A mixture design with nanoplastics and bisphenol A: cytotoxicity and genotoxicity assessment

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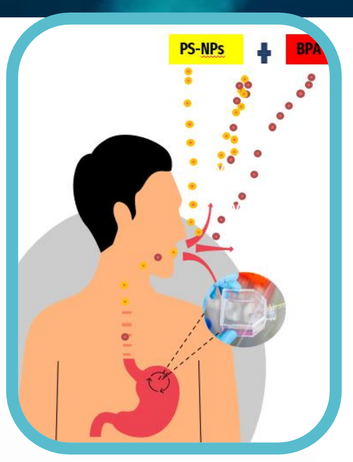
Aim

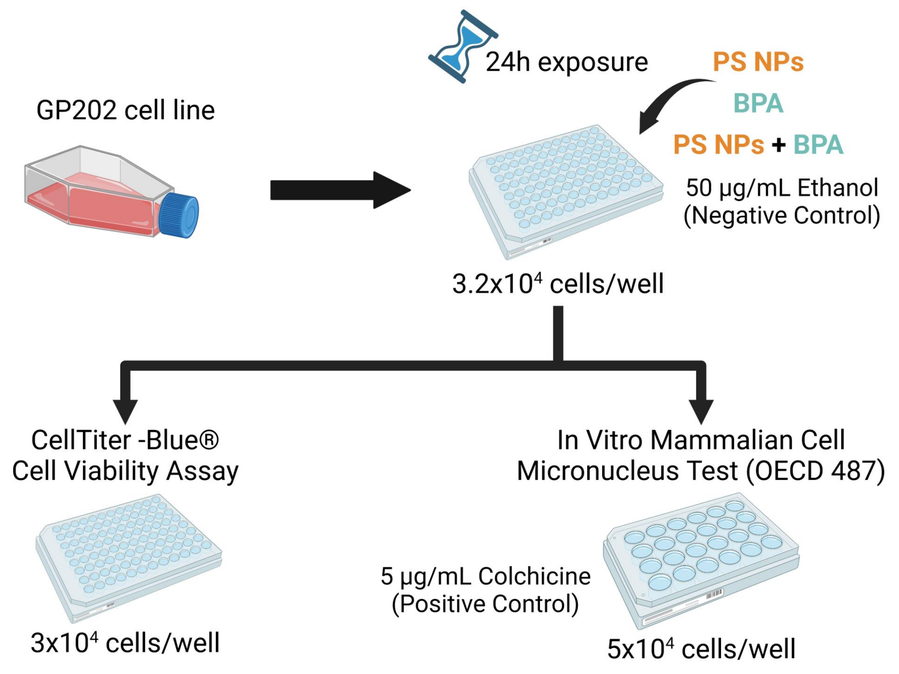
The aim of this *in vitro* study was to investigate the cytotoxicity and genotoxicity of polystyrene (PS)-NPs and BPA *per se* and in combined exposures on the gastric GP202 cell line. For that, environmental relevant concentrations previously tested in literature were selected, for PS-NPs - 20, 100 and 200 µg/mL and for BPA - 0.1, 1 ng/mL, and 0.04 ng/mL as the new reference value proposed by EFSA.



Introduction

The widespread circulation of nanoplastics (NPs) and bisphenols (BPs) in the environment, inevitably results in complex interactions and associated detrimental effects, which have the potential to reach the human body by multiple routes. Considering the levels of plastic oral exposure via ingestion and the absorption of these substances in gastric tissues, NPs endorse hazardous potential given the evidence of toxic effects on a cellular level. Furthermore, the ability of acting as a vector for other pollutants and its ability to be internalized by cells potentiate long term effects. Bisphenol A (BPA) is a xenoestrogen, exhibiting hormone-like properties that mimic the effects of estrogen in the body and is widely used in consumer products.

Methods



$$CBPI = \frac{[\text{Number of mononucleate cells}] + 2\times[\text{Number of binucleate cells}] + 3\times[\text{Number of multinucleate cells}]}{[\text{Total number of cells}]}$$

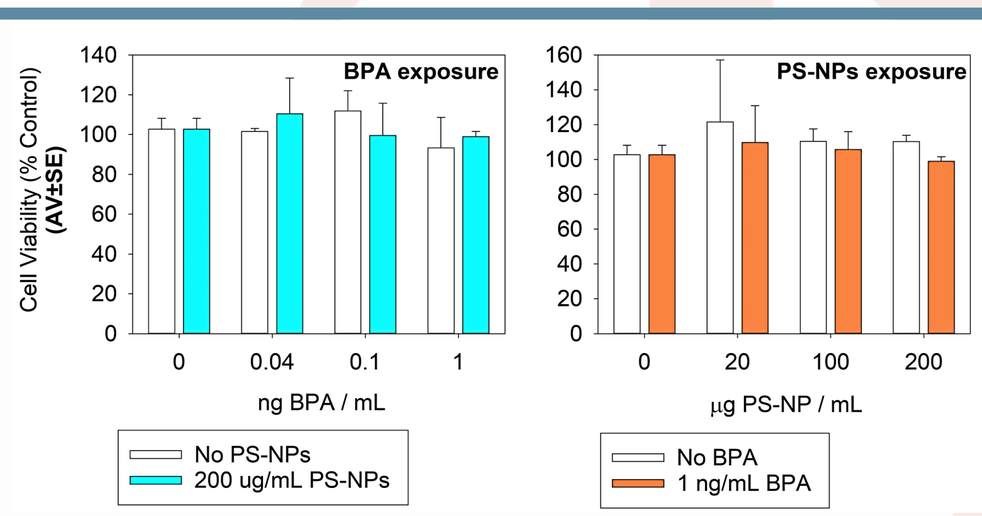
Data Analysis:

One-way ANOVA + post-hoc Dunnet's (individual exposure)
Two-way ANOVA + post-hoc Tukey test (combined exposure)

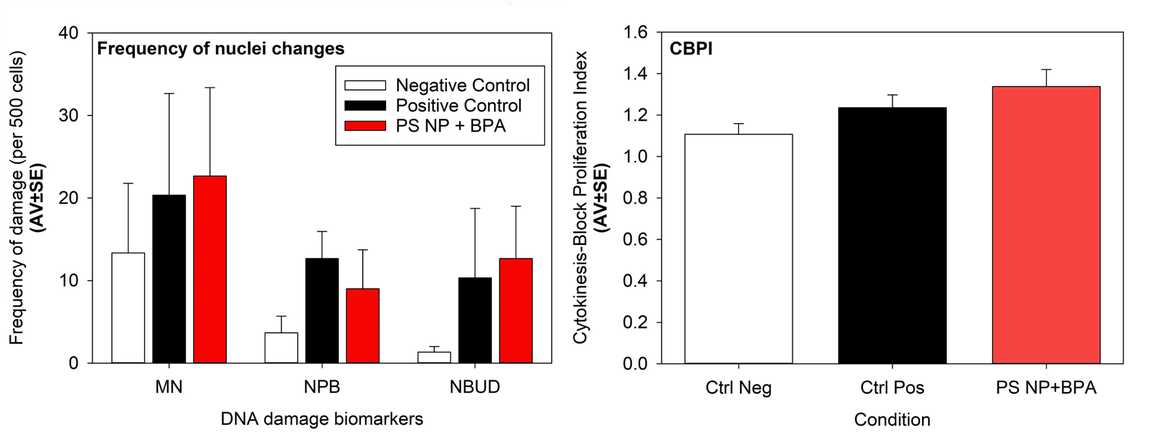
Table 1: Concentrations used for individual exposures (I) and combined exposures of PS-NPs and BPA (C) for cytotoxicity assays and for genotoxicity assays (G).

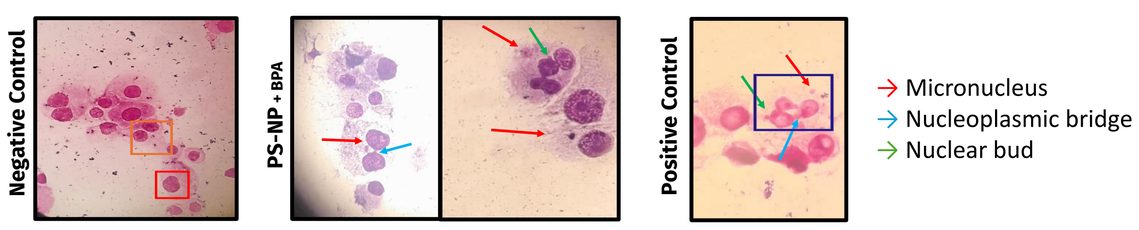
PS-NPs (µg/mL) \ BPA (ng/mL)	0	0.04	0.1	1
0	I	I	I	I+C
20	I	I	I	I+C
100	I	I	I	I+C
200	I+C	I+C	I+C	I+C+G

Results



No significant differences were observed in both individual and combined exposures to PS-NPs and BPA.





Combined exposure at the highest concentrations (200 µg/mL PS-NPs and 1 ng/mL BPA) increased **micronuclei** frequency.

Conclusions

- ✓ No cytotoxic effects associated to either individual or combined exposures.
- ✓ Genotoxicity was tested for the worst-case scenario of combined exposure, being all the endpoints – micronucleus, nucleoplasmic bridges and nuclear buds, significantly increased (200 µg/mL PS NPs + 1 ng/mL BPA) (69±6.15, 26±2.73 and 37±3.15) in comparison with negative control (40±6.56, 11±1.53 and 4.0±0.58).
- ✓ These results highlight the importance of further studies with combined exposures of NPs with BPA and other chemicals of concern, since NPs are known to act as vectors.

Aknowledgements

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