In Vitro Toxicity Evaluation of Aerosol from Seven Flavors from a Temperature Regulated Nicotine Salt-Based Connected ENDS Product

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Poster #22

ABSTRACT

The Glas G2 ENDS is a temperature-regulated nicotine salt pre-filled disposable pod connected system (Glas system) designed to minimize the combustion byproducts across a range of operating conditions. Aerosol generated from Glas system was evaluated for cytotoxicity, mutagenicity and genotoxicity potential. Testing was conducted by an accredited ISO 17025 laboratory using validated methods. Seven different flavored e-liquids (three tobacco, two menthol, two fruit; 50 mg/ml nicotine) were vaped following CRM81 non-intense puffing conditions and aerosol collected mass (ACM) was prepared using an ethanol extraction methodology. For toxicological assys, ACM exposure was conducted in a dose-dependent manner. The cytotoxicity was assessed by the neutral red uptake in vitro assay in BALBc/3T3 cells (OECD, TG 129). The mutagenicity was assessed by bacteria reverse mutation assay (OECD TG 471) using 5 tester strains of (TA98, TA100, TA102, TA1535, and TA1537) in the presence and absence of rat liver S9 fraction metabolic activation system. The genotoxicity was assessed by MN assay (OECD TG 487) in human lymphoblast TK6 cells. Appropriate negative and positive controls were included in assays. In conclusion, under the experimental conditions and based on the criteria for evaluation of various assays, no aerosol mediated cytotoxicity, mutagenicity or genotoxicity was observed at any of the tested Glas flavors. EC50 for the Glas aerosol could not be calculated for any assay because the lack of dose-response. This in vitro toxicological analysis of aerosol generated with seven Glas e-liquid products in combination testing with the Glas G2 device did not induce cytotoxic, mutagenic or genotoxic response.

STUDY OVERVIEW

- All testing were conducted at Enthalpy Analytical LLC with locations in Richmond, VA.
- The Glas flavors, flavor characterization and nicotine concentrations tested for in vitro toxicological evaluations are listed in Table 1.

Table 1. Products Tested

	Test Articles	Flavor	Nicotine Concentration
1	BLUE TOBACCO	Tobacco	50 MG/ML
2	BLONDE TOBACCO	Tobacco	50 MG/ML
3	SIGNATURE TOBACCO	Tobacco	50 MG/ML
4	FRESH MENTHOL	Menthol	50 MG/ML
5	CLASSIC MENTHOL	Menthol	50 MG/ML
6	SAPPHIRE	Non characterizing	50 MG/ML
7	GOLD	Non characterizing	50 MG/ML

METHODS

AEROSOL GENERATION

For aerosol generation, Glas G2 device was used following CORESTA CRM 81 puffing regime; Puff volume: 55 mL, Puff duration: 3 seconds, Puff frequency: 30 seconds, Puff profile: Square-wave, Vent blocking: Not applied. 3 treatment plates for each assay were analyzed.

(CRM 81: CORESTA recommended method nº 81. Routine analytical machine for e-cigarette aerosol generation and collection – definitions and standard conditions (June 2015).

E-VAPOR CONDENSATE COLLECTION

E-vapor condensate was generated by collecting e-vapor condensate on a pre-weighed 55 mm Cambridge filter pad followed in series by an impinger filled with 20 mL of USP ethanol. The ethanol from the impinger was used to extract the pad to produce the e-vapor condensate solution. An appropriate number of devices was vaped per sample to generate an adequate amount of e-vapor condensate concentration (60-80 mg/mL). Number of puffs and pad weight was recorded. The devices were primed prior to e-vapor condensate collection to ensure the wick is completely saturated prior to condensate collection.

CYTOTOXICITY TESTING USING NRU ASSAY

The cytotoxicity was measured using neutral red dye uptake assay according to OECD guideline Test No.129 in BALBc/3T3 cells. Eight different concentrations of e-vapor condensate ranging from 20 to 300 µg/mL were tested.

(OECD (2010). Guidance document on using cytotoxicity tests to estimate starting doses for acute oral systemic toxicity tests (No. 129).

MUTAGENICITY TESTING USING THE AMES ASSAY

The Bacteria Reverse Mutation assay was conducted according to OECD guideline Test No. 471 using 5 tester strains of Salmonella typhimurium (TA98, TA100, TA102, TA1535, and TA1537) in the presence and absence of rat liver S9 fraction metabolic activation system. Six non-zero concentrations of aerosol condensate ranging from 50 to 2500 µg/plate were tested.

(OECD (1997). Bacterial Reverse Mutation Test (No. 471). Organisation for Economic Co-operation and Development.)

GENOTOXICITY TESTING USING IN VITRO MICRONUCLEUS (MN) ASSAY

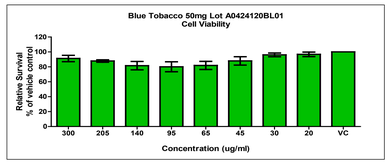
The MN assay was conducted according to OECD guideline Test No.487 using human lymphoblast TK6 cells (TK6, ATCC® CRL-8015™). Five different concentrations of e-vapor condensate ranging from 50 to 600 µg/mL were tested under the short-term conditions of the assay (presence and absence of rat liver S9 metabolic activation for 4 hrs), while concentrations ranging from 20 to 200 µg/mL were tested under the long-term treatment conditions of the assay (absence of rat liver S9 metabolic activation for 27 hrs).

(OECD (2016). In Vitro Mammalian Cell Micronucleus Test (No. 487). Organisation for Economic Co-operation and Development. 29).

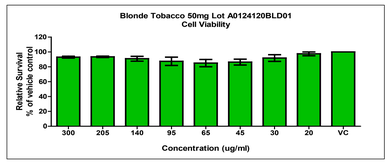
RESULTS

CYTOTOXICITY

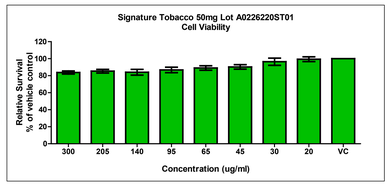
Blue Tobacco



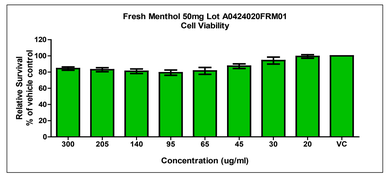
Blonde Tobacco



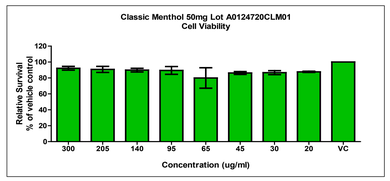
Signature Tobacco



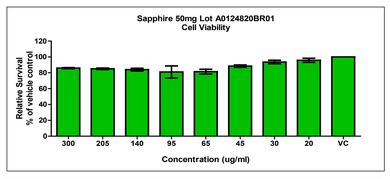
Fresh Menthol



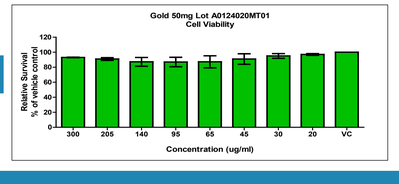
Classic Menthol



Sapphire



Gold



MUTAGENECITY

		-S9		+S9		
		Colony Fold Increase (max.)	Dose-dependent	Colony Fold Increase (max.)	Dose-dependent	Mutagenic
Blue Tobacco	TA98	1.1	No	1.1	No	No
	TA100	1.2	No	1.2	No	No
	TA102	1.0	No	1.2	No	No
	TA1535	1.8	No	0.9	No	No
	TA1537	1.3	No	2.4	No	No

		-S9		+S9		
		Colony Fold Increase (max.)	Dose-dependent	Colony Fold Increase (max.)	Dose-dependent	Mutagenic
Blonde Tobacco	TA98	1.0	No	1.1	No	No
	TA100	1.0	No	1.1	No	No
	TA102	1.0	No	1.0	No	No
	TA1535	1.4	No	0.9	No	No
	TA1537	1.1	No	0.7	No	No

		-S9		+S9		
		Colony Fold Increase (max.)	Dose-dependent	Colony Fold Increase (max.)	Dose-dependent	Mutagenic
Signature Tobacco	TA98	1.1	No	1.1	No	No
	TA100	1.3	No	1.2	No	No
	TA102	1.0	No	1.0	No	No
	TA1535	1.5	No	1.7	No	No
	TA1537	2.1	No	1.5	No	No

		-S9		+S9		
		Colony Fold Increase (max.)	Dose-dependent	Colony Fold Increase (max.)	Dose-dependent	Mutagenic
Fresh Menthol	TA98	1.1	No	1.2	No	No
	TA100	1.1	No	1.5	No	No
	TA102	1.1	No	1.2	No	No
	TA1535	1.5	No	1.7	No	No
	TA1537	1.3	No	1.1	No	No

		-S9		+S9		
		Colony Fold Increase (max.)	Dose-dependent	Colony Fold Increase (max.)	Dose-dependent	Mutagenic
Classic Menthol	TA98	1.6	No	0.9	No	No
	TA100	1.1	No	1.2	No	No
	TA102	1.0	No	1.1	No	No
	TA1535	1.9	No	1.4	No	No
	TA1537	0.7	No	1.4	No	No

		-S9		+S9		
		Colony Fold Increase (max.)	Dose-dependent	Colony Fold Increase (max.)	Dose-dependent	Mutagenic
Sapphire	TA98	1.3	No	1.1	No	No
	TA100	1.1	No	1.1	No	No
	TA102	1.3	No	1.1	No	No
	TA1535	1.2	No	1.6	No	No
	TA1537	1.6	No	1.5	No	No

		-S9		+S9		
		Colony Fold Increase (max.)	Dose-dependent	Colony Fold Increase (max.)	Dose-dependent	Mutagenic
Gold	TA98	1.2	No	1.1	No	No
	TA100	1.3	No	1.5	No	No
	TA102	1.1	No	1.1	No	No
	TA1535	1.6	No	1.6	No	No
	TA1537	2.5	No	1.5	No	No

GENOTOXICITY

	Short-term	% Cytotoxicity	MN Fold Increase	t-test	Chi² test for trend	Genotoxic
Blue Tobacco	-S9	17	1.9	NS	NS	No
	+S9	25	1.5	NS	NS	
	Long-term					
	-S9	24	1.7	NS	NS	

	Short-term	% Cytotoxicity	MN Fold Increase (max.)	t-test	Chi² test for trend	Genotoxic
Blonde Tobacco	-S9	7	1.4	NS	NS	No
	+S9	10	1.4	NS	NS	
	Long-term					
	-S9	8	1.3	NS	NS	

	Short-term	% Cytotoxicity	MN Fold Increase (max.)	t-test	Chi² test for trend	Genotoxic
Signature Tobacco	-S9	39	1.6	NS	NS	No
	+S9	33	1.8	NS	NS	
	Long-term					
	-S9	34	1.9	NS	NS	

	Short-term	% Cytotoxicity	MN Fold Increase (max.)	t-test	Chi² test for trend	Genotoxic
Fresh Menthol	-S9	27	1.8	NS	NS	No
	+S9	20	1.6	NS	NS	
	Long-term					
	-S9	18	1.4	NS	NS	

	Short-term	% Cytotoxicity	MN Fold Increase (max.)	t-test	Chi² test for trend	Genotoxic
Classic Menthol	-S9	26	1.6	NS	NS	No
	+S9	31	1.6	NS	NS	
	Long-term					
	-S9	26	1.4	NS	NS	

	Short-term	% Cytotoxicity	MN Fold Increase (max.)	t-test	Chi² test for trend	Genotoxic
Sapphire	-S9	20	1.6	NS	NS	No
	+S9	19	1.4	NS	NS	
	Long-term					
	-S9	20	2.1	NS	NS	

	Short-term	% Cytotoxicity	MN Fold Increase (max.)	t-test	Chi² test for trend	Genotoxic
Gold	-S9	38	1.4	NS	NS	No
	+S9	40	1.6	NS	NS	
	Long-term					
	-S9	52	1.5	NS	NS	

CONCLUSIONS

- The cytotoxicity as measured by NRU assay demonstrated that the aerosol from seven Glas e-liquid test article products were not considered cytotoxic across the tested concentrations of aerosol condensate in comparison to vehicle control. No EC50 could be calculated for any Glas test articles test products since no dose-dependent cytotoxicity was observed.
- The mutagenicity as measured by Ames plate incorporating assay demonstrated that the aerosol from seven Glas e-liquid test article products were not considered mutagenic across the tested concentrations of aerosol condensate in comparison to vehicle control.
- The genotoxicity as measured by in vitro Micronucleus assay demonstrated that under both short-term and long-term conditions of the assay, the aerosol condensates generated from seven Glas e-liquids test articles are considered not genotoxic.

75th TSRC Conference 2022, New Orleans, USA