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Comparison of tobacco heating products and conventional cigarette: a systematic review

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Abstract

Heat-not-burn products, which are supposed to reduce the harmful effects of exposure to cigarette smoke components (harm reduction approach), are under development. Comprehensive evaluation of the newest available on the market tobacco heating products (THPs) in comparison with conventional cigarettes (CC) within pre-clinical and clinical studies. A systematic review of the literature was performed in MEDLINE, EMBASE, The Cochrane Library, Center for Reviews and Dissemination databases. Primary clinical studies from the highest level of credibility (randomized controlled clinical trials), evaluating the use of THP compared to the use of a CC by smokers were searched. Additionally in order to study impact of passive smoking on health, pre-clinical studies and studies evaluating indoor air quality were included. In the review 9 randomized clinical trials, 37 pre-clinical studies and 6 studies evaluating the impact of heat-not-burn products on indoor air quality were included. Studies demonstrated that switching from CC to THP is associated with reduction of harmful and potentially harmful constituents' exposure and probable less harm in clinical risk markers in comparison with the continuation of smoking conventional cigarettes while maintaining comparable nicotine delivery. Results suggest no negative impact on indoor air quality when using THP in an indoor environment. THPs compared to CC smoking (within the analyzed risk factors) shows a tendency to limit negative health influence. The assessment of heat-not-burn product impact on the risk of smoking-related diseases requires further research and long-term observations.

Background

Smoking has been recognized by the World Health Organization (WHO) as a health problem caused by tobacco addiction (ICD-10: F17 - Mental and behavioural disorders due to use of tobacco).[1],[2] Addiction to smoking is caused by nicotine and behavioural addictions. Nicotine addiction is associated with the need to maintain specific concentrations of nicotine in the blood serum, while behavioural addiction depends on psychological, environmental, cultural and social factors.[3] There are around 1.1 billion smokers in the world.[4] The tobacco epidemic is one of the world's greatest threats to public health. According to data from the WHO, over 6 million deaths per year is a direct result of smoking, while approx. 890,000 deaths occur due to tobacco smoke exposure on non-smokers.[4] According to estimates of the WHO in 2025, the prevalence of cigarette smoking will reach 18.9% in the world population.[5] Unfortunately less than 5-10% of smokers who tried to quit are smoking free for 6 months or longer.[6],[7]

Smoking causes many serious diseases, including cardiovascular diseases, lung cancer and chronic obstructive pulmonary disease.[8],[9],[10] It is widely recognized that the adverse effects of smoking are not primarily caused by nicotine, but by toxic substances released during the combustion of tobacco.[11] Cigarette smoke is a very complex mixture in which over 6,000 chemicals have been identified. Among them, about 100 compounds are thought to contribute to smoking-related diseases.[12] Epidemiological studies showed that inhalation of tobacco smoke by non-smokers (so-called passive smoking) is associated with a serious health risk.[13]

Harm reduction approach

The main strategies to reduce health-related harm associated with smoking are prevention of starting smoking and promotion of smoking cessation. In the treatment of smoking addiction, methods are used to strengthen motivation and to speed up the decision to give up the addiction, support the patient in the actions taken and reduce the symptoms of withdrawal.[14]

Heat-not-burn systems and e-cigarette

Heat-not-burn products and e-cigarettes, which are supposed to reduce the harmful effects of exposure to cigarette smoke components (harm reduction approach), are under development as alternatives to cigarettes for smokers who are not able or not willing to quit smoking. A recent review indicated that limited evidence suggests that e-cigarettes may be effective in reducing cigarette use among adult smokers willing to quit.[15]

Heat-not-burn products are tobacco products that produce an aerosol containing nicotine and other chemicals that are inhaled by the user. Tobacco heating products imitate the action of traditional cigarettes and are not e-cigarettes, because THPs heat tobacco to release nicotine, while in the e-cigarette a liquid, which may contain nicotine is heated.[16] The main components of liquids heat-

ed in the e-cigarette are nicotine (in nicotine-containing products), propylene glycol (± glycerol) and flavours.

THPs heat tobacco up to 240-350°C (dependent on the device), which is much lower than the combustion temperature, to aerosolize nicotine from specially designed cigarettes, or a heated sealed chamber, to aerosolize nicotine directly from tobacco leaf. Hybrid THPs fuses tobacco heating and vaping technology. Examples of THPs include IQOS (Tobacco Heating System 2.2, THS 2.2; Philip Morris International), Ploom TECH (Japan Tobacco International), Glo (Tobacco Heating Product 1.0, THP 1.0; British American Tobacco), iFUSE (British American Tobacco) and PAX (PAX Labs).[16], [17]

We aimed to compare the newest available on the market THPs with conventional cigarettes in terms of clinical harm.

Methods

In order to compare THPs with a conventional cigarette, a systematic review of the literature was carried out in database systems: MEDLINE, EMBASE, The Cochrane Library and Center for Reviews and Dissemination. Keywords included i.e.: "tobacco", "tobacco products", "heated", "heating", "modified risk" and "heat-not-burn." The review was carried out with a cut-off date of August 2, 2018.

Primary clinical studies with the highest level of reliability (randomized controlled clinical trials), published in full-text or as conference abstracts, evaluating switching from conventional cigarettes to newest available on the market THPs in comparison with continued smoking conventional cigarettes, were searched. Additionally in order to study mainstream aerosol, toxicology and impact of passive smoking on health, pre-clinical studies and studies evaluating indoor air quality were included.

Randomized trials assessing in particular clinically relevant endpoints, exposure to harmful and potentially harmful constituents (HPHCs) as defined by WHO[18] and Food and Drug Administration (FDA)[19] guidelines, clinical risk markers and safety were searched.

Within pre-clinical evaluation studies assessing aerosol chemistry, toxicology and in vitro studies, in which newest available on the market THPs were compared to conventional cigarettes, were included. Exclusion criteria were: older devices (for which the next upgraded version is available), prototypes, devices not commercially available. The detailed scope and search strategy are shown in the appendixes A and B.

Results

The review and selection of studies were carried out independently by two reviewers. The first stage of selection was based on abstracts, and then on full texts of the publications. Stages of review and selection of studies are presented in the PRISMA diagram [20] (see appendix C).

The review included:

- 37 pre-clinical studies (32 studies for THS 2.2, 10 studies for THP 1.0, 1 studies for Ploom TECH, 3 studies for iFUSE);
- 9 randomized clinical trials (8 trials for THS 2.2, 2 trials for THP 1.0, 1 trial for Ploom TECH);
- 6 studies evaluating indoor air quality (5 studies for THS 2.2, 1 studies for THP 1.0).

Pre-clinical studies

Assessment of pre-clinical studies is presented in appendix H. Conclusions and discussion are presented in appendix I.

Clinical trials

The review included 9 randomized clinical trials evaluating the use of THPs compared to smoking CCs. Characteristics of the included randomized clinical trials is presented in appendix F. Detailed numerical results of the studies included in the analysis are presented in appendixes J and K.

The analysis of the included studies indicates a high risk of bias in one domain (blinding of participants and personnel). Additionally, for the studies Brossard 2017a, Brossard 2017b (Brossard 2017[21], Yuki 2017[22] and Gee 2017[23], the "other factors" domain also indicates a high risk of bias (crossover study), but these studies were aimed at assessing pharmacokinetics or puffing topography, so the selection of study type is justified. The analysis of other domains did not show a high risk of bias (see Appendix D).

Exposure to harmful and potentially harmful constituents (HPHC), nicotine concentration and pharmacokinetics

The exposure to HPHC was assessed in five trials[24],[25],[26],[27],[28] The list of evaluated harmful or potentially harmful constituents assessed in the included trials (abbreviations explained in the appendix G) covers a wide range of chemical classes and organ toxicity classes as defined by the FDA (carcinogen, cardiovascular toxicant, respiratory toxicant, reproductive and developmental toxicant, addiction potential).[24],[25],[29]

Five studies showed that switching from CC to THP in smokers was associated with a reduction in exposure to all 18 analyzed HPHC as compared to the continuation of smoking CCs both after 5 and 90 days (Table 1) regardless of cigarettes type (menthol and non-menthol).[24,25,26,27,28] The concentrations of exposure markers in the THP groups were comparable to those observed in the smoking cessation group. Comparable concentrations of total nicotine equivalents, nicotine and cotinine were observed in the analysed groups in most of included studies (Table 2). Only in the Gale 2018 study, the total concentration of nicotine equivalents after 5 days was statistically significantly lower in the THP groups than in the continuation of smoking group, both for comparisons with non-menthol (THS 2.2 vs CC, THP 1.0 vs CC) and menthol cigarettes (THP 1.0 vs CC).[26]

Table 1. Markers of exposure to harmful or potentially harmful constituents - THP vs conventional cigarette.

HPHC	Day	THP vs CC				
		Ludicke 2018	Haziza 2016d	Haziza 2016a	Haziza 2016b	Gale 2018
NNAL	5	▼	▼	▼	▼	▼
	90	▼	▼	NA	NA	NA
NNN	5	▼	▼	▼	▼	▼
	90	▼	▼	NA	NA	NA
COHb	5	▼	▼	▼	▼	NA
	90	▼	▼	NA	NA	NA
eCO	5	NA	NA	NA	NA	▼
	90	NA	NA	NA	NA	NA
MHBMA	5	▼	▼	▼	▼	▼
	90	▼	▼	NA	NA	NA
3-HPMA	5	▼	▼	▼	▼	▼
	90	▼	▼	NA	NA	NA
S-PMA	5	▼	▼	▼	▼	▼
	90	▼	▼	NA	NA	NA
1-OHP	5	▼	▼	▼	▼	▼
	90	▼	▼	NA	NA	NA
4-ABP	5	▼	▼	▼	▼	▼
	90	▼	▼	NA	NA	NA
1-NA	5	▼	▼	▼	▼	NA
	90	▼	▼	NA	NA	NA
2-NA	5	▼	▼	▼	▼	▼
	90	▼	▼	NA	NA	NA
o-toluidine	5	▼	▼	▼	▼	▼
	90	▼	▼	NA	NA	NA
CEMA	5	▼	▼	▼	▼	▼
	90	▼	▼	NA	NA	NA
HEMA	5	▼	▼	▼	▼	▼
	90	▼	▼	NA	NA	NA
3-HMPMA	5	▼	▼	▼	▼	▼
	90	▼	▼	NA	NA	NA
3-OH-B[a]P	5	▼	▼	▼	▼	NA
	90	▼	▼	NA	NA	NA
AAMA	5	NA	NA	NA	NA	▼
	90	NA	NA	NA	NA	NA
GAMA	5	NA	NA	NA	NA	▼
	90	NA	NA	NA	NA	NA

NA – not available

▼ *Reduction of concentration; result in favour of THP (no information on statistical significance)*

▼ *Reduction of concentration; statistically significant result in favour of THP*

NNAL - 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNN - N-nitrosonornicotine); COHb – carboxyhemoglobin; eCO - exhaled carbon monoxide; MHBMA - monohydroxybutenyl mercapturic acid; 3-HPMA - 3-hydroxypropylmercapturic acid; S-PMA - S-phenylmercapturic acid; 1-OHP - 1-hydroxypyrene; 4-ABP - 4-aminobiphenyl; 1-NA - 1-aminonaphthalene; 2-NA - 2-aminonaphthalene; CEMA - 2-cyanoethylmercapturic acid; HEMA - 2-hydroxyethylmercapturic acid; 3-HMPMA - 3-hydroxy-1-methylpropylmercapturic acid; 3-OH-B[a]P - 3-hydroxy-benzo(a)pyrene; AAMA - N-acetyl-S-(2-carbamoylethyl)cysteine); GAMA - N-acetyl-S-(2-hydroxy-2-carbamoylethyl)cysteine.
S-BMA - S-benzylmercapturic acid;.

Table 2. Concentrations of nicotine, cotinine and nicotine equivalent - THP vs conventional cigarette.

	Day	THP vs CC				
		Ludicke 2018	Haziza 2016d	Haziza 2016a	Haziza 2016b	Gale 2018
Nicotine equivalent	5	▬	NA	▬	▬	▼
	90	▬	NA	NA	NA	NA
Nicotine	5	NA	NA	▬	▬	NA
	90	NA	NA	NA	NA	NA
Cotinine	5	NA	NA	▬	▬	NA
	90	NA	NA	NA	NA	NA

NA – not available

▼ *Reduction of concentration; statistically significant result in favour of THP*

▬ *Result statistically insignificant*

Three studies (Brossard 2017a and Brossard 2017b[21], Yuki 2017)[22] compared pharmacokinetics of nicotine between THPs and CCs. In both Brossard 2017[21] studies the plasma concentration profile of nicotine was comparable for THP and CC, suggesting similar absorption of nicotine. However, Yuki 2017[22] study showed that THP evaluated in this study delivered lower maximum observed plasma nicotine concentration and total exposure to nicotine compared to a conventional cigarette (45,7% and 68,3%, respectively). These results can be explained by differences in nicotine intake, as participants used the assessed THP (Ploom TECH) for the first time, and puff duration and number were fixed by study researchers.

In three studies[24, 25, 23] puffing topography were evaluated (results not included in this publication).

Clinical risk markers

The results in terms of clinical risk markers are presented in Table 6. In the Ludicke 2018 study[27] statistically significant changes were observed in favor of THP vs CC for endothelial dysfunction, oxidative stress, inflammation markers and lipid metabolism. Clinical risk markers were also evaluated in the Haziza 2016d study but not fully reported.[28]

Studies primarily focused on exposure to HPHCs were not powered to estimate clinical risk markers differences.

Table 3. Clinical risk markers at 90 days - THP vs conventional cigarette.

Outcome	THP vs CC
	Ludicke 2018
Endothelial dysfunction	
sICAM-1	▼
Oxidative stress	
8-epi-PGF2α	▼
Platelet activity	
11-DTX-B2	▬
Cardiovascular risk/function	
Fibrinogen	▬
Homocysteine	▬
hs-CRP	▬
Systolic blood pressure	▬
Diastolic blood pressure	▬
Metabolic syndrome	
Blood glucose	▬
HbA1c	▬
Body weight	▬
Waist circumference	▬
Inflammation	
WBC	▼
Lipid metabolism	
LDL cholesterol	▬
HDL cholesterol	▲
Triglycerides	▬
Total cholesterol	▬
Lung function	
FEV_1	▬

▼ *Decrease; statistically significant result in favour of THP*
▲ *Increase; statistically significant result in favour of THP*
▬ *Result statistically insignificant*

Indoor air quality

No randomized studies evaluating indoor air quality were found. Summary of findings on indoor air quality assessment is presented in appendix L.

Discussion

Nine randomized clinical trials were included in the analysis. Randomized trials have shown that the use of THPs is associated with a reduction in exposure to 18 out of 93 constituents, most of which were identified as HPHCs by FDA,[30], [31] compared to continuing CC smoking.

Our results are consistent with most recent independent review, evaluating THPs secondhand emissions and its use by humans.[32] The review also included studies on older versions of devices (for which the next version is available) as well as non-randomized studies and case reports. However, the review does not cover all pre-clinical studies, but only those that evaluated mainstream emissions. Findings showed the use of THPs is associated with exposure to toxic substances, but at substantially lower levels than CCs.

According to the German Federal Institute for Risk Assessment, levels of major carcinogens are markedly reduced (by about 80-99%) in the analyzed THP products' emissions in comparison to conventional cigarettes. Substantial reductions of toxicant levels might be regarded as a discrete benefit compared to the use of conventional cigarettes, even if potential consequences for human health still need to be explored.[33] Similarly, according to Public Health England (PHE) and Rijksinstituut voor Volksgezondheid en Milieu (RIVM) opinions, THPs may be considerably less harmful than tobacco cigarettes.[34], [35] Korean Ministry of Food and Drug Safety (KFDA) suggest that THPs also contain carcinogens, however their levels are significantly (by more than 90%) reduced.[36] According to the Toxicology Committee of the British Health Ministry, the risk associated with the use of THPs cannot be quantified due to shortcomings in the available information and the uncertain relationship between the concentration of harmful constituents and potential negative health outcomes. Moreover, concentrations of particular aerosol compounds differ from those observed in CC smoke, so it is not possible to extrapolate from epidemiological data on smoking risks, in particular after considering the complexity of interactions that occur between chemical compounds in producing adverse health effects. However, according to Committee the use of THPs is probably less harmful than the use of CCs, but the best way to limit the harmful health effect is smoking cessation.[37],[38]

The lack of blinding in included randomized studies results from the differences in the appearance of the tested products (THPs and CC) and the lack of technical possibility of using an identical device. However, Ludicke 2018 and Gale 2018 study[26],[27] indicated that the laboratories were blinded to the randomization scheme. Main limitation is lack of studies focused on clinical outcomes, such as the occurrence of smoking-related diseases and death due to smoking-related diseases. However, the assessment of the occurrence of cardiovascular diseases, lung cancer and chronic obstructive pulmonary disease (COPD) would require a significant extension of the follow up in clinical trials, as smoking-related symptoms and deaths usually occur after a long asymptomatic period.[39] Therefore it is important to evaluate surrogate endpoints suitable for risk assessment in short-term trials to determine the risk reduction profile and the potential long-term effect.[40] Clinical risk markers assessed in the studies were selected from multiple clinical risk components across several biological processes and mechanisms associated with smoking-related diseases, including inflammation markers, oxidative stress, platelet activation, lipid metabolism and lung function. The selection was based on epidemiological evidence on relationship between the clinical risk endpoint and at least one known smoking-related health outcome, clinical evidence linking smoking to the clinical risk endpoint (consistent with the epidemiological evidence) and clinical evidence linking smoking cessation to the reversibility of the endpoint.[40] The interpretation of results in the context of reducing smoking-related disease risk by switching to THP can only be made indirectly and its confirmation in direct studies requires long-term observations and evaluation of clinically relevant endpoints.

Results of pre-clinical studies evaluating aerosol chemistry and physics indicate about 90% reduction of combustion markers and harmful or potentially harmful constituents in THPs aerosol compared to CCs smoke. Both in vitro and in vivo standard toxicology studies as well as data from systems toxicology assessment indicate that THPs are less toxic than CCs (**see appendixes H and I**).

Results of included studies suggest that there is no negative impact on indoor air quality when using THP in an indoor environment, which can affect the risks associated with passive smoking (**see appendix L**).

All randomized trials and majority of pre-clinical studies as well as studies evaluating indoor air quality were sponsored by the THPs manufacturers. Independent evidence are needed to validate current findings, although cited review found largely similar results for independent and industry-funded studies.[32]

Conclusions

It has been shown that switching to THPs from smoking CCs is associated with reduced exposure to some HPHCs and likely improvement of clinical risk markers related to oxidative stress, endothelial dysfunction, lipid metabolism, inflammation and lung function as compared to continuing smoking of CCs while maintaining similar concentrations of nicotine. The impact on smoking related diseases needs to be explored in long term follow up clinical trials, but THPs certainly should not be perceived as alternative approach to smoking cessation.

The identified studies revealed no negative impact of heat-not-burn product on indoor air quality, which might reduce the risks associated with passive smoking of CC.

Summary

THPs are intended for use by people addicted to nicotine who refused or failed smoking cessation, and can be an opportunity to reduce the negative effects of exposure to HPHCs contained in traditional cigarette smoke.

THPs compared to smoking CCs (within the analyzed risk factors) can offer reduction of HPHCs exposure. Its impact on smoking-related diseases risk requires further long-term studies.

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38. Committee on Toxicity: Toxicological evaluation of novel heat-not-burntobacco products –non-technical summary, 2017 [cited 13.05.2019]. Available from: https://cot.food.gov.uk/sites/default/files/heat_not_burn_tobacco_summary.pdf

39. Narodowy Fundusz Zdrowia: Program profilaktyki chorób odtytoniowych – palenie jest uleczalne [cited 13.05.2019]. Available from: http://www.nfz.gov.pl/download/gfx/nfz/pl/defaultaktualnosci/293/1669/1/54_2005_zal.pdf

40. Lüdicke F, Picavet P, Baker G et al. Effects of Switching to the Menthol Tobacco Heating System 2.2, Smoking Abstinence, or Continued Cigarette Smoking on Clinically Relevant Risk Markers: A Randomized, Controlled, Open-Label, Multicenter Study in Sequential Confinement and Ambulatory Settings (Part 2). Nicotine Tob Res. 2018 Jan 5;20(2):173-182.

SUPPLEMENT/APPENDIX

Appendix A. Scope

Table 1. Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
1. Primary clinical studies: · randomized controlled clinical trials, · published in full-text or as the conference abstracts, · evaluating switching from conventional cigarettes to newest available on the market tobacco heating products (THPs) by smokers in comparison with continuation of smoking conventional cigarettes, · studies assessing in particular clinically relevant endpoints, exposure to harmful and potentially harmful constituents, considered in the World Health Organization (WHO) and Food and Drug Administration (FDA) guidelines regarding their reporting, clinical risk markers and safety, 2. Pre-clinical studies: · studies assessing aerosol chemistry, toxicology and in vitro studies, in which newest available on the market THPs were compared to conventional cigarettes, 3. Indoor air quality: · studies assessing indoor air quality, in which newest available on the market THPs were compared to conventional cigarettes.	· older versions of devices (for which the next version is available), · prototypes, · devices not commercially available.

Appendix B. Search strategy

Table 2. Search strategy for studies on the effectiveness and safety of Tobacco Heating System in the MEDLINE (PubMed) database system - 08/02/2018.

Query	Key word	Results
1	tobacco	117 015
2	"Tobacco Products"[Mesh]	5 994
3	#1 OR #2	117 015
4	heated	20 463
5	heating	51 758
6	„modified risk”	255
7	heat-not-burn OR „heat not burn”	55
8	3T	6 764
9	glo	1 310
10	iFuse	20
11	THP	12 129
12	Tobacco Heating Product	85
13	IQOS	21
14	THS	1 588
15	Tobacco Heating System	81
16	iSmoke	0
17	Lil	290
18	Pax	2 624
19	Ploom	11
20	ZeroStyle	0
21	V2 AND Pro	101
22	#4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21	91 845
23	#3 AND #22	732

Table 3. Search strategy for studies on the effectiveness and safety of Tobacco Heating System in the Embase (Elsevier) database system - 08/02/2018.

Query	Key word	Results
1	'tobacco'/exp OR tobacco	142 419
2	'tobacco products'	4 145
3	#1 OR #2	142 419
4	heated	23 025
5	heating	61 692
6	‘modified risk’	383
7	‘heat-not-burn’ OR ‘heat not burn’	66
8	3T	17 100
9	glo	5 329
10	iFuse	42
11	THP	16 889
12	Tobacco Heating Product	125
13	IQOS	16
14	THS	2 324
15	Tobacco Heating System	135
16	iSmoke	0
17	Lil	862
18	Pax	3 903
19	Ploom	13
20	ZeroStyle	0
21	V2 AND Pro	319
22	#4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21	124 928
23	#3 AND #22	1 054

Table 4. Search strategy for studies on the effectiveness and safety of Tobacco Heating System in the Cochrane Library database system - 08/02/2018.

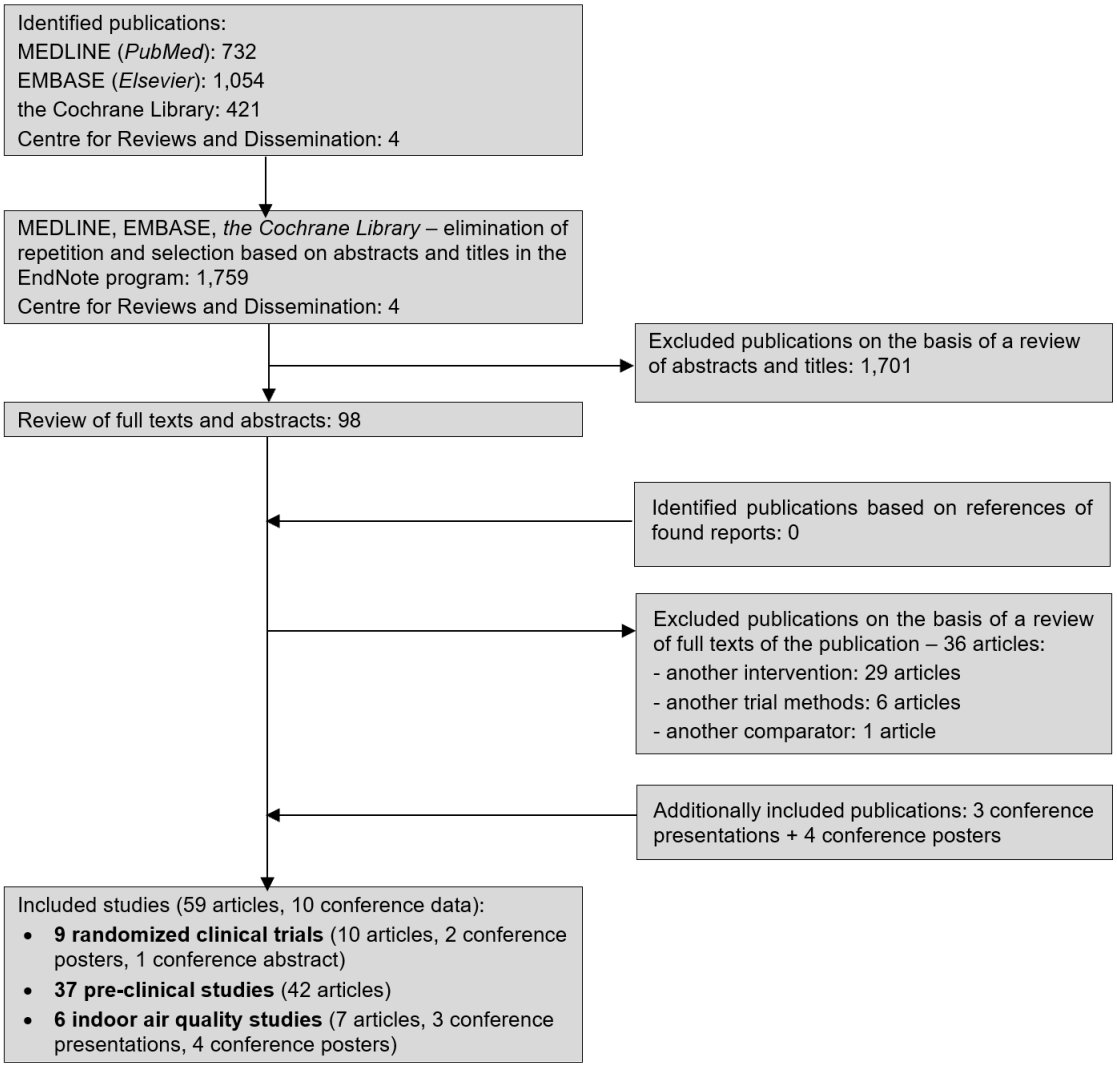
Query	Key word	Results
1	MeSH descriptor: [tobacco] explode all trees	147
2	tobacco	13 261
3	#1 OR #2	13 261
4	heated	961
5	heating	1 103
6	'modified risk'	9 408
7	'heat-not-burn' OR 'heat not burn'	6 322
8	3T	613
9	glo	71
10	iFuse	14
11	THP	199
12	Tobacco Heating Product	32
13	IQOS	4
14	THS	119
15	Tobacco Heating System	47
16	iSmoke	0
17	Lil	12
18	Pax	45
19	Ploom	0
20	ZeroStyle	0
21	V2 AND Pro	36
22	#4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21	17 768
23	#3 AND #2	421
24	#23 in Cochrane Reviews	259
25	#23 in Other Reviews	3
26	#23 in Clinical Trials	150
27	#23 in Economic Evaluations	7
28	#23 in Cochrane Groups	2

Table 5. Search strategy for studies on the effectiveness and safety of Tobacco Heating System in the Centre for Reviews and Dissemination database system - 08/02/2018.

Query	Key word	Results
1	MeSH DESCRIPTOR Tobacco Products EXPLODE ALL TREES	13
2	tobacco	468
3	#1 OR #2	468
4	heated	39
5	heating	42
6	modified risk	2
7	heat-not-burn OR heat not burn	174
8	3T	3
9	glo	1
10	iFuse	4
11	THP	1
12	Tobacco Heating Product	0
13	IQOS	0
14	THS	2
15	Tobacco Heating System	0
16	iSmoke	0
17	Lil	1
18	Pax	1
19	Ploom	0
20	ZeroStyle	0
21	V2 AND Pro	0
22	#4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21	248
23	#3 AND #22	4

Appendix C. PRISMA diagram

Figure 1. Scheme of subsequent stages of search and selection of studies for tobacco heating products (PRISMA diagram [[1]]).



1 Moher D, Liberati A, Tetzlaff J et al. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Medicine 2009; 6(7): e1000097.

Appendix D. Assessment of the risk of bias

Table 6. Assessment of the risk of bias in included studies using the Cochrane Risk of Bias Tool.[2]

Study	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data addressed	Selective reporting	Other factors
Ludicke 2018	low	unclear	high	low*	low	low	low
Haziza 2016d***	low	unclear	high	unclear	low	low	low
Haziza 2016a	low	unclear	high	unclear	low	low	low
Haziza 2016b	low	unclear	high	unclear	low	low	low
Gale 2018	low	unclear	high	low*	low	low	low
Brossard 2017#	low	unclear	high	unclear	low	low	high**
Brossard 2017#	low	unclear	high	unclear	low	low	high**
Yuki 2017#	low	unclear	high	unclear	low	low	high**
Gee 2017##	low	unclear	high	unclear	low	low	high**

** laboratories were blinded to randomization scheme; ** crossover study; *** unpublished study, characteristics and results available in the form of a conference poster; # study aimed at assessing pharmacokinetics; ## study aimed at assessing puffing topography.*

Appendix E. List of included and excluded studies

Table 7. List of publications included in the analysis.

Nr	Symbol	Publication
Pre-clinical studies – aerosol chemistry and physics		
1	Bekki 2017	Bekki K, Inaba Y, Uchiyama S, Kunugita N. Comparison of Chemicals in Mainstream Smoke in Heat-not-burn Tobacco and Combustion Cigarettes. J UOEH. 2017;39(3):201-207. doi: 10.7888/juoeh.39.201.
2	Crooks 2018	Crooks I, Neilson L, Scott K, Reynolds L, Oke T, Forster M, Meredith C, McAdam K, Proctor C. Evaluation of flavourings potentially used in a heated tobacco product: Chemical analysis, in vitro mutagenicity, genotoxicity, cytotoxicity and in vitro tumour promoting activity. Food Chem Toxicol. 2018 Aug;118:940-52.
3	Eaton 2018	Eaton D, Jakaj B, Forster M, Nicol J, Mavropoulou E, Scott K, Liu C, McAdam K, Murphy J, Proctor CJ. Assessment of tobacco heating product THP1.0. Part 2: Product design, operation and thermophysical characterisation. Regul Toxicol Pharmacol. 2018 Mar;93:4-13.
4	Farsalinos 2017	Farsalinos KE, Yannovits N, Sarri T, Voudris V, Poulas K. Nicotine Delivery to the Aerosol of a Heat-Not-Burn Tobacco Product: Comparison With a Tobacco Cigarette and E-Cigarettes. Nicotine Tob Res. 2017.
5	Farsalinos 2018	Farsalinos KE, Yannovits N, Sarri T, Voudris V, Poulas K, Leischow SJ. Carbonyl emissions from a novel heated tobacco product (IQOS): comparison with an e-cigarette and a tobacco cigarette. Addiction. 2018 Nov;113(11):2099-106.
6	Forster 2018	Forster M, Fiebelkorn S, Yurteri C, Mariner D, Liu C, Wright C, McAdam K, Murphy J, Proctor C. Assessment of novel tobacco heating product THP1.0. Part 3: Comprehensive chemical characterisation of harmful and potentially harmful aerosol emissions. Regul Toxicol Pharmacol. 2018 Mar;93:14-33.
7	Jaccard 2017	Jaccard G, Tafin Djoko D, Moennikes O, Jeannet C, Kondylis A, Belushkin M. Comparative assessment of HPHC yields in the Tobacco Heating System THS2.2 and commercial cigarettes. Regul Toxicol Pharmacol. 2017 Nov;90:1-8.
8	Jaccard 2018	Jaccard G, Kondylis A, Gunduz I, Pijnenburg J, Belushkin M. Investigation and comparison of the transfer of TSNA from tobacco to cigarette mainstream smoke and to the aerosol of a heated tobacco product, THS2.2. Regul Toxicol Pharmacol. 2018 Aug;97:103-9.
9	Li 2018	Li X, Luo Y, Jiang X, Zhang H, Zhu F, Hu S, Hou H, Hu Q, Pang Y. Chemical Analysis and Simulated Pyrolysis of Tobacco Heating System 2.2 Compared to Conventional Cigarettes. Nicotine Tob Res. 2018 Jan 8.
10	Poynton 2017	Poynton S, Sutton J, Goodall S, Margham J, Forster M, Scott K, Liu C, McAdam K, Murphy J, Proctor C. A novel hybrid tobacco product that delivers a tobacco flavour note with vapour aerosol (Part 1): Product operation and preliminary aerosol chemistry assessment. Food Chem Toxicol. 2017 Aug;106(Pt A):522-32.
11	Pratte 2017_1	Pratte P, Cosandey S, Goujon Ginglinger C. Innovative methodology based on thermo-denuder principle for the detection of combustion related solid particles or high boiling point droplets: Applications to cigarette and the Tobacco Heating System THS 2.2. Journal of Aerosol Science.
12	Pratte 2017_2	Pratte P, Cosandey S, Goujon Ginglinger C. Investigation of solid particles in the mainstream aerosol of the Tobacco Heating System THS2.2 and mainstream smoke of a 3R4F reference cigarette. Hum Exp Toxicol. 2017 Nov;36(11):1115-20.
13	Savareear 2017	Savareear B, Lizak R, Brokl M, Wright C, Liu C, Focant JF. Headspace solid-phase microextraction coupled to comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry for the analysis of aerosol from tobacco heating product. J Chromatogr A. 2017 Oct 20;1520:135-42.
14	Schaller 2016_1	Schaller JP, Keller D, Poget L, Pratte P, Kaelin E, McHugh D, Cudazzo G, Smart D, Tricker AR, Gautier L, Yerly M, Reis Pires R, Le Bouhellec S, Ghosh D, Hofer I, Garcia E, Vanscheeuwijck P, Maeder S. Evaluation of the Tobacco Heating System 2.2. Part 2: Chemical composition, genotoxicity, cytotoxicity, and physical properties of the aerosol. Regul Toxicol Pharmacol. 2016 Nov 30;81 Suppl 2:S27-47..
15	Schaller 2016_2	Schaller JP, Pijnenburg JPM, Ajithkumar A, Tricker AR .Evaluation of the Tobacco Heating System 2.2. Part 3: Influence of the tobacco blend on the formation of harmful and potentially harmful constituents of the Tobacco Heating System 2.2 aerosol. Regul Toxicol Pharmacol. 2016 Nov 30;81 Suppl 2:S48-58.
16	Uchiyama 2018	Uchiyama S, Noguchi M, Takagi N, Hayashida H, Inaba Y, Ogura H, Kunugita N. Simple Determination of Gaseous and Particulate Compounds Generated from Heated Tobacco Products. Chem Res Toxicol. 2018 Jul 16;31(7):585-93.
Pre-clinical studies – standard toxicology assessment		
1	Schaller 2016	Schaller J.P, Keller D, Poget L, et al. Evaluation of the Tobacco Heating System 2.2. Part 2: Chemical composition, genotoxicity, cytotoxicity, and physical properties of the aerosol; Regulatory Toxicology and Pharmacology; 81; 2016; S27 - S47.
2	Breheny 2017	Breheny D, Adamson J, Azzopardi D. A novel hybrid tobacco product that delivers a tobacco flavour note with vapour aerosol (Part 2): In vitro biological assessment and comparison with different tobacco-heating products; Food and Chemical Toxicology; 106; 2017; 533 – 546.
3	Jaunky 2018	Jaunky T, Adamson J, Santopietro S. Assessment of tobacco heating product THP1.0. Part 5: In vitro dosimetric and cytotoxic assessment; Regulatory Toxicology and Pharmacology; 93; 2018; 52 – 61.
4	Thorne 2018	Thorne D, Breheny D, Proctor C, Gaca M. Assessment of novel tobacco heating product THP1.0. Part 7: Comparative in vitro toxicological evaluation; Regulatory Toxicology and Pharmacology; 93; 2018; 71 – 83.
5	Crooks 2018	Crooks I, Neilson L, Scott K, et al. Evaluation of flavourings potentially used in a heated tobacco product: Chemical analysis, in vitro mutagenicity, genotoxicity, cytotoxicity and in vitro tumour promoting activity, Food Chem Toxicol. 2018; 118: 940 - 952.

Table 7. List of publications included in the analysis.		
Nr	Symbol	Publication
6	Wong 2016	Wong E.T, Kogel U, Veljkovic E, et al. Evaluation of the Tobacco Heating System 2.2. Part 4: 90-day OECD 413 rat inhalation study with systems toxicology endpoints demonstrates reduced exposure effects compared with cigarette smoke; Regulatory Toxicology and Pharmacology; 81; 2016; S59 - S81.
		Sewer A, Kogel U, Talikka M, et al. Evaluation of the Tobacco Heating System 2.2 (THS2.2). Part 5: microRNA expression from a 90-day rat inhalation study indicates that exposure to THS2.2 aerosol causes reduced effects on lung tissue compared with cigarette smoke; Regulatory Toxicology and Pharmacology; 81; 2016; S82 - S92.
7	Oviedo 2016	Oviedo A, Lebrun S, Kogel U, et al. Evaluation of the Tobacco Heating System 2.2. Part 6: 90-day OECD 413 rat inhalation study with systems toxicology endpoints demonstrates reduced exposure effects of a mentholated version compared with mentholated and non-mentholated cigarette smoke; Regulatory Toxicology and Pharmacology; 81; 2016; S93 - S122.
		Kogel U, Titz B, Schlage W.K, et al. Evaluation of the Tobacco Heating System 2.2. Part 7: Systems toxicological assessment of a mentholated version revealed reduced cellular and molecular exposure effects compared with mentholated and non-mentholated cigarette smoke; Regulatory Toxicology and Pharmacology; 81; 2016; S123 - S138.
Pre-clinical studies – systems toxicology assessment		
1	Gonzalez-Suarez 2016	Gonzalez-Suarez I, Martin F, Marescotti D, Guedj E, Acali S, Johne S, Dulize R, Baumer K, Peric D, Goedertier D, Frentzel S, Ivanov NV, Mathis C, Hoeng J, Peitsch MC. In Vitro Systems Toxicology Assessment of a Candidate Modified Risk Tobacco Product Shows Reduced Toxicity Compared to That of a Conventional Cigarette. Chem Res Toxicol. 2016 Jan 19;29(1):3-18.
2	Haswell 2018	Haswell LE, Corke S, Verrastro I, Baxter A, Banerjee A, Adamson J, Jaunky T, Proctor C, Gaça M, Minet E. In vitro RNA-seq-based toxicogenomics assessment shows reduced biological effect of tobacco heating products when compared to cigarette smoke. Sci Rep. 2018 Feb 5;8(1):1145.
3	Iskandar 2017a	Iskandar AR, Mathis C, Martin F, Leroy P, Sewer A, Majeed S, Kuehn D, Trivedi K, Grandolfo D, Cabanski M, Guedj E, Merg C, Frentzel S, Ivanov NV, Peitsch MC, Hoeng J. 3-D nasal cultures: Systems toxicological assessment of a candidate modified-risk tobacco product. ALTEX. 2017;34(1):23-48
4	Iskandar 2017b	Iskandar AR, Mathis C, Schlage WK, Frentzel S, Leroy P, Xiang Y, Sewer A, Majeed S, Ortega-Torres L, Johne S, Guedj E, Trivedi K, Kratzer G, Merg C, Elamin A, Martin F, Ivanov NV, Peitsch MC, Hoeng J. A systems toxicology approach for comparative assessment: Biological impact of an aerosol from a candidate modified-risk tobacco product and cigarette smoke on human organotypic bronchial epithelial cultures. Toxicol In Vitro. 2017 Mar;39:29-51.
5	Iskandar 2017c	Iskandar AR, Titz B, Sewer A, Leroy P, Schneider T, Zanetti F, Mathis C, Elamin A, Frentzel S, Schlage WK, Martin F, Ivanov NV, Peitsch MC, Hoeng J. Systems toxicology meta-analysis of in vitro assessment studies: biological impact of a candidate modified-risk tobacco product aerosol compared with cigarette smoke on human organotypic cultures of the aerodigestive tract. Toxicol Res (Camb). 2017 May 29;6(5):631-653.
6	Iskandar 2017d	Iskandar AR, Martinez Y, Martin F, Schlage WK, Leroy P, Sewer A, Torres LO, Majeed S, Merg C, Trivedi K, Guedj E, Frentzel S, Mathis C, Ivanov NV, Peitsch MC, Hoeng J. Comparative effects of a candidate modified-risk tobacco product Aerosol and cigarette smoke on human organotypic small airway cultures: a systems toxicology approach. Toxicol Res (Camb). 2017 Sep 28;6(6):930-946.
7	Jaunky 2018	Jaunky T, Adamson J, Santopietro S, Terry A, Thorne D, Breheny D, Proctor C, Gaça M. Assessment of tobacco heating product THP1.0. Part 5: In vitro dosimetric and cytotoxic assessment. Regul Toxicol Pharmacol. 2018 Mar;93:52-61.
8	Lo Sasso 2016	Lo Sasso G, Titz B, Nury C, Boué S, Phillips B, Belcastro V, Schneider T, Dijon S, Baumer K, Peric D, Dulize R, Elamin A, Guedj E, Buettner A, Leroy P, Kleinhans S, Vuillaume G, Veljkovic E, Ivanov NV, Martin F, Vanscheeuwijck P, Peitsch MC, Hoeng J. Effects of cigarette smoke, cessation and switching to a candidate modified risk tobacco product on the liver in Apoe -/- mice--a systems toxicology analysis. Inhal Toxicol. 2016 Apr;28(5):226-40.
9	Malinska 2018	Malinska D, Szymański J, Patalas-Krawczyk P, Michalska B, Wojtala A, Prill M, Partyka M, Drabik K, Walczak J, Sewer A, Johne S, Luettich K, Peitsch MC, Hoeng J, Duszyński J, Szczepanowska J, van der Toorn M, Wieckowski MR. Assessment of mitochondrial function following short- and long-term exposure of human bronchial epithelial cells to total particulate matter from a candidate modified-risk tobacco product and reference cigarettes. Food Chem Toxicol. 2018 May;115:1-12.
10	Philips 2016	Phillips B, Veljkovic E, Boué S, Schlage WK, Vuillaume G, Martin F, Titz B, Leroy P, Buettner A, Elamin A, Oviedo A, Cabanski M, De León H, Guedj E, Schneider T, Talikka M, Ivanov NV, Vanscheeuwijck P, Peitsch MC, Hoeng J. An 8-Month Systems Toxicology Inhalation/Cessation Study in Apoe-/- Mice to Investigate Cardiovascular and Respiratory Exposure Effects of a Candidate Modified Risk Tobacco Product, THS 2.2, Compared With Conventional Cigarettes. Toxicol Sci. 2016 Feb;149(2):411-32.
		Titz B, Boué S, Phillips B, Talikka M, Vihervaara T, Schneider T, Nury C, Elamin A, Guedj E, Peck MJ, Schlage WK, Cabanski M, Leroy P, Vuillaume G, Martin F, Ivanov NV, Veljkovic E, Ekroos K, Laaksonen R, Vanscheeuwijck P, Peitsch MC, Hoeng J. Effects of Cigarette Smoke, Cessation, and Switching to Two Heat-Not-Burn Tobacco Products on Lung Lipid Metabolism in C57BL/6 and Apoe-/- Mice-An Integrative Systems Toxicology Analysis. Toxicol Sci. 2016 Feb;149(2):441-57.
11	Poussin 2016	Poussin C, Laurent A, Peitsch MC, Hoeng J, De Leon H. Systems toxicology-based assessment of the candidate modified risk tobacco product THS2.2 for the adhesion of monocytic cells to human coronary arterial endothelial cells. Toxicology. 2016 Jan 2;339:73-86.
12	Taylor 2018	Taylor M, Thorne D, Carr T, Breheny D, Walker P, Proctor C, Gaça M. Assessment of novel tobacco heating product THP1.0. Part 6: A comparative in vitro study using contemporary screening approaches. Regul Toxicol Pharmacol. 2018 Mar;93:62-70.
13	van der Toorn 2015	van der Toorn M, Frentzel S, De Leon H, Goedertier D, Peitsch MC, Hoeng J. Aerosol from a candidate modified risk tobacco product has reduced effects on chemotaxis and transendothelial migration compared to combustion of conventional cigarettes. Food Chem Toxicol. 2015 Dec;86:81-7.

Table 7. List of publications included in the analysis.

Nr	Symbol	Publication
14	van der Toorn 2018	van der Toorn M, Sewer A, Marescotti D, Johne S, Baumer K, Bornand D, Dulize R, Merg C, Corciulo M, Scotti E, Pak C, Leroy P, Guedj E, Ivanov N, Martin F, Peitsch M, Hoeng J, Luettich. The biological effects of long-term exposure of human bronchial epithelial cells to total particulate matter from a candidate modified-risk tobacco product. Toxicol In Vitro. 2018 Aug;50:95-108.
15	Zanetti 2016	Zanetti F, Sewer A, Mathis C, Iskandar AR, Kostadinova R, Schlage WK, Leroy P, Majeed S, Guedj E, Trivedi K, Martin F, Elamin A, Merg C, Ivanov NV, Frentzel S, Peitsch MC, Hoeng J. Systems Toxicology Assessment of the Biological Impact of a Candidate Modified Risk Tobacco Product on Human Organotypic Oral Epithelial Cultures. Chem Res Toxicol. 2016 Aug 15;29(8):1252-69.
16	Zanetti 2017	Zanetti F, Titz B, Sewer A, Lo Sasso G, Scotti E, Schlage WK, Mathis C, Leroy P, Majeed S, Torres LO, Keppler BR, Elamin A, Trivedi K, Guedj E, Martin F, Frentzel S, Ivanov NV, Peitsch MC, Hoeng J. Comparative systems toxicology analysis of cigarette smoke and aerosol from a candidate modified risk tobacco product in organotypic human gingival epithelial cultures: A 3-day repeated exposure study. Food Chem Toxicol. 2017 Mar;101:15-35.
Randomized controlled trials		
1	Ludicke 2018 (NCT01970995)	Lüdicke F, Picavet P, Baker G, Haziza C, Poux V, Lama N, Weitkunat R. Effects of Switching to the Tobacco Heating System 2.2 Menthol, Smoking Abstinence, or Continued Cigarette Smoking on Biomarkers of Exposure: A Randomized, Controlled, Open-Label, Multicenter Study in Sequential Confinement and Ambulatory Settings (Part 1). Nicotine Tob Res. 2018 Jan 5;20(2):161-172.
		Lüdicke F, Picavet P, Baker G, Haziza C, Poux V, Lama N, Weitkunat R. Effects of Switching to the Menthol Tobacco Heating System 2.2, Smoking Abstinence, or Continued Cigarette Smoking on Clinically Relevant Risk Markers: A Randomized, Controlled, Open-Label, Multicenter Study in Sequential Confinement and Ambulatory Settings (Part 2). Nicotine Tob Res. 2018 Jan 5;20(2):173-182.
		Haziza C, Lama N, Donelli A, Picavet P, Baker G, Ancerewicz J, Benzimra M, Franzon M, Endo M, Ludicke F. Reduced Exposure to Harmful and Potentially Harmful Constituents After 90 Days of Use of Tobacco Heating System 2.2 Menthol in Japan: A Comparison with Continued Cigarete Use or Smoking Abstinence. SRNT – 22nd Annual Meeting, Chicago, USA, 2-5 March 2016. Plakat.
		Picavet P, Haziza C, Lama N, Donelli A, Baker G, Ancerewicz J, Benzimra M, Franzon M, Masahiro Endo MD, Ludicke F. Reduced exposure to harmful and potentially harmful constituents after 90 days of use of tobacco heating system 2.2 in Japan: A comparison with continued combustible cigarette use or smoking abstinence. Toxicology Letters. Volume 259, Supplement, 10 October 2016, Page S141.
2	Haziza 2016d (NCT01989156)	Haziza C, de La Bourdonnaye G, Picavet P, Baker G, Skiada D, Merlet S, Franzon M, Farmer F, Lewis W, Ludicke F. Reduced Exposure to Harmful and Potentially Harmful Constituents After 90 Days of Use of Tobacco Heating System 2.2 Menthol in the U.S.: A Comparison with Continued Cigarete Use or Smoking Abstinence. SRNT – 22nd Annual Meeting, Chicago, USA, 2-5 March 2016. Plakat.
3	Haziza 2016a (NCT01959932)	Haziza C, de La Bourdonnaye G, Skiada D, Ancerewicz J, Baker G, Picavet P, Lüdicke F. Evaluation of the Tobacco Heating System 2.2. Part 8: 5-Day randomized reduced exposure clinical study in Poland. Regul Toxicol Pharmacol. 2016 Nov 30;81 Suppl 2:S139-S150.
		Haziza C, de La Bourdonnaye G, Skiada D, Ancerewicz J, Baker G, Picavet P, Lüdicke F. Biomarker of exposure level data set in smokers switching from conventional cigarettes to Tobacco Heating System 2.2, continuing smoking or abstaining from smoking for 5 days. Data Brief. 2016 Nov 18;10:283-293.
		Martin F, Talikka M, Ivanov NV, Haziza C, Hoeng J, Peitsch MC. Evaluation of the tobacco heating system 2.2. Part 9: Application of systems pharmacology to identify exposure response markers in peripheral blood of smokers switching to THS2.2. Regul Toxicol Pharmacol. 2016 Nov 30;81 Suppl 2:S151-S157.
4	Haziza 2016b (NCT01970982)	Haziza C, de La Bourdonnaye G, Merlet S, Benzimra M, Ancerewicz J, Donelli A, Baker G, Picavet P, Lüdicke F. Assessment of the reduction in levels of exposure to harmful and potentially harmful constituents in Japanese subjects using a novel tobacco heating system compared with conventional cigarettes and smoking abstinence: A randomized controlled study in confinement. Regul Toxicol Pharmacol. 2016 Nov;81:489-499.
5	Gale 2018	Gale N, McEwan M, Eldridge A.C et al. Changes in Biomarkers of Exposure on Switching From a Conventional Cigarette to Tobacco Heating Products: A Randomized, Controlled Study in Healthy Japanese Subjects. Nicotine & Tobacco Research, 2018, 1–8.
6, 7	Brossard 2017 (NCT01959607, NCT01967706)	Brossard P, Weitkunat R, Poux V, Lama N, Haziza C, Picavet P, Baker G, Lüdicke F. Nicotine pharmacokinetic profiles of the Tobacco Heating System 2.2, cigarettes and nicotine gum in Japanese smokers. Regul Toxicol Pharmacol. 2017 Oct;89:193-199.
8	Yuki 2017	Yuki D, Sakaguchi C, Kikuchi A, Futamura Y. Pharmacokinetics of nicotine following the controlled use of a prototype novel tobacco vapor product. Regulatory Toxicology and Pharmacology 87 (2017) 30e35.
9	Gee 2017	Gee J, Prasad K, Slayford S, Gray A, Nother K, Cunningham A, Mavropoulou E, Proctor C. Assessment of tobacco heating product THP1.0. Part 8: Study to determine puffing topography, mouth level exposure and consumption among Japanese users. Regul Toxicol Pharmacol. 2018 Mar;93:84-91.

Table 7. List of publications included in the analysis.		
Nr	Symbol	Publication
Indoor air quality studies		
1	Mitova 2016	Mitova MI, Campelos PB, Goujon-Ginglinger CG, Maeder S, Mottier N, Rouget EG, Tharin M, Tricker AR. Comparison of the impact of the Tobacco Heating System 2.2 and a cigarette on indoor air quality. Regul Toxicol Pharmacol. 2016 Oct;80:91-101.
		Mottier N, Tharin M, Cluse C, Crudo JR, Lueso MG, Goujon-Ginglinger CG, Jaquier A, Mitova MI, Rouget EG, Schaller M, Solioz J. Validation of selected analytical methods using accuracy profiles to assess the impact of a Tobacco Heating System on indoor air quality. Talanta. 2016 Sep 1;158:165-78.
		Mitova M, Campelos P, Goujon-Ginglinger C, Maeder S, Mottier N, Rouget E, Tharin M, Smith M, Tricker A. Indoor Air Chemistry Assessment of environmental aerosol generated by Tobacco Heating System 2.2. ACT 36th Annual Meeting. Red Rock Resort. Summerlin, Nevada, November 8-11, 2015. Plakat konferencyjny. https://www.pmiscience.com/resources/docs/default-source/library-documents/mitova.pdf?sfvrsn=1b17f606_0
		Goujon-Ginglinger C, Campelos P, Maeder S, Mitova M, Mottier N, Rouget E, Tharin M, Tricker A, Smith M. Indoor Air Chemistry Comparative study between conventional cigarette and heat-not-burn technology. Plakat konferencyjny. https://www.pmiscience.com/resources/docs/default-source/library-documents/coujon_poster.pdf?sfvrsn=517f606_2
		Goujon-Ginglinger C. Air Quality assessment during indor use of the Tobacco Heating System 2.2. Japan Society for Environment Chemistry. June, 7-9. Shizuoka (Japan). Prezentacja konferencyjna. https://www.pmiscience.com/resources/docs/default-source/Presentations_Latest/jsfec-2017_ggoujon_air-quality-assessment-during-indoor-use-of-ths-2-2.pdf?sfvrsn=722dca06_0
		Goujon-Ginglinger C, Mitova M, Maeder S, Smith M. Air quality assessment during indor use of the Tobacco Heating System THS 2.2. EUROTOX 2017, Bratislava. September 10-13, 2017. Plakat konferencyjny. https://www.pmiscience.com/resources/docs/default-source/Posters_Latest/eurotox-2017-goujonginglinger-air-quality-assessment-during-in-door-use-of-ths-2-2.pdf?sfvrsn=f8d3cd06_0
		Goujon-Ginglinger C, Mitova M, Maeder S. Indoor Air Quality Assessment of the Tobacco Heating System THS 2.2, Electronic Cigarettes and Cigarette using a dedicated Exposure Room Atmos'Fair, 7th Edition. October, 12 2016. Prezentacja konferencyjna. https://www.pmiscience.com/resources/docs/default-source/library-documents/goujon_atmos_fair_2016_indoor_air_quality_assessment.pdf?sfvrsn=62aef706_2
		Mitova M, Goujon-Ginglinger C, Rotach M, Maeder S. Air Quality assessment during indor use of the Tobacco Heating System 2.2. CORESTA 2017, October, 8-12 2017. Kitzbuhel (Austria). Prezentacja konferencyjna. https://www.pmiscience.com/resources/docs/default-source/Presentations_Latest/coresta-2017-mitova-air-quality-assessment-during-indoor-use.pdf?sfvrsn=30bccc06_0
		Tharin M, Bielik N, Rouget E, Rotach M, Glabasnia A. Assessment of the total volatile organic compounds in indoor air during use of the Tobacco Heating System THS 2.2. Smoke Science and Product Technology (SSPT 2017), Kitzbühel, Austria. 08. –12. October 2017. Plakat konferencyjny. https://www.pmiscience.com/resources/docs/default-source/Posters_Latest/coresta-2017-glabasnia-assessment-of-the-total-volatile-organic-compoundsf4b4a5852f88696a9e88ff040043f5e9.pdf?sfvrsn=243ccc06_0
2	Pratte 2017	Pratte P, Cosandey S, Goujon Ginglinger C. Investigation of solid particles in the mainstream aerosol of the Tobacco Heating System THS2.2 and mainstream smoke of a 3R4F reference cigarette. Human and Experimental Toxicology 2017, Vol. 36(11) 1115–1120.
3	Protano 2016	Protano C, Manigrasso M, Avino P, Sernia S, Vitali M. Second-hand smoke exposure generated by new electronic devices (IQOS® and e-cigs) and traditional cigarettes: submicron particle behaviour in human respiratory system, Ann Ig 2016; 28: 109-112.
4	Protano 2017	Protano C, Manigrasso M, Avino P, Vitali M. Second-hand smoke generated by combustion and electronic smoking devices used in real scenarios: Ultrafine particle pollution and age-related dose assessment. Environment International 107 (2017) 190–195.
5	Ruprecht 2017	Ruprecht A.A, De Marco C, Saffari A, et al. Environmental pollution and emission factors of electronic cigarettes, heat-not-burn tobacco products, and conventional cigarettes. Aerosol Science and Technology 2017, 51:6, 674-684.
6	Forster 2018	Forster M, McAughey J, Prasad K, Mavropoulou E, Proctor C. Assessment of tobacco heating product THP1.0. Part 4: Characterisation of indoor air quality and odour. Regulatory Toxicology and Pharmacology 93 (2018) 34e51.

Table 8. List of publications excluded from the analysis.

Nr	Publication	Reason for exclusion
	Pre-clinical studies	
1	Lindson-Hawley N, Hartmann-Boyce J, Fanshawe TR, Begh R, Farley A, Lancaster T. Interventions to reduce harm from continued tobacco use. Cochrane Database Syst Rev. 2016 Oct 13;10:CD005231.	Another intervention
2	Adamson J, Jaunky T, Thorne D, Gaça MD. Characterisation of the borgwaldt LM4E system for in vitro exposures to undiluted aerosols from next generation tobacco and nicotine products (NGPs). Food Chem Toxicol. 2018 Mar;113:337-344.	Another comparator
3	Ansari, S., K. Baumer, et al. (2016). Comprehensive Systems Biology Analysis of a 7-Month Cigarette Smoke Inhalation Study in C57bl/6 Mice. Sci Data 3: 150077.	Another intervention
4	Bombick, B. R., J. T. Avalos, et al. (1998). Comparative Studies of the Mutagenicity of Environmental Tobacco Smoke from CigaE rettes That Burn or Primarily Heat Tobacco. Environmental and Molecular Mutagenesis 31(2): 169-175.	Another intervention
5	Bombick, B. R., H. Murli, et al. (1998). Chemical and Biological Studies of a New Cigarette That Primarily Heats Tobacco. Part 2. In Vitro Toxicology of Mainstream Smoke Condensate. Food and Chemical Toxicology 36(3): 183-190.	Another intervention
6	Bombick D.W., Ayres P.H., Putnam K., Bombick B.R., Doolittle D.J. Chemical and biological studies of a new cigarette that primarily heats tobacco. Part 3. In vitro toxicity of whole smoke. Food and Chemical Toxicology 1998 36:3 (191-197)	Another intervention
7	Bombick, D. W., K. Putnam, et al. (1998). Comparative Cytotoxicity Studies of Smoke Condensates from Different Types of Cigarettes and Tobaccos. Toxicology in Vitro 12(3): 241-249.	Another intervention
8	Camacho, O. M., J. Sommarstrom, et al. (2016). Reference Change Values in Concentrations of Urinary and Salivary Biomarkers of Exposure and Mouth Level Exposure in Individuals Participating in an Ambulatory Smoking Study. Pract Lab Med 5: 47-56.	Another intervention
9	Coggins, C. R., P. H. Ayres, et al. (1989). Ninety-Day Inhalation Study in Rats, Comparing Smoke from Cigarettes That Heat Tobacco with Those That Burn Tobacco. Fundam Appl Toxicol 13(3): 460-483.	Another intervention
10	Davis B, Williams M, Talbot P. iQOS: evidence of pyrolysis and release of a toxicant from plastic. Tob Control. 2018 Mar 13. pii: tobaccocontrol-2017-054104. doi: 10.1136/tobaccocontrol-2017-054104. [Epub ahead of print]	Another trial methods
11	Doolittle, D. J., C. K. Lee, et al. (1990). Genetic Toxicology Studies Comparing the Activity of Sidestream Smoke from Cigarettes Which Burn or Only Heat Tobacco. Mutation Research 240(2): 59-72.	Another intervention
12	Elamin, A., B. Titz, et al. (2016). Quantitative Proteomics Analysis Using 2d-Page to Investigate the Effects of Cigarette Smoke and Aerosol of a Prototypic Modified Risk Tobacco Product on the Lung Proteome in C57bl/6 Mice. Journal of Proteomics 145: 237-245.	Another intervention
13	Fields W, Fowler K, Hargreaves V, Reeve L, Bombick B. Development, qualification, validation and application of the neutral red uptake assay in Chinese Hamster Ovary (CHO) cells using a VITROCELL® VC10® smoke exposure system; Toxicology in Vitro; 40; 2017; 144–152.	Another intervention
14	Forster, M., C. Liu, et al. (2015). An Experimental Method to Study Emissions from Heated Tobacco between 100-200 Degrees C. Chem Cent J 9: 20.	Another intervention
15	Fujimoto, H., H. Tsuji, et al. (2015). Biological Responses in Rats Exposed to Mainstream Smoke from a Heated Cigarette Compared to a Conventional Reference Cigarette. Inhalation Toxicology 27(4): 224-236.	Another intervention
16	Ishikawa, S., Y. Kanemaru, et al. (2016). Assessing the Mutagenic Activities of Smoke from Different Cigarettes in Direct Exposure Experiments Using the Modified Ames Salmonella Assay. Mutation Research - Genetic Toxicology and Environmental Mutagenesis 803-804: 13-21	Another intervention
17	Kogel, U., I. Gonzalez Suarez, et al. (2015). Biological Impact of Cigarette Smoke Compared to an Aerosol Produced from a Prototypic Modified Risk Tobacco Product on Normal Human Bronchial Epithelial Cells. Toxicology in Vitro 29(8): 2102-2115.	Another intervention
18	Kogel, U., W. K. Schlage, et al. (2014). A 28-Day Rat Inhalation Study with an Integrated Molecular Toxicology Endpoint Demonstrates Reduced Exposure Effects for a Prototypic Modified Risk Tobacco Product Compared with Conventional Cigarettes. Food and Chemical Toxicology 68: 204-217.	Another intervention
19	Lopez, A. A., M. Hiler, et al. (2016). Expanding Clinical Laboratory Tobacco Product Evaluation Methods to Loose-Leaf Tobacco Vaporizers. Drug and Alcohol Dependence 169((Lopez A.A.; Hiler M.; Maloney S.; Eissenberg T.; Breland A.B., abbrelan@vcu.edu) Virginia Commonwealth University, Department of Psychology and Center for the Study of Tobacco Products, Richmond, United States): 33-40.	Another trial methods
20	Mallock N, Böss L, Burk R, Danziger M, Welsch T, Hahn H, Trieu HL, Hahn J, Pieper E, Henkler-Stephani F, Hutzler C, Luch A. Levels of selected analytes in the emissions of "heat not burn" tobacco products that are relevant to assess human health risks. Arch Toxicol. 2018 Jun;92(6):2145-2149.	Another trial methods
21	McKarns, S. C., D. W. Bombick, et al. (2000). Gap Junction Intercellular Communication and Cytotoxicity in Normal Human Cells after Exposure to Smoke Condensates from Cigarettes That Burn or Primarily Heat Tobacco. Toxicology in Vitro 14(1): 41-51.	Another intervention
22	McKarns, S. C. and D. J. Doolittle (1991). A Quantitative Approach to Assessing Intercellular Communication: Studies on Cigarette Smoke Condensates. Toxicology and Applied Pharmacology 111(1): 58-68.	Another intervention
23	Murphy J, Liu C, McAdam K, Gaça M, Prasad K, Camacho O, McAughey J, Proctor C. Assessment of tobacco heating product THP1.0. Part 9: The placement of a range of next-generation products on an emissions continuum relative to cigarettes via pre-clinical assessment studies. Regul Toxicol Pharmacol. 2018 Mar;93:92-104.	Another trial methods
24	Patskan, G. and W. Reininghaus (2003). Toxicological Evaluation of an Electrically Heated Cigarette. Part 1: Overview of Technical Concepts and Summary of Findings. Journal of Applied Toxicology 23(5): 323-328.	Another intervention
25	Phillips, B., E. Veljkovic, et al. (2015). A 7-Month Cigarette Smoke Inhalation Study in C57bl/6 Mice Demonstrates Reduced Lung Inflammation and Emphysema Following Smoking Cessation or Aerosol Exposure from a Prototypic Modified Risk Tobacco Product. Food Chem Toxicol 80: 328-345.	Another intervention

Table 8. List of publications excluded from the analysis.

Nr	Publication	Reason for exclusion
26	Poussin, C., V. Belcastro, et al. (2017). Crowd-Sourced Verification of Computational Methods and Data in Systems Toxicology: A Case Study with a Heat-not-burn Candidate Modified Risk Tobacco Product. Chem Res Toxicol 30(4): 934-945.	Another trial methods
27	Roemer, E., R. Stabbert, et al. (2004). Chemical Composition, Cytotoxicity and Mutagenicity of Smoke from Us Commercial and Reference Cigarettes Smoked under Two Sets of Machine Smoking Conditions. Toxicology 195(1): 31-52.	Another intervention
28	Roemer, E., R. Stabbert, et al. (2008). Reduced Toxicological Activity of Cigarette Smoke by the Addition of Ammonium Magnesium Phosphate to the Paper of an Electrically Heated Cigarette: Smoke Chemistry and in Vitro Cytotoxicity and Genotoxicity. Toxicol In Vitro 22(3): 671-681.	Another intervention
29	Stabbert, R., P. Voncken, et al. (2003). Toxicological Evaluation of an Electrically Heated Cigarette. Part 2: Chemical Composition of Mainstream Smoke. Journal of Applied Toxicology 23(5): 329-339.	Another intervention
30	Stapleton, J. A., M. A. Russell, et al. (1998). Nicotine Availability from Eclipse Tobacco-Heating Cigarette. Psychopharmacology (Berl) 139(3): 288-290.	Another intervention
31	Stephens, W. E. (2017). Comparing the Cancer Potencies of Emissions from Vapourised Nicotine Products Including E-Cigarettes with Those of Tobacco Smoke. Tobacco Control 2018;27:10-17.	Another trial methods
32	Stinn, W., A. Berges, et al. (2013). Towards the Validation of a Lung Tumorigenesis Model with Mainstream Cigarette Smoke Inhalation Using the a/J Mouse. Toxicology 305: 49-64.	Another intervention
33	Terpstra, P. M., A. Teredesai, et al. (2003). Toxicological Evaluation of an Electrically Heated Cigarette. Part 4: Subchronic Inhalation Toxicology. Journal of Applied Toxicology 23(5): 349-362.	Another intervention
34	Tewes, F. J., T. J. Meisgen, et al. (2003). Toxicological Evaluation of an Electrically Heated Cigarette. Part 3: Genotoxicity and Cytotoxicity of Mainstream Smoke. Journal of Applied Toxicology 23(5): 341-348.	Another intervention
35	Tsuji, H., C. Okubo, et al. (2014). Comparison of Dermal Tumor Promotion Activity of Cigarette Smoke Condensate from Prototype (Heated) Cigarette and Reference (Combusted) Cigarette in Sencar Mice. Food Chem Toxicol **72**: 187-194.	Another intervention
36	van der Toorn, M., S. Frentzel, et al. (2015). A Prototypic Modified Risk Tobacco Product Exhibits Reduced Effects on Chemotaxis and Transendothelial Migration of Monocytes Compared with a Reference Cigarette. Food and Chemical Toxicology 80: 277-286.	Another intervention

Appendix F. Characteristics of included randomized clinical trials

Table 9. Characteristics of included randomized clinical trials.

Study	Sponsor	Study method	Study type	Number and location of centers	Population size	Observation scheme	Population	Compared interventions with the number of patients (N)
Ludicke 2018 (NCT01970995)	Philip Morris Products	Single-centre, randomized, open-label clinical trial*	Parallel	1 centre in Japan	160	90 days + 28 days of safety follow-up	Participants aged 23-65 who smoke ≥10 conventional menthol cigarettes/day for ≥3 years	THS 2.2 menthol, N=78; CC menthol, N=42; Smoke cessation, N=40.
Haziza 2016d** (NCT01989156)	Philip Morris International	Randomized, open-label clinical trial	Parallel	USA, information about the number of centres not mentioned	160	90 days	Participants smoking ≥10 conventional menthol cigarettes/day for ≥3 years	· THS 2.2 menthol, N=80; · CC menthol, N=41; · Smoke cessation, N=39.
Haziza 2016a (NCT01959932)	Philip Morris International	Single-centre, randomized, open-label clinical trial	Parallel	1 centre in Poland	160	5 days + 7 days of safety follow-up	Participants aged 21-65 who smoke ≥10 conventional non-menthol cigarettes/day (max 1 mg of nicotine/cigarette) for ≥3 years	· THS 2.2 non-menthol, N=80; · CC non-menthol, N=41; · Smoke cessation, N=39.
Haziza 2016b (NCT01970982)	Philip Morris Products	Single-centre, randomized, open-label clinical trial	Parallel	1 centre in Japan	160	5 days + 7 days of safety follow-up	Participants aged 23-65 who smoke ≥10 conventional non-menthol cigarettes/day (max 1 mg of nicotine/cigarette) for ≥3 years	· THS 2.2 non-menthol, N=80; · CC non-menthol, N=40; · Smoke cessation, N=40.
Gale 2018	British American Tobacco	Randomized, open-label clinical trial*	Parallel	2 centres in Japan	180	5 days + 2 days of pharmacokinetic follow-up + 5-7 days of safety follow-up	Participants aged 23-55 who smoke ≥10 (max. 30) conventional cigarettes/day for ≥3 years	· THS 2.2, N=30; · non-menthol THP 1.0, N=30; · menthol THP 1.0, N=30; · CC non-menthol, N=30; · CC menthol, N=30; · Smoke cessation, N=30.
Brossard 2017a (NCT01959607)	Philip Morris Products	Randomized, open-label clinical trial	Cross-over	Japan, information about the number of centres not mentioned	62	1 day (single use)	Participants aged 23-65 who smoke ≥10 conventional cigarettes/day (max 1 mg of nicotine/cigarette) for ≥3 years	· THS 2.2 non-menthol -> CC, N=22; · CC -> THS 2.2 non-menthol, N=22; · THS 2.2 non-menthol -> NRT (chewing gum), N=9; · NRT (chewing gum) -> THS 2.2 non-menthol, N=9.
Brossard 2017b (NCT01967706)	Philip Morris Products	Randomized, open-label clinical trial	Cross-over	Japan, information about the number of centres not mentioned	62	1 day (single use)	Participants aged 23-65 who smoke ≥10 conventional cigarettes/day (max 1 mg of nicotine/cigarette) for ≥3 years	· THS 2.2 menthol -> CC, N=22; · CC -> THS 2.2 menthol, N=22; · THS 2.2 menthol -> NRT (chewing gum), N=9; · NRT (chewing gum) -> THS 2.2 menthol, N=9.
Yuki 2017	Japan Tobacco Inc	Single-centre, randomized, open-label clinical trial	Cross-over	1 centre in Japan	24	1 day (single use)	Participants aged 21-65 who smoke >11 conventional cigarettes/day for ≥12 months	· Ploom TECH -> CC, N=24; · CC -> Ploom TECH, N=24.
Gee 2017	British American Tobacco	Single-centre, randomized, open-label clinical trial	Cross-over	1 centre in Japan	208	4 days	Participants aged 21-64 who smoke ≥5 conventional cigarettes/day for >6 months or who were using THPs ≥5 sessions/day for ≥3 months	· CC -> THP 1.0 -> THS 2.2, N=52; · CC menthol -> THP 1.0 menthol, N=52; · THP 1.0 -> THS 2.2, N=52; · THP 1.0, N=52.

THS 2.2 - Tobacco Heating System 2.2 (IQOS); THP 1.0 – Tobacco Heating Product 1.0 (glo); CC - conventional cigarette; NRT - nicotine replacement therapy; * laboratories were blinded to randomization scheme; ** unpublished study, characteristics and results available in the form of a conference poster.

Appendix G. Exposure markers evaluated in randomized trials

Table 10. Markers of exposure to harmful or potentially harmful constituents [3], [4], [5]

Acronym	Exposure marker	Harmful or potentially harmful constituents	Toxicity
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol	4-(methylnitrosamino)-1-(3-piridyl)-1-butanon (NNK)	CA
NNN	N-nitrosonornicotine	N-nitrosonornicotine (NNN)	CA
MHBMA	monohydroxybutenyl mercapturic acid	1,3-butadiene	CA, RT, RDT
3-HPMA	3-hydroxypropylmercapturic acid	acrolein	RT, CT
S-PMA	S-phenylmercapturic acid	benzene	CA, CT, RDT
COHb	carboxyhemoglobin	carbon monoxide	RDT
eCO	carbon monoxide	carbon monoxide	RDT
1-OHP	1-hydroxypyrene	pyrene	NA
3-OH-B[a]P	3-hydroxy-benzo(a)pyrene	benzo(a)pyrene	CA
4-ABP	4-aminobiphenyl	4-aminobiphenyl	CA
1-NA	1-aminonaphthalene	1-aminonaphthalene	CA
2-NA	2-aminonaphthalene	2-aminonaphthalene	CA
o-toluidine	o-toluidine	o-toluidine	CA
CEMA	2-cyanoethylmercapturic acid	acrylonitrile	CA, RT
HEMA	2-hydroxyethylmercapturic acid	ethylene oxide	CA, RT, RDT
3-HMPMA	3-hydroxy-1-methylpropylmercapturic acid	crotonaldehyde	CA
AAMA	N-acetyl-S-(2-carbamoylethyl)-cysteine	acrylamide	CA
GAMA	N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-cysteine	acrylamide	CA

CA – carcinogen; RT – respiratory toxicant; RDT – reproductive or developmental toxicant; CT – cardiovascular toxicant; NA – not available.

Appendix H. Pre-clinical studies – methodology and results

Table 11. Characteristics of included pre-clinical studies.

Study	Aim	Research material	Sponsor	Observation scheme	End points	Interventions
Aerosol chemistry and physics						
Bekki 2017	To compare levels of nicotine and HPHC in mainstream THP emissions from regular and menthol tobacco sticks with those in mainstream cigarette smoke	Mainstream smoke and tobacco fillers	Partially supported by Grants from Ministry of Health, Labour and Welfare of the Japanese Government, and the practical research project from Japan Agency for Medical Research and Development	55 mL of puff volume with 2 s puff duration and 30 s puff interval, ventilation closed; 9/11 puffs per one CC/HnB; each sampling was performed by 3 CC or HnB	· Concentration of nicotine, tar and CO in mainstream smoke and filler of THS 2.2 · Concentration of TSNAs (NNN, NAT, NAB, NNK) in mainstream smoke and filler of THS 2.2	· THS 2.2 regular · THS 2.2 menthol · CC (3R4F) · CC (1R5F)
Crooks 2018	To test if flavour ingredients in THP do no increase the risk of using it. To compare the emission chemistry between 3R4F and THP.	Smoke/aerosol	British American Tobacco	Three regimens: - 35 mL of puff volume with 2 s puff duration and 60 s puff interval, open ventilation; - 45 mL of puff volume with 2 s puff duration and 30 s puff interval, ventilation 50% blocked; - 55 mL of puff volume with 2 s puff duration and 30 s puff interval, ventilation closed	· Chemical analysis: concentration of 77 analytes	· THP 1.0 · CC

Table 11. Characteristics of included pre-clinical studies.

Study	Aim	Research material	Sponsor	Observation scheme	End points	Interventions
Eaton 2018	To compare levels of HPHC in mainstream glo emissions with those in mainstream cigarette smoke	Mainstream smoke/aerosol and	British American Tobacco	Heating programme (3 replicates per sample): 1) initial ramp at 5 °C/min from ambient to 240 °C, holding for 5 min at 240 °C; 2) next ramp: 5 °C/min to 900 °C. Machine-puffing: 55 mL of puff volume with 2 s puff duration and 30 s puff interval (8 puffs in total).	· Levels of combustion markers · Levels of tobacco smoke toxicants	· THP 1.0 · CC
Farsalinos 2017	To measure levels of nicotine in tobacco and aerosol of THP compared to ECs CCs	Smoke/aerosol	No funding	55 mL of puff volume with2 s puff duration, 27.5mL/s puff flow rate and 30 s puff interval	· Levels of nicotine per gram of tobacco · Levels of nicotine delivered to the aerosol	· THS 2.2 · ECs · CC
Farsalinos 2018	To measure emissions of carbonyl from THP compared to ECs CCs	Smoke/aerosol	Mayo Clinic, National Cancer Institute CCSG Cancer Center Grant	Puffing regimens: - 12 puffs: 55 mL of puff volume with 2 s puff duration, 30 s puff interval; - 12 puffs: 80 mL of puff volume with 3 s puff duration, 30 s puff interval; - 14 puffs: 90 mL of puff volume with 3 s puff duration, 25 s puff interval.	· Levels of carbonyl emissions per unit of CC and THS 2.2 · Levels of carbonyl emissions per mg of nicotine emission to the aerosol (nicotine yield)	· THS 2.2 · ECs · CC
Forster 2018	To compare levels of toxicant emissions in the aerosol of THP and smoke of CC.	Smoke/aerosol	British American Tobacco	Puffing regime: 55 mL of puff volume with 2 s puff duration, 30 s puff interval and a bell-shaped puff profile	Levels of: · TPM, nicotine, water and NFDPM · Oxygen-containing substances · Nitrogenous species · Metals · Phenols · Glycols · Hydrocarbons · Nitrosamines · Nicotine-related compounds · Carbonyl compounds	· THP 1.0 (T) · THP 1.0 (M) · CC
Jaccard 2017	To compare HPHC yields in THS2.2 and CCs	Smoke/aerosol	Philip Morris International	Puffing regime: 55 mL of puff volume with 2 s puff duration and 30 s puff interval	· HPHC analysis (yields and percentage reductions)	· THS 2.2 · CC
Jaccard 2018	To compare TSNA transfer from tobacco to the aerosol of THS 2.2 and mainstream smoke of CC.	Mainstream smoke and tobacco fillers	Philip Morris International	Puffing regime: 55 mL of puff volume with 2 s puff duration and 30 s puff interval	· TSNA, nitrate and minor alkaloids levels in cigarette cut fillers · Mainstream smoke analyses · Transfer from tobacco cut filler to mainstream smoke	· THS 2.2 · CC

Table 11. Characteristics of included pre-clinical studies.

Study	Aim	Research material	Sponsor	Observation scheme	End points	Interventions
Li 2018	To test a comprehensive list of chemical releases from THS2.2 and compared to those from CC (3R4F).	Mainstream smoke and tobacco fillers	National Natural Science Foundation of China, Science Innovation Foundation of China National Tobacco Quality and Supervision and Test Center and State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences	Smoking regimens: - 35 mL of puff volume with 2 s puff duration and 60 s puff interval, open ventilation; -55 mL of puff volume with 2 s puff duration and 30 s puff interval, closed ventilation.	· Yields of chemical emitted from CC and THS2.2 (basic analytes, CO, carbonyls, VOCs, aromatic amines, ammonia, hydrogen cyanide, phenol, N-nitrosamines, PAH). · Chemical constituents emitted by THS2.2 and CC in simulated pyrolysis conditions.	· THS 2.2 · CC
Poynton 2017	To describe the characteristics of a novel THP – to present aerosol chemistry data and compare with a control product (without the tobacco insertion) and CC.	Smoke/aerosol	British American Tobacco	55 mL of puff volume with 3 s puff duration and 30 s puff interval	· Physical characterization of the novel product · In-house sensory panel characterization · Aerosol chemistry	· Glo iFUSE · CC · ECs
Pratte 2017_1	To verify a Dekati thermo-denuder method of particles and droplets detection THS2.2 aerosol and CC smoke were compared.	Smoke/aerosol	Philip Morris Products	55 mL of puff volume with 1 s puff duration and 30 s puff interval - 12 puffs for CC and 10 puffs for THP	· Solid particle penetration and wall losses · Liquid droplet penetration and thermo-denuder removal efficiency · Coating removal experiments to test the thermo-denuder efficiency	· THS 2.2 · CC
Pratte 2017_2	To compare solid particle number and chemical composition of THS2.2 aerosol and mainstream smoke of CC (3R4F).	Smoke/aerosol	Philip Morris Products	55 mL of puff volume with 1 s puff duration and 30 s puff interval - 12 puffs for CC and 10 puffs for THP	· Number of solid particles · Chemical composition of THS2.2 aerosol and mainstream smoke of CC	· THS 2.2 · CC
Savareear 2017	To develop a method for the analysis of volatile and semi-volatile compounds present in the particulate phase of aerosol emitted from heating tobacco – this method was used to compare CC and THP.	Smoke/aerosol	Centre for Analytical Research and Technologies, British American Tobacco, Restek Corporation, SGE international and Gerstel Japan	55 mL of puff volume with 2 s puff duration and 30 s puff interval, bell-shaped puffs.	· Compounds identified in THP aerosol and distribution of the major chemical classes which were identified in aerosol of THP using analysed method · Analysis of particulate phases fraction of CC smoke · Comparison of the VOC/SVOC profile of THP aerosol and CC smoke (apex plots)	· THP 1.0 · CC

Table 11. Characteristics of included pre-clinical studies.

Study	Aim	Research material	Sponsor	Observation scheme	End points	Interventions
Schaller 2016_1	To compare levels of HPHC in mainstream aerosol of THP and mainstream smoke of CC.	Smoke/aerosol	Philip Morris International	different puff regimens, incl. bell-shaped puffs: 55 mL of puff volume with 2 s puff duration and 30 s puff interval – 12 puffs	Chemical composition of THS2.2 aerosol and CC smoke, eg.: · Levels of nicotine delivered to the aerosol · Levels of combustion markers · Levels of toxicants	· THS 2.2 · CC
Schaller 2016_2	To assess the variations in HPHC yields caused by using different tobacco blends in THS2.2 tobacco plugs	Smoke/aerosol	Philip Morris International	55 ml puff every 30 s, 2 s puff duration	· The concentrations of HPHC of mainstream aerosol · Markers of combustion · Nitrogen-containing HPHCs · Other HPHCs · Effects of the blend used on the generation of HPHCs	· THS 2.2 · CC
Uchiyama 2018	To determinate of compounds (gaseous and particulate) generated from HTPs	Smoke/aerosol	Health and Labour Science Research Grants from Ministry of Health, Labour and Welfare of the Japanese Government and the Practical Research Project from Japan Agency for Medical Research and Development	Puffing regimens: - 55 mL of puff volume with 2 s puff duration and 30 s puff interval and ventilation blocked; - 35 mL of puff volume with 2 s puff duration and 60 s puff interval and open ventilation.	· Chromatographic profiles of target compounds · LOD, LOQ and reproducibility · Chemical compounds analysis in mainstream smoke of HTPs and CCs using GF-CX°ᵛᵛ Cartridge Followed by Two-Phase Elution · Chemical compounds generated on each puff and at the temperature of tobacco leaves	· THS 2.2 · THP 1.0 · Ploom TECH · CC (CM6, 3R4F, 1R5F)
Standard Toxicology Assessment						
Schaller 2016	In vitro genotoxicity and cytotoxicity of the aerosol assessment	Mouse embryonic fibroblast cell line Balb/c 3T3, Salmonella typhimurium, L5178Y tk± cell line	Philip Morris International	NRU assay, Ames assay, mouse lymphoma assay, test matrices generated by smoking machines	· cytotoxicity · mutagenicity	· THS 2.2 FR1 (regular) · THS 2.2 D2 (regular) · THS 2.2 FR1 M (menthol) · THS 2.2 D2 (menthol) · CC
Breheny 2017	In vitro toxicological assessment	Salmonella typhimurium, NCI-H292 human bronchial epithelial cells, BEAS-2B human bronchial epithelial cells, Bhas 42 mouse embryo fibroblast cells, human umbilical vein endothelial cells (HUVEC),	British American Tobacco	Ames assay, NRU assay, H2AX assay, Bhas assay, GSH:GSSG assay, DCF assay, ARE-reporter cell assay, Apolive-Glo assay, scratch wound assay, test matrices generated by smoking machines	· nicotine in whole aerosole (WA) emission and total particulate matter (TPM) · carbonyls in aqueous aerosol extracts (AqE) · mutagenicity · cytotoxicity · DNA damage · tumour-promoting potential · cell viability and apoptosis · intercellular oxidative stress · endothelial wound repair	· iFUSE · THS 2.2 · CC

Table 11. Characteristics of included pre-clinical studies.

Study	Aim	Research material	Sponsor	Observation scheme	End points	Interventions
Jaunky 2017	In vitro cytotoxicity assessment	H292 human bronchial epithelial cells	British American Tobacco	NRU assay, exposed for 1h at the air-liquid interface and 24 h post-exposure recovery period, test matrices generated by smoking machines	· nicotine concentration · cell viability	· THS 2.2 · THP1.0 · CC
Thorne 2017	In vitro mutagenicity, cytotoxicity and tumour-promoting activity assessment	Mouse fibroblast cells, Salmonella typhimurium, Mouse lymphoma cells, Bhas 42 mouse fibroblast cell	British American Tobacco	NRU assay, Ames assay, mouse lymphoma assay, test matrices generated by smoking machines	· mutagenicity · cytotoxicity · tumour-promoting activity	· THS 2.2 · THP1.0 · CC
Crooks 2018	In vitro toxicity and risk related to flavor ingredients assessment	Salmonella typhimurium, L5178Y tk+/− cells, Chinese Hamster lung fibroblasts, Bhas 42 cells, Balb/c 3T3 mouse fibroblasts	British American Tobacco	Ames assay, mouse lymphoma assay, micronucleus test, Bhas 42 assay, NRU assay, test matrices generated by smoking machines	· concentration of toxicants · mutagenicity · cytotoxicity · tumour-promoting activity	· THP flavoured Neostick · THP unflavoured NEOstick · CC
Wong 2016	In vivo toxicity and respiratory effects from sub-chronic inhalation of test atmospheres assessment	Sprague Dawley rats	Philip Morris International	Exposition for a period of 90 days, at an exposure regimen of 6h, 5 days per week + 42 days to assess reversibility or persistence of findings	· test atmosphere composition · general conditions and health · carboxyhemoglobin in blood · urine nicotine metabolites and biomarkers of exposure · respiratory physiology · hematology and clinical chemistry · lung analysis · organ weights · histopathological assessment · transcriptomics assessment	· Filtered air · THS 2.2 aerosol at 3 target concentrations of nicotine · CC smoke at 3 target test astmosphere concentrations of nicotine
Oviedo 2016	In vivo toxicity and respiratory effects from sub-chronic inhalation of test atmospheres assessment	Sprague Dawley rats	Philip Morris International	Exposition for a period of 90 days, at an exposure regimen of 6h, 5 days per week + 42 days to assess reversibility or persistence of findings	· test atmosphere composition · carboxyhemoglobin in blood · urine nicotine metabolites and · biomarkers of exposure in blood · respiratory physiology · histopathological assessment · lung analysis · organ weights · transcriptomics assessment · proteomics assessment · lipidomics analysis	· Filtered air · THS ۲,۲ aerosol at ۳ target concentrations of nicotine · CC smoke at ۲ target test astmosphere concentrations of nicotine
Systems Toxicology Assessment						
Gonzalez-Suarez 2016	In Vitro Systems Toxicology Assessment	Normal human bronchial epithelial cells	Philip Morris International	Bd	· HPHC levels · cell viability · toxicity · impact on cell transcriptome	· THS 2.2 · CC

Table 11. Characteristics of included pre-clinical studies.

Study	Aim	Research material	Sponsor	Observation scheme	End points	Interventions
Haswell 2018	In vitro Systems Toxicology Assessment	3D airway tissue	British American Tobacco	Acute exposure, observation 48 h post-exposure	· RNA-seq-based toxicogenomics assessment	· THP 1.0 · THS 2.2 · CC
Iskandar 2017a	In Vitro Systems Toxicological Assessment	Nasal organotypic epithelial tissue cultures	Philip Morris International	Exposure for 28 min, observation 72h post exposure	· smoke/aerosol exposure · cytotoxicity and tissue morphology · immunostaining analysis · cilia beating frequency · CYP1A1/CYP1B1 activity · profiles of secreted pro-inflammatory mediators · exposure impact on global mRNA/miRNA profiles	· THS 2.2 · CC
Iskandar 2017b	In Vitro Systems Toxicology Assessment	Human organotypic bronchial epithelial cultures	Philip Morris International	Exposure for 28 min, observation 72h post exposure	· cytotoxicity and tissue morphology · immunostaining analysis · cilia beating frequency · secretion of pro-inflammatory mediators · network-based differential gene expression analysis · expression of selected genes regulating cell stress and inflammation · alterations in miRNA profiles	· THS 2.2 · CC
Iskandar 2017d	In Vitro Systems Toxicology Assessment	Organotypic human small airway culture model	Philip Morris International	Exposure for 28 min, observation up to 72h post exposure	· nicotine concentrations · effects on cytotoxicity and culture morphology · effects on ciliary beating function · inflammatory responses following exposure · global mRNA and miRNA alterations	· THS 2.2 · CC
Taylor 2018	In Vitro Systems Toxicology Assessment	Human bronchial epithelial cells	British American Tobacco	6 or 24h exposure	· multiparametric toxicity · oxidative stress	· THP 1.0 · THS 2.2 · CC
Van der Toorn 2018	In Vitro Systems Toxicology Assessment	Human bronchial epithelial cell line	Philip Morris International	12 weeks of exposure	· nicotine · epithelial cell adhesion · gene and miRNA expression changes · protein analysis · cell transformation · epithelial cell invasion · gene and miRNA expression changes	· THS 2.2 · CC

Table 11. Characteristics of included pre-clinical studies.

Study	Aim	Research material	Sponsor	Observation scheme	End points	Interventions
Malinska 2018	In Vitro Systems Toxicology Assessment	Human bronchial epithelial cell line	Philip Morris International	1-12 weeks exposure	· ATP levels · oxygen consumption · cytosolic reactive oxygen species levels · mitochondrial superoxide levels · oxidatively modified proteins · analysis of transcriptomics data	· THS 2.2 · CC
Jaunky 2018	In Vitro Systems Toxicology Assessment	Human bronchial epithelial cells	British American Tobacco	Exposure for 1h, observation 24h post exposure	· nicotine · cell viability	· THP 1.0 · THS 2.2 · CC
Zanetti 2016	In Vitro Systems Toxicology Assessment	Organotypic buccal epithelial cultures	Philip Morris International	Exposure for 28 min, observation 72h post exposure	· cytotoxicity and tissue morphology · CYP1A1/CYP1B1 activity · profiles of secreted proinflammatory mediators · exposure impact on mRNA/miRNA profiles	· THS 2.2 · CC
Zanetti 2017	In Vitro Systems Toxicology Assessment	Organotypic human gingival epithelial cultures	Philip Morris International	3-day repeated 28 min exposure, observation 24h post exposure	· viability and morphology · biological impact based on transcriptional changes · oxidative stress · xenobiotic metabolism · expression and secretion of proinflammatory mediators · keratinization and cell-cell adhesion	· THS 2.2 · CC
Van der Toorn 2015	In Vitro Systems Toxicology Assessment	Monocytic cell line and human coronary arterial endothelial cells	Philip Morris International	18h exposure	· chemical analyses of CC and THS 2.2 extracts · cytotoxicity · inflammation · chemotaxis and transendothelial migration · integrity of an endothelial monolayer	· THS 2.2 · CC
Poussin 2016	In Vitro Systems Toxicology Assessment	Monocytic cells to human coronary arterial endothelial cells	Philip Morris International	2h exposure	· chemical analysis of aqueous CC smoke and THS 2.2 aerosol extracts · Mono Mac 6 cells adhesion · inflammatory markers · RNA transcriptome analysis	· THS 2.2 · CC

Table 11. Characteristics of included pre-clinical studies.

Study	Aim	Research material	Sponsor	Observation scheme	End points	Interventions
Iskandar 2017c	In Vitro Systems Toxicology Assessment – meta-analysis	Human organotypic cultures of the aerodigestive tract	Philip Morris International	28min-18h	· cytotoxicity and ciliary beating · functionality · perturbation of molecular mechanisms · xenobiotic metabolism and oxidative stress responses · inflammatory responses · alterations of microRNAs	· THS 2.2 · CC
Philips 2016, Titz 2016	In Vivo Systems Toxicology Assessment	The Apoe–/– mouse model, 828 mice	Philip Morris International	8 months	· in-life observations and biomarkers of exposure · hematology · clinical chemistry · aortic arch plaque formation · plasma and aortic arch lipidomics · lung function and lung volume · histopathology of the respiratory tract · transcriptomics of lung tissue · lung proteomics · histopathology of the liver and kidney	· THS 2.2 · CC · Sham (fresh air) · Cessation · Switch (CC->THS 2.2)
Lo Sasso 2016b	In Vivo Systems Toxicology Assessment	The Apoe–/– mouse model, 828 mice	Philip Morris International	8 months	· liver tissue histopathology · liver proteome · liver transcriptome · liver and plasma lipidomes	· THS 2.2 · CC · Sham (fresh air) · Cessation · Switch (CC->THS 2.2)

CC – combustible cigarette (eg. 3R4F, 1R5F); ECs - E-cigarettes; HnB – heat-not-burn; HPHC – harmful and potentially harmful constituents; LOD - Limit of Detection; LOQ - Limit of Quantitation; (M) = mentholated consumable variant; NAB – N'-nitrosoanabasine; NAT – N'-nitrosoanatabine; NNK – 4-(Methylnitrosamino)-l-(3-pyridyl)-l-butanone; NNN - N'-nitrosonornicotine; PAH – polyaromatic hydrocarbons; (T) = non-mentholated consumable variant; TSNAs - tobacco-specific nitrosamines; THP – tobacco heating product; THS – tobacco heating system; VOCs – votatile organic chemicals; SVOC - semi-volatile organic compounds.

Table 12. Main results and conclusions from included pre-clinical studies (direct results and conclusions from publications).		
Study	Main results	Author's conclusions (cited as in original paper)
Aerosol chemistry and physics		
Bekki 2017	· Nicotine concentration in the fillers of THP were very similar to CCs; nicotine levels in the mainstream smoke of THP were comparable with 1R5F and relatively lower than 3R4F; transfer rates of nicotine in THP are more effective than in CCs (above 23% vs about 11%). · Tar concentration in the mainstream smoke of THP was ≤50% than in CCs. THP emitted CO at lower concentrations than CCs (one-hundredth of that emitted by CCs, most likely due to heating mechanism in THP) · Despite slightly higher transfer rates of NNN, NAT and NNK in THP than in CCs, significantly lower concentration levels of TSNAs were detected in tobacco fillers and mainstream smoke of THP compared to CCs.	The results of the study indicate that 'the concentration levels of hazardous compounds in the mainstream smoke of iQOS are much lower than those in conventional combustion cigarettes. Although it is low concentration, toxic compounds are definitely included in the mainstream smoke of iQOS.'
Crooks 2018	· The concentrations of detectable analytes (ie. 43/77) were significantly reduced for Neostiks (flavoured and unflavoured) compared to 3R4F, with one exception (glycidol - marginally higher in Neostiks).	Because of the low heating temperature of the THPs the concentration and amounts of measured toxicants in emissions were reduced in THPs compared to CC (3R4F) cigarettes.
Eaton 2018	· Levels of CO, CO2, NO and NOx (combustion markers) in the aerosol of THP1.0 were reduced by more than 90% compared with cigarette smoke.	'The results of the study indicate that for THP1.0 the primary mechanisms of aerosol formation are distillation and evaporation and that there is very little or no combustion.'
Farsalinos 2017	· Lower level of nicotine in THP than in CC – nicotine was delivered in IQOS at 2 s and 4 s puffing regimens at levels 0.46±0.06 mg and 0.86±0.08 mg, respectively ; the level of nicotine delivered by CC was 1.99±0.20 mg per cigarette.	*'HnB deliver less nicotine to the aerosol compared to the smoke of a tobacco cigarette under the puffing regimes tested.'*
Farsalinos 2018	· Levels of carbonyl emissions from THP were reduced by 91.6%, for formaldehyde, 84.9% for acetaldehyde, 90.6% for acrolein, 89.0% for propionaldehyde and 95.3% for crotonaldehyde compared to CC in case of Health Canada Intense puffing regime. At more intense puffing regimes levels of formaldehyde were 3-4 times lower than in CC.	THP emits substantially lower levels of carbonyls compared to CC.
Forster 2018	· The mean reductions in THP1.0 aerosol of nine toxicants - TobReg priority constituents (1,3-Butadiene, Acetaldehyde, Acrolein, Benzene, Benzo[a]pyrene, CO, Formaldehyde, NNK, NNN) – were 90.6-99.9% per consumable and overall reduction was 97.1%. · The mean reductions in THP1.0 aerosol for the abbreviated list of HPHCs of smoke (1,3-Butadiene, 1-Aminonaphthalene, 2-Aminonaphthalene, 4-Aminobiphenyl, Acetaldehyde, Acrolein, Acrylonitrile, Ammonia, Benzene, Benzo[a]pyrene, CO, Crotonaldehyde, Formaldehyde, Isoprene, Nicotine, NNK, NNN, Toluene) were 84.6-99.9% per and overall reduction was 97.5%.	The levels of emitted toxicant were significantly reduced in THP1.0 compared to CC (3R4F) across all chemical classes.
Jaccard 2017	· The mean reduction of HPHC in aerosol of THS2.2 and CC is very similar in all countries analysed in this study. · For THS2.2 compared to CC 90% market mean reduction is observed across a broad range of HPHCs.	Results if this study 'confirm that the average reduction in aerosol yields shown for the THS2.2 in comparison to the 3R4F reference cigarette are equally valid when considering commercially available cigarette products from diverse markets worldwide.'
Jaccard 2018	· The transfer of nicotine and TSNA from tobacco to the aerosol in THS2.2 was similar to that observed for cigarettes in case of nicotine and was 2–3 times lower than in cigarettes in case of TSNA. · The total median transfer rate of TSNA under intense analytical smoking/puffing conditions varies between 7 and 19% for THS2.2 and between 17 and 67% for cigarettes.	In THS2.2 *'the transfer of TSNA from tobacco to aerosol is reduced in comparison with cigarettes tobacco to mainstream smoke, due to a combination of lower evaporating transfer and the limitation of pyrosynthesis and pyrorelease induced by the lower temperature applied to THS2.2 tobacco part.'*
Li 2018	· The chemical releases from THS2.2, other than some carbonyls, NAB and ammonia, were ≥80% lower than those from CC (3R4F). · The levels of nicotine and tar in THS2.2 and CC were very similar.	'THS 2.2 delivered fewer harmful constituents than the conventional cigarette 3R4F.'
Poynton 2017	· In a targeted analysis of 113 compounds, only 26 and 87 were quantifiable in the aerosol of THP and CC, respectively (a further 19 and 5 were detected but not quantifiable, respectively). · Levels of acetaldehyde in the CC (2R4F) were about 200-fold higher than in THP (~1700 mg per stick vs ~8.22 mg in puff block 1-100, and ~8.53 mg in puff block 101-200). · For the nine TobReg toxicants, THP showed a mean reduction of 91.0% per-product and a 99.5%per-puff compared with CC (3R4F) without blank correction and with blank correction 97.0% and 99.8%,, respectively.	The emission levels from THP were 92-99% lower on a per-puff basis than those from CC. 'These data demonstrate that the aerosol from the novel hybrid product is compositionally much less complex than cigarette smoke and contains significantly lower levels of toxicant compounds.'

Table 12. Main results and conclusions from included pre-clinical studies (direct results and conclusions from publications).

Study	Main results	Author's conclusions (cited as in original paper)
Pratte 2017_1	· The quantity of solid particles or high boiling point droplets were about 100 times larger than the LLOQ in CC mainstream smoke In the case of THS 2.2 mainstream aerosol the penetration was overlapping with the LLOQ, reflecting the experimental uncertainty.	*'No combustion related particles were released and transferred in the mainstream of THS2.2'* in contrast to CC.
Pratte 2017_2	· In case of CC about 80% of TPM was neither evaporated nor removed by the thermodenuder and in case of THS2.2 solid particles were not detected after passing through the thermodenuder (at 300°C).	THPs 'neither generate nor transfer solid particles in the mainstream aerosol when considering' experimental conditions of this study ' in contrast to CC.
Savareear 2017	· The chromatogram of THP aerosol was much less complex than the chromatogram of CC smoke (mean total numbers of peaks were 723 and 1995, respectively). · For THP aerosol and CC smoke 56% and 31% of detected analytes were identified with reasonable confidence, respectively.	'Compared to combustible product PP, THP sample chromatograms were significantly less complex, illustrating the greater chemical complexity of volatiles and semi-volatiles emitted from combustible products and the associated technical challenge of characterizing whole smoke emissions.'
Schaller 2016_1	· The majority of analysed HPHCs were reduced by more than 90% in THS2.2 compared to CC (no change in mass median aerodynamic diameter of the aerosol). · At intense puffing regimens the HPHC yields remained lower for THS2.2 compared to CC.	'The low operating temperature of THS2.2 results in significantly lower concentrations of HPHCs in the mainstream aerosol compared with the mainstream smoke of the 3R4F reference cigarette when expressed on either a per-Tobacco Stick/cigarette or a per-mg nicotine basis, while the MMAD of both aerosols remains similar. The reductions in the concentrations of most HPHCs in the THS2.2 aerosol were greater than 90% when compared with 3R4F, and were not affected by machine-smoking of THS2.2 under extreme climatic conditions.'
Schaller 2016_2	· For most of the analyzed HPHCs the blend composition had minimal effect on their yields. · 43 different experimental tobacco plug blends produced aerosols in the THS2.2 which contained significantly lower concentrations of HPHCs compared to the mainstream smoke of CC (3R4F).	The concentrations of HPHCs were significantly lower in THS2.2 mainstream aerosol than in smoke produced by CC.
Uchiyama 2018	· No considerable difference was noted between HTPs and CC in respect to TGPM (for HTPs 18-42 µg/stick depending of product and 31 µg/stick for CC) · HTPs generated lower nicotine levels than CC (for HTPs 230-1200 µg/stick depending of product and 1900 µg/stick for CC). · HTPs generated higher acetaldehyde levels than CC (for HTPs 360-5900 µg/stick depending of product and 18 µg/stick for CC). · The mean heating temperatures were lower for HTPs (23-210 °C) compared to CC (460 °C).	' With respect to the total gaseous and particulate compounds, no considerable difference is noted between HTPs and traditional cigarettes'. 'The generated chemical compounds depend on the temperature of tobacco leaves in HTPs.'
Standard Toxicology Assessment		
Schaller 2016	· Cytotoxicity of each tested version of THP were reduced by 85%-95% in the NRU assay as compared with the CC; · Ames assay did not show significant mutagenicity of the tested versions of THP aerosols, while fractions from CC showed significant mutagenicity. · Mouse Lymphoma Assay showed that each THP aerosol fraction is mutagenic. However, lower in vitro mutagenic potency of the THP aerosol fractions was demonstrated in comparison with CC smoke.	'The mutagenic and cytotoxic potencies of the mainstream aerosol fractions from THS2.2, when evaluated by the Ames, mouse lymphoma, and NRU assays were reduced by at least 85%e95% compared with the mainstream smoke aerosol of 3R4F.'
Breheny 2017	· The tested THPs achieved 87-90% reduction of cytotoxicity in comparison to CC; · Exposure to aerosol from the CC elicited a genotoxic response, while aerosols from the THPs were non-genotoxic. · THPs were less active than the conventional cigarette across the assays, apart from the DCF assay.	'All the THPs tested demonstrated significantly reduced responses in these in vitro assays when compared to 3R4F. The findings suggest these products have the potential for reduced health risks..'
Jaunky 2018	· Cell media nicotine concentration for CC ranged from 413 ± 260 ng/ml to 7863 ± 672 ng/ml (air control value of 84 ± 69 ng/m), while for tested THPs this concentration ranged from 1161 ± 362 ng/ml to 15,050 ± 2387 ng/ml for one of the products (air control value of 131 ± 24 ng/ml), and from 2095 ± 943 ng/ml to 28,150 ± 3594 ng/ml for the second tested product (air control value of 165 ± 71 ng/ml). · Results indicate that, in comparison with CC smoke, a greater exposure concentration of THP aerosols are required to elicit reductions in cell viability. It means that the test products are less cytotoxic than the reference product.	'The two THPs demonstrated a statistically similar, substantially reduced biological response as compared with tobacco smoke, as indicated by a shift in the dose-response curve induced by the THPs relative to the viability profile obtained from 3R4F cigarette exposure.'
Thorne 2018	· Both tested THPs showed no biological response to all used test. Results confirm reduced in vitro biological and toxicological effects of THPs as compared to CC smoke.	'This study has clearly demonstrated that compared to cigarette smoke at equivalent doses in TPM and WA techniques, THPs demonstrate significantly reduced in vitro toxicological activity.'

Table 12. Main results and conclusions from included pre-clinical studies (direct results and conclusions from publications).		
Study	Main results	Author's conclusions (cited as in original paper)
Crooks 2018	· Concentrations of toxicants in THPs aerosols were reduced in comparison to CC. · CC showed positive response in all biological tests used in study, while both THPs showed no response to most tests and only showed weak activity in the micronucleus assay, which was significantly lower in comparison with conventional cigarette.	Results show significantly differences in cytotoxicity, genotoxicity and mutagenicity of THP in comparison to cigarette. Flavor ingredients added to THP did not cause changes in results of biological tests and concentrations of measured toxicants.
Wong 2016	· Histopathological alterations and lung inflammation observed in rats exposed to tested THP aerosol were less pronounced in comparison to rats exposed to CC smoke. · Transcriptomics assessment of respiratory tract organs showed concentration-dependent differential gene expression following CC exposure, which was less pronounced in the rats exposed to THP. · Other toxicological endpoints did not show exposure-related effects.	'Toxicological changes observed in the respiratory tract organs of THS2.2 aerosol-exposed rats were much less pronounced than in 3R4F-exposed rats while other toxicological endpoints either showed no exposure-related effects or were comparable to what was observed in the 3R4F-exposed rats.'
Oviedo 2016	· Histopathological alterations and pulmonary inflammation observed in rats exposed to tested mentholated THP aerosol were less pronounced in comparison to rats exposed to mentholated and non-mentholated CC smoke. · Transcriptomics assessment of respiratory tract organs showed lower effects of mentholated THP aerosol on rat nose and lung tissue in comparison with reference cigarette. · In comparison with CC, molecular changes to THP aerosol exposure were much weaker and limited mostly to the highest concentration of aerosol in female rats.	'Heating tobacco rather than burning it leads to a remarkable reduction in toxicologically-relevant constituents in the produced aerosols and the test atmospheres, resulting in notable and significantly smaller biological effects.'
Systems Toxicology Assessment		
Gonzalez-Suarez 2016	· Reduced HPHC levels in mainstream THS 2.2 aerosol compared to levels observed in CC smoke; · Normal human bronchial epithelial cells exposed to THS 2.2 aerosol demonstrated Increased cell viability compared to cells exposed to CC smoke; · Reduced toxicity in normal human bronchial epithelial cells exposed to THS 2.2 aerosol compared to that of CC smoke; · THS 2.2 aerosol fractions exposure had lover impact on the normal primary human bronchial epithelial cell transcriptome compared to that of CC smoke fractions; · Lower biological impact in the normal primary human bronchial epithelial cell transcriptome after THS 2.2 aerosol exposure compared to that of CC smoke was observed.	'THS 2.2 aerosol is less toxic than combustible cigarette smoke and thus may have the potential to reduce the risk for smoke-related diseases.'
Haswell 2018	· The relationship between the identified RNA features and gene ontologies were mapped showing · A strong association with stress response xenobiotics metabolism, and COPD-related terms was shown for CC by mapping the relationship between the identified RNA features and gene ontologies. There were fewer ontologies found for THPs aerosols; · A pro-inflammatory effect was confirmed for CC smoke but not for THPs.	'THPs have a reduced impact on gene expression compared to 3R4F.'
Iskandar 2017a	· In case of THS2.2 aerosol exposure, concentrations of various carbonyls were lower, as compared with exposure to CC smoke; · In the CC group greater cytotoxicity levels were observed compared with the THS2.2 group; · No substantial alterations in CYP1A1/CYP1B1 activity were observed in THS2.2 groups; · The CC-induced impact on nasal cultures was greater than the THS2.2-induced impact;	'For all tested concentrations the impact of 3R4F smoke was substantially greater than that of THS 2.2 aerosol in terms of cytotoxicity levels, alterations in tissue morphology, secretion of pro-inflammatory mediators, impaired ciliary function, and increased perturbed transcriptomes and miRNA expression profiles.'
Iskandar 2017b	· Lower cytotoxicity was observed after THS 2.2 aerosol exposure than after CC smoke exposure; · After exposure to THS 2.2 aerosol at nicotine concentration ≤three times that of CC smoke no morphological change was observed; · Exposure to THS 2.2 aerosol elicited a reduced levels of secreted mediators and fewer miRNA alterations compared to CC smoke. · The impact of THS 2.2 (0.14 mg nicotine/L) on gene expression changes (Cell Fate, Cell Proliferation, Cell Stress, Inflammatory Network Models) at 4 h post-exposure was significantly lower (7.6%) than the impact of CC (0.13 mg nicotine/L; 100%).	'At similar nicotine concentrations, aerosol of the heat-not-burn product THS2.2 elicited a reduced biological impact in organotypic bronchial cultures, as compared with 3R4F smoke exposure across all measured endpoints.'

Table 12. Main results and conclusions from included pre-clinical studies (direct results and conclusions from publications).

Study	Main results	Author's conclusions (cited as in original paper)
Iskandar 2017d	· Lower cytotoxicity levels were observed after THS 2.2 aerosol exposure than after CC smoke exposure; · THS 2.2 aerosol exposure was associated with lower changes in the secreted pro-inflammatory mediators than CC smoke; · Lower transcriptome-induced biological impact was observed after THS2.2 aerosol exposure than after CC smoke; · The effects of THS 2.2 aerosol exposure, if observed, were mostly transient and diminished more rapidly after exposure than those of CC smoke. · THS 2.2 aerosol exposure was associated with mostly transient effect, which diminished more rapidly than in case of CC smoke exposure.	'The aerosol from the candidate MRTP THS 2.2 elicited lower impact in all measured endpoints in the human small airway cultures.'
Taylor 2018	· THPs total particulate matter exposure were associated with no or a little activity in all assays, while CC total particulate matter exposure stimulated significant increases in antioxidant response element activation and moderate activity in high content screening cell-based assays.	'The lack of biological responses from both THPs reported in this study may be attributed to the reduction in measurable chemical species and toxicants in the aerosol from the THPs compared to 3R4F smoke.'
Van der Toorn 2018	· In cells treated to CC total particulate matter increased levels of inflammatory mediators were observed., while in cells treated to ≤ 5 times higher concentrations of THS 2.2 total particulate matter there were no increased levels of inflammatory mediators; · THS 2.2 total particulate matter at concentration 20 times higher increased oxidative stress and DNA damage and caused reversible epithelial to mesenchymal transition; · In cells treated to CC or a high concentration of THS 2.2 total particulate matter an anchorage-independent growth was observed; · THS 2.2 total particulate matter-derived clones were not invasive in contrast to CC total particulate matter-derived clones.	'Repeated exposure of BEAS-2B cells to TPM from the aerosol of THS 2.2, in comparison with TPM from CC smoke of the 3R4F reference cigarette, induced ongoing alterations in gene expression, as well as phenotypic changes such as EMT and anchorage independence, both indicators of cellular transformation. Long-term exposure to TPM from the THS 2.2 heat-not-burn tobacco product had a lower dose-dependent biological impact on human bronchial epithelial cells in comparison with TPM from combusted tobacco product.'
Malinska 2018	· Strong inhibitory effect on mitochondrial basal and maximal oxygen consumption rates were observed after 1-week exposure of total particulate matter from CC compared to total particulate matter from THS 2.2; · To disturb cellular function to a similar extent a concentration of total particulate matter from THS 2.2 aerosol 20 times higher than for CC was required.	'Reducing levels of HPHCs by heating rather than combusting tobacco could reduce mitochondrial dysfunction and oxidative stress-related diseases associated with smoking combustible tobacco products.'
Jaunky 2017	· A reduced in vitro cytotoxicity in H292 human bronchial epithelial cells at the aireliquid interface were observed for THPs in comparison with CC exposure; · Biological response were statistically better for both THPs compared to CC at a common aerosol dilution (1:40, aerosol:air).	Study results shows 'safety and risk reduction potential of next-generation tobacco products relative to cigarettes.'
Zanetti 2016	· No relevant signs of toxicity were observed after organotypic cultures exposure to the THS 2.2 aerosol at any of the concentration tested. The only exception was a light desquamation with exposure to the higher doses; · Exposure to THS 2.2 was associated with a lower alteration of the level of E-cadherin; · In cultures exposed to THS 2.2 after 24 h CYP activity was significantly higher than in cultures exposed to comparable concentrations of CC smoke; · Exposure to THS 2.2 was associated with a lower alteration of the level of secreted proinflammatory mediators; · Cultures exposed to the THS 2.2 aerosol showed lower impact on transcriptomics (both mRNA and miRNA) data.	'Compared with CS exposure, THS 2.2 aerosol exposure had an overall significantly lower impact on buccal epithelial physiology (except for a transiently higher CYP1A1/1B1 activity), as indicated by histopathological, inflammatory and transcriptomics (both mRNA and miRNA) data.'
Zanetti 2017	· After THS 2.2 aerosol exposure only minor histopathological alterations and minimal cytotoxicity were observed compared to CC smoke (at the high concentration: 1% for THS 2.2 aerosol vs. 30% for CC smoke); · After THS 2.2 exposure only 5 among the 14 analyzed proinflammatory mediators exhibited significant alterations compared with 11 after CC smoke exposure; · After THS 2.2 aerosol exposure ~79% lower biological impact was observed than the impact observed for CC smoke; · 13 metabolites significantly perturbed for THS 2.2 vs. 181 for CC smoke.	'Study indicates that exposure to THS 2.2 aerosol had a lower impact on the pathophysiology of human gingival organotypic cultures than CS.'

Table 12. Main results and conclusions from included pre-clinical studies (direct results and conclusions from publications).

Study	Main results	Author's conclusions (cited as in original paper)
Van der Toorn 2015	· Extracts from CC are much more cytotoxic and inflammatory than those from THS 2.2; · Extracts from CC more potently inhibit chemotaxis and transendothelial migration than those from THS 2.2; · THS 2.2 extract was associated with lower decrease of integrity of an endothelial monolayer than CC extract.	'For all examined endpoints, the extract from 3R4F showed more than one order of magnitude stronger effects than that from THS 2.2 extract. These data indicate the potential of a heat not burn tobacco product to reduce the risk for cardiovascular disease compared to combustible cigarettes.'
Poussin 2016	· At matched nicotine concentrations in the aqueous extract of the THS 2.2 aerosol reduced carbonyl levels compared with for CC smoke were observed; · After THS 2.2 aqueous extract exposures no significant increase of human monocytic MM6 cell-human coronary arterial endothelial cells adhesion was observed at concentrations showing maximal adhesion with CC aqueous extract; · In human coronary arterial endothelial cells exposed to THS 2.2 a reduced gene expression changes were induced versus CC; · From the systems response profiles of human coronary arterial endothelial cells and MM6 cells exposed to THS 2.2 a reduced network perturbation amplitudes and biological impact factors were computed in comparison with CC; · THS2.2 aqueous extract has lower than CC extract effect on gene expression changes in MM6 cells, as well as on the release of inflammatory marker proteins.	'Our systems toxicology study demonstrated reduced effects of an aqueous aerosol extract from the candidate MRTP, THS 2.2, using the adhesion of monocytic cells to human coronary endothelial cells as a surrogate pathophysiologically relevant event in atherogenesis.'
Iskandar 2017c	· After exposure to THS 2.2 aerosol lower toxicity in all cultures was observed in comparison with CC smoke; · At comparable nicotine concentrations, THS 2.2 aerosol elicited reduced and more transient effects on xenobiotic, oxidative stress and inflammatory responses than CC smoke; · THS2.2 aerosol showed reduced cellular stress responses compared with CC smoke in the nasal culture.	'The results show consistently across all three in vitro models—buccal, bronchial, and nasal—that THS 2.2 aerosol exposure had a considerably reduced and more transient biological impact on these in vitro models compared with equivalent exposures to 3R4F CS.'
Philips 2016, Titz 2016	· The chronic exposure to THS 2.2 aerosol had minimal biological impact on disease endpoints compared to CC smoke for similar nicotine concentration; · THS 2.2 aerosol had weak effects on molecular endpoints; · The 2-month exposure to CC smoke resulted in early manifestations of emphysema and atherosclerosis endpoints, while both switching to THP and smoking cessation resulted in a partial or even complete recovery to sham-exposed levels in context of lung function, plaque area, and lung morphometry and pulmonary inflammation.	'In this mouse model cessation or switching to THS 2.2 retarded the progression of CS-induced atherosclerotic and emphysematous changes, while THS 2.2 aerosol alone had minimal adverse effects.'
Lo Sasso 2016b	· Livers of Apoe-/- mice exposed to CC smoke did exhibit specific molecular responses which were less affected in the THS 2.2, smoking cessation and switching to THS groups; · In mice exposed to THS 2.2 and in the cessation and switching groups most proteomic and transcriptomic changes were lower compared to the CC group.	'TH S2.2 aerosol has reduced biological effects, as compared with CS, on the livers of Apoe-/- mice.'

CS – cigarette smoke; EMT - epithelial to mesenchymal transition; WA – whole aerosol; THP - tobacco heating product; 3R4F - University of Kentucky 3R4F Reference Cigarette; % Redn - reduction in concentration as a percentage of the level in 3R4F MSS; BDL - below detection limit; CC - combustible cigarette; CO – carbon monoxide; CO2 - carbon dioxide; LLOQ/LLQ - lower limit of quantification; (M) - mentholated consumable variant; NAB – N'-nitrosoanabasine; NAT – N'-nitrosoanatabine; NNK – 4-(Methylnitrosamino)-l-(3-pyridyl)-l-butanone; NNN - N'-nitrosonornicotine; NO – nitrogen oxide; NOx - oxides of nitrogen; NQ - not quantified; (T) - non-mentholated consumable variant; TGPM - total gaseous and particulate matter; TPM – total particular matter; TSNAs - tobacco-specific nitrosamines.

Appendix I. Pre-clinical studies – summary and discussion

Methodology of included pre-clinical studies are presented in appendix E.

Aerosol chemistry and physics

We focused on carcinogens or substances toxic to the cardiovascular and respiratory systems, i.e. harmful and potentially harmful constituents (HPHCs), detected in aerosols produced by new THPs and in combustible cigarettes (CCs) smoke.[6] Comparison of the aerosol and smoke chemistry was made using standardized and validated analytical methods and is based on 16 studies included in the analysis of aerosol chemistry and physics, of which more than half were sponsored by the tobacco industry.

Because THPs operate without combustion there should be no solid carbon-based particles in the mainstream aerosol.[7] Results of pre-clinical studies confirm that combustion-related particles were not detected in the mainstream aerosol of THPs.[8],[7] Furthermore, the comparison of volatile compounds in the THPs aerosol and CCs smoke indicates less complex chemical composition of THPs aerosol than CCs smoke.[9] According to one of the studies included in the analysis, there are no significant differences in total gaseous and particulate compounds between THPs and CCs but differences are noticeable when comparing levels of specific constituents.[10]

Detailed analysis of the composition of the aerosol indicates that concentrations of combustion markers (e.g. CO, NO) are reduced in THPs aerosol compared to CCs smoke due to heating mechanism in THPs.[11],[12] According to Eaton et al. 2018, the levels of these markers were 90% lower in THPs than in CCs.[12] Also, tobacco-specific nitrosamines (TSNA) levels were lower in tobacco fillers and mainstream aerosol of THPs compared to CCs.[11] The percentage transfer of tobacco-specific nitrosamines from tobacco to THP aerosol is 2-3 times lower than to cigarette smoke.[13] THPs also emit lower levels of carbonyls compared to CCs.[14] The concentrations of nicotine in the fillers or THPs aerosol were lower or at comparable level with CCs, depending on the type of reference cigarette [11, [15], [16]]. Transfer rates of nicotine were higher for THPs than CCs.[11] The concentration of tar (ie. the total weight of solid and liquid smoke residue without water and nicotine, both toxic and non-toxic ones) in THPs aerosol was lower or comparable to CCs smoke.[11, 16]

The concentration of hazardous compounds or toxic constituents were lower in THPs aerosol than in CCs smoke[11, 14, 16,17,18,19] According to pre-clinical studies, concentrations of broad range of different HPHCs are reduced on average by 90% in THPs aerosol compared to CCs smoke.[20, 21] The blend compositions did not affect significantly HPHCs yields.[22]

In conclusion, the results of pre-clinical studies evaluating aerosol chemistry and physics indicate about 90% reduction of combustion markers and harmful or potentially harmful constituents in THPs aerosol compared to CCs smoke.[12, 14, 16, 17, 20, 21]

Standard toxicology assessment

Seven studies concerning standard toxicity of the THPs aerosols in comparison with CCs smoke were identified.[19, 21,23,24,25,26,27] All studies selected for the analysis were funded by THPs' manufacturers. Toxicological evaluation was performed using both in vitro and in vivo tests. Regarding the in vitro cytotoxicity and genotoxicity assessment, standard in vitro assays such as Neutral Red Uptake assay, Ames test or Mouse Lymphoma assay were used in five studies. Cell systems, also human cells, used in these assays are specific for each endpoint, while test matrices were generated by smoking machines according to modified Health Canada intense smoking conditions. The identified in vivo studies were conducted on rats with 90 days of nose-only inhalation exposure according to the Organization for Economic Co-operation and Development (OECD) testing guidelines.

The results of the in vitro studies showed that each assessed THP aerosol is less cytotoxic and less genotoxic than CCs smoke.[19, 21, 23, 24, 25] In one of the selected studies, the mutagenic and cytotoxic potential of the THP aerosol was reduced by 85% to 95% in comparison with CCs smoke.[21] In three other studies two different THPs were assessed in comparison with CCs.[23, 24, 25] Each of these studies showed that tested THPs demonstrate comparable biological response, which was reduced in comparison with CCs. Assessment of tested tobacco products at a common aerosol to air ratio of 1:40 in one study showed that one of THPs achieved 90% reduction of cytotoxicity in comparison to CCs, while another tested THP showed no cytotoxicity.[23] Similar assessment performed in another study revealed that tested THPs showed >87% cell viability, while CCs demonstrated a complete loss of viability. In fact, for both heat-not-burn products assessed in this study, complete cytotoxicity was not achieved even at the highest aerosol to air ratio (1:2).[24] In the third study, both assessed

THPs showed no response to biological tests used, while CCs showed only positive responses.[25] According to the authors, results suggest at least parity between evaluated heat-not-burn products.[25] Assessment of flavor ingredients to THP associated risk in comparison to unflavored THP and CCs cigarette showed that the use of both THPs was associated with no response to most biological tests, while CCs showed positive response to all tests.[19] Only in one test, micronucleus assay, THPs showed activity, although it was lower than in direct comparison with CCs. Authors suggested that used flavor ingredients do not increase the risk of THP.[19]

Two in vivo studies also demonstrated smaller biological effects of THPs in direct comparison with CCs.[26, 27] Histopathological alterations, lung inflammation as well as gene expression observed in rats exposed for 90 days to the tested THP aerosol were less pronounced in comparison to rats exposed to CCs smoke.[26, 28] Other endpoints assessed in this study did not show difference between tested products. Another study showed that mentholated version of the THP also have significantly smaller toxic effects on respiratory tract organs in comparison with both mentholated and non-mentholated CCs.[27, 29]

In conclusion, both in vitro and in vivo standard toxicology studies indicate that THPs are less toxic than CCs.

Systems toxicology assessment

In 14 studies in vitro systems toxicology assessment of the impact of CCs smoke and THPst aerosol on organotypic human epithelial cultures was evaluated. Based on the evaluation of parameters such as cytotoxicity, inflammation, mRNA/miRNA transcription and oxidative stress, it was demonstrated that THP aerosol has an overall lower impact on buccal[30], bronchial[31,32,33,34], gingival[35] and nasal[36] epithelial physiology. These results were also confirmed in a meta-analysis that showed significantly lower and more transient biological effect on nasal, oral and bronchial in vitro models after THP aerosol exposure.[37] Lower biological impact was also shown on organotypic human small airway culture model[38], while in Haswell 2018 it was shown that exposure of a 3D airway tissue to tobacco heating products aerosol is associated with reduced impact on gene expression compared to CC cigarette smoke exposure.[39] Results regarding the effect of THP aerosol on respiratory system were also confirmed in studies with long-term exposure (up to 12 weeks).[40, 41]

Data from studies assessing the impact of CC smoke and THP aerosol on human coronary arterial endothelial cells indicate the potential of a heat not burn tobacco product to reduce the risk for cardiovascular disease compared to combustible cigarettes.[42, 43]

Systems toxicology assessment was evaluated in an animal model of disease (Apoe-/- mouse).[44, 45, 46] The results of these 8-month exposure studies have shown that either switching to THP or cessation after 2-month exposure to CC smoke was associated with a reduction in the risk of developing atherosclerosis and emphysema when compared to continued smoking.

The data from systems toxicology assessment suggest that THPs aerosols are less toxic than CC smoke and thus may have the potential to reduce the risk of smoke-related diseases compared to CC.

Appendix J. Exposure markers - numerical results

Table 13. Exposure markers in Ludicke 2018 (NCT01970995) and Haziza 2016d (NCT01989156) studies.

Outcome	Ludicke 2018 (NCT01970995)			Haziza 2016d (NCT01989156)		
	THS, N=70, mean* (95%CI)	**CC, N=41, mean* (95%CI)**	**THS-CC,** %**	**THS, N=80, mean (95%CI)**	**CC, N=41, mean (95%CI)**	**THS-CC,*** %**
NNAL, pg/mg creatinine						
Baseline	85,64 (72,96; 100,51)	84,77 (68,88; 104,33)		NA	NA	
Day 5	37,90 (32,29; 44,48)	85,94 (70,93; 104,13)	-56	NA	NA	-56
Day 90	23,23 (19,34; 27,91)	95,03 (77,31; 116,82)	-77 (68,9; 82,6)	NA	NA	-74
NNN, pg/mg creatinine						
Baseline	4,45 (3,38; 5,86)	3,97 (2,87; 5,47)		NA	NA	
Day 5	1,20 (0,97; 1,49)	4,10 (2,94; 5,73)	-73	NA	NA	-86
Day 90	1,40 (1,13; 1,73)	4,28 (3,03; 6,05)	-71	NA	NA	-82
COHb, %						
Baseline	5,11 (4,75; 5,49)	5,17 (4,70; 5,70)		NA	NA	
Day 5	2,48 (2,40; 2,57)	5,55 (5,06; 6,08)	-55 (52,0; 57,9)	NA	NA	-62
Day 90	2,97 (2,88; 3,06)	5,73 (5,24; 6,25)	-48	NA	NA	-53
MHBMA, pg/mg creatinine						
Baseline	653,78 (530,04; 806,39)	737,29 (554,67; 980,04)		NA	NA	
Day 5	81,71 (75,52; 88,41)	622,58 (454,60; 852,64)	-87 (83,4; 89,0)	NA	NA	-87
Day 90	141,74 (120,62; 166,57)	785,27 (576,82; 1069,04)	-81	NA	NA	-81
3-HPMA, ng/mg creatinine						
Baseline	667,53 (599,28; 743,54)	642,20 (552,68; 746,21)		NA	NA	
Day 5	304,68 (284,63; 326,14)	591,33 (507,72; 688,69)	-49 (42,8; 55,1)	NA	NA	-54
Day 90	386,37 (356,30; 418,97)	695,58 (602,43; 803,13)	-46	NA	NA	-48
S-PMA, pg/mg creatinine						
Baseline	1058,84 (857,94; 1306,79)	1096,79 (823,05; 1461,57)		NA	NA	
Day 5	118,36 (107,37; 130,48)	1096,47 (805,13; 1493,22)	-89 (87,0; 90,7)	NA	NA	-87
Day 90	145,58 (121,67; 174,18)	1157,25 (848,59; 1578,17)	-87	NA	NA	-78
1-OHP, pg/mg creatinine						
Baseline	153,98 (138,85; 170,75)	164,33 (143,20; 188,58)		NA	NA	
Day 5	46,36 (41,68; 51,55)	122,90 (104,71; 144,26)	-61	NA	NA	-52
Day 90	85,47 (76,64; 95,33)	167,38 (146,23; 191,58)	-48	NA	NA	-34
4-ABP, pg/mg creatinine						
Baseline	9,33 (8,44; 10,32)	8,75 (7,44; 10,29)		NA	NA	
Day 5	1,97 (1,76; 2,21)	9,50 (8,15; 11,07)	-80	NA	NA	-81
Day 90	2,07 (1,82; 2,36)	9,62 (8,12; 11,39)	-79	NA	NA	-71
1-NA, pg/mg creatinine						
Baseline	61,45 (55,12; 68,52)	57,24 (49,04; 66,80)		NA	NA	
Day 5	3,14 (2,85; 3,46)	53,27 (45,86; 61,89)	-94	NA	NA	-96
Day 90	3,55 (2,96; 4,26)	55,34 (46,21; 66,26)	-94	NA	NA	-86

Table 13. Exposure markers in Ludicke 2018 (NCT01970995) and Haziza 2016d (NCT01989156) studies.

Outcome	Ludicke 2018 (NCT01970995)			Haziza 2016d (NCT01989156)		
2-NA, pg/mg creatinine						
Baseline	15,49 (13,82; 17,37)	15,32 (13,13; 17,87)		NA	NA	
Day 5	1,97 (1,80; 2,15)	14,23 (12,18; 16,62)	-86	NA	NA	-87
Day 90	2,34 (2,11; 2,59)	14,84 (12,63; 17,44)	-85	NA	NA	-84
o-toluidine, pg/mg creatinine						
Baseline	128,19 (112,28; 146,36)	136,04 (107,42; 172,27)		NA	NA	
Day 5	51,64 (45,52; 58,59)	127,28 (103,27; 156,88)	-56	NA	NA	-51
Day 90	68,35 (53,91; 86,67)	125,64 (96,13; 164,20)	-41	NA	NA	-57
CEMA, ng/mg creatinine						
Baseline	75,32 (66,47; 85,36)	75,19 (62,27; 90,80)		NA	NA	
Day 5	12,43 (11,12; 13,90)	68,17 (56,39; 82,40)	-62	NA	NA	-83
Day 90	7,91 (6,74; 9,29)	83,98 (69,17; 101,95)	-91	NA	NA	-86
HEMA, pg/mg creatinine						
Baseline	3203,95 (2699,53; 3802,62)	3148,47 (2465,16; 4021,17)		NA	NA	
Day 5	1137,96 (995,50; 1300,81)	2235,37 (1742,88; 2867,03)	-50	NA	NA	-61
Day 90	1741,53 (1510,19; 2008,30)	3739,46 (2858,39; 4892,12)	-55	NA	NA	-62
3-HMPMA, ng/mg creatinine						
Baseline	300,07 (266,94; 337,32)	298,73 (256,46; 347,96)		NA	NA	
Day 5	124,47 (115,36; 134,30)	286,80 (251,37; 327,21)	-57	NA	NA	-62
Day 90	154,30 (137,07; 173,70)	299,41 (260,62; 343,97)	-50	NA	NA	-50
3-OH-B[a]P, fg/mg creatinine						
Baseline	83,73 (70,69; 99,18)	82,00 (67,42; 99,71)		NA	NA	
Day 5	20,72 (18,61; 23,07)	75,10 (62,60; 90,08)	-73	NA	NA	-71
Day 90	30,02 (25,29; 35,65)	86,92 (71,78; 105,27)	-67	NA	NA	-57
Nicotine equivalent, mg/mg creatinine						
Baseline	5,71 (5,08; 6,41)	5,56 (4,64; 6,65)		NA	NA	
Day 5	6,16 (5,55; 6,83)	5,22 (4,35; 6,27)	16 (-1,1; 36,0)	NA	NA	NA
Day 90	6,85 (5,96; 7,88)	6,33 (5,11; 7,84)	104 (66,7; 163,2)^	NA	NA	NA
CYP1A2 activity, %						
Baseline	NA	NA		NA	NA	
Day 5	NA	NA	p=ns	NA	NA	NA
Day 90	NA	NA	-28,04	NA	NA	NA
Mutagenicity,## rev/24h			-30,91			
	mean (SD)/N	mean (SD)/N				
Baseline	17294 (12543)/65	15132 (10702)/38		NA	NA	
Day 5	7500 (8886)/73	13477 (7826)/40	NA	NA	NA	NA
Day 90	6761 (6689)/70	17204 (12258)/40	NA	NA	NA	NA

* geometric mean; ** part of the data from the Haziza 2016c conference poster; *** data from the Haziza 2016d conference poster; ^ THS/CC; ## estimated based on the Ames test; rev – revertant.

Result in favor of THS 2.2 (no information on the statistical significance of the result)
Result statistically significant in favor of THS 2.2
Result statistically insignificant

Table 14. Exposure markers in the Haziza 2016a (NCT01959932) study.			
Outcome	Haziza 2016a (NCT01959932)		
	THS, N=80, mean* (95%CI)	CC, N=41, mean* (95%CI)	THS/CC,** %, mean (95%CI)
Nicotine equivalent, mg/g creatinine			
Baseline	9,01 (8,09; 10,03)	8,69 (7,51; 10,04)	104,9 (92,0; 119,6)
Day 5	10,60 (9,34; 12,04)	9,76 (8,54; 11,15)	
Change %	22,95 (13,92; 31,98)	14,78 (7,04; 22,53)	
Nicotine, ng/ml			
Baseline	14,16 (12,62; 15,89)	14,03 (11,90; 16,53)	112,9 (91,3; 139,5)
Day 5	20,74 (17,46; 24,62)	19,01 (16,52; 21,87)	
Change %	35,98 (19,19; 52,77)	19,68 (1,74; 37,62)	
Cotinine, ng/ml			
Baseline	208,54 (188,61; 230,58)	211,26 (183,05; 243,82)	111,0 (90,8; 135,7)
Day 5	239,99 (211,30; 272,58)	219,73 (190,21; 253,83)	
Change %	11,94 (4,05; 19,84)	-0,31 (-8,24; 7,63)	
NNAL, pg/mg creatinine			
Baseline	111,01 (95,44; 129,13)	105,05 (84,10; 131,21)	43,5 (39,3; 48,2)
Day 5	49,65 (42,47; 58,05)	107,04 (85,90; 133,37)	
Change %	-53,98 (-56,69; -51,27)	3,85 (-2,84; 10,54)	
NNN, pg/mg creatinine			
Baseline	4,81 (3,99; 5,78)	4,34 (3,56; 5,28)	24,1 (17,7; 32,8)
Day 5	1,55 (1,17; 2,05)	5,99 (4,94; 7,26)	
Change %	-69,75 (-53,26; -82,75)#	29,93 (12,84; 74,96)	
COHb, %			
Baseline	4,65 (4,29; 5,04)	4,68 (4,22; 5,18)	23,5 (22,0; 25,0)
Day 5	1,06 (1,03; 1,08)	4,51 (4,05; 5,01)	
Change %	-76,20 (-78,11; -74,29)	-1,16 (-8,31; 5,99)	
MHBMA, pg/mg creatinine			
Baseline	1888,27 (1542,95; 2310,86)	2317,31 (1861,41; 2884,85)	8,4 (6,8; 10,2)
Day 5	192,93 (174,90; 212,83)	2399,40 (1884,60; 3054,83)	
Change %	-84,98 (-88,32; -81,63)	6,89 (-1,37; 15,15)	
3-HPMA, ng/mg creatinine			
Baseline	841,84 (745,88; 950,14)	799,37 (693,71; 921,13)	41,6 (37,7; 46,0)
Day 5	402,26 (366,55; 441,45)	931,01 (825,73; 1049,72)	
Change %	-49,68 (-54,32; -45,04)	20,28 (10,41; 30,14)	
S-PMA, pg/mg creatinine			
Baseline	2394,03 (1982,83; 2890,50	2765,20 (2227,38; 3432,88)	6,0 (5,2; 6,9)
Day 5	164,45 (144,45; 187,22)	2922,81 (2362,80; 3615,54)	
Change %	-92,03 (-93,20; -90,85)	9,48 (-0,48; 19,45)	
1-OHP, pg/mg creatinine			
Baseline	217,69 (197,88; 239,48)	218,41 (196,28; 243,03)	44,3 (39,8; 49,4)
Day 5	81,22 (74,82; 88,16)	182,85 (161,24; 207,37)	
Change %	-60,17 (-63,70; -56,65)	-13,52 (-21,11; -5,92)	
4-ABP, pg/mg creatinine			
Baseline	13,25 (11,85; 14,81)	13,11 (11,33; 15,16)	14,9 (12,8; 17,4)
Day 5	1,9 (1,70; 2,12)	12,58 (11,03; 14,34)	
Change %	-82,12 (-85,59; -78,64)	-1,66 (-8,59; 5,26)	
1-NA, pg/mg creatinine			
Baseline	73,83 (66,48; 81,99)	77,84 (66,32; 91,36)	3,7 (3,1; 4,5)
Day 5	3,30 (2,89; 3,78)	89,37 (77,81; 102,64)	
Change %	-94,16 (-95,49; -92,82)	19,17 (9,08; 29,26)	

Table 14. Exposure markers in the Haziza 2016a (NCT01959932) study.

Outcome	Haziza 2016a (NCT01959932)		
2-NA, pg/mg creatinine			
Baseline	24,54 (22,12; 27,22)	24,14 (21,18; 27,51)	11,5 (10,0; 13,3)
Day 5	2,96 (2,67; 3,28)	25,32 (22,27; 28,79)	
Change %	-85,39 (-87,95; -82,82)	7,19 (0,41; 13,97)	
o-toluidyna, pg/mg creatinine			
Baseline	135,20 (122,75; 148,90)	131,32 (115,72; 149,01)	41,7 (36,0; 48,3)
Day 5	51,15 (46,10; 56,75)	121,16 (105,07; 139,71)	
Change %	-50,96 (-61,87; -40,05)	-3,08 (-12,17; 6,01)	
CEMA, ng/ng creatinine			
Baseline	98,03 (85,10; 112,92)	98,46 (83,81; 115,67)	13,2 (11,5; 15,0)
Day 5	13,18 (11,37; 15,17)	99,48 (85,79; 115,35)	
Change %	-86,10 (-87,04; -85,17)	4,21 (-4,18; 12,61)	
HEMA, pg/mg creatinine			
Baseline	4161,66 (3409,70; 5079,44)	4718,48 (3582,18; 6215,23)	32,0 (27,1; 37,8)
Day 5	1342,40 (1140,44; 1580,12)	4504,00 (3506,73; 5784,88)	
Change %	-60,71 (-68,31; -53,11)	0,74 (-9,86; 11,34)	
3-HMPMA, ng/mg creatinine			
Baseline	479,34 (435,40; 527,72)	460,52 (407,39; 520,59)	22,5 (20,1; 25,3)
Day 5	86,65 (80,31; 93,49)	376,78 (329,54; 430,80)	
Change %	-80,58 (-82,48; -78,68)	-14,53 (-22,49; -6,57)	
3-OH-B[a]P, fg/mg creatinine			
Baseline	161,17 (142,28; 182,57)	149,47 (128,96; 173,23)	27,5 (23,2; 32,6)
Day 5	37,07 (33,25; 41,32)	130,29 (110,17; 154,07)	
Change %	-71,43 (-76,65; -66,21)	-8,92 (-17,00; -0,84)	
CYP1A2 activity following coffee intake, %			
Baseline			-33,60 (-40,59; -26,61) ***
Day 5	91,35 (NA)	124,95 (NA)	
Change %			
Mutagenicity,##,### rev/24h			
Baseline	19 681 (0; 107 250)	15 775 (0; 72 216)	
Day 5	8 823 (0; 39 600)	21 689 (0; 63 840)	
Change %	-57	29	NA

** geometric mean for baseline and day 5 values, arithmetic mean for change from baseline; ** geometric least squares means ratio (exposure markers estimated as concentrations adjusted for creatinine concentration, similar values were obtained for exposure markers estimated quantitatively within 24 hours); *** last squares mean difference THS-CC; # change from baseline estimated on the basis of medians; ## estimated based on the Ames test; ### median (min; max), median change from baseline; rev – revertant.*

Result in favour of THS 2.2 (no information on the statistical significance of the result)
Result statistically significant in favour of THS 2.2
Result statistically insignificant

Table 15. Exposure markers in the Haziza 2016b (NCT01970982) study.			
Outcome	Haziza 2016b (NCT01970982)		
	THS, N=80, mean* (95%CI)	CC, N=40, mean* (95%CI)	THS/CC,** %, mean (95%CI)
Nicotine equivalent, mg/g creatinine			
Baseline	5,21 (4,49; 6,04)	5,30 (4,41; 6,38)	
Day 5	5,44 (4,61; 6,41)	5,52 (4,58; 6,66)	
Change %	16,94 (2,85; 31,03)	9,49 (-1,76; 20,73)	104,98 (92,03; 119,55)
Nicotine,# ng/ml			
Baseline	15,14 (12,79; 17,91)	18,28 (15,58; 21,44)	
Day 5	19,13 (15,60; 23,46)	21,34 (18,56; 24,55)	
Change %	22,47 (64,47)^	3,18 (44,47)^	112,91 (91,36; 139,54)
Cotinine,# ng/ml			
Baseline	140,37 (118,61; 166,10)	147,32 (117,68; 184,43)	
Day 5	161,00 (131,19; 197,57)	164,30 (130,93; 206,17)	
Change %	16,14 (59,09)^	6,63 (27,67)^	96,14 (70,73; 130,67)
NNAL, pg/mg creatinine			
Baseline	77,86 (64,20; 94,42)	77,29 (58,68; 101,81)	
Day 5	37,77 (31,43; 45,38)	76,55 (59,76; 98,04)	
Change %	-48,04 (-52,73; -43,36)	8,82 (-6,42; 24,07)	49,03 (41,95; 57,30)
NNN, pg/mg creatinine			
Baseline	4,08 (3,26; 5,10)	4,57 (3,42; 6,11)	
Day 5	1,31 (1,06; 1,61)	4,64 (3,51; 6,12)	
Change %	-59,81 (-66,73; -52,89)	18,35 (-4,02; 40,73)	30,06 (23,74; 38,06)
COHb,## %			
Baseline	5,15 (4,80; 5,52)	5,4 (4,88; 5,97)	
Day 5	2,39 (2,32; 2,46)	5,14 (4,66; 5,66)	
Change %	-51,13 (-54,86; -47,41)	-2,98 (-9,37; 3,41)	47,10 (44,30; 50,08)
MHBMA, pg/mg creatinine			
Baseline	538,46 (416,79; 695,65)	490,97 (344,01; 700,72)	
Day 5	107,39 (97,24; 118,60)	450,19 (300,07; 675,42)	
Change %	-66,41 (-74,24; -58,58)	4,90 (-13,60; 23,39)	23,09 (18,41; 28,95)
3-HPMA, ng/mg creatinine			
Baseline	661,25 (582,13; 751,13)	685,45 (602,02; 780,44)	
Day 5	311,08 (279,59; 346,12)	599,67 (511,70; 702,76)	
Change %	-47,33 (-53,47; -41,18)	-7,24 (-17,50; 3,02)	52,86 (45,67; 61,17)
S-PMA, pg/mg creatinine			
Baseline	911,15 (715,17; 1160,83)	784,67 (570,61; 1079,02)	
Day 5	143,77 (126,08; 163,93)	850,02 (620,40; 1164,63)	
Change %	-77,24 (-83,40; -71,08)	21,22 (2,70; 39,75)	15,68 (13,09; 18,78)
1-OHP, pg/mg creatinine			
Baseline	189,9 (171,66; 210,07)	177,51 (158,88; 198,33)	
Day 5	73,02 (65,19; 81,79)	149,62 (132,68; 168,72)	
Change %	-58,57 (-62,65; -54,49)	-14,31 (-19,42; -9,21)	46,44 (41,32; 52,19)
3-OH-B[a]P, fg/mg creatinine			
Baseline	115,78 (98,47; 136,14)	106,17 (89,08; 126,54)	
Day 5	29,52 (26,01; 33,50)	96,42 (80,55; 115,41)	
Change %	-64,76 (-72,17; -57,35)	-2,75 (-13,51; 8,02)	29,99 (24,84; 36,20)
4-ABP, pg/mg creatinine			
Baseline	7,72 (6,75; 8,83)	8,4 (7,14; 9,87)	
Day 5	1,53 (1,37; 1,70)	8,57 (7,11; 10,34)	
Change %	-74,08 (-79,46; -68,69)	13,50 (-3,30; 30,30)	18,21 (15,29; 21,69)

Table 15. Exposure markers in the Haziza 2016b (NCT01970982) study.			
Outcome	Haziza 2016b (NCT01970982)		
1-NA, pg/mg creatinine			
Baseline	48,61 (42,45; 55,66)	55,41 (47,36; 64,83)	
Day 5	2,47 (2,23; 2,72)	57,08 (48,55; 67,11)	
Change %	-93,12 (-94,98; -91,26)	11,23 (-3,21; 25,67)	4,44 (3,80; 5,18)
2-NA, pg/mg creatinine			
Baseline	13,22 (11,52; 15,16)	13,88 (11,66; 16,52)	
Day 5	2,33 (2,10; 2,59)	13,38 (10,93; 16,37)	
Change %	-75,84 (-81,56; -70,13)	6,31 (-8,86; 21,48)	17,62 (14,72; 21,08)
o-toluidine, pg/mg creatinine			
Baseline	111,82 (95,48; 130,96)	106,51 (87,32; 129,91)	
Day 5	50,4 (44,64; 56,91)	98,18 (82,69; 116,57)	
Change %	-44,23 (-52,58; -35,89)	4,66 (-12,50; 21,83)	50,52 (42,28; 60,38)
CEMA, ng/ng creatinine			
Baseline	57,12 (48,28; 67,59)	63,19 (52,60; 75,90)	
Day 5	10,61 (9,17; 12,29)	54,19 (43,47; 67,55)	
Change %	-79,42 (-81,75; -77,08)	-7,83 (-18,77; 3,11)	21,21 (18,10; 24,86)
HEMA, pg/mg creatinine			
Baseline	2391,21 (1984,68; 2881,02)	2299,17 (1776,20; 2976,12)	
Day 5	997,76 (866,57; 1148,82)	2099,41 (1614,33; 2730,24)	
Change %	-50,99 (-57,47; -44,51)	3,26 (-15,67; 22,18)	46,50 (39,53; 54,69)
HMPMA, ng/mg creatinine			
Baseline	194,95 (168,47; 225,59)	195,1 (163,02; 233,49)	
Day 5	59,51 (53,40; 66,30)	157,83 (128,07; 194,51)	
Change %	-60,61 (-68,59; -52,64)	-12,85 (-23,15; -2,55)	37,71 (31,57; 45,05)
S-BMA, pg/mg creatinine			
Baseline	2957,22 (2599,76; 3363,83)	2703,81 (2268,89; 3222,09)	
Day 5	2098,09 (1833,19; 2401,26)	2354,17 (1968,07; 2816,02)	
Change %	-20,57 (-29,28; -11,85)	-2,42 (-19,44; 14,59)	NA
CYP1A2 activity after coffee intake, %			
Baseline			
Day 5	56,56 (NA)	76,50 (NA)	
Change %	-27,36 (-30,51; -24,22)	NA	-21,65 (-25,49; -17,81) ***

** geometric mean for baseline and day 5 values, arithmetic mean for change from baseline; ** geometric least squares means ratio; *** last squares mean difference THS-CC; # weighted mean concentration over 24h; ## measured between 8.00 and 10.00 PM; ^ arithmetic mean (SD).*

Result in favour of THS 2.2 (no information on the statistical significance of the result)
Result statistically significant in favour of THS 2.2
Result statistically insignificant

Table 16. Exposure markers in the Gale 2018 study – part 1.			
Outcome	Gale 2018		
	THS 2.2, N=30, mean (95%CI)	Non-menthol CC, N=30, mean (95%CI)	THS 2.2 - non-menthol CC,** mean (95%CI)
Nicotine equivalent, mg/24h			
Baseline	8,20 (NA)	7,53 (NA)	
Day 5	7,58 (NA)	8,33 (NA)	
Change*	-0,63 (-1,36; 0,10)	0,80 (-0,03; 1,64)	-1,43 (-2,63; -0,23)
eCO, ppm			
Baseline	23,18 (NA)	24,48 (NA)	
Day 5	3,40 (NA)	20,30 (NA)	
Change*	-19,78 (-22,84; -16,73)	-4,18 (-7,42; -0,94)	-15,60 (-19,90; -11,30)
1-OHP, ng/24h			
Baseline	236,52 (NA)	186,99 (NA)	
Day 5	50,18 (NA)	172,86 (NA)	
Change*	-186,34 (-213,75; -158,92)	-14,13 (-27,40; -0,87)	-172,21 (-203,63; -140,79)
2-AN, ng/24h			
Baseline	17,11 (NA)	17,79 (NA)	
Day 5	1,72 (NA)	17,80 (NA)	
Change*	-15,39 (-18,52; -12,27)	0,01 (-1,57; 1,59)	-15,41 (-18,78; -12,03)
3-HPMA, µg/24h			
Baseline	1021,58 (NA)	1177,15 (NA)	
Day 5	639,21 (NA)	1448,93 (NA)	
Change*	-382,37 (-504,80; -259,93)	271,78 (139,64; 403,91)	-654,14 (-853,53; -454,75)
4-ABP, ng/24h			
Baseline	10,35 (NA)	11,79 (NA)	
Day 5	2,25 (NA)	10,86 (NA)	
Change*	-8,10 (-9,52; -6,67)	-0,93 (-1,76; -0,09)	-7,17 (-9,15; -5,19)
AAMA, µg/24h			
Baseline	117,01 (NA)	129,62 (NA)	
Day 5	65,76 (NA)	111,65 (NA)	
Change*	-51,26 (-59,81; -42,70)	-17,97 (-28,19; -7,76)	-33,28 (-46,93; -19,64)
CEMA, µg/24h			
Baseline	128,96 (NA)	153,78 (NA)	
Day 5	16,54 (NA)	159,04 (NA)	
Change*	-112,43 (-133,33; -91,52)	5,26 (-8,82; 19,34)	-117,69 (-144,33; -91,05)
GAMA, µg/24h			
Baseline	19,05 (NA)	19,27 (NA)	
Day 5	13,75 (NA)	17,24 (NA)	
Change*	-5,30 (-7,02; -3,58)	-2,03 (-3,66; -0,40)	-3,27 (-5,74; -0,81)
HEMA, µg/24h			
Baseline	6,44 (NA)	6,27 (NA)	
Day 5	2,60 (NA)	5,08 (NA)	
Change*	-3,84 (-5,06; -2,61)	1,20 (-2,04; -0,35)	-2,64 (-4,40; -0,89)
HMPMA, µg/24h			
Baseline	333,61 (NA)	356,38 (NA)	
Day 5	79,63 (NA)	385,50 (NA)	
Change*	-253,98 (-309,32; -198,64)	29,11 (-3,89; 62,12)	-283,10 (-349,25; -216,95)
MHBMA, ng/24h			
Baseline	754,16 (NA)	750,78 (NA)	
Day 5	118,38 (NA)	770,64 (NA)	
Change*	-635,78 (-927,54; -344,03)	19,86 (-123,66; 163,38)	-655,64 (-1011,42; -299,86)

Table 16. Exposure markers in the Gale 2018 study – part 1.			
Outcome	Gale 2018		
S-PMA, µg/24h			
Baseline	1,86 (NA)	2,09 (NA)	
Day 5	0,19 (NA)	2,25 (NA)	
Change*	-1,67 (-2,12; -1,23)	0,17 (-0,05; 0,38)	-1,84 (-2,55; -1,13)
NNAL, ng/24h			
Baseline	174,33 (NA)	188,43 (NA)	
Day 5	80,35 (NA)	197,85 (NA)	
Change*	-93,98 (-115,46; -72,51)	9,42 (-10,29; 29,13)	-103,40 (-135,41; -71,39)
NNN, ng/24h			
Baseline	9,13 (NA)	15,32 (NA)	
Day 5	1,06 (NA)	15,36 (NA)	
Change*	-8,07 (-10,77; -5,38)	0,04 (-2,75; 2,83)	-8,12 (-11,35; -4,88)
o-toluidine, ng/24h			
Baseline	107,96 (NA)	129,80 (NA)	
Day 5	54,81 (NA)	153,21 (NA)	
Change*	-53,16 (-76,33; -29,99)	23,41 (-2,97; 49,80)	-76,57 (-103,33; -49,81)

** least squares mean difference Day 5-Baseline; ** least squares mean difference THP-CC.*

Result statistically significant in favour of THP
Result statistically insignificant

Table 17. Exposure markers in the Gale 2018 study – part 2.

Outcome	Gale 2018					
	Non-menthol THP 1.0, N=30, mean (95%CI)	Menthol THP 1.0, N=30, mean (95%CI)	Non-menthol CC, N=30, mean (95%CI)	Menthol CC, N=30, mean (95%CI)	Non-menthol THP 1.0 - non-menthol CC,** mean (95%CI)	Menthol THP 1.0 - menthol CC,** mean (95%CI)
Nicotine equivalent, mg/24h						
Baseline	8,17 (NA)	9,29 (NA)	7,53 (NA)	8,25 (NA)		
Day 5	6,15 (NA)	5,75 (NA)	8,33 (NA)	9,77 (NA)		
Change*	-2,02 (-3,03; -1,01)	-3,54 (-4,33; -2,76)	0,80 (-0,03; 1,64)	1,51 (0,87; 2,16)	-2,82 (-4,02; -1,63)	-5,06 (-6,26; -3,86)
eCO, ppm						
Baseline	26,67 (NA)	27,00 (NA)	24,48 (NA)	24,55 (NA)		
Day 5	3,40 (NA)	2,80 (NA)	20,30 (NA)	20,07 (NA)		
Change*	-23,27 (-27,02; -19,51)	-24,20 (-28,18; -20,22)	-4,18 (-7,42; -0,94)	-4,48 (-6,07; -2,89)	-19,08 (-23,38; -14,78)	-19,72 (-24,02; -15,42)
1-OHP, ng/24h						
Baseline	211,33 (NA)	239,46 (NA)	186,99 (NA)	237,81 (NA)		
Day 5	75,58 (NA)	63,46 (NA)	172,86 (NA)	195,19 (NA)		
Change*	-135,74 (-158,56; -112,93)	-176,00 (-207,07; -144,93)	-14,13 (-27,40; -0,87)	-42,61 (-55,98; -29,25)	-121,61 (-153,03; -90,19)	-133,39 (-164,81; -101,97)
2-AN, ng/24h						
Baseline	18,57 (NA)	19,58 (NA)	17,79 (NA)	17,62 (NA)		
Day 5	1,74 (NA)	1,92 (NA)	17,80 (NA)	17,65 (NA)		
Change*	-16,83 (-19,52; -14,14)	-17,66 (-20,91; -14,42)	0,01 (-1,57; 1,59)	0,03 (-1,11; 1,17)	-16,84 (-20,21; -13,47)	-17,70 (-21,07; -14,32)
3-HPMA, µg/24h						
Baseline	1208,79 (NA)	1281,90 (NA)	1177,15 (NA)	1136,31 (NA)		
Day 5	568,66 (NA)	656,99 (NA)	1448,93 (NA)	1422,37 (NA)		
Change*	-640,13 (-824,77; -455,49)	-624,91 (-799,23; -450,59)	271,78 (139,64; 403,91)	286,05 (212,25; 359,86)	-911,91 (-1111,30; -712,52)	-910,96 (-1110,35; -711,51)
4-ABP, ng/24h						
Baseline	12,61 (NA)	12,76 (NA)	11,79 (NA)	10,89 (NA)		
Day 5	2,45 (NA)	2,31 (NA)	10,86 (NA)	10,44 (NA)		
Change*	-10,17 (-11,73; -8,60)	-10,45 (-12,73; -8,18)	-0,93 (-1,76; -0,09)	-0,46 (-1,06; 0,14)	-9,24 (-11,22; -7,26)	-9,99 (-11,97; -8,01)
AAMA, µg/24h						
Baseline	133,92 (NA)	132,81 (NA)	129,62 (NA)	115,05 (NA)		
Day 5	91,75 (NA)	88,82 (NA)	111,65 (NA)	114,96 (NA)		
Change*	-42,18 (-55,32; -29,04)	-43,99 (-52,30; -35,68)	-17,97 (-28,19; -7,76)	-0,09 (-7,85; 7,67)	-24,21 (-37,85; -10,56)	-43,90 (-57,54; -30,26)
CEMA, µg/24h						
Baseline	165,75 (NA)	172,51 (NA)	153,78 (NA)	159,65 (NA)		
Day 5	17,84 (NA)	21,03 (NA)	159,04 (NA)	165,62 (NA)		
Change*	-147,91 (-173,47; -122,36)	-151,48 (-175,62; -127,35)	5,26 (-8,82; 19,34)	5,96 (-7,54; 19,46)	-153,17 (-179,81; -126,54)	-157,45 (-184,08; -130,81)
GAMA, µg/24h						
Baseline	20,34 (NA)	19,32 (NA)	19,27 (NA)	17,67 (NA)		
Day 5	15,68 (NA)	15,36 (NA)	17,24 (NA)	16,40 (NA)		
Change*	-4,66 (-6,96; -2,36)	-3,95 (-5,82; -2,09)	-2,03 (-3,66; -0,40)	-1,26 (-2,88; 0,36)	-2,63 (-5,10; -0,17)	-2,69 (-5,16; -0,23)
HEMA, µg/24h						

Table 17. Exposure markers in the Gale 2018 study – part 2.						
Outcome	Gale 2018					
Baseline	5,65 (NA)	7,23 (NA)	6,27 (NA)	8,60 (NA)		
Day 5	2,46 (NA)	2,84 (NA)	5,08 (NA)	7,13 (NA)		
Change*	-3,19 (-4,37; -2,01)	-4,39 (-5,61; -3,16)	1,20 (-2,04; -0,35)	-1,48 (-2,85; -0,10)	-1,99 (-3,75; -0,24)	-2,91 (-4,67; -1,16)
HMPMA, µg/24h						
Baseline	372,98 (NA)	383,91 (NA)	356,38 (NA)	342,03 (NA)		
Day 5	79,00 (NA)	73,23 (NA)	385,50 (NA)	362,45 (NA)		
Change*	-293,99 (-355,77; -230,20)	-310,68 (-368,56; -252,79)	29,11 (-3,89; 62,12)	20,43 (-12,06; 52,91)	-323,10 (-389,25; -256,95)	-331,10 (-397,25; -264,95)
MHBMA, ng/24h						
Baseline	574,54 (NA)	935,36 (NA)	750,78 (NA)	942,61 (NA)		
Day 5	49,87 (NA)	98,40 (NA)	770,64 (NA)	1010,18 (NA)		
Change*	-524,67 (-769,55; -279,79)	-836,96 (-1157,18; -516,57)	19,86 (-123,66; 163,38)	67,57 (-151,04; 286,17)	-544,53 (-900,31; -188,75)	-904,53 (-1260,31; -548,75)
S-PMA, µg/24h						
Baseline	1,84 (NA)	2,66 (NA)	2,09 (NA)	2,68 (NA)		
Day 5	0,20 (NA)	0,20 (NA)	2,25 (NA)	2,81 (NA)		
Change*	-1,63 (-2,17; -1,10)	-2,46 (-3,26; -1,65)	0,17 (-0,05; 0,38)	0,13 (-0,21; 0,48)	-1,80 (-2,51; -1,10)	-2,59 (-3,30; -1,89)
NNAL, ng/24h						
Baseline	198,10 (NA)	237,06 (NA)	188,43 (NA)	188,59 (NA)		
Day 5	128,63 (NA)	149,38 (NA)	197,85 (NA)	167,02 (NA)		
Change*	-69,47 (-90,09; -48,85)	-87,68 (-119,14; -56,22)	9,42 (-10,29; 29,13)	-21,57 (-38,70; -4,44)	-78,89 (-110,89; -46,88)	-66,11 (-98,12; -34,10)
NNN, ng/24h						
Baseline	11,55 (NA)	11,58 (NA)	15,32 (NA)	8,04 (NA)		
Day 5	5,85 (NA)	5,57 (NA)	15,36 (NA)	9,62 (NA)		
Change*	-5,71 (-7,46; -3,95)	-6,00 (-9,28; -2,72)	0,04 (-2,75; 2,83)	1,58 (0,29; 2,87)	-5,75 (-8,98; -2,51)	-7,58 (-10,82; -4,35)
o-toluidine, ng/24h						
Baseline	114,26 (NA)	106,89 (NA)	129,80 (NA)	114,96 (NA)		
Day 5	58,52 (NA)	39,39 (NA)	153,21 (NA)	119,04 (NA)		
Change*	-55,74 (-70,84; -40,64)	-67,50 (-81,25; -53,74)	23,41 (-2,97; 49,80)	4,08 (-19,29; 27,46)	-79,15 (-106,12; -52,17)	-71,58 (-97,62; -45,54)

* least squares mean difference Day 5-Baseline; ** least squares mean difference THP-CC.

Result statistically significant in favour of THP
Result statistically insignificant

Appendix K. Clinical risk markers - numerical results

Table 18. Clinical risk markers in the Ludicke 2018 (NCT01970995) study - part 1.

Outcome	mTHS, geometric mean (95%CI)	mCC, geometric mean (95%CI)	mTHS vs mCC, least square mean ratio, % (95%CI), p
Endothelial dysfunction			
sICAM-1, ng/ml			
Baseline	222,92 (205,10; 242,28)	198,70 (171,01; 230,86)	
Day 90	188,43 (176,13; 201,59)	188,40 (163,69; 216,83)	91,28 (85,06; 97,95), p=0,0116
Oxidative stress			
8-epi-PGF2α, pg/mg creatinine			
Baseline	201,95 (186,30; 218,92)	202,65 (183,33; 224,00)	
Day 90	194,40 (177,99; 212,32)	222,48 (203,07; 243,75)	87,29 (78,19; 97,45), p=0,0159
Platelet activity			
11-DTX-B2, pg/mg creatinine			
Baseline	580,41 (531,09; 634,32)	533,13 (487,32; 583,24)	
Day 90	498,22 (447,54; 554,63)	515,18 (466,99; 568,35)	91,02 (80,48; 102,94), p=0,1327
Cardiovascular risk/function			
Fibrinogen, mg/dl			
Baseline	279,19 (266,68; 292,28)	276,16 (259,93; 293,40)	
Day 90	275,91 (262,37; 290,14)	286,14 (267,36; 306,24)	94,58 (87,87; 101,80), p=0,1360
Homocysteine, micromole/l			
Baseline	10,39 (9,30; 11,61)	10,94 (9,39; 12,75)	
Day 90	11,57 (10,37; 12,90)	12,05 (10,31; 14,08)	100,66 (93,35; 108,54), p=0,8638
hs-CRP, mg/l			
Baseline	0,20 (0,15; 0,25)	0,17 (0,13; 0,23)	
Day 90	0,24 (0,18; 0,32)	0,25 (0,16; 0,37)	93,59 (62,23; 140,75), p=0,7487
Metabolic syndrome			
Glucose, mg/dl			
Baseline	84,9 (83,0; 86,9)	85,4 (83,5; 87,3)	
Day 90	89,8 (87,7; 91,8)	91,1 (89,1; 93,1)	98,98 (96,42; 101,60), p=0,4370

hs-CRP - high-sensitivity C-reactive protein; sICAM-1 - soluble intercellular adhesion molecule-1; 8-epi-PGF2α - 8-epi-prostaglandin F2α; 11-DTX-B2 - 11-dehydro-thromboxan B2.

Result statistically significant in favour of THS 2.2

Result statistically insignificant

Table 19. Clinical risk markers in the Ludicke 2018 (NCT01970995) study - part 2.			
Outcome	mTHS, arithmetic mean (95%CI)	mCC, arithmetic mean (95%CI)	mTHS vs mCC, least square mean difference, (95%CI), p
Inflammation			
WBC, GI/l Baseline Day 90	 5,90 (5,60; 6,19) 5,54 (5,24; 5,83)	 5,76 (5,34; 6,20) 6,04 (5,54; 6,54)	-0,57 (-1,03; -0,1), p=0,0173
Lipid metabolism			
LDL cholesterol, mg/dl Baseline Day 90	 121,3 (113,0; 129,7) 113,4 (104,7; 122,1)	 123,3 (111,3; 135,2) 114,1 (104,7; 123,6)	0,9 (-6,6; 8,3), p=0,8162
HDL cholesterol, mg/dl Baseline Day 90	 56,9 (53,8; 60,0) 60,3 (56,5; 64,2)	 60,0 (55,0; 65,1) 58,5 (53,8; 63,3)	4,5 (1,1; 7,9), p=0,0084
Triglicerydes, mg/dl Baseline Day 90	 139,5 (123,1; 156,0) 138,5 (120,4; 156,7)	 131,5 (115,3; 147,7) 137,2 (123,0; 151,5)	-6,3 (-21,2; 8,7), p=0,4095
Total cholesterol, mg/dl Baseline Day 90	 197,5 (188,9; 206,1) 191,1 (181,9; 200,3)	 201,4 (188,2; 214,6) 192 (181,7; 202,3)	2,0 (-6,7; 10,7), p=0,6499
Metabolic syndrome			
HbA1c, % Baseline Day 90	 5,17 (5,10; 5,25) 5,17 (5,09; 5,26)	 5,23 (5,14; 5,32) 5,20 (5,09; 5,32)	0,02 (-0,06; 0,10), p=0,5866
Body weight, kg Baseline Day 90	 62,35 (59,70; 65,01) 62,67 (60,00; 65,34)	 62,01 (58,74; 65,29) 62,41 (59,12; 65,71)	-0,09 (-0,75; 0,57), p=0,7926
Waist circumference, cm Baseline Day 90	 87,1 (81,0; 93,1) 81,0 (77,6; 106,9)	 111,0 (107,8; 114,2) 105,5 (101,3; 109,6)	1,6 (-2,4; 5,6), p=0,4251
Cardiovascular risk/function			
Systolic blood pressure, mmHg Baseline Day 90	 110,2 (107,4; 112,9) 104,2 (101,5; 106,9)	 111,0 (107,8; 114,2) 105,5 (101,3; 109,6)	-0,59 (-3,80; 2,62), p=0,7157
Diastolic blood pressure, mmHg Baseline Day 90	 67,0 (64,8; 69,3) 62,8 (60,7; 64,9)	 67,5 (65,2; 69,8) 63,9 (61,0; 66,8)	-0,68 (-3,04; 1,69), p=0,5705
Lung function			
FEV_1, % Baseline Day 90	 94,08 (92,25; 95,92) 95,54 (93,63; 97,44)	 93,46 (89,94; 96,96) 94,02 (91,18; 96,85)	1,91 (-0,14; 3,97), p=0,0669

FEV_1 - forced expiratory volume in 1 second; HDL – high-density lipoprotein); LDL – low-density lipoprotein); WBC – white blood cell count.

Result statistically significant in favour of THS 2.2

Result statistically insignificant

Appendix L. Indoor air quality – summary

No randomized studies evaluating indoor air quality were found.

Six studies which assessed indoor air quality when using THPs in comparison with CC, were identified[47, 48, 49, 50, 51, 52] All included studies assessed the occurrence of particulate matter, while three of them also assessed levels of HPHCs or other organic compounds and metals.

Two studies, which were funded by one of the manufacturer of THPs (IQOS), showed no particulate matter in THP emission.[47, 48] Another study, funded by another manufacturer, detected particle mass and number more than 98-99% lower than in CC smoke and particle diameters similar for both assessed interventions.[52] Three other studies were independent. [49, 50, 51] According to Protano 2016 study, concentration of particulate matter related to smoking CCs are 4-times higher than those related to using heat-not-burn products.[49] Also, Protano 2017 study showed that particles uptake of subject passively exposed to smoking is much lower for assessed THP than CCs.[50] Ruprecht 2017 study showed that polycyclic aromatic hydrocarbons were mostly non-detectable in the THP smoke, and level of carcinogenic aldehyde compounds were substantially lower compared to conventional cigarettes. However, certain n-alkanes, organic acids and levoglucosan were still emitted in substantial levels.[51]

The results of Mitova 2016 study showed that for THP the concentrations of most HPHCs did not exceed background levels set in a non-smoking room in equivalent conditions with exception for acetaldehyde (below the minimum risk level for chronic exposure - 140 µg/m3 according to the California Office of Environmental Health Hazard Assessment 2008[53] and 200 µg/m3 in the European Union[54] and nicotine (significantly below the minimum level of occupational exposure - 500 µg/m3 in the European Union[55] and the United States[56]).[47] The use of a CC was associated with an increase in the concentration of acetaldehyde and nicotine as well as other substances evaluated in each of the analysed environments. Alike Forster 2018 study found most assessed compounds did not exceed background levels and only concentration of nicotine, acetaldehyde and formaldehyde were increased (>90% lower compared to CC).[52] Independent study showed concentrations of tested HPHCs (acrolein, acetaldehyde and formaldehyde) in THP aerosol were lower than in CC smoke (equivalent to about 1,8-2,3%, 5,0-5,8% and 6,9-7,1% of the indoor concentration during CCs smoking, respectively).[51] Results regarding metals and organic compounds also indicate that THPs have lower emission of these compounds, but still these devices are not risk-free.

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