Reproductive and developmental toxicity of parabens and their mixtures

Preliminary results

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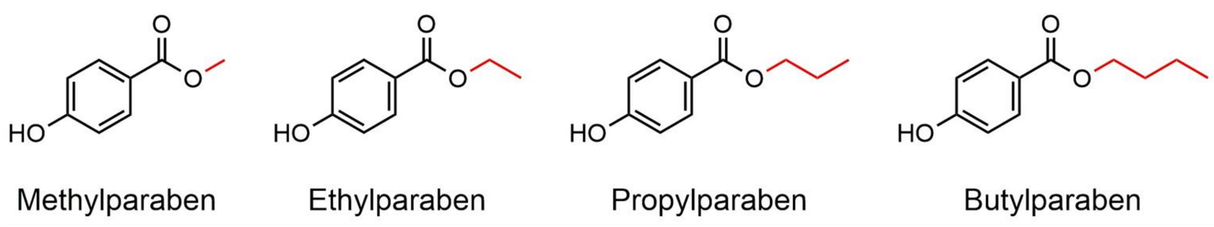
Background

Male reproductive health disorders, such as testicular cancer, malformation of sex organs in newborn boys, and general poor sperm quality among young men, are increasing in the human population. The reason for this trend is unknown, but it is hypothesized that prenatal exposure of the fetus to **endocrine disrupting chemicals** may contribute to the development of these health problems [1]. Parabens are known to be able to **antagonize the androgen receptor** (AR) – one of the key molecular initiating events leading to adverse outcomes on male reproductive health [1, 2]. Importantly, humans are not exposed to single chemicals at any given time, but rather to complex mixtures of a variety of chemicals, which combined may potentiate their toxicity [3].

The question is: are parabens able to affect the very **early development of the embryo**? And what is **the total mixture effect** to these parabens during development and on reproductive health?



Methods

The 4 parabens methyl-, ethyl-, propyl and butylparaben (**MP, EP, PP** and **BP,** respectively) were initially run as single chemicals on 2 cell-based assays, AR EcoScreen™ and PluriLum assays.

The **AR EcoScreen™** human AR transactivation reporter gene assay validated by OECD were used to test for AR activity [4]. Cells were exposed to the parabens in the presence of 0.1 nM of the AR agonist R1881. Data was normalised to the reference compound for AR antagonism, hydroxyflutamide (OHF).

The **PluriLum** 3D reporter gene assay was used to test for developmental toxicity. The assay is based on human induced pluripotent stem cells (hiPSCs) with a cardiac endpoint assessing the embryotoxic potential of chemicals [5]. Cells were exposed to the 4 parabens on day 1, 2 and 3 of the differentiation protocol (fig. 2A).

Results

Results so far show that the **single parabens** are able to **antagonize the human AR** in similar way (Fig 1a), where **MP**, the paraben with the shortest side chain, appears as the **least potent** (i.e., higher IC_{50}), while **cytotoxicity increases with the length of the sidechain** (i.e., BP > PP > EP > MP; Fig 1B)**.** Importantly**, levels of PP found in human samples** (adult and cord blood samples) by suspect screening analysis within the **EU H2020 PANORAMIX project** (https://panoramix-h2020.eu/) **falls within its effective/toxic range** (Fig. 1B).

Preliminary results from the **PluriLum assay** indicate that the single parabens are able to interfere with **stem cell viability** (Fig 2B), **cardiomyocyte differentiation** (Fig. 2C) and **contractility** (Fig. 2D), following a structure dependent profile of potency (i.e., BP > PP > EP > MP).

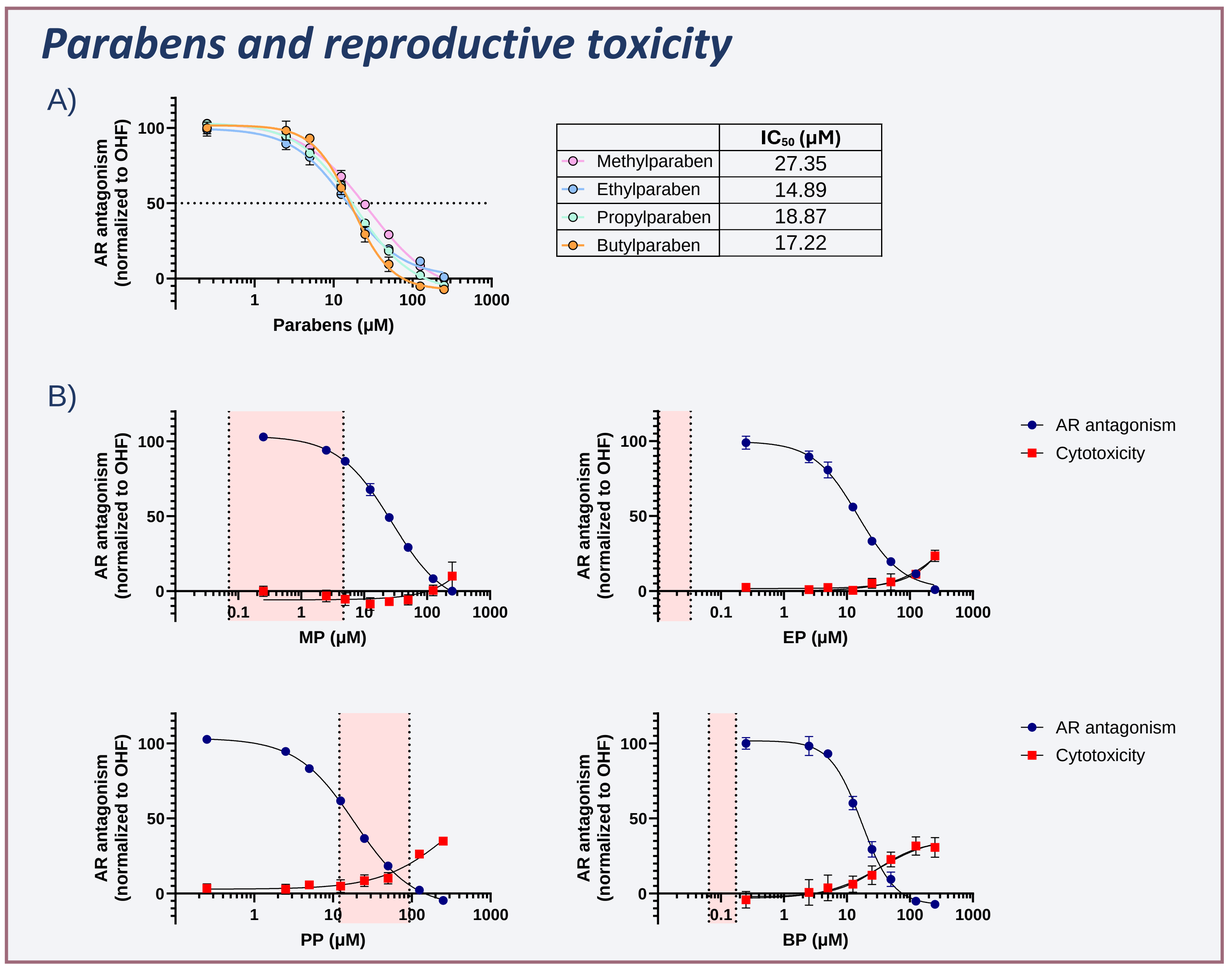


***Figure 1**: A) Concentration-response curves for AR antagonism of the 4 parabens. B) Concentration-response curve for AR antagonism for the individual parabens, methyl-, ethyl-, propyl, and butylparaben (MP, EP, PP, BP). The pink bar shows the range of concentration on levels found in human samples.*

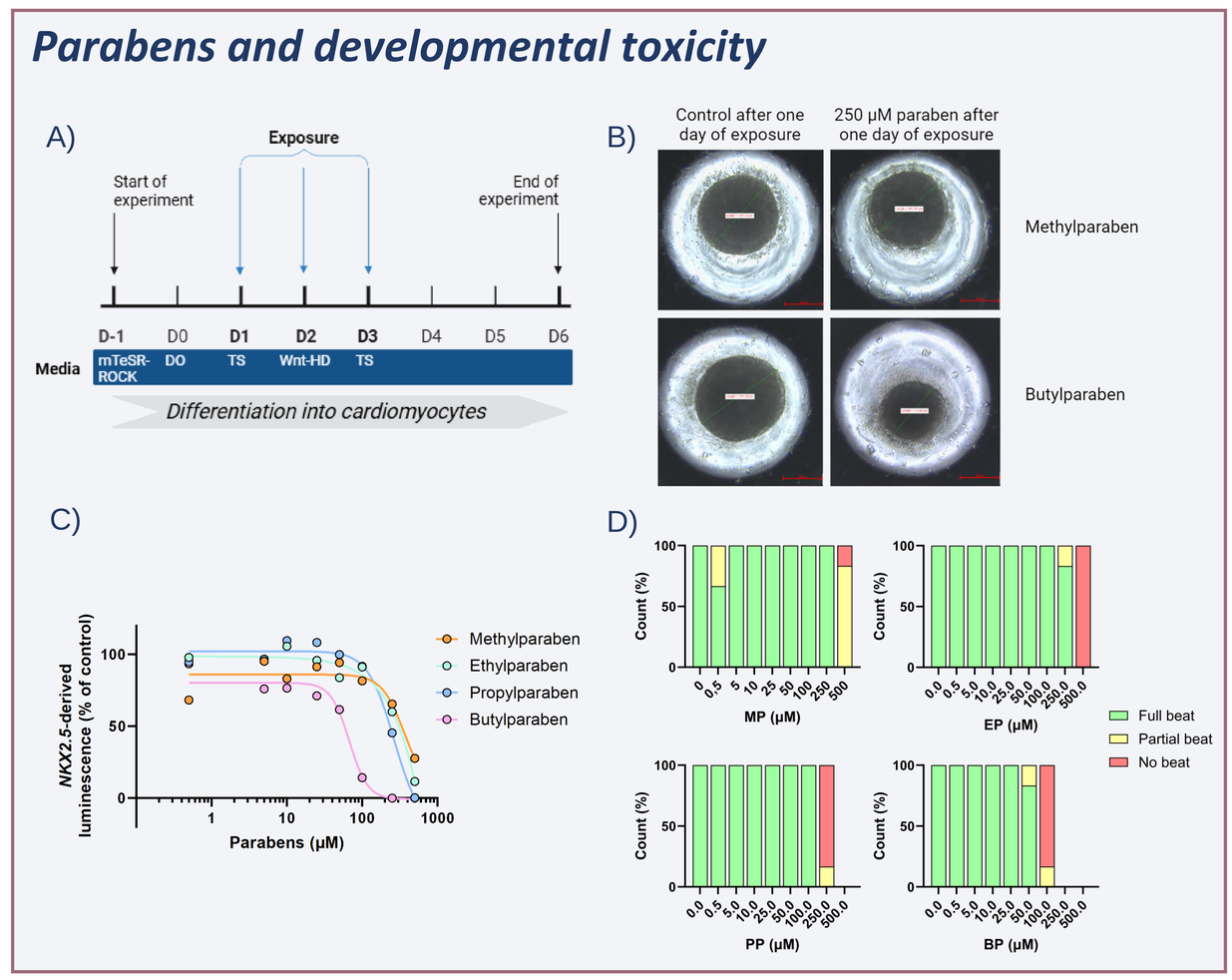


***Figure 2**: A) Schematic overview of the PluriLum assay. B) Images of embryoid bodies after one day of exposure with methyl- or butylparaben. C) Concentration-response curve of NKX2.5 derived luminescence for of the 4 parabens. D) Beat scoring of the embryoid bodies for methyl-, ethyl-, propyl- and butylparaben.*

Conclusions

- Single parabens are able to antagonize the androgen receptor in the AR EcoScreen™ assay and affect cell differentiation in the PluriLum assay.
- The potency of the parabens appear to be related to length of the sidechain, with BP being the most toxic.
- The levels of parabens previously found in human samples fall close or even within the tested range of concentration, with PP being well within toxic ranges

Next steps

Different mixtures of parabens will be prepared based on real-life concentrations quantified (targeted analysis in progress) in samples from the PANORAMIX project and tested in the same models to reflect mixture effects arising from exposure from everyday life.

References

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