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blu bar® kit aerosol shows substantially lower biological activity in CORESTA and ToxTracker® in vitro assays compared to cigarette smoke

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Álvaro Marinas-Lacasta[1], Roman Wieczorek[2], Sarah Jean Pour[2], Edgar Trelles Sticken[2], Christiana Kasim[1], Konstantinos Papikinos[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

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INTRODUCTION

Smoking is a serious cause of 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[1].

Electronic vapour products (EVPs) are non-combustible nicotine delivery systems intended for adult smokers who would otherwise continue smoking cigarettes. By generating an aerosol through heating e-liquid rather than burning tobacco, EVPs have the potential to reduce exposure to many toxicants found in cigarette smoke. The biological activity of EVP aerosol can be assessed to measure the harm reduction potential of EVPs relative to cigarette smoke.

In vitro toxicological testing, along with weight-of-evidence assessments, is a key component of our product stewardship for next-generation nicotine products. Techniques assessing cytotoxicity, mutagenicity, and genotoxicity provide insight into potential biological hazards, while mechanistic approaches can help contextualise any biological responses.

EVPs are available in a range of flavours and nicotine concentrations to meet the needs of adults who smoke. Although nicotine concentrations are limited to 20 mg/mL in the European Union, higher-strength formulations remain commercially available in several international markets. Characterising all product variants is important to ensure there are no changes in biological activity which may impact the overall risk profile.

This study evaluated the cytotoxic, mutagenic, and genotoxic potential (CORESTA battery) of blu bar® kit aerosols of different flavours and nicotine strengths relative to 1R6F cigarette smoke, and assessed the utility of ToxTracker® for mechanistic interpretation of observed responses.

METHOD

1. Exposure

Aerosol and smoke generation were performed as described in **Table 1**.

2. Neutral Red Uptake (NRU)

BEAS-2B human bronchial epithelial cells were exposed to fresh aerosol or smoke at the air-liquid interface (ALI) and each test was repeated three times in duplicate.

3. Ames test

Five strains of *S. typhimurium* (TA98, TA100, TA102, TA1535, and TA1537) in 10mL of PBS had freshly generated aerosol or smoke bubbled through them then plated with and without S9 metabolic mix.

4. In vitro micronucleus test (IVM)

Adherent hamster V79 lung fibroblast cells were treated with aerosol or smoke, then recovered for the long term condition, or treated with S9 for 3h before recovery, in two independent test days.

5. ToxTracker® Assay[4]

ToxTracker is a stem cell-based reporter assay that identifies the genotoxic properties of chemicals by combining six fluorescent reporter genes that are selectively activated by different cellular signalling responses. The stem cell lines for DNA damage, oxidative stress, and protein damage were exposed to neat e-liquid of either tobacco or orange flavour with and without S9 metabolic mix. Reactive oxygen species scavengers N-acetyl cysteine and reduced L-glutathione helped reduce oxidative stress to assess potential for direct DNA damaging activity.

Product	blu bar® kit with orange flavour & blu bar® kit with tobacco flavour			1R6F reference cigarette[2]		
Nicotine content	27 mg/ml			1.896 mg/cig (in smoke)	0.721 mg/cig (in smoke)	1.896 mig/cig (in smoke)
Assay	NRU	Ames	IVM	NRU	Ames	IVM
System	SAEIVS 2	Smoking robot VC 10 S-Type	SAEIVS[3]	SAEIVS 2	Smoking robot VC 10 S-Type	SAEIVS[3]
Puffing regime	ISO 20768			ISO 20778	ISO 3308	ISO 20778
Conditioning	N/A			ISO 3402		
Dilution factor	N/A			N/A		1:8/1:7
Vent blocking	N/A			Yes	N/A	Yes

Table 1 – Methodology of aerosol and smoke generation per assay type denoting the exposure of relevant *in-vitro* systems. Selected EVPs are meant to encompass fruit and tobacco flavour directions.

RESULTS

1. Neutral Red Uptake

Both EVP aerosols exhibited cytotoxicity, however, they were 42 times less cytotoxic than diluted 1R6F cigarette smoke. See **Figure 1**.

2. Ames assay

Neither e-liquid exhibited mutagenic activity in any of the bacterial strains. See **Figure 2**.

3. In-vitro micronucleus test

The orange e-liquid exhibited negative results in all conditions of the assay. For tobacco, the +S9 condition yielded negative results on both test days. The -S9 condition had a dose dependent and statistically significant increase in MN, and one with a dose dependent increase that did not reach statistical significance on different test days. No indication of genotoxicity was visible in the average results. Fresh smoke from 1R6F yields a clear genotoxic response. See **Figure 3**.

4. ToxTracker® assay

Both the orange and tobacco e-liquids were classified as non-genotoxic. The no observed genotoxic effect levels (NOGELs) for both were 40µg/mL. This yields a margin of exposure (MOE) of 1143; which represents how much higher the NOGEL is when compared to a maximum blood level nicotine concentration of 35ng/mL, as reported by Schneider et al (2001)[5].

For the orange e-liquid; no activation of any reporter line was observed, except for oxidative stress and unfolded protein response at the highest concentration. For comparison, these results are displayed in **Table 2**.

For the tobacco e-liquid; the DNA damage, p53 activation and the Blvrb-GFP oxidative stress tests were negative. The results for the Srxn1-GFP oxidative stress reporter and the Ddit3-GFP protein stress response are displayed in **Table 2**. These findings show that addition of NAC or GSH reduced the oxidative stress response in the -S9 condition, but not the protein stress response. As with the NOGELs, the protein stress response was observed at supra-physiological levels of dosing, and the oxidative stress response would be expected to occur in line with the +GSH and +NAC conditions. See **Figure 4** for neat e-liquid results.

	Srxn1-GFP reporter (Oxidative Stress)		Ddit3-GFP reporter (Unfolded Protein Response)	
	Orange fl.	Tobacco fl.	Orange fl.	Tobacco fl.
-S9	Negative	Positive	Negative	Positive
+S9	Positive	Negative	Negative	Negative
-S9 +NAC	Negative	Negative	Negative	Positive
+S9 +NAC	Positive	Negative	Negative	Negative
-S9 +GSH	Negative	Equivocal	Negative	Positive
+S9 +GSH	Equivocal	Negative	Positive	Equivocal

Table 2 – ToxTracker® oxidative & protein stress results.

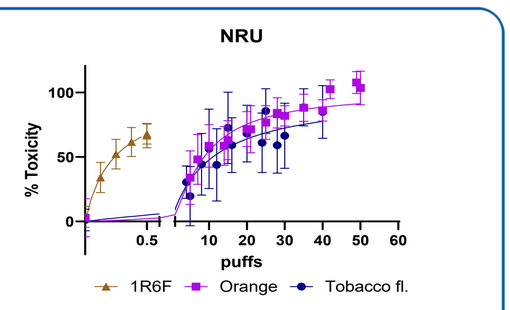

Figure 1 – Dose response of blu bar® kit with orange, tobacco e-liquids and 1R6F in NRU test.

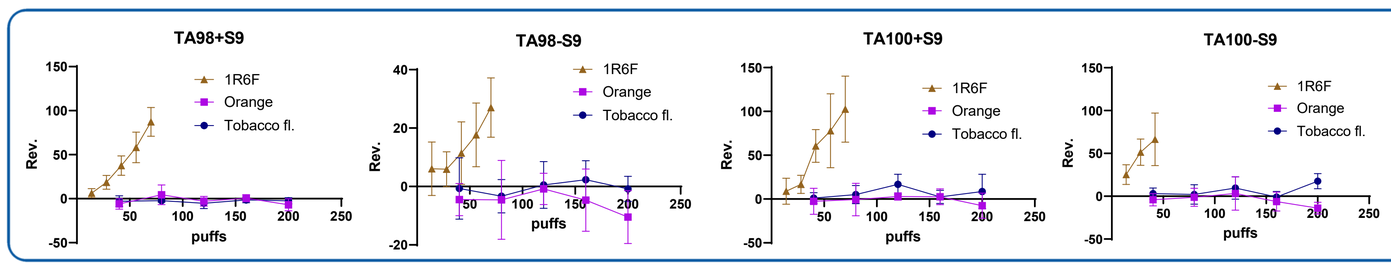

Figure 2 – Dose response of blu bar® kit with tobacco, orange e-liquid, or 1R6F in Ames test.

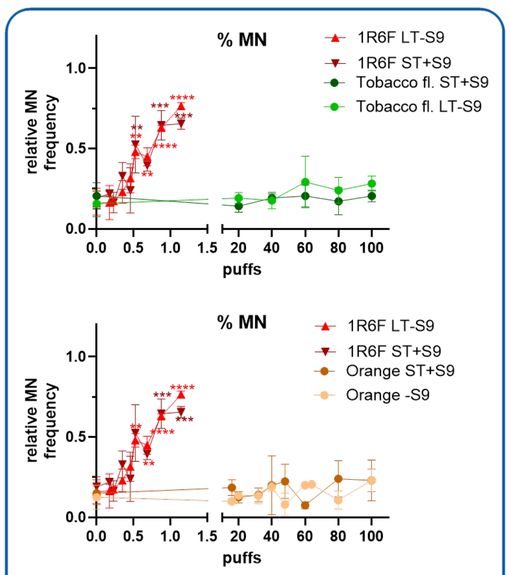

Figure 3 – Dose response of blu bar® kit with tobacco or orange flavour and 1R6F in IVM test. ST = short treatment; LT = long treatment.

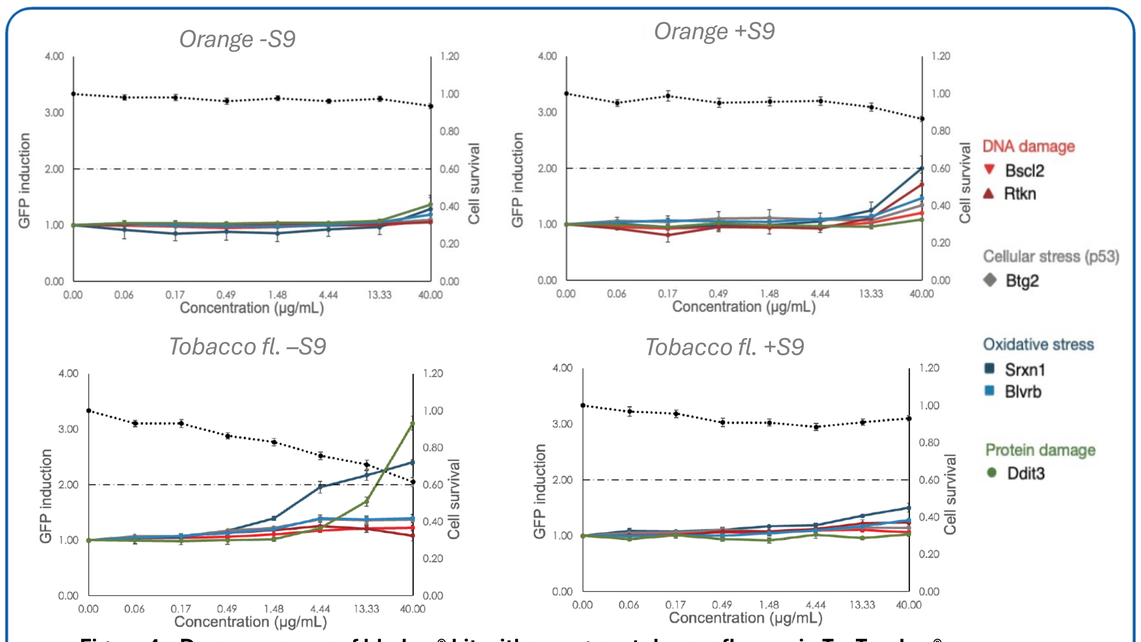

Figure 4 – Dose response of blu bar® kit with orange or tobacco flavour in ToxTracker®.

CONCLUSIONS

1. EVP aerosols from an orange and a tobacco e-liquid did not display mutagenicity or genotoxicity in vitro, and showed a marked reduction in cytotoxicity compared to fresh smoke from a reference cigarette. These results suggest that blu bar kit aerosol potentially offers a harm reduced alternative to smoking cigarettes and the potential to make a meaningful contribution to tobacco harm reduction.
2. Aerosol from e-liquids with a nicotine content of 27 mg/mL did not display any evidence of causing increased biological activity when compared with 18mg/mL e-liquids[6, 7].
3. ToxTracker® functions as a valuable supplement to robust toxicological risk-assessment methodologies by providing information on the mode of action[8]. As observed in the results here, ToxTracker allows for the calculation of an MOE by providing a NOGEL for both e-liquids. Furthermore, it shows that no direct DNA damage was observed, oxidative stress is reduced with the addition of NAC or GSH, and any positive responses occur at supraphysiological levels, and therefore would not be expected to increase the risk profile of the e-liquids.

REFERENCES

1. Centers for Disease Control and Prevention. (2024, September 17). Health effects of cigarettes: Cancer. U.S. Department of Health and Human Services. https://www.cdc.gov/tobacco/about/cigarettes-and-cancer.html
2. University of Kentucky, Center for Tobacco Reference Products. (2024, October 31). Certificate of analysis: 1R6F certified reference cigarette (Certificate No. 2024-005CTRP). University of Kentucky, Center for Tobacco Reference Products.
3. Wieczorek, R., Trelles Sticken, E., Pour, S. J., Chapman, F., Röwer, K., Otte, S., ... & 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), 1050-1063.
4. OECD (2026), Test No. 446A: ToxTracker gene reporter assay for the identification of genotoxic hazard and genotoxic/nongenotoxic mechanism of action, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/f2d51b5b-en.
5. Schneider, N. G., Olmstead, R. E., Franzon, M. A., & Lunell, E. (2001). The nicotine inhaler: clinical pharmacokinetics and comparison with other nicotine treatments. Clinical pharmacokinetics, 40(9), 661-684.
6. Oltean, M.-A., Wieczorek, R., Pour, S. J., Trelles Sticken, E., Kasim, C., & Papikinos, K. (2026). Toxicological evaluation of blu Bar Kit aerosol compared with cigarette smoke: Evidence as part of responsible product stewardship [Conference poster]. Imperial Brands Science Research Archive. https://imperialbrandsscience.com/research-archive/
7. Kasim, C., Iheke, E., Oltean, M.-A., Komini, O., & Papikinos, K. (2026). Integrated aerosol chemistry and in vitro assessment provide a foundation for an evidence-based, responsible product development framework [Conference poster]. Imperial Brands Science Research Archive. https://imperialbrandsscience.com/research-archive/
8. Czekala, L., Chapman, F., Simms, L., Rudd, K., Trelles Sticken, E., Wieczorek, R., ... & Walele, T. (2021). The in vitro ToxTracker and Aneugen Clastogen Evaluation extension assay as a tool in the assessment of relative genotoxic potential of e-liquids and their aerosols. Mutagenesis, 36(2), 129-142.

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