Genotoxic potential of several polybrominated diphenyl ethers (PBDEs) and hydroxylated PBDEs (OH-PBDEs) in chicken DT40 mutant cells

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Introduction

● PBDEs have been widely used in electronic equipment, casing for personal computers and television sets, and for a variety of other plastic products as flame retardant
● In spite of the widespread occurrence of PBDEs in the environment, limited information is available on the extent and mechanism of genotoxicity of PBDEs and OH-PBDEs
● The aim of the present study is to investigate the genotoxicity of PBDEs and OH-PBDEs using chicken DT40 cells, supplying data for the mechanisms of genotoxic effects

Materials & Methods

● Test compounds

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<thead>
<tr>
<th>Test compounds</th>
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● ATP assays

- Cells were plated onto 24-well plates (1 ml/well), and incubated at 39.5°C for 3 days
- To evaluate the involvement of reactive oxygen species (ROS) in DNA damage induction by PBDEs, we pretreated DT40 cells with 1 mM NAC, an ROS scavenger, 2 hour before PBDEs treatment
- ATP assays were carried out with 96-well plates using a Luminescent Cell Viability Assay Kit

● Chromosomal aberration assay

- Analysis of chromosome aberrations: 11 autosomal chromosomes and the Z chromosome in Giemsa-stained metaphase cells (50 cells) per treatment/condition

Results & Discussion

Genotoxicity of PBDEs

- After exposure to BDE-47 and BDE-49 for 72 hr, cell viability of Polβ- and REV3- was significantly affected compared to wild-type cells
- However, cells exposed to BDE-99, BDE-138, and BDE-209 did not show any significant impact on survival of both mutants

Genotoxicity of PBDEs vs. OH-PBDEs

- Tetra-OH-BDEs were more genotoxic than tetrabDEs
- 6-OH-BDE-47 was the most potent chemical that decreased cell viability of Polβ- mutants by ~99% at 400 μg/L

Genotoxicity mechanism of PBDEs and OH-PBDEs

- Chromosomal aberrations barely detectable in non-exposed cells, whereas both chromatid- and chromosome-type breaks were frequently occurred in cells exposed to BDE-47 and 6-OH-BDE-47
- In REV3- DT40 cells, γ-H2AX focus formation increased significantly after 1 h treatment of BDE-47, BDE-49, 6-OH-BDE-47, and 4-OH-BDE-49, compared to the wild-type cells, indicating that tetra-PBDEs and tetra-OH-PBDEs indeed induce DSBs
- Pretreatment with NAC significantly reversed the cellular sensitivity of Polβ- and REV3- mutants, suggesting that PBDEs and OH-PBDEs may generate DNA damage induced by ROS leading to replication blocks and subsequent chromosomal breaks

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- Measurement of γ-H2AX foci induction: to confirm the double strand repair, we observed more than 200 cells and recorded number of foci

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Conclusions

- We have shown that
  1) exposure to tetrabDEs and tetra-OH-PBDEs resulted in hypersensitivity in chicken DT40 cells deficient in base excision repair and translesion DNA synthesis pathway
  2) PBDEs and OH-PBDEs generate DNA damage induced by ROS, leading to replication blocks and subsequent chromosomal breaks
  3) Potential consequences of such genotoxicity in vivo should warrant further investigation

For questions or comments please email me at jksh20@kaist.ac.kr