

Model matters: Comparative toxicity of BPA and BPS-MAE in 2D and 3D HepG2 models

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Background: The phase-out of Bisphenol A (BPA) led to the introduction of analogues such as Bisphenol S 4-allyl ether (BPS-MAE), yet concerns remain about their safety. Evaluating their genotoxicity is paramount to hazard assessment and regulation. New Approach Methodologies (NAMs), including three-dimensional (3D) cell culture models, are increasingly adopted in toxicology and enhance human relevance and reduce reliance on animal testing, gaining trust and acceptability in regulatory frameworks like the European Partnership for Assessment of Risk from Chemicals (PARC).

Aim: To comparatively assess the cytotoxicity and genotoxicity potential of BPA and BPS-MAE across 2D and 3D HepG2 cell culture systems, following the characterization of the HepG2 3D spheroid model established via liquid overlay technique.

Methods: HepG2 spheroids (2,500 cells) were maintained for 13 days and characterized by total cell counting, metabolic activity (Alamar Blue assay), and morphological analysis. Cytotoxicity and genotoxicity of BPA and BPS-MAE were assessed in 2D and 3D HepG2 cell cultures via Cytokinesis-Block Micronucleus (CBMN) assay.

Results: Preliminary characterization showed stable spheroid morphology over 13 days, and an observed increase in metabolic activity in 3D versus 2D cultures. Preliminary cytotoxicity data revealed model-dependent responses. Both compounds exhibited higher cytotoxicity in 2D than in 3D cultures: BPA: \approx 55% at 160 μ M in 2D vs. 35% in 3D cultures; BPS-MAE: \approx 55% at 60 μ M in 2D and at 80 μ M \approx 28% in 3D cultures. Additionally, BPS-MAE exhibited higher cytotoxicity than BPA in both models. Genotoxicity assessment is underway.

Conclusions: Spheroids showed higher tolerance to both compounds, likely attributable to diffusion barriers, structural complexity, or differential metabolic activity, possibly related to cells organization in a tissue-like structure. The fact that BPS-MAE was more toxic to liver cells than BPA suggest that a regrettable substitution may have occurred. These findings underscore the critical importance of NAMs development in genotoxicity.

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