Adverse (geno)toxic effects of BPA and its analogues BPS, BPAP, BPAF, BPFL, and BPC in a 3D HepG2 cell model

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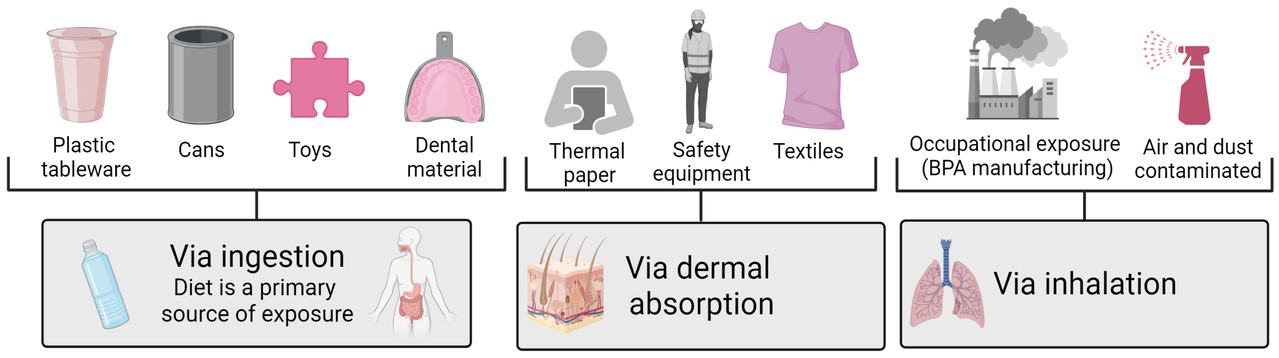
Background

Bisphenol A (BPA) is widely used chemical for manufacturing a range of products for human applications. It is known to be an **endocrine disruptor**, and there is a growing evidence that it is **genotoxic**. The hazardous properties of BPA led the EU to **restrict its use to protect human health and the environment.** As a result, there has been a **gradual shift towards development and usage of presumably safer analogues.** However, our knowledge of toxicological profiles of BPA analogues **is scarce.**



AIM

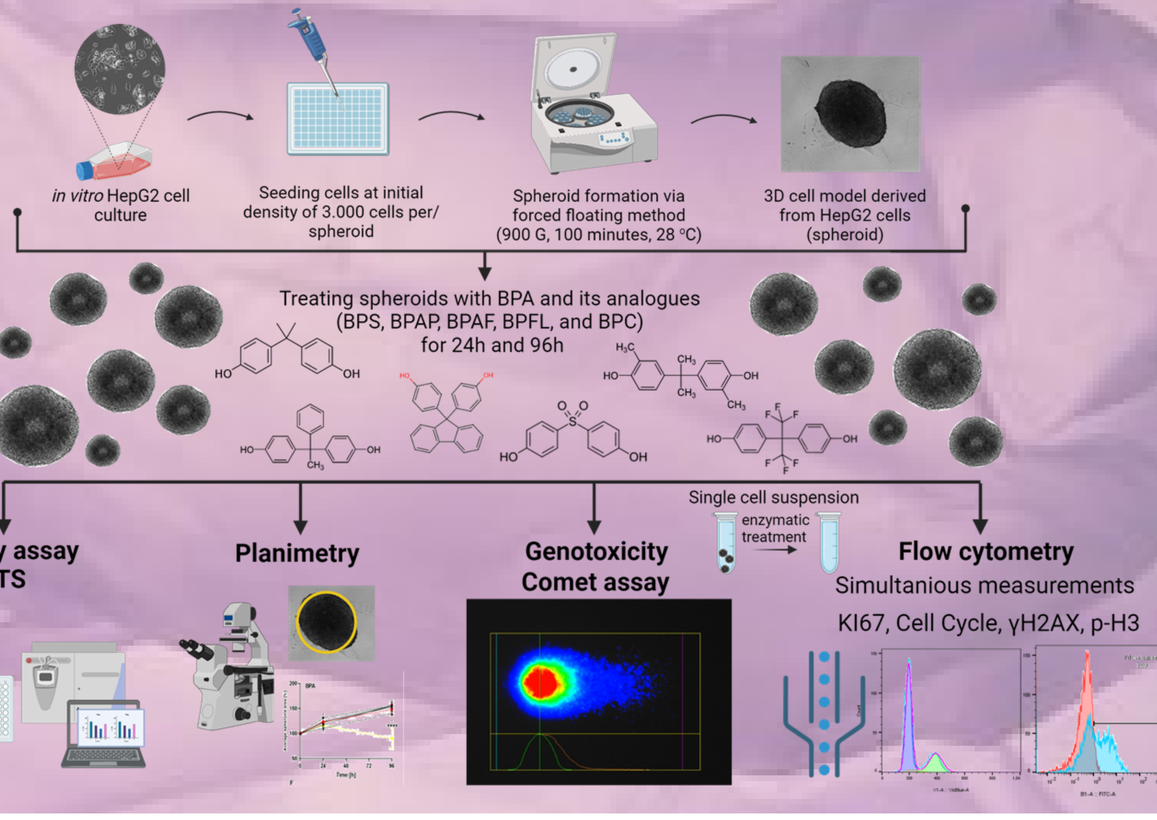
To investigate the adverse toxic effects of BPA and its analogues with emphasis on their (geno)toxic activities after short and prolonged exposure in an *in vitro* hepatic 3D cell model developed from HepG2 cells.

More about our research on bisphenols:

Are the alternatives to BPA safer?

BPAP, BPAF, BFL, BPS, and BPC analogues cannot be considered as safer alternatives to BPA due to their similar cytotoxic and genotoxic activities.

Further studies are needed to evaluate the adverse effects and mechanisms of BPA analogues, along with comprehensive research to assess their risks and ensure human safety.



GENOTOXICITY RESULTS

Comet assay (SSB)

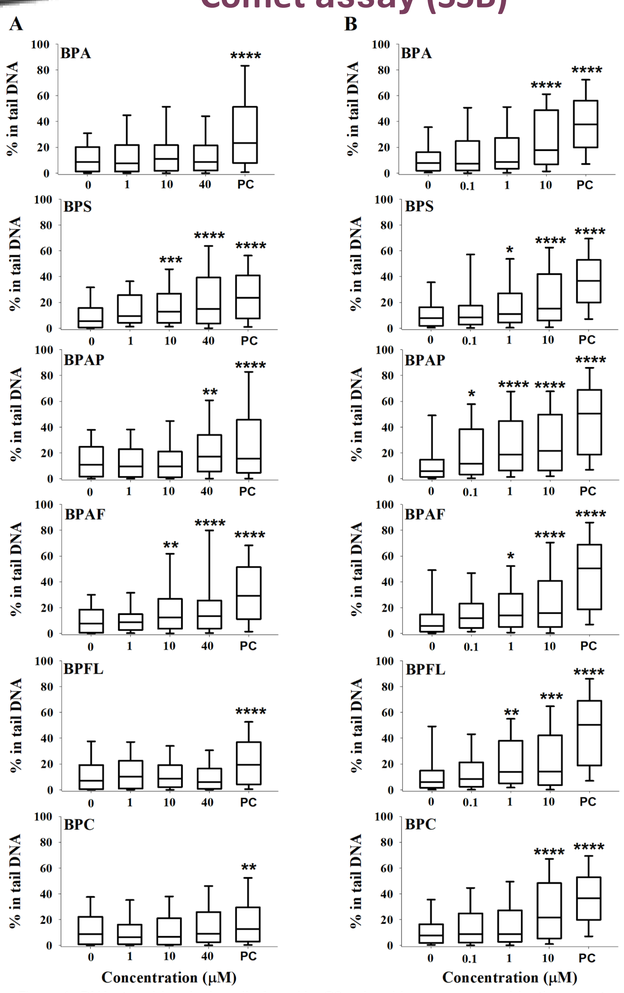


Figure 1: DNA strand breaks in cells from HepG2 spheroids were assessed by the comet assay after 24 (A) and 96-h (B) of exposure to selected BPs. Etoposide (17 μM) was considered as a positive control (PC). Fifty nuclei were measured per experimental point and presented in box plots using SigmaPlot.11 software. The experiments were repeated three times independently. Statistical analysis was performed by two-way ANOVA [p<0.05 (*), p<0.001 (**), p<0.0001 (0.0001) and p<0.00001 (****).

GammaH2AX (DBS)

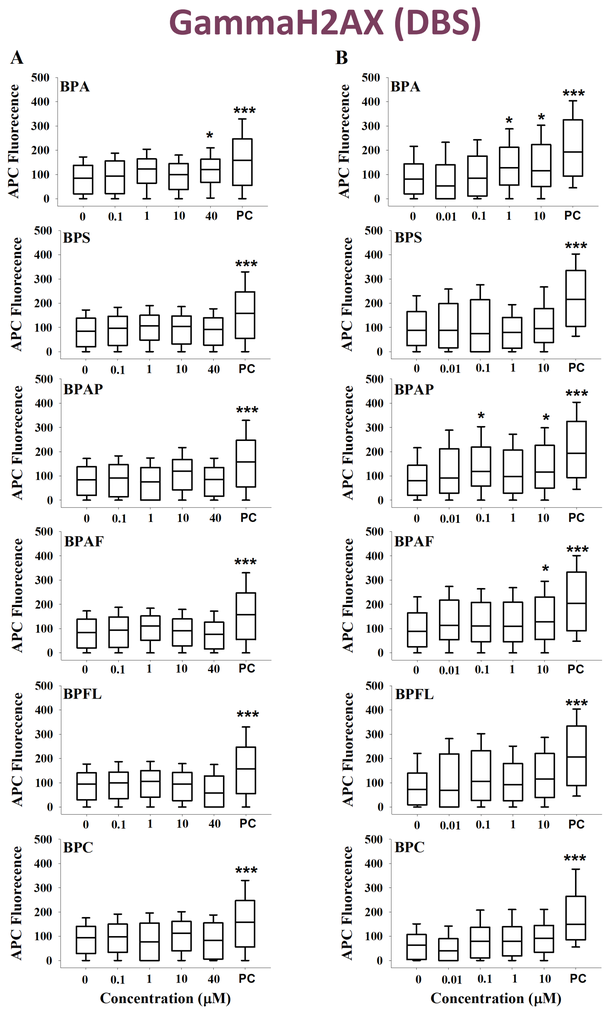


Figure 2: DNA double-strand breaks (assessed by γH2AX using flow cytometry) in HepG2 cells from spheroids after exposure to selected BPs for 24 (A) and 96-h (B). Etoposide (1.7 μM) served as a positive control (PC). The distribution of data is presented in box-plots using SigmaPlot.11. Significant differences between treated samples and the solvent control (0) for γH2AX were tested using R software by the Mixed Effects Models (nlme) package by REML [p<0.05 (*), p<0.001 (**), p<0.0001 (***)]. The experiments were repeated three times independently.

pH3 (mitotic cells)

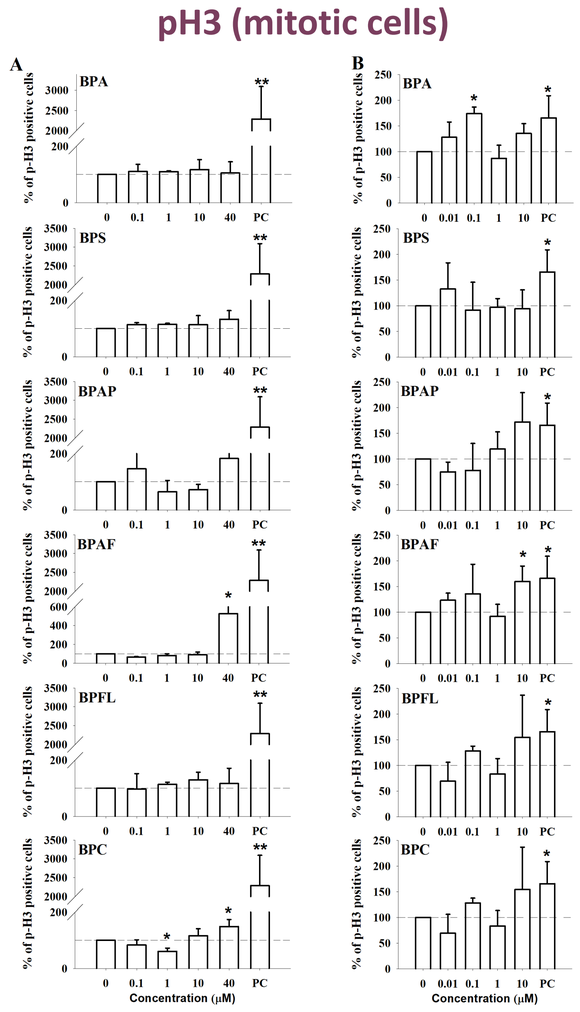


Figure 3: Percentage of p-H3 positive cells compared with solvent control in HepG2 cells from spheroids after 24 (A) and 96-h (B) exposure to selected BPs, as measured by flow cytometry. Colchicine (0.1 μM) served as a positive control (PC). Results are presented in bar charts as means (N=3) and statistical analysis was performed using SigmaPlot.11 software by Two-way ANOVA with a Dunnett's post hoc test [p<0.05 (*), p<0.001 (**)]. The experiments were repeated three times independently.

Cell cycle distribution

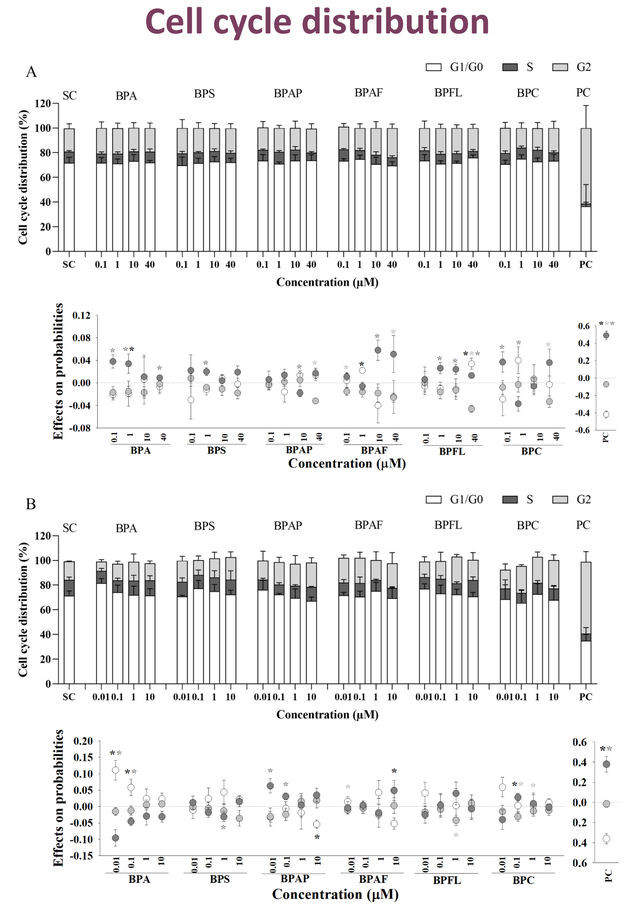


Figure 4: Distribution of HepG2 cells from spheroids across cell cycle phases after exposure to selected BPs for 24 (A) and 96-h (B) and calculated effects of predicted probabilities with 95 % CIs after 24-h (A.1) and 96 h (B.2) of exposure in respect to SC. Cells were labelled with Hoechst dye and analysed by flow cytometry. Etoposide (1.7 μM) was considered as a positive control (PC). The % of cell cycle distribution is presented as mean (N=3) in SigmaPlot.11 and statistical analysis was performed by multinomial logistic regression in STATA15. Asterisks show significant differences for G1 (black asterisks), S (dark grey asterisks) and G2 (light grey asterisks) phases compared to the solvent control [p<0.05 (*)]. The experiments were repeated three times independently.

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