- Potential risks associated with disruption of T4 transport to the developing foetus (e.g negative consequences for fetal neurological development) should be considered in further studies.

Thank You!!!!! Questions????

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Related Publications

- Wan Y., Wiseman S., Chang H., Zhang X.W., Jones P.D., Hecker M., Kannan K., Tanabe S., Hu J.Y., Lam M.H.W., Giesy J.P. Origin of hydroxylated brominated diphenyl ethers: natural compounds or man-made flame retardants? ***Environmental Science & Technology*** 43, 7536-7542, 2009.
- Wan Y., Choi K., Kim S., Ji K., Chang H., Wiseman S., Jones P.D., Khim J., Park S., Park J., Giesy J.P. Hydroxylated polybrominated diphenyl ethers and bisphenol A in pregnant women and their matching fetuses: placental transfer and potential risks. ***Environmental Science & Technology***, In press.
- Chang H., Wan Y., Naile J., Zhang X.W., Wiseman S., Hecker M., Lam M.H.W., Giesy J.P., Jones P.D. Simultaneous quantification of multiple classes of phenolic compounds in blood plasma by liquid chromatography-electrospray tandem mass spectrometry. ***Journal of Chromatography A***, 1217, 506-513, 2010.
- Wan Y., Jones P.D., Wiseman S., Chang H., Chorney D., Kannan K., Khim J.S., Tanabe S., Lam M.H.W., Giesy J.P., Contribution of Anthropogenic and Naturally Occurring Organobromine Compounds to Bromine Mass in Marine Organisms ***Environmental Science & Technology***, Submitted.
- Wan Y., Liu F.Y., Wiseman S., Zhang X.W., Chang H., Hecker M., Jones P.D., Lam M.H.W., Giesy J.P., Toxic Hydroxylated PBDEs: New Evidence for Natural Origins. ***PNAS***, Submitted.