The genotoxicological assessment of a tobacco heating product aerosol relative to cigarette smoke

BAT
A BETTER TOMORROW

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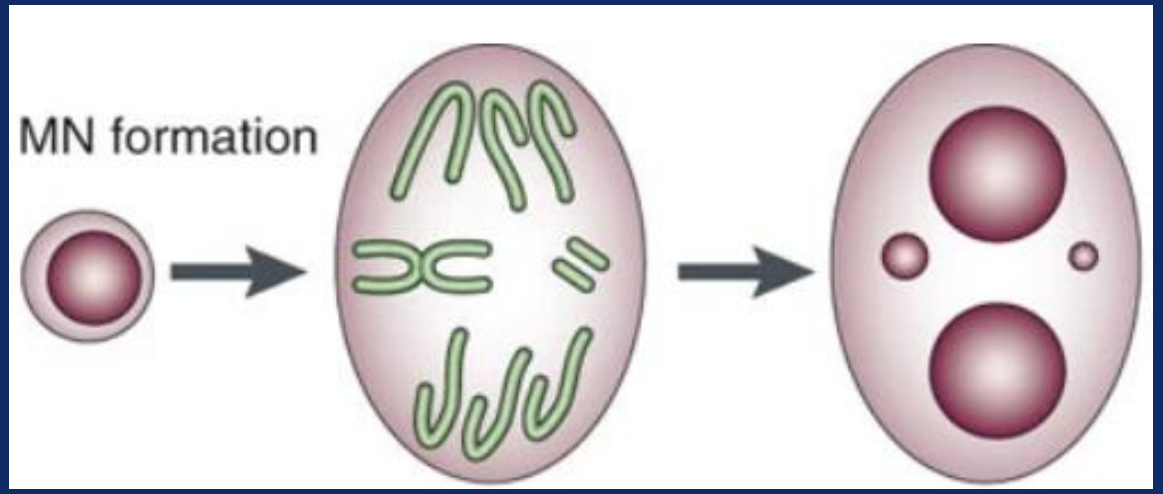
Micronucleus test overview

IVMN

Measure of genotoxicity

The ability to measure the potential for genetic damage



Following exposure to a genotoxic substance, damage to the genetic material can occur

The damaged genetic material is left behind as ‘micronuclei’

Chromosomal damage is an important stage in carcinogenesis and other diseases

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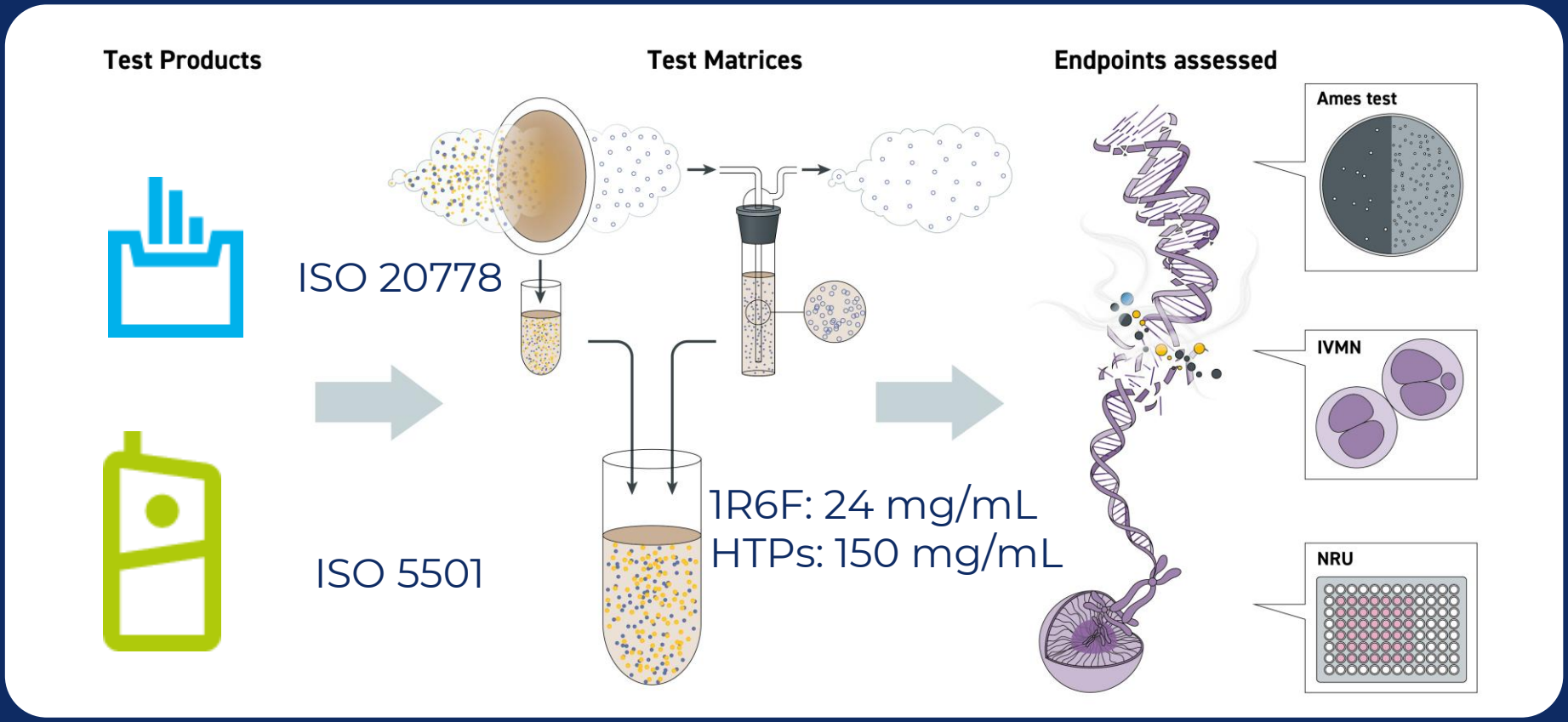
Approach to testing

Unflavoured HTP
Reconstituted Virginia tobacco plus glycerol (14.8%)

Flavoured HTP
Reconstituted Virginia tobacco plus glycerol (14.8%), and flavourings

3R4F
Kentucky reference cigarette



Crooks, I, et al. https://doi.org/10.1016/j.fct.2018.05.058

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The *in vitro* micronucleus test

V79 cells seeded in 75 cm^3 flasks (4 replicate cultures per concentration), using the cytochalasin B cytokinesis block method

Cells treated with a range of at least 5 concentrations for 3 hours with a 21-hour recovery period in the absence and presence of S9, and for 24 hours with no recovery period in the absence of S9.

Positive controls used were cyclophosphamide in the 3h+S9 treatments, mitomycin C in the 3h –S9 treatment and vinblastine in the 24h –S9 treatment

Following the treatment/recovery period, slides were prepared with staining for manual scoring

An initial dose range finder was used to identify the appropriate concentration range to test, to avoid concentrations that exceeded 60% cytotoxicity

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Acceptance & evaluation criteria

Acceptance Criteria

Vehicle controls are within the historical control ranges

Positive controls induced statistically significant increases in micronucleated binucleate cells

At least 50% of cells had gone through one cell division

Evaluation Criteria

The TPM/ACM induced statistically significant increases in micronuclei

A concentration related increase was seen

The induction of micronucleated binucleate cells exceeded the historic vehicle control ranges

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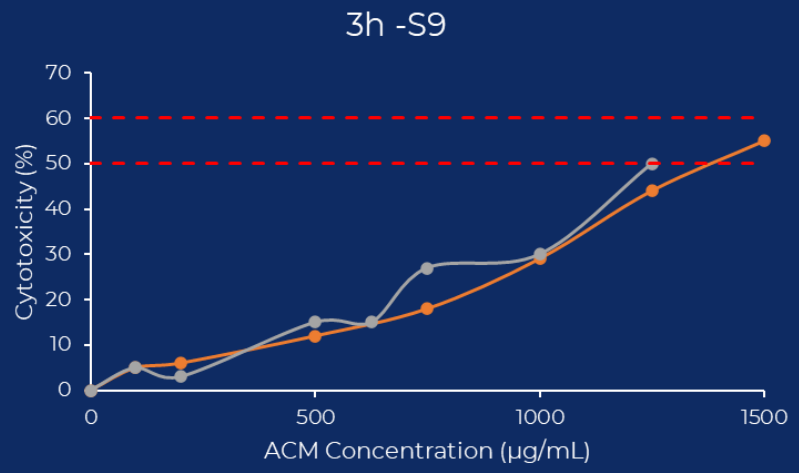
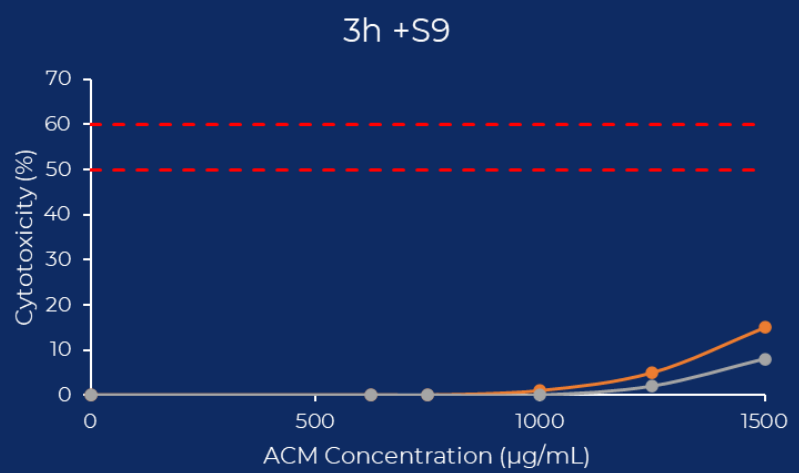
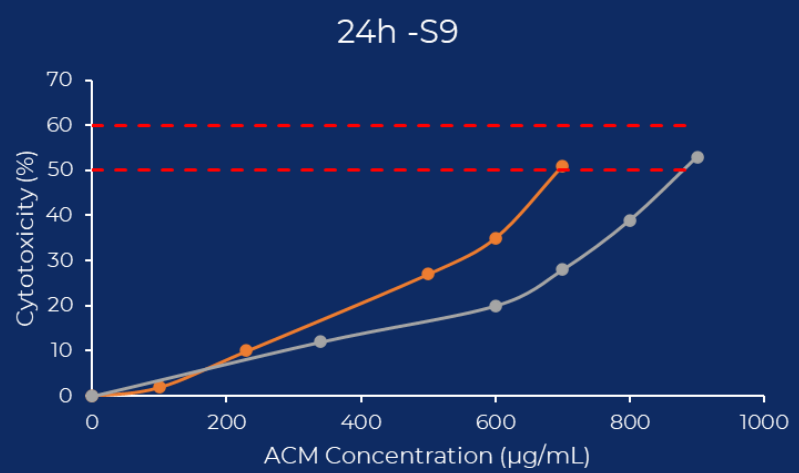
Results - cytotoxicity







Clear differences in the range of concentrations used in each treatment condition
Only the 3h+S9 did not reach adequate levels of cytotoxicity, despite testing at the maximum feasible concentration

Flavoured
Unflavoured
3R4F

Crooks, I, et al. https://doi.org/10.1016/j.fct.2018.05.058

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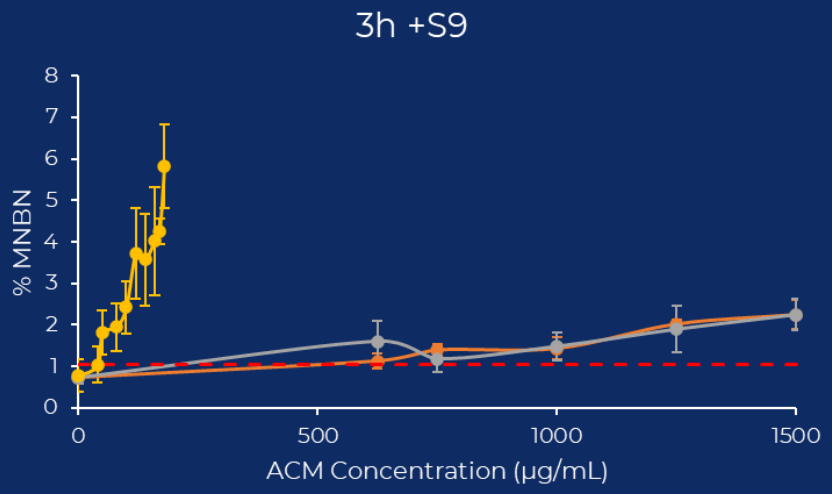
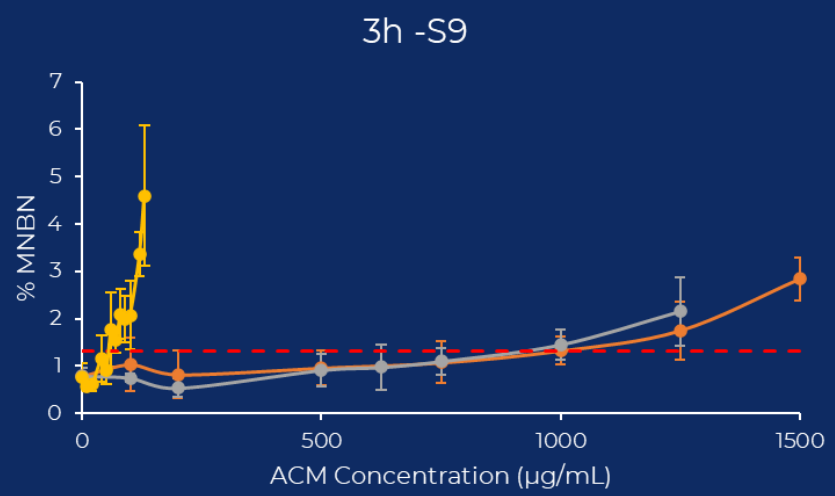
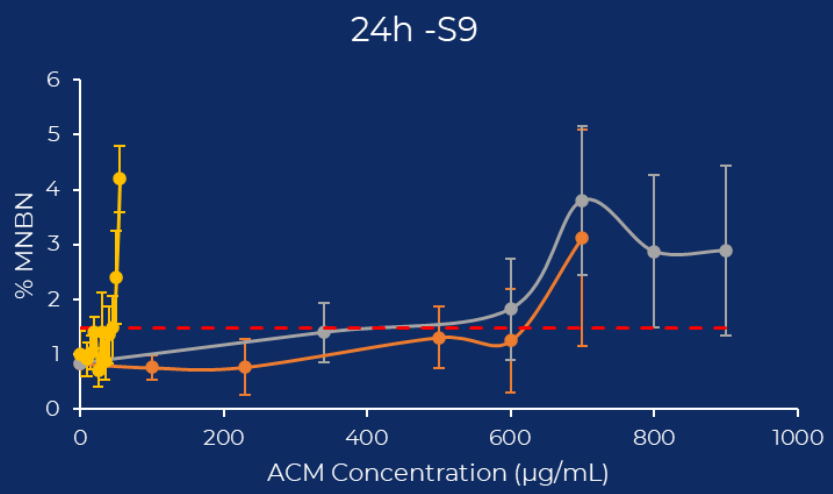
Results – micronuclei induction







Both the unflavoured and flavoured HTP induced increases in micronuclei in all treatments
The response of the unflavoured and unflavoured HTP were similar to each other

Flavoured
Unflavoured
3R4F

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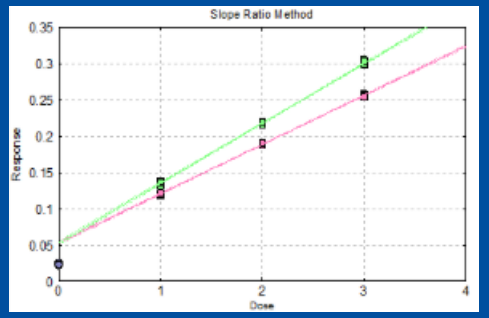
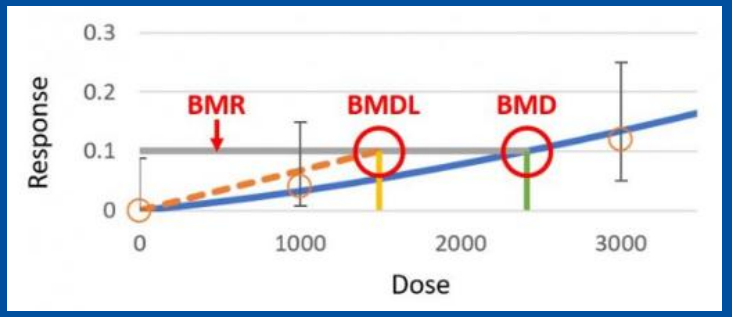
Approaches to analysis

Comparative analysis was only applied if both products to be compared provided dose related increases that were statistically significant and biologically relevant

Slope analysis



BMD analysis



BMR: Benchmark Reference
BMD: Benchmark Dose
BMDL: lower bound 95% confidence limit

https://www.epa.gov/bmds/frequent-questions-about-bmds-modeling-tools
https://www.unistat.com/guide/bioassay-analysis-slope-ratio-method/

Focus on BMD approach

TPMs were compared using a benchmark dose (BMD) approach with BMDS 2.6 software (www.epa.gov/bmds)

The application was run using default the wizard settings (BMDS Wizard 1.10)

The benchmark reference was set as the 95% upper limit of the vehicle control as the threshold and the selected model was based on the recommendation of the wizard and the lowest Akaike Information Criteria (AIC).

The BMD was defined as the concentration of the test item to induce a response that was equal to the 95% upper limit of the historic vehicle control range and the BMDL was defined as the lower bound 95% confidence limit.

Focus on BMD approach

	3h –S9				3h +S9				24h –S9			
	TPM		Nicotine		TPM		Nicotine		TPM		Nicotine	
	BMD	BMDL	BMD	BMDL	BMD	BMDL	BMD	BMDL	BMD	BMDL	BMD	BMDL
3R4F	46.5	40.3	2.22	1.92	17.7	11.7	0.84	0.56	44.4	41.6	2.12	1.99
Flavoured	946	848	21.58	19.35	463	329	10.56	7.51	572	474	13.05	10.81
Unflavoured	875	795	20.05	17.98	341	68.6	7.81	1.57	356	273	8.16	6.26

Conclusions

The IVMN is a valuable tool for evaluating tobacco and nicotine-containing products

The sensitivity of the cell line used needs to be taken into consideration, as does the highest concentration of the respective test articles, and their preparation

Several methods exist to valuate the data, including slope analysis and benchmark dosing

In all instances, the methods/software and parameters used should be documented, and the rationale for the BMD justified