NEXT GENERATION PRODUCTS

Toxicological Evaluation of blu Bar Kit Aerosol Compared with Cigarette Smoke: Evidence as part of Responsible Product Stewardship

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INTRODUCTION

Smoking is a cause of serious diseases, including lung cancer and cardiovascular disease. However, next generation products (NGP) such as e-vapour products (EVP) offer potentially reduced harm forms of nicotine delivery. They feature pre-filled cartridges, removable and rechargeable batteries and a variety of flavour options. Electronic vaping products gained significant popularity among adults who smoke, seeking alternatives to conventional cigarettes.

As part of responsible product development, manufacturers should characterise the biological activity of EVP aerosols using internationally recognised toxicological methods.
The toxicological methods incorporating biological testing alongside an expert desk-based assessment provide valuable evidence of the EVP's potential for reduced harm when compared with conventional cigarettes.

This study evaluated aerosol generated from the blu bar® kit containing a fruit-flavoured e-liquid with 20 mg/mL nicotine salt formulation and compared results with reference cigarette smoke (1R6F).
Biological activity was assessed using cytotoxicity (neutral red uptake, NRU), mutagenicity (Ames), and genotoxicity (in vitro micronucleus, IVM) assays.

METHODS

Test Articles

- blu bar® kit containing a fruit-flavoured e-liquid with 20 mg/mL nicotine salt formulation (Imperial Brands PLC)
- for reference 1R6F Reference Cigarette (University of Kentucky)

Smoke and aerosol generation and exposure

Product	Assay	Guideline	Puff volume (ml)	Puff duration (seconds)	Puff Interval (seconds)	Venting blocking	Puff profile
EVP Aerosol	AMES, NRU & IVM	ISO 20768:2018	55	3	30	n/a	Square shaped
1R6F	NRU & IVM	ISO 20778:2018	55	2	30	yes	Bell shaped
	Ames	ISO 3308:2012	35	2	60	n/a	Bell shaped

Table 1. Guidelines description based on product category and in vitro testing used.

Aerosol and whole smoke were generated using two exposure systems:
SAEIVS (for NRU and IVM): a five-port smoking machine integrated with an air–liquid interface exposure module, compatible with 24- and 96-well plates [1].
VITROCELL® VC 10® S-Type Smoking Robot (for Ames): whole smoke or aerosol was bubbled directly through bacterial suspensions.

In Vitro assays

Ames (Mutagenicity)
Salmonella typhimurium strains TA98, TA100, TA102, TA1535 and TA1537 were exposed to freshly generated aerosol or 1R6F smoke in accordance with OECD TG 471 using the VC 10 Smoking Robot with the pre-incubation method [2]. Tests were performed in two independent replicates per strain with and without metabolic activation.

IVM (Genotoxicity)
V79 Chinese hamster lung fibroblasts were exposed at the air–liquid interface to undiluted aerosol and 1:10-diluted 1R6F smoke. Following exposure, the IVM test was performed in accordance with OECD TG 487, with and without metabolic activation [3]. Tests were performed in two independent replicates per treatment.

NRU (Cytotoxicity)
BEAS-2B cells were exposed at the air–liquid interface to undiluted aerosol and 1:16-diluted 1R6F reference cigarette smoke using the SAEIVS system.

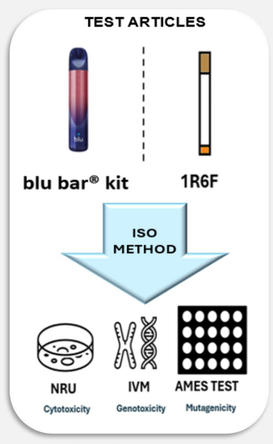

Figure 1:Overview of study methods

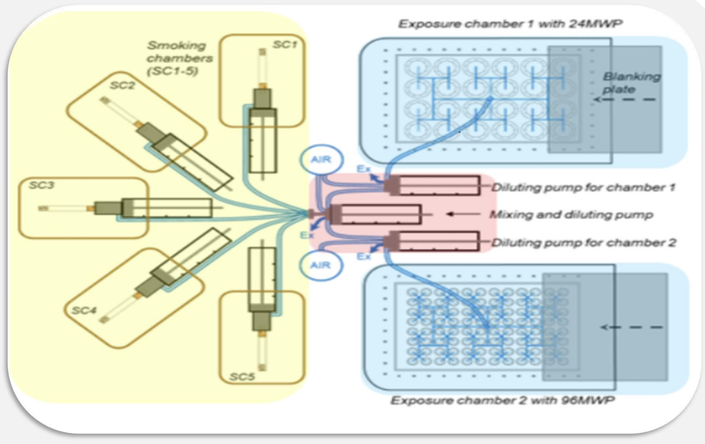

Figure 2: Smoke Aerosol Exposure In Vitro System (SAEIVS)

RESULTS

Ames results

Fresh smoke from the 1R6F reference cigarette produced clear mutagenic activity, with statistically significant, dose-related increases in revertant counts and positive responses in key strains such as TA98 and TA100. In contrast, exposure to 200 puffs of undiluted aerosol from the fruit-flavoured blu bar® kit did not induce statistically significant increases in any of the tested strains, and no strain demonstrated a reproducible slope, resulting in an overall negative mutagenicity outcome for the blu bar® kit in the Ames assay.

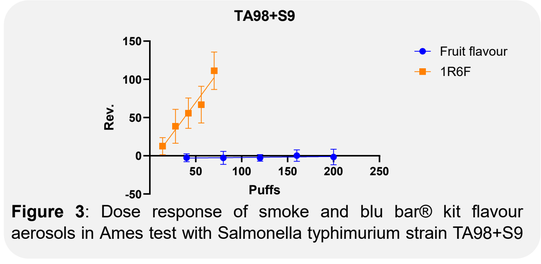

Figure 3: Dose response of smoke and blu bar® kit flavour aerosols in Ames test with Salmonella typhimurium strain TA98+S9

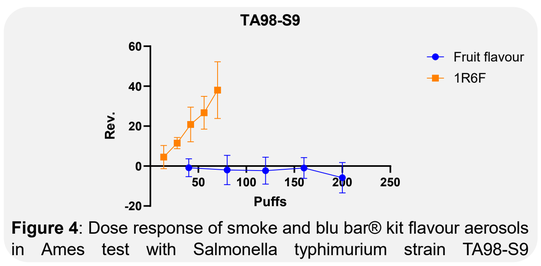

Figure 4: Dose response of smoke and blu bar® kit flavour aerosols in Ames test with Salmonella typhimurium strain TA98-S9

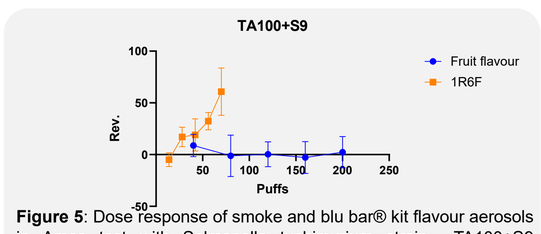

Figure 5: Dose response of smoke and blu bar® kit flavour aerosols in Ames test with Salmonella typhimurium strain TA100+S9

IVM results

A 1:10 dilution of 1R6F cigarette smoke produced a statistically significant increase in micronucleus frequency after a single puff on every test day, whereas, in contrast, the undiluted fruit-flavour blu bar® kit aerosol was classified as equivocal because a rise in micronucleus frequency was seen in one of the replicates at the highest puff numbers in the presence of S9.

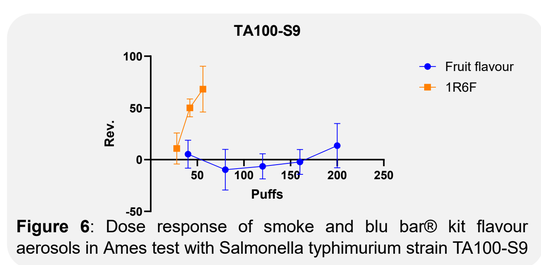

Figure 6: Dose response of smoke and blu bar® kit flavour aerosols in Ames test with Salmonella typhimurium strain TA100-S9

NRU results

In the NRU assay, 1R6F cigarette smoke was highly cytotoxic, with an EC_{50} of 0.221 puffs at a 1:16 dilution, whereas in contrast, the undiluted fruit-flavour blu bar® kit aerosol showed markedly lower cytotoxicity, with an EC_{50} of 13.9 puffs, representing a 98.4% reduction in cytotoxicity relative to 1R6F under the same assay conditions.

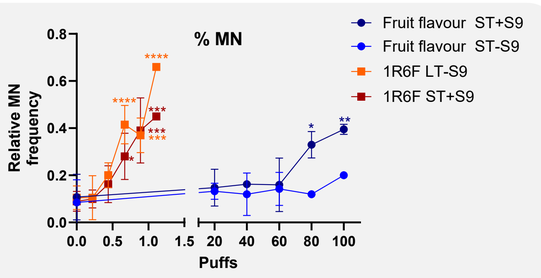

Figure 7: Dose response of smoke and blu bar® kit flavour aerosol in IVM with V79 hamster lung fibroblast, both with and without metabolic activation

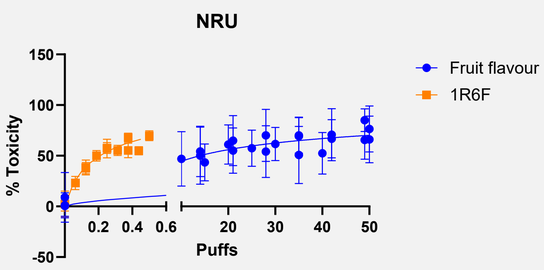

Figure 8: NRU viability curve for 1R6F smoke and blu bar® kit flavour aerosols

CONCLUSIONS

Across three well established in vitro assays, aerosol from the blu bar® kit containing a fruit flavoured e-liquid with 20 mg/mL nicotine salt consistently demonstrated substantially lower biological activity compared with smoke from the 1R6F reference cigarette. Cigarette smoke produced clear mutagenic, genotoxic and cytotoxic responses, whereas blu bar® kit aerosol showed no mutagenicity in the Ames assay, 98% reduction of cytotoxicity in the NRU assay, and at most an equivocal response in the in vitro micronucleus assay under metabolically active conditions at the highest puff numbers. Summarising, these findings indicate that the blu bar® kit aerosol has a significantly reduced in vitro toxicological profile relative to conventional cigarette smoke. This study provides robust evidence to support a reduced harm potential for the blu bar® kit compared with combustible cigarettes.
As a responsible manufacturer, we apply a rigorous Product Stewardship Framework that integrates multiple lines of evidence to assess our products, from ingredient risk assessment all the way to whole product testing as demonstrated here.

REFERENCES

[1] 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.
[2] OECD, 1997. Test No. 471: Bacterial Reverse Mutation Test, OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. OECD Publishing
[3] OECD, 2016. Test No. 487. In vitro Mammalian Cell Micronucleus Test, OECD Guidelines for the testing of chemicals, Section 4: Health effects. OECD Publishing

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