Integrated aerosol chemistry and *in vitro* assessment provide a foundation for an evidence-based, responsible product development framework

British Toxicology Society| 27th – 29th April 2026

Christiana Kasim[1] , Emmanuel Iheke[2] , Maria-Andrea Oltean[1], Ourania Komini[1], Konstantinos Papikinos[1]
[1] Imperial Brands PLC, 121 Winterstoke Road, Bristol, BS3 2LL, UK | [2] Imperial Brands, Liverpool Physics Road, Speke, Liverpool L24 9HP UK

FIND OUT MORE

INTRODUCTION

Cigarette smoking is a cause of serious disease including lung cancer, cardiovascular disease and emphysema. The greatest risk of smoking-related diseases comes from burning tobacco and inhaling smoke containing around 7,000 chemicals. In contrast, Next Generation Products (NGP) such as E-Vapour Products (EVP) deliver nicotine without burning tobacco so have the potential to play a role in tobacco harm reduction. The category continues to progress rapidly through ongoing formulation changes, flavour developments and device innovations shaped by consumer expectations and evolving regulatory requirements. To support responsible and evidence-based product development, aerosol chemistry and mechanistic *in vitro* assessments were integrated into a structured, rapidly deployable evaluation framework for EVP. To illustrate this rapid framework, we present data on an EVP, blu bar™ kit EVP (fruit flavour, 20 mg/mL nicotine) was assessed using complementary aerosol characterisation and biological testing to illustrate how these combined data streams can inform consumer-safety-focused decision making. Aerosol emissions were analysed for harmful and potentially harmful constituents, including carbonyls and metals, using internationally recognised analytical methods and were compared with regulatory limits. The corresponding e-liquid was evaluated using the ToxTracker® assay, Including the extended antioxidant version (GSH/NAC Supplementation), to detect potential genotoxic or oxidative stress-related responses to the mixture. Considered together, these complementary approaches provide a coherent understanding of both exposure and mechanistic hazard potential.

METHODS

Test Articles

- 1R6F Reference cigarette (University of Kentucky)
- Electronic Vapour Product (EVP) blu bar™ kit, Imperial Brands PLC (fruit flavour 20mg/ml nicotine)

Emission testing

Emission testing was performed in accordance with Article 20 of the Tobacco Product Directive (2014/40/EU). Nicotine was measured using GC-FID or GC-MS; aldehydes (formaldehyde, acetaldehyde, acrolein and crotonaldehyde) were measured using UHPLC. Elements (chromium, nickel, lead, cadmium, arsenic, antimony, mercury, iron, aluminium) were measured using ICP-MS. Tobacco-Specific Nitrosamine (NNN, NNK, NAB and NAT) were measured using LC-MS-MS. Volatile Organic Compounds (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 of the neat e-liquid was performed by Toxys B.V. The ToxTracker genotoxicity assay utilises 6 green fluorescent protein (GFP) reporter cell lines measuring DNA damage, p53 activation, oxidative stress and protein damage (see figure 1). These cell lines were exposed to the test articles for 24h±S9. To assess reactive oxygen species (ROS) production and oxidative stress, the test article was exposed for 24h±ROS scavengers N-acetyl Cysteine (NAC) and reduced L-Glutathione (GSH) at concentrations of 10mM.

Following these exposure periods, the differential induction of the GFP reporters, as well as cytotoxicity (cell survival) 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. Cytotoxicity was expressed as a percentage of intact cells after the exposure period.

RESULTS

Emission Assessment

From the toxicants assessed in the EVP aerosol, 10 out of 13 were below their limits of detection (acrolein, crotonaldehyde, lead, cadmium, benzene, 1,3-butadiene, NNN, NNK, NAB and NAT). Only formaldehyde, acetaldehyde and toluene were detected but at concentrations below their respective limits of quantification. For the analytes below their LOQ, it was calculated that the reduction in TPD analytes compared to 1R6F reference cigarette ranged from 99.7-99.9% (see figure 2).

- **Antimony, arsenic, chromium, iron, mercury and nickel** for the EVP were below the inhalation permitted daily exposure (PDE) as recommended by the ICH guideline Q3D (R2) on elemental impurities.
- **Aluminium** for the EVP was below the Occupational Safety and health administration (OSHA) PEL-Time Weighted-Average (TWA) with a margin of safety of over 41000.
- **2,3-butanedione** for the EVP 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 safety of 19.
- **2,3-pentanedione** for the EVP was below its respective limit of detection and below the German Social Accident Insurance Information System for Hazardous Substances (GESTIS) Time-Weighted Average (TWA) with a calculated margin of safety of 133.

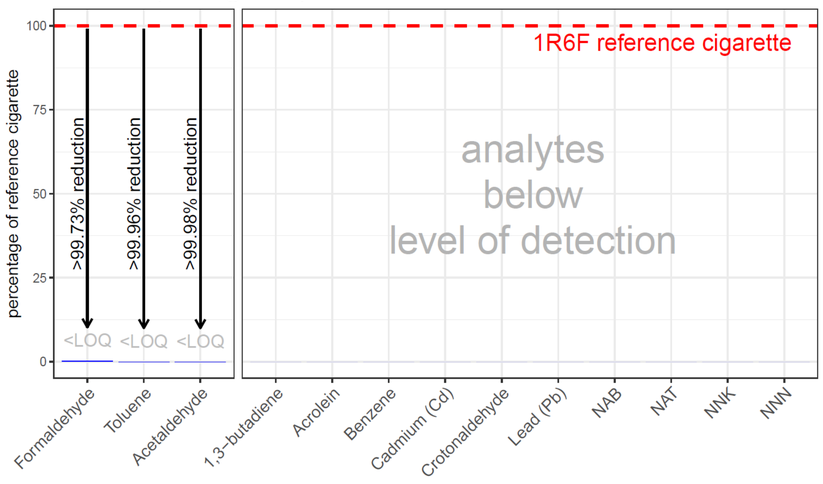

Figure 2: Overview of the emission assessment for EVP

Biological Assessment

The EVP neat e-liquid was classified as non-genotoxic in the ToxTracker assay due to no activation of the ToxTracker reporters in either the absence or presence of S9 (see figures 4-6). The neat e-liquid was given a NOGEL (No Observed Genotoxic Effect Level) of 40µg/ml, the highest nicotine concentration tested. When compared to the maximum blood nicotine concentrations which were reported by O'Connell *et al* (2019) - 12ng/ml - the NOGEL of 40µg/ml is over 3000 times higher than the maximum blood nicotine concentration.

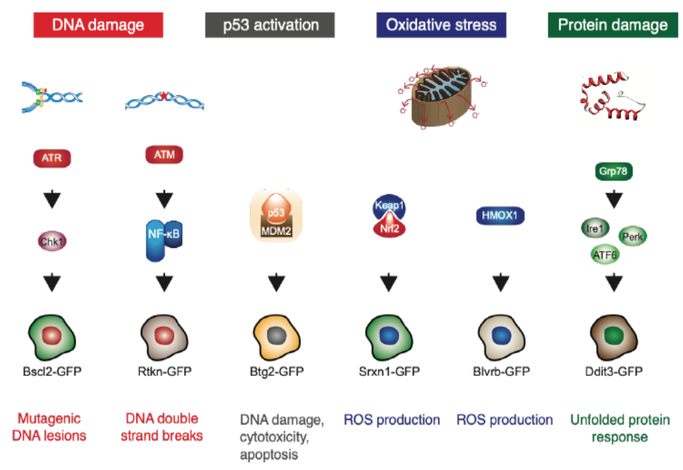

Figure 1: Overview of the stress pathways and biomarkers assessed by the ToxTracker reported assay.

EVP Neat e-liquid

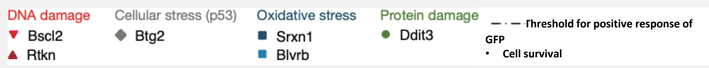

Figure 3: Key showing the cellular events for the below graphs.

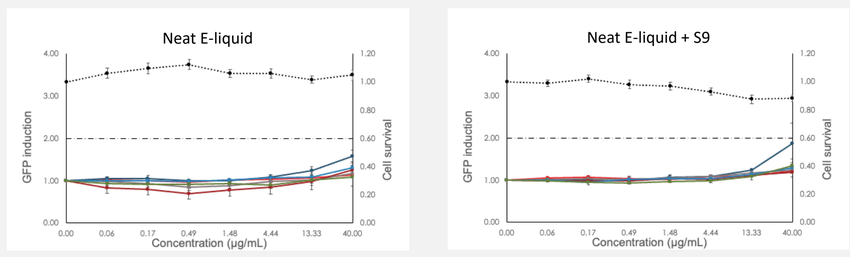

Figure 4: Overview of the ToxTracker results in the presence and absence of S9 activation

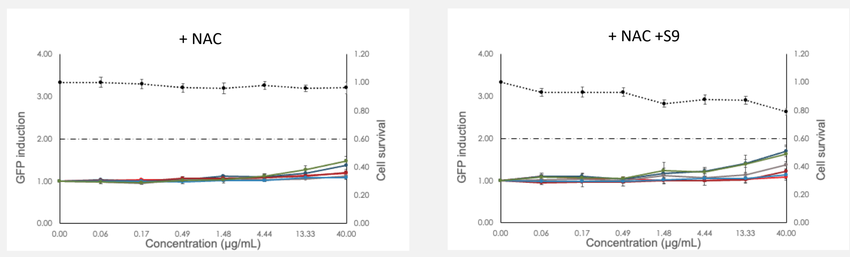

Figure 5: Overview of the ToxTracker results with NAC, in the presence and absence of S9 activation

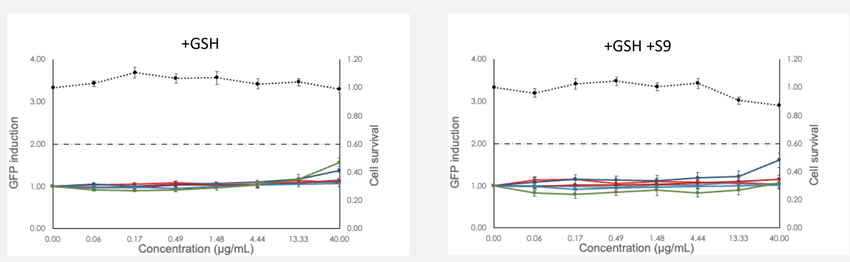

Figure 6: Overview of the ToxTracker results with GSH, in the presence and absence of S9 activation

CONCLUSIONS

Formaldehyde, acetaldehyde and toluene were below their respective limits of quantification with reductions ranging from 99.7-99.9% lower than 1R6F reference cigarette. Acrolein, crotonaldehyde, lead, cadmium, benzene, 1,3-butadiene, NNN, NNK, NAB and NAT were below their limits of detection, which further illustrates the reduced toxicants present in the EVP.

Antimony, arsenic, chromium, iron, mercury and nickel were below the ICH inhalation PDE limits. Aluminium, 2,3-butanedione and 2,3-pentanedione were below their OSHA PEL-TWA, ACGIH and GESTIS limits, respectively. The Tox Tracker assay classified the neat e-liquid to be non-genotoxic. There was no induction of DNA damage, oxidative stress, and p53 activation, with a NOGEL of 40µg/ml (highest concentration tested).

The testing of aerosol chemistry and mechanistic *in vitro* assays provides a rigorous and complementary stewardship assessment framework for rapid product characterisation, strengthening evidence-based evaluation and supporting responsible, science-led EVP development.

REFERENCES

1. Czekala L, Chapman F, Simms L, Rudd K, Trelles Sticken E, Wieczorek R, Bode LM, Pani J, Moelijker N, Derr R, Brandsma I, Hendriks G, Stevenson M, Walele T. 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. 2021 May 31;36(2):129-142. doi: 10.1093/mutage/geaa033.
2. ICH, International Council for Harmonisation. Q3D(R2) Guideline for Elemental Impurities. (EMA/CHMP/ICH/353369/2013).
3. OSHA Occupational Safety and Health Authority. Chemical data: CAS 74-83-9. U.S. Department of Labor. Retrieved from https://www.osha.gov/chemicaldata/496 Accessed on 19th of March 2026
4. ACGIH: Documentation of the Threshold Limit Values (TLVs) and Biological Exposure Indices (BEIs) - Diacetyl. Accessed on 18th March 2026
5. Record of 2,3-pentandione in the GESTIS Substance Database of the Institute for Occupational Safety and Health. Retrieved from https://limitvalue.ifa.dguv.de/limitvalues/47682 Accessed on 18th of March 2026
6. O'Connell G, Pritchard JD, Prue C, Thompson J, Verron T, Graff D, Walele T. A randomised, open-label, cross-over clinical study to evaluate the pharmacokinetic profiles of cigarettes and e-cigarettes with nicotine salt formulations in US adult smokers. Intern Emerg Med. 2019 Sep;14(6):853-861. doi: 10.1007/s11739-019-02025-3. Epub 2019 Feb 2. PMID: 30712148; PMCID: PMC6722145.

IMPERIAL BRANDS SCIENCE blu®