

# Multiple In Vitro Bioassays Support Reduced Toxicological Potential of PULZE™ iSENZIA™ & iD™ Aerosols Relative to 1R6F Cigarette Smoke

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## INTRODUCTION

Heated tobacco products (HTP) are an increasingly important category within next-generation nicotine technologies and are intended as alternatives to cigarettes for adult who smoke due to their tobacco harm reduction potential. Comprehensive *in vitro* biological evaluation is an important component of product assessment, providing information on the potential toxicological properties of product aerosols.

As part of Imperial Brands' commitment to responsible manufacturing, the PULZE™ heated system and its associated consumables undergo an extensive *in vitro* biological evaluation programme.

There are two consumables designed for use with the PULZE™ device: iD™, a tobacco-based consumable and iSENZIA™, which contains a tea-based substrate.

Whilst emissions analysis provides useful information on aerosol composition, biological testing enables assessment of the potential biological responses associated with product aerosols. This work represents the biological assessment component complementing the emissions evaluation reported by Holmes *et al*, 2026 (EUROTOX – See poster ID: 1283).

Biological activity was assessed using cytotoxicity,

mutagenicity, and genotoxicity assays.

The objective of the present study was to compare the toxicological responses induced by aerosols from iD™ and iSENZIA™ consumables with those produced smoke from the 1R6F reference cigarette using OECD-aligned *in vitro* assays conducted under ISO 17025-accredited conditions. By integrating biological and emissions data, this research contributes to the overall weight of evidence supporting the reduced harm potential of PULZE aerosols compared with 1R6F cigarette smoke.

## METHODS

### Test Articles

- **1R6F Reference Cigarette (University of Kentucky)**
- **Commercially available Heated system PULZE™, two stick variants: iD™ Rich Bronze & iSENZIA™ Fruity Flavour.**

### In Vitro Assays

The following regulatory *in vitro* toxicology assays were conducted: the Neutral Red Uptake (NRU) assay to assess cytotoxicity in BEAS-2B cells, performed according to standard protocols under ISO 17025 accreditation; the *Salmonella typhimurium* reverse mutation assay (Ames test) to evaluate mutagenicity, in accordance with OECD Test Guideline 471 (OECD, 1997); and the *in vitro* micronucleus (IVM) assay using V79 cells with and without metabolic activation ( $\pm$ S9) to assess genotoxicity, following OECD Test Guideline 487 (OECD, 2016). For the NRU and IVM assays, cells were exposed to smoke or aerosol at the air-liquid interface using the in-house Smoke Aerosol Exposure *In Vitro* System (SAEIVS; Burghart Tabaktechnik, Wedel, Germany). The Ames assay was conducted using exposures generated with a Vitrocell VC10S smoking machine (VITROCELL Systems GmbH, Waldkirch, Germany).

### Smoke & Aerosol Generation

| Sample | Puffing Regime | Puff Volume (ml) | Puff Duration (Seconds) | Puff Interval (Seconds) | Vent Blocking | Puff Profile |
|--------|----------------|------------------|-------------------------|-------------------------|---------------|--------------|
| HTP    | ISO 20778      | 55               | 2                       | 30                      | N/A           | Bell shaped  |
| HHP    | ISO 20778      | 55               | 2                       | 30                      | N/A           | Bell shaped  |
| 1R6F   | ISO 20778      | 55               | 2                       | 30                      | Yes           | Bell shaped  |

Table 1. Puffing parameters description based on product category and *in vitro* testing used.

For the NRU and IVM assays, fresh aerosol or whole smoke was generated using the bespoke SAEIVS smoking machine (Figure 1). The SAEIVS is a five-port smoking machine directly coupled to the exposure system and fitted with smoke distribution manifolds for 24- and 96- well plates. For the Ames assay, whole smoke or aerosol was delivered into bacterial cultures by bubbling it through the medium using the Vitrocell VC 10 S-Type Smoking Robot (Wiecezorek *et al.*, 2023).

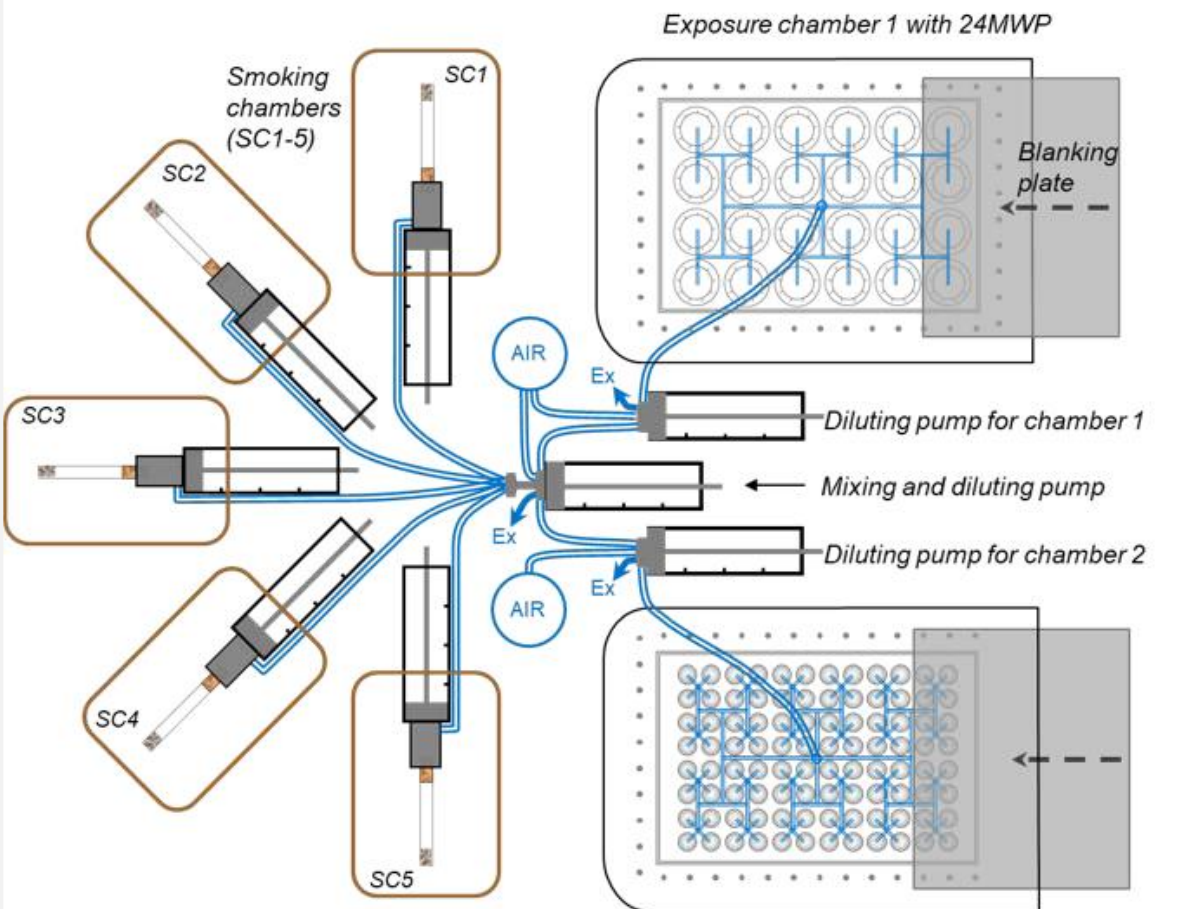


Figure 1 Smoke Aerosol Exposure *In Vitro* System (SAEIVS)

## RESULTS

### Ames

Smoke generated from the 1R6F reference cigarette, tested at 70 puffs of undiluted aerosol (due to the high cytotoxicity), caused a statistically significant and reproducible increase in the number of revertants for TA100  $\pm$  S9 mix and was therefore classified as mutagenic under the test conditions. In TA100 ( $\pm$  S9 mix), the mutagenic potential of 200 puffs of undiluted PULZE™ & iD™ Rich Bronze aerosol was 4-9 times lower than cigarette smoke with variability dependant upon replicate. Exposure to 200 puffs of undiluted aerosol from the iSENZIA™ Fruity Flavour did not induce statistically significant increases in any of the tested strains. Therefore, this product meets the criteria to be classified as not mutagenic under the conditions applied in the study.

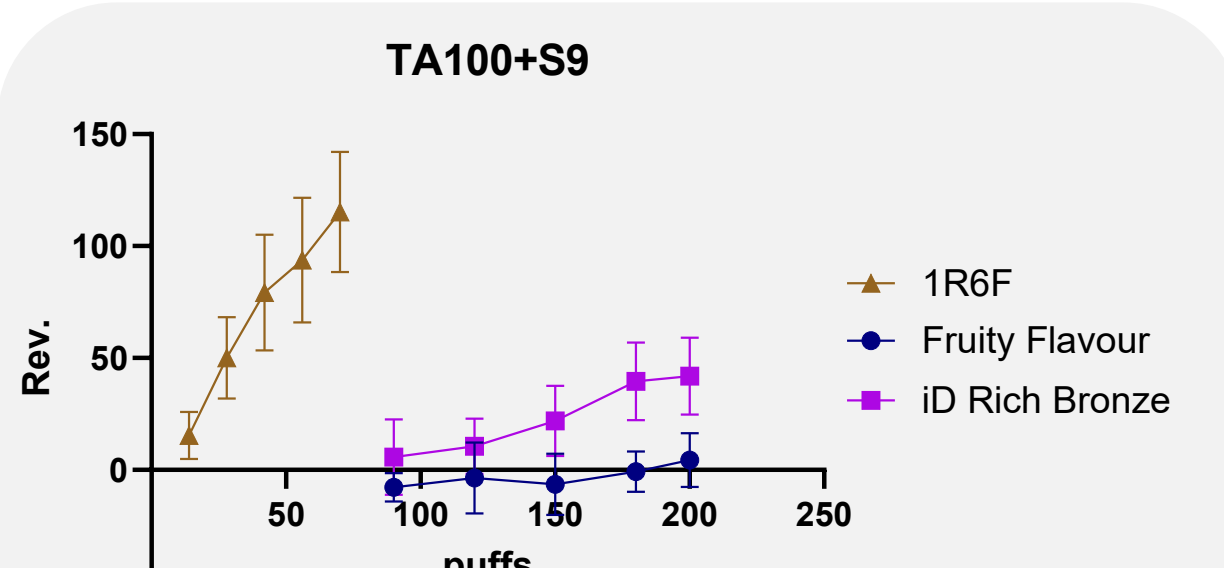


Figure 2: Dose response of smoke and different aerosols in Ames test with *Salmonella typhimurium* strain TA100+S9

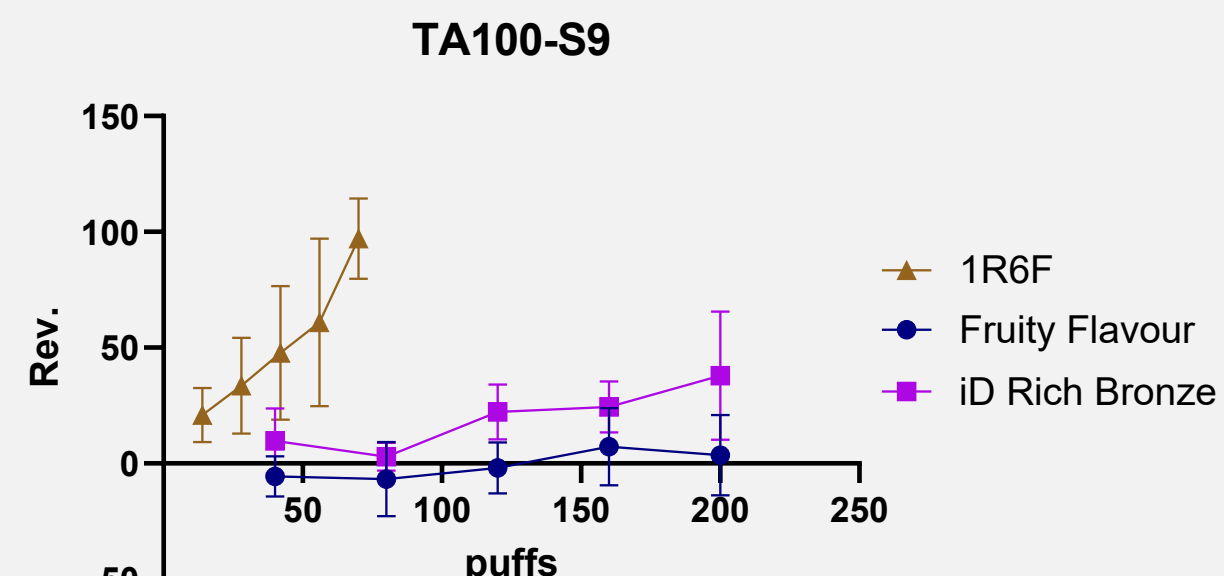


Figure 3: Dose response of smoke and different aerosols in Ames test with *Salmonella typhimurium* strain TA100-S9

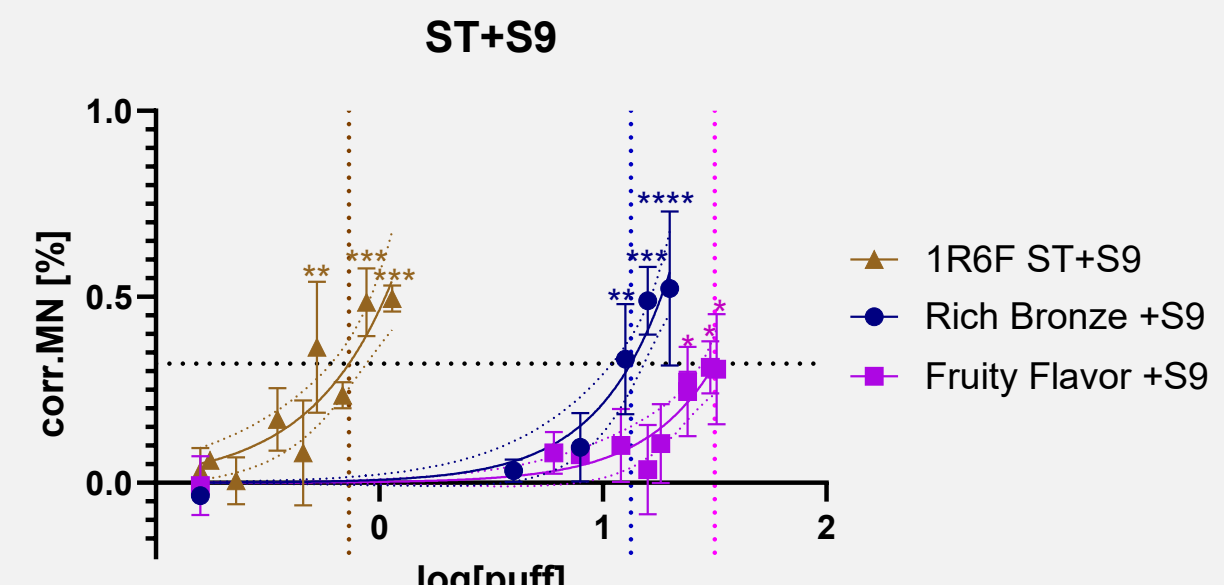


Figure 4: Dose-response curves for smoke and test aerosols in the IVM assay (+S9). The dotted line indicates a 3-fold increase in micronucleus frequency above background.

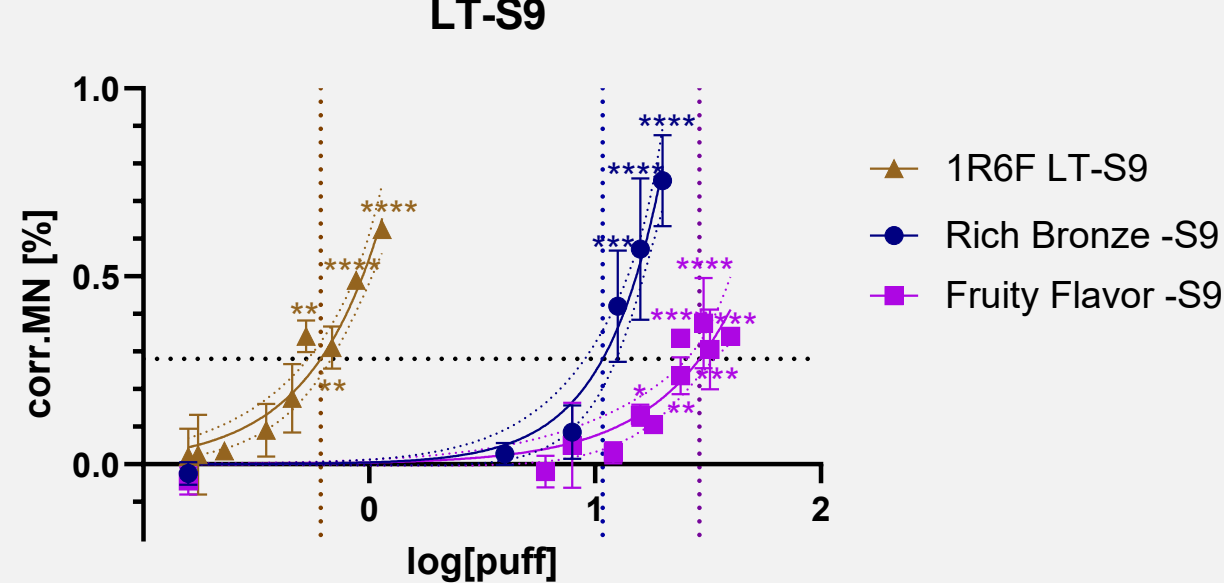


Figure 5: Dose-response curves for smoke and test aerosols in the IVM assay (-S9). The dotted line indicates a 3-fold increase in micronucleus frequency above background.

### IVM

Dose-dependent, reproducible, and statistically significant increases in micronucleus frequency were observed for all products tested regardless of metabolic activation status. However, PULZE™ & iD™ Rich Bronze aerosol was 16.7 times and 14.3 times less genotoxic than 1R6F smoke in the presence and absence of S9, respectively. Similarly, compared with 1R6F smoke, 29.8-fold (with S9) and 35.6-fold (without S9) more puffs of iSENZIA™ Fruity flavour aerosol were required to induce a 3-fold increase in micronucleus frequency above background levels.

### NRU

1R6F cigarette smoke was highly cytotoxic, with an EC<sub>50</sub> of 0.24 puffs. In contrast, iD™ Rich Bronze aerosol showed markedly lower cytotoxicity, with an EC<sub>50</sub> of 7.7 puffs. Furthermore, iSENZIA™ Fruity Flavour aerosol exhibited an even lower cytotoxicity, with an EC<sub>50</sub> of 15.33 puffs; representing a 98.5% reduction in cytotoxicity relative to 1R6F cigarette smoke.

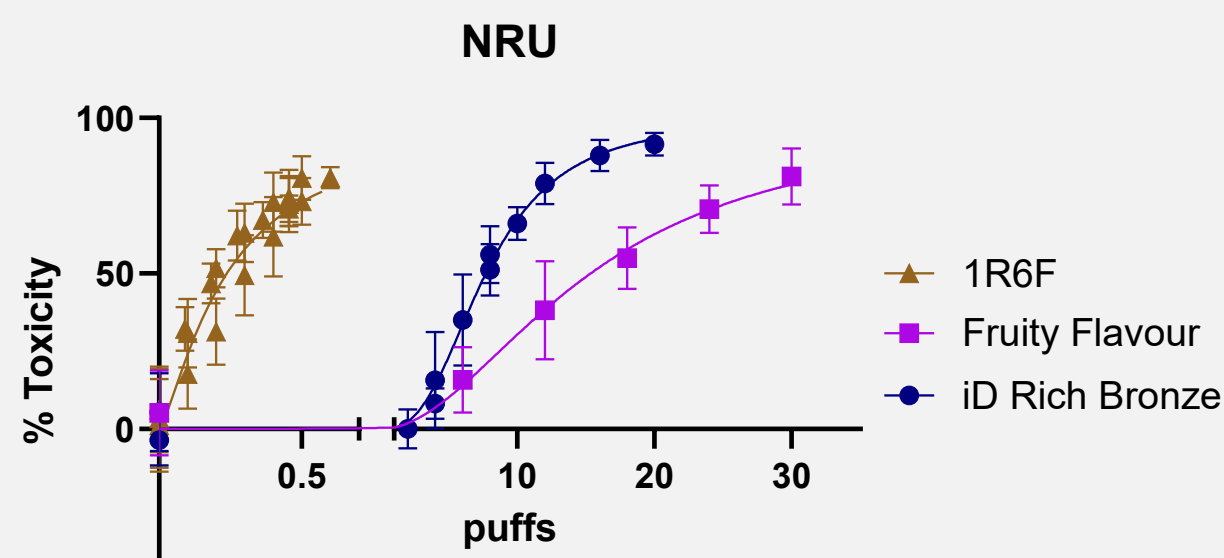


Figure 6: NRU viability curve for 1R6F smoke, iD™ Rich Bronze & iSENZIA™ Fruity Flavour aerosols.

## CONCLUSIONS

Across all assays, both iD™ Rich Bronze and iSENZIA™ Fruity Flavour aerosols demonstrated substantial reductions in cytotoxic, genotoxic and mutagenic activity compared with 1R6F reference cigarette smoke. The reduced biological activity observed for both consumables was consistent with the reduced toxicant emissions reported by Holmes *et al*, 2026 with all measured biological endpoints remaining markedly lower than those observed for cigarette smoke. Together, these data provide complementary evidence that the PULZE™

heated tobacco system generates substantially lower biological responses than conventional cigarettes under the test conditions employed.

As a responsible manufacturer, Imperial Brands is committed to providing adults who smoke with potentially less harmful alternatives to continued cigarette smoking. As seen with the above results, these findings reinforce that the substantial reductions in toxicant emissions observed with the PULZE™ system

using either iD™ Rich Bronze or iSENZIA™ Fruity Flavour consumables are associated with corresponding reductions in *in vitro* biological activity. Under the conditions of these studies, the results suggest that PULZE™ with iD™ Rich Bronze and iSENZIA™ Fruity Flavour has the potential to offer a harm-reduced alternative to cigarette smoking and contribute meaningfully to tobacco harm reduction.

## REFERENCES

- [1] Wiecezorek, 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.
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- [4] Holmes G, 2026, Comparative Assessment of PULZE Heated Tobacco and Herbal Aerosols Demonstrates Substantially Lower Toxicant Levels On Average (95%) Than Cigarette, Imperial Brands, Bristol.



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