Bioaccumulation, biotransformation and toxicity of BDE-47, 6-OH-BDE-47 and 6-MeOH-BDE-47 in early-life stages of zebrafish (Danio rerio)

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I. Determine the time-course of accumulation, biotransformation, and toxicity of BDE-47, 6-OH-BDE-47, and 6-MeOH-BDE-47 in zebrafish larvae.

A. Bioaccumulation

1. The concentration of BDE-47 increased substantially coinciding with the hatching period of zebrafish embryos (48 hpf). In the late developmental periods of the larvae (after 96 hpf), the bioaccumulation of BDE-47 and 6-MeO-BDE-47 stopped increasing and reached a plateau, which might be due to an increase in metabolism and/or excretion activities.

2. 6-OH-BDE-47 had the greatest accumulation potential throughout the exposure experiment, and 6-MeO-BDE-47 accumulated last into the body, though it was the most toxic.

B. Biotransformation

1. The clustering results of three compounds correlated well with their respective accumulation potential, indicating the great importance of the usage of internal dose to assess the dose-response relationship for studies of PBDEs, especially MeO-PBDEs.

2. Further analyses of molecular toxicity pathways indicated general disruption of receptor pathways by all three BDE congeners, which correlated well with the observed teratogenic effects in zebrafish. Specifically, 6-OH-BDE-47 altered the expression of AhR, ER, and MR receptor-mediated pathways, while AhR, ER, and GR were the primary pathways altered by BDE-47. Yet, exposure to the more bioaccumulative 6-MeO-BDE-47 affected AhR, ER, AR, GR, and TR pathways.

3. Our findings provided valuable insights into the interactions of these compounds with steroid hormone receptor pathways, which provided novel clues for their in vivo mechanisms of endocrine disruption and developmental toxicities.

C. Toxicity

1. Among the three compounds, 6-OH-BDE-47 was most potent to zebrafish embryo-larvae.

2. The time points during which accumulation of BDE-47 and 6-MeO-BDE-47 increased substantially coincide with the hatching period of zebrafish embryos (48 hpf). In the late developmental periods of the larvae (after 96 hpf), the bioaccumulation of BDE-47 and 6-MeO-BDE-47 stopped increasing and reached a plateau, which might be due to an increase in metabolism and/or excretion activities. 6-OH-BDE-47 had the greatest accumulation potential throughout the exposure experiment, and 6-MeO-BDE-47 accumulated last into the body, though it was the most toxic.

3. The clustering results of three compounds correlated well with their respective accumulation potential, indicating the great importance of the usage of internal dose to assess the dose-response relationship for studies of PBDEs, especially MeO-PBDEs.

4. Further analyses of molecular toxicity pathways indicated general disruption of receptor pathways by all three BDE congeners, which correlated well with the observed teratogenic effects in zebrafish. Specifically, 6-OH-BDE-47 altered the expression of AhR, ER, and MR receptor-mediated pathways, while AhR, ER, and GR were the primary pathways altered by BDE-47. Yet, exposure to the more bioaccumulative 6-MeO-BDE-47 affected AhR, ER, AR, GR, and TR pathways.

5. Our findings provided valuable insights into the interactions of these compounds with steroid hormone receptor pathways, which provided novel clues for their in vivo mechanisms of endocrine disruption and developmental toxicities.

Methods

Exposure Design

Family eggs from adult zebrafish (AB strain, 7-month-old) were randomly distributed into 25 ml glass beaker containing 20 ml of exposure solution and were exposed to 4 from 120 hour post-fertilization (hpf).

RNA extraction and RT-PCR

After exposure for 120 hpf the larvae were anesthetized with MS-222, and were preserved in RNAlater RNA Stabilization Reagents until total RNA isolation. Quantitative RT-PCR was performed by an Applied Biosystems StepOne Plus Real-Time PCR system to study the effects of expression of genes involved in eight receptor-mediated pathways.

Analytical Approach

For Bioavailability analysis and QA/QC, detailed protocols for extraction, clean up, identification and quantification, and quality assurance and quality control (QA/QC) are provided in previous studies (Zheng et al., 2012).

Data evaluation

Nuclear receptor pathways were integrated and visualized as one network by Cytoscape. Hierarchical cluster analysis of changes in gene expression upon chemical exposures was performed by the “complete” method in R software version 3.10 (R Core Team, Vienna, Austria). A heatmap of the gene expression results was implemented by “pheatmap” package version 0.7.1 in R.

Figure 1. Effects of 6-MeO-BDE-47 and BDE-47 on morphologies of several development stages of zebrafish-embryo. A) normal zebrafish at 72 hpf; B) neural tube curvature caused by 6-MeO-BDE-47 at 72 hpf; C) severe edema caused by 6-MeO-BDE-47 at 72 hpf; D) arrested development caused by 6-MeO-BDE-47 at 72 hpf; E) normal zebrafish at 120 hpf; F) severe edema and spinal curvature caused by 6-MeO-BDE-47 at 120 hpf, EC50=1.6 μM, LC50=0.09 μM; G) severe edema and spinal curvature caused by 6-OH-BDE-47 at 120 hpf, EC50=0.15 μM, LC50=0.85 μM; H) edema caused by BDE-47 at 120 hpf, NOEC=0.15 μM.

Figure 2. Changes in concentrations of BDE-47, 6-MeO-BDE-47, or 6-OH-BDE-47 in exposure medium (μg/l) and in zebrafish embryos-larvae (μg/g, wet) after exposure to 300 μg/l over the duration of the experiment.

Figure 3. Bioconcentration factors (BCF) calculated for zebrafish embryos after exposure to 300 μg/l of BDE-47, 6-MeO-BDE-47, and 6-OH-BDE-47 over the duration of the experiment.

Figure 4. Concentrations of biotransformed 6-OH-BDE-47 detected in zebrafish embryos-larvae (μg/g, wet) after exposure to 300 μg/l of 6-MeO-BDE-47 over the duration of the experiment.

Figure 5. A). Heatmap of gene expression profiles was generated using the average gene expression values of the exposure in zebrafish larvae at 120 hpf. Genes involved in different receptor pathways are given different colors above the heatmap; B) The dendrogram of hierarchical cluster analysis was calculated using the average gene expression values of exposure in zebrafish larvae at 120 hpf. C) The heatmap cluster analysis of gene expression was used to identify the degree of clustering of genes in different receptor pathways.

Figure 6. Interaction network of genes in NR pathways. Edges are either protein-protein or protein-DNA interactions. Statistically significant changes in gene expression following different concentrations of treatment of BDE-47, 6-MeO-BDE-47, and 6-OH-BDE-47 at 120 hpf are given in the respective boxes.