



OH-PBDEs and MeO-PBDEs: Methods, Sources and Consequences

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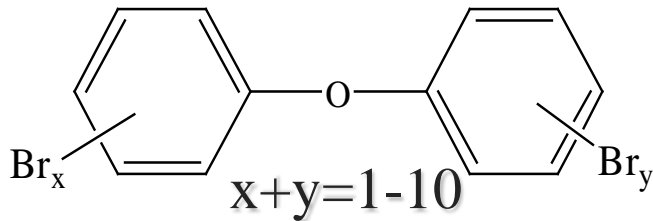


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Visiting Professor Program

[PBDEs and Structurally Related Compounds]

■ PBDEs

- **Synthetic flame retardants**
- **Ubiquitous environmental distribution**
- **Neurotoxins**
- **Endocrine disruption**
- **Moderately toxic at high concentrations**



E-Waste handling



[PBDEs and Structurally Related Compounds]

■ Hydroxylated PBDEs

○ Natural Products

■ 2-OH-PBDE-47

■ 6-OH-PBDE-47

○ Metabolite of PBDEs

■ Variety of Effects

○ Endocrine disruption

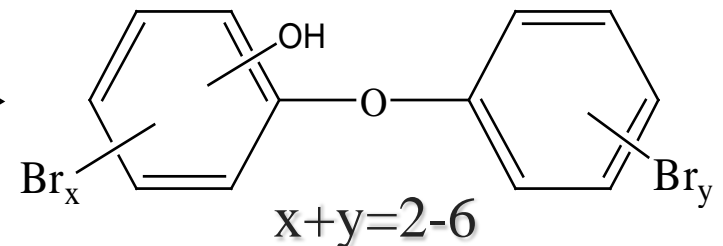
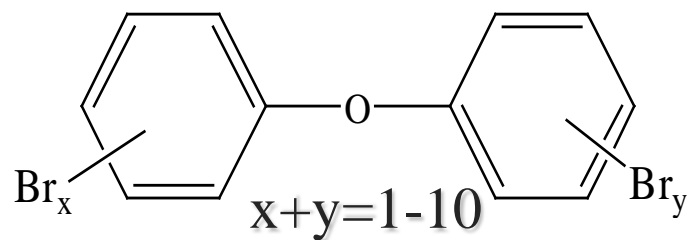
○ Disruption of oxidative phosphorylation

■ 6-OH-PBDE is acutely toxic to Zebrafish

Marine Sponge



Red algae

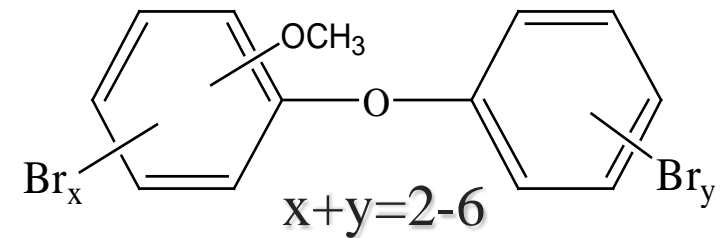
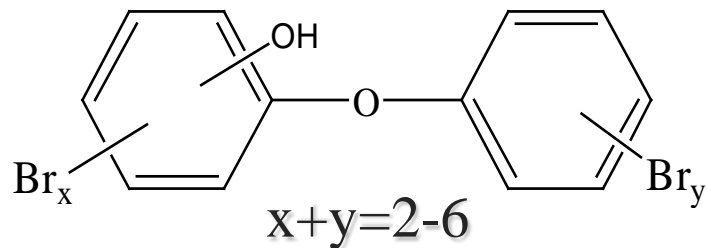


[PBDEs and Structurally Related Compounds]

■ Methoxylated PBDEs

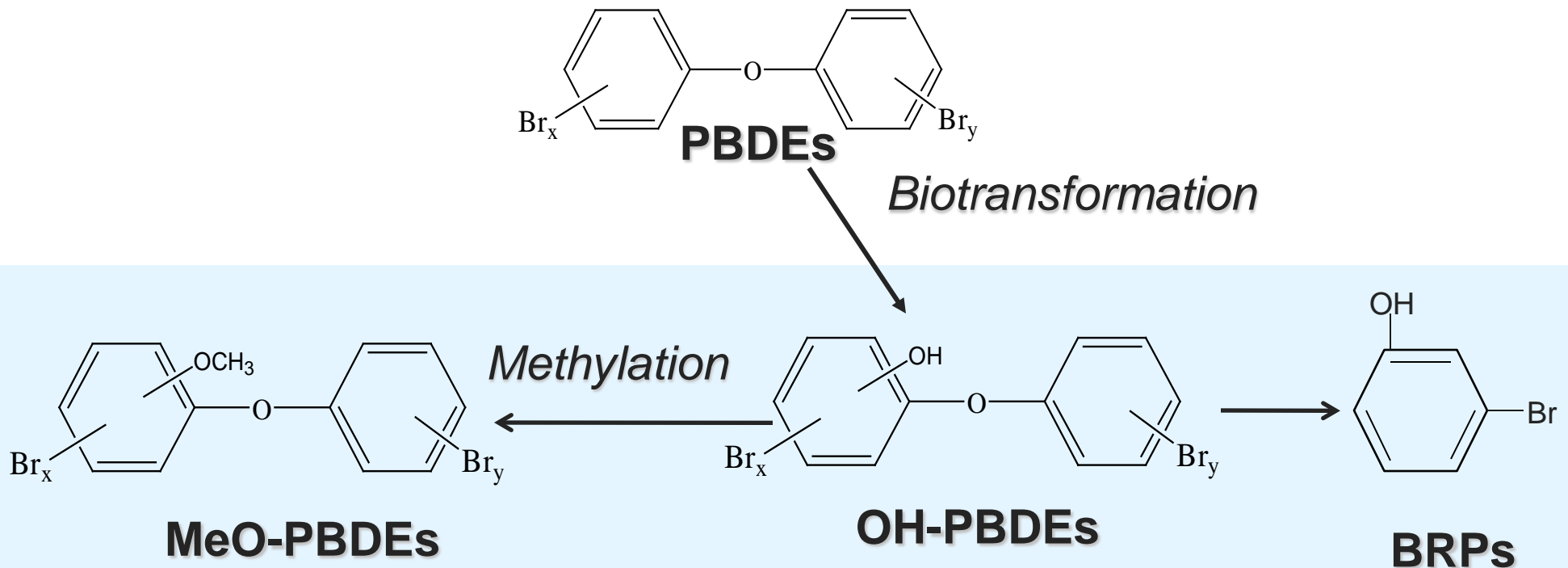
- Concentrations sometimes greater than PBDE
- Two abundant congeners are natural products
 - *2-MeO-PBDE-68*
 - *6 MeO-PBDE-47*
- Suggested that they may be form from OH-PBDE metabolism
- No reported toxicity

Fish oil



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 levels of PBDEs in *in vitro* or *in vivo* studies were high (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 high 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

- Our recent results of *in vitro* biotransformation in hepatic microsomes indicate MeO-PBDEs were the major sources of OH-PBDEs (*Environ. Sci. Technol.* 43:7536-7542)
- Current studies 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**

Analytical Method

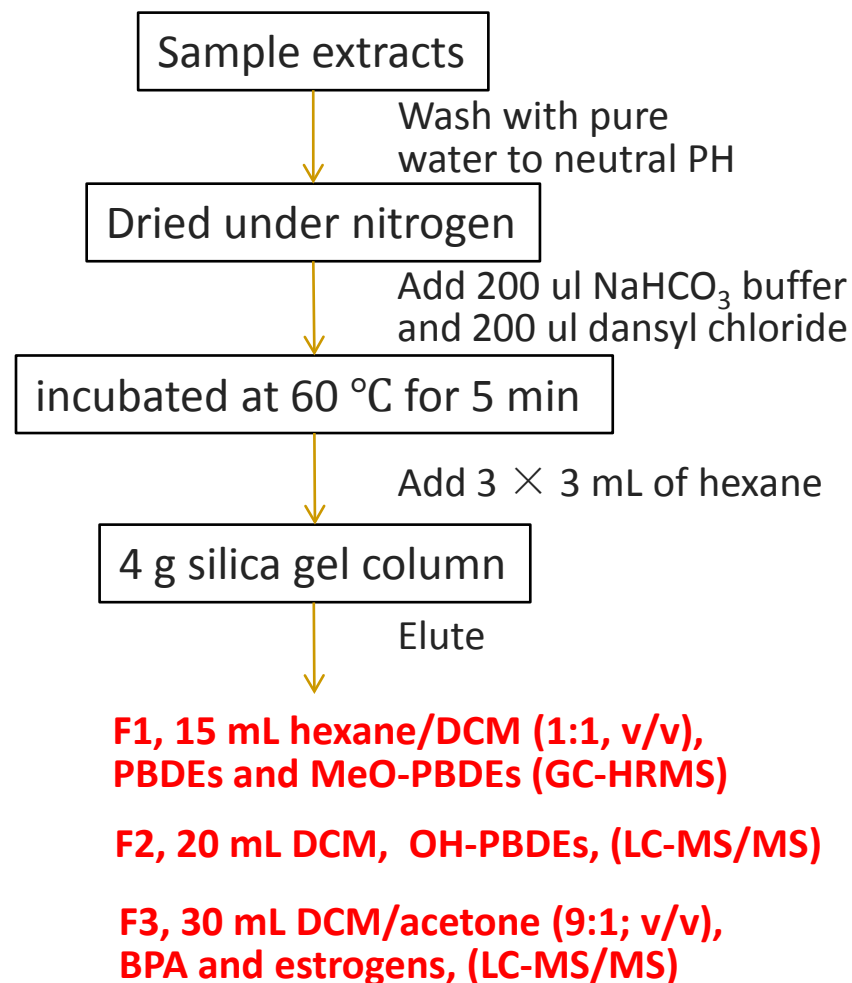
■ Target compounds

- 7 PBDEs, 12 MeO-PBDEs, 8 OH-PBDEs, BPA, 4 estrogens

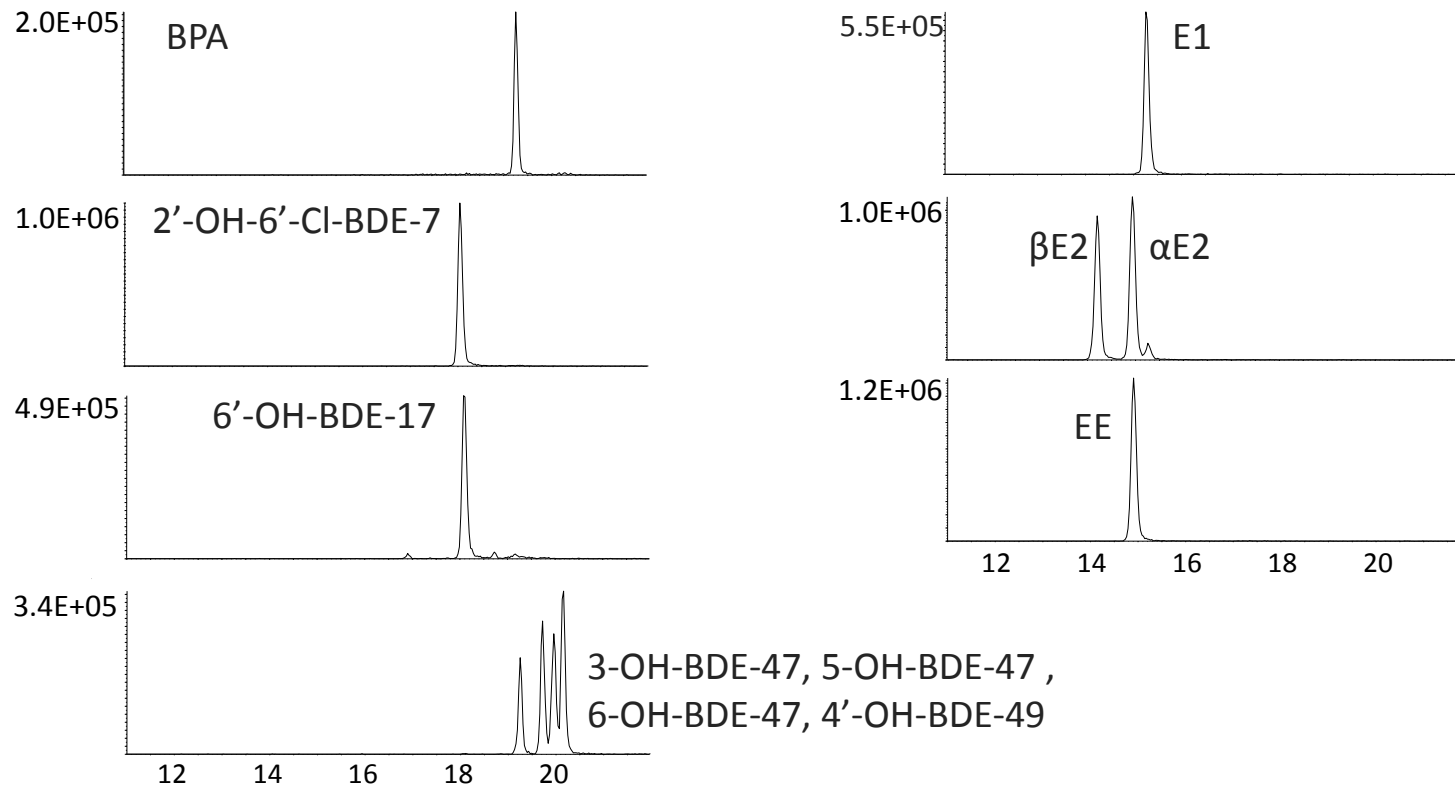
■ Derivatization

- Partition with KOH in 50% ethanol is the traditional way to separate the neutral and phenolic compounds
- Dansyl chloride was used for analysis of phenolic compounds
- neutral and phenolic compounds can be separated in silica gel column after derivatization

Journal of Chromatography A,
1217, 506-513, 2010.



Chromatographic separation of target phenolic compounds



**Separated on a 100×2.1 mm,
i.d.; Waters X-Bridge column**

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 |

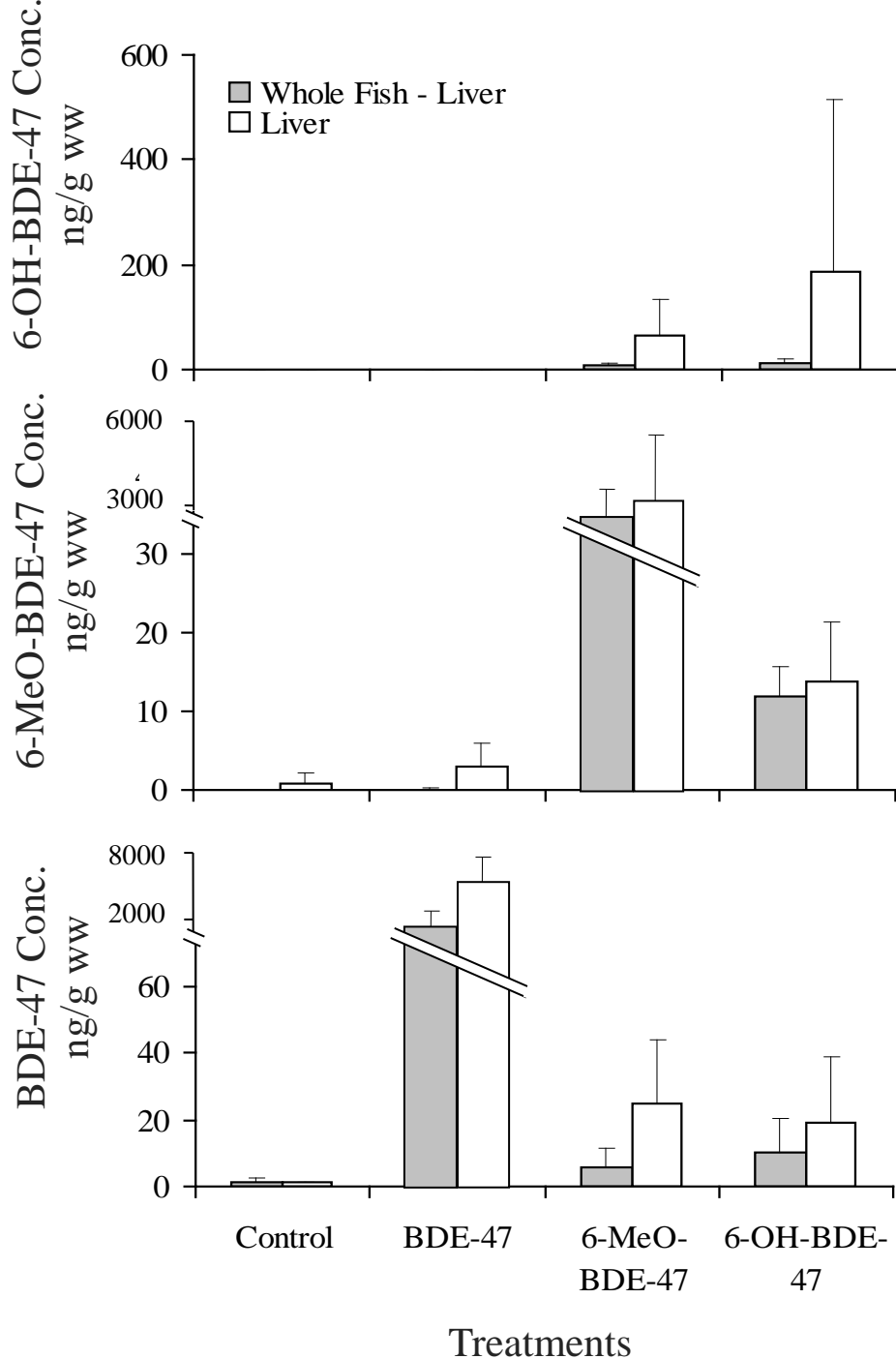
N.D.: not detected.

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

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

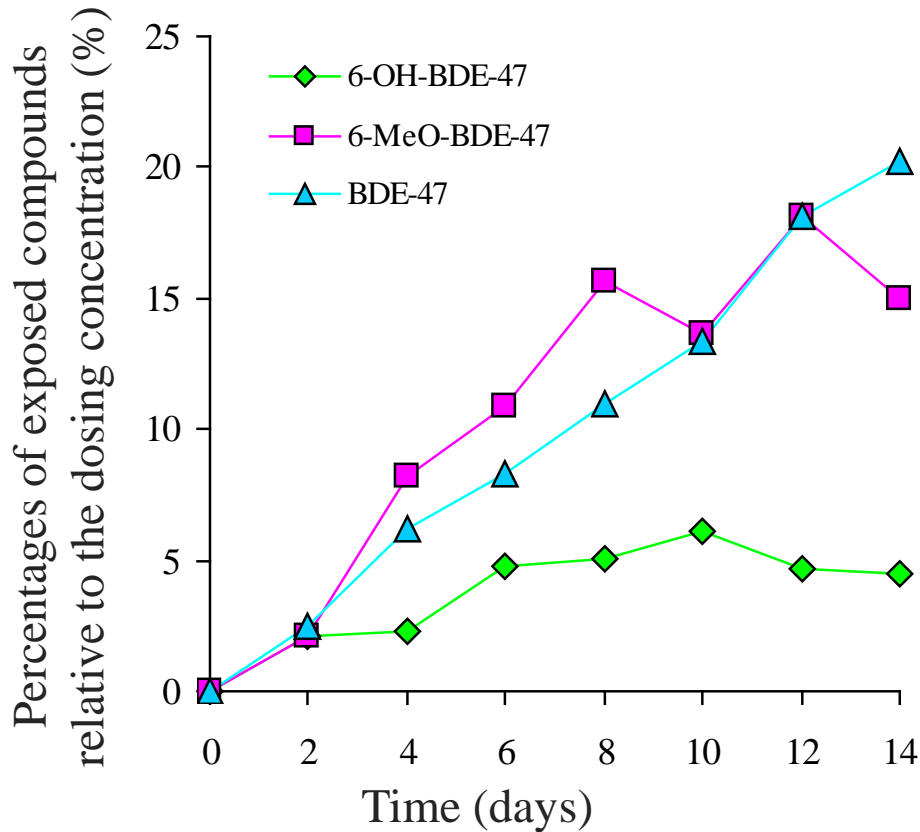
- **Fish**
 - **Freshwater Japanese Medaka (*Oryzias latipes*)**
- **Exposed groups**
 - **Control**
 - **BDE-47:**
 - **6-MeO-BDE-47**
 - **6-OH-BDE-47**
- **Exposure duration**
 - **Exposure via food for 2 weeks**

Concentrations of Target Compounds in Exposed Medaka



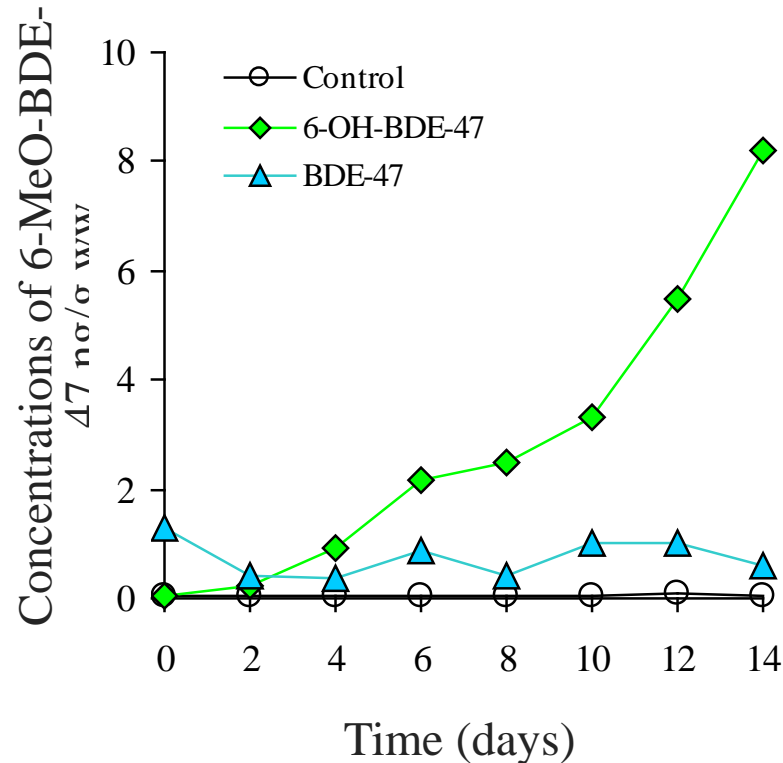
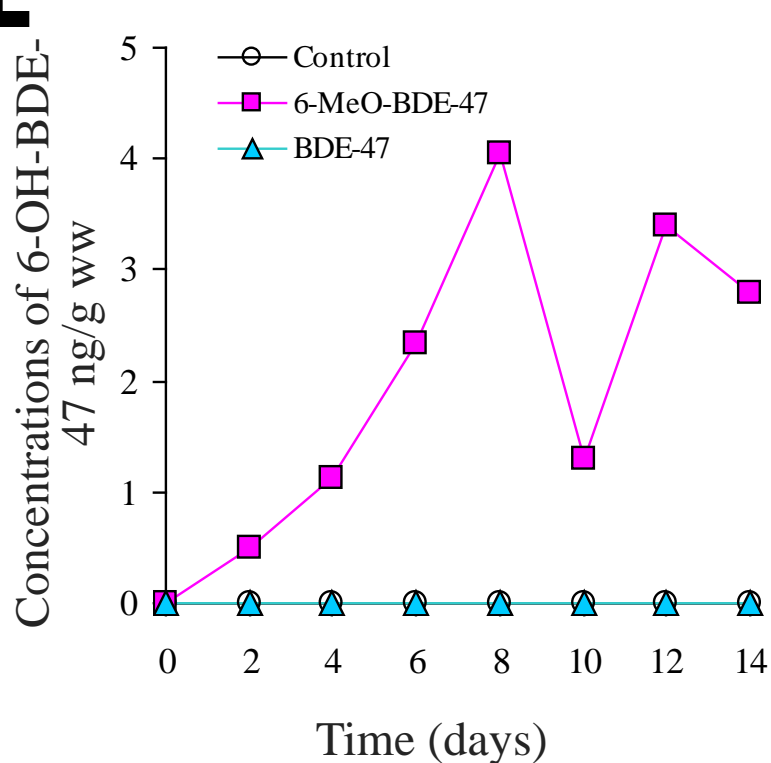
- Significant concentrations of 6-OH-BDE-47 were detected in medaka exposed to 6-MeO-BDE-47, but not BDE-47
- 6-MeO-BDE-47 was formed from 6-OH-BDE-47 in medaka
- BDE-47 observed in medaka exposed to 6-MeO-BDE-47 and 6-OH-BDE-47 is likely due to BDE-47 impurities in the stock standard solutions.

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.**

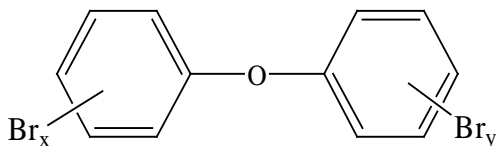
Biotransformation Products in Eggs



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

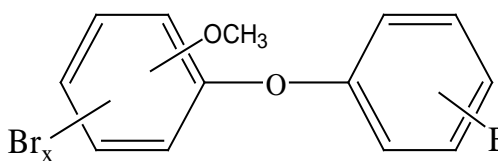
Biotransformation of 6-OH-BDE-47 to 6-MeO-BDE-47 did not occur in hepatic microsomal fraction

Proposed metabolic relationships among brominated compounds

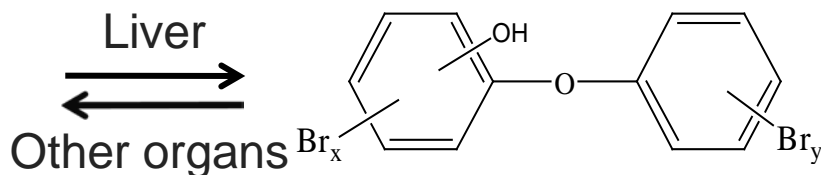


PBDEs

PBDEs are synthetic flame retardant, but OH-PBDEs in environment are not mainly from biotransformation of PBDEs



MeO-PBDEs



OH-PBDEs

Liver
Other organs

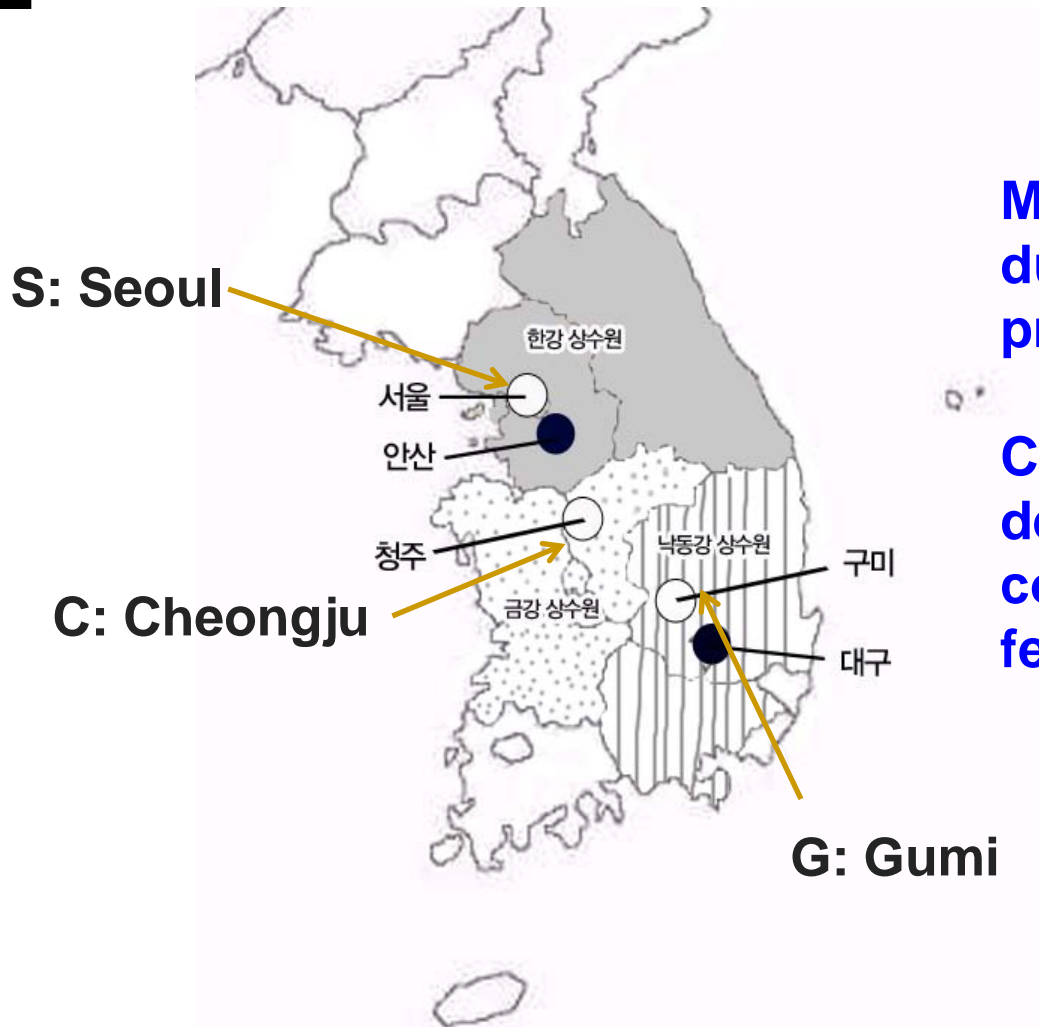
MeO-PBDEs are naturally occurring compounds
(Science 2005, 307, 917-920)

Interconversion exists between MeO-PBDEs and OH-PBDEs

Placental transfer and potential effects of OH-PBDEs

- 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 have 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

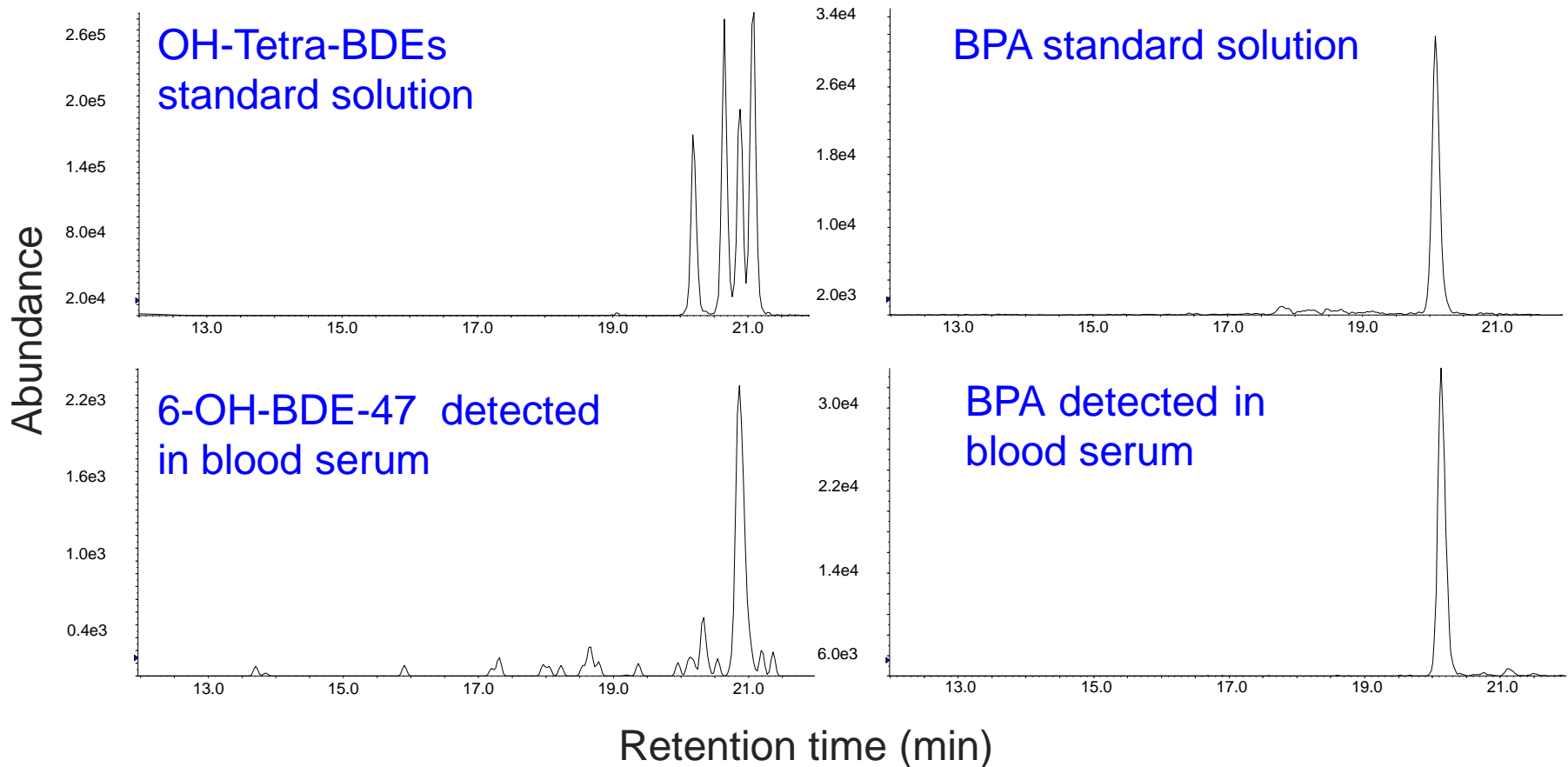
G: Gumi

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.

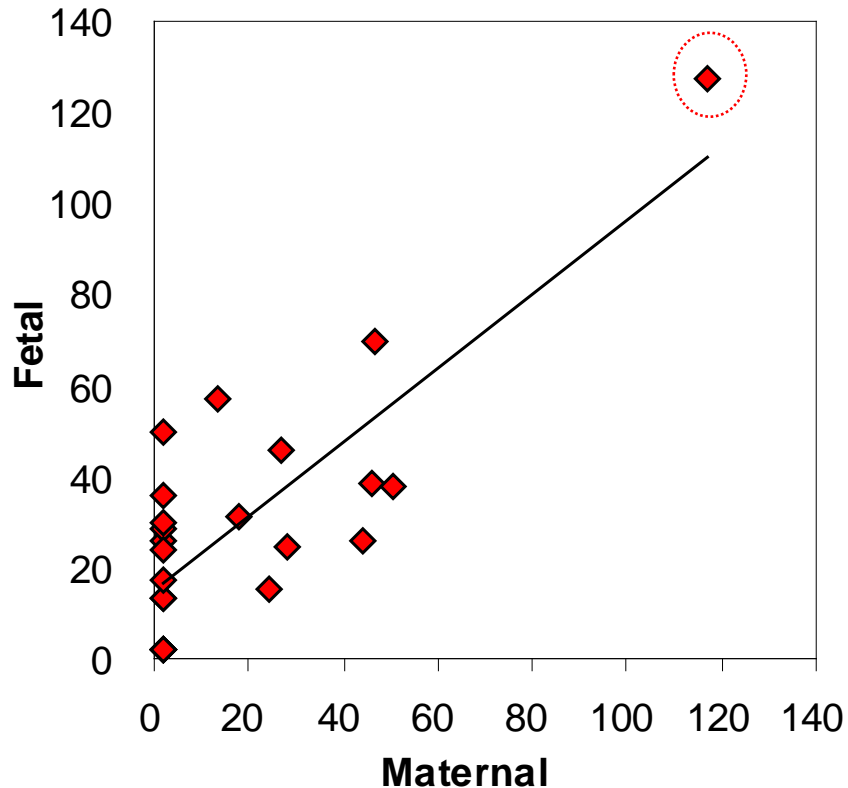
LC-MS/MS chromatographic profiles of OH-Tetra-BDEs and BPA



Concentrations of 6-OH-BDE47 in people worldwide

| | 6-OH-BDE-47 | | | | | Region | Ref |
|---|-------------|-------|-------------------|----------|--------|------------------|--------------------------|
| | n | n>LOD | Mean \pm SD | Range | Median | | |
| Fetal | 25 | 20 | 30.2 \pm 27.1 | <4-127 | 26 | 2008-2009, Korea | This study |
| | 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 |
| <p><u><i>OH-PBDEs in Korean pregnant women originating primarily from natural sources (marine food)</i></u></p> | | | | | | | |
| <p><u><i>Pregnant South Korean women are exposed to relatively great concentrations of OH-PBDEs compared with people in other geographical regions</i></u></p> | | | | | | | |
| | 4 | 4 | 7.4 \pm 4.1 | 4.1-12.9 | 6.3 | 2002, Nicaragua | Athanasiadou et al. 2008 |
| Children | 10 | 10 | 8.9 \pm 8.7 | 1.7-25.7 | 6.8 | 2002, Nicaragua | Athanasiadou et al. 2008 |

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?

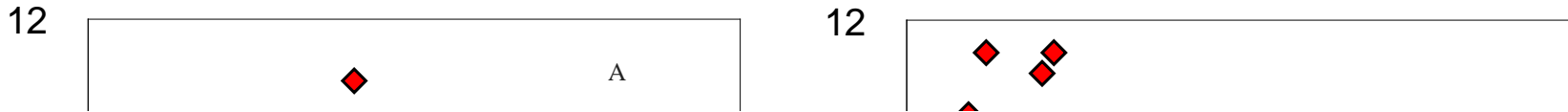
The ratios were greater than that of OH-PCBs (Netherlands: 0.6-0.7, and Japan: 0.1-0.9).

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 hormone effects??

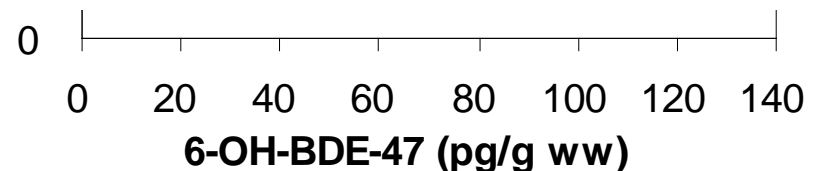
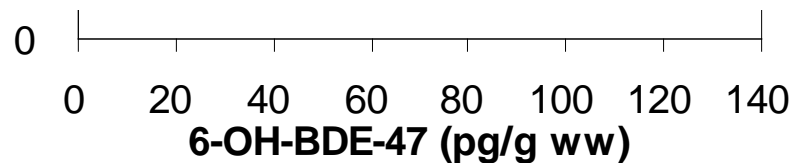
6-OH-BDE-47 vs E2 and T4 in Fetal Serum



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

- Hydroxylation of synthetic PBDEs to OH-PBDEs was negligible
- Significant production of OH-PBDEs from biotransformation MeO-PBDEs
- 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).
- A major effect of OH-PBDE exposure might be a decrease in serum T4 concentrations.

[Thank You!!!! Questions????]

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