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Robustness of HPHC Reduction in THS 2.2 Aerosol Relative to 3R4F Reference Cigarette Smoke under Extreme Climatic Conditions*

by

Laurent Poget, Catherine Goujon, Samuel Kleinhans, Serge Maeder, and Jean-Pierre Schaller

PMI R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland

SUMMARY

In order to assess robustness for the reduction of harmful and potentially harmful constituent (HPHC) levels generated by the Tobacco Heating System 2.2 (THS 2.2), a heated tobacco product, we compared the aerosol of this product with mainstream smoke from the 3R4F reference cigarette under different conditions of temperature and humidity. The desired climatic conditions were achieved by using an air-conditioning system coupled with the smoking-machine housing. Two extreme climatic conditions were selected, representing a "Hot and Dry" climate (30 °C and 35% relative humidity RH) and a "Hot and Very Humid" climate (30 °C and 75% RH). In addition, aerosol and smoke were generated using the standard conditions recognized for smoking-machine analyses of tobacco products (22 °C and 60% RH), which were close to the climatic conditions defined for "Subtropical and Mediterranean" environments (25 °C and 60% RH). The experimental conditions were chosen to simulate the use of THS 2.2 and cigarettes under extreme conditions of temperature and humidity. HeatSticks and cigarettes taken from freshly opened packs were subjected to short-term conditioning from two to a few more days under the same experimental conditions. We analyzed 54 HPHCs in THS 2.2 aerosol and 3R4F cigarette smoke, generated in accordance with the Health Canada Intense (HCI) standard, using modified temperature and humidity conditions for sample conditioning and machine-smoking experiments. We used a volume-adjusted approach for comparing HPHC reductions across the different climatic conditions investigated. Although a single puffing regimen was used, the total puff volume recorded for the 3R4F cigarette smoke varied due to the influence of temperature and humidity on combustion rate, which justified the use of a volume-adjusted approach. Volume-adjusted yields were derived from HPHC yields expressed in mass-per-tobacco stick normalized per total puff volume.

The results indicated that, regardless of the considered climatic conditions, the HPHC levels investigated in THS 2.2 aerosol were reduced by at least 90%, on average, when compared with the concentrations in 3R4F cigarette mainstream smoke. This confirmed the robustness in performance for THS 2.2 to deliver reduced levels of HPHCs under the extreme climatic conditions investigated in this study. In order to further characterize the robustness of these reductions, the lowest reduction performance achieved for individual HPHCs across all climatic conditions was used to define the threshold for a robust reduction. The majority of the 54 HPHCs investigated in THS 2.2 aerosol showed more than 90% reduction. Calculations derived from nicotine-adjusted yields also confirmed robust reductions for all investigated HPHCs. The small differences in absolute reduction between the volume- and nicotine-adjusted approaches were predominantly attributed to a combination of the differences in both nominal nicotine deliveries and total puff volumes between THS 2.2 and 3R4F cigarettes; however, this did not influence the determination of robustness. Our findings confirm the value of this approach for assessing the robustness of a product's performance under different climatic conditions.
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KEYWORDS

Tobacco Heating System, THS 2.2, reduced-risk product, harmful and potentially harmful constituent, HPHC aerosol chemistry

ZUSAMMENFASSUNG

Ziel der vorliegenden Studie war die Überprüfung der Verlässlichkeit der Reduzierung von schädlichen und potenziell schädlichen Bestandteilen (HPHCs) im Aerosol von THS 2.2, einem Tabakerhitzerprodukt, gegenüber dem Hauptstromrauch einer 3R4F-Referenz-Zigarette bei unterschiedlichen Temperatur- und Luftfeuchtigkeitsbedingungen. Die gewünschten Umgebungsbedingungen wurden mittels eines an das Gehäuse einer Rauchmaschine angeschlossenen Lüftungssystems generiert. Die Studie berücksichtigte ein "heisses und tockenes" Klima (30 °C und 35% RH), ein "heisses und feuchtes" Klima (30 °C und 75% RH), sowie ein "subtropisches und mediterranes" Klima (25 °C und 60% RH). Da letzteres Klima den beim maschinellen Abrauchen von Tabakprodukten standardmässig verwendeten Umgebungsbedingungen (22 °C und 35% RH) sehr ähnlich ist, wurden stattdessen diese verwendet. Um einen möglichst realen Gebrauch von THS 2.2 und 3R4F-Referenz-Zigaretten unter den extremen Temperatur- und Luftfeuchtigkeitskonditionen zu simulieren, wurden beide Tabakprodukte nach der Entnahme aus den frisch geöffneten Packungen zunächst für eine Dauer von zwei oder wenigen Tagen bei den jeweiligen Umgebungsbedingungen konditioniert. Anschliessend wurden beide Produkte nach Health Canada Intense (HCI) Standardparametern mit modifizierten Temperatur- und Luftfeuchtigkeitsbedingungen maschinell abgeraucht und das Aerosol zuletzt auf die Gehalte von 54 HPHCs untersucht.

Es wurden volumennormalisierte Werte für den Vergleich der HPHC-Reduzierung zwischen unterschiedlichen Umgebungsbedingungen herangezogen. Obwohl ein Einzel-Zugverfahren verwendet wurde, variierte das für den 3R4F-Zigarettenrauch erfasste Gesamtzugvolumen, aufgrund des Einflusses von Temperatur und Luftfeuchtigkeit auf die Verbrennungsrate, was die Verwendung eines volumenbasierten Ansatzes rechtfertigte. Die volumenbasierten Ausbeuten wurden von den HPHC-Ausbeuten abgeleitet, in Masse pro Tabakstick ausgedrückt und auf das Gesamtzugvolumen normalisiert. Unabhängig von den untersuchten Umgebungsbedingungen hat sich gezeigt, dass die im Durchschnitt gemessenen HPHC-Gehalte im THS 2.2-Aerosol mindestens 90% unterhalb der Gehalte im 3R4F-Hauptstromrauch lagen. Dies bestätigte die Verlässlichkeit der Leistungsfähigkeit von THS 2.2 in der Reduzierung von HPHC-Gehalten unter den in dieser Studie untersuchten extremen Umgebungsbedingungen.

Um die Verlässlichkeit der Reduzierung der einzelnen HPHCs zu charakterisieren, wurde zur Festlegung eines Schwellenwertes für die Verlässlichkeit jenes Ergebnis berücksichtigt, das die geringste Reduzierung unter allen untersuchten Umgebungsbedingungen gezeigt hatte. Die Mehrheit der 54 untersuchten HPHCs im THS 2.2-Aerosol wies eine Reduzierung um mehr als 90% auf. Berechnungen auf der Basis von auf Nikotin normalisierten Werten bestätigten ebenfalls die robuste Reduzierung für alle analysierten HPHCs. Obwohl die absoluten Reduzierungen zwischen volumennormalisierten und auf Nikotin normalsierten Werten eine geringe Differenz aufwiesen, die weitestgehend auf die unterschiedlichen Nikotingehalte und Gesamtzugvolumen im THS 2.2-Aerosol gegenüber dem 3R4F-Zigarettenrauch zurückgeführt werden konnten, hatte dieser Unterschied keinen Einfluss auf die Reduzierungsverlässlichkeit. Unsere Resultate bestätigen die Aussagekraft des gewählten Ansatzes zur Charakterisierung der Robustheit der Produkteigenschaften unter verschiedenen klimatischen Bedingungen. [Contrib. Tob. Nicotine Res. 30 (2021) 109–126]

RESUME

Dans le but d'évaluer la robustesse au niveau réduction des niveaux de constituants nocifs et potentiellement nocifs (HPHC en anglais), l'aérosol du THS 2.2, un produit de tabac chauffé, a été comparé avec la fumée principale de la cigarette de référence 3R4F, ceci sous diverses conditions de température et d'humidité. Un système de climatisation couplé à l'enceinte de la machine à fumer a été utilisé afin d'atteindre les conditions ambiantes souhaitées. Deux zones climatiques ont été étudiées comme climat chaud et sec (30 °C et 35% RH), climat chaud et très humide (30 °C et 75% RH) et une troisième zone climatique simulée comme climat subtropical et méditerranéen (25 °C et 60% RH). Cette troisième condition étant très proche des conditions ambiantes indiquées dans les normes reconnues pour les analyses réalisées à partir de machines à fumer analytiques (22 °C et 60% RH), a été substituée par cette dernière. Nous avons défini les conditions expérimentales pour simuler l'utilisation du THS 2.2 et de la cigarette de référence 3R4F dans des conditions de température et d'humidité extrêmes après que les sticks de produits soient sortis des emballages fraîchement ouverts et soumis à un bref conditionnement d'au moins deux jours à quelques jours supplémentaires dans les mêmes conditions expérimentales. Cinquante-quatre HPHCs ont été analysés dans l'aérosol THS 2.2 et la fumée de cigarette 3R4F générées selon la norme Heath Canada Intense (HCI) dont seules les conditions de température et d'humidité ont été modifiées pour le conditionnement des échantillons et les fumages analytiques. Un ajustement des taux par le volume a été utilisé dans la présente étude pour comparer la réduction des HPHCs dans différentes conditions ambiantes. Même si un seul régime de bouffées a été considéré, le volume total de bouffées n'était pas constant pour la cigarette 3R4F, en raison de l'influence de la température et de l'humidité sur le taux de combustion, ce qui justifiait l'approche de l'ajustement par le volume. Le niveau des HPHCs ajusté par le volume découle de la masse des HPHCs exprimée par stick de tabac et normalisée par le volume total de bouffées. Nous avons comparé les réductions de concentrations moyennes d'HPHCs (rendements ajustés par le volume) entre les conditions climatiques étudiées. Les résultats ont indiqué qu'indépendamment des conditions climatiques considérées les niveaux moyens des HPHCs étudiés dans les aérosols THS 2.2 ont été réduits d'au moins 90% par rapport aux concentrations générées dans la fumée principale des cigarettes 3R4F. Ceci confirme la performance robuste du THS 2.2 à réduire les taux de HPHCs dans les conditions climatiques extrêmes explorées dans cette étude. Afin de caractériser individuellement la robustesse pour chaque HPHC, la performance de réduction la plus basse observée parmi toutes les conditions climatiques testées a été utilisée pour définir le seuil limite de robustesse de réduction. La majorité des 54 HPHCs étudiés dans les aérosols THS 2.2 ont montré une réduction de plus de 90%. Les calculs effectués à partir des rendements ajustés par la nicotine ont également confirmé la bonne robustesse de réduction sur l'ensemble des HPHCs étudiés. Une légère différence de réduction absolue entre l'ajustement par la nicotine et l'ajustement par le volume a été attribuée principalement à l'influence conjuguée des niveaux respectifs de nicotine et du nombre total de bouffées entre l'aérosol THS 2.2 et la fumée de cigarette 3R4F, toutefois sans remettre en cause l'appréciation de la robustesse. Nos résultats confirment la pertinence de cette approche pour évaluer la robustesse de performance d'un produit dans diverses conditions ambiantes. [Contrib. Tob. Nicotine Res. 30 (2021) 109–126]

ABBREVIATIONS

ANOVA:	Analysis of variance;
As:	Arsenic;
Cd:	Cadmium;
CO:	Carbon monoxide;
Cr:	Chromium;
HCI:	Health Canada Intense;
HCN:	Hydrogen cyanide;
Hg:	Mercury;
HPHC:	Harmful and potentially harmful constituent;
ISO:	International Organization for Standardization;
LOD:	Limit of detection;
LOQ:	Limit of quantitation;
MEK:	Methyl ethyl ketone;
NAB:	*N*-nitrosoanabasine;
NAT:	*N*-nitrosoanatabine;
NFDPM:	Nicotine-free dry particulate matter;
Ni:	Nickel;
NNK:	4-(*N*-nitrosomethylamino)-1-(3-pyridyl)-1-butanone;
NNN:	*N*-nitrosonornicotine;
NO:	Nitrogen oxide;
NO_x:	Nitrogen oxides;
Pb:	Lead;
RH:	Relative humidity
Se:	Selenium;
SD:	Standard deviation
THS 2.2:	Tobacco Heating System 2.2

INTRODUCTION

The Tobacco Heating System 2.2 (THS 2.2), developed by Philip Morris Products S.A. and commercialized under the brand name IQOS®, uses a precisely controlled heating device into which specially designed tobacco sticks (HeatSticks) are inserted and heated to generate an aerosol. When a tobacco substrate is heated instead of being burned, the levels of harmful and potentially harmful constituents (HPHCs) released into the aerosol are significantly reduced, and average reductions in the range of 90–95% on a per-stick basis relative to cigarette smoke have been observed (1). By exposing users to lower levels of HPHCs, THS 2.2 has the potential to offer a less harmful alternative for adult smokers who would otherwise continue to smoke, as has been widely published (2–12). Since THS 2.2 is a product designed to be made available to all markets in the world where cigarettes are currently sold, it is therefore important to understand how the product behaves under different climatic conditions, in terms of its ability to maintain reductions in the emission of recognized HPHCs. It is known that the moisture content of a cigarette tends to equilibrate with ambient conditions once the packet has been opened, and the effect of moisture on the composition of cigarette smoke has been comprehensively presented in a study published by Zha and Moldoveanu (13). Therefore, it is reasonable to assume that ambient temperature and humidity conditions could also affect the composition of heated tobacco aerosol when such products are used under different climatic conditions.

The aim of this study was to characterize the performance of THS 2.2 in its ability to reduce the levels of known HPHCs in aerosol relative to cigarette smoke, represented by the 3R4F reference cigarette over a range of different climatic conditions. Considering that the THS 2.2 heating element is positioned in the center of the tobacco substrate plug during use, and that the temperature is controlled electronically, the average tobacco temperature is lower in a reduced temperature environment when compared with standard conditions, resulting in lower HPHC yields in THS 2.2 aerosol. Accordingly, our focus was upon climatic conditions that were higher in temperature than standard ISO conditions (14), with either increased or decreased humidity, to best represent worst-case scenarios for product usage. Relative reductions using the average of all investigated HPHCs, and also the levels of reduction for each HPHC individually with respect to predefined "robustness" limits, were determined.

Analytical smoking machines capable of controlling climatic conditions were used to generate aerosol and smoke under different conditions of temperature and humidity. Tobacco sticks and cigarettes were conditioned under the same conditions prior to aerosol generation in order to evaluate how short-term exposure to extreme climatic conditions could influence the performance of THS 2.2 in terms of reducing the levels of HPHCs compared with cigarette smoke. While the duration of common stability studies may extend over several months - for example, when determining a product's shelf life - in this instance, the goal was to assess the performance of tobacco sticks taken from a freshly opened pack, which are then exposed to extreme climatic conditions for a few days before being used to generate aerosol under the same conditions. To compare the performance of THS 2.2 sticks under extreme scenarios, two climatic conditions representing zones identified in the World Health Organization (WHO) guidelines (15) as "Hot and Dry" climate (30 °C and 35% RH) and "Hot and Very Humid" climate (30 °C and 75% RH) were introduced. In place of the WHO-defined conditions for "Subtropical and Mediterranean" climates (25 °C and 60% RH), temperature and humidity conditions set forth in ISO 3402, which has a lower temperature stipulation (22 °C), were used. Atmospheric pressure was excluded from climatic parameters, hence the range 86–106 kPa as required by ISO 3402 was assumed for all climatic conditions investigated.

Analysis of an established list of principal aerosol/smoke constituents and 54 relevant HPHCs was used for comparative assessment of levels between THS 2.2 aerosol and mainstream smoke of the 3R4F reference cigarette (9). As applied in a previous investigation (16), our approach was again to use puff volume adjustment to express the yields for each individual constituent. Although the total puff volume is fixed for THS 2.2, we anticipated that the combustion rate of the tobacco rod of the 3R4F reference cigarette could change with climatic conditions, and therefore influence the total number of available puffs. In such an eventuality, this volume-adjusted approach offers a means to compare the two products on the basis of equivalent total puff volume. Moreover, we intentionally retained the same approach for determining the average HPHC reduction levels across climatic conditions, which not only considers yields expressed on a total puff volume basis, but also yields adjusted in relation to nicotine delivery (nicotine-adjusted yield, expressed as HPHC yield per milligram of nicotine), which is commonly used to express the level of toxicants to which cigarette smokers would be exposed (17–18). Furthermore, we used the concept of including a threshold of robust reduction, which was also introduced in our previous article (16).

EXPERIMENTAL PROCEDURES

Reference cigarette

The reference cigarette used was 3R4F, supplied by the University of Kentucky (University of Kentucky Center for Tobacco Reference Products, Lexington, KY, USA; http://www.ca.uky.edu/refcig/).

THS 2.2 tobacco sticks

Commercially produced non-mentholated (regular) tobacco sticks, known as HeatSticks or HEETS, designed for use with the THS 2.2 device, were used. Previous studies (11, 19) have described in detail the design and functionality principles of THS 2.2.

Generation of THS 2.2 aerosol and 3R4F cigarette smoke under standard and non-standard ambient conditions

We generated THS 2.2 aerosol and 3R4F reference cigarette smoke under different ambient temperature and RH conditions to reproduce "Subtropical and Mediterranean", "Hot and Dry", and "Hot and Very Humid" climatic conditions (Table 1).

According to the study on tobacco product conditioning published by CHEN *et al.* (20), under non-forced air flow condition (0 m/s), flue-cured and blended cigarettes which had high, low or routine level of water content took 23 h for conditioning in a constant climate laboratory (22 °C ± 1 °C and 60% ± 3% RH). By analogy, we have considered that our current practice of conditioning THS 2.2 sticks and cigarettes for 48 h was also appropriate for non-standard atmosphere and reflected well few days of usage of a freshly open pack. Therefore, the cigarettes and tobacco sticks were conditioned for at least 48 h, maximum 10 days, in accordance with ISO 3402:1999 (14).

For all analytes (except elements), THS 2.2 aerosol and 3R4F cigarette smoke were generated by using a linear smoking machine prototype SM405XR (Cerulean Molins PLC, Milton Keynes, UK) under the Health Canada Intense (HCI) machine-smoking regimen (21).

The SM405XR smoking machine was housed in a conditioned air cabinet (temperature range, 10–35 °C; humidity range, 10–80% RH) fitted with a Delta 335 air-conditioning unit (Design Environmental Ltd., Ebbw Vale, UK). The atmosphere of the cabinet was constantly refreshed with conditioned air. The temperature and RH in the cabinet were monitored using a TH1 datalogger (ELPRO-Buchs AG, Buchs, Switzerland).

The reference cigarette 3R4F was smoked to a butt length of 35 mm with a bell-shaped puff profile and 100% blocking of ventilation holes. Aerosol from THS 2.2 tobacco sticks was generated to a defined puff count of 12 puffs using a bell-shaped puff profile. The limitation to 12 puffs is based on the fixed settings of the THS 2.2 device - which is programmed to finish heating after a maximum period of 6 min - and the puff interval of the HCI regimen (30 s).

Stick and cigarette conditioning and subsequent machine-smoking experiments were carried out in accordance with the HCI standard conditions mentioned above. The same machine-smoking parameters were maintained throughout the study, and only the sample conditioning and ambient conditions under which the smoking machine operated were modified to produce "Hot and Dry" and "Hot and Very Humid" conditions, as specified in Table 1. Whenever Cambridge glass-fiber filter pads were used, they were conditioned for 48 h under the respective climatic conditions specified for testing the cigarettes and sticks.

Five replicates were used for the analyses, unless specified otherwise.

Table 1. Ambient conditions representing the climatic zones considered for the study.

Conditions (abbreviation)	Temperature (°C)	Relative humidity (% RH)
ISO [a] (S)	22 ± 2	60 ± 5
Hot and Very Humid (H)	30 ± 2	75 ± 5
Hot and Dry (D)	30 ± 2	35 ± 5

[a] Close to "Subtropical and Mediterranean" conditions 25 °C and 60% RH.

Aerosol/smoke chemical analysis

Except for analysis of elements, the analytical methods used for quantifying the considered analytes in THS 2.2 aerosol and 3R4F reference cigarette smoke are described in the supplementary material published by SCHALLER *et al.* (9).

Aerosol/smoke generation and element analysis

The set-up for analysis of elements (i.e., arsenic, cadmium, chromium, lead, mercury, nickel, and selenium) did not fit the design of the smoking-machine housing. Therefore, the test was limited to the influence of conditioning temperature and humidity on product performance. The samples were conditioned for a minimum of 48 h directly after being removed from the pack, using the same absolute tolerance of humidity and temperature as that for ISO conditioning. Immediately after conditioning, and for the three tested conditions, the samples were transiently stored in airtight plastic boxes, and the machine-smoking experiments were subsequently carried out under standard HCI ambient conditions (ISO 3402 ambient conditions). Aerosol/smoke was generated with a linear smoking machine for mercury and a rotary smoking machine for the other elements. The aerosol/smoke generation and analysis were performed at Labstat International ULC (Kitchener, Ontario, Canada), an ISO 17025 (/22) laboratory accredited for all mandated tobacco-related Health Canada methods (21), with the modifications described in Supplementary Information Table S1.

Calculation of nicotine-adjusted yields

Nicotine-adjusted HPHC yields were calculated as mass-per-tobacco stick normalized for the corresponding nicotine yield for each product, constituent, climatic condition, and replicate. These data were used to calculate the average reductions for THS 2.2 *versus* 3R4F per climatic condition by using the combined data for all HPHCs.

Calculation of aerosol/smoke concentration

The HPHC aerosol/smoke concentrations were calculated as mass-per-stick normalized per total puff volume for each product, constituent, climatic condition and replicate. The number of puffs multiplied by the volume of puffs determined the total puff volume. The number of puffs averaged among all items of a replicate was assigned to the 3R4F cigarette, and the number of 12 puffs predefined for the HCI regimen was used for THS 2.2. These data were used for the entire evaluation process as described below.

Data evaluation

Descriptive statistics per product and machine-smoking condition were computed for all HPHCs investigated in this study.
Relative reduction was defined as the reduction in HPHC concentrations in THS 2.2 aerosol compared with those in 3R4F cigarette smoke. Reductions were calculated within the same climatic conditions for both THS 2.2 and 3R4F and also between each of the conditions used for testing THS 2.2 relative to the ISO conditions for 3R4F. By crossing conditions this way, the comparison was based on a total of five combinations.
Statistical analysis: Bayesian analysis of variance (ANOVA) (23–24) was performed to assess the reduction in HPHCs across the different machine-smoking conditions. The mean concentrations and respective 0.95 confidence intervals for each HPHC were calculated for each of the three conditions tested for 3R4F, and each of the three conditions tested for THS 2.2. Relative reductions in the levels of individual HPHCs and their respective 0.95 confidence intervals were calculated for the five combinations of conditions by using Markov chains produced by the Bayesian model (23, 25).
For each of the five combinations, the average reductions were calculated for both nicotine-adjusted and volume-normalized yields by using the combined data of all measured HPHCs.
For a subset of HPHCs in which levels were impacted by climatic conditions, mean differences of volume-adjusted yields in extreme conditions relative to ISO climatic conditions were calculated for both THS 2.2 and 3R4F.
Predefined standard distributions (i.e., non-informative priors) were assigned to all Bayesian model parameters to match the classical frequentist modelling results. The Bayesian approach allowed computation of uncertainty around complex statistical estimates such as ratios of means with heterogeneous dispersions (heteroscedasticity).
SAS Enterprise Guide 7.1 was used for the statistical treatments, including procedures MCMC and MIXED (26–27) for the computation aspects.

Calculation of relative reduction including determination limits

When aerosol and smoke constituent values were below the limit of detection (LOD) and/or lower limit of quantitation (LOQ) of the analytical method for THS 2.2, the values used for calculating the relative reduction between THS 2.2 and 3R4F were imputed as follows: for results < LOD, the value was estimated by the LOD; for results < LOQ but > LOD, the value was estimated by the LOQ.
When, across all conditions, more than two replicate values used for calculating reduction were below the LOQ for a compound, the 0.95 confidence intervals were not reported for that compound. The number of acceptable values below the LOQ were considered individually in THS 2.2 and 3R4F emissions.
No result of reduction was reported when more than two out of five replicates measured for a constituent in 3R4F reference cigarette mainstream smoke were below the LOQ.

Threshold of robust reduction

Each HPHC was categorized to one of eleven individual thresholds of robust reduction. The first threshold category was established for cases demonstrating a minimum of 40% reduction, and the next successive limits followed a progression using 5% increments until the last threshold, which was fixed at 99%.
A particular HPHC reduction belongs to a category if the lower-bound of the 0.95 confidence interval of this HPHC reduction exceeds or equals the threshold value defining the corresponding category. This statistical test, which is illustrated in Figure 1, is unilateral and can detect non-inferiority with at least 0.975 probability. For each investigated HPHC, the closest threshold demonstrating the non-inferiority of reduction for all considered climatic

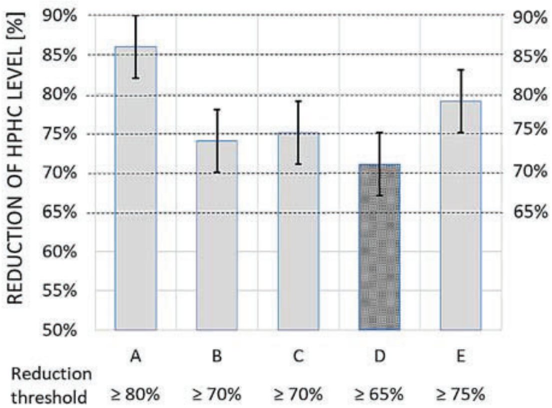

Figure 1. Example of the non-inferiority test used in the determination of threshold of robust reduction across A, B, C, D and E conditions. Error bars delimit 0.95 confidence intervals of the means. The lowest-bound (D) is right above 65% and the considered HPHC therefore belongs to the corresponding threshold category (i.e., ≥65%).

conditions served to determine the threshold of robust reduction. The threshold of robust reduction is defined by the lowest lower-bound across all confidence intervals of considered conditions.

RESULTS

The dataset of aerosol/smoke constituent yields is available in Supplementary Information Table S2. The descriptive statistics for the concentrations of the investigated constituents generated in THS 2.2 aerosol and 3R4F cigarette mainstream smoke are compiled in Supplementary information Table S3 and Supplementary information Table S4, respectively. The means and standard deviations of the yields of the constituents investigated in mainstream aerosol/smoke were calculated among the replicates analyzed for each product and condition and are reported in Appendix A. The means and standard deviations calculated from nicotine-adjusted and volume-adjusted yields are reported in Appendixes B and C, respectively. These tables summarize the results for 57 analytes, including nicotine, nicotine-free dry particulate matter (NFDPM), and glycerol, and 54 HPHCs for all tested conditions.

Table 2. Puff count for 3R4F as function of climatic conditions.

Conditions (abbreviation)	Puff number mean ± SD (n)
ISO (S)	10.58 ± 0.40 (83)
Hot and Very Humid (H)	12.26 ± 1.09 (83)
Hot and Dry (D)	9.18 ± 0.62 (84)

From respective means calculated over all test replicates and reported in Table 2, the changes of puff numbers in 3R4F calculated relatively to ISO conditions decreased by 13% under "Hot and Dry" conditions, whereas the number increased by 16% under "Hot and Very Humid" conditions. The relative reductions in HPHC concentrations in THS 2.2 aerosol were calculated as percentages of the concentrations in 3R4F cigarette mainstream smoke. Out of the 54 HPHCs investigated in THS 2.2 aerosol, 14 presented various proportions of determined values falling below the LOQ (1-aminonaphthalene, 2-aminonaphthalene, 3-aminobiphenyl, 4-aminobiphenyl, arsenic, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, cadmium, chromium, lead, nickel, quinoline, selenium, and vinyl chloride). These cases required particular attention because of computational limitations. Supplementary Information Table S5 presents the imputed compounds and the proportions of > LOQ cases per product. The preponderance of imputed values resulted in a computational optimization shortage, such that the 0.95 confidence intervals of reduction were not calculated. Instead, the results reported in Supplementary Information Table S6 for 11 of these compounds are the minimum and maximum reduction means observed over the combinations. Because more than 90% of the values were below the LOQ for both THS 2.2 and the 3R4F cigarette, the calculation of reduction was not applicable for chromium, nickel, and selenium. Excluding these three HPHCs, the average reductions in HPHC concentrations were calculated across all smoking-machine climatic conditions used to generate THS 2.2 aerosol and 3R4F smoke. For lead and quinoline, no reductions were calculated for conditions where more than two out of five 3R4F replicates had values below the LOQ. Consequently, the number of HPHCs used for calculating the average reductions varied among the conditions (n = 50 or 51). Figure 2 (reprinted with permission from (1)) summarizes the average HPHC reductions calculated for each combination

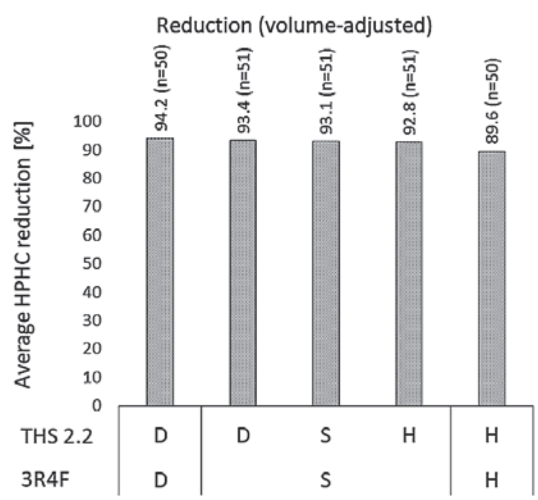

Figure 2. Average reduction in HPHC in volume-adjusted yields. Comparison of overall relative reduction across various ambient conditions using volume-adjusted values. THS 2.2 aerosol levels are calculated as a percentage of 3R4F cigarette mainstream smoke levels. D, S, and H stand for Dry (Hot and Dry), ISO (Mediterranean), and Humid (Hot and Very Humid) conditions. The respective conditions identified by D, S, and H are described in Table 1. The number of HPHCs (n) considered for calculation of the average reductions varied among the different combinations of conditions.

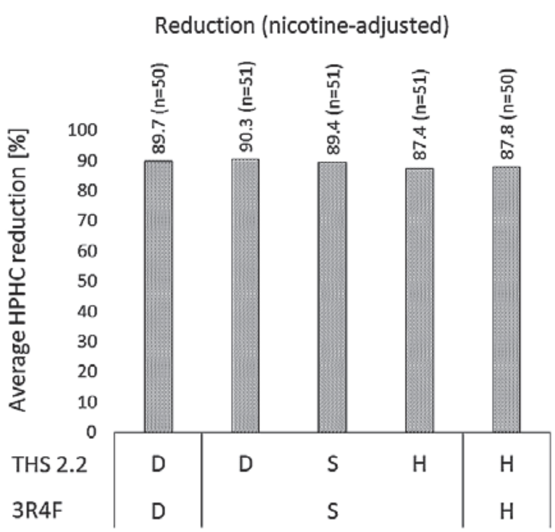

Figure 3. Average reduction in nicotine-adjusted HPHC yields. Comparison of overall relative reduction across various ambient conditions using nicotine-adjusted values. THS 2.2 aerosol levels are calculated as a percentage of 3R4F cigarette mainstream smoke levels. D, S, and H stand for Dry (Hot and Dry), ISO (Mediterranean), and Humid (Hot and Very Humid) conditions. The respective conditions identified by D, S, and H are described in Table 1. The number of HPHCs (n) considered for calculation of the average reductions varied among the different combinations of conditions.

of climatic conditions and is based on puff volume-adjusted yields (range 89.6–94.2%). Figure 3 (reprinted with permission from (1)) summarizes the average HPHC reductions calculated for each combination of climatic conditions and is based on nicotine-adjusted yields (range 87.4–90.3%).

For aerosol generated by THS 2.2 under the three investigated ambient conditions, the average reduction in puff volume-adjusted HPHC concentrations relative to 3R4F was in the range of 92.8–93.4% when compared only against the ISO ambient conditions used for the 3R4F cigarette (range 87.4–90.3%, by using nicotine-adjusted data).

The lowest reduction performance achieved for individual HPHCs across all climatic conditions was used to define the threshold for robust reduction. The 0.95 confidence intervals for reductions used to calculate the low boundaries of reduction associated with the lowest conditions are reported in Supplementary Information Table S7. In the cases where it was not possible to calculate confidence intervals, for each imputed compound, we used the lowest mean value of reduction observed within the compared conditions.

The threshold of reduction was derived from volume-adjusted yields. Table 5 presents a summary of the thresholds of robust reduction of THS 2.2 tested under various climatic conditions, but compared with 3R4F tested under ISO climatic conditions. The results are presented for 51 compounds, the calculation of reduction for three compounds being not applicable. Comparing with 3R4F under ISO climatic conditions showed that the reduction of HPHC levels in THS 2.2 aerosol was greater than 90% for 39 compounds. The thresholds of reduction for the other compounds ranged from 40% to 85%.

Using 3R4F values generated under ISO conditions as a reference, the seven compounds presented in Table 3 were characterized by changes of threshold values when THS 2.2 processed under ISO climatic conditions was compared with extreme climatic conditions. For HCN, *m*-cresol and toluene the threshold values changed from 99% to 95%. For the three compounds, the diminution of relative reduction means did not exceeded 0.3%. The changes of relative reduction means were more prominent for acetone (−0.5%), acetamide (−1.2%), mercury (−3.5%) and ammonia (−6%). In these five compounds, ammonia showed the highest diminution of threshold value with a difference of 10% whereas the three remainders equally decreased by 5%. "Hot and Dry" condition dominantly influenced ammonia, HCN and mercury whereas the effect of "Hot and Very Humid" conditions was more pronounced for *m*-cresol, toluene, acetone and acetamide.

Table 3. Mean relative reduction ($CI_{95\%}$) and threshold calculated with unvaried 3R4F conditions (ISO climatic). HPHCs (n = 7) impacted by change of reduction threshold between ISO and extreme climatic conditions.

Compound	THS 2.2 and 3R4F ISO cond. (Threshold)	THS 2.2 lowest [a] 3R4F ISO cond. (Threshold)	Lowest [b] bound
HCN	99.4 ± 0.1% (≥99)	99.1 ± 0.2% (≥95)	D
m-Cresol	99.2 ± 0.1% (≥99)	99.0 ± 0.2% (≥95)	H
Toluene	99.2 ± 0.1% (≥99)	99.0 ± 0.1% (≥95)	H
Acetone	95.7 ± 0.6% (≥95)	95.2 ± 0.6% (≥90)	H
Acetamide	78.4 ± 2.0% (≥75)	77.16 ± 2.2% (≥70)	H
Ammonia	68.1 ± 2.7% (≥65)	62.1 ± 3.1% (≥55)	D
Hg	52.7 ± 4.8% (≥45)	49.2 ± 5.1% (≥40)	D

[a] Relative reduction corresponding to extreme climatic condition used for testing THS 2.2 which resulted in the lowest-bound value
[b] Climatic condition used for testing THS 2.2 which resulted in the lowest-bound value.
Abbreviations: $CI_{95\%}$ = 95% confidence interval; cond. = conditions; HCN = hydrogen cyanide; Hg = mercury; HPHC = harmful and potentially harmful constituent; D = Hot and Dry; H = Hot and Very Humid

In contrast to Table 5, Table 6 includes results that show the influence of 3R4F subjected to extreme climatic conditions. It presents a summary of the thresholds of robust reduction resulting from non-inferiority tests conducted over the five climatic combinations considered in the comparison of THS 2.2 and 3R4F. Here, 38 compounds demonstrated a robust reduction greater than 90%. In the low range, mercury, acrylamide, and phenol demonstrated a reduction not exceeding 40%. The remaining compounds showed threshold reduction values distributed between 50% and 85%.

Reduction threshold values decreased for fifteen HPHCs with the introduction of comparisons with differing 3R4F conditions. For this group of constituents, percent relative differences derived from volume-adjusted yields were calculated in "Hot and Very Humid" relative to ISO climatic conditions. Results in Table 4 show significant decreases of means in the range of 11–76% for 3R4F

Table 4. Mean differences ($CI_{95\%}$) of volume-adjusted yields in "Hot and Very Humid" relative to ISO climatic conditions.

Compound	3R4F (%)	THS 2.2 (%)
Acetaldehyde	−11 ± 7	14 ± 15 [a]
Acetamide	−33 ± 9	6 ± 8 [a]
Acrylamide	−49 ± 10	−4 ± 9 [a]
Ammonia	−22 ± 9	3 ± 7 [a]
Butyraldehyde	−13 ± 8	6 ± 11 [a]
Ethylene oxide	−11 ± 6	33 ± 11
Formaldehyde	−55 ± 15	−11 ± 24 [a]
Hg	−18 ± 11	7 ± 7.1 [a]
MEK	−13 ± 8	27 ± 23
NAB	−21 ± 7	1 ± 9 [a]
Nitrobenzene	−59 ± 9	−35 ± 6
o-Cresol	−62 ± 12	40 ± 26
p-Cresol	−65 ± 11	35 ± 23
Phenol	−76 ± 14	25 ± 23
Pyridine	−18 ± 7	−3 ± 5 [a]

[a] Uncertainty of the mean equals or exceeds the mean value
Abbreviations: $CI_{95\%}$ = 95% confidence interval; MEK = methyl ethyl ketone; Hg = mercury; NAB = *N*-nitrosoanabasine

smoke. In the low range, values can be associated with the volume-adjustment corresponding to the relative difference of 16% in puff number, whereas higher values can be predominantly attributed to the change in 3R4F smoke composition. Focusing on THS 2.2, when the uncertainty of the mean equaled or exceeded the mean value, which was observed for nine aerosol constituents, then the statistical resolution did not lead to a reliable conclusion about variations within the calculated relative differences. To lower this uncertainty and obtain more accurate results, additional replicates would have been needed. Nevertheless, for ethylene oxide, methyl ethyl ketone, *o*-cersol, *p*-cresol and phenol, the statistical resolution was found acceptable to conclude that changes in reduction thresholds resulted from volume-adjusted yields decreasing in 3R4F smoke and increasing in THS 2.2 aerosol. Regardless statistical resolution, the high levels of relative differences found for the six other HPHCs suggested a dominant influence of 3R4F in decreases of reduction thresholds.

The greatest differences between reduction threshold categories linked to the change in 3R4F reference conditions were observed for acetamide (−10%), formaldehyde (−20%), and nitrobenzene (−20%). In a group of compounds falling below the threshold of 40%, important changes were also observed for acrylamide (−40%) and mercury (−15%). Phenol was the most impacted with a threshold dropping from 85% down to below 40% with the introduction of varied 3R4F conditions. The overlap of the confidence intervals of the means ($CI_{95\%}$) of the volume-adjusted yields between THS 2.2 (2.8 ± 0.3 ng/mL) and 3R4F (5.7 ± 3.2 ng/mL) explained the substantial drop in reduction threshold value for phenol.

The examples for individual HPHCs combined in Figure 4 present graphics of volume-adjusted yields placed contiguously to graphics of respective relative reductions. Data are extracted from Supplementary Information Tables S 3, S 4 and S 7. Means and 0.95 confidence intervals of volume-adjusted yields for THS 2.2 and 3R4F are also included to show the diversity between products and conditions and to identify cases that would dominantly influence changes in relative reductions. This representation can be used for the identification of factors, across climatic conditions, which had dominant influences on volume-adjusted yields and relative reductions. The figures provide visual evidence of the substantial influence of extreme climatic conditions on the levels of volume-adjusted yields of acetamide, acrylamide, formaldehyde, nitrobenzene, mercury, and phenol in 3R4F smoke. Also, the relative reduction means and the threshold lines presented in the corresponding graphs clearly highlighted the influence of climatic conditions on relative reduction which can be linked to the respective volume-adjusted yields.

DISCUSSION

Robustness of average reduction

By using ISO climatic conditions and considering 51 constituents, the average reduction in puff volume-adjusted HPHC concentrations in THS 2.2 aerosol relative to 3R4F cigarette smoke was found to be 93.1% (89.4% using nicotine-adjusted values). This is in line with published results (11), where the majority of HPHCs measured in THS 2.2 aerosol were reduced by more than 90%. When retaining ISO climatic conditions for the 3R4F reference cigarette and applying "Hot and Dry" and "Hot and Very Humid" conditions for THS 2.2, the average reductions based on puff volume-adjusted concentrations for THS 2.2 were in the range of 92.8–93.4%. These results demonstrated the very robust performance of THS 2.2 for overall HPHC reduction. When using the five combinations of conditions considered in this study - that is, when the 3R4F cigarette results produced under extreme climatic conditions were included - the good performance of THS 2.2 was also demonstrated by relative reductions in a slightly broader range of 89.6–94.2%. When considering the nicotine-adjusted approach, irrespective of the conditions used for the 3R4F reference cigarette, the performance of HPHC reduction in THS 2.2 was maintained in the range of 87.4–90.3%.

The overall HPHC reduction values obtained in both the volume-adjusted and nicotine-adjusted approaches were within narrow ranges. Although the nicotine-adjusted approach showed lower reduction values than the volume-adjusted approach for the same conditions, this difference can only be attributed to the two different methodologies and not to the robustness of performance of THS 2.2. In the most general sense, the robustness of the performance of THS 2.2 is reflected in the values obtained through the comparison of HPHC reduction across different climatic conditions and not by the absolute values of reduction.

When the 3R4F conditions were kept constant (i.e., "Mediterranean"), the reduction determined with the volume-adjusted approach resulted in lower variability (max – min: 0.6%) than that with the nicotine-adjusted approach (max – min: 2.9%). Indeed, in contrast with the first case, where the total puff volume was invariant, the higher variability observed in the second case can simply be explained by the influence of the variability of nicotine

Table 5. HPHCs categorized by thresholds of robust reduction: THS 2.2 over all climatic conditions *vs.* 3R4F ISO climatic conditions.

Compounds	n	Threshold
1-Aminonaphthalene, 2-aminonaphthalene, 3-aminobiphenyl, 1,3-butadiene, acrylonitrile, benzene, Cd, ethylene oxide, isoprene, *p*-cresol	10	≥99
4-Aminobiphenyl, CO, crotonaldehyde, HCN, MEK, *m*-cresol, NAB, NNK, NNN, NAT, NO, NO_x, *o*-cresol, *o*-toluidine, Pb, quinoline, resorcinol, styrene, toluene, vinyl chloride	20	≥95
Acetone, acrolein, benzo[*a*]anthracene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, formaldehyde, hydroquinone, propylene oxide, pyrene	9	≥90
As, acetaldehyde, propionaldehyde, phenol	4	≥85
Catechol, pyridine	2	≥80
Butyraldehyde, nitrobenzene	2	≥75
Acetamide	1	≥70
–	0	≥65
Acrylamide	1	≥60
Ammonia	1	≥55
–	0	≥50
–	0	≥45
Hg	1	≥40

Table 6. HPHCs categorized by thresholds of robust reduction: THS 2.2 *vs.* 3R4F over all climatic conditions.

Compounds	n	Threshold
1-Aminonaphthalene, 2-aminonaphthalene, 3-aminobiphenyl, 1,3-butadiene, acrylonitrile, benzene, Cd, isoprene	8	≥99
4-Aminobiphenyl, CO, crotonaldehyde, ethylene oxide, HCN, *m*-cresol, NNK, NNN, NAT, NO, NO_x, *o*-toluidine, *p*-cresol, Pb, quinoline, resorcinol, styrene toluene, vinyl chloride	19	≥95
Acetone, acrolein, benzo[*a*]anthracene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, hydroquinone, MEK, NAB, *o*-cresol, propylene oxide, pyrene	11	≥90
As, propionaldehyde,	2	≥85
Acetaldehyde, catechol, formaldehyde	3	≥80
Pyridine	1	≥75
Butyraldehyde	1	≥70
–	0	≥65
Acetamide	1	≥60
Nitrobenzene	1	≥55
Ammonia	1	≥50
–	0	≥45
–	0	≥40
Acrylamide, Hg, phenol	3	<40

Abbreviations: As = arsenic; CO = carbon monoxide; HCN = hydrogen cyanide; Hg = mercury; HPHC = harmful and potentially harmful constituent; MEK = methyl ethyl ketone; NAB = *N*-nitrosoanabasine; NAT = *N*-nitrosoanatabine; NNK = 4-(*N*-nitrosomethylamino)-1-(3-pyridyl)-1-butanone; NNN = *N*-nitrosonornicotine; NO = nitrogen oxide; No_x = nitrogen oxides; Pb = lead

deliveries in THS 2.2 (see nicotine results in Appendix A). It should also be highlighted that the variability of nicotine deliveries cannot only be attributed to climatic conditions but also partly to the variability of the analytical method.

When the 3R4F results produced in "Hot and Dry" and "Hot and Very Humid" conditions were included in the evaluation, we observed that, on average, the range of reductions calculated with the volume-adjusted approach (max – min: 4.6%) exceeded that with the nicotine-adjusted approach (max – min: 2.9%). On the basis of these results, we concluded that the volume-adjusted approach is as sensitive as the nicotine-adjusted approach and, therefore, appropriately reflects the climatic influence on the actual reduction of HPHCs. It allows HPHC reduction rates to be determined independently of nicotine variations and nicotine levels specific to individual products and offers a practical approach for comparing the two products.

These results confirm our confidence in using the volume-adjusted approach, considering that the robustness of the nicotine yield could be addressed separately if this kind of assessment should ever be needed. For this reason, we have focused the discussion on the robustness of reduction of individual HPHCs, presented in the next section on the volume-adjusted approach.

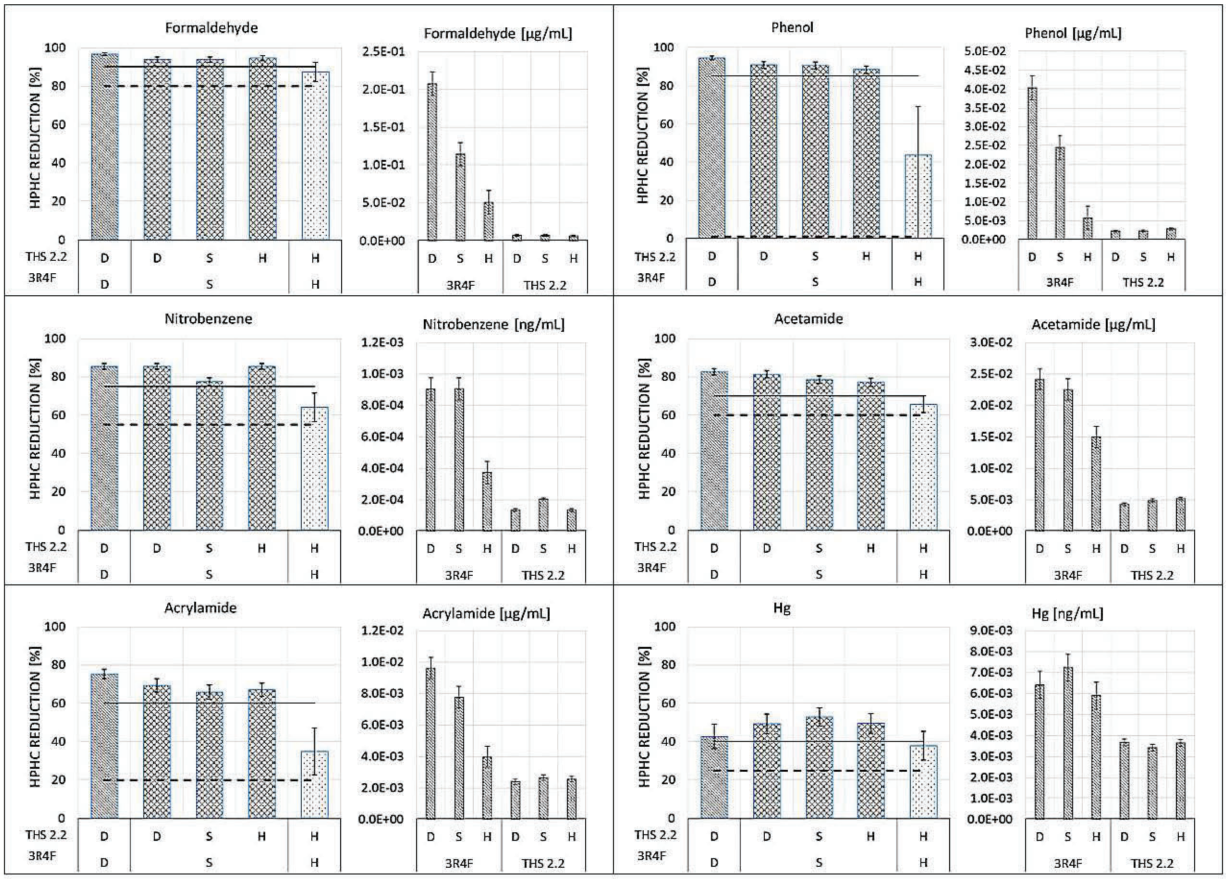

Figure 4. Cases substantially impacted by change in the climatic conditions used for smoking the 3R4F reference cigarette. Error bars delimit 0.95 confidence intervals of the respective means. The dashed lines indicate the closest threshold category sitting below the lowest-bound of the confidence intervals considered across all conditions. With the same principle, the solid lines indicate the threshold category defined for THS 2.2 relative to 3R4F under ISO climatic conditions. Threshold categories consist of a series of predefined discrete values. Contiguous graphs were paired by compounds, with relative reduction presented on the left and volume-adjusted yields on the right. The respective conditions identified by D, S, and H are described in Table 1.
Abbreviations: HPHC = harmful and potentially harmful constituent; Hg = mercury.

Individual HPHC and threshold of reduction

We could reasonably anticipate that moisture is a decisive factor that would influence the composition of THS 2.2 aerosol. Focusing on results produced by comparison with 3R4F values generated under ISO conditions as a reference, decreases of 1–5% in reduction thresholds were observed for a limited number of seven HPHCs, whereas others were left unchanged. THS 2.2 exposed to “Hot and Very Humid” conditions caused significant decreases in reduction thresholds for four compounds, whereas three compounds were impacted by “Hot and Dry” conditions. Considering that the same temperature of 30 °C was set for the two extreme climatic conditions, results indicated that decreases in reduction thresholds observed with extreme climatic conditions, keeping 3R4F unvaried, can be attributed to moisture changes caused by humidity.

Focusing on a comparison across all conditions, the decreases in reduction thresholds was mainly caused by substantial influence of variant puff numbers used in calculations of volume-adjusted yields, which in some cases, were amplified by a lack of robustness of the composition of the 3R4F mainstream smoke. Indeed the results demonstrated that decreases in reduction thresholds, which were observed for fifteen constituents when THS 2.2 and 3R4F were both compared under “Hot and Very Humid” conditions, can be predominantly attributed to changes in volume-adjusted HPHC yields in 3R4F smoke.

It is important to mention that reduction thresholds derived from volume-adjusted yields, which is the approach carried out in this study, can compare to results derived from yields-per-stick only if all products count the same puff numbers, regardless of the conditions. The volume-adjusted yield is an approach proposed in absence of standards to compare aerosol and/or smoke yields across different product categories (e.g., cigarettes, e-cigarettes, heated tobacco products).The number of puffs delivered by the THS 2.2 is constant, meaning that volume-adjusted yields produced under HCI with various climatic conditions were not influenced by changes in puff numbers. In contrast to THS 2.2, climatic conditions had a substantial effect on the number of puffs delivered by the 3R4F cigarette which in turn amplified the variability of volume-adjusted yields across conditions. In 3R4F smoke, this effect can be attributed to a lack of proportionality between puff number and yield per cigarette and was considered as the cause of decreases in reduction thresholds observed under certain conditions for a small number of HPHCs.

Although the differences observed in overall reduction were marginal, they were nonetheless a little more pronounced when the investigation focused on individual compounds. The small number of imputed compounds and the minor variation in their individual absolute reductions substantiated the good robustness of the performance of THS 2.2 for the reduction of the HPHCs investigated under various climatic conditions. The limited number of individual compounds that were considerably impacted by climatic conditions explains the slight difference that is also observed from overall reduction perspective.

CONCLUSIONS

The present study has demonstrated the robustness of THS 2.2, a heat-not-burn tobacco product, in reducing the levels of HPHCs in the aerosol produced under extreme temperature and humidity conditions corresponding to actual climatic zones where the product could be used. The study was designed to evaluate the robustness of the product used in normal conditions as opposed, for instance, to assessment of its shelf life. Therefore, the test setup allowed us to simulate situations where THS 2.2 and 3R4F reference cigarettes were used in extreme temperature and humidity conditions after freshly opened packs were exposed for at least 48 h to the same experimental conditions. We used two climatic zones identified in the WHO guidelines as "Hot and Dry" climate (30 °C and 35% RH) and "Hot and Very Humid" climate (30 °C and 75% RH) to simulate extreme climatic conditions. In the context of this study, we accepted that the temperature and humidity used in accordance with ISO 3402 (22 °C and 60% RH) were sufficiently close to the defined "Subtropical and Mediterranean" climate zone (25 °C and 60% RH), to simulate a third category of climatic conditions. For both THS 2.2 and 3R4F reference cigarettes, the puffing intensity used was in accordance with the HCI standard, which was modified with regard to the temperature and humidity conditions considered in this study.

Evaluation of the robustness of reduction over all HPHCs in THS 2.2 aerosol in proportion to the levels measured in 3R4F smoke indicated that the volume-adjusted approach was comparable with the nicotine-adjusted approach. The general point to be made is that using volume-adjusted yields is an appropriate approach for comparing products of different categories, regardless of the possible nicotine levels. Therefore, volume-adjusted yields allow comparison of actual HPHC yields between different products and conditions, while nicotine-adjusted values more specifically address the users' adaption of puffing with regard to nicotine yields. The effectiveness of the approach presented here means that its application could confidently be envisaged within the broader scope of the evolution of this category of product.

Machine-smoking testing is useful for characterizing tobacco product emissions for design and regulatory purposes. The demonstration of product robustness substantiates the evidence package provided in the due diligence assessment of THS 2.2. In this context, the study demonstrated that, overall, the concentrations of investigated HPHCs were reduced by at least 90% under HCI and extreme climatic conditions, and this was established with a conservative approach including various climatic conditions for generating 3R4F cigarette mainstream smoke, which was used as a reference.

The concept of thresholds applied for robust concentration reduction was introduced in a previous study (16) to categorize each HPHC investigated in THS 2.2 as a function of its demonstrated level of robust reduction. The same methodology was adopted in this study, and, as expected, THS 2.2 showed a robust performance in terms of HPHC reductions.

More interestingly, we should highlight that the thresholds were, in all cases, defined by the statistically determined lowest level of reduction among the three climatic conditions applied in this study. This approach, combined with the level of confidence of the non-inferiority test (0.975) used in the statistical computations, clearly affirms that, for the three climatic zones - which include extreme temperature and humidity conditions - we have minimized the risk of overestimating the performance of THS 2.2.

SUPPORTING INFORMATION

The Supporting Information is available free of charge on the Publications website:
Supplementary Information Table S1 (PDF)
Supplementary Information Tables S2–S7 (XLXS)

AUTHOR INFORMATION

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Corresponding author:

Laurent Poget
PMI R&D, Philip Morris Products S.A.
Quai Jeanrenaud 5
CH-2000 Neuchâtel, Switzerland
E-mail:laurent.poget@pmi.com

Appendix A. Mean (± SD) of compound deliveries investigated in 3R4F mainstream smoke and THS 2.2 aerosol, measured under various climatic conditions.

Item	3R4F			THS 2.2			Unit
Identification	35% RH / 30	60% RH / 22	75% RH / 30	35% RH / 30	60% RH / 22	75% RH / 30	
ISO parameters and product-specific constituents							
Glycerol	2.28 ± 0.15	2.37 ± 0.21	2.26 ± 0.19	4.25 ± 0.20	4.68 ± 0.29	4.16 ± 0.16	[mg/item]
Nicotine	2.01 ± 0.14	1.91 ± 0.14	1.41 ± 0.14	1.46 ± 0.04	1.42 ± 0.04	1.24 ± 0.06	[mg/item]
NFDPM	30.9 ± 1.0	31.2 ± 1.9	38.3 ± 1.5	11.3 ± 1.1	12.2 ± 0.9	12.6 ± 1.2	[mg/item]
Carbon monoxide	26.9 ± 0.7	30.5 ± 0.9	31.7 ± 1.1	0.45 ± 0.04	0.61 ± 0.02	0.47 ± 0.02	[mg/item]
Ammonia	37.8 ± 2.9	29.2 ± 1.9	29.1 ± 2.1	12.5 ± 0.4	10.5 ± 0.4	10.8 ± 0.7	[µg/item]
Carbonyls							
Formaldehyde	100.9 ± 11.8	64.9 ± 5.1	31.7 ± 1.5	4.69 ± 0.99	4.67 ± 0.44	4.12 ± 0.99	[µg/item]
Acetaldehyde	1730 ± 50	1670 ± 90	1630 ± 100	190 ± 16	212 ± 20	240 ± 28	[µg/item]
Acetone	758 ± 23	746 ± 49	707 ± 45	36.3 ± 4.8	37.2 ± 3.8	41.7 ± 5.3	[µg/item]
Acrolein	191 ± 6	186 ± 11	169 ± 13	9.22 ± 1.39	9.86 ± 1.45	13.25 ± 2.85	[µg/item]
Propionaldehyde	134 ± 3	133 ± 9	131 ± 8	12.4 ± 1.5	13.9 ± 1.5	17.3 ± 2.3	[µg/item]
Crotonaldehyde	51.9 ± 2.3	50.9 ± 3.9	47.8 ± 3.3	1.73 ± 0.23	1.81 ± 0.20	2.18 ± 0.39	[µg/item]
Methyl ethyl ketone	204 ± 6	209 ± 17	199 ± 13	7.38 ± 1.02	7.96 ± 0.97	10.01 ± 1.45	[µg/item]
Butyraldehyde	63.6 ± 2.7	62.3 ± 4.7	59.5 ± 4.3	13.0 ± 1.0	14.4 ± 1.2	15.2 ± 1.2	[µg/item]
Hydrogen cyanide	438 ± 20	475 ± 19	467 ± 25	4.74 ± 0.33	3.09 ± 0.44	3.04 ± 0.18	[µg/item]
Mercury	3.70 ± 0.22	4.44 ± 0.32	4.08 ± 0.48	2.42 ± 0.10	2.25 ± 0.06	2.41 ± 0.15	[ng/item]
Trace metals (except mercury)							
Cadmium	93.9 ± 6.6	101.7 ± 7.1	116.6 ± 6.0	0.13*	0.17*	0.20*	[ng/item]
Lead	26.4*	26.9 ± 1.1	30.5 ± 1.6	0.94*	0.49*	1.17*	[ng/item]
Chromium	11.9*	17.4*	17.4*	3.31*	3.31*	3.31*	[ng/item]
Nickel	12.9*	18.9*	12.9*	15.9*	15.9*	15.9*	[ng/item]
Arsenic	8.23 ± 0.67	8.63 ± 0.78	8.94 ± 0.38	1.24*	1.20*	1.20*	[ng/item]
Selenium	4.42*	4.42*	4.42*	0.60*	0.71*	0.83*	[ng/item]
Nitrogen oxides							
NO	446 ± 26	489 ± 16	539 ± 56	16.3 ± 0.7	18.3 ± 1.0	18.2 ± 0.2	[µg/item]
NO_x	483 ± 25	538 ± 19	601 ± 66	17.3 ± 0.7	19.5 ± 1.2	19.0 ± 0.4	[µg/item]
Volatiles							
1,3-Butadiene	63.6 ± 1.9	62.4 ± 3.2	70.0 ± 2.7	0.25 ± 0.01	0.28 ± 0.03	0.32 ± 0.02	[µg/item]
Isoprene	804 ± 28	841 ± 50	919 ± 37	1.83 ± 0.14	2.19 ± 0.17	2.60 ± 0.20	[µg/item]
Acrylonitrile	30.6 ± 1.1	32.4 ± 1.1	32.7 ± 1.2	0.18 ± 0.02	0.17 ± 0.03	0.19 ± 0.03	[µg/item]
Benzene	97.8 ± 3.4	101.8 ± 3.8	106.5 ± 3.2	0.59 ± 0.03	0.60 ± 0.03	0.61 ± 0.02	[µg/item]
Toluene	185 ± 7	198 ± 7	209 ± 8	1.85 ± 0.12	2.11 ± 0.16	2.25 ± 0.15	[µg/item]
Semi-volatiles							
Pyridine	35.7 ± 3.1	38.3 ± 1.3	34.7 ± 2.2	5.76 ± 0.34	7.47 ± 0.25	7.26 ± 0.23	[µg/item]
Quinoline	0.51 ± 0.05	0.48 ± 0.03	0.32*	0.013*	0.013*	0.013*	[µg/item]
Styrene	23.0 ± 1.1	25.7 ± 0.9	26.5 ± 1.3	0.62 ± 0.03	0.64 ± 0.03	0.69 ± 0.03	[µg/item]
Nitrobenzene	0.43 ± 0.03	0.52 ± 0.04	0.30 ± 0.02	0.087 ± 0.004	0.134 ± 0.008	0.087 ± 0.007	[µg/item]
Phenols and acid derivatives							
Hydroquinone	76.2 ± 4.3	84.1 ± 1.4	96.5 ± 2.1	5.45 ± 0.85	7.75 ± 0.55	8.67 ± 0.81	[µg/item]
Resorcinol	1.70 ± 0.08	1.87 ± 0.05	1.94 ± 0.03	0.029 ± 0.004	0.039 ± 0.004	0.056 ± 0.008	[µg/item]
Catechol	91.7 ± 6.1	89.7 ± 1.6	79.2 ± 1.4	11.7 ± 1.3	14.9 ± 0.8	15.6 ± 1.3	[µg/item]
Phenol	19.69 ± 2.67	13.94 ± 1.25	3.39 ± 0.45	1.45 ± 0.32	1.50 ± 0.17	1.86 ± 0.12	[µg/item]
p-Cresol	11.77 ± 1.44	9.80 ± 0.70	3.60 ± 0.35	0.069 ± 0.013	0.071 ± 0.008	0.096 ± 0.009	[µg/item]
m-Cresol	4.57 ± 0.55	3.81 ± 0.28	1.43 ± 0.12	0.033 ± 0.006	0.035 ± 0.004	0.046 ± 0.003	[µg/item]
o-Cresol	5.95 ± 0.77	4.91 ± 0.41	1.94 ± 0.19	0.067 ± 0.015	0.082 ± 0.009	0.114 ± 0.012	[µg/item]
Acetamide	11.80 ± 1.00	12.84 ± 0.51	8.85 ± 0.99	2.78 ± 0.26	3.21 ± 0.11	3.38 ± 0.17	[µg/item]
Acrylamide	4.69 ± 0.50	4.42 ± 0.26	2.34 ± 0.29	1.57 ± 0.15	1.75 ± 0.09	1.68 ± 0.07	[µg/item]
Polycyclic aromatic hydrocarbons							
Pyrene	89.1 ± 7.0	80.8 ± 2.6	102.5 ± 10.5	7.27 ± 1.31	5.66 ± 0.49	5.85 ± 1.02	[ng/item]
Benzo[*a*]anthracene	28.5 ± 1.7	26.8 ± 1.9	30.2 ± 2.5	1.51 ± 0.28	1.39 ± 0.08	1.39 ± 0.16	[ng/item]
Benzo[*a*]pyrene	14.5 ± 0.9	12.9 ± 1.0	16.1 ± 1.6	1.03*	1.00*	1.00*	[ng/item]
Dibenz[*a,h*]anthracene	1.47 ± 0.14	1.43 ± 0.13	1.53 ± 0.18	0.10*	0.10*	0.10*	[ng/item]

Appendix A. Continued.

Item	3R4F			THS 2.2			Unit
Identification	35% RH / 30	60% RH / 22	75% RH / 30	35% RH / 30	60% RH / 22	75% RH / 30	
Epoxides and vinyl chloride							
Vinyl chloride	76.8 ± 4.2	83.0 ± 2.7	86.9 ± 4.2	3.73*	3.73*	3.73*	[ng/item]
Ethylene oxide	32.5 ± 1.4	36.0 ± 1.2	37.1 ± 2.6	0.27 ± 0.01	0.27 ± 0.01	0.35 ± 0.03	[µg/item]
Propylene oxide	1.47 ± 0.04	1.64 ± 0.06	1.73 ± 0.05	0.13 ± 0.00	0.15 ± 0.01	0.16 ± 0.02	[µg/item]
Aromatic amines							
1-Aminonaphthalene	17.1 ± 1.1	18.7 ± 1.0	20.8 ± 1.6	0.070*	0.070*	0.070*	[ng/item]
2-Aminonaphthalene	10.7 ± 0.4	11.9 ± 0.9	16.0 ± 1.3	0.017*	0.016*	0.088*	[ng/item]
3-Aminobiphenyl	3.41 ± 0.16	3.80 ± 0.27	5.13 ± 0.61	0.029*	0.029*	0.029*	[ng/item]
4-Aminobiphenyl	2.58 ± 0.10	2.96 ± 0.17	4.08 ± 0.55	0.032 ± 0.013	0.047*	0.073 ± 0.018	[ng/item]
o-Toluidine	96.3 ± 16.4	94.7 ± 9.2	78.5 ± 3.3	0.65 ± 0.26	1.14 ± 0.09	0.62 ± 0.48	[ng/item]
Tobacco-specific nitrosamines							
NNN	321 ± 37	279 ± 9	219 ± 17	4.91 ± 0.30	5.38 ± 0.28	5.17 ± 0.30	[ng/item]
NAT	326 ± 12	282 ± 14	226 ± 17	11.3 ± 0.9	11.8 ± 0.7	10.6 ± 0.5	[ng/item]
NAB	40.1 ± 1.9	34.2 ± 2.1	28.1 ± 1.7	1.73 ± 0.13	1.75 ± 0.11	1.77 ± 0.09	[ng/item]
NNK	278 ± 22	276 ± 21	214 ± 17	5.52 ± 0.55	5.90 ± 0.52	5.47 ± 0.33	[ng/item]

Abbreviations: NAB = *N*-nitrosoanabasine; NAT = *N*-nitrosoanatabine; NNK = 4-(*N*-nitrosomethylamino)-1-(3-pyridyl)-1-butanone; NNN = *N*-nitrosonornicotine; NO = nitrogen oxide; NO_x = nitrogen oxides; NFDPM = nicotine free dry particulate matter; SD = standard deviation
For results <LOD, the values used in the calculation of mean and standard deviation were estimated by the LOD; for results <LOQ but >LOD, the values were estimated by the LOQ.
An asterisk (*) indicates when more than two out of five replicate values were below LOQ and standard deviation is not reported.

Appendix B. Mean (± SD) of nicotine-adjusted yields of compounds investigated in 3R4F mainstream smoke and THS 2.2 aerosol, measured under various climatic conditions.

Item	3R4F			THS 2.2			Unit
Identification	35% RH / 30	60% RH / 22	75% RH / 30	35% RH / 30	60% RH / 22	75% RH / 30	
ISO parameters and product-specific constituents							
Glycerol	1.13 ± 0.08	1.24 ± 0.11	1.61 ± 0.13	2.91 ± 0.14	3.29 ± 0.20	3.37 ± 0.13	[mg/item/mg nicotine]
Nicotine	1.00 ± 0.07	1.00 ± 0.07	1.00 ± 0.10	1.00 ± 0.03	1.00 ± 0.03	1.00 ± 0.05	[mg/item/mg nicotine]
NFDPM	15.4 ± 0.5	16.4 ± 1.0	27.2 ± 1.1	7.73 ± 0.79	8.60 ± 0.64	10.22 ± 0.99	[mg/item/mg nicotine]
Carbon monoxide	13.4 ± 0.4	16.0 ± 0.5	22.5 ± 0.8	0.31 ± 0.03	0.43 ± 0.01	0.38 ± 0.02	[mg/item/mg nicotine]
Ammonia	18.8 ± 1.4	15.3 ± 1.0	20.6 ± 1.5	8.53 ± 0.27	7.40 ± 0.28	8.72 ± 0.54	[µg/item/mg nicotine]
Carbonyls							
Formaldehyde	50.2 ± 5.9	34.0 ± 2.7	22.5 ± 1.1	3.21 ± 0.68	3.29 ± 0.31	3.34 ± 0.80	[µg/item/mg nicotine]
Acetaldehyde	863 ± 24	875 ± 46	1154 ± 74	130 ± 11	149 ± 14	194 ± 22	[µg/item/mg nicotine]
Acetone	378 ± 11	391 ± 26	502 ± 32	24.9 ± 3.3	26.2 ± 2.7	33.7 ± 4.3	[µg/item/mg nicotine]
Acrolein	95.1 ± 3.0	97.5 ± 5.9	120.0 ± 9.3	6.31 ± 0.95	6.94 ± 1.02	10.72 ± 2.31	[µg/item/mg nicotine]
Propionaldehyde	66.9 ± 1.3	69.7 ± 4.9	92.8 ± 5.8	8.46 ± 1.01	9.81 ± 1.04	13.98 ± 1.84	[µg/item/mg nicotine]
Crotonaldehyde	25.9 ± 1.1	26.7 ± 2.1	33.9 ± 2.3	1.19 ± 0.16	1.27 ± 0.14	1.77 ± 0.32	[µg/item/mg nicotine]
Methyl ethyl ketone	102 ± 3	110 ± 9	141 ± 9	5.05 ± 0.70	5.60 ± 0.68	8.10 ± 1.17	[µg/item/mg nicotine]
Butyraldehyde	31.7 ± 1.3	32.6 ± 2.5	42.2 ± 3.1	8.90 ± 0.65	10.12 ± 0.81	12.30 ± 0.97	[µg/item/mg nicotine]
Hydrogen cyanide	218 ± 10	249 ± 10	331 ± 18	3.24 ± 0.23	2.17 ± 0.31	2.46 ± 0.14	[µg/item/mg nicotine]
Mercury	1.84 ± 0.11	2.33 ± 0.17	2.89 ± 0.34	1.66 ± 0.07	1.58 ± 0.04	1.95 ± 0.12	[ng/item/mg nicotine]
Trace metals (except mercury)							
Cadmium	46.8 ± 3.3	53.3 ± 3.7	82.7 ± 4.2	0.088*	0.117*	0.165*	[ng/item/mg nicotine]
Lead	13.1*	14.1 ± 0.6	21.6 ± 1.1	0.64*	0.34*	0.95*	[ng/item/mg nicotine]
Chromium	5.93*	9.14*	12.38*	2.27*	2.33*	2.68*	[ng/item/mg nicotine]
Nickel	6.42*	9.92*	9.15*	10.9*	11.2*	12.9*	[ng/item/mg nicotine]
Arsenic	4.10 ± 0.33	4.52 ± 0.41	6.34 ± 0.27	0.85*	0.84*	0.97*	[ng/item/mg nicotine]
Selenium	2.20*	2.32*	3.14*	0.41*	0.50*	0.67*	[ng/item/mg nicotine]

Appendix B. Continued.

Item	3R4F			THS 2.2			Unit
Identification	35% RH / 30	60% RH / 22	75% RH / 30	35% RH / 30	60% RH / 22	75% RH / 30	
Nitrogen oxides							
NO	222 ± 13	256 ± 9	382 ± 40	11.2 ± 0.5	12.8 ± 0.7	14.8 ± 0.2	[µg/item/mg nicotine]
NO_x	241 ± 12	282 ± 10	426 ± 47	11.8 ± 0.5	13.7 ± 0.8	15.4 ± 0.3	[µg/item/mg nicotine]
Phenols and acid derivatives							
Hydroquinone	38.0 ± 2.2	44.1 ± 0.7	68.5 ± 1.5	3.73 ± 0.58	5.46 ± 0.39	7.02 ± 0.65	[µg/item/mg nicotine]
Resorcinol	0.85 ± 0.04	0.98 ± 0.02	1.38 ± 0.02	0.020 ± 0.003	0.027 ± 0.003	0.046 ± 0.006	[µg/item/mg nicotine]
Catechol	45.7 ± 3.0	47.0 ± 0.8	56.2 ± 1.0	8.03 ± 0.88	10.50 ± 0.55	12.61 ± 1.01	[µg/item/mg nicotine]
Phenol	9.81 ± 1.33	7.30 ± 0.65	2.41 ± 0.32	0.99 ± 0.22	1.06 ± 0.12	1.51 ± 0.09	[µg/item/mg nicotine]
p-Cresol	5.86 ± 0.72	5.13 ± 0.37	2.55 ± 0.25	0.047 ± 0.009	0.050 ± 0.005	0.078 ± 0.007	[µg/item/mg nicotine]
m-Cresol	2.28 ± 0.28	2.00 ± 0.15	1.01 ± 0.09	0.022 ± 0.004	0.024 ± 0.003	0.037 ± 0.003	[µg/item/mg nicotine]
o-Cresol	2.96 ± 0.38	2.58 ± 0.21	1.38 ± 0.13	0.046 ± 0.011	0.058 ± 0.006	0.092 ± 0.010	[µg/item/mg nicotine]
Acetamide	5.87 ± 0.50	6.73 ± 0.27	6.28 ± 0.71	1.90 ± 0.18	2.26 ± 0.08	2.74 ± 0.14	[µg/item/mg nicotine]
Acrylamide	2.34 ± 0.25	2.32 ± 0.13	1.66 ± 0.21	1.08 ± 0.11	1.23 ± 0.06	1.36 ± 0.06	[µg/item/mg nicotine]
Polycyclic aromatic hydrocarbons							
Pyrene	44.4 ± 3.5	42.4 ± 1.4	72.7 ± 7.4	4.98 ± 0.90	3.98 ± 0.35	4.74 ± 0.82	[ng/item/mg nicotine]
Benzo[*a*]anthracene	14.2 ± 0.9	14.1 ± 1.0	21.4 ± 1.8	1.04 ± 0.19	0.98 ± 0.06	1.12 ± 0.13	[ng/item/mg nicotine]
Benzo[*a*]pyrene	7.21 ± 0.47	6.74 ± 0.53	11.46 ± 1.12	0.70*	0.70*	0.81*	[ng/item/mg nicotine]
Dibenz[*a,h*]anthra-cene	0.73 ± 0.07	0.75 ± 0.07	1.08 ± 0.13	0.068*	0.070*	0.081*	[ng/item/mg nicotine]
Volatiles							
1,3-Butadiene	31.7 ± 1.0	32.7 ± 1.7	49.7 ± 1.9	0.17 ± 0.01	0.20 ± 0.02	0.26 ± 0.02	[µg/item/mg nicotine]
Isoprene	401 ± 14	441 ± 26	652 ± 26	1.25 ± 0.10	1.54 ± 0.12	2.10 ± 0.16	[µg/item/mg nicotine]
Acrylonitrile	15.2 ± 0.6	17.0 ± 0.6	23.2 ± 0.9	0.12 ± 0.01	0.12 ± 0.02	0.15 ± 0.02	[µg/item/mg nicotine]
Benzene	48.7 ± 1.7	53.3 ± 2.0	75.6 ± 2.3	0.40 ± 0.02	0.42 ± 0.02	0.50 ± 0.02	[µg/item/mg nicotine]
Toluene	92.3 ± 3.4	104.0 ± 3.9	148.4 ± 6.0	1.26 ± 0.08	1.48 ± 0.11	1.82 ± 0.12	[µg/item/mg nicotine]
Semi-volatiles							
Pyridine	17.8 ± 1.5	20.1 ± 0.7	24.6 ± 1.6	3.95 ± 0.23	5.26 ± 0.18	5.88 ± 0.18	[µg/item/mg nicotine]
Quinoline	0.25 ± 0.02	0.25 ± 0.01	0.23*	0.0089*	0.0091*	0.0105*	[µg/item/mg nicotine]
Styrene	11.5 ± 0.5	13.5 ± 0.5	18.8 ± 0.9	0.42 ± 0.02	0.45 ± 0.02	0.56 ± 0.02	[µg/item/mg nicotine]
Nitrobenzene	0.21 ± 0.02	0.27 ± 0.02	0.21 ± 0.01	0.060 ± 0.003	0.094 ± 0.005	0.071 ± 0.005	[µg/item/mg nicotine]
Epoxides and vinyl chloride							
Vinyl chloride	38.2 ± 2.1	43.5 ± 1.4	61.7 ± 3.0	2.55*	2.62*	3.02*	[ng/item/mg nicotine]
Ethylene oxide	16.2 ± 0.7	18.9 ± 0.6	26.4 ± 1.8	0.18 ± 0.01	0.19 ± 0.01	0.29 ± 0.02	[µg/item/mg nicotine]
Propylene oxide	0.73 ± 0.02	0.86 ± 0.03	1.23 ± 0.03	0.088 ± 0.003	0.109 ± 0.006	0.126 ± 0.012	[µg/item/mg nicotine]
Aromatic amines							
1-Aminonaphthalene	8.50 ± 0.54	9.79 ± 0.50	14.73 ± 1.17	0.048*	0.049*	0.057*	[ng/item/mg nicotine]
2-Aminonaphthalene	5.34 ± 0.22	6.26 ± 0.46	11.34 ± 0.90	0.011*	0.011*	0.071*	[ng/item/mg nicotine]
3-Aminobiphenyl	1.70 ± 0.08	1.99 ± 0.14	3.64 ± 0.43	0.020*	0.020*	0.023*	[ng/item/mg nicotine]
4-Aminobiphenyl	1.29 ± 0.05	1.55 ± 0.09	2.90 ± 0.39	0.022 ± 0.009	0.033*	0.059 ± 0.014	[ng/item/mg nicotine]
o-Toluidine	47.9 ± 8.2	49.6 ± 4.8	55.7 ± 2.3	0.44 ± 0.18	0.81 ± 0.06	0.50 ± 0.38	[ng/item/mg nicotine]
Tobacco-specific nitrosamines							
NNN	160 ± 19	146 ± 5	155 ± 12	3.36 ± 0.21	3.79 ± 0.20	4.19 ± 0.25	[ng/item/mg nicotine]
NAT	162 ± 6	148 ± 8	160 ± 12	7.75 ± 0.63	8.34 ± 0.46	8.57 ± 0.37	[ng/item/mg nicotine]
NAB	20.0 ± 0.9	17.9 ± 1.1	19.9 ± 1.2	1.18 ± 0.09	1.23 ± 0.08	1.43 ± 0.08	[ng/item/mg nicotine]
NNK	138 ± 11	145 ± 11	152 ± 12	3.78 ± 0.38	4.15 ± 0.37	4.43 ± 0.27	[ng/item/mg nicotine]

Abbreviations: NAB = *N*-nitrosoanabasine; NAT = *N*-nitrosoanatabine; NNK = 4-(*N*-nitrosomethylamino)-1-(3-pyridyl)-1-butanone; NNN = *N*-nitrosonornicotine; NO = nitrogen oxide; NO_x = nitrogen oxides; NFDPM = nicotine free dry particulate matter; SD = standard deviation
For results <LOD, the values used in the calculation of mean and standard deviation were estimated by the LOD; for results <LOQ but >LOD, the values were estimated by the LOQ.
An asterisk (*) indicates when more than two out of five replicate values were below LOQ and standard deviation is not reported.

Appendix C. Mean (± SD) of volume-adjusted yields of compounds investigated in 3R4F mainstream smoke and THS 2.2 aerosol, measured under various climatic conditions.

Item	3R4F			THS 2.2			Unit
Identification	35% RH / 30	60% RH / 22	75% RH / 30	35% RH / 30	60% RH / 22	75% RH / 30	
ISO parameters and product-specific constituents							
Glycerol	4.56 ± 0.38	4.13 ± 0.38	3.17 ± 0.21	6.43 ± 0.30	7.09 ± 0.44	6.30 ± 0.25	[µg/mL]
Nicotine	4.02 ± 0.31	3.32 ± 0.25	1.97 ± 0.15	2.21 ± 0.06	2.15 ± 0.07	1.87 ± 0.09	[µg/mL]
NFDPM	61.8 ± 2.4	54.3 ± 2.2	53.8 ± 3.9	17.1 ± 1.7	18.5 ± 1.4	19.1 ± 1.9	[µg/mL]
Carbon monoxide	53.8 ± 2.7	53.0 ± 0.8	44.4 ± 2.0	0.69 ± 0.06	0.93 ± 0.02	0.71 ± 0.03	[µg/mL]
Ammonia	71.2 ± 4.7	49.9 ± 3.2	38.8 ± 2.9	18.9 ± 0.6	15.9 ± 0.6	16.3 ± 1.0	[ng/mL]
Carbonyls							
Formaldehyde	207.6 ± 25.8	114.1 ± 9.5	50.7 ± 3.9	7.10 ± 1.50	7.08 ± 0.67	6.25 ± 1.49	[ng/mL]
Acetaldehyde	3560 ± 200	2930 ± 110	2600 ± 150	287 ± 24	321 ± 30	363 ± 42	[ng/mL]
Acetone	1560 ± 100	1310 ± 60	1130 ± 70	55.1 ± 7.3	56.4 ± 5.8	63.2 ± 8.1	[ng/mL]
Acrolein	393 ± 19	327 ± 18	270 ± 18	14.0 ± 2.1	14.9 ± 2.2	20.1 ± 4.3	[ng/mL]
Propionaldehyde	277 ± 19	233 ± 11	209 ± 13	18.7 ± 2.2	21.1 ± 2.2	26.2 ± 3.4	[ng/mL]
Crotonaldehyde	106.8 ± 6.4	89.4 ± 5.5	76.2 ± 5.6	2.63 ± 0.35	2.74 ± 0.30	3.31 ± 0.59	[ng/mL]
Methyl ethyl ketone	420 ± 22	367 ± 22	317 ± 21	11.2 ± 1.5	12.1 ± 1.5	15.2 ± 2.2	[ng/mL]
Butyraldehyde	130.8 ± 7.8	109.3 ± 5.5	95.0 ± 6.7	19.7 ± 1.4	21.8 ± 1.8	23.0 ± 1.8	[ng/mL]
Hydrogen cyanide	998 ± 255	807 ± 67	720 ± 28	7.18 ± 0.51	4.68 ± 0.67	4.61 ± 0.27	[ng/mL]
Mercury	6.40 ± 0.46	7.23 ± 0.77	5.89 ± 0.71	3.67 ± 0.16	3.41 ± 0.09	3.65 ± 0.23	[pg/mL]
Trace metals (except mercury)							
Cadmium	182 ± 14	176 ± 13	176 ± 11	0.19*	0.25*	0.31*	[pg/mL]
Lead	51.0*	43.5 ± 8.5	46.0 ± 2.5	1.43*	0.74*	1.77*	[pg/mL]
Chromium	23.0*	30.2*	26.1*	5.02*	5.02*	5.02*	[pg/mL]
Nickel	25.0*	32.8*	19.5*	24.1*	24.1*	24.1*	[pg/mL]
Arsenic	15.9 ± 1.2	14.9 ± 1.4	13.5 ± 0.6	1.87*	1.82*	1.82*	[pg/mL]
Selenium	8.55*	7.64*	6.67*	0.91*	1.08*	1.26*	[pg/mL]
Nitrogen oxides							
NO	814 ± 60	820 ± 39	752 ± 64	24.7 ± 1.0	27.7 ± 1.5	27.6 ± 0.3	[ng/mL]
NO_x	882 ± 59	901 ± 42	839 ± 77	26.2 ± 1.0	29.6 ± 1.8	28.9 ± 0.5	[ng/mL]
Phenols and acid derivatives							
Hydroquinone	156 ± 8	147 ± 3	163 ± 4	8.25 ± 1.29	11.75 ± 0.84	13.14 ± 1.22	[ng/mL]
Resorcinol	3.48 ± 0.21	3.28 ± 0.09	3.26 ± 0.03	0.044 ± 0.006	0.058 ± 0.005	0.086 ± 0.012	[ng/mL]
Catechol	188 ± 12	157 ± 4	133 ± 3	17.8 ± 1.9	22.6 ± 1.2	23.6 ± 1.9	[ng/mL]
Phenol	40.25 ± 5.03	24.41 ± 2.41	5.73 ± 0.89	2.20 ± 0.49	2.28 ± 0.27	2.82 ± 0.18	[ng/mL]
p-cresol	24.06 ± 2.73	17.15 ± 1.38	6.07 ± 0.75	0.10 ± 0.02	0.11 ± 0.01	0.15 ± 0.01	[ng/mL]
m-Cresol	9.35 ± 1.04	6.67 ± 0.55	2.41 ± 0.26	0.050 ± 0.010	0.053 ± 0.006	0.070 ± 0.005	[ng/mL]
o-Cresol	12.17 ± 1.46	8.61 ± 0.79	3.28 ± 0.40	0.10 ± 0.02	0.12 ± 0.01	0.17 ± 0.02	[ng/mL]
Acetamide	24.1 ± 2.0	22.5 ± 1.1	14.9 ± 2.0	4.21 ± 0.39	4.86 ± 0.17	5.13 ± 0.25	[ng/mL]
Acrylamide	9.60 ± 0.94	7.74 ± 0.49	3.95 ± 0.58	2.38 ± 0.23	2.65 ± 0.14	2.55 ± 0.11	[ng/mL]
Polycyclic aromatic hydrocarbons							
Pyrene	161 ± 8	139 ± 7	148 ± 15	11.02 ± 1.98	8.57 ± 0.75	8.87 ± 1.54	[pg/mL]
Benzo[*a*]anthracene	51.6 ± 1.4	46.0 ± 4.1	43.6 ± 3.7	2.29 ± 0.42	2.11 ± 0.12	2.10 ± 0.25	[pg/mL]
Benzo[*a*]pyrene	26.2 ± 1.0	22.1 ± 2.2	23.3 ± 2.3	1.55*	1.52*	1.52*	[pg/mL]
Dibenz[*a,h*]anthracene	2.66 ± 0.19	2.45 ± 0.26	2.20 ± 0.26	0.15*	0.15*	0.15*	[pg/mL]
Volatiles							
1,3-Butadiene	134 ± 4	109 ± 4	111 ± 4	0.38 ± 0.02	0.42 ± 0.04	0.48 ± 0.03	[ng/mL]
Isoprene	1690 ± 60	1470 ± 80	1450 ± 20	2.78 ± 0.22	3.32 ± 0.26	3.93 ± 0.31	[ng/mL]
Acrylonitrile	64.3 ± 1.6	56.8 ± 1.5	51.7 ± 2.6	0.27 ± 0.03	0.25 ± 0.04	0.29 ± 0.04	[ng/mL]
Benzene	206 ± 6	178 ± 5	169 ± 8	0.90 ± 0.04	0.91 ± 0.05	0.93 ± 0.04	[ng/mL]
Toluene	390 ± 13	347 ± 7	331 ± 20	2.80 ± 0.18	3.20 ± 0.24	3.41 ± 0.22	[ng/mL]
Semi-volatiles							
Pyridine	74.9 ± 3.6	67.1 ± 1.5	54.9 ± 4.7	8.73 ± 0.51	11.32 ± 0.38	11.01 ± 0.34	[ng/mL]
Quinoline	1.07 ± 0.08	0.83 ± 0.03	0.50*	0.020*	0.020*	0.020*	[ng/mL]
Styrene	48.4 ± 2.0	45.0 ± 1.2	42.0 ± 2.8	0.94 ± 0.05	0.97 ± 0.04	1.05 ± 0.05	[ng/mL]
Nitrobenzene	0.90 ± 0.10	0.90 ± 0.08	0.37 ± 0.02	0.13 ± 0.01	0.20 ± 0.01	0.13 ± 0.01	[ng/mL]

Appenix C. Continued.

Item	3R4F			THS 2.2			Unit
Identification	35% RH / 30	60% RH / 22	75% RH / 30	35% RH / 30	60% RH / 22	75% RH / 30	
Epoxides and vinyl chloride							
Vinyl chloride	156 ± 9	142 ± 4	128 ± 6	5.65*	5.65*	5.65*	[pg/mL]
Ethylene oxide	66.2 ± 3.2	61.6 ± 1.8	54.7 ± 3.1	0.41 ± 0.02	0.40 ± 0.02	0.54 ± 0.04	[ng/mL]
Propylene oxide	3.35 ± 0.08	2.82 ± 0.06	2.64 ± 0.16	0.20 ± 0.01	0.23 ± 0.01	0.24 ± 0.02	[ng/mL]
Aromatic amines							
1-Aminonaphthalene	34.7 ± 2.1	31.6 ± 1.6	29.6 ± 2.5	0.11*	0.11*	0.11*	[pg/mL]
2-Aminonaphthalene	21.8 ± 0.9	20.2 ± 1.5	22.7 ± 1.5	0.025*	0.024*	0.133*	[pg/mL]
3-Aminobiphenyl	6.94 ± 0.36	6.44 ± 0.45	7.29 ± 0.72	0.044*	0.044*	0.044*	[pg/mL]
4-Aminobiphenyl	5.26 ± 0.18	5.02 ± 0.28	5.80 ± 0.67	0.048 ± 0.020	0.071*	0.111 ± 0.027	[pg/mL]
o-Toluidine	196 ± 34	160 ± 17	112 ± 5	0.98 ± 0.39	1.73 ± 0.14	0.94 ± 0.72	[pg/mL]
Tobacco-specific nitrosamines							
NNN	635 ± 74	479 ± 24	363 ± 29	7.44 ± 0.46	8.15 ± 0.43	7.84 ± 0.46	[pg/mL]
NAT	645 ± 27	484 ± 32	375 ± 30	17.1 ± 1.4	18.0 ± 1.0	16.1 ± 0.7	[pg/mL]
NAB	79.3 ± 2.9	58.6 ± 4.5	46.5 ± 3.0	2.62 ± 0.19	2.65 ± 0.16	2.68 ± 0.14	[pg/mL]
NNK	550 ± 44	474 ± 45	355 ± 29	8.36 ± 0.84	8.94 ± 0.79	8.29 ± 0.50	[pg/mL]

Abbreviations: NAB = *N*-nitrosoanabasine; NAT = *N*-nitrosoanatabine; NNK = 4-(*N*-nitrosomethylamino)-1-(3-pyridyl)-1-butanone; NNN = *N*-nitrosonornicotine; NO = nitrogen oxide; NO_x = nitrogen oxides; NFDPM = nicotine free dry particulate matter; SD = standard deviation
For results <LOD, the values used in the calculation of mean and standard deviation were estimated by the LOD; for results <LOQ but >LOD, the values were estimated by the LOQ.
An asterisk (*) indicates when more than two out of five replicate values were below LOQ and standard deviation is not reported.