Heated tobacco and e-vapour products induce lower biological activity compared to cigarettes using the ToxProfiler assay

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1. INTRODUCTION

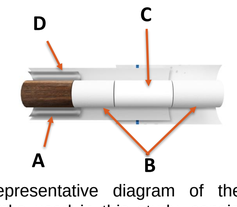
With the rapid pace of innovation with Next Generation Products (NGP), there is a need for quick, sensitive, and mechanistically insightful *in vitro* techniques for product stewardship assessment and evaluation of the harm reduction potential[1]. Here we assessed a commercially available new approach method (NAM), the ToxProfiler reporter assay for the assessment of NGP. This method consists of a panel of 7 unique human liver reporter cell lines that can be applied to visualize and quantify specific cellular stress response pathways (oxidative stress, cell cycle stress, ER stress, autophagy, ion stress, protein stress and inflammation).

2. METHODS

Test Articles

- 1R6F Reference Cigarette (University of Kentucky)
- Heated Tobacco Product (HTP), "Pulze" with "iD stick" (iD Regular) (see Figure 1)
- E-vapour (EVP), "blu 2.0" (Tobacco) (see Figure 2)

Representative image of the HTP device used in this study (reconstituted tobacco stick inserted).



Representative diagram of the HTP sticks used in this study, consisting of reconstituted tobacco (A), filters (B), a cardboard tube (C) and outer paper with aluminium foil (D).

Figure 1: HTP diagram

Representative image of the EVP device used in this study.

Representative diagram of the EVP device used in the study

Figure 2: EVP diagram

Smoke / Aerosol Extract Generation method

Smoke and aerosol from test products was generated with a Vitrocell VC10s (Vitrocell, Munich, Germany) smoking machine. Smoke or aerosol extracts were prepared by bubbling the sample aerosol into 3 in-line Impingers each containing 10 mL Phosphate Buffered Saline (PBS) solution (see Figure 3). A total stock solution of 30 mLs per test article was used: 1.8 puffs per mL for 1R6F cigarette and 4 puffs per mL for the HTP/ EVP.

Figure 3: Bubbling smoke/vapor exposure system

Trapped nicotine and carbonyls were quantified within the aerosol and smoke bubbled PBS (bPBS) samples. Nicotine was quantified using liquid chromatography with tandem mass spectrometry (LC-MS/MS) with an AB Sciex API 6500 QTRAP (SCIEX, Framingham, MA, USA) using nicotine-d4 as the internal standard. For the analysis of Carbonyls, bPBS samples were diluted with 2,4-dinitrophenylhydrazine (DNPH). The carbonyl-DNPH derivates were then quantified using high performance liquid chromatography with a diode-array detector (HPLC-DAD, Agilent Technologies 1100 Series).

Biological Assessment

The ToxProfiler assay was performed by Toxys B.V.

The ToxProfiler assay determines activation of seven specific cellular stress response pathways (oxidative stress, cell cycle stress, ER stress, autophagy, ion stress, protein stress, inflammation) utilising 7 stable genetically engineered human liver HepG2 cell lines[2,3] (see Figure 4). Each one of these cell lines contain a fluorescent reporter for a specific cellular stress signal transduction pathway. These cell lines were exposed to the test articles for 24h±S9. Cells were imaged using an Operetta CLS imager at 24h after treatment. The ToxProfiler assay is considered to have a positive response when a Point of Departure is calculated.

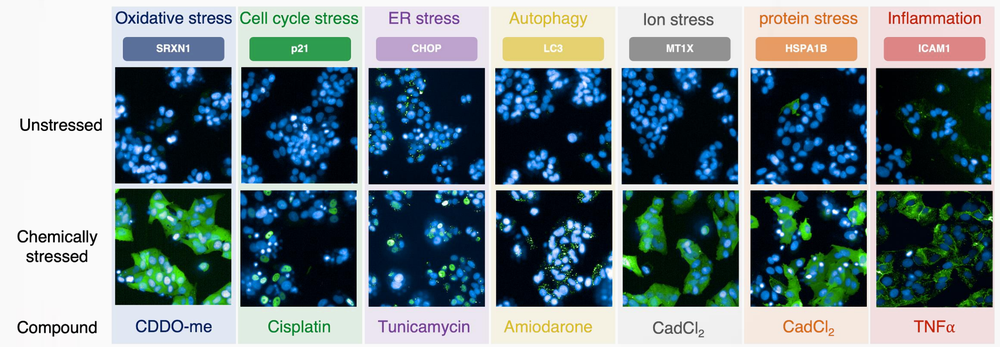


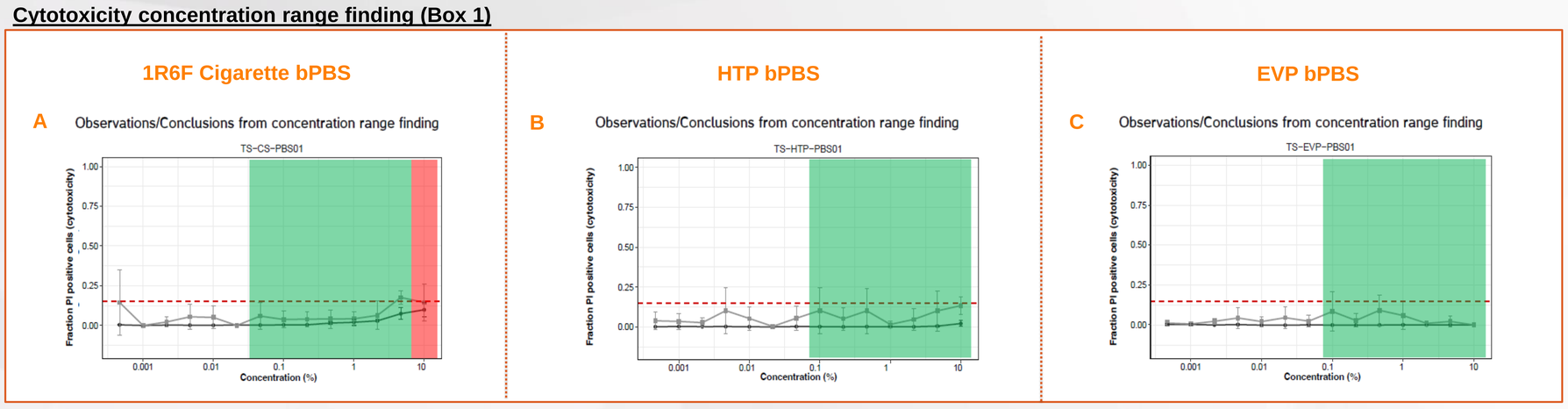
Figure 4: Overview of the stress pathways and biomarkers covered by the ToxProfiler reporter system

3. RESULTS

Dosimetry Nicotine and carbonyl levels in bubbled PBS extracts

- For each test article, PBS from all three impingers was combined to generate 30mls stock for analysis.
- Nicotine and eight carbonyls (Formaldehyde, Acetaldehyde, Acetone, Acrolein, Propionaldehyde, Crotonaldehyde, 2-Butanone and n-Butyraldehyde) were quantified in the bPBS matrix.
- The 1R6F sample delivered the highest levels of nicotine to the PBS (217µg/ml whereas EVP delivered 181µg/ml and for HTP 166µg/ml).
- The 1R6F bPBS samples contained the highest level of carbonyls (levels ranging from 1.64 – 173.09µg/ml). In contrast, the total quantified carbonyls were greatly reduced for the HTP bPBS (94%) and for EVP bPBS (97%).

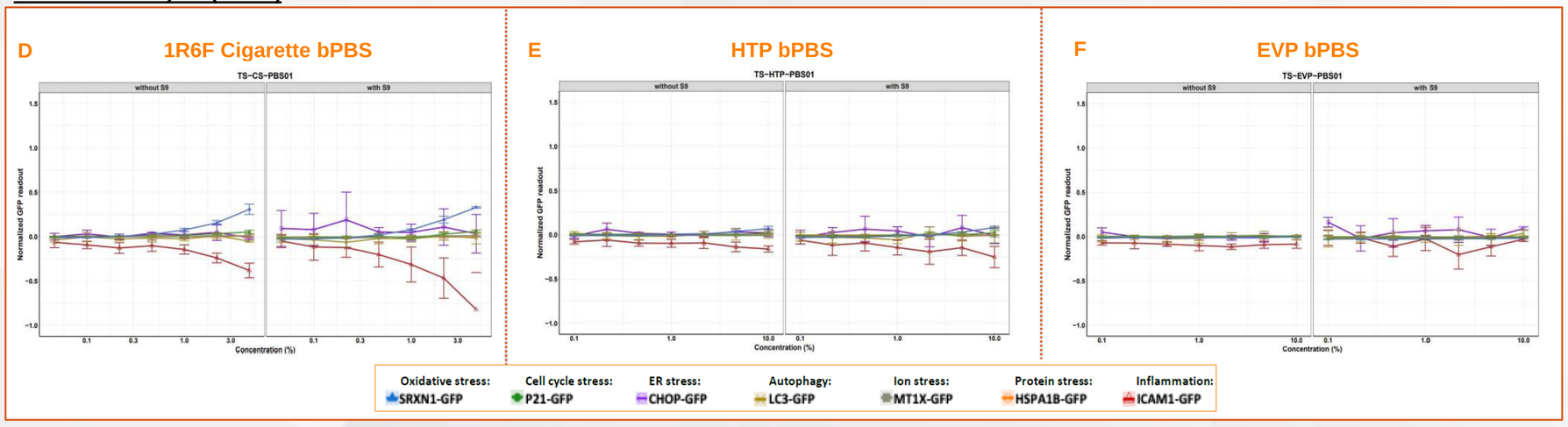
Cytotoxicity concentration range finding (Box 1)



Key – Normalized concentration response plots of cell death data of all compounds exposed in absence (black) or presence (grey) of S9 included in this project at timepoint 24h as measured with the PI stain in the parental HepG2 (wild type) line. Green shaded area: the 7 selected concentrations for the reporter assay, Red shaded area: concentrations excluded due to cytotoxicity. The red line indicates the used threshold of cytotoxicity; 15% PI positive cells (see Box 1).

A – Concentration range of 1R6F Cigarette bPBS, chosen for the reporter assay = 0.046 - 4.6%
B – Concentration range of HTP bPBS, chosen for the reporter assay = 0.1 - 10%
C – Concentration range of EVP bPBS, chosen for the reporter assay = 0.1 - 10%

Biomarker analysis (Box 2)



Key – Point of departures (PoDs) were determined for all samples, with the lowest POD indicating the primary response (see Box 2).

D – 1R6F cigarette bPBS induced an oxidative stress response in a concentration dependent manner at concentrations of 0.32% without S9 and 0.23% with S9. Cell cycle stress was induced in a concentration dependent manner at concentrations of 1% without S9 and 0.8% plus S9.
E - HTP bPBS induced an oxidative stress response in a concentration dependent manner at concentrations of 1% without S9 and 0.7% with S9.
F - EVP bPBS no biomarker response was observed at any concentration with or without S9.

4. CONCLUSIONS

- ToxProfiler detected oxidative stress from the 1R6F cigarette bPBS at low concentrations, whilst HTP bPBS induced this endpoint at 3-fold higher concentrations, whereas the EVP bPBS induced no oxidative stress.
- The reduced biological activity of both the EVP and HTP aerosols relative to combustible cigarette smoke, using bPBS extracts, in the ToxProfiler assay adds to the growing evidence that these products have harm reduction potential.
- The ToxProfiler assay has proven to be a rapid and mechanistically informative assay and has the potential to be part of future assessment strategies for next generation products.

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