

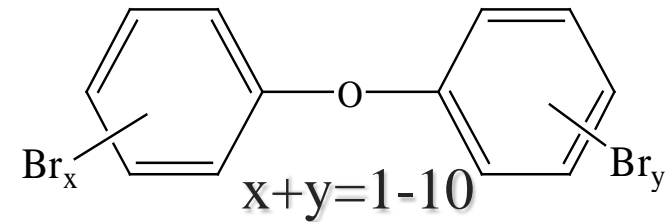
**Origin of Hydroxylated Brominated Diphenyl Ethers:  
Natural Compounds or Man-made Flame Retardants?**

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Shinsuke Tanabe, Jianying Hu,  
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# [ PBDEs and Structurally Related Compounds ]

## ■ PBDEs

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



# [PBDEs and Structurally Related Compounds]

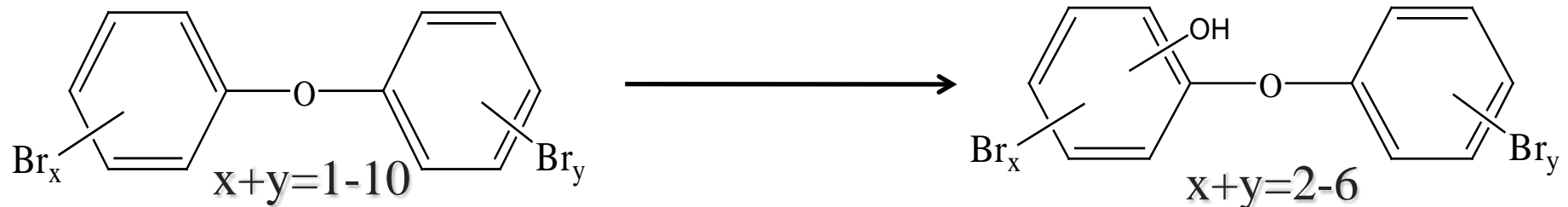
## ■ Hydroxylated PBDEs

- Produced naturally
- Metabolite of PBDEs
- Atmospheric hydroxylation of PBDE



## ■ Variety of Effects

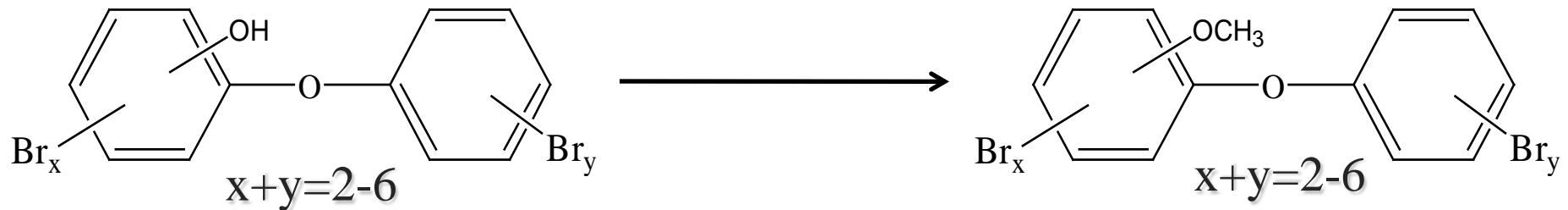
- Compete with T4 for TTR
- Disruption of ER signaling
- Disruption of oxidative phosphorylation
  - *6-OH-PBDE is acutely toxic to Zebrafish*



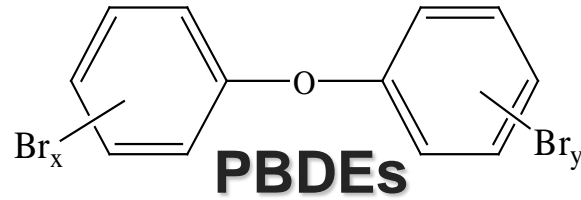
# [PBDEs and Structurally Related Compounds]

## ■ Methoxylated PBDEs

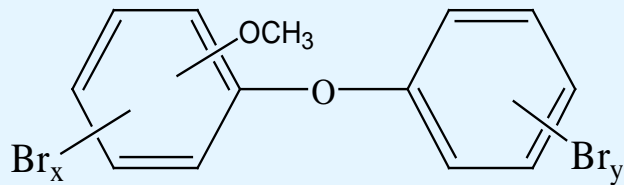
- Concentrations sometimes greater than PBDE
- Two abundant congeners are natural products
  - 2-MeO-PBDE-68
  - 6 MeO-PBDE-47
- Suggested that they are formed from metabolism of OH-PBDEs.
- No reported toxicity



# Sources and Relationships

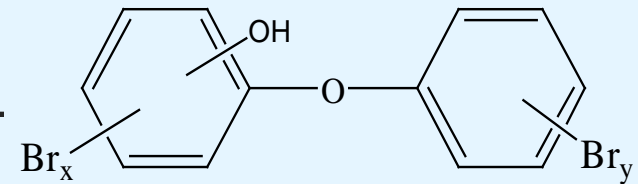


*Metabolism*



**MeO-PBDEs**

*Methylation*



**OH-PBDEs**

*both have natural sources*

# PBDEs as Precursors of OH-PBDEs ?

- Exposure levels of PBDEs in *in vitro* or *in vivo* studies were great (ppm), but OH-PBDEs occurred at trace levels (<0.01-1% of PBDEs)
- Relatively great concentrations of OH-PBDEs were found in marine organisms, suggesting the existence of other sources

*What are the sources of OH-PBDEs ?*

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

# Experimental Goals -1

- Determine levels of PBDEs, MeO-PBDEs, OH-PBDEs and bromophenols in livers of tuna, five albatross species and polar bear collected from remote marine locations
  - Levels of these compounds
  - Investigate relationships among PBDEs, MeO-PBDEs, OH-PBDEs and bromophenols

# Sample details

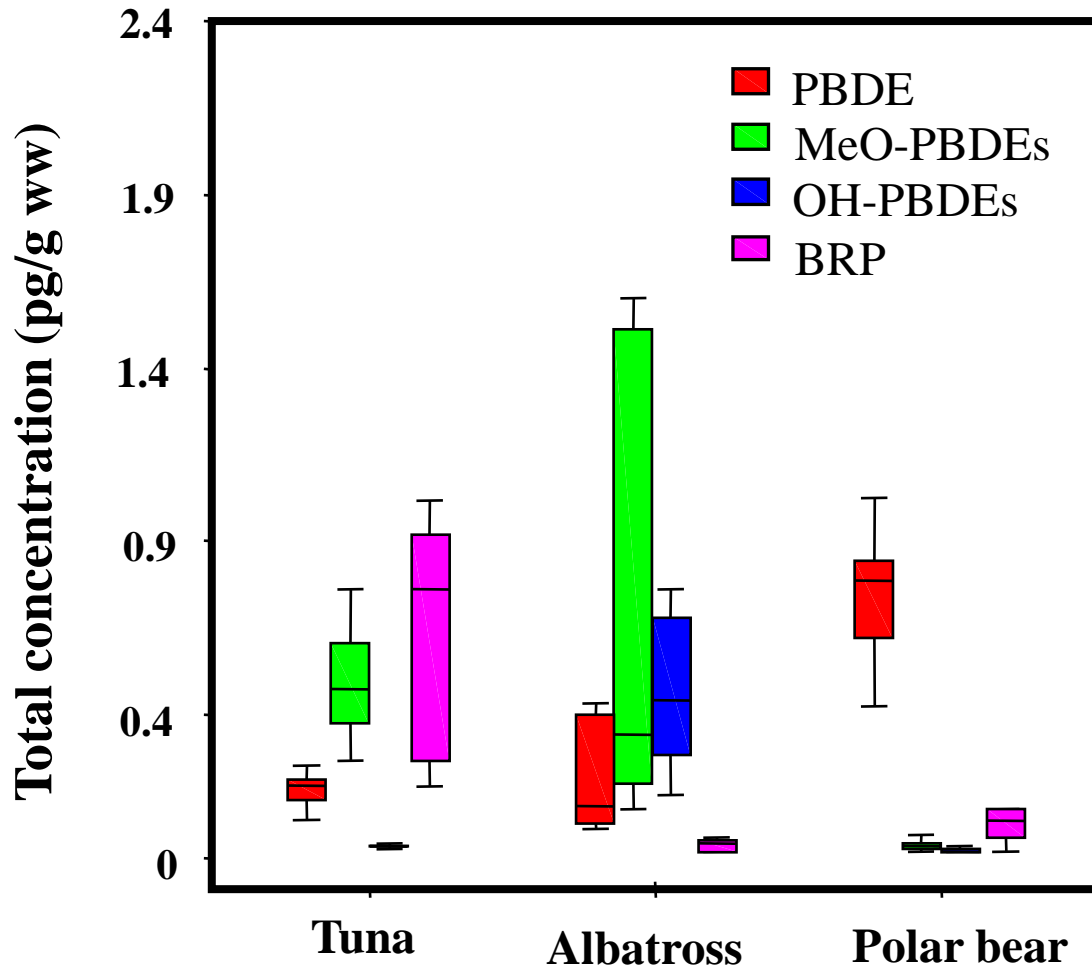
<b>Collection Date</b>	<b>#</b>	<b>Species Name</b>	<b>Location</b>
1999	10	Pacific tuna	North Pacific Ocean
1992-1996	6	Black-browed albatross	Indian Ocean, South Pacific Ocean
1994-1996	3	Grey-headed albatross	Indian Ocean
1995	1	Light-mantled sooty albatross	South Atlantic Ocean
1995-1996	2	Shy albatross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



# Analytical Method

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

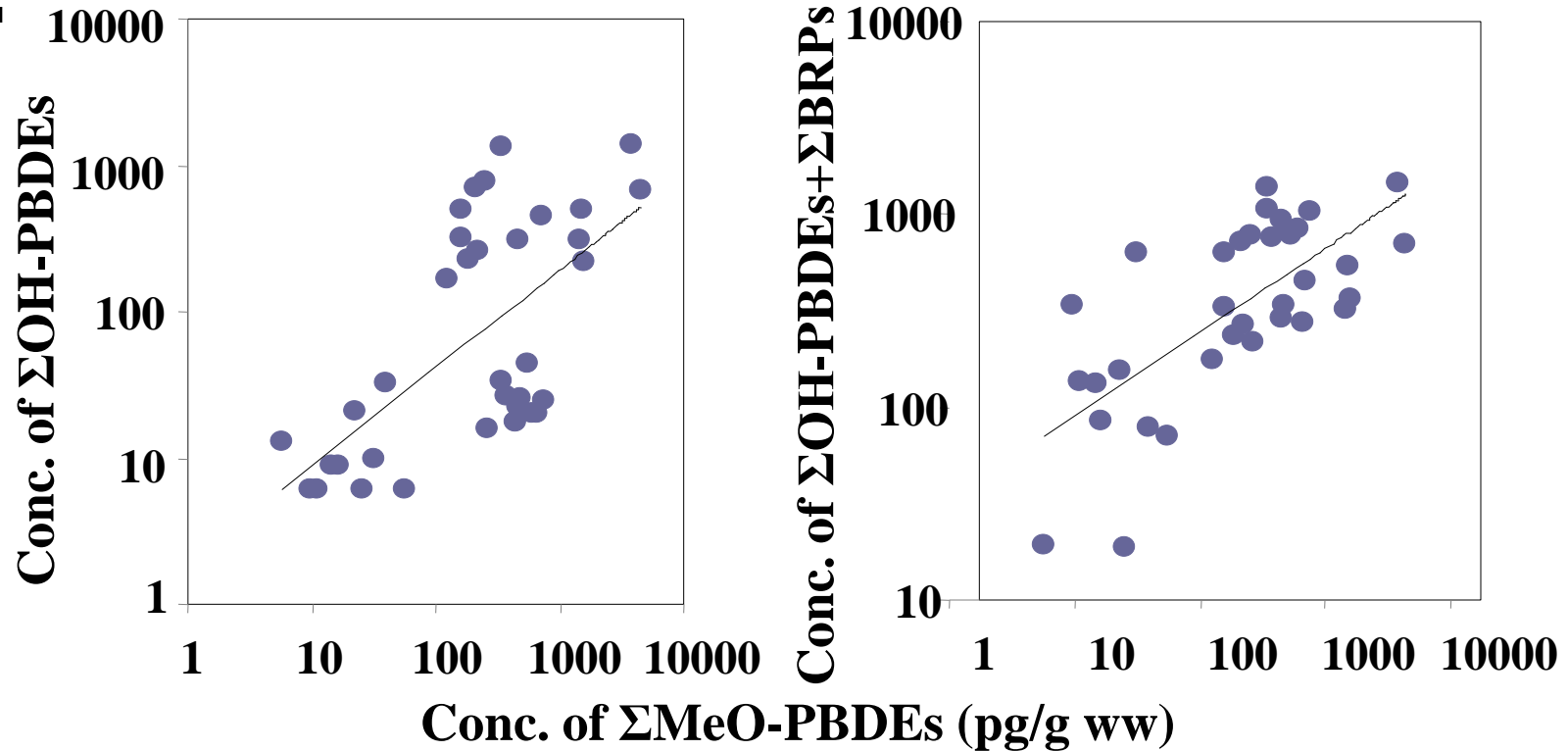
# Levels of PBDEs, MeO-PBDEs, OH-PBDEs and BRPs in Marine Organisms



Concentration of  $\Sigma$ PBDEs not related to those of  $\Sigma$ OH-PBDEs

Possible relationships between MeO-PBDEs and OH-PBDEs

# Correlations between MeO-PBDEs, OH-PBDEs and BRPs



*No significant relationships between  $\Sigma$ PBDEs and  $\Sigma$ OH-PBDEs*

*Significant correlations between  $\Sigma$ MeO-PBDEs and OH-PBDEs*

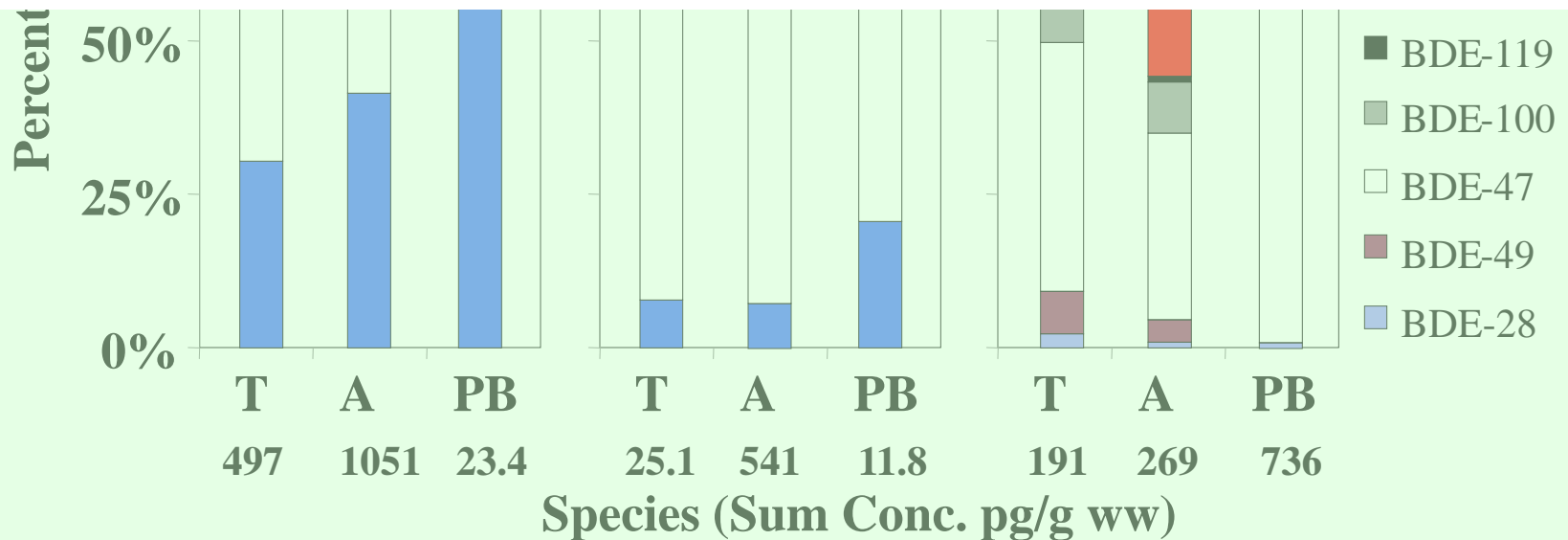
*More significant correlations between  $\Sigma$ MeO-PBDEs and  $\Sigma$ OH-PBDEs +  $\Sigma$ BRPs*

# Profiles

■ 5'-MeO-BDE-100     ■ 4'-OH-BDE-49

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

**Significant correlations for compounds with similar structures suggest methylation of OH-PBDEs to MeO-PBDEs.**



# [ Experimental Goals -2 ]

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

**Study the metabolism of the compounds**

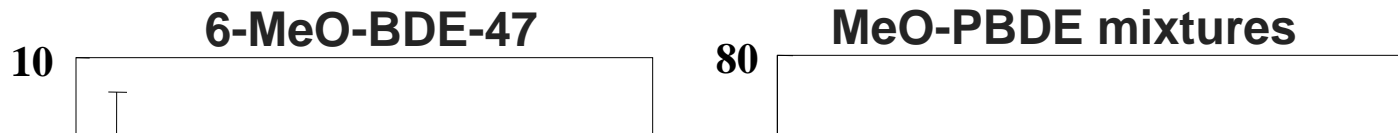
**Gain insight into sources of each compound**

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

- **Microsomes**
  - **Microsomal fractions of 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

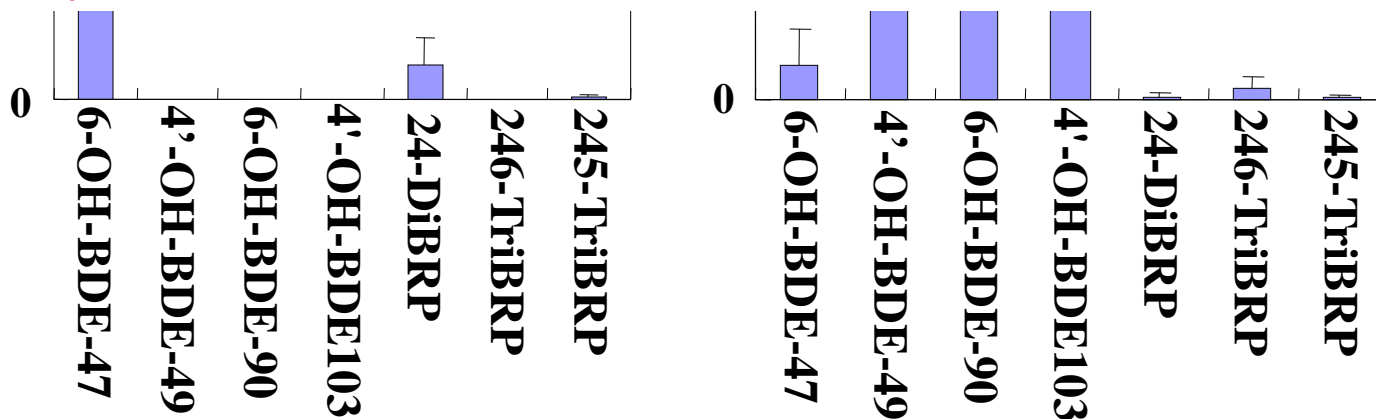
# Percentage of OH-PBDEs and BRPs in PBDEs and MeO-PBDEs exposed microsomes

OH-PBDEs and BRPs were not detected in PBDE exposed microsomes



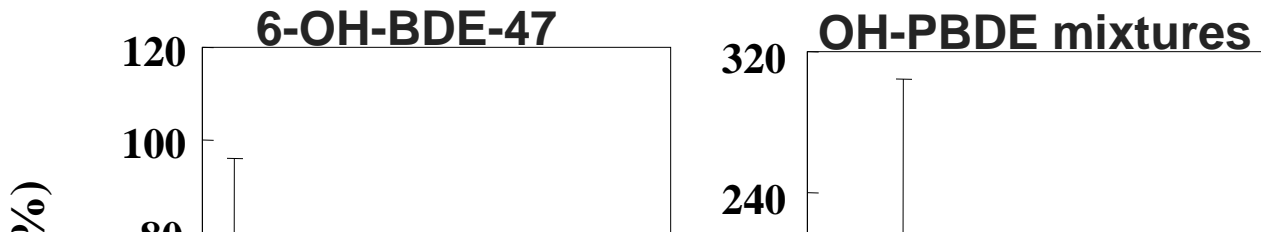
Significant amounts of 6-OH-BDE-47 were generated from 6-MeO-BDE-47, and more OH-PBDE congeners were detected when additional MeO-PBDE congeners were incubated with microsomes, even at lesser concentrations

Demonstrating the biotransformation of MeO-PBDEs to OH-PBDEs at environmentally relevant concentrations



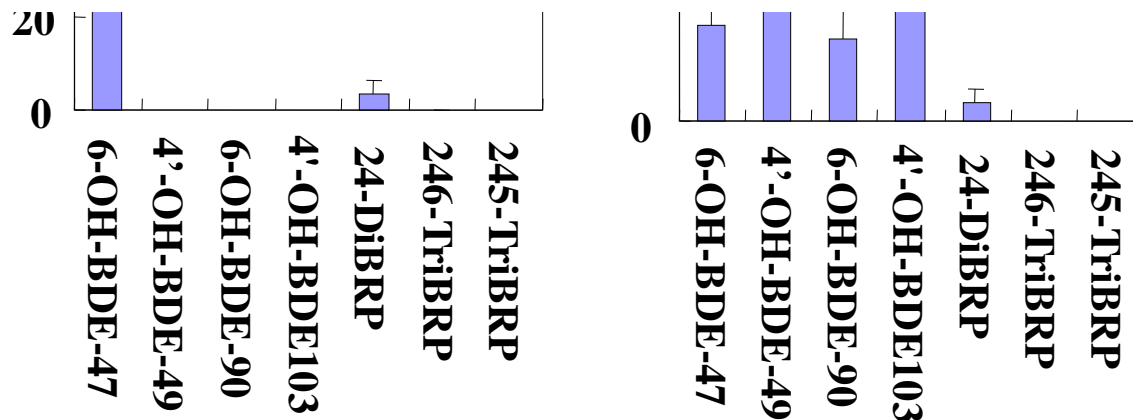
# Percentage of OH-PBDEs and BRPs in OH-PBDEs exposed microsomes

- MeO-PBDEs were not detected in OH-PBDEs exposed microsomes, indicating a lack of methylation of OH-PBDEs to MeO-PBDEs, as has been suggested previously

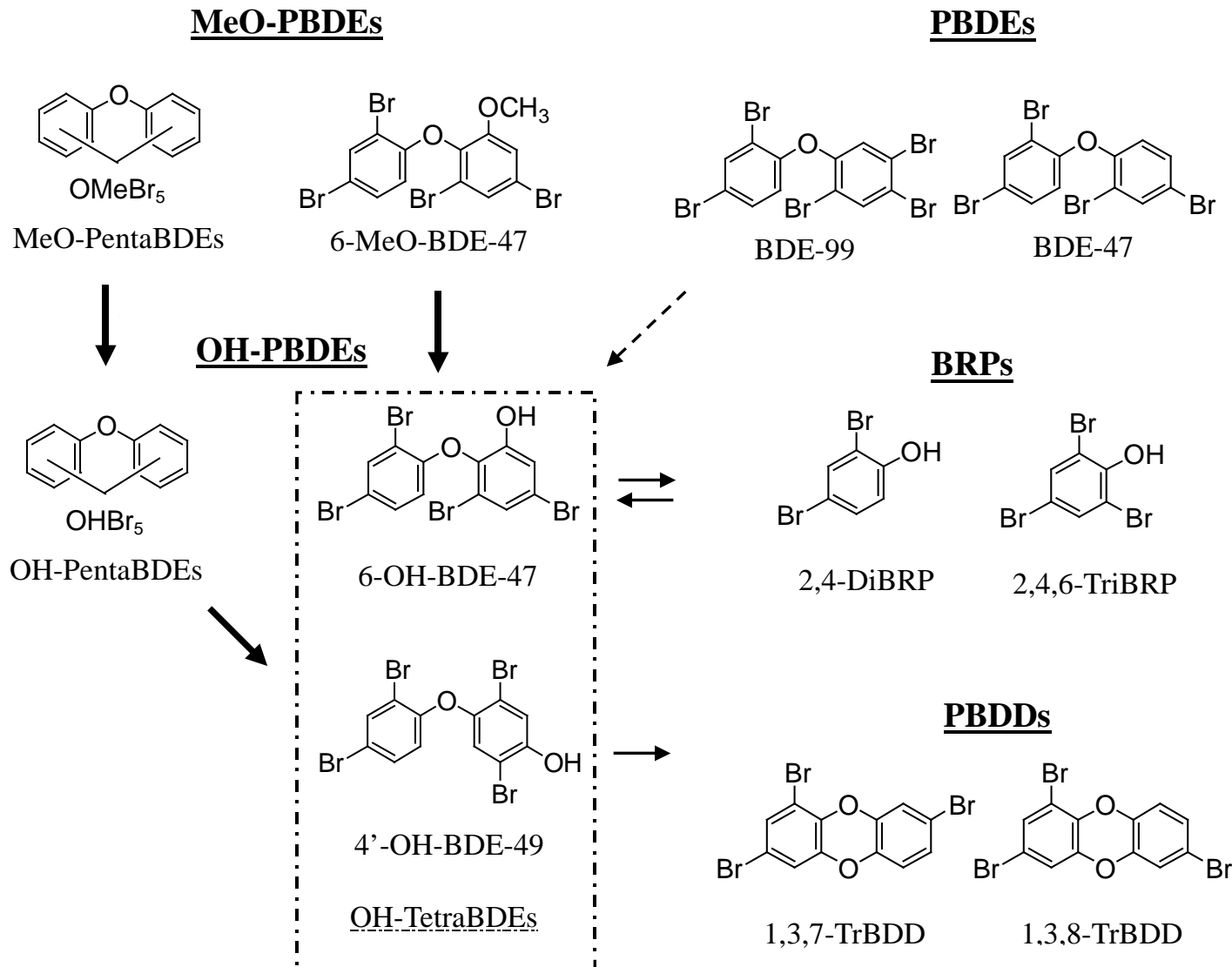


**2,4-DiBRP was the major BRP congener of OH-PBDE metabolism**

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



# Proposed metabolic relationships among brominated compounds



# Summary

- Significant metabolic production of OH-PBDEs from MeO-PBDEs, while hydroxylation of synthetic PBDEs to OH-PBDEs was negligible
- MeO-TetraBDEs could be an important contributor for the occurrence of *ortho* substituted OH-PBDEs found in wildlife from remote areas
- MeO-PentaBDEs could be an important contributor for the occurrence of *para* and *meta* substituted OH-PBDEs
- risk assessment paradigms for PBDEs and their metabolites need reevaluation and that human exposure to MeO-PBDEs that occur naturally in marine organisms should be considered

# Sample Prep and Analysis

