Investigating the Links Between the Effect of Bisphenol Variants in an Engineered Breast Cancer Model

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Introduction

Bisphenols are endocrine-disrupting chemicals commonly occurring in environments and are detected in 90% of the urine samples tested globally [1]. Found in food packaging, toys, water pipes, and medical tubing, it is an environmental health concern due to its potential toxicity. During prolonged exposures, they inactivate tumour-suppressing genes and lead to the activation of oncogenes contributing to breast cancer, one of the most frequently diagnosed among women in developed countries [2,3]. In this pilot study, bisphenol-A (BPA) and its analogues bisphenol-S (BPS) and bisphenol-F (BPF) were tested for their effects on proliferation, migration, and viability, in wildtype human adenocarcinoma cells MCF-7 and a CRISPR-engineered clone B4 lacking the expression of the newly identified breast cancer gene *ZFP36L1* [4]. The gene located on the long arm of human chromosome 14 has been implicated in regulating inflammatory pathways, potentially suppressing tumour development and progression [5]. Our findings indicate that the *ZFP36L1* knock-out clone B4 exhibited increased sensitivity to both BPA and its analogues suggesting that BPA exerts the most pronounced toxicity on the cells. The observation is supported by alterations in clonal viability and migration in the presence of BPA, BPS, and BPF consistent with the trends observed in chemosensitivity tests. We anticipate that our subsequent research in a non-cancerous model MCF12A will enhance the comprehensive understanding of the link between bisphenols and cancer progression in the context of the absence of a cancer driver gene.

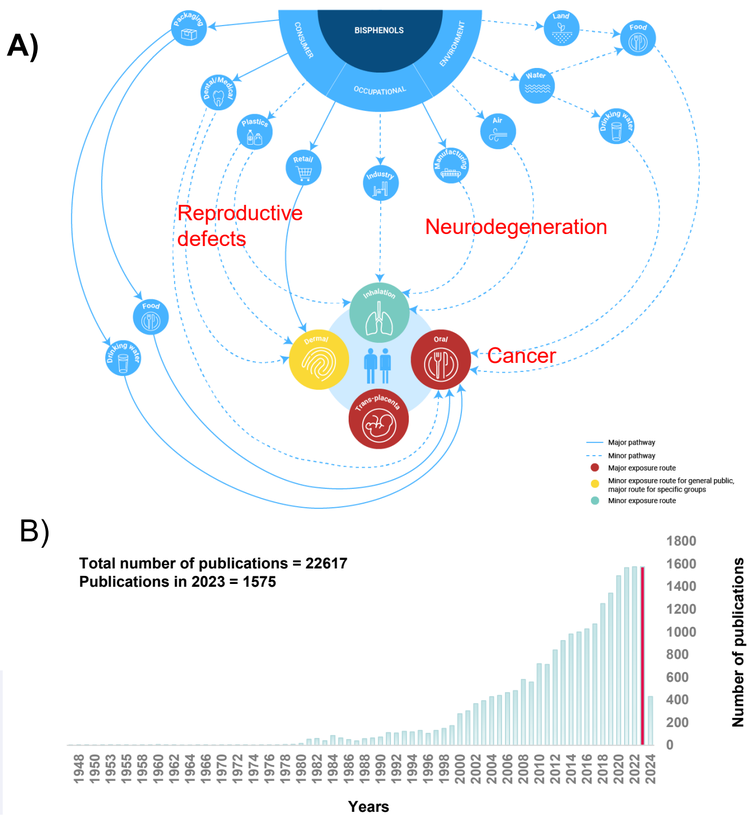
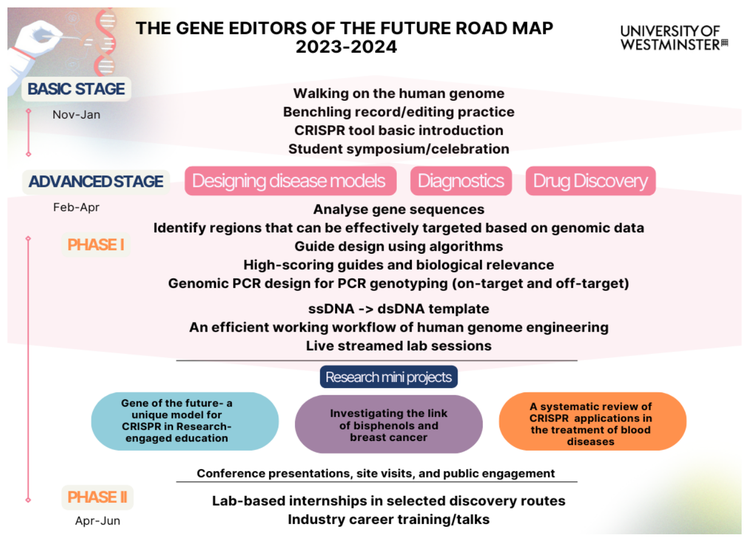


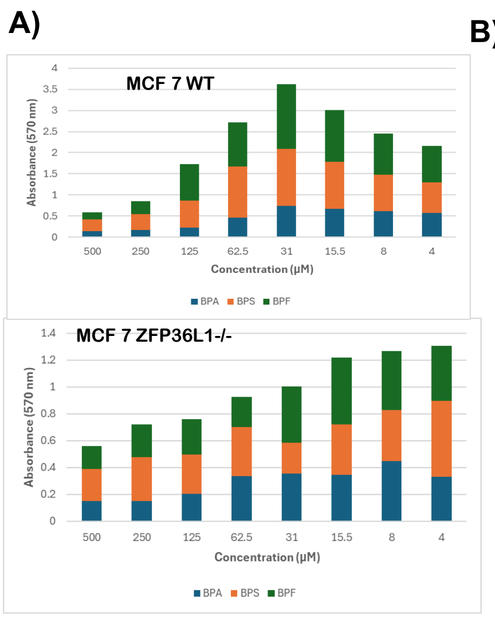
Figure 1. A) Overview of exposure sources and routes for bisphenols and potential health risks. BPA has been found in various foods, with detection rates ranging from 36% to 91% and concentrations ranging from <0.14 to 730 ng/g wet weight. Other bisphenol analogues BPB and BPS were detected in canned foods and beverages at concentrations ranging from <2.3 to 170 ng/g. B) Increase research awareness in Bisphenols in 2023 as indicated by the number of publications on PubMed

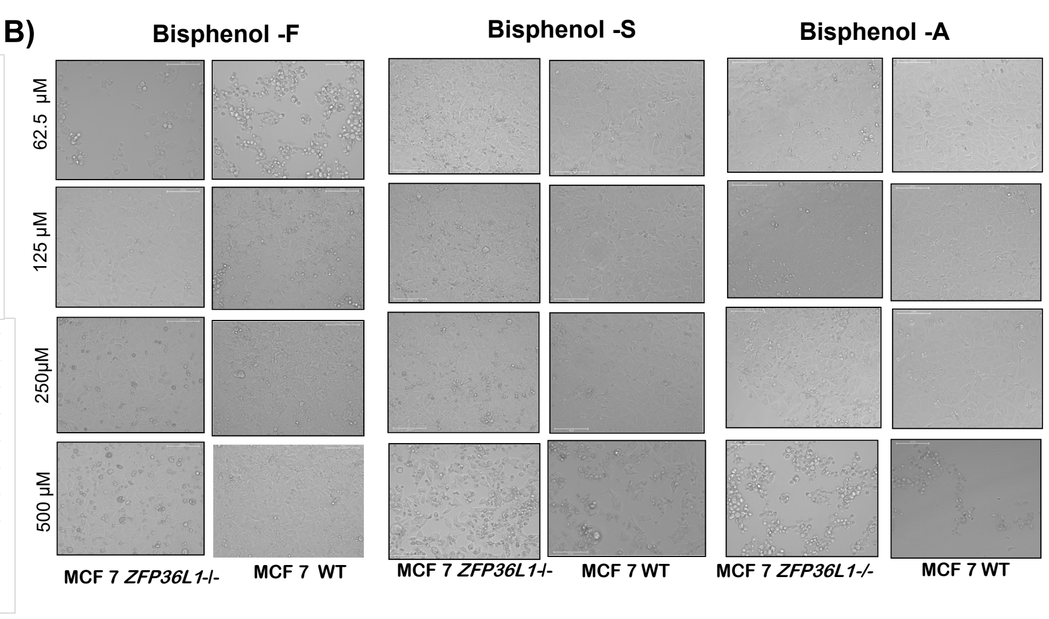


The revolutionising CRISPR-Cas systems, a result of decades of research, have transformed several areas of biotechnology and biomedical sciences in the last 10 years. The work on bisphenols presented here is derived from the initial stages of a mini-project on Gene Editors of the Future, an extracurricular initiative of the School of Life Sciences at the University of Westminster. This initiative is built around the Nobel Prize-winning technology in Chemistry, bringing cutting-edge technologies closer to students of all levels and exploring various aspects of innovation.

Gene Editors of the Future in Research – Engaged Education and at the crossroads of curricular and extracurricular activity

Analysing the effects of Bisphenol variants in a CRISPR-engineered breast cancer model





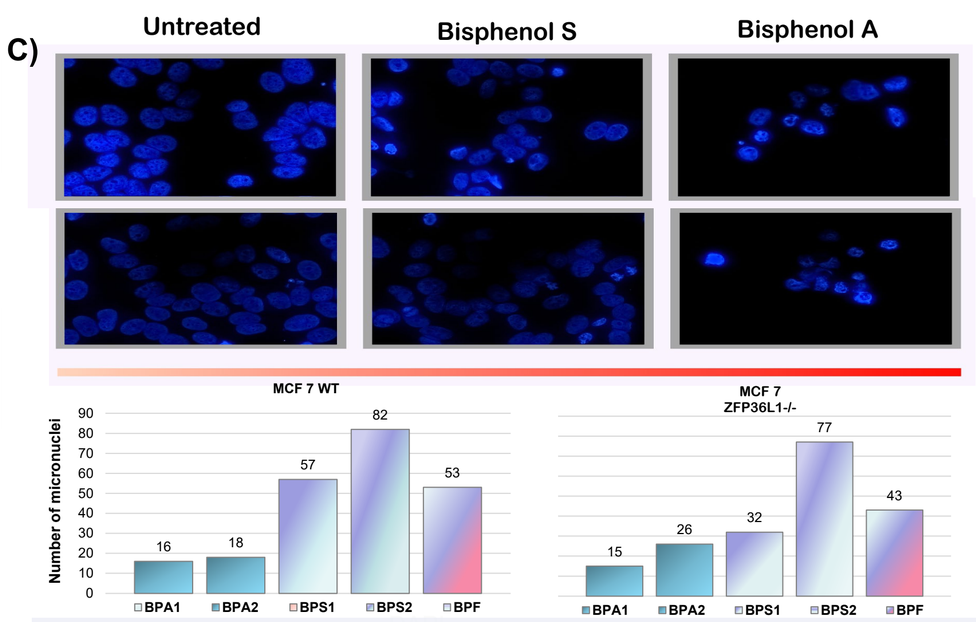


Figure 3A Crystal Violet assay was performed in WT MCF7 and *ZFP36L1* -/- clones B4 and F7 to determine the effects of bisphenols at the indicated concentrations, for 48 hours. This was followed by a brief washing step and staining with 100 µl of Crystal Violet in a solution containing 20% methanol. **(A)** represents the absorbance determined at 570nm. The data represent the mean values. **(B)** shows representative images acquired after 48-hour treatment with rutin under the EVOS cell imaging system.

Figure 3C Analysis of nuclear morphology post bisphenol treatment. 400,000 cells were plated on glass coverslips and allowed to attach for 48 hours. Cells were either left untreated or treated with 31 µM of indicated for 72 hours and subsequently mounted and stained with Prolong Antifade DAPI (Invitrogen) for analysis under 20x objective of an Olympus BX41 microscope coupled to the Elite Micropix digital camera. Bar graphs indicate quantification of micronuclei.

Bisphenols slowed the proliferation of breast cancer cell lines

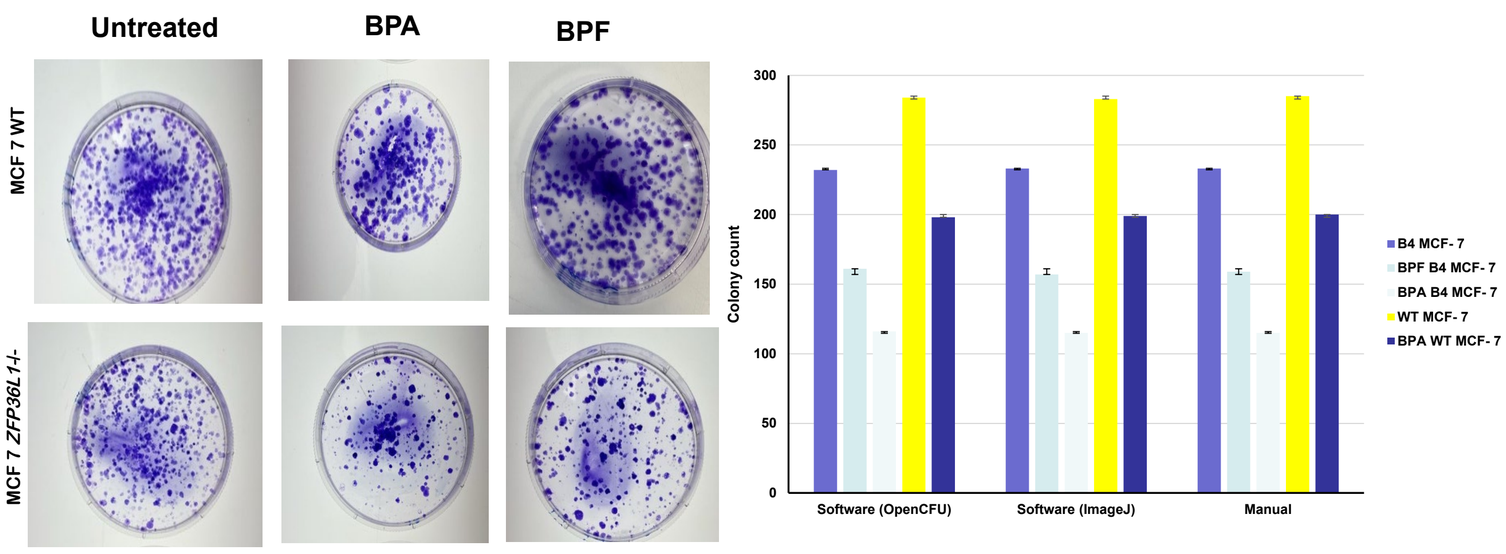


Figure 4 500 cells per well were plated in 10 cm plates and treated with similar concentrations of bisphenols investigating the long-term and short-term proliferative potential and survival capabilities of the plated cells in the presence and absence of the indicated compounds. The observed sensitivity in the ZFP36L1 ablated clone B4 suggests the potential nature of toxicity of the bisphenols used which can be demonstrated precisely in a genetically manipulated cell population lacking the expression of a cancer driver gene..

Conclusions

- Research-engaged education is crucial for developing critical thinking skills, enhancing learning experiences, and fostering creativity and innovation. It equips students with practical skills, prepares them for further education and careers, and empowers them to contribute to knowledge and society.
- The sensitivity profile of MCF7 cell variants underscores the diverse responses to different bisphenols in human cells. *ZFP36L1-/-* B4 cells exhibit more sensitivity compared to wild-type counterparts.
- Clonogenic assay results reveal distinct proliferation patterns among precisely edited MCF7 cell variants. While both the wild-type (WT) and *ZFP36L1-/-* B4 cells demonstrated robust proliferation in a population of 500 cells, *ZFP36L1-/-* cells showed lesser plating efficiency, possibly due to impaired cell-to-cell contact or other unknown features in the presence of bisphenols A and F.
- Further investigation is crucial to unravel the sensitivity of normal cells to specifically evaluate the direct involvement of *bisphenols* in a variety of potential damages occurring to cells.
- Gene Editors of the Future has fostered an environment that cultivates a multifaceted skill set among undergraduates, postgraduates, and PhD students—hands-on research experiences equipped students with critical thinking abilities and interpreting data. Students exhibited proficiency in experimental design, data analysis, and laboratory techniques, fostering a robust foundation in scientific inquiry.
- Teamwork, communication, and collaboration with peers and mentors further refine their interpersonal skills, keeping time management at the top of their learning experience.

References

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