

# Hazard effects associated with microplastics mixed with *Pseudomonas lurida* in human lung alveolar A549 cells

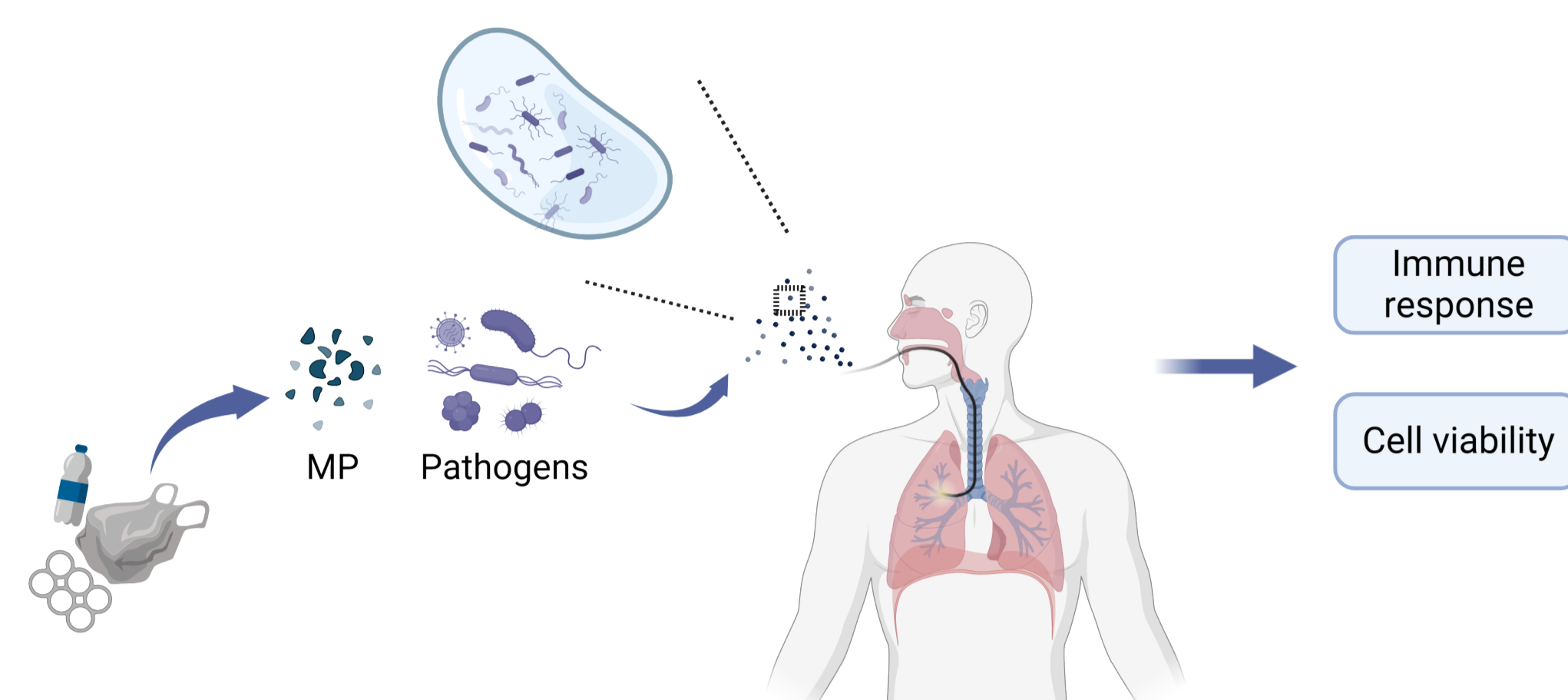
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## INTRODUCTION

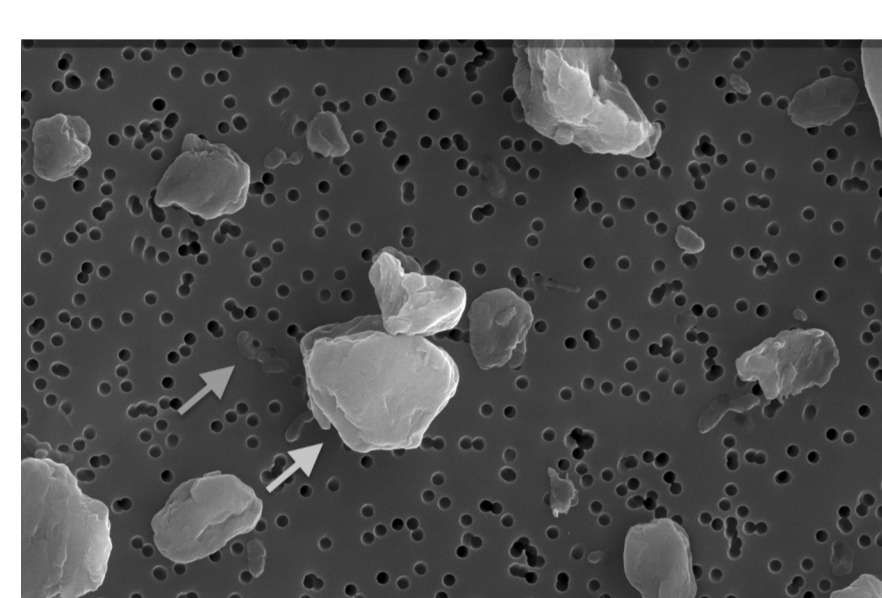
Plastic particles are ubiquitous and known to be persistent in the environment. Micro- and nano-plastics (MNPs) can serve as carriers for microbial pathogens and chemical compounds. Our knowledge on the potential health hazards associated with MNPs contaminated by bacterial particles remains limited.

Here, we report the toxic effects of high-density polyethylene microparticles (HDPE-MPs) mixed with heat-inactivated *Pseudomonas Lurida* (PL) in human lung alveolar type II cells (A549).

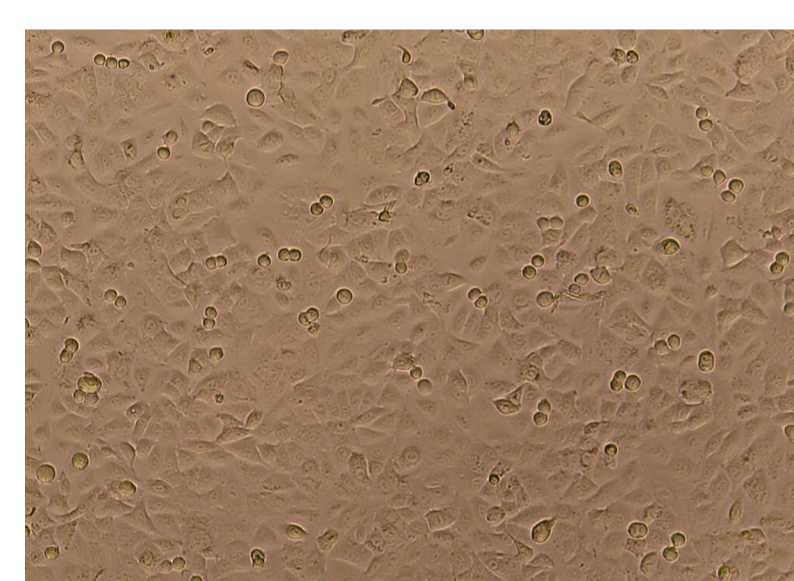


Plastic polymers degrade into MNPs, which can continue to exist in the environment. These particles may serve as a carrier for pathogens and cause potential harm. Exposure to MNPs via the respiratory route can potentially lead to inflammation, cell damage, oxidative stress and necrosis.

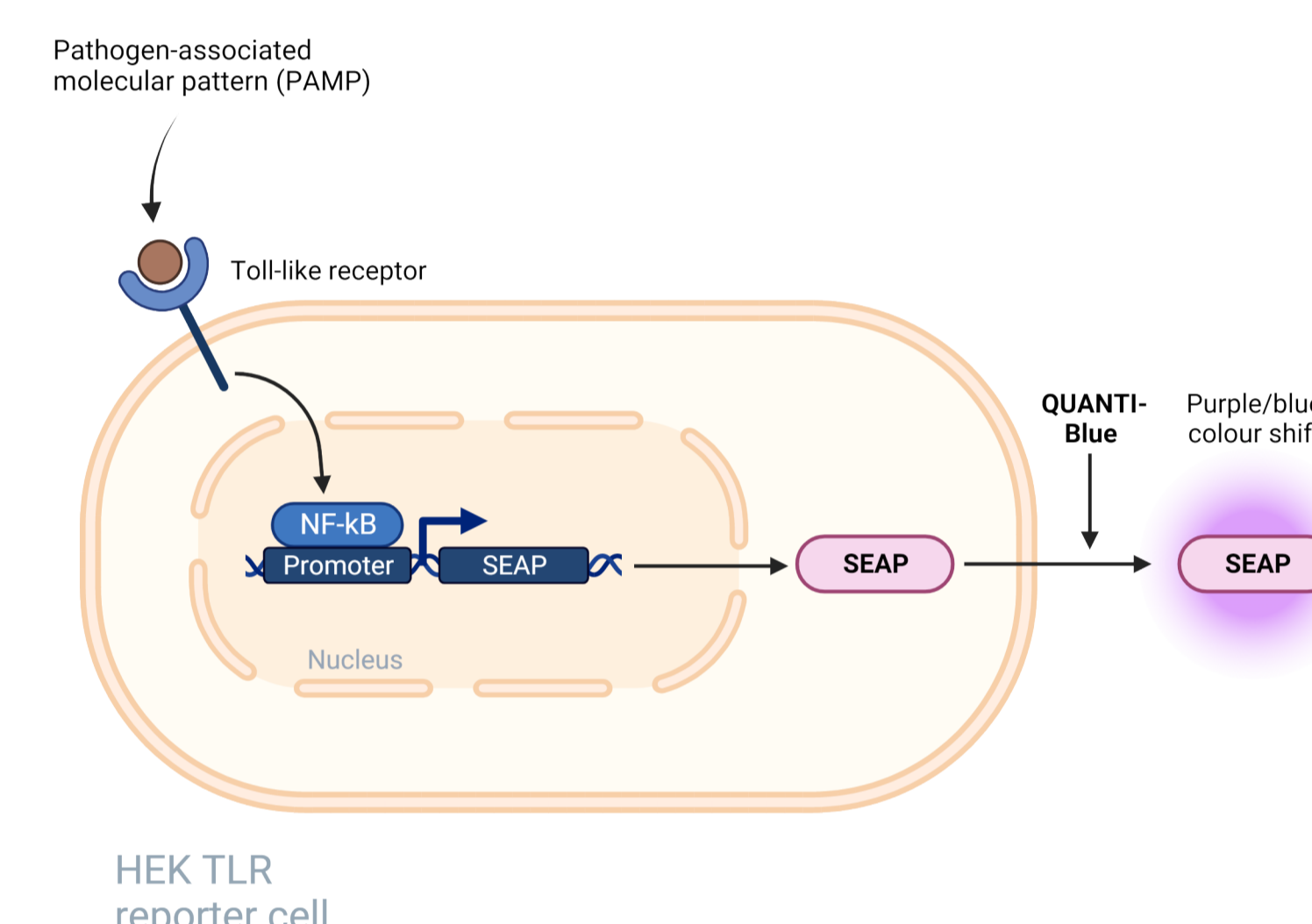
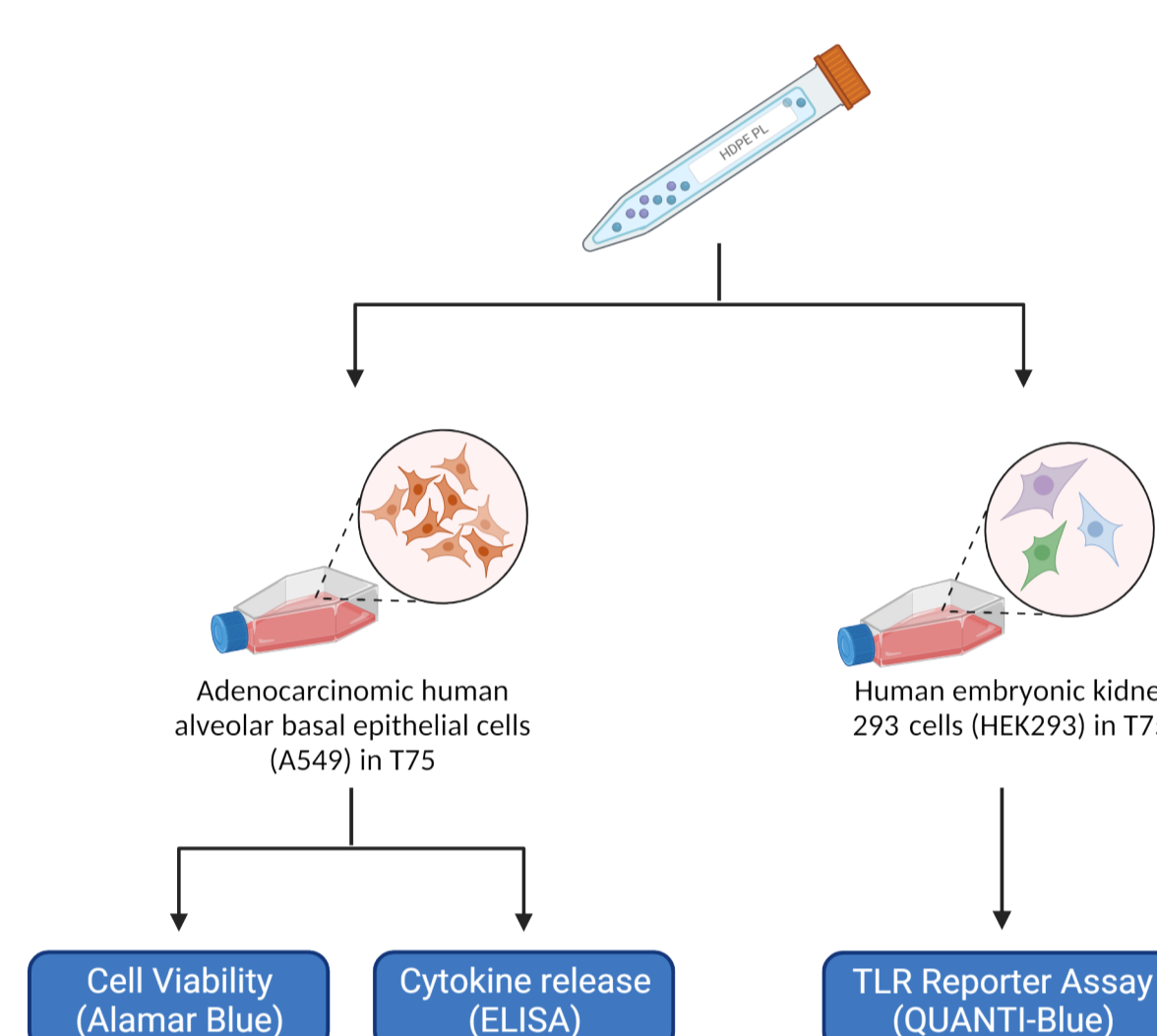
## MATERIAL & METHODS



High resolution field scanning electron microscopy (FESEM) image of HDPE-MP (lighter arrow) and *P. Lurida* (darker arrow)



Human alveolar type II cells A549 were used as a model system for the investigation of cytotoxic effects and inflammatory responses in this study.

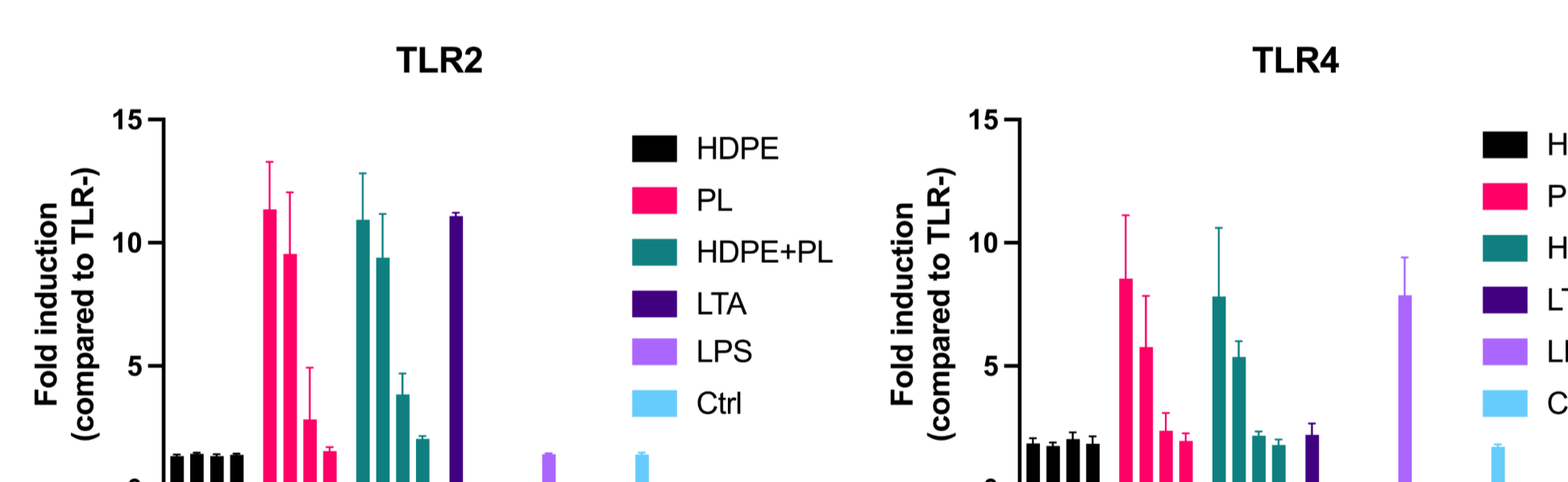


A549 cells were exposed to HDPE-MPs (d 50= 5µm) mixed with heat-inactivated *Pseudomonas Lurida* at doses between 0 and 200µg/mL for the plastic particles and 0 and 2x10<sup>6</sup> bacterial particles for PL.

The immune response to the HDPE-MP + PL mixture was investigated by the activation of toll-like receptor (TLR) 2 and 4 in HEK293 reporter cells.

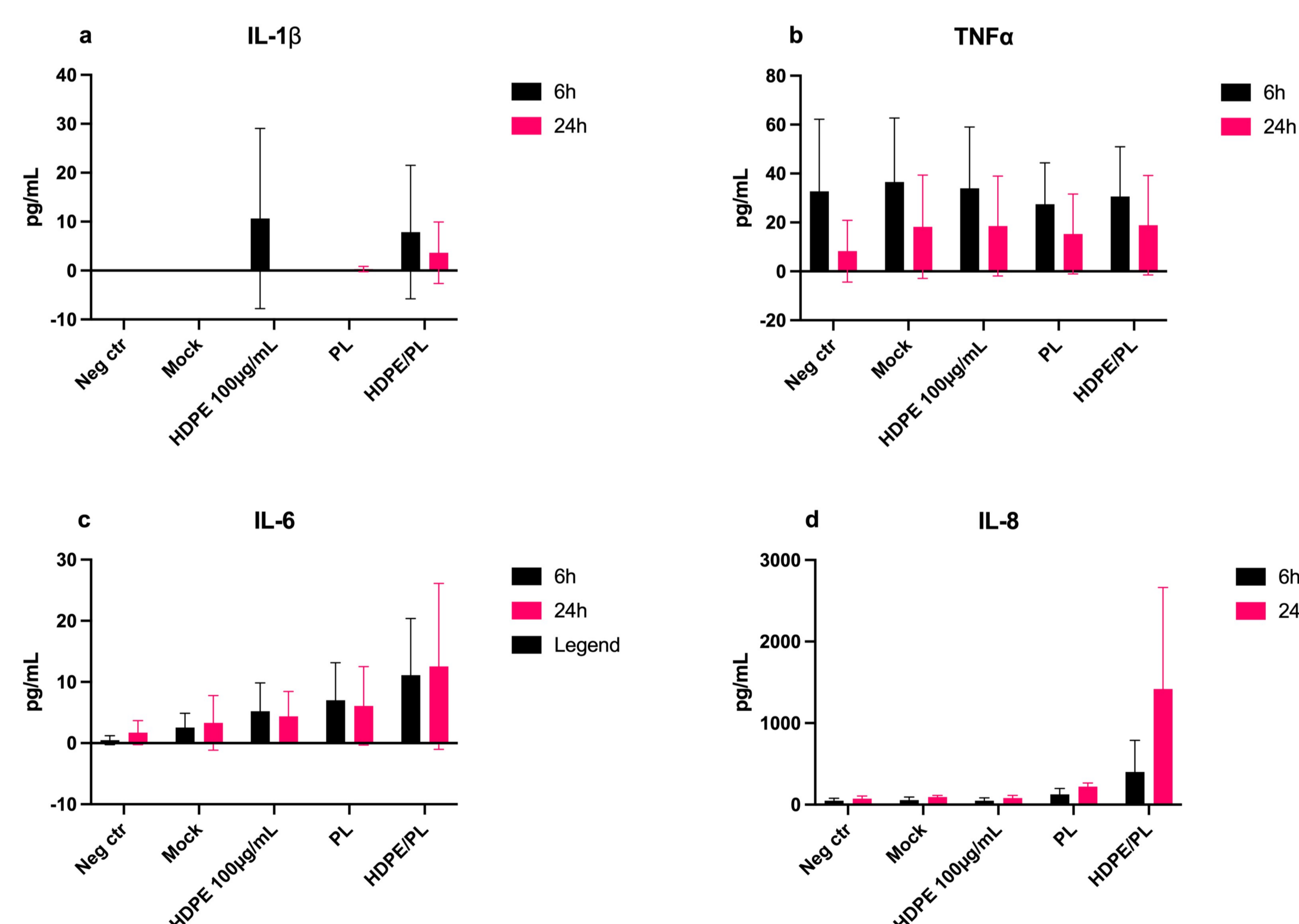
## RESULTS

### HEK-TLR Reporter Assay



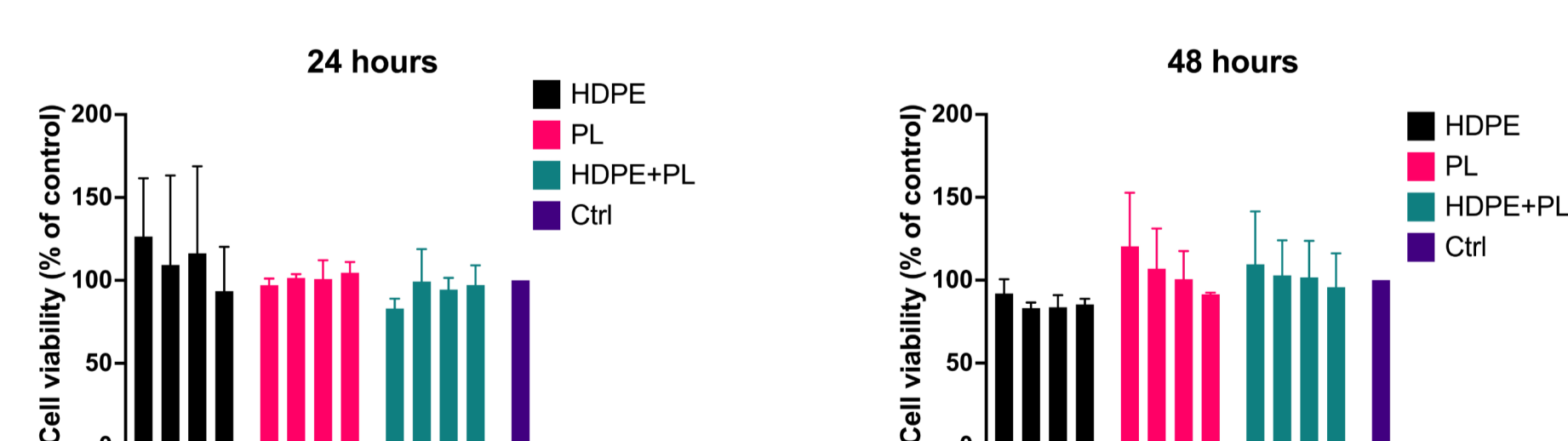
HEK reporter assay with HEK293 hTLR2 cell line (from left) and hTLR4 cell line to the right. From left black bars HDPE 200 µg/mL, 100 µg/mL, 50 µg/mL and 1000 µg/mL. Pink bars from left; PL particles 2x10<sup>6</sup>, 2x10<sup>5</sup>, 2x10<sup>4</sup> and 2x10<sup>3</sup> and green bars HDPE 100 µg/mL mixed with 2x10<sup>6</sup>, 2x10<sup>5</sup>, 2x10<sup>4</sup> and 2x10<sup>3</sup>. Controls; violet bar; LTA 10 µg/mL purple bar; LPS 10µg/mL and blue bar; negative control.

### Cytokine release after Exposure of HDPE-PL



ELISA cytokine release (pg/mL) after 6h and 24h exposure with HDPE 100 µg/mL, 2x10<sup>5</sup> bacterial particles and HDPE 100 µg/mL mixed with 2x10<sup>5</sup> *P. Lurida*.

### Cell viability Assay after 24h and 48h exposure to HDPE+PL



Cell viability assay (AlamarBlue) in A549 cells after 24h/48h exposure to HDPE-MPs (d 50= 5µm), PL particles and mixture of HDPE+PL. From left black bars HDPE 200 µg/mL, 100 µg/mL, 50 µg/mL and 1000 µg/mL. Pink bars from left; PL particles 2x10<sup>6</sup>, 2x10<sup>5</sup>, 2x10<sup>4</sup> and 2x10<sup>3</sup> and green bars HDPE 100 µg/mL mixed with 2x10<sup>6</sup>, 2x10<sup>5</sup>, 2x10<sup>4</sup> and 2x10<sup>3</sup> and purple bar; negative control.

## CONCLUSION

Preliminary results indicate no significant cytotoxic effect of HDPE-MP and PL, or the mixture. Moreover, HDPE-MP alone does not activate TLR2 or TLR4 and induces no release of pro-inflammatory marker. However, the mixture HDPE-MP +PL activates TLR2 and TLR4 and induces the release of IL8 after 6h and 24h exposure.