NEXT GENERATION PRODUCTS

Non-combustible next generation products induced lower biological activity than conventional cigarettes in the ToxProfiler assay

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Matthew Stevenson[1]*, Ourania Komini[1], Bas ter Braak[2], Liesanne Wolters[2], Edgar Trelles Sticken[3], Roman Wieczorek[3], Sarah Jean Pour[3], Fiona Chapman[1], Liam Simms[1]

[1]Imperial Brands PLC, 121 Winterstoke Road, Bristol, BS3 2LL, UK; [2]Toxys B.V., De limes 7, 2342 DH, Oegstgeest, The Netherlands; [3]Reemtsma Cigarettenfabriken GmbH, An Imperial Brands PLC company, Albert-Einstein-Ring-7, D-22761, Hamburg, Germany

*Corresponding author's e-mail: matthew.stevenson@impbrands.com

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INTRODUCTION

Imperial Brands understands that smoking is a cause of serious disease in smokers including lung cancer, heart disease and emphysema. The greatest risk of smoking related diseases comes from burning tobacco and inhaling smoke containing around 7,000 chemicals. While science suggests that nicotine is addictive and not risk-free, Public Health experts worldwide have concluded that it is the toxicants in conventional cigarette smoke generated by burning tobacco, and not nicotine, which is the primary cause of smoking-related disease.

Tobacco Harm Reduction (THR) refers to strategies designed to reduce the health risks associated with tobacco smoking. Next Generation Products (NGP), like Heated Tobacco Products (HTP) and E-Vapour Products (EVP), deliver nicotine without burning tobacco so have the potential to play a role in THR.

With the rapid pace of innovation in Next Generation Products, there is an increasing need for quick, sensitive, and mechanistically insightful *in vitro* techniques for product stewardship assessment and evaluation of their harm reduction potential[1].

In this study, we evaluated a commercially available new approach method (NAM), the ToxProfiler reporter assay, for assessing non-combustible next generation products. The ToxProfiler assay includes a panel of seven unique human liver reporter cell lines that visualize and quantify specific cellular stress response pathways such as oxidative stress, cell cycle stress, ER stress, autophagy, ion stress, protein stress and inflammation.

We exposed the model to conventional cigarette smoke or NGP aerosol fractions and assessed the resulting biological response. Test samples included 1R6F reference cigarette, heated tobacco product "Pulze + iD sticks" (HTP), and an e-vapour product "blu 2.0" (EVP). Cigarette smoke and HTP/EVP aerosols were bubbled through a series of impingers containing PBS (bPBS), generating stock solution concentrations of 1.8 puffs/ml for the 1R6F and 4.8 puffs/ml for EVP and HTP samples.

The ToxProfiler cells were exposed to a maximum bPBS concentration of 10% and live cell confocal imaging was performed 24 hours post exposure. The conventional cigarette smoke bPBS caused a significant response in some cell stress pathways, including oxidative stress and cell cycle stress; with effects appearing from 0.23% bPBS concentrations. In contrast, HTP bPBS only induced oxidative stress with 0.7% bPBS. EVP extracts did not induce significant changes in cell stress pathway activation at the maximum concentration of 10% bPBS.

These results further substantiate the harm reduction potential of the NGPs assessed relative to continued smoking of conventional cigarettes. The ToxProfiler assay is a rapid and mechanistically informative tool that could be integrated to future assessment strategies for non-combustible next generation products.

METHODS

Test articles

- 1R6F Reference Cigarette (University of Kentucky)
- Heated Tobacco Product (HTP), "Pulze" with "iD stick" (iD Regular)
- Electronic-Vapour Product (EVP), "blu 2.0" (Tobacco pods)

Smoke / Aerosol Extract Generation method

Smoke and aerosol from test products was generated with a Vitrocell VC10s (Vitrocell, Waldkirch, 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. 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. 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 trapped 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).

Smoke / Aerosol Extract Generation method

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 seven stable genetically engineered human liver HepG2 cell lines[2,3] (see Figure 1). 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.

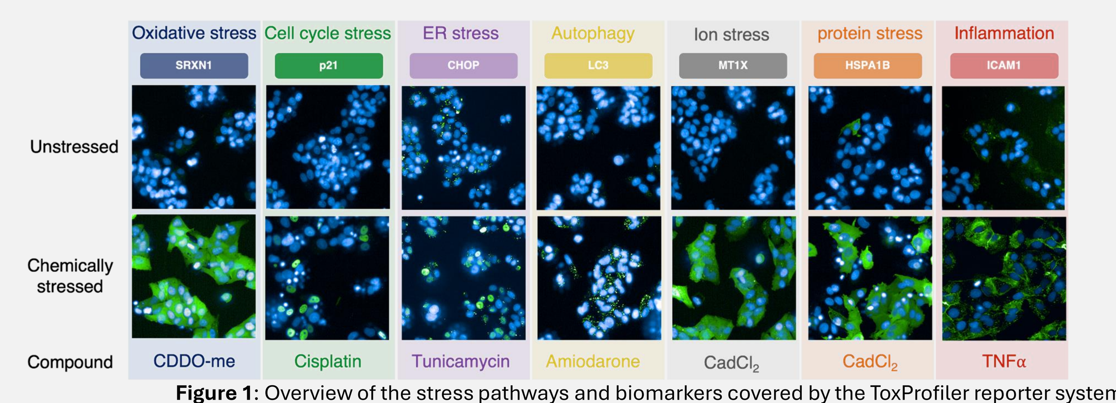

Figure 1: Overview of the stress pathways and biomarkers covered by the ToxProfiler reporter system

RESULTS

Dosimetry Nicotine and carbonyl levels in bubbled PBS extracts

- For each test article, PBS from all three impingers were 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 and Biomarker analysis in bubbled PBS extracts (Figure 2, 3)

- 1R6F cigarette bPBS induced an oxidative stress response in a concentration-dependent manner, starting at concentrations of 0.32% without S9 and 0.23% with S9. Cell cycle stress was also induced in a concentration-dependent manner beginning at 1% without S9 and 0.8% with S9.
- For the HTP bPBS, an oxidative stress response was observed in a concentration-dependent manner at 1% without S9 and 0.7% with S9. This endpoint was induced at 3-fold higher concentration compared to 1R6F.
- No biomarker response was observed for the EVP bPBS at any concentration, with or without S9.

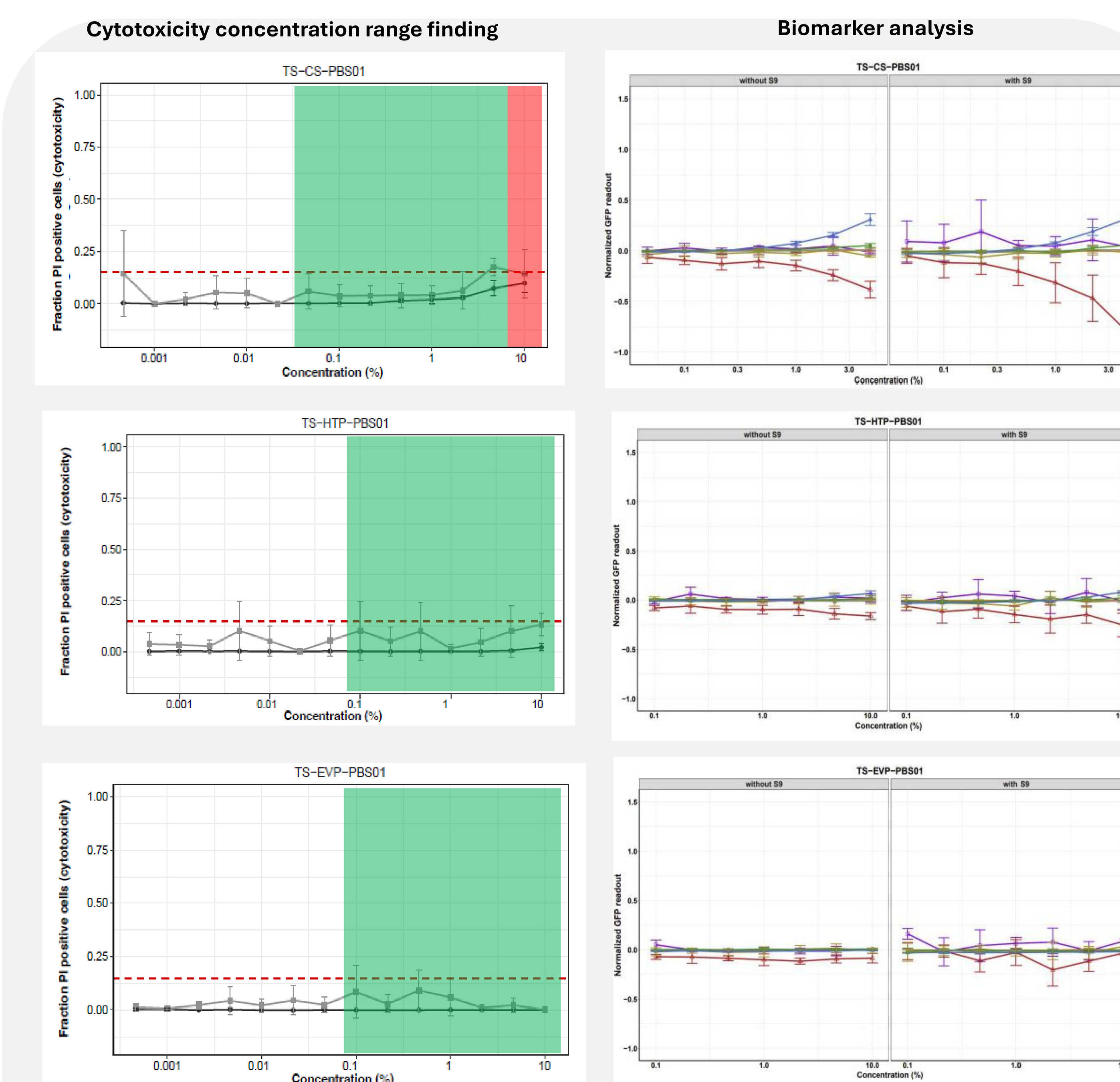

Figure 2: Cytotoxicity concentration range (left) (top: 1R6F; middle: HTP; bottom: EVP)

Figure 3: Biomarker analysis (right) (top: 1R6F; middle: HTP; bottom: EVP)

Blue: Oxidative stress; Green: Cell cycle stress; Purple: ER stress; Yellow: Autophagy; Grey: Ion stress; Orange: Protein stress; Red: Inflammation

CONCLUSIONS

The ToxProfiler assay detected oxidative stress from the 1R6F cigarette bPBS at low concentrations, while HTP bPBS induced this endpoint at 3-fold higher concentrations. In contrast, the EVP bPBS did not induce oxidative stress up to the maximum concentration of 10% bPBS.

The reduced biological activity of both the EVP and HTP aerosol extracts, compared to conventional cigarette smoke, in the ToxProfiler assay, supports the growing evidence that these products have tobacco harm reduction potential.

Finally, the nicotine and carbonyls analysis demonstrated that both EVP and HTP delivered comparable levels of nicotine but with a significant reduction, of more than 90%, in the carbonyls measured, when compared to the reference cigarette.

The ToxProfiler assay has proven to be a rapid and mechanistically informative tool, with the potential to be integral to future assessment strategies for next generation products.

Under the conditions of the test, these results indicate that both the EVP and the HTP have the potential to offer a reduced harm alternative to smoking conventional cigarettes and the potential to make a meaningful contribution to tobacco harm reduction.

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