P30-11

Assessment of Disposable of e-vapour products using New Approach Methodologies shows marked reductions of toxicity relative to cigarettes

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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 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 E-Vapour Products (EVP), deliver nicotine but do not contain or burn tobacco, so have the potential to play a role in THR.

In the present study, we characterised the aerosol of a blu bar™ 1000 e-liquid (Blueberry Ice) and tested the e-liquid with innovative in vitro techniques. The EVP aerosol and tobacco smoke were characterized for multiple analytes including carbonyls, heavy metals, tobacco specific nitrosamines, and volatile organic compounds. The e-liquid was tested with ToxTracker anti-oxidant (AO) assay and compared to 1R6F reference cigarette. The ToxTracker AO assay is a panel of six validated GFP-based mouse embryonic stem (mES) reporter cell lines that monitors activation of specific cellular signalling pathways for detection of the biological reactivity of compounds. The mES GFP reporter cell lines represent four distinct biological responses that are associated with carcinogenesis, i.e. general cellular stress, DNA damage, oxidative stress and the unfolded protein response.

METHODS

Test articles

- 1R6F Reference cigarette (University of Kentucky)
- Electronic Vapour Product (EVP), blu bar™ 1000 Blueberry Ice

Emission testing

The emission testing was performed according to Article 20 of the Tobacco Product Directive (2014/40/EU). The tests were performed according to Clause 5.4.7 of AFNOR XP D 90-300-3:2021. Nicotine was measured using GC-FID or GC-MS; aldehydes (formaldehyde, acetaldehyde, acrolein and crotonaldehyde) were measured using LC-DAD. Elements (chromium, nickel, lead, cadmium, arsenic, antimony, mercury, iron, aluminium) were measured using ICP-OES. VOCs (toluene, benzene, 1,3-butadiene,isoprene) were performed according to section 4 of ISO 20768:2018 using GC-MS.

Biological Assessment

The biological assessment was performed by Toxys B.V. The ToxTracker genotoxicity assay utilises 6 green fluorescent protein (GFP) reporter cell lines measuring DNA damage, oxidative stress, p53 activation and protein damage (Figure 1). These cell lines were exposed to the test articles for 24h±S9. Following this exposure period, the differential induction of the green fluorescent protein reporters, as well as cytotoxicity was determined using flow cytometry. Reporters with a greater than 2- fold induction of GFP fluorescence compared to controls were deemed to be a positive signal.

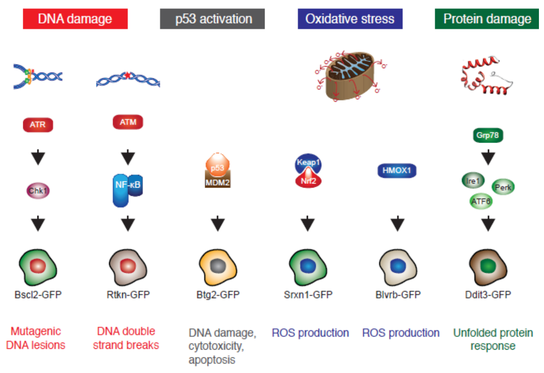


Figure 1: Overview of the stress pathways and biomarkers assessed by the ToxTracker reporter assay

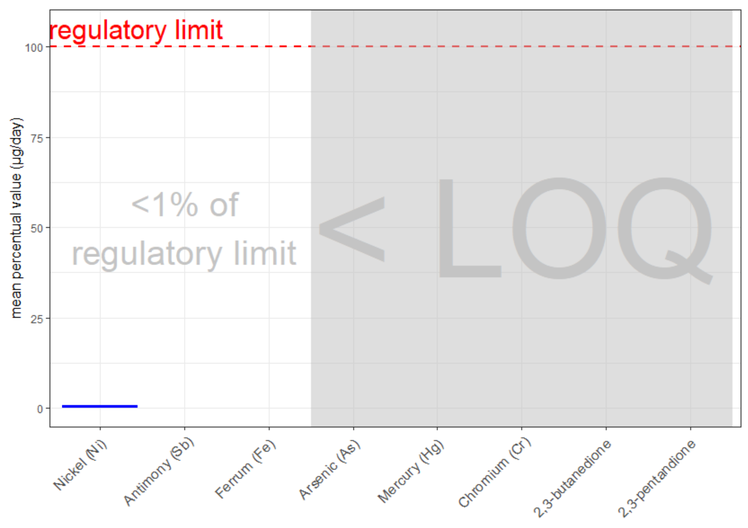
Smoke extract generation method for biological assessment

Smoke from 1R6F reference cigarette was generated with a Vitrocell VC10s (Vitrocell, Munich, Germany) smoking machine. Smoke extract was prepared by bubbling the sample aerosol into 3 in-line Impingers each containing 10 mL of Phosphate Buffered Saline (PBS) solution. A total stock solution of 30 mLs was used.

blu bar™ 1000 Blueberry Ice was directly added to the cell cultures.

RESULTS

Emission Assessment



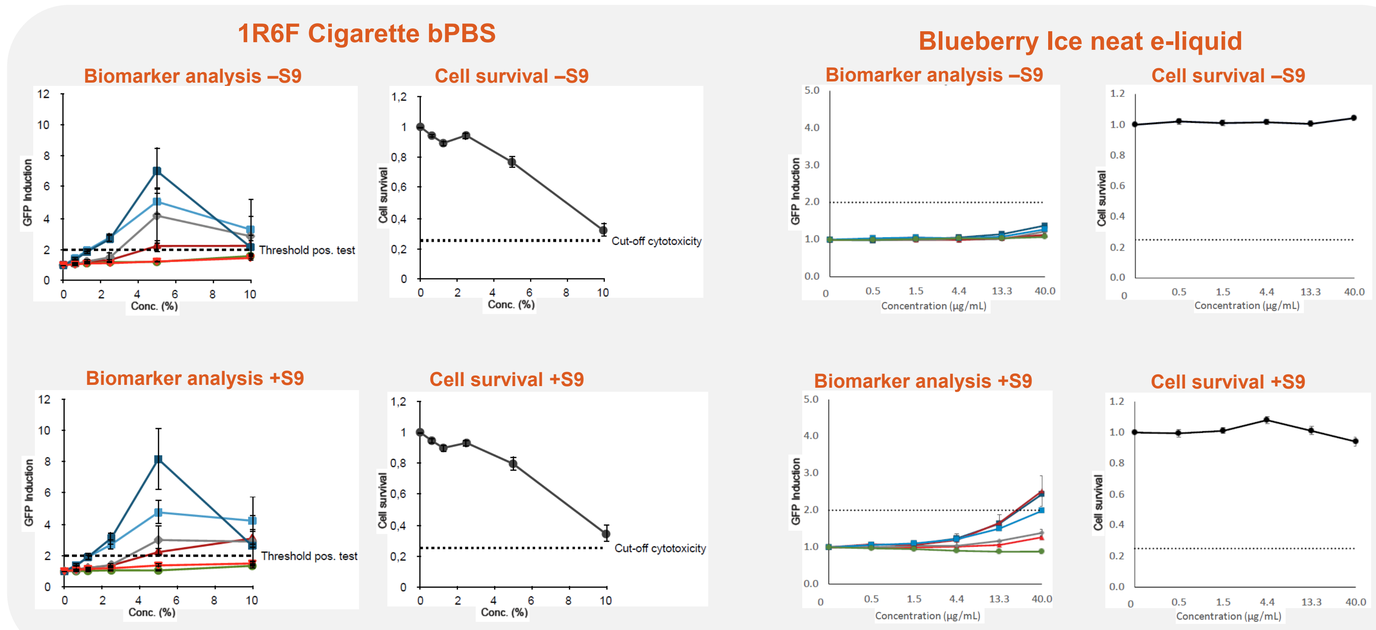
Nickel, iron, antimony, arsenic, and mercury were below the inhalation permitted daily exposure (PDE) as recommended by the ICH guideline Q3D (R2) on elemental impurities.

2,3-butanedione was below its respective limit of quantification and below the American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Value (TLV©)- Time-Weighted Average (TWA) with a calculated margin of exposure of 13.6.

2,3-pentanedione was below its respective limit of quantification and below the German Social Accident Insurance Information System for Hazardous Substances (GESTIS) Time-Weighted Average (TWA) with a calculate margin of exposure of 28.6.

Of the toxicants tested for in blu bar™ 1000 Blueberry Ice aerosol, formaldehyde, acetaldehyde, acrolein, crotonaldehyde, lead, cadmium, NNN, NNK, toluene, benzene and 1,3-butadiene were below their respective limits of quantification and at substantially reduced levels when compared to 1R6F smoke (with reductions ranging from 95-99.9%).

Biological Assessment



Key-The concentrations of the blueberry Ice neat liquid tested 0, 0.49, 1.48, 4.44, 13.3, 40 µg/ml is equivalent to 0, 0.0025, 0.0075, 0.022, 0.067, 0.2%

The 1R6F cigarette bPBS induced cytotoxicity with increasing dose, with 50% cytotoxicity observed from 8% bPBS. A >2 fold GFP induction for Rtkn-GFP, Btg2-GFP, Srxn1-GFP and Blvrb-GFP was observed for increasing concentration of 1R6F cigarette bPBS. 1R6F cigarette bPBS did not induce either Bscl2 or Ddit3 GFPs (±S9) above the threshold. The 1R6F cigarette bPBS was classified as being genotoxic at 2.8 µg/ml or 2.5% under the conditions of this test. The Blueberry Ice neat e-liquid was classified as genotoxic in the ToxTracker assay at 40 µg/ml or 0.2%. However, when compared to the maximum blood nicotine concentrations observed by O'Connell et al., 2019 (C_{max}= 12ng/ml), which is over a 3000-fold less potent in terms of genotoxicity.

CONCLUSIONS

In contrast to 1R6F, the toxicants investigated were below the limit of detection in the disposable e-vapour product's aerosol. Formaldehyde, acetaldehyde, acrolein, crotonaldehyde, lead, cadmium, NNN, NNK, toluene, benzene and 1,3-butadiene were at substantially reduced levels when compared to 1R6F smoke, with reductions ranging from 95-99.9%.

The ToxTracker assay detected the genotoxic potential for both 1R6F and blu bar™ 1000 Blueberry Ice however, the results showed that the neat e-liquid was over a 3000-fold less potent in terms of genotoxicity when compared to the maximum blood nicotine concentrations. Both induced DNA damage, oxidative stress, and p53 activation, however, the bPBS 1R6F NOGEL (No Observed Genotoxicity Effect Level) was 2.8 µg/ml and the neat e-liquid NOGEL was 40 µg/ml.

Under the conditions of the test, these results suggest that EVP has the potential to offer a reduced harm alternative to smoking cigarettes and the potential to make a meaningful contribution to tobacco harm reduction.

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