VAPE

Disposable E-Vapour Products Exhibit Lower in vitro toxicological Activity Than Cigarettes

P30-06 | EUROTOX | September 14th-17th 2025

Maria-Andrea Oltean[1], Roman Wieczorek[2], Sarah Jean Pour[2], Edgar Trelles Sticken[2], Konstantinos Papikinos[1], Christiana Kasim[1], Ourania Komini[1], Matthew Stevenson[1], Liam Simms[1]
1 Imperial Brands PLC, 121 Winterstoke Road, Bristol, BS3 2LL, UK
2 Reemtsma Cigarettenfabriken GmbH, An Imperial Brands PLC Company, Albert-Einstein-Ring-7, D-22761, Hamburg, Germany

FIND OUT MORE

INTRODUCTION

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.[1]

In the present study, we evaluate the in vitro biological activity of aerosol generated from three variants of the blu bar™ 1000 disposable e-vapour product (Watermelon Ice, Strawberry Ice, and Blueberry Ice) and compare their cytotoxicity and genotoxicity potential to that of 1R6F reference cigarette smoke. We used the CORESTA battery, which includes Bacterial Reverse Mutation test (Ames test), In Vitro Micronucleus test (IVM), and Neutral Red Uptake (NRU) assay that provide insights into the biological activity relative to cigarette smoke. This study sought to generate robust scientific evidence supporting the harm reduction potential of e-vapour products and provide critical insights into their relative biological activity.

METHODS

Test Articles

blu bar™ 1000 e-vapour products 20 mg nicotine strength (Imperial Brands PLC)

- Watermelon ice
- Strawberry ice
- Blueberry ice

For reference 1R6F Reference Cigarette (University of Kentucky)

Smoke and aerosol generation and exposure

Fresh aerosol generation was carried out according to ISO 20768:2018 puffing regime, whereas 1R6F cigarette smoke was generated according to ISO 20778:2018 using Smoke Aerosol Exposure In Vitro System (SAEIVS) (NRU and IVM) & Smoking Robot VC 10® S-Type (Ames)

In Vitro assays

- Mutagenic potential was evaluated using Ames test with *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 with and without metabolic activation in compliance with OECD test Guideline 471.[2] Exposure was realised by direct bubbling of bacteria suspension with fresh aerosol/ smoke, which we call air liquid transfer (ALT).
- Genotoxicity was determined using IVM test with V79 hamster lung fibroblasts following direct exposure to fresh aerosol/ smoke at the air-liquid interface (ALI). The test was performed using short-term treatment in the presence of S9 and long-term treatment in the absence of S9 metabolic activation. Due to technical limitations, no short-term treatment in the absence of S9 was carried out, deviating from OECD test Guideline 487 [3] .
- The potential cytotoxicity was determined using the NRU assay with BEAS-2B human bronchial cells in accordance to ISO 17025:2017 following fresh smoke/aerosol exposure at the ALI.

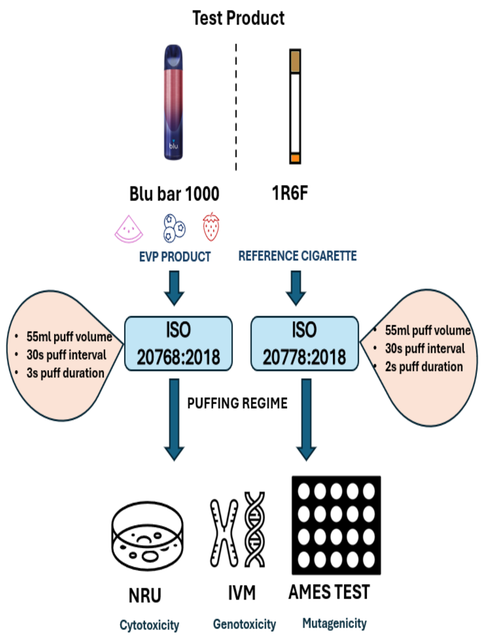

Figure 1:Overview of study methods.

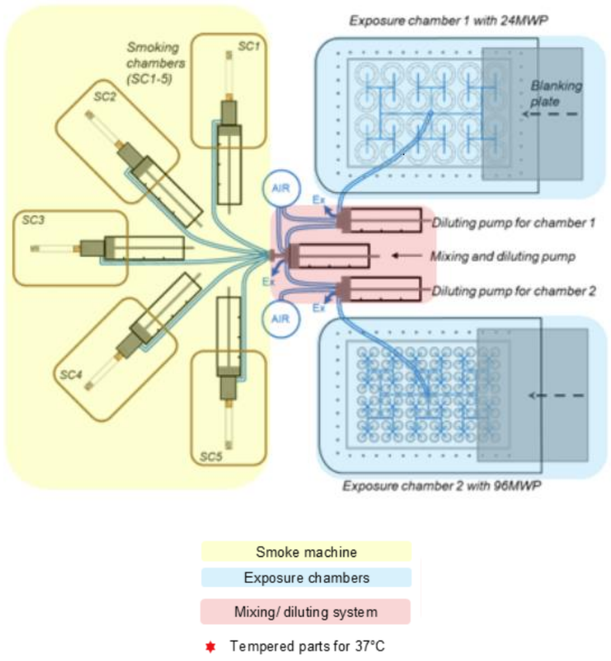

Figure 2: Smoke Aerosol Exposure In Vitro System (SAEIVS)

RESULTS

No mutagenicity observed for e-vapour products in the Ames test

200 puffs of undiluted aerosol from blu bar™ 1000 did not induce a statistically significant increase in the revertants, either in the presence or the absence of S9 metabolic activation. In contrast, after just 40 puffs of undiluted cigarette smoke from 1R6F resulted in a statistically significant increase of revertant in the strain TA100.

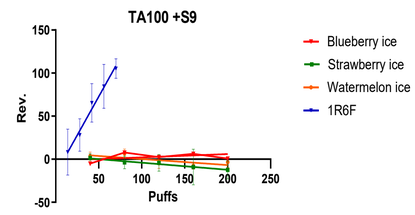

Figure 3: Dose response of smoke and blu bar™ 1000 flavour aerosols in Ames test with Salmonella typhimurium strain TA100+S9

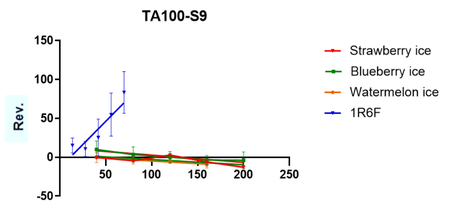

Figure 4: Dose response of smoke and blu bar™ 1000 flavour aerosols in Ames test with Salmonella typhimurium strain TA100-S9

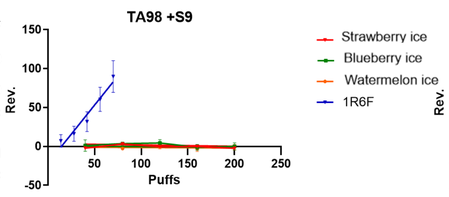

Figure 5: Dose response of smoke and blu bar™ 1000 flavour aerosols in Ames test with Salmonella typhimurium strain TA98+S9

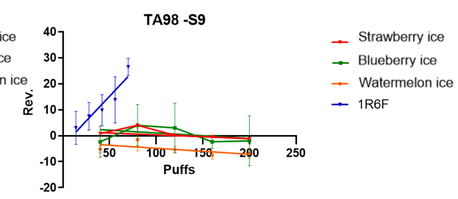

Figure 6: Dose response of smoke and blu bar™ 1000 flavour aerosols in Ames test with Salmonella typhimurium strain TA98-S9

No genotoxicity observed for e-vapour products in IVM assay

The undiluted aerosol from blu bar™ 1000 (100 puffs) did not induce a statistically significant increase in the micronuclei frequency, either in the presence or the absence of S9 metabolic activation. In contrast, 1 puff of cigarette smoke from 1R6F, diluted at 1:10, resulted in a statistically significant increase in the micronuclei frequency.

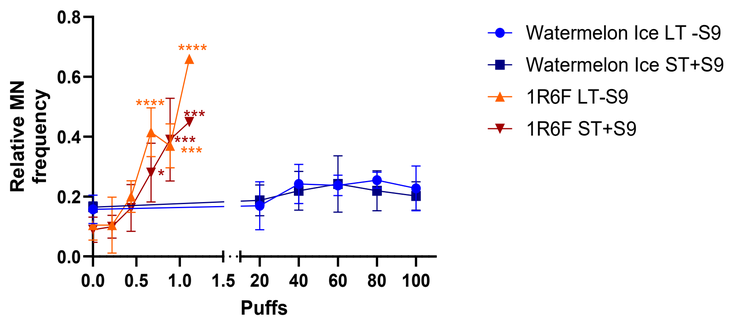

Figure 7: Dose response of smoke and blu bar™ 1000 Blueberry ice aerosol in IVM test with v79 hamster lung fibroblast both with and without metabolic activation

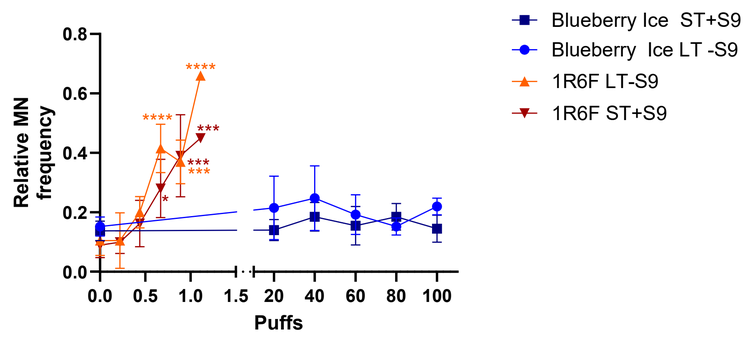

Figure 8: Dose response of smoke and blu bar™ 1000 Watermelon ice aerosol in IVM test with v79 hamster lung fibroblast both with and without metabolic activation

An average of 96.8% reduction in cytotoxicity when compared to 1R6F reference cigarette smoke using Neutral Red Uptake Assay

100 undiluted puffs of blu bar™ 1000 showed an average of 96.8% reduction in cytotoxicity compared to 8-9 puffs of 1R6F reference cigarette, which was diluted at 1: 12.

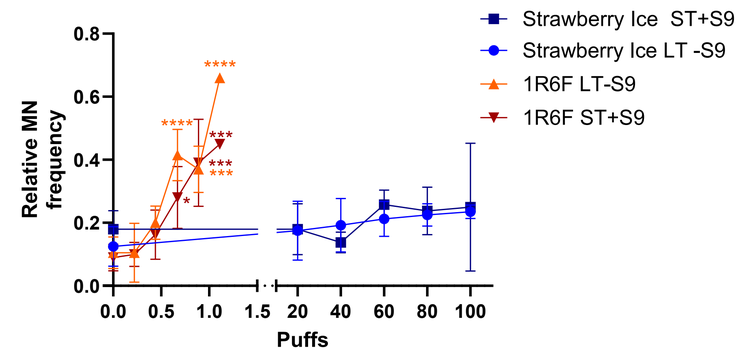

Figure 9: Dose response of smoke and blu bar™ 1000 Strawberry Ice aerosol in IVM test with v79 hamster lung fibroblast both with and without metabolic activation

CONCLUSIONS

The blu bar™ 1000 undiluted aerosols demonstrated noticeably reduced biological activity compared to the 1R6F reference cigarette diluted smoke across all in vitro assays evaluated. In the NRU assay, cytotoxicity was reduced by an average of 96.8%, indicating minimal cellular toxicity. The Ames assay showed no mutagenic potential, with no significant increase in revertant colonies observed either with or without metabolic activation. Likewise, the in vitro micronucleus (IVM) assay revealed no significant elevation in micronuclei frequency, suggesting an absence of genotoxic effects. Under the conditions of this study, these findings support the conclusion that blu bar™ 1000 variants Watermelon Ice, Strawberry Ice, and Blueberry Ice may represent a reduced harm alternative to conventional cigarette smoking and hold promise as contributors to tobacco harm reduction strategies.

REFERENCES

[1] Rudd, K., Stevenson, M., Wieczorek, R., Pani, J., Trelles-Sticken, E., Dethloff, O., ... & Walele, T. (2020). Chemical Composition and In Vitro Toxicity Profile of a Pod-Based E-Cigarette Aerosol Compared to Cigarette Smoke. Applied In Vitro Toxicology, 6(1), 11-41.
[2] Wieczorek, R., Trelles Sticken, E., Pour, S.J., Chapman, F., Röwer, K., Otte, S., Stevenson, M. & Simms, L. (2023) Characterisation of a smoke/aerosol exposure in vitro system (SAEIVS) for delivery of complex mixtures directly to cells at the air-liquid interface. Journal of Applied Toxicology, 43(7). DOI:10.1002/jat.4442.
[3] OECD, 1997. Test No. 471: Bacterial Reverse Mutation Test, OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. OECD Publishing
[4] OECD, 2016. Test No. 487. In vitro Mammalian Cell Micronucleus Test, OECD Guidelines for the testing of chemicals, Section 4: Health effects. OECD Publishing

IMPERIAL BRANDS SCIENCE | blu®