

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

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 tetra-BDEs
 - 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 observed 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

Materials & Methods

Test compounds

	Tetra-BDEs	Penta-BDEs	Octa-BDEs	Deca-BDEs
PBDEs	BDE-47 BDE-49	BDE-99	BDE-138	BDE-209
OH-PBDEs	6-OH-BDE-47 4-OH-BDE-49			

ATP assays

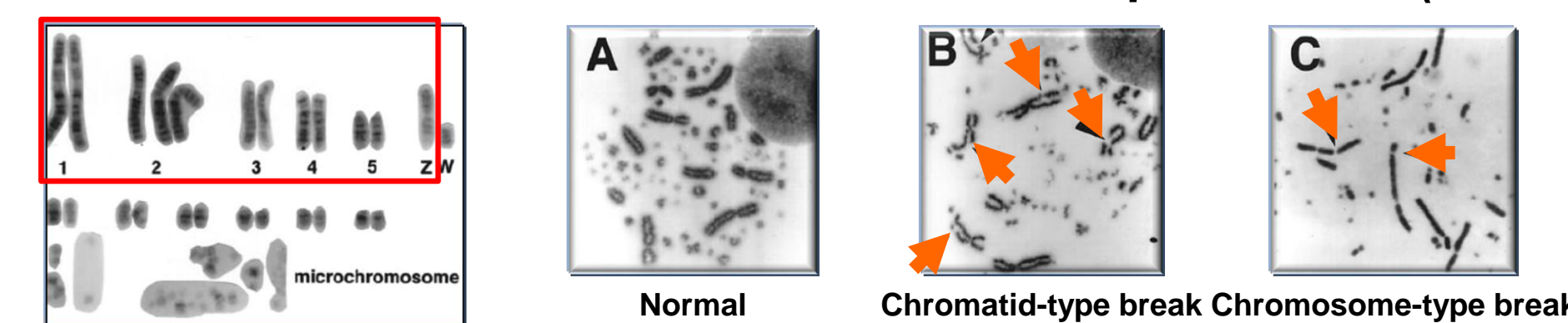
- Cells were plated onto 24-well plates (1 ml/well), and incubated at 39.5°C for 3 days
- To evaluate the involvement of reactive oxygen species (ROS) in DNA damage induction by PBDEs, we pretreated DT40 cells with 1 mM NAC, an ROS scavenger, 2 hour before PBDEs treatment
- ATP assays were carried out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

Gene Function

<i>KU70</i>	Non-homologous end-joining
<i>Polβ</i>	Base excision repair, which repairs single-strand breaks
<i>RAD54</i>	Homologous recombination, which repairs double-strand breaks
<i>REV3</i>	Translesion DNA synthesis
<i>XPA</i>	Nucleotide excision repair, which eliminates bulky base damage

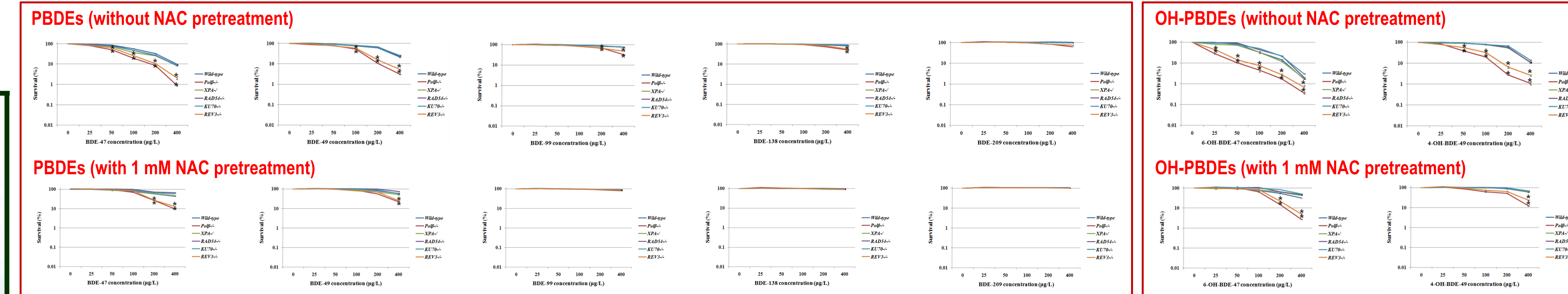
Chromosomal aberration assay

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



γ-H2AX test

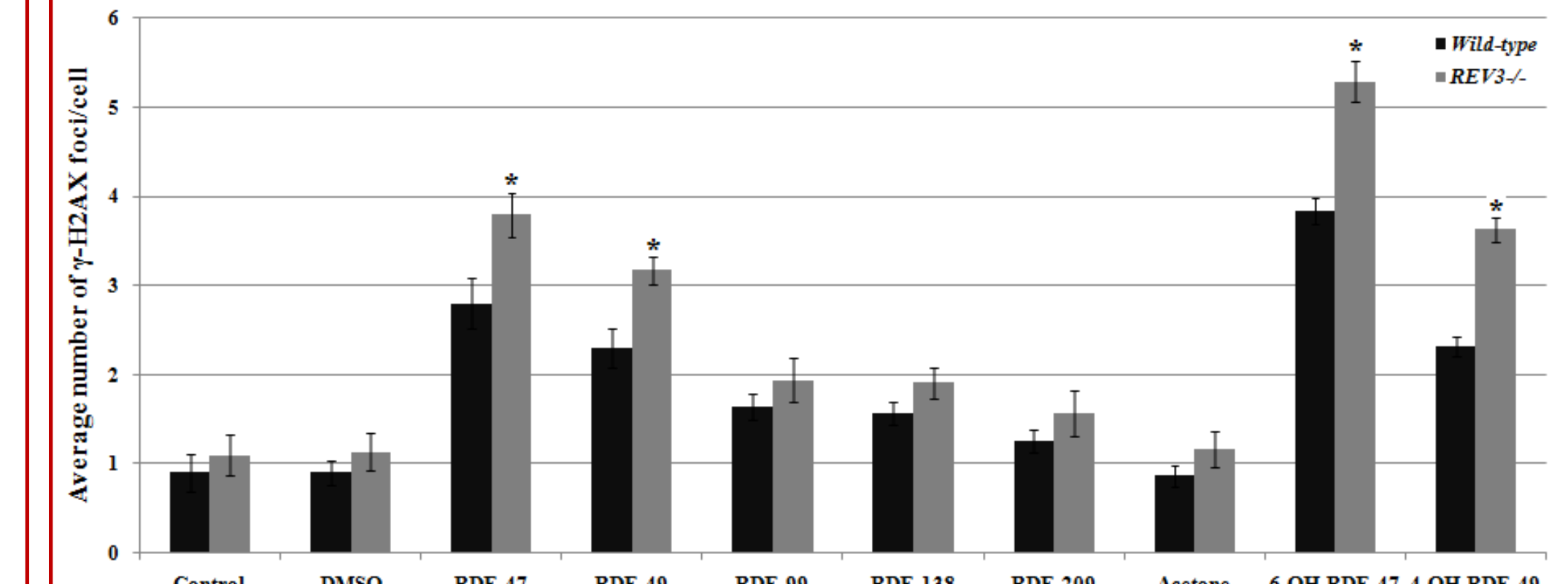
- Measurement of γ-H2AX foci induction: to confirm the double strand repair, we observed more than 200 cells and recorded number of foci



Frequency of chromosomal aberration in DT40 cells after the exposure to BDE-47 and 6-OH-BDE-47

Cell type	Chemicals (μg/L)	NAC (mM)	Chromatid breaks	Chromosome breaks	No.(%) of cells with chromosome aberrations
Wild-type	Control	0	1	0	1(2)
	Control	1	1	0	1(2)
	BDE-47 200	0	4	3	7(14)
	BDE-47 200	1	3	0	3(6)
	6-OH-BDE-47 200	0	8	3	11(22)
6-OH-BDE-47 200	1	3	1	4(8)	
<i>REV3</i> ^{-/-}	Control	0	1	1	2(4)
	Control	1	1	0	1(2)
	BDE-47 200	0	7	4	11(22)
	BDE-47 200	1	2	2	4(8)
	6-OH-BDE-47 200	0	9	6	15(30)
6-OH-BDE-47 200	1	3	2	5(10)	

PBDEs and OH-PBDEs induced γ-H2AX subnuclear foci formation in wild-type and *REV3*^{-/-} cells



Conclusion

- We have shown that
 - 1) exposure to tetra-PBDEs 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
- Potential consequences of such genotoxicity *in vivo* should warrant further investigation