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Comparative assessment of HPHC yields in the Tobacco Heating System THS2.2 and commercial cigarettes

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ABSTRACT

There has been a sustained effort in recent years to develop products with the potential to present less risk compared with continued smoking as an alternative for adult smokers who would otherwise continue to smoke cigarettes. During the non-clinical assessment phase of such products, the chemical composition and toxicity of their aerosols are frequently compared to the chemical composition and toxicity of the smoke from a standard research cigarette – the 3R4F reference cigarette. In the present study, it is demonstrated that results of these analytical comparisons are similar when considering commercially available cigarette products worldwide. A market mean reduction of about 90% is observed on average across a broad range of harmful and potentially harmful constituents (HPHC) measured in the aerosol of a candidate modified risk tobacco product, the Tobacco Heating System 2.2 (THS2.2), compared against the levels of HPHC of cigarettes representative of selected markets; this mean reduction is well in line with the reduction observed against 3R4F smoke constituents in previous studies.

1. Introduction

In recent years, much research and development has focused on products that provide an acceptable alternative to continued smoking of cigarettes while having the potential to present a reduced risk compared with continued cigarette smoking. Examples of such products are electronic cigarettes and a novel class of heated tobacco products – products which heat tobacco to temperatures well below that required for combustion, thereby substantially reducing the formation of harmful and potentially harmful constituents (HPHC) compared to the mainstream smoke of cigarettes.

One such product – the Tobacco Heating System 2.2 (THS2.2) was described recently (Smith et al., 2016). It has been extensively characterized in non-clinical and clinical studies, and has been demonstrated to provide lower HPHC yields and a lower *in vitro* toxicity of the aerosol in comparison to the smoke of a 3R4F reference cigarette (Schaller et al., 2016). In addition a substantial reduction in exposure to HPHC excluding nicotine for adult smokers switching to THS2.2 as compared to continued smoking of commercially available cigarettes was reported recently (Haziza et al., 2016a; Ludicke et al., 2017a).

It is important to consider that such products offer an alternative to continued cigarette smoking, and as such they should be evaluated in a comparative manner against cigarettes, i.e. there must be a starting level against which a reduction is to be achieved. For most assays in the non-clinical assessment steps, there is a need to select one specific cigarette comparator and we selected the 3R4F reference cigarette (Smith et al., 2016).

This cigarette is frequently used in non-clinical studies as a comparator, it is a standard cigarette designed and manufactured for research purposes. It is distributed by the Center for Tobacco Reference Products of the University of Kentucky (Anonymous, 2013). Due to the single point in time manufacturing of the 3R4F cigarettes from a single set of tobacco lots, as well as controlled storage conditions, it has been shown to elicit long-term variations in HPHC yields significantly lower than those observed in commercial cigarette products (Eldridge et al., 2015; Belushkin et al., 2015). Due to the standardized design and consistency of mainstream smoke deliveries, the choice of the 3R4F reference cigarette as a ubiquitous comparator is reasonable.

A range of different HPHC yields is typically observed in commercial products (Bodnar et al., 2012; Piadé et al., 2013; Eldridge et al., 2017). It is due to differences in terms of cigarette designs, which can impact the mainstream smoke yields (Siu et al., 2013; Piadé et al., 2013; Hearn et al., 2010).

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This is also reported to be the case in biological assays, although the discriminatory power of chemical analysis of HPHC in cigarette smoke is higher (Oldham et al., 2012), it has been shown that the *in vitro* toxicological activity of cigarette smoke is also influenced by cigarette design parameters. The impact of the tobacco type and blend is well established (Bombick et al., 1998; Roemer et al., 2012; Schramke et al., 2006; Yauk et al., 2012) and for example cigarette diameter, filter ventilation or presence of activated charcoal in the filter have been shown to also have an impact (McAdam et al., 2016; Rickert et al., 2007).

This raises the question, however, of how representative the results of aerosol composition and toxicity comparisons against the 3R4F reference cigarette are when considering commercially available cigarette products.

To address the question above, we assessed the aerosol composition of THS2.2 compared against the mainstream smoke composition of 3R4F and commercial cigarettes from selected countries, on the basis of common lists of HPHC, using the Health Canada intense smoking regime (Health Canada, 1999) to generate the aerosol/smoke.

The comparative assessment of composition that was performed is based on the Health Canada list of HPHC (Health Canada, 2000) and subsets of this list: The WHO Study Group on Tobacco Product Regulation list (TobReg) (World Health Organization, 2015), the U.S. Food and Drug Administration abbreviated list (FDA, 2012), the Health Canada list of HPHC, HPHC which are classified as International Agency for Research on Cancer (IARC) group 1 carcinogenic compounds and the list of first priority toxicants proposed by TobReg (Burns et al., 2008). Those lists include 44, 39, 19, 12 and 9 HPHC respectively. The Health Canada intense machine smoking regime (Health Canada, 2000) was selected based on the recommendation of TobReg to assess cigarette smoke yields under such conditions (Burns et al., 2008), and because it provides a more meaningful basis for the comparison of emissions between the two different product categories. Countries were selected to be representative of major tobacco blends and cigarette designs, with Australia (an essentially Virginia/flue-cured blended products market), Germany, selected European Union countries grouped together (essentially American blended products, containing a mix of flue-cured and air-cured tobaccos markets), South Korea (a market with a high proportion of low 'tar' cigarettes), Japan, and Russia (two diversified markets in terms of cigarette designs, ie use of filters containing activated charcoal, reduced diameter cigarettes).

Although the comparative assessment was calculated on a per article basis and on nicotine-adjusted basis to cope with the different reporting requirements (e.g. Canada, USA, Brazil versus TobReg), it is more appropriate to use the data on a per article basis for the comparison of THS2.2 and commercial cigarettes smoke constituents' reduction: According to the results obtained in a 3-month switching clinical study (Ludicke et al., 2017b), the subjects switching to THS2.2 arm and in the continued smoking of commercial cigarettes arm had no significant difference in their mean consumption of articles (of THS2.2 and commercial cigarettes respectively).

2. Methods

2.1. Commercial cigarettes samples

Samples of commercial cigarette products were purchased between 2008 and 2016 in so-called Market Map studies at the point of sale. Products were selected to be representative of the market in terms of different manufacturers, blend types, ISO 'tar' level and cigarette designs (cigarette diameter, filter type). The number of samples is provided in Table 1.

2.2. HPHC analysis

The analyses of the constituents of mainstream smoke in commercial cigarettes, 3R4F reference cigarettes and in the aerosol of THS2.2 were conducted by Labstat International (Kitchener, Ont., Canada), an independent ISO 17025 accredited tobacco testing laboratory, under contract to Philip Morris International. For commercial cigarettes, the list of constituents mandated for regulatory reporting by Health Canada ("Health Canada list") was assessed. For THS2.2, the PMI-58 list of HPHC and analytes (Schaller et al., 2016) was assessed; this list includes the Health Canada list of constituents. The comparison of emissions of THS2.2 and commercial cigarettes is therefore based on the Health Canada list (Health Canada, 2000) – currently the most extensive active regulatory reporting list for cigarette smoke constituents worldwide.

The generation and collection of THS2.2 aerosol necessitates adequate adaptations to smoking machines: For aerosol collection, only linear smoking machines could be used in order to accommodate the THS2.2 stick holders. The puff number on smoking machines was set to 12 puffs which corresponds to the 6 min duration of the heat stick holder battery while applying a Heath Canada Intense smoking regime, instead of using butt length detection. The linear smoking machine was equipped with an activation bar which activated the heat sticks holder by pressing all activation buttons simultaneously at the start of the process. An interval of 30 s was taken between the device activation and the first puff.

The quantification of constituents in the aerosol also requires specific considerations, since the composition of the THS2.2 aerosol is distinctly different to the composition of cigarette smoke. With respect to ISO parameters, due to the high water content of the THS2.2 aerosol, accurate water measurements cannot be obtained with the ISO standard methods due to its evaporation and condensation (Ghosh and Jeannet, 2014). As such, water is not considered further. Nicotine-free dry particulate matter (NFDPM), is not considered in the comparisons, because this quantity (International Organization for Standardization, 2000), was developed specifically in the context of cigarette smoke analyses and is not meaningful for product categories that do not involve combustion of tobacco and smoke generation. Indeed, the THS2.2 aerosol has a very high water content which requires special methodologies deviating from the ISO standard methods for its quantification, and the overall composition of the aerosol is distinctively different from cigarette smoke (Schaller et al., 2016). It is mainly composed of water and glycerin, the latter acting as an aerosol former. Thus even if the appropriate analytical methodology for the quantification of water in the THS2.2 aerosol were

Table 1
Sampling information.

Sampling year	Market	Number of products
2008	South Korea	13
2010	South Korea	23
2012	South Korea	17
2015	South Korea	35
2008–2016	Germany	59
2008–2015	Russia	204
2008–2016	Japan	169
2010–2016	Australia	44
2015–2016	EU	111

Note: EU (European Union) in the table is limited to the following EU countries: Germany, Denmark, France, Italy, Netherlands, Poland, Portugal, Romania, Slovenia and Sweden.

applied, the resulting value would largely reflect the glycerin content of the aerosol, and could not be interpreted in the same manner as NFDPM for cigarettes. Therefore, NFDPM is not considered further.

For commercial cigarettes, all analyses were performed according to the official Health Canada methods (Health Canada, 2000), with the exception of the analysis of tobacco-specific nitrosamines, which was performed as of 2010 by a liquid chromatography-tandem mass spectrometry method, according to the Labstat International internal method TMS-135. Analyses were performed in triplicate, except for mainstream cigarette smoke yields of 'tar', nicotine and carbon monoxide (CO) for which 8 replicates were performed.

For THS2.2 and 3R4F cigarettes, all analyses performed were based on official Health Canada methods (Health Canada, 2000), with the exception of two methods: analysis of tobacco-specific nitrosamines, which was performed by a liquid chromatography-tandem mass spectrometry method according to the Labstat International internal method TMS-135, and the analysis of polycyclic aromatic hydrocarbons (e.g. benzo[a]pyrene), which was performed by a gas chromatography-mass spectrometry method according to the Labstat International internal method TMS-120. The official Health Canada methods were slightly modified to either enhance detection limits or extend the number of compounds determined by the method. For THS2.2, analyses were performed in triplicate, on each of three samples, and the aggregated results are reported. For 3R4F, average results across more than a year of analysis are reported. All modified methods have been validated and are part of Labstat ISO 17025 scope of accreditation.

2.3. Data treatment

The comparison of chemical composition focuses on the HPHC which are part of the Health Canada list mandated for regulatory reporting in Canada or subsets of this list. Aerosol constituents of THS2.2 have been assessed against the yield of each smoke constituent of commercial cigarette products sampled in each specific market for a given year. Within each market, the first step consisted of calculating an average yield reduction between THS2.2 and each cigarette product, as the average in the reductions among the individual HPHC levels in the aerosol of THS2.2 compared to their levels in cigarette smoke. In a second step, we calculated the aggregate mean, median value, and selected additional percentiles (2.5th, 25th, 75th, and 97.5th) of the distribution of the products' average yield reduction values. The summary statistics of average yield reduction of THS2.2 aerosol constituents are reported for completeness on a per article and on a nicotine-adjusted basis, however as previously stated the most relevant comparison is the one on per article basis.

Additionally, the assessment of the average yield percentage difference among the Health Canada list of HPHC in THS2.2 was calculated against the weighted (according to the number of products) yearly median yields of smoke constituents in commercial cigarettes by country, using all data obtained from 2008 to 2016.

2.3.1. Treatment of limit of detection/quantification values

For several HPHC, the reported yields for some or all replicates were below the limit of detection (<LOD) or limit of quantification (<LOQ) of the laboratory analytical methods. Some HPHC are below LOQ only for THS2.2, such as cadmium, lead, hydrogen cyanide, resorcinol (see also Table 2 for a complete list), some HPHC are below LOQ for both commercial cigarettes and THS2.2, such as nickel, chromium, selenium, while some HPHC are very close to LOQ for THS2.2 only (for example the aromatic amines) or in both commercial cigarettes and THS2.2 and may be quantifiable or not (for example mercury). This may be due to the analytical variability, which is higher when the levels are close to LOQ, or to tobacco lot variability, and may result in large differences in terms of percentage reduction for such constituents. In addition, especially considering the large time period from 2008 to 2016 throughout which data were obtained, the LOD and LOQ values differ between different years for some constituents, both for commercial cigarettes as well as for the 3R4F reference cigarette. In all cases in which a given HPHC yield was reported <LOD or <LOQ for either or both cigarette product, reference cigarette, or THS2.2, an estimate of the median value across the replicates is reported for the individual HPHC, however the HPHC was omitted from percentage difference or further quantitative computations. This approach results in a conservative estimation of the average percentage reduction of HPHC in THS2.2 towards commercial cigarettes.

3. Results

3.1. Comparisons to the 3R4F reference cigarette

The results for the Kentucky reference cigarette 3R4F and THS2.2 for the individual HPHC are provided in Table 2. Except for nicotine, there is a reduction of more than 90% for most HPHC of the Health Canada list, covering a broad range of chemical classes, with an average reduction of about 92% on a per article basis.

3.2. Comparisons to commercial products

The comparison of HPHC in the THS2.2 aerosol and the mainstream smoke of commercial cigarettes was performed on one hand for South Korea with datasets obtained for cigarettes bought in 2008 and in 2015 to assess the potential stability of average reduction results over time, and on the other hand for a number of countries (list of countries and number of products provided in Table 1) for cigarettes bought from 2008 to 2016.

3.2.1. Korean market analysis

The comparison of the THS2.2 aerosol HPHC content with commercial Korean cigarette products sampled and analyzed in 2008 and 2015 is summarized and reported in Table 3. HPHC are grouped in five major HPHC lists, and the resulting reductions are calculated and reported for each HPHC list in a separate column in Table 3. HPHC reductions are based either on per article basis comparisons or on nicotine-adjusted yield comparisons. The estimated reductions are statistically analyzed using the average as well as five percentile values covering the whole range of the observed reductions.

The reductions observed in the levels of THS2.2 aerosol HPHC compared with the HPHC in the mainstream smoke of commercial cigarettes bought in 2008 and 2015 are very similar, considering both results obtained on a per article basis or with nicotine-adjusted results, whatever list of HPHC is taken into account. Using the Health Canada list of HPHC as an example, the mean reduction observed in Korea, on per article basis is 90 and 89% against the cigarettes bought in 2015 and in 2008, while the mean reduction observed, on nicotine-adjusted yields is 88% and 86% for the cigarettes bought in 2008 and 2015, respectively. Those results are well in line with the average percentage reductions calculated against the weighted median yields obtained by combining the results for commercial cigarettes in South Korea and bought in 2008, 2010, 2012 and 2014 (average reduction of 90 and 86% on a per article basis or on nicotine-adjusted basis respectively, using the Health Canada list, see Table 4).

The range of reduction expressed by the 2.5th and 97.5th

Table 2
HPHC yields of THS2.2 expressed as a percentage of the HPHC yields of the 3R4F. Comparison of percentage reduction per article according to the Health Canada list, under HCI smoking regime.

HPHC[a]	3R4F Reference Cigarette Mean	3R4F Reference Cigarette SD[b]	THS2.2 Mean	THS2.2 SD	THS2.2% Reduction vs. 3R4F, Stick Basis
Nicotine (mg/article)	1.86	0.175	1.14	0.0332	-[c]
Mercury (ng/article)	4.77	0.669	2.04	0.104	57.1%
Ammonia (μg/article)	29.3	2.88	10.5	1.63	64.3%
Butyraldehyde (μg/article)	83.5	5.55	20.3	0.586	75.6%
Pyridine (μg/article)	29.7	6.01	6.14	0.423	79.3%
Catechol (μg/article)	89.8	7.14	14.4	0.68	84.0%
Acetaldehyde (μg/article)	1641	258	217	7.85	86.8%
Propionaldehyde (μg/article)	123	7.75	13.6	0.662	89.0%
Formaldehyde (μg/article)	85.2	16.7	7.98	0.504	90.6%
Hydroquinone (μg/article)	89.1	6.65	7.2	0.391	91.9%
Phenol (μg/article)	14	1.86	1.12	0.0849	92.0%
Styrene (μg/article)	15.4	3.23	1.05	0.145	93.1%
N-nitrosoanabasine (NAB) (ng/article)	30.2	2.61	1.92	0.182	93.6%
Benzo[a]pyrene (ng/article)	15	1.3	0.939	0.0796	93.7%
Acrolein (μg/article)	156	25.4	9.63	0.703	93.8%
N-nitrosoanatabine (NAT) (ng/article)	270	22.9	14	1.13	94.8%
Acetone (μg/article)	690	37.5	35.5	1.84	94.9%
Methyl-ethyl-ketone (MEK) (μg/article)	185	12.3	7.59	0.456	95.9%
N-Nitrosonornicotine (NNN) (ng/article)	283	27.8	10.2	0.486	96.4%
Nitric oxide (NO) (μg/article)	504	29	13.8	0.967	97.3%
4-(Methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (ng/article)	264	26.4	6.75	0.493	97.4%
Nitrogen oxides (NOx) (μg/article)	560	30.6	14.1	0.943	97.5%
Carbon monoxide (CO) (mg/article)	30.2	2.76	0.436	0.0811	98.6%
Toluene (μg/article)	137	16.9	1.82	0.163	98.7%
o-Cresol (μg/article)	4.15	0.494	0.0393	0.00649	99.1%
Benzene (μg/article)	81.1	8.78	0.544	0.0312	99.3%
Acrylonitrile (μg/article)	24.5	3.52	0.158	0.0122	99.4%
1,3-Butadiene (μg/article)	98.5	9.8	0.342	0.0347	99.7%
4-Aminobiphenyl (ng/article)	2.83	0.434	0.00958	0.0014	99.7%
3-Aminobiphenyl (ng/article)	4.18	0.773	0.0112	0.0031	99.7%
Isoprene (μg/article)	894	76.7	2.15	0.202	99.8%
1-Aminonaphthalene (ng/article)	21.6	2.28	0.0407	0.0103	99.8%
2-Aminonaphthalene (ng/article)	16.2	2.54	0.0277	0.00909	99.8%
Crotonaldehyde (μg/article)	50.5	9.42	<3.29	*[d]	> 93.5
Lead (ng/article)	32.1	4	<1.62	*	> 95
Quinoline (μg/article)	0.431	0.0416	<0.011	*	> 97.4
Hydrogen cyanide (μg/article)	365	31.2	<4.37	*	> 98.8
m+p-Cresol (μg/article)	12.1	0.897	<0.0646	*	> 99.5
Cadmium (ng/article)	92.9	10.4	<0.28	*	> 99.7
Arsenic (ng/article)	7.9[e]	*	<1.2	*	NA[f]
Resorcinol (μg/article)	1.79[g]	*	<0.055	*	NA
Chromium (ng/article)	<LOQ[h]	*	ND[i]	*	NA
Nickel (ng/article)	<LOQ[j]	*	ND	*	NA
Selenium (ng/article)	1.95[k]	*	1.57	0.123	NA
Average reduction /Health Canada List					92.27%

Notes: LOQ – Limit of Quantification. The calculations can be reported on yields per unit mass nicotine, by dividing each HPHC yield by the average nicotine concentration. The data show that THS2.2 reduced HPHC levels on average, by more than 90% considering their concentrations on a per article basis, and by about 87% considering their concentrations on nicotine basis relative to the reference cigarette.

LOQ – Limit of Quantification.

[a] The reported Health Canada list of constituents does not include pH, tar, and Total Particulate Matter (TPM).

[b] Standard deviation.

[c] THS2.2 is designed to deliver similar levels of nicotine as cigarettes.

[d] A '*' in place of the standard deviation indicates that constituent levels for some or all replicates were below the limit of quantification of the analytical method. In these cases, the median is shown instead of the mean.

[e] 10% of values below the limit of quantification. Median value reported in place of the mean.

[f] Not applicable – could not be quantified in this study.

[g] Few values below the limit of quantification. Median value reported in place of the mean.

[h] 80% values below the limit of quantification.

[i] Not detected (below the limit of detection of the method).

[j] 90% values below the limit of quantification.

[k] 20% of values below the limit of quantification. Median value reported in place of the mean.

percentiles is between 88% and 92% for the per article basis results and between 81% and 92% for the nicotine-adjusted results (using the Health Canada list). The range of reduction between the HPHC in the THS2.2 aerosol and the HPHC in the mainstream smoke of cigarettes is also illustrated in Figs. 1 and 2.

3.2.2. Worldwide markets analysis

The results on the reduction of the levels of THS2.2 aerosol HPHC as compared to HPHC in the mainstream smoke of commercial cigarettes worldwide are reported in Table 5. Results are based on both aerosol/smoke yields measured on a per article basis, as well as yields based on nicotine-adjusted yields. The results

Table 3
Observed percentage reduction of HPHC in THS2.2 compared with commercial cigarettes in South Korean market sampled and analyzed in 2008 and in 2015. Reported percentage reductions are based on aerosol/smoke yields measured on both per article (top) and a nicotine-adjusted (bottom) basis.

	2008					2015				
	Per Article basis					Per Article basis				
	Health Canada	WHO 39	WHO-9	FDA 18	IARC	Health Canada	WHO 39	WHO-9	FDA 18	IARC
97.5th Percentile	92%	92%	94%	94%	97%	92%	92%	94%	95%	97%
75th Percentile	91%	91%	93%	94%	96%	91%	90%	93%	94%	95%
Median	*89%*	*89%*	*93%*	*93%*	*96%*	*90%*	*90%*	*92%*	*93%*	*95%*
Mean	*90%*	*89%*	*92%*	*93%*	*96%*	*90%*	*89%*	*92%*	*93%*	*95%*
25th Percentile	89%	88%	92%	92%	95%	88%	88%	91%	92%	94%
2.5th Percentile	88%	87%	90%	91%	94%	88%	87%	90%	91%	93%
	Nicotine basis					Nicotine basis				
	Health Canada	WHO 39	WHO-9	FDA 18	IARC	Health Canada	WHO 39	WHO-9	FDA 18	IARC
97.5th Percentile	92%	92%	95%	95%	97%	89%	89%	93%	93%	96%
75th Percentile	91%	91%	93%	93%	96%	87%	86%	90%	91%	93%
Median	*88%*	*88%*	*92%*	*92%*	*95%*	*86%*	*85%*	*89%*	*90%*	*93%*
Mean	*88%*	*88%*	*91%*	*92%*	*95%*	*86%*	*85%*	*89%*	*90%*	*93%*
25th Percentile	88%	87%	90%	92%	95%	85%	84%	88%	90%	92%
2.5th Percentile	83%	82%	86%	88%	92%	81%	80%	85%	87%	88%

Lists of HPHC according to Health Canada (Health Canada, 2000), WHO TobReg nine first priority toxicants (Burns et al., 2008), WHO TobReg non-exhaustive priority list (World Health Organization, 2015), FDA abbreviated list (FDA, 2012) and IARC group 1 carcinogens.

Table 4
Observed percentage reduction of HPHC in THS2.2 compared with commercial cigarettes sampled and analyzed between 2008 and 2015, using Health Canada list of HPHC. Reported percentage reductions are based on aerosol/smoke yields measured on both per article (left) and a nicotine-adjusted (right) basis and on weighted median smoke constituents' yields in commercial cigarettes.

Country	Average % reduction on per article basis	Average % reduction on nicotine basis
South Korea	90	86
Japan	91	87
Russia	91	88
Germany	92	86
Australia	90	83

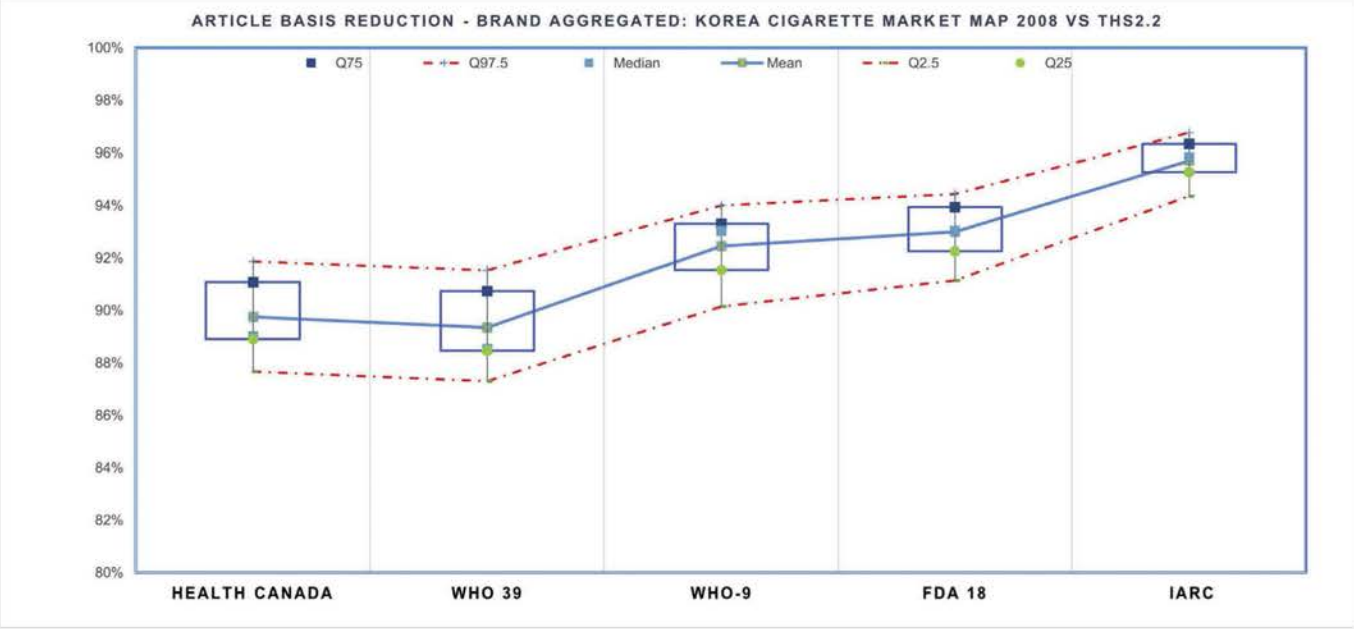

Fig. 1. Box plots summarizing the distribution property of THS2.2 average HPHC yield reductions on a per article basis, against the average yields of individual commercial cigarette products sampled in 2008 from South Korean Market.

provided in Table 4 are calculated from the weighted median yields obtained per country using all data from 2008 to 2016.

The results provided in Table 5 are statistically assessed using the average yield reduction of THS2.2 aerosol HPHC across all

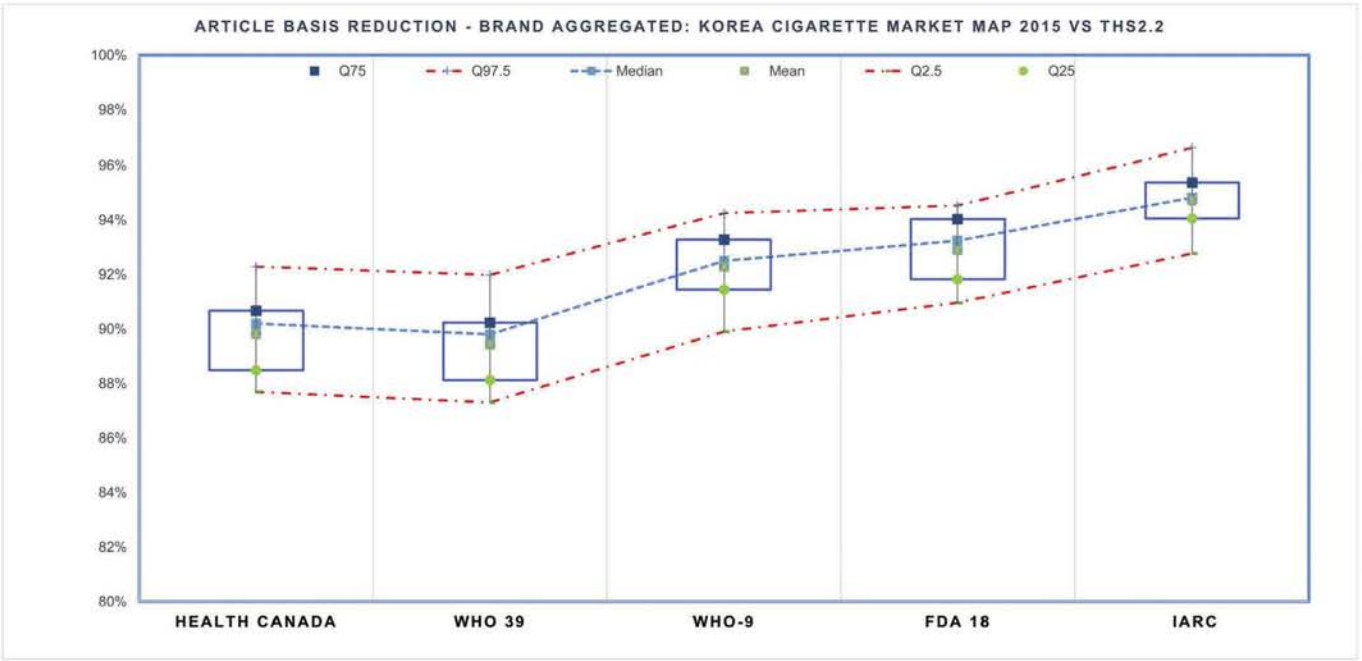

Fig. 2. Box plots summarizing the distribution property of THS2.2 average HPHC yield reductions on a per article basis, against the average yields of individual commercial cigarette products sampled in 2015 from South Korean Market.

Table 5
Observed percentage reduction of HPHC in THS2.2 compared with commercial cigarettes in major markets worldwide in 2015–2016. Reported percentage distribution reduction statistics are based on aerosol/smoke yields measured on a per article basis (left) and on nicotine-adjusted smoke yields (right).

		Per article basis					Nicotine basis				
		Health Canada	WHO 39	WHO-9	FDA 18	IARC	Health Canada	WHO 39	WHO-9	FDA 18	IARC
Australia	97.5th Percentile	93%	93%	94%	95%	96%	86%	86%	90%	91%	94%
	75th Percentile	91%	91%	93%	93%	95%	85%	84%	88%	88%	92%
	Median	*91%*	*90%*	*92%*	*93%*	*94%*	*82%*	*81%*	*84%*	*86%*	*87%*
	Mean	*91%*	*90%*	*92%*	*93%*	*93%*	*82%*	*81%*	*84%*	*86%*	*87%*
	25th Percentile	90%	89%	90%	91%	92%	80%	79%	81%	83%	84%
	2.5th Percentile	89%	88%	89%	91%	90%	78%	76%	78%	81%	80%
Japan	97.5th Percentile	93%	93%	95%	95%	97%	89%	89%	93%	93%	96%
	75th Percentile	92%	91%	94%	95%	96%	87%	86%	91%	91%	94%
	Median	*91%*	*91%*	*93%*	*93%*	*95%*	*85%*	*85%*	*90%*	*90%*	*93%*
	Mean	*90%*	*90%*	*93%*	*93%*	*95%*	*85%*	*84%*	*88%*	*89%*	*91%*
	25th Percentile	89%	89%	92%	92%	94%	84%	83%	88%	89%	91%
	2.5th Percentile	86%	86%	89%	90%	90%	76%	75%	74%	80%	78%
Russia	97.5th Percentile	92%	91%	94%	95%	97%	91%	91%	95%	94%	97%
	75th Percentile	91%	91%	94%	94%	96%	87%	87%	91%	92%	94%
	Median	*91%*	*90%*	*94%*	*94%*	*96%*	*87%*	*86%*	*90%*	*91%*	*94%*
	Mean	*91%*	*90%*	*93%*	*94%*	*96%*	*87%*	*87%*	*91%*	*91%*	*94%*
	25th Percentile	91%	90%	93%	93%	95%	86%	86%	90%	91%	94%
	2.5th Percentile	90%	90%	92%	93%	95%	85%	85%	89%	90%	93%
EU	97.5th Percentile	93%	93%	95%	96%	97%	90%	90%	93%	93%	95%
	75th Percentile	92%	92%	94%	95%	96%	87%	87%	91%	91%	94%
	Median	*92%*	*91%*	*94%*	*94%*	*96%*	*86%*	*85%*	*90%*	*91%*	*93%*
	Mean	*92%*	*91%*	*94%*	*94%*	*96%*	*86%*	*86%*	*89%*	*90%*	*93%*
	25th Percentile	91%	90%	93%	94%	95%	85%	84%	88%	90%	92%
	2.5th Percentile	89%	89%	91%	92%	94%	82%	82%	85%	88%	89%

cigarette products. Five critical percentiles covering the whole range of the observed reductions are also provided.

The mean reduction observed for the THS2.2 aerosol HPHC towards commercial cigarettes bought in 2015 and 2016, using the Health Canada list is very similar in all countries with values between 90% and 92% on a per article basis or between 82% and 87% with nicotine adjusted values.

4. Discussion

The assessment program for THS2.2 was recently described (Smith et al., 2016) and followed by a series of publications providing the results of the non-clinical and parts of the clinical assessment of THS2.2. Investigations on differences in aerosol composition compared to the smoke composition of cigarettes, and differences in biological effects in *in vitro* assays conducted in the non-clinical part of the THS2.2 assessment used the University of Kentucky 3R4F cigarette as a reference cigarette (Schaller et al., 2016). There are several arguments in favor for that selection: The availability of the reference cigarette from an independent source; its widespread use for tobacco research purposes, internally and externally, which provides a solid data base of results for HPHC analysis and effects in biological assays supported by a homogeneity of the reference cigarettes usually exceeding that of commercial products. There are however also limitations, inter alia the fact that the 3R4F has been designed as reference for an American blend type cigarette. Consequently, it remained to be demonstrated that the commercially available THS2.2 achieves a comparable overall reduction in yields of selected HPHC whether compared with the 3R4F or commercial cigarettes from various markets.

In this work, in comparison with 3R4F cigarettes mainstream smoke HPHC, the average reduction over all analyzed HPHC in THS2.2 covering a wide range of chemical classes is found to be 92% on a per article basis and 87% on a nicotine-adjusted basis. The results are consistent with what was reported previously for THS2.2 and 3R4F (Schaller et al., 2016) and with recently published data related to 3R4F mainstream smoke yields (Margham et al., 2016; Pazo et al., 2016; Eldridge et al., 2015; Roemer et al., 2012).

In comparison with the HPHC in the mainstream smoke of commercial cigarettes in specific markets, the mean reduction observed for the THS2.2 aerosol HPHC is very similar in all markets and very close to the reduction for 3R4F (e.g., 90%–92% reduction for the per article basis results using the Health Canada list in individual markets and 83% and 88% reduction for the nicotine-adjusted results, using the weighted median values for commercial cigarettes as a comparison). These results confirm that the use of 3R4F reference cigarette as a comparator provides a value for the average reduction in aerosol/smoke yields which is representative of commercial cigarettes available in various markets in studies with potentially modified risk tobacco products, such as THS2.2.

The generally lower reduction observed for the nicotine-adjusted results against the results obtained on a per article basis is mainly due to the average higher machine smoking yields of nicotine from cigarettes. The range of reduction observed varies between around 75% and 97% for individual products for nicotine-adjusted results and between 86% and 97% on a per article basis. The observed range among different commercial cigarettes in a given country is due to differences in terms of cigarette designs, which can impact the mainstream smoke yields of commercial cigarettes. Typically, the influence of the cigarette diameter (Siu et al., 2013; McAdam et al., 2016), the blend types (Piadé et al., 2013) or the filter types (Hearn et al., 2010; Piadé et al., 2015; Shin et al., 2009) have been pointed out.

If we take the same comparative approach for the HPHC yields between different commercial cigarette products, we obtain for example a mean decrease in terms of HPHC of 12% on a per article basis (or 10% on nicotine-adjusted results) between the cigarettes from Australia (a typical Virginia blended market) and the cigarettes from selected EU countries (American blended cigarette markets) in this study, using the 2015-2016 median values of each HPHC in both markets. This difference is driven mainly by the TSNA, ammonia and aromatic amines levels which are lower in Virginia blends than in American blends (Piadé et al., 2013). Considering further differences in biological activity of smoke from cigarettes with different tobacco blends (Belushkin et al., 2014), it is well known, for example, that Total Particulate Matter (TPM) from Virginia tobacco is consistently less mutagenic than Burley tobacco in the Ames bacterial mutagenicity assay (Roemer et al., 2004). On the contrary, in the mammalian cell based Mouse Lymphoma genotoxicity assay, TPM from Virginia tobacco cigarettes is more mutagenic than that from experimental all Burley tobacco cigarettes (Schramke et al., 2006). Those differences do not, however, appear to reflect any difference in terms of cancer and COPD occurrence in markets consisting essentially of Virginia blended cigarettes (such as Australia or UK) or essentially American blended cigarettes (such as Germany) as shown in a review of epidemiological data (Lee et al., 2009). This is also true for the charcoal containing filter cigarettes which have lower amounts of mostly volatile and semi-volatile compounds but not particulate phase compounds in cigarette mainstream smoke, when compared with cellulose acetate containing filter cigarettes (Shin et al., 2009; Hearn et al., 2010). In a clinical study with carbon-filtered cigarettes with a high loading of charcoal and test cigarettes with regular acetate tow filters, there were no significant difference in the measures of biological effect which were performed (Sarkar et al., 2008), even though there was a significant decrease of the HPHC present in the gas phase. A review of published data, including non-clinical, clinical and epidemiological studies, concluded that current charcoal filter techniques alone may not be sufficient to reduce smoking-related disease (Coggins and Gaworski, 2008).

In addition, it was recently reported that there was no consistent change of biomarkers of effect in smokers of cigarettes which were specifically designed for reduced toxicant emissions and delivered around 50% less on average for a wide range of HPHC than reference cigarettes (Dittrich et al., 2014; Proctor et al., 2014).

In the case of THS2.2, due to absence of combustion, a significant decrease in formation of about 90% is observed on average across a broad range of chemical compounds when compared against 3R4F reference cigarettes and commercial cigarettes available in a number of countries. The reduced formation for this technology is also consistent with results on toxicity reductions. In comparison with 3R4F a significant reduction of the cytotoxicity determined by the neutral red uptake assay and the mutagenic potency in the mouse lymphoma assay has been observed, while THS2.2 aerosol was not mutagenic in the Ames assay (Schaller et al., 2016). Furthermore, a significant reduced exposure to HPHC (close to the levels observed for smoking abstinent subjects) has been observed for subjects switching to this product in comparison with continuing to smoke commercially available cigarettes in clinical trials (Haziza et al., 2016a, 2016b; Ludicke et al., 2017a).

In summary, our results 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. This leads to the twofold conclusion, firstly that about 90% average HPHC emission reductions of the THS2.2 in comparison to the 3R4F are sufficiently representative of commercial market means and secondly, that the 3R4F is a reasonable comparator for the assessment of aerosols of potentially modified risk tobacco products such as the THS2.2 in the non-clinical phase of evaluation.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.yrtph.2017.08.006.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.yrtph.2017.08.006.

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