Interconversion of Hydroxylated and Methoxylated Polybrominated Diphenyl Ethers in Japanese Medaka

University of Saskatchewan

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INTRODUCTION

- **Polybrominated diphenyl ethers (PBDEs) and structurally related hydroxylated (OH-) and methoxylated (MeO-) PBDEs are ubiquitous in the environment.**
- **Several origins for OH-PBDEs and MeO-PBDEs have been suggested.**
  - ***Ortho*-substituted OH-PBDEs and MeO-PBDEs are formed as naturally occurring compounds in the marine environment.**
  - **Result from biotransformation of synthetic PBDEs**
- **Demethylation of natural MeO-PBDEs is a major contributor of OH-PBDEs.**
- **We have previously demonstrated, *in vitro*, that 6-MeO-BDE-47 is a precursor for 6-OH-BDE-47. In this same study no 6-OH-BDE-47 was generated during the microsomal metabolism of BDE-47. Currently no direct *in vivo* evidence of this pathway of OH-PBDE formation.**

OBJECTIVES

Objective 1: Establish relationships between BDE-47, 6-MeO-BDE-47 and 6-OH-BDE-47 *in vivo*.

Objective 1: Establish maternal transfer of BDE-47, 6-MeO-BDE-47 and 6-OH-BDE-47 , and metabolites, *in vivo*.

MEDAKA EXPOSURE

- **Sexually mature Japanese medaka (*Oryzias latipes*)(8 females and 4 males) randomly assigned to 10L tanks containing 6L of dechlorinated tap water.**
- **All exposures were performed in duplicate tanks.**
- **Medaka were fed diets of food spiked with BDE-47, 6-OH-BDE-47 or 6-MeO-BDE47, or acetone (vehicle control) for 14 days.**
- **Eggs were collected each morning (days 0-14) during the exposure period.**
- **On day 14 six female fish were collected from each tank and liver and liver-free carcass were collected for analysis of target chemical concentrations.**

RESULTS

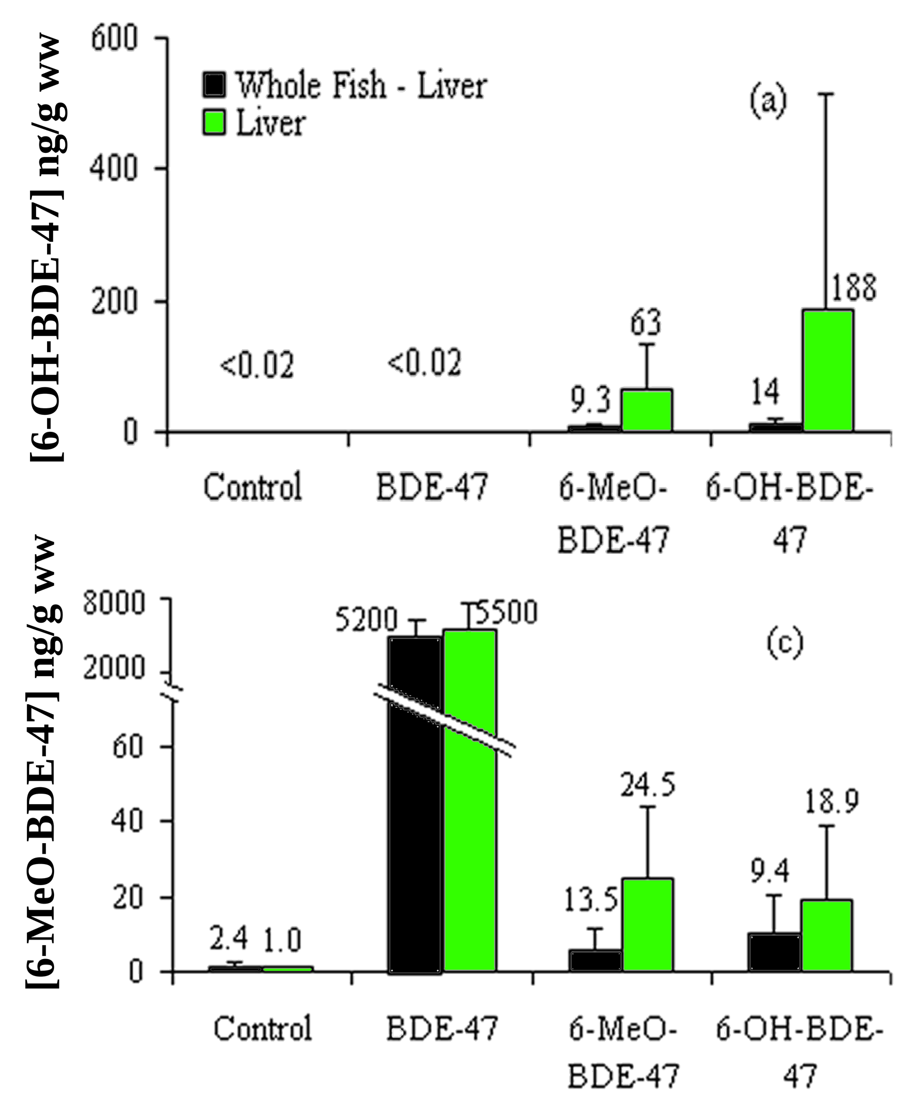
Purity of Dosing Solutions

Table 1: Concentrations of 6-OH-BDE-47, 6-MeO-BDE-47 and BDE-47 in Spiked Food (ng.g dry weight) and Stock Standard Solutions (ng/ml).

Sample	6-OH-BDE-47	6-MeO-BDE-47	BDE-47
Control Food	< 0.02	0.1	1.6
6-OH-BDE-47 Food	900	0.2	15
6-MeO-BDE-47 Food	<0.02	0.2	28.3
BDE-47 Food	<0.02	0.2	21,000
6-OH-BDE-47 Stock	1,500,000	4,300	1,900
6-MeO-BDE-47 Stock	<0.8	1,300,000	4,800
BDE-47 Stock	<0.8	<2.0	50,000

- ***6-OH-BDE-47 not an impurity in BDE-47 or 6-MeO-BDE-47 food.***
- ***6-MeO-BDE-47 was detected in the fish food, but did not affect conclusions.***
- ***Purity tests were not reported in previous exposure studies, however the possible contribution of impurities of MeO-PBDEs in commercial rat food containing fish or shrimp cannot be neglected.***

Accumulation of Parent Compounds and Metabolites (in vivo)



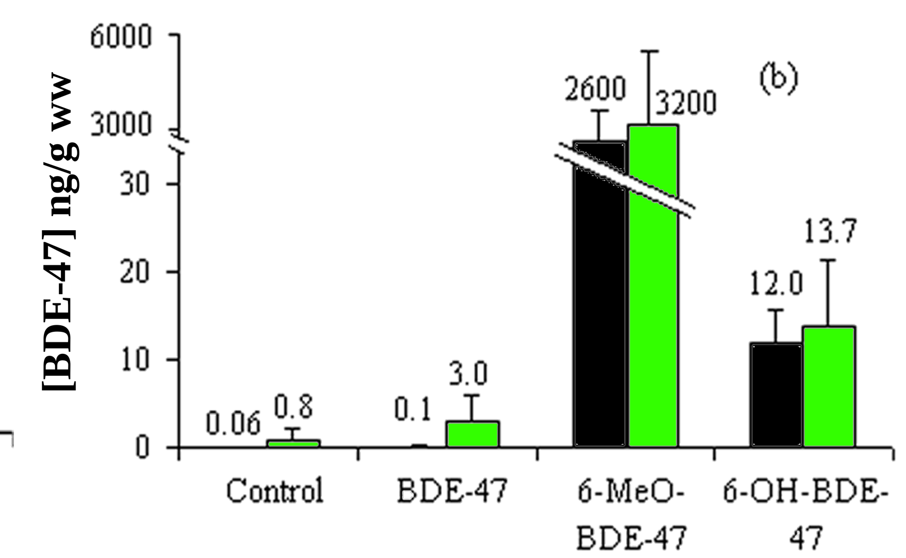


Fig 1. Concentrations of BDE-47, 6-MeO-BDE-47 and 6-OH-BDE-47 in liver and liver free residual carcass from female Medaka after 14 days of exposure. Numbers above the error bars are mean concentrations of target compounds (ng/g ww).

- ***Significant concentrations of 6-OH-BDE-47 were measured in medaka exposed to 6-MeO-BDE-47, but not BDE-47.***
- ***6-MeO-BDE-47 was formed in Medaka exposed to 6-OH-BDE-47.***
- ***Comparable concentrations of BDE-47 were observed in female medaka exposed to 6-MeO-BDE-47 and 6-OH-BDE-47, which is due to BDE-47 impurities in food.***

Maternal Transfer of Metabolites

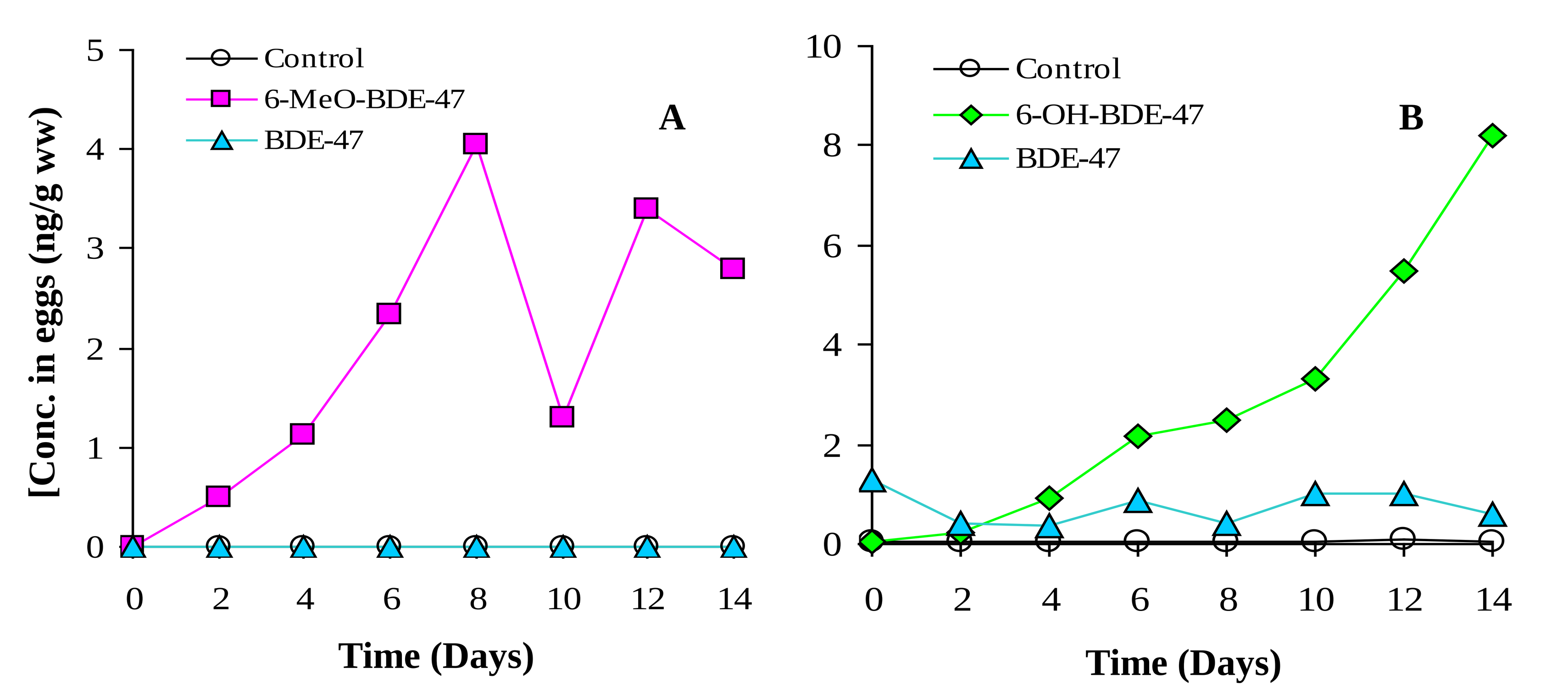


Fig 3. Accumulation of a) 6-OH-BDE-47 and b) 6-MeO-BDE-47 as metabolites in eggs during dietary exposure to feed spiked with solvent (control, panel a and b), BDE-47 (panel a and b), 6-MeO-BDE-47 (panel a) or 6-OH-BDE-47 (panel b).

- ***Both 6-OH-BDE-47 and 6-MeO-BDE-47 occurred in eggs as biotransformation products of 6-MeO-BDE-47 and 6-OH-BDE-47, respectively.***
- ***Neither transformation product was detected in eggs collected from medaka exposed to BDE-47***

In vitro Metabolism of Target Compounds

Table 3: Concentrations of target compounds after metabolism with medaka microsomes exposed to BDE-47, 6-MeO-BDE-47, and 6-OH-BDE-47 (ng/mL). The dosing concentrations for all chemicals were 2 µg/mL.

	Analyzed Chemical		
Exposed Chemical	BDE-47	6-MeO-BDE-47	6-OH-BDE-47
6-MeO-BDE-47	< 1.6	710 ± 72	62.8 ± 9.9
6-OH-BDE-47	< 1.6	< 0.05	680 ± 110
BDE-47	620	< 0.05	< 0.02

- ***6-MeO-BDE-47 is a contributor to formation of 6-OH-BDE-47***
- ***Biotransformation of 6-OH-BDE-47 to 6-MeO-BDE-47 was not observed in vitro.***
- ***Neither 6-OH-BDE-47 nor 6-MeO-BDE-47 were detected in medaka microsomes exposed to BDE-47.***

Maternal Transfer of Parent Compounds

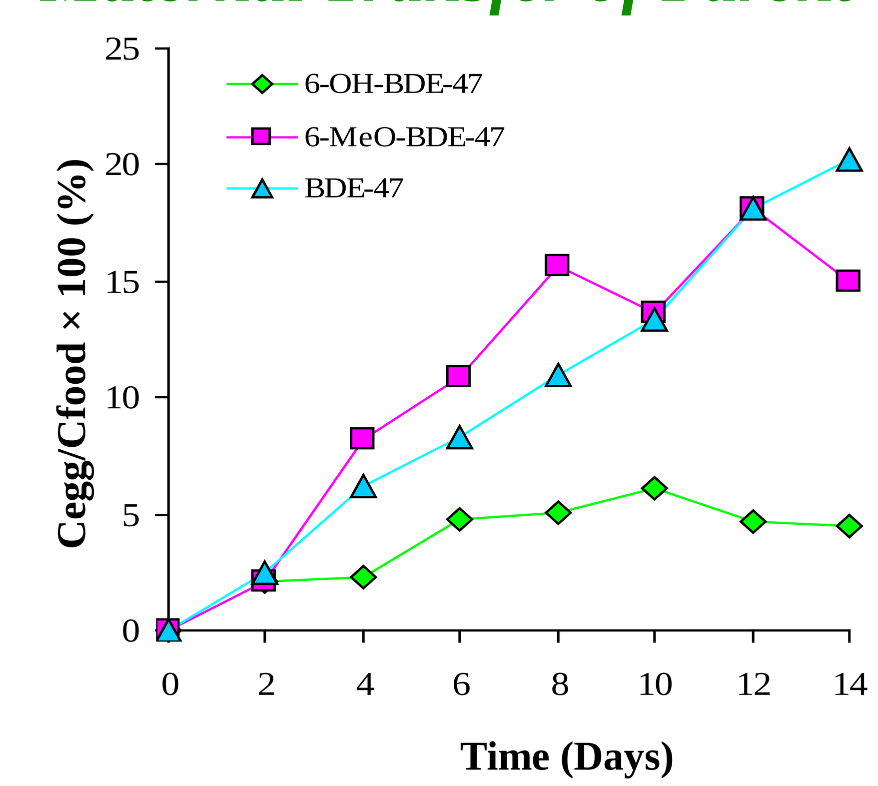


Fig 2. Accumulation of BDE-47, 6-OH-BDE-47 and 6-MeO-BDE-47 in eggs during the 14-day dietary exposure to BDE-47, 6-MeO-BDE-47 or 6-OH-BDE-47. C_{egg} is the concentration of target compounds in eggs, and C_{food} is the concentration in dosing food.

- ***Accumulation of BDE-47 did not reach steady-state after 14d of exposure.***
- ***Relatively great assimilation efficiencies were observed for 6-MeO-BDE-47 and BDE-47 in contrast to 6-OH-BDE-47.***
- ***Depuration rate of BDE-47 is less than that of 6-MeO-BDE-47***

CONCLUSIONS

- **This study presents 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 was demonstrated *in vivo*, but the conversion was not observed *in vitro* (liver microsomes).**
- **The previously hypothesized formation of OH-PBDEs from synthetic BDE-47 did not occur.**
- **Biotransformation products formed in female medaka were transferred to eggs.**

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