A mini-literature review on the science of tobacco heating product THP1.0

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Abstract: A novel tobacco heating product (THP1.0) that heats tobacco below 245 ℃, is described. It is designed to eliminate tobacco combustion, while heats tobacco to release nicotine, tobacco volatiles and glycerol to form its aerosol. Recently, a series of preclinical studies have been published on THP1.0. This mini-review summarises the main findings and highlights the research areas for further consideration. The key findings are: 1) THP1.0 produces its aerosol mainly by evaporation and distillation, and not by combustion or pyrolysis. A validation process is proposed which may be used to check the heating-not-burning characteristics of a THP product. 2) In comparison with 3R4F mainstream smoke, toxicant levels in the THP1.0 emissions are significantly reduced using a range of regulatory proposed cigarette smoke toxicant lists. 3) Using total particulate matter and whole aerosol from 3R4F cigarette and THP1.0, *in vitro* mutagenicity, cytotoxicity and tumour-promoting activities are significantly reduced in THP1.0. 4) In a puffing topography study conducted in Japan, mouth level exposure and average daily consumption of Japanese consumers using THP1.0 are found to be significantly lower when using THP1.0. 5) No consumer lip blocking while using THP1.0 is found in the consumer study. These results serve as a part of broad scientific evidence pack to assess the harm reduction potential of THP1.0.

Keywords: Tobacco heating product; THP1.0; Emission; Indoor air quality; *In vitro* assessment; Pre-clinical study

Introduction

THP1.0 is one of the recent commercial tobacco heating products. It is a rechargable battery powered heating product, used in combination with tobacco consumable rods. In comparison to two other commercially available tobacco heating products (iQOS and Eclipse), there has not been any systematic scientific study conducted on THP1.0. In this mini-review, we introduce some key findings conducted on this product that have just been published, covering its design, aerosol emission measurements, and *in vitro* toxicological assessment.

1 Design and operation of THP1.0

In a study by Eaton et al.[1], THP1.0's design principle is described. THP1.0 comprises two functional parts: an electronic handheld device with a heating chamber, and a specially designed tobacco rod that works with the heater. The electronic heating device (Fig.1) contains a rechargeable Li-ion battery, and a resistively heated tube which in turn is divided into two heater segments, running with by separate temperature program. The battery capacity allows for up to 30 repeated use cycles from a single charge. The superslim tobacco rod has a length of 82 mm, with a 42-mm long tobacco section. A user inserts the tobacco rod into the heating chamber, and on pressing the activation button the heating chamber heats the tobacco rod to less than 245 ℃ for up to 3.5 minutes. The tobacco consumable is a blended Virginia tobacco, and processed in a paper-style reconstitution with ca. 14.5% glycerol as its main aerosol agent. The overall mass of the tobacco material is about 260 mg.

When the product is puffed, the temperature experienced by the tobacco is measured and reported[1]. For example, see Fig.2, at Location 1 the temperature increases quickly as the moment heat is applied. Each puff leads to a significant decrease in temperature, this is a feature different to that of a burning cigarette. From ca. 100 s onwards, the proximal tobacco section reaches a temperature > 200 ℃ and remains broadly at

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this temperature for the remainder of the heating cycle. For the second section of the tobacco rod (Location 3), its temperature exceeds 200 ℃ for only the last four puffs. The maximum temperature experienced by both sections of the tobacco rod is less than the maximum heater temperature. There is no evidence of self-sustained energy release (or autorun-off), which would otherwise be the case if the tobacco is experience combustion. Further information about the thermophysics of THP1.0 can be found in this study [1].

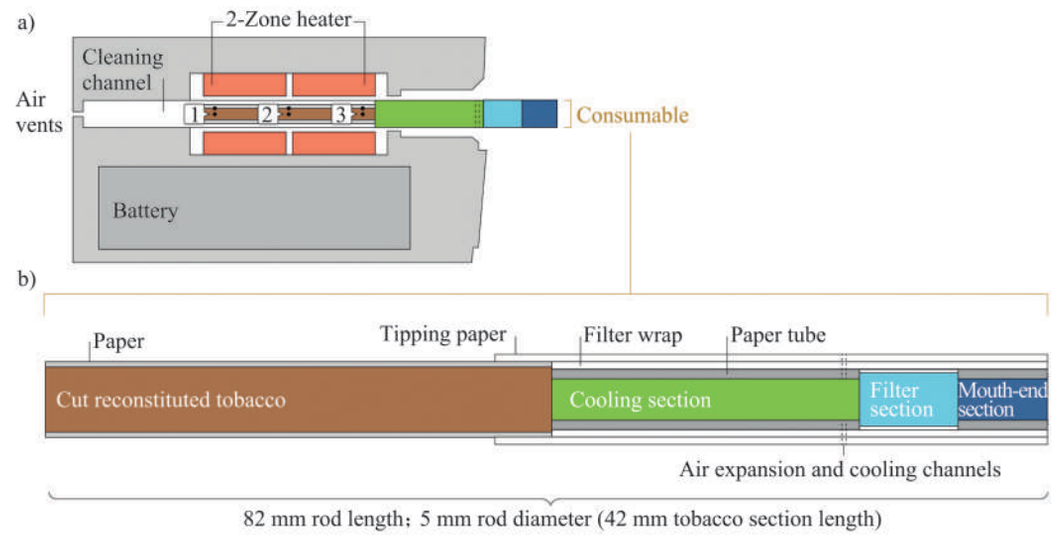

Fig.1 Schematic drawing of THP1.0 device (a) and a tobacco consumable (b).

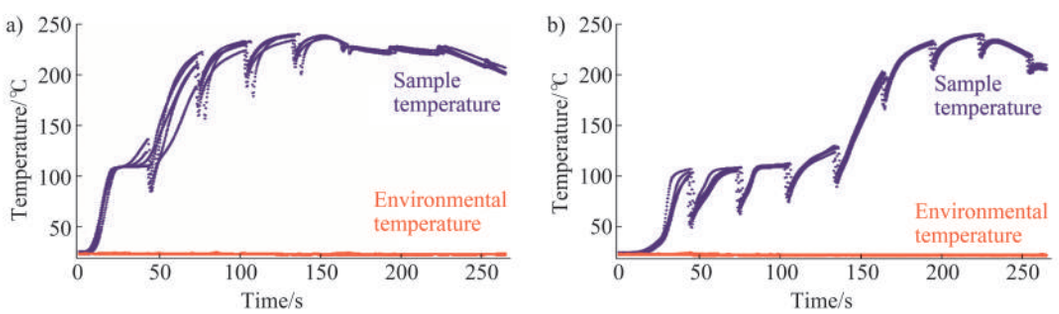

Fig.2 Thermocouple temperature profiles at two locations inside the tobacco rod with machine puffing at 55 mL, 2 s duration and once every 30 s. (a) Location 1; (b) Location 3.

2 Aerosol emissions: THP1.0 vs 3R4F reference cigarette

A comprehensive aerosol emission analysis is described in a separate study [2]. The analytes measured in this study comprise the lists of priority compounds proposed for tobacco product regulation [3-7], additional substances based on knowledge from previous analysis of vapour product aerosols and the likely thermal decomposition products of glycerol [8]. The analytical methods used by the testing laboratory are based on the Health Canada methods for cigarette smoke analysis and are accredited to ISO/IEC 17025: 2005 [9] for all reported constituents of mainstream cigarette smoke and vapour product aerosols. The methods are not accredited for THP emissions but additional validation has been undertaken by the testing laboratory to assure compatibility with THP1.0 aerosol matrix.

An example of the THP1.0 aerosol chemistry from this study is given in Table 1. The extensive reduction on these combustion-driven compounds (acrolein, 1,3-butaniene) and sugar decomposition reactions (acetaldehyde, formaldehyde) demonstrating that the main mechanism of tobacco heating produces significantly lowers the levels of tobacco toxic constituents than those are present in mainstream cigarette smoke. For some substances the results are below the limit of quantification or detection of the method, illustrating that some of the analytical methods, which have been extended from methods for cigarette smoke, may have limitation for this new class of tobacco product [2]. A range of other aerosol chemistry implications from THP1.0 has been described in this paper.

In addition to the machine-puffing emission levels, the impact on indoor air quality (IAQ) when THP1.0 is

Table 1 3R4F cigarette mainstream smoke yields and THP1.0 emission yields for the 9 TobReg priority constituents presented on a per consumable basis.

Parameter	Unit	3R4F	THP1.0(T)		THP1.0(M)	
		Mean per consumable	Mean per consumable	%Red[a] per consumable	Mean per consumable	%Red[a] per consumable
1,3-Butadiene	µg	108	BDL (0.029)	>99.9	BDL (0.029)	>99.9
Acetaldehyde	µg	2 200	111	95.0	115	94.8
Acrolein	µg	157	2.22	98.6	2.50	98.4
Benzene	µg	78.6	NQ (0.056)	>99.9	NQ (0.056)	>99.9
Benzo[a]pyrene	ng	12.9	NQ (0.354)	97.7	0.356	97.2
Carbon Monoxide	mg	32.0	NQ (0.223)	99.8	NQ (0.223)	99.6
Formaldehyde	µg	54.10	3.29	93.9	3.51	93.5
4-(*N*-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK)	ng	281	6.61	97.7	5.32	98.1
Nitrosonornicotine (NNN)	ng	263	24.7	90.6	19.1	92.8
Average				97.0		97.1

Values calculated using replicate data per analyte (N = 5).
BDL: Below Detection Limit (LOD); NQ: Not Quantified (below LOQ).

keen topic for consumer and regulators alike. A separate study using controlled indoor environments has been published to detail the method and the results comparing THP1.0 using and commercial cigarette comparators[10]. THP1.0 is found to show a significantly lower IAQ impact using a range of chemical and aerosol physics indicators as compared with smoking combustible cigarettes in the same controlled environmental conditions, driven by a significantly lower emissions profile of both aerosol particles and chemical emissions from THP1.0. In this study, aerosol particle size measurements on THP1.0 demonstrate the aerosol particle distribution is respirable.

3 *In vitro* dosimetric and cytotoxic assessment

Adequate *in vitro* dosimetry is the cornerstone of a valid *in vitro* assessment on a novel product like THP1.0, because the markedly different aerosol chemistry may cause preferential evaporation and condensation of aerosol constituents and therefore presents a distorted picture at the site of *in vitro* exposure[11]. In the study performed by Jaunky et al.[11], nicotine is used as a marker compound to assess the dosimetry. An example of which is given in Fig.3 as a Boxplot between cell media nicotine concentration after 1 hour cellular exposure. Seven aerosol dilutions are performed, ranging from 1∶20 to 1∶10,000 (aerosol∶air,

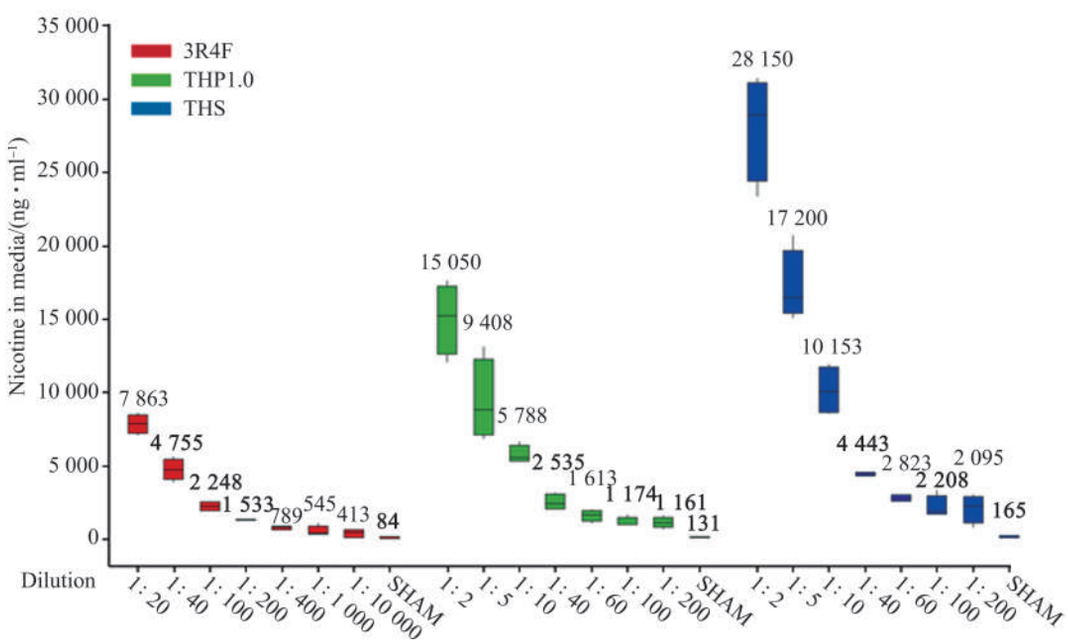

Fig.3 Nicotine concentration in the cell culture media after aerosol exposures demonstrating controlled aerosol dosage from three different tobacco products.

v∶*v*) for the 3R4F reference cigarette and from 1∶2 to 1∶200 (the same 7 dilutions) for THP 1.0. In the study, another commercial tobacco heating product (THS) is also used as a comparator. The results confirm that the *in vitro* dosimetry system used in the study is able to deliver the aerosol from THP1.0 in a range of controlled dosages[11].

As a result of the controlled aerosol dosimetry, Neutral Red Uptake (NRU), as determined by cell viability of NCI-H292 bronchial epithelial cells after 1 hour exposure to a range of aerosol dilutions of the three test products are given in Fig.4 as an example, which shows a statistical difference in the biological response among the 3 products (p<0.000 1) but no difference between the two THPs against aerosol dilution (p=0.015 2).

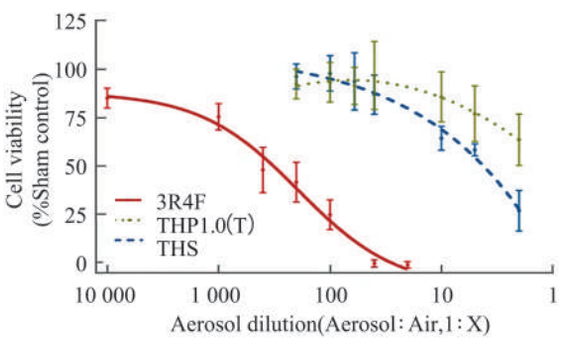

Fig.4 Neutral red uptake (NRU)-cell viability of H292 lung epithelium after 1 hour exposure to a range of dilutions for the three test products. Error bars are standard deviations for replicates at the same dilution, and the type of curve fitting applied to the data is a non-linear regression.

4 Multiparametric toxicity and oxidative stress assessments on THP1.0

In Taylor et al.[12] study, effects of TPM from 3R4F and THP1.0 are investigated using multiparametric high-content screening (HCS) assays, with a total of 10 endpoints assessed over serial dilutions at 4 and 24 h exposures. The results show that exposure of NHBE cells to TPM from 3R4F for a short 4h exposure induces a moderate reduction in cellular availability of the antioxidant glutathione, ROS generation and ATP, an impaired mitochondrial membrane potential, decreases in cellular nuclear size and DNA structure, in combination with an increased incidence of DNA damage. These responses are modest with majority of effects occurring at the higher doses of TPM (80–120 μg/mL). These responses indicate initial cellular oxidative stress responses most likely as a result, from the generation of superoxide intermediates that may deplete/oxidise glutathione and cause oxidative damage to mitochondria or disrupt the electron transfer chain. By contrast exposure to the TPM generated from THP1.0 does not induce a positive signal in the HCS endpoints assessed in this study (see Fig.5 as an example). These assays are an extended *in vitro*

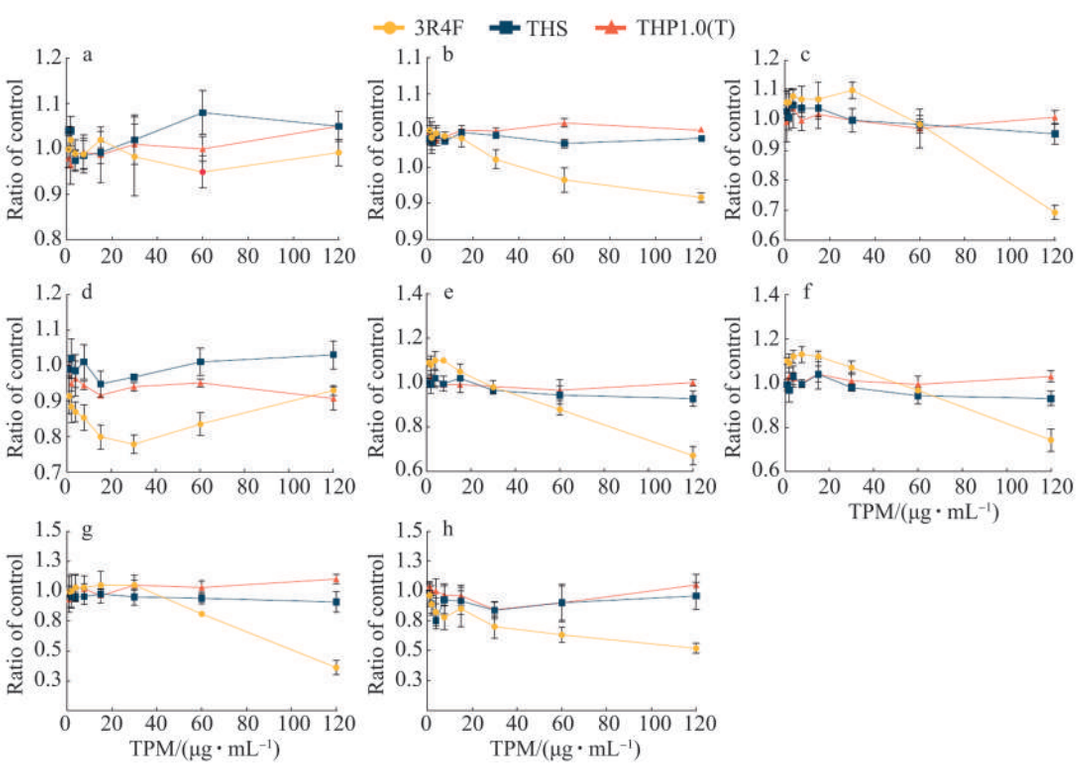

Fig.5 High content screening approaches; cell health endpoint activation following 4 h exposure to 3R4F, THP1.0 or THS. (a) Cell count, (b) Nuclear size, (c) DNA structure, (d) Mitochondrial mass, (e) Mitochondrial membrane potential, (f) ROS formation, (g) Glutathione content, (h) Cellular ATP.

assessment tools that are useful, as a part of weight-of-evidence assessment, to evaluate any toxicological effect of the THP1.0 aerosol.

In Thorne et al.'s study[13], cytotoxicity, mutagenicity and tumour promoting activity assays are used to compare cigarette smoke and THP1.0 aerosol. Acute cytotoxicity is assessed using the ICCVAM NRU protocol in BALB/c 3T3 mouse fibroblasts (an example is given in Fig.6). As it can be seen, 3R4F TPM is shown to be cytotoxic in a range of 20–140 μg/mL, whiles THP1.0 TPM shows no decrease in viability beyond that of the control and an IC50 could not be defined even at the highest achievable dose of 240 μg/mL. Therefore, under the test conditions THP1.0 can be classified as non-cytotoxic.

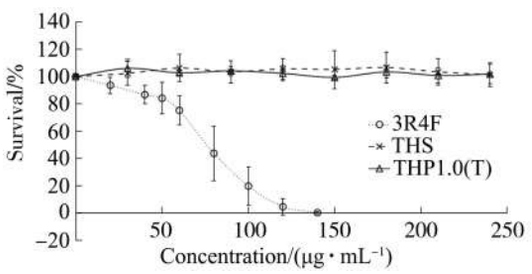

Fig.6 Acute cytotoxicity in NRU assay using BALB/c 3T3s. A positive cytotoxic response was observed with 3R4F cigarette smoke, in contrast to the non-cytotoxic response from THP1.0.

Bhas 42 cell transformation assay can be used to predict carcinogenic potential, one of such example from Thorne et al.[13] is given in Fig.7. Exposure to TPM from 3R4F elicites a concentration-dependent response and is deemed to be tumour-promoting. In comparison, THP1.0 does not produce a positive response in the assay, up to 120 μg/mL.

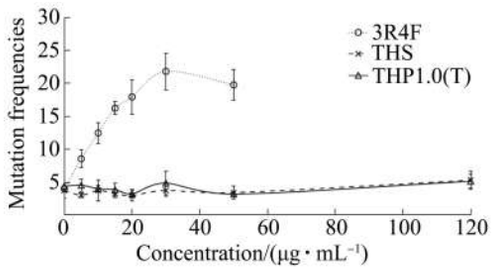

Fig.7 Effect of TPM from 3R4F and THP1.0 on *in vitro* promotion in Bhas 42 cell transformation assay.

5 Puffing topography and mouth level exposure among Japanese users

For a novel tobacco heating product like THP1.0, puffing topography measurements are essential to assess, as close as possible to real usage conditions, the puffing behaviours from consumer. In the paper published by Gee et al.[14], mean values for total puff volume, mean puff volume, puff duration, puff number and inter-puff interval are reported and shown in Table 2. It can be seen that different groups of participants take different puff volumes when using THP1.0 compared with their usual cigarette (coded T189). Puff intervals are significantly shorter for THP1.0 compared with the cigarette. Another group (Group 2) display a similar behaviour with respect to the higher mean puff volume and shorter puff interval for THP1.0 menthol variant as compared with their menthol cigarette (coded M322). More studies are needed to see how a wider population of users would behalf for THP1.0 over a longer period of usage time.

In the paper by Gee et al.[14], used THP1.0 mouthpieces are collected and analysed by the application of a ninhydrin solution. For a total of 104 mouthpieces collected, 3 samples are marked as inconclusive, and the rest of the samples show no evidence of blocking of the air inlet holes. The air inlet holes on the tobacco sticks designed for THP1.0 are placed 18 mm from the mouth end. Fig.8 shows that the mean maximum mouth insertion depth is (7.7 ±

Table 2 Comparison of puffing topography.

Product	User Group	Total puff volume/mL		Mean puff volume/mL		Puff number/*n*		Mean puff duration/s		Mean puff interval/s	
		Mean±SD	Tukey's ranking*	Mean±SD	Tukey's ranking*	Mean±SD	Tukey's ranking*	Mean±SD	Tukey's ranking*	Mean±SD	Tukey's ranking*
T189	1	489.0±177.7	b	48.9±14.8	b	10.7±5.0	a	1.8±0.6	a	9.7±3.4	a
THP1.0(T)		736.4±415.8	a	66.7±23.7	a	10.9±5.6	a	1.8±0.6	a	7.4±2.7	b
THS		668.1±322.6	ab	63.5±20.3	a	10.3±3.6	a	1.8±0.6	a	8.3±3.0	b
M322	2	493.7±192.4	a	51.1±16.0	b	10.0±3.7	a	2.0±0.5	a	9.9±3.4	a
THP1.0(M)		618.2±389.6	a	62.2±32.8	a	10.0±4.5	a	1.8±0.5	a	8.1±3.0	b
THP1.0(T)	3	773.5±545.7	a	60.9±24.8	a	12.3±7.3	a	1.8±0.7	a	7.7±3.9	a
THS		588.0±360.0	a	55.1±23.9	b	10.8±5.1	a	1.8±0.7	a	8.6±3.1	a

*Same letter indicates no statistical difference ($p>0.05$) within user groups, SD, standard deviation.

3.4）mm, and the maximum mouth insertion depth observed from all the samples analysed are 17 mm（Fig.8）.

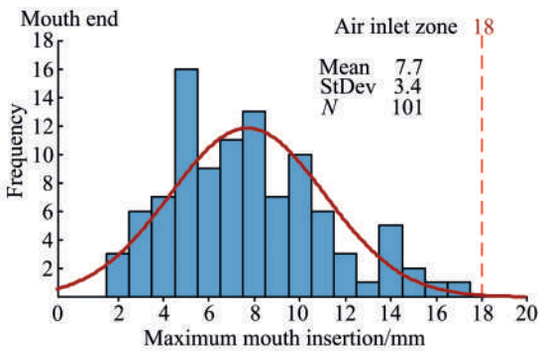

Fig.8 Distribution of mouth insertion depths using THP1.0.

6 Summary

In this mini-literature review, we summarise the published results on a tobacco heating product THP1.0. These studies serve to highlight the main mechanism of aerosol formation in THP1.0 is distillation and evaporation and that there is very little or no combustion. Compared with 3R4F mainstream smoke, the toxicant levels in the THP1.0 emissions are significantly reduced across all chemical classes. A range of regulatory *in vitro* toxicological responses from THP1.0 aerosol demonstrate a statistically significant reduced biological response as compared with cigarette smoke. Puffing topography and the extent of lip blocking while using THP1.0 find no evidence users using their lip to block the air inlet holes of the THP1.0 tobacco rods. The puffing topography results support the machine puffing regime used to generate toxicant emissions data and *in vitro* toxicology testing. More studies are needed to fully evaluate the risk reduction potential of THP1.0 as an alternative product to cigarette smoking.

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