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Comprehensive Air Quality Assessment of the Tobacco Heating System 2.2 under Simulated Indoor Environments

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Abstract: Despite the growing popularity of heated tobacco products, there are few comprehensive studies on their environmental aerosols. Therefore, the impact of the Tobacco Heating System 2.2 (THS 2.2) on indoor air quality was evaluated on the basis of a comprehensive list of 31 airborne constituents along with targeted screening of the gas–vapor and particulate phases of the environmental aerosol. The assessments were conducted at three ventilation rates. Indoor use of THS 2.2 increased the levels of nicotine, acetaldehyde, glycerin, and (if mentholated products were used) menthol relative to background levels, with a corresponding increase in total volatile organic compounds (TVOC) values. Moreover, a temporary increase in ultrafine particles was observed when two or more tobacco sticks were used simultaneously or with a short time lapse between usages, but the concentrations returned to close to background levels almost immediately. This is because THS 2.2 generates an aerosol of liquid droplets, which evaporate quickly. Nicotine, acetaldehyde, glycerin, and TVOC levels were measured in the low $\mu g/m^3$ range and were below the existing guideline limits. A comparison of airborne constituent levels during indoor THS 2.2 use with emissions from combustion products and common everyday activities revealed a substantially lower impact of THS 2.2 on the indoor environment.

Keywords: indoor air quality; nicotine; carbonyls; volatile organic compounds; particulate matter; ultrafine particles; Tobacco Heating System; *IQOS*; electrically heated tobacco product

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Citation: Mitova, M.I.; Cluse, C.; Correia, D.; Goujon-Ginglinger, C.G.; Kleinhans, S.; Poget, L.; Sendyk, S.S. Comprehensive Air Quality Assessment of the Tobacco Heating System 2.2 under Simulated Indoor Environments. *Atmosphere* **2021**, *12*, 989. https://doi.org/10.3390/atmos12080989

Academic Editor: Luca Stabile

Received: 17 June 2021
Accepted: 26 July 2021
Published: 31 July 2021

1. Introduction

In recent years, a variety of products have appeared on the market which are potentially less-harmful alternatives to continued cigarette smoking [1]. Among others, electrically heated tobacco products have become increasingly popular since the introduction of the Tobacco Heating System 2.2 (THS 2.2, marketed as *IQOS*®) on the market in 2014 (Supplementary Figure S1). Previous studies have shown that the environmental aerosols emitted by electrically heated tobacco products have a substantially lower impact on indoor environments than the environmental tobacco smoke generated from cigarettes [2–19]. The lower emission levels are explained by the underlying mechanism of aerosol formation, which is based on heating below temperatures required for both tobacco combustion and high-temperature pyrolysis, leading, in turn, to a significant reduction in the generation of harmful and potentially harmful constituents (HPHC) [20]. Furthermore, electrically heated tobacco products do not contain a smoldering tip that releases sidestream smoke, thereby eliminating the major contributor to indoor pollution caused by cigarettes [17].

Over the last few years, several studies have evaluated the impact of the use of electrically heated tobacco products on indoor air quality in model environments with limited control of environmental parameters [2,10–12,14,21,22] and in environmentally controlled rooms or walk-in exposure chambers [4,8,13,17–19]. All these investigations were conducted with volunteers using electrically heated tobacco products under different experimental settings, including volume of the experimental location, ventilation rate, number of tobacco sticks, and distance between users and collection/measurement points. Furthermore, one study used THS 2.2 emissions generated by machine puffing in a small exposure chamber (0.2 m^3) to predict the impact of THS 2.2 on indoor air quality in residential and public environments [5]. A smaller number of studies have assessed the impact of electrically heated tobacco product use in real-life environments, such as public catering and entertainment environments [7,23], residential settings [6,16], and cars [6,24]. In addition, earlier investigations have recently been reviewed by several groups [3,9,17].

Most of these studies have investigated the indoor concentrations of particulate matter (PM). In particular, PM below or equal to 2.5 µm in aerodynamic diameter ($PM_{2.5}$) has been extensively studied [2,4,6,10,13,14,16–19,22–24]. Most of these investigations also measured a broader range of mass concentrations (PM_1–PM_{10}: aerodynamic diameters $\leq$1 µm to $\leq$10 µm) [2,4,6,13,14,16–18,22,23]. The experimental results of these assessments differed to a certain extent, and this can be partially explained by the differences in methodology, experimental settings, number of tobacco sticks, and distance between users and measurement equipment. One study reported extreme PM values related to neither of the following: intense product use, very close distance between users of electrically heated tobacco products and measurement equipment, or small volume of experimental location combined with very low ventilation [22].

Furthermore, some studies have shown that electrically heated tobacco product use led to a rapid increase in the levels of ultrafine particles (UFP; here, the term is used to describe particles with diameters below 500 nm in accordance with the definition of Baldauf and coauthors [25]) above background levels, followed by a rapid decrease to almost background levels within seconds [4,8,11,12,16,23,24].

A few investigations have studied the concentrations of airborne nicotine in environmental aerosols of electrically heated tobacco products [4,7,10,13,17–19,23,24]. Likewise, some studies have focused on determining the indoor concentrations of formaldehyde and/or acetaldehyde or other carbonyls [2,4,7,13,17–19,23,24].

Several studies have evaluated the indoor concentrations of some volatile organic compounds (VOC) during electrically heated tobacco product use [13,18,19,21,24]. A small number of studies have investigated the indoor concentrations of some other airborne constituents, including gases [4,6,7,13,18,19], tobacco-specific nitrosamines (TSNA) [13,18], black carbon [2,6], benzo[*a*]pyrene and some other polycyclic aromatic hydrocarbons [7,21], and metals/trace elements [2].

The vast majority of the above-mentioned studies evaluated the impact of THS 2.2 use on indoor air quality. A smaller number of these studies investigated the levels of airborne constituents during indoor use of Glo® (British American Tobacco, London, UK) [10,13,14,22]. One study evaluated particle number concentrations and distribution during indoor use of Pulze® (Imperial Brands PLC, Bristol, UK) [8]. Even if these and other yet-to-be evaluated electrically heated tobacco products have some differences in their design, in general, they all generate an inhalable aerosol in a similar manner. More specifically, specially processed tobacco leaves are distilled at temperatures that are below those leading to combustion and high-temperature pyrolysis byproducts [1,8,26]. Therefore, it is expected that there will be similarities in the environmental aerosols of these products, with some common trends in their impact on air quality depending (in absolute numbers) on the composition of their mainstream aerosols and the background levels of the measured constituents. For example, during indoor use of electrically heated tobacco products, an increase in airborne nicotine in the low µg/m^3 range is plausible. As a matter of fact, an increase in nicotine levels has been reported to be attributable to indoor use of THS 2.2 and Glo, with some differences in the indoor concentrations of nicotine depending on the evaluated product and differences in experimental settings [4,10,13,17–19]. Yet, in a study with a high consumption rate of THS 2.2 (total of 80 tobacco sticks, 1 h, ventilation switched off), nicotine was not detected in indoor air [7]. Furthermore, for certain constituents and, in particular, for PM, the discrepancies in study findings could be substantial, as briefly mentioned above.

Most of the discussed studies on electrically heated tobacco products evaluated a limited number of airborne constituents. A few were based exclusively on real-time measurements with sensors, and therefore only evaluated airborne constituents for which such techniques are available [6,8,11,12,14,16,22]. Indeed, comprehensive analyses of the levels of airborne constituents during indoor use of electrically heated tobacco products remain scarce. The purpose of the present study is to fill this gap and to examine some concerns regarding the current lack of a full understanding of the impact of electrically heated tobacco products on indoor air quality [5,9]. The assessments were conducted during indoor use of THS 2.2, a Philip Morris International electrically heated tobacco product currently commercialized in more than 60 countries. To achieve a comprehensive assessment of the impact of THS 2.2 on indoor air quality, this study evaluated an extended list of airborne constituents. In addition to the list of 24 compounds assessed during the previous study [18], this study considered seven additional constituents, thus quantifying a total of 31 airborne compounds (see Tables 1 and 2 in the Results Section). Moreover, an assessment of the constituents of the gas–vapor phase (30 compounds, Supplementary Tables S11–S13) and particulate phase (36 compounds, Supplementary Table S6) of the environmental aerosol was also performed. The airborne constituents investigated during this study included compounds from the established U.S. Food and Drug Administration list of HPHCs [27], compounds from the World Health Organization (WHO) priority list of toxicants [28], constituents with relevance for air quality, and product-specific markers (Supplementary Table S17). The experiments were conducted under three ventilation conditions: ventilation condition representative of a residential environment ("Residential category III", 0.5 h^{-1}) and two sets of ventilation conditions typical of public environments ("Store", 2.4 h^{-1}, and "Restaurant", 4.3 h^{-1}).

2. Materials and Methods

2.1. Study Design

The study was designed to compare the environmental aerosol generated during THS 2.2 use with background air. Scenarios representing "Residential category III" (37 m^3/h, 0.5 h^{-1}), "Store" (175 m^3/h, 2.4 h^{-1}), and "Restaurant" (312 m^3/h, 4.3 h^{-1}) environments were simulated in a walk-in, environmentally controlled room, the so-called "indoor air quality room" (IAQ room, size: 24.1 m^2, 72.3 m^3), equipped with an air lock (Supplementary Figure S2, refer to Section 2.4). The occupant density was set at 6 m^2/person, which corresponded to the presence of three volunteer panelists and one Philip Morris International (PMI) staff member (moderator). The ventilation rates were based on the European ventilation performance standard EN 15251 [29] and ASHRAE 62-1 and 62-2 [30,31] (Supplementary Table S1).

Real-world scenarios were simulated during the experiments, with several restrictions being implemented to improve experimental reproducibility and to reduce contamination levels. First, the panelists and all staff who accessed the IAQ room were required to only use products provided in a special personal care kit (hypoallergenic personal care kit containing unscented or only slightly scented products) during the days they participated in the test. Furthermore, these persons were asked to not use perfume, aftershave, or makeup or wear new clothes (i.e., clothes that had yet not been washed after purchase) or new footwear during the sessions in order to minimize, as much as possible, the emission of pollutants that could disrupt the background levels. Second, prior to their participation, dual cigarette/THS 2.2 users agreed to refrain from cigarette smoking for at least 1 h 30 min before the start of the background sessions. Third, with the exception of water, no consumption of food or beverages was allowed during the experiments. A meal was served outside the room during a 60-min lunch break between the background ("background") and product-use sessions ("product"). Lastly, after entering the IAQ room, the volunteer panelists and moderator were not allowed to leave the IAQ room till the end of the experimental session.

Each set of experiments was performed on a separate day, starting at approximately 9:30, with a 2-h evaluation of the background (panelists present; no product use allowed), followed by a 60-min break with no panelists in the room (break) and a subsequent 2-h product-use session (product). Four replicates were foreseen for each assessment type, and air sampling was performed for 2 h, starting at time t = 0 min.

Each of the three panelists was free to choose his/her preferred brand variant of tobacco sticks from among the five different variants available on the Swiss market. Each panelist used four tobacco sticks at an ad libitum rate during the 2 h of the product session, which thus corresponded to a total of 12 sticks for the 2-h experiment. The consumption pattern of each panelist was recorded, and their respective puffing intensities of the tobacco sticks were verified by determining the user's mouth-level exposure (MLE) to nicotine [32].

Experiments under the store simulated environment were interrupted because of safety measures implemented following the COVID-19 outbreak in March 2020. Three sessions were conducted separately during June 2020, with glass panels being introduced between the volunteer panelists (Supplementary Figure S3). This change in the design of the IAQ room brought about differences in the levels of some of the airborne constituents (Supplementary Table S2). These results were, therefore, treated separately.

The ventilation rate in the IAQ room was verified using a tracer gas method [33] based on the International Organization for Standardization (ISO) standard method [34]. Specifically, the room was flooded with carbon dioxide (CO_2) up to a concentration of 1%, and the decay rate of CO_2 was measured over 13 h by using a non-dispersive infrared instrument (X-Stream™ Process Gas Analyzer, Emerson Electric Co.).

The IAQ room was air-washed at the maximum flow rate of filtered fresh air (750 m^3/h) for 15 min after the background session and overnight between the individual assessments. The ventilation was stabilized for 45 min prior to the product session depending on the simulated environment at one of the following rates: 0.5 h^{-1} ("Residential category III"), 2.4 h^{-1} ("Store"), and 4.3 h^{-1} ("Restaurant"). Before commencing the study and at the end of each week, the walls, floor, ceiling, and furniture were washed with a water/ethanol mixture (80:20 v/v).

2.2. Test Products

Detailed information on the concentrations of 58 analytes in the mainstream aerosol of THS 2.2 generated under the Health Canada Intense (HCI) testing method [35] has been reported in comparison with the concentrations present in 3R4F reference cigarette smoke [36,37], as well as in comparison with smoke from commercial cigarettes [38].

All tobacco sticks (commercialized under the brand name *HEETS®*) were used with the tobacco heating device *IQOS* 3 DUO (Supplementary Figure S1). Data on the different brands of tobacco sticks used during the study are summarized in Supplementary Table S3 and show that the most frequently selected flavors in this study were Sienna (42%) and Turquoise (39%), followed by Bronze (13%).

2.3. Subjects

Adult *IQOS* users for the THS 2.2 sessions were recruited by an external consumer panel recruiting agency (RANDOM SA, Morges, Switzerland). The panelists fulfilled the following requirements: they were aged between 21 and 65 years, healthy, asymptomatic, not taking any medication, and not pregnant or breast feeding.

The adult *IQOS* users had consumed HEETS for at least 3 months prior to the recruitment interview, with a minimum consumption rate of 5–20 HEETS per day within the last month. Most of the volunteers reported that they smoked cigarettes in addition to using *IQOS* (refer to panelists profile in Supplementary Table S4).

The panelists were informed both orally and in writing of who the sponsor was, the aims of the study and how it would unfold, and the voluntary nature of their participation.

All panelists were also informed that the products tested were not risk-free. The panelists gave their written informed consent for their participation prior to commencement of the study and were free to discontinue their participation in the study at any time. None of the panelists resigned.

A moderator was present during all assessments to assist the panelists and to ensure that the test product was used in accordance with the established protocol. The moderators for the THS 2.2 assessments were occasional *IQOS* users and neither smoked nor used any test product during the assessments.

Data on the panelist profiles (sex, age, and smoking/vaping profile) are summarized in Supplementary Table S4, and the distances between their position in the IAQ room and the different sampling positions are presented in Supplementary Table S5.

2.4. Environmentally Controlled Room

A detailed description of the environmentally controlled room (IAQ room, Supplementary Figure S2) was previously published [18,39]. It has a surface area of 24.1 m^2 and a cubic volume of 72.3 m^3 and is adjacent to the technical room (panel h), from which trapping flow rates and environmental conditions are controlled (ventilation and temperature) or monitored (humidity). It is equipped with a variable-mixing ventilation system allowing fresh air to be supplied between 37 and 879 m^3/h, and is furnished with a table, chairs and cupboards. The ventilation rate is maintained by adjusting the flow of inlet air, which is controlled by sensors. All simulations are performed using 100% fresh filtered outdoor air, purified by sequentially passing the air through a filter assembly to remove particles and VOCs: pre-filter (class G4), particles filter (class M6), activated charcoal filter (class C8F7), fine particles (class F9), and EPA filter (class E11). Filtered air enters the environmentally controlled room through two ventilation ducts (a and b) located in the ceiling at diametrically opposed corners of the room (Supplementary Figure S2). Two exhaust ducts (c and d) positioned in the other ceiling corners remove air from the room. Two electric fans are used to mix and circulate the indoor air (e and f).

An evaluation of the efficiency of the filter system to remove or decrease the levels of the airborne constituents was published [40]. This evaluation was performed by comparing the levels of airborne constituents in an empty IAQ room with either the filter system removed, or with the filter system in operation. Analysis of the results indicated that the filter system of the IAQ room provides low background levels of organic airborne compounds and particulate matter necessary to correctly run the experiments [40]. The filter system is not efficient in reducing the concentration of inorganic gases such as CO, NO and NO_x [40]. In fact, to obtain a reduction in NO_2 and NH_3 concentrations in the incoming air flow, a specific filter is required, and to the best of our knowledge no filters exist for CO and NO. That is why, on each experimental day, we measured the levels of the airborne constituents, including those of the gases.

In addition, an assessment of the human presence and selected activities such as use of personal care products on the levels of airborne constituents in the IAQ room was conducted [40]. Based on these results and a critical evaluation of published studies in the field, we provided several recommendations for the design of indoor air quality studies with electrically heated tobacco products [17]. Accordingly, several restrictions on the subjects participating in the study were implemented in this and previous studies [17,18] together with regular experimental day-per-experimental day monitoring of the baseline levels of the airborne constituents in the IAQ room (Tables 1 and 2, Supplementary Tables S2 and S7–S9).

2.5. Determination of Indoor Air Constituents

The analytical methods for quantification of ultraviolet PM (UVPM), fluorescent PM (FPM), solanesol, gas-phase tobacco-specific markers (3-ethenylpyridine and nicotine), VOCs (acrylonitrile, benzene, 1,3-butadiene, isoprene, and toluene), low-molecular weight carbonyls (acetaldehyde, acrolein, crotonaldehyde, and formaldehyde), total VOCs (TVOC), nicotine-derived nitrosamine ketone (NNK), *N*-nitrosonornicotine (NNN), glycerin, propylene glycol, and gases (CO, NO, and combined oxides of nitrogen (NO_x)) as well as online measurement of PM (PM 1 µm (PM_1) and PM 2.5 µm ($PM_{2.5}$)) have been previously published [18,39,41,42]. Detailed descriptions of these methods are available online [43–51]. Outlines of the remaining methods are given below.

The methods used in this study were accredited in accordance with ISO 17025 [52] by the Swiss Accreditation Service (Accreditation number STS 0045, SAS, Bern, Switzerland). Exceptions were the methods for online measurement of UFP and O_3, targeted analysis of airborne PM, and the method for the assessment of MLE.

2.5.1. Description of the Method for Quantification of Catechol and Hydroquinone

Catechol and hydroquinone were sampled on Cambridge filter pads (44 mm, Borgwaldt) for 120 min at a nominal flow rate of 2.0 ± 0.2 L/min. The contents of the pads were then extracted using 50 µL of 2-butanone and 4000 µL of extraction solution containing catechol-d_6 and hydroquinone-d_6, each at a concentration of 72 ng/mL. The extractions were performed for 30 min on an orbital shaker operating at 200 rpm. Aliquots of extracted solutions (100 µL) were subsequently derivatized with BSTFA (*N*,*O*-*bis*(trimethylsilyl)trifluoroacetamide) and TMCS (trimethylsilyl chloride; 50 µL).

A 1-µL aliquot was then injected in splitless mode into a gas chromatograph coupled with a triple-quadrupole mass spectrometer operating in electron impact ionization mode (GC–EI-MS/MS; TQ8030, Shimadzu Corporation). Separation was performed on a 30-m × 0.25-mm × 0.25-µm ZB-5HT inferno capillary column (Phenomenex) in linear velocity mode (44.4 cm/s) by using a temperature program of 50 °C for 1.0 min, programmed to increase to 170 °C at 10 °C/min and to 350 °C at 50 °C/min and be held at 350 °C for 5.0 min. The injector temperature, transfer line, and ion source were set at 225 °C, 340 °C, and 225 °C, respectively. Catechol and hydroquinone were detected in the multiple reaction monitoring mode and quantified using the following parameters: catechol at 10.90 min, m/z 254 > 239 (CE 10), m/z 254 > 73 (CE 15); hydroquinone at 12.00 min, m/z 254 > 239 (CE 10), m/z 254 > 73 (CE 15); catechol-d_6 at 10.88 min, m/z 258 > 243 (CE 10), m/z 258 > 73 (CE 15); hydroquinone-d_6 at 11.99 min, m/z 258 > 243 (CE 10), m/z 258 > 73 (CE 15).

The GC–EI-MS/MS system was calibrated for both compounds using authentic standards. The 8-point linear calibration curve for catechol had a typical range of 8.70–1760 ng/$m^{3,}$ and that for hydroquinone was 19.0–3840 ng/m^3. LOD and LLOQ were 2.0 ng/m^3 and 8.70 ng/m^3, respectively for catechol, and 1.9 ng/m^3 and 19.0 ng/m^3, respectively for hydroquinone. The uncertainty on the mean results at the 95% confidence interval was set at ±22% for a 4-replicate sample.

2.5.2. Description of the Method for Quantification of CO_2

CO_2 was detected and quantified online by a non-dispersive infrared sensor (x-stream, Emerson). Measurements were performed every 5 s over the entire duration of the sampling period.

The CO_2 sensor was calibrated, and its performance was verified via a system suitability test with certified CO_2. The calibrations were conducted using Tedlar bags (Supelco) containing either pure nitrogen (Alphagaz 2 N60, Carbagas) or certified CO_2 standard (Carbagas) at a concentration of 1500 ppm. The system suitability test was performed at 200 ppm once per week during the study. The concentrations measured during the system suitability test had to be within the limits of the control chart established on the basis of the data acquired during validation.

2.5.3. Description of the Method for Quantification of NH_3

NH_3 was detected and quantified online by a time-resolved measurement system that uses a laser to quantify the spectral features of gas phase molecules in an optical cavity (G2103, Picarro). The system uses a highly sensitive optical spectroscopic technique that enables measurement of the absolute optical extinction by samples that scatter and absorb light. It measures the time required to decay the light to 1/e of its initial intensity, and this "ringdown time" can be used to calculate the concentration of the absorbing substance in the gas mixture inside the cavity. Measurements were performed every 5 s over the entire duration of the sampling period.

The NH_3 sensor was calibrated by the supplier in comparison to a reference instrument, and its performance was verified via a system suitability test with proxy-certified CO_2, as NH_3 is too unstable. The system suitability test was conducted using Tedlar bags (Supelco) containing a certified CO_2 standard (Carbagas) at a concentration of 0.5 %, once per week during the study. The concentrations measured during the system suitability test had to be within the limits of the control chart established on the basis of the bias observed (during validation) between the measured value and the certified concentration of the gas specified by the supplier.

2.5.4. Description of the Method for Quantification of O_3

O_3 was detected and quantified online by absorption of UV light at 254 nm using a mercury lamp (205 Model Dual Beam Monitor, 2B Technologies). The acquisition rate was set at 2 s, with the datapoints being averaged over 1 min throughout the sampling duration.

The O_3 sensor used in this study was calibrated on a yearly basis by METAS (Federal Institute of Metrology, Switzerland). Its performance was verified on a monthly basis via zero check by using an ozone scrubber connected to the instrument inlet. The average concentration of O_3 must be within defined criteria, failing which the drift must be corrected.

2.5.5. Description of the Method for Determination of Ultrafine Particulate Matter

UFP matter was detected and quantified online on the basis of the principle of electrical charging of aerosols (DiSCmini UFP counter, Testo), which simultaneously measures the particle number concentration in the range of 10–700 nm and the average particle diameter in the range of 10–300 nm. Measurements were performed every 1 s over the sampling duration.

2.5.6. Targeted Analysis of Airborne Particulate Matter

The PM fraction of THS 2.2 environmental aerosol was subjected to semi-quantitative analysis by liquid chromatography coupled with high-resolution mass spectrometry (LC–HRMS) targeting 36 compounds selected from a list of known harmful and potentially harmful constituents of tobacco smoke [27] and from other chemicals identified in THS 2.2 aerosol [53].

Samples for LC–HRMS analysis were prepared by transferring 250 µL of the solution used for UVPM/FPM/solanesol analysis into a 2-mL vial containing 300 µL of an internal standard solution (Supplementary Table S6).

Chromatographic separation was performed on a Vanquish UHPLC system (Thermo Scientific) using two sequentially coupled columns: an anion exchange column (Biobasic AX, 5 µm, 50 × 2.1 mm; Thermo Scientific) and a pentafluorophenyl column with trimethylsilyl endcapping (Kinetex, 2.6 µm PFP, 100 Å, 150 × 2.1 mm; Phenomenex). A solvent gradient of aqueous ammonium formate 10 mM adjusted to pH 3.5 with formic acid, and acetonitrile was applied. The analytes were detected on a Q Exactive mass spectrometer (Thermo Scientific) as $[M+H]^+$ pseudomolecular ions after positive electrospray ionization. Semi-quantitative concentrations were calculated from the ratio of analyte to internal standard peak areas by using a 10-point calibration curve. The retention times and respective internal standards used for each analyte are detailed in Supplementary Table S6a–e.

2.6. MLE to Nicotine

The consumption pattern of each panelist was recorded, and their respective puffing intensities of the tobacco sticks were verified by determining their MLE to nicotine, which was also compared with the nicotine yields produced by machine puffing of the tobacco sticks under various regimens described in Supplementary Table S14. The MLE values were subsequently used as input to estimate the levels of inert airborne nicotine, assuming that nicotine would be an inert and stable gas, ideally mixed with and extracted from the room air only by the ventilation and not retained by the panelists or adsorbed on surfaces. The relative difference between the airborne nicotine concentrations calculated with the inert model and the measured values was used to estimate the relative nicotine loss.

The MLE was derived from the UV absorbance (measured at 310 nm) of the methanol extracts of the mouthpiece filters collected from used sticks. Quinoline was used as a surrogate standard for quantifying nicotine in the filters. Each brand of tobacco sticks was machine-puffed under various regimens (Supplementary Table S14). Linear regression was calculated for calibrating the nicotine yield (GC–FID (flame ionization detector)) in the aerosol collected from the tobacco sticks as a function of the quinoline equivalent concentration (measured by UV spectrophotometry) in the respective mouthpiece filter extracts. The method of measuring MLE to nicotine was first introduced for THS 2.2, with quantitation of nicotine in mouthpiece filter extracts [32]. However, we found a similar method, which is based on UV absorbance and which was used in the present study, to be more appropriate after comparing the two methods by using samples produced by machine-puffing experiments under various regimens.

2.7. Data Treatment

SAS 9.4 and R 3.6.2 software were used for statistical treatment. All data were reported if the measured values were between the lower working range limit (LWRL) and upper working range limit (UWRL) of the analytical method. In cases where analyte levels were between the lower limit of quantification (LLOQ) and LWRL, the values were reported as "<LWRL" (method uncertainty range). If analyte concentrations were below the LLOQ, the LLOQ was reported.

Descriptive statistics are given for all target constituents evaluated per simulated environment in this study. All concentrations are presented as the number of values, arithmetic mean, standard deviation, and minimum and maximum values observed. Furthermore, values below quantification limits (LWRL, LLOQ, and/or LOD (limit of detection)) are also reported.

The main objective of the statistical analysis was to assess the impact of THS 2.2 use on the background indoor air quality (i.e., concentrations of measured indoor air constituents) in an environmentally controlled room. This assessment was performed by mixed-effects analysis of variance. The model parameters were estimated using the restricted maximum likelihood objective function. In cases where a heterogeneous variance pattern was observed between the background and THS 2.2 conditions, one of several additional covariance parameters was introduced into the model to adjust for the variability, and the Satterthwaite approximation for degrees of freedom was used.

To determine the difference between the THS 2.2 and background mean values, both the 0.9 and 0.95 confidence intervals (CI) were calculated. The 0.9 CI was compared with a ±25% equivalence limit threshold by using the background mean value as the reference, whereas the 0.95 CI was used for testing null differences between the same conditions.

For all the statistical analyses described above, outliers were detected by using studentized residuals calculated within each model. In certain sessions, some outlying values were immediately evident (Supplementary Table S2), and therefore these results were treated separately.

The outcome of the statistical modeling is summarized in Supplementary Materials-Statistical Modeling.

Sample size considerations (number of days, number of replicates, etc.) were not theoretically determined, but set in accordance with common practice in such studies to match workload capacities.

3. Results

3.1. Results for Offline Analytes

Table 1 and Supplementary Table S2 summarize the data obtained for the measurement of airborne constituents measured offline in the background air and the environmental aerosol of THS 2.2 per simulated environment. In addition, Table 1 presents data from previous studies. Overall, the results of this study and those of previous assessments conducted under the simulated Residential category III and Restaurant environments are in good agreement (Table 1).

The first point to highlight is that nicotine (Figure 1), acetaldehyde (Figure 2), glycerin (Figure 3), and TVOC (Figure 4) levels had all increased above the background levels in THS 2.2 use sessions under all simulations. This finding was supported by the statistical results indicating that the 95% CIs for the differences in the mean concentrations of acetaldehyde (all simulations), nicotine (0.5 h^{-1}), and TVOCs (0.5 h^{-1} and 2.4 h^{-1}) between the THS 2.2 sessions and corresponding background sessions excluded 0 (Supplementary Material–Statistical Modeling). Moreover, for all these compounds, the 90% CIs of the differences in mean concentrations were outside the equivalence limits in all simulations (refer to Supplementary Material–Statistical Modeling). Regarding glycerin, it was not possible to perform statistical processing of the data for any of the simulated environments. Similarly, statistical processing was not possible for nicotine at 2.4 and 4.3 h^{-1}. The reason for this is that the mean concentrations of both these compounds in the background sessions were below the reporting limits of their respective quantification methods.

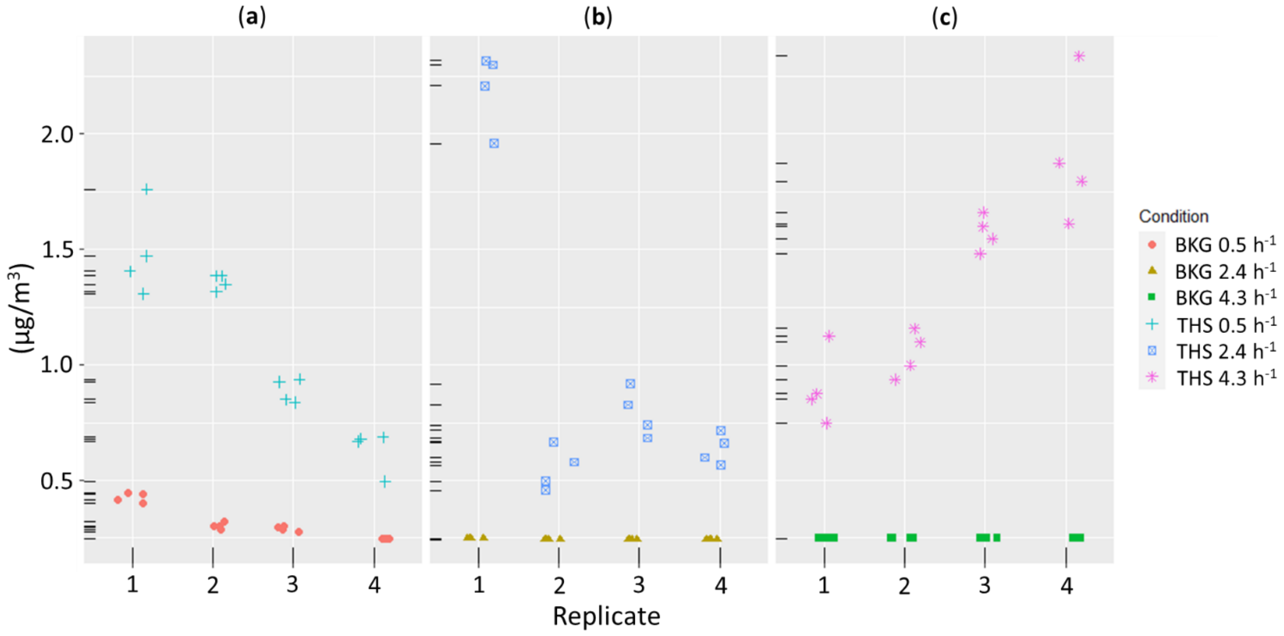

Figure 1. Comparison of the concentrations of airborne nicotine (μg/m^3) in the background (BKG) and THS 2.2 use sessions (THS) under simulated (**a**) Residential category III (0.5 h^{-1}), (**b**) Store (2.4 h^{-1}), and (**c**) Restaurant (4.3 h^{-1}) environments.

Table 1. Summary of mean concentrations of airborne constituents measured offline in the background and THS 2.2 environmental aerosol under simulated Residential category III (0.5 air changes/h), Store (2.4 air changes/h), and Restaurant environmental conditions (4.3 air changes/h).

Variable [1,2]	BKG	THS	BKG	THS	BKG	THS	BKG	THS	BKG	THS
	2020	2020	2016	2016	2020	2020	2020	2020	2018	2018
	Residential Category III				Store		Restaurant			
Air changes/h	0.5	0.5	0.5	0.5	2.4	2.4	4.3	4.3	4.3	4.3
Persons/users [N]	4/3	4/3	3/2	3/2	4/3	4/3	4/3	4/3	4/3	4/3
Sticks [N]	0	12	0	12	0	12	0	12	0	8
Replicate	4	4	3	3	3	3	4	4	1	1

Table 1. *Cont.*

Variable [1,2]		BKG	THS	BKG	THS	BKG	THS	BKG	THS	BKG	THS
		2020	2020	2016	2016	2020	2020	2020	2020	2018	2018
		Residential Category III				Store		Restaurant			
UVPM-THBP	Mean	<LWRL	<LWRL	<LLOQ	<LWRL	nm	nm	<LWRL	<LWRL	nm	nm
[µg/m^3]	SD	na	na	na	na	na	na	na	na	nm	nm
FPM-scopoletin	Mean	<LWRL	<LWRL	<LLOQ	<LLOQ	nm	nm	<LOD	<LOD	nm	nm
[µg/m^3]	SD	na	na	na	na	na	na	na	na	nm	nm
PM-solanesol	Mean	<LOD	<LWRL	<LOD	<LLOQ	nm	nm	<LOD	<LLOQ	<LOD	<LLOQ
[µg/m^3]	SD	na	na	na	na	na	na	na	na	na	na
3-Ethenylpyridine	Mean	<LOD [3]	<LOD [3]	<LOD [3]	<LOD [3]	<LOD	<LOD	<LOD[3]	<LOD	<LOD	<LOD
[µg/m^3]	SD	na	na	na	na	na	na	na	na	na	na
Nicotine	Mean	0.317	1.09	0.330	1.48	<LLOQ	0.659	<LLOQ	1.36	0.484	1.14
[µg/m^3]	SD	0.069	0.371	0.047	0.685	na	0.132	na	0.448	0.014 [5]	0.077 [5]
Acetaldehyde	Mean	2.19 [4]	5.87	3.32 [4]	6.76	3.19 [4]	4.41	1.10 [4,6]	2.10 [4,6]	1.46 [4]	2.21 [4]
[µg/m^3]	SD	0.140	0.271	0.280	0.760	0.174	0.297	0.129	0.173	0.035 [5]	0.023 [5]
Acrolein	Mean	<LLOQ	<LWRL	<LLOQ	<LWRL	<LLOQ	<LLOQ	<LOD	<LLOQ	<LLOQ	<LLOQ
[µg/m^3]	SD	na	na	na	na	na	na	na	na	na	na
Crotonaldehyde	Mean	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
[µg/m^3]	SD	na	na	na	na	na	na	na	na	na	na
Formaldehyde	Mean	10.5	10.2	13.0	10.8	7.49 [4]	7.55 [4]	3.33 [4]	3.32 [4]	4.76 [4]	4.94 [4]
[µg/m^3]	SD	1.19	1.27	2.24	1.05	0.273	0.633	0.240	0.203	0.036 [5]	0.118 [5]
Acrylonitrile	Mean	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
[µg/m^3]	SD	na	na	na	na	na	na	na	na	na	na
Benzene	Mean	0.628	0.639	1.00	0.943	0.361	0.315	0.384	0.364	0.171 [4]	0.182 [4]
[µg/m^3]	SD	0.101	0.101	0.127	0.119	0.044	0.049	0.065	0.072	0.008 [5]	0.014 [5]
1,3-Butadiene	Mean	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
[µg/m^3]	SD	na	na	na	na	na	na	na	na	na	na
Isoprene	Mean	9.16	10.6	8.65	9.85	4.26	4.54	2.23	2.48	2.22	2.31
[µg/m^3]	SD	0.766	1.11	0.614	1.32	0.433	0.578	0.161	0.224	0.154 [5]	0.133 [5]
Toluene	Mean	1.11 [4]	1.03 [4]	2.68	2.32	1.48 [4]	1.36 [4]	0.718 [4]	0.701 [4]	0.658 [4]	0.659 [4]
[µg/m^3]	SD	0.226	0.165	0.280	0.306	0.105	0.394	0.152	0.181	0.027 [5]	0.049 [5]
TVOC	Mean	35.3	57.8	20.4	22.2	4.44	14.4	3.12	7.23	14.0	11.8
[µg/m^3]	SD	15.9	20.3	0.632	0.933	1.29	3.52	1.83	3.66	1.58 [5]	0.079 [5]
TVOC w/t flavors	Mean	30.7	40.6	na	na	4.44	14.4	3.12	4.33	nm	nm
[µg/m^3]	SD	11.2	17.5	na	na	1.29	3.52	1.83	2.29	nm	nm
Catechol	Mean	<LLOQ	<LLOQ	nm	nm	nm	nm	<LLOQ	<LLOQ	nm	nm
[µg/m^3]	SD	na	na	na	na	na	na	na	na	nm	nm
Hydroquinone	Mean	<LLOQ	<LLOQ	nm	nm	nm	nm	<LOD	<LLOQ	nm	nm
[µg/m^3]	SD	na	na	na	na	na	na	na	na	nm	nm
Glycerin	Mean	<LLOQ	20.1	<LLOQ	13.3	<LLOQ	13.6	<LLOQ	15.8	<LLOQ	8.59
[µg/m^3]	SD	na	4.26	na	3.39	na	3.02	na	4.00	na	2.87 [5]
Propylene glycol	Mean	27.6	38.8	<LLOQ	<LWRL	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
[µg/m^3]	SD	16.7	34.2	na	na	na	na	na	na	na	na
NNK	Mean	<LOD	<LLOQ	<LOD	<LOD	nm	nm	<LLOQ	<LLOQ	nm	nm
[µg/m^3]	SD	na	na	na	na	na	na	na	na	nm	nm
NNN	Mean	<LOD	<LLOQ	<LOD	<LLOQ	nm	nm	<LLOQ	<LLOQ	nm	nm
[µg/m^3]	SD	na	na	na	na	na	na	na	na	nm	nm

[1] Abbreviations: BKG, background; FPM, fluorescent particulate matter; LLOQ, lower limit of quantification; LWRL, low working range limit; LOD, limit of detection; na, not applicable; nm, not measured; NNK, nicotine-derived nitrosamine ketone; NNN, *N*-nitrosonornicotine; SD, standard deviation; THS, Tobacco Heating System 2.2; TVOC, total volatile organic compounds; UVPM-THBP, ultra-violet particulate matter-2,2′,4,4′-tetrahydroxybenzophenone; [2] Mean values reported for the Store environment for replicates 2–4; average values per day for each simulated environment are summarized in Supplementary Materials, Table S2; [3] Few replicates at LLOQ < x < LOD; [4] Below LWRL; [5] Standard deviation reported for replicates in a single session; [6] Outlying results for background acetaldehyde levels on day 3 under the Restaurant simulated environment. Accordingly, the results for acetaldehyde on day 3 were excluded from calculation of the mean and standard deviation values.

In addition, to have better comparability between these TVOC results and those from previous studies that used a single tobacco flavor [18], the data were analyzed with and without subtraction of flavor compounds. Specifically, menthol or limonene and menthol peaks were subtracted from THS 2.2 samples, while eucalyptol, limonene, and menthol peaks were subtracted from background samples under the Residential category III environment (0.5 h^{-1}; Table 1). As a result, the values for TVOCs decreased by 10–55% in the Residential category III environment (0.5 h^{-1}) and 28–66% in the Restaurant environment (4.3 h^{-1}). Notably, the insertion of glass panels in replicates 2–4 in the store environment (2.4 h^{-1}) led to a significant decrease in airborne menthol levels. Thus, for these sessions, only one value for TVOCs was reported (Table 1). Overall, the concentrations of TVOCs after subtraction of flavor compounds varied among the different sessions and ventilation conditions, from remaining at background levels to rising slightly above the background level (Figure 5).

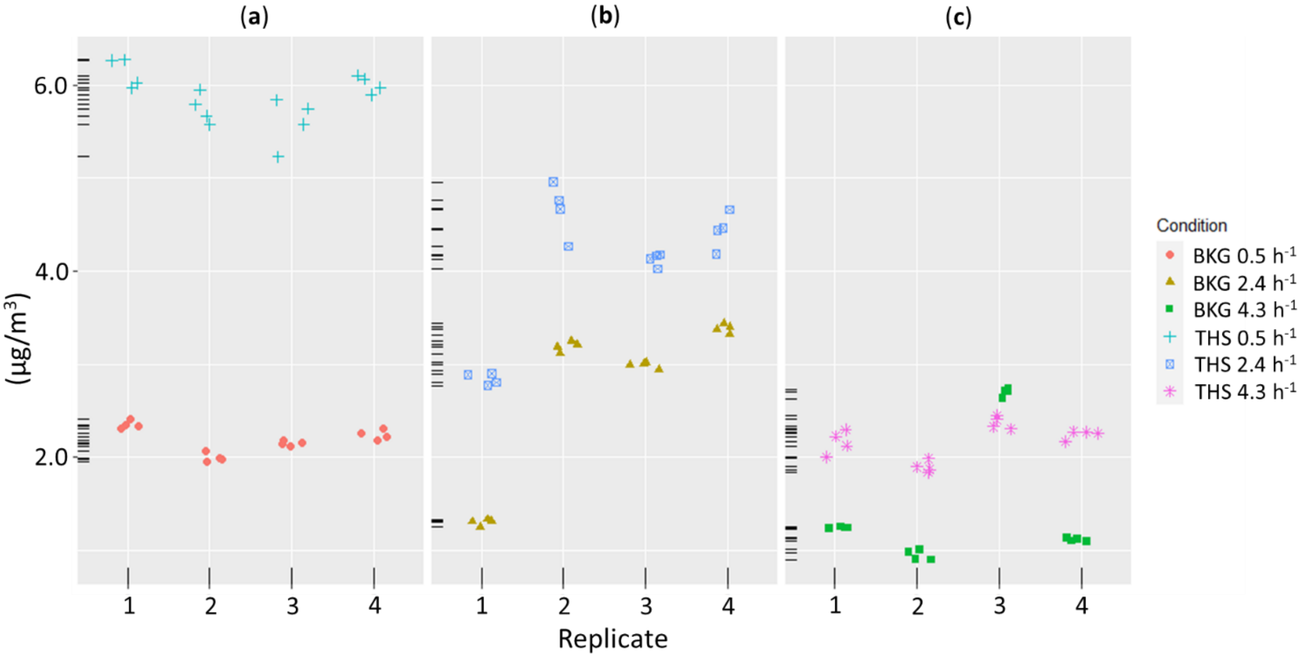

Figure 2. Comparison of the concentrations of airborne acetaldehyde (μg/m^3) in the background (BKG) and THS 2.2 use sessions (THS) under simulated (**a**) Residential category III (0.5 h^{-1}), (**b**) Store (2.4 h^{-1}), and (**c**) Restaurant (4.3 h^{-1}) environments.

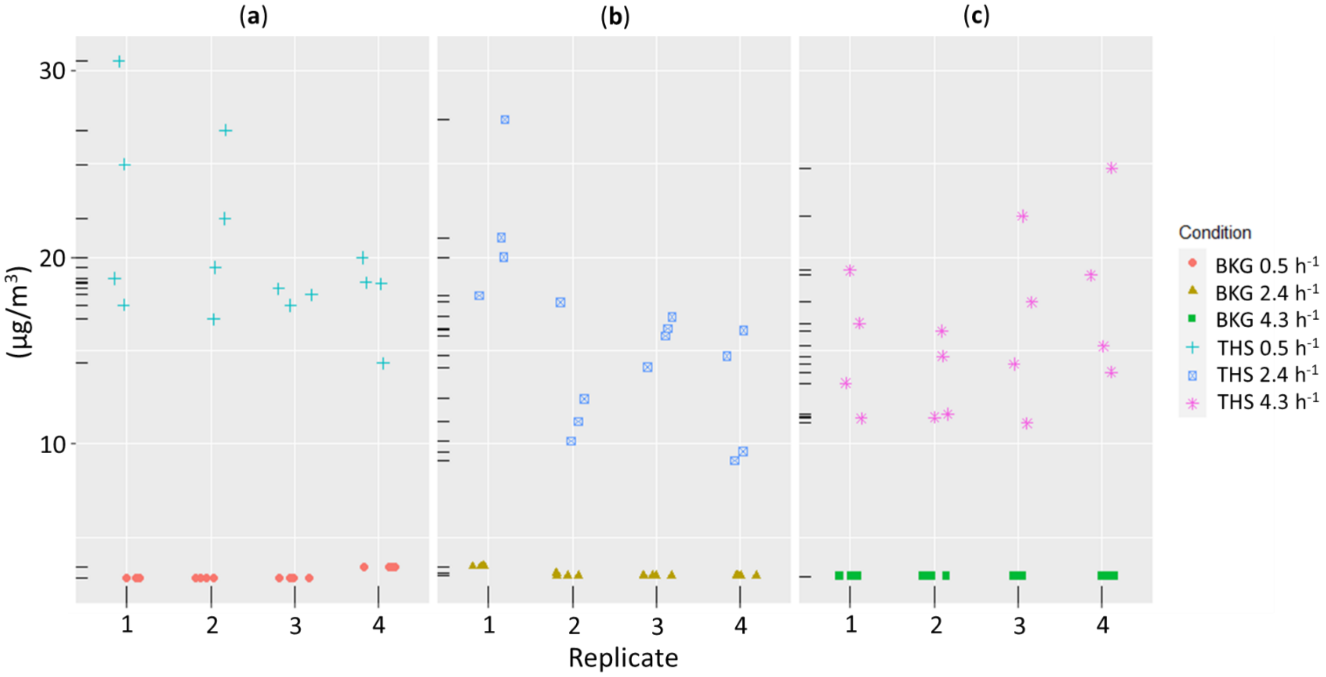

Figure 3. Comparison of the concentrations of airborne glycerin (μg/m^3) in the background (BKG) and THS 2.2 use sessions (THS) under simulated (**a**) Residential category III (0.5 h^{-1}), (**b**) Store (2.4 h^{-1}), and (**c**) Restaurant (4.3 h^{-1}) environments.

The levels of 3-ethenylpyridine, acrylonitrile, 1,3-butadiene, crotonaldehyde, hydroquinone, NNK, and NNN were consistently below the LOD or LLOQ for all background and THS 2.2 sessions irrespective of the ventilation rate (Table 1; Supplementary Table S2). In the case of acrolein, UVPM, FPM, and solanesol, the indoor concentrations remained below quantification limits in both background and THS 2.2 sessions at 2.4 h^{-1} and 4.3 h^{-1} as well in the background at 0.5 h^{-1}. It was seen that, at 0.5 h^{-1}, the concentrations of these compounds in the THS 2.2 sessions were slightly higher than those in the background sessions, with the values remaining in the method uncertainty range of LLOQ < x < LWRL

(Background LOD < x < LWRL). Catechol and propylene glycol (Table 1; Supplementary Table S2) both had indoor concentrations varying around the LLOQ in background and THS 2.2 sessions at 0.5 h^{-1} and below the LLOQ in background and THS 2.2 sessions at 2.4 h^{-1} and 4.3 h^{-1} (Supplementary Table S2).

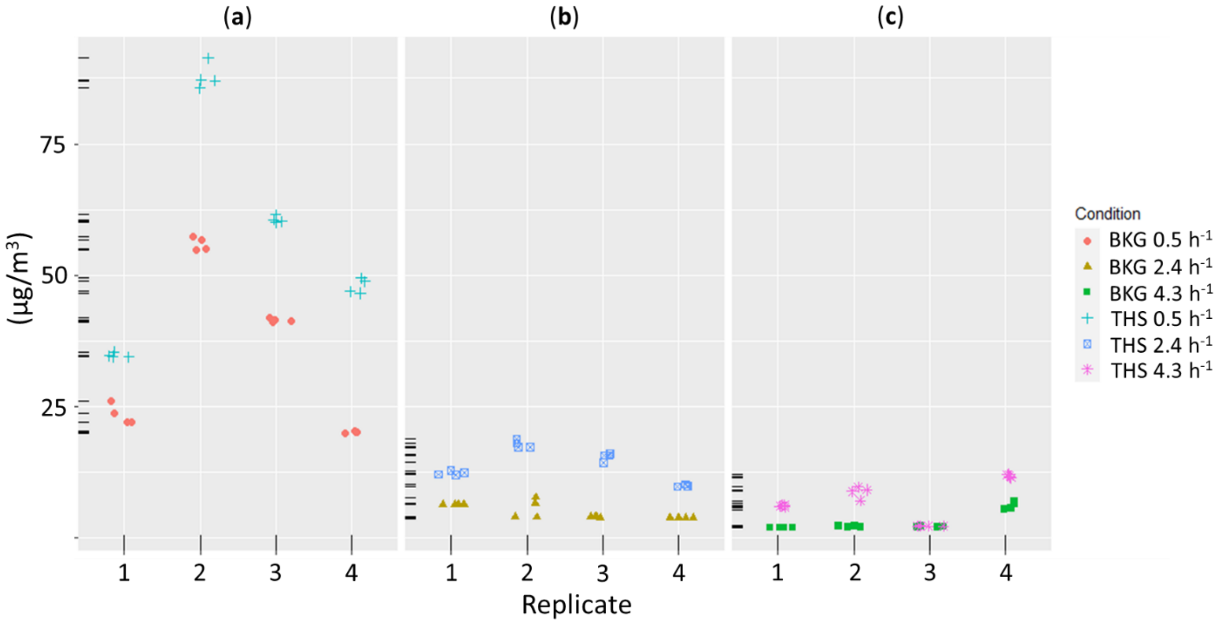

Figure 4. Comparison of the concentrations of airborne TVOCs (μg/m^3) in the background (BKG) and THS 2.2 use sessions (THS) under simulated (**a**) Residential category III (0.5 h^{-1}), (**b**) Store (2.4 h^{-1}), and (**c**) Restaurant (4.3 h^{-1}) environments.

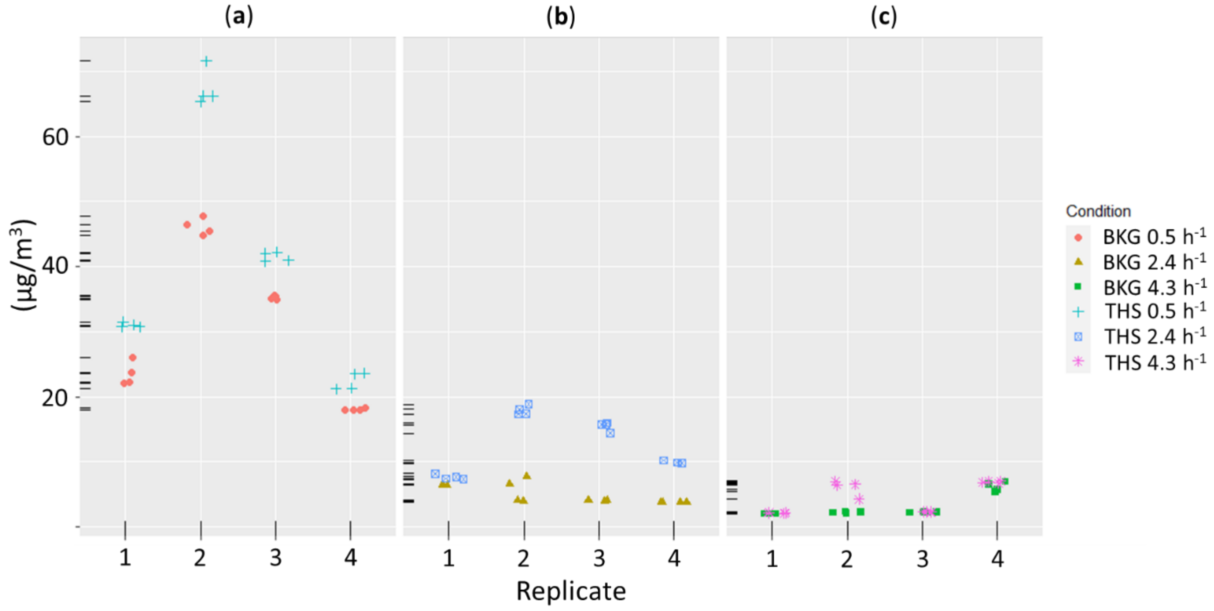

Figure 5. Comparison of the concentrations of airborne TVOCs with flavor compounds subtracted (μg/m^3) in the background (BKG) and THS 2.2 use sessions (THS) under simulated (**a**) Residential category III (0.5 h^{-1}), (**b**) Store (2.4 h^{-1}), and (**c**) Restaurant (4.3 h^{-1}) environments.

Concerning formaldehyde, benzene, and toluene, the levels of these compounds in sessions with THS 2.2 remained within background variations irrespective of the ventilation rate (Table 1; Supplementary Table S2). Overall, isoprene levels were within the range of background variations in the THS 2.2 sessions and showed a slight increase relative to background in some THS 2.2 sessions at 0.5 h^{-1} and 2.4 h^{-1} (Supplementary Table S2).

The mean concentrations of benzene, formaldehyde, isoprene, and toluene in the THS 2.2 sessions and corresponding background sessions were found to be equivalent as the 90% confidence intervals of the corresponding differences in mean concentrations were included within the equivalence limits (Supplementary Material–Statistical Modeling).

3.2. Results for Online Analytes

Table 2 summarizes the data obtained for the measurement of airborne constituents, measured online, in the background air and the environmental aerosol of THS 2.2 per simulated environment together with data from previous studies.

Consistent with the results of previous studies, the levels of CO, NO, and NO_x (Table 2; Supplementary Tables S7–S9; Figure 6) in the THS 2.2 sessions remained within background variations irrespective of the ventilation rate.

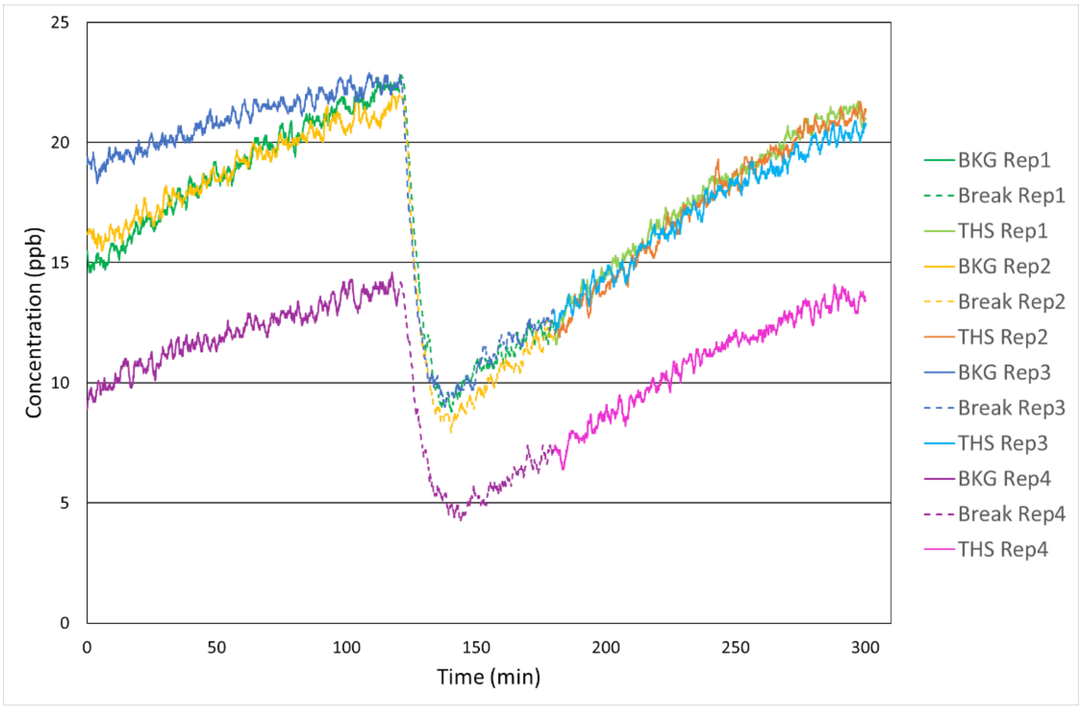

Figure 6. Comparison of NO_x traces (ppb) during the background (BKG), break, and THS 2.2 sessions (THS) under the simulated Residential category III environment (0.5 h^{-1}).

Table 2. Summary of mean concentrations of airborne constituents measured online in the background and THS 2.2 environmental aerosol under simulated Residential category III (0.5 air changes/h), Store (2.4 air changes/h), and Restaurant environmental conditions (4.3 air changes/h).

Variable [1,2,3]		**BKG**	**THS**	**BKG**	**THS**	**BKG**	**THS**	**BKG**	**THS**	**BKG**	**THS**
		2020	**2020**	**2016**	**2016**	**2020**	**2020**	**2020**	**2020**	**2018**	**2018**
		Residential Category III				**Store**		**Restaurant**			
Air changes/h		0.5	0.5	0.5	0.5	2.4	2.4	4.3	4.3	4.3	4.3
Persons/users [N]		4/3	4/3	3/2	3/2	4/3	4/3	4/3	4/3	4/3	4/3
Sticks [N]		0	12	0	12	0	12	0	12	0	8
Replicate		4	4	3	3	3	3	4	4	1	1
PM_1 window	Mean	<LOD	<LLOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
[$\mu g/m^3$]	SD	na	na	na	na	na	na	na	na	na	na
PM_1 door	Mean	<LOD	<LLOQ	nm	nm	<LOD	<LOD	<LOD	<LOD	nm	nm
[$\mu g/m^3$]	SD	na	na	na	na	na	na	na	na	nm	nm
$PM_{2.5}$ window	Mean	<LOD	<LLOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
[$\mu g/m^3$]	SD	na	na	na	na	na	na	na	na	na	na
$PM_{2.5}$ door	Mean	<LOD	<LLOQ	nm	nm	<LOD	<LOD	<LOD	<LOD	nm	nm
[$\mu g/m^3$]	SD	na	na	na	na	na	na	na	na	nm	nm
UFP window	Mean	316	14199	nm	nm	120	6319	79	6024	nm	nm
[$count/cm^3$]	SD	158	1101	na	na	10	1498	14	1125	nm	nm

Table 2. *Cont.*

Variable [1,2,3]		**BKG**	**THS**	**BKG**	**THS**	**BKG**	**THS**	**BKG**	**THS**	**BKG**	**THS**
		2020	**2020**	**2016**	**2016**	**2020**	**2020**	**2020**	**2020**	**2018**	**2018**
		Residential Category III				**Store**		**Restaurant**			
UFP door	Mean	320	17993	nm	nm	148	7773	61	5156	nm	nm
[count/cm^3]	SD	194	974	na	na	47	1177	8	3690	nm	nm
UFP window	Mean	9	113	nm	nm	7	107	0	100	nm	nm
[nm]	SD	14	9	na	na	3	9	0	9	nm	nm
UFP door	Mean	8	79	nm	nm	6	79	0	55	nm	nm
[nm]	SD	13	6	na	na	3	4	0	37	nm	nm
CO	Mean	0.289	0.287	0.495	0.444	0.235	0.230	0.177	0.172	0.127	0.138
[ppm]	SD	0.055	0.041	0.052	0.037	0.030	0.021	0.023	0.023	na	na
CO_2	Mean	1318	1477	nm	nm	770	821	634	678	nm	nm
[ppm]	SD	52	43	na	na	10	24	16	22	nm	nm
NH_3	Mean	23.6	38.1	nm	nm	20.4	19.4	18.2	20.0	17.7	16.1
[ppb]	SD	4.1	3.7	na	na	2.7	1.9	6.3	6.1	na	na
NO	Mean	12.9	11.7	35.8	23.9	2.65	2.57	5.14	4.90	2.39	2.30
[ppb]	SD	3.89	2.99	10.7	4.19	0.15	0.27	0.47	0.36	na	na
NO_x	Mean	17.8	15.8	42.2	30.0	5.53	5.18	7.74	7.43	2.78	2.54
[ppb]	SD	3.90	3.28	11.4	5.00	0.43	0.35	0.57	0.43	na	na
O_3 [4]	Mean	<LOD	<LOD	nm	nm	<LOD	<LOD	<LOD	<LOD	nm	nm
[μg/m^3]	SD	na	na	na	na	na	na	na	na	nm	nm

[1] Abbreviations: BKG, background; LLOQ, lower limit of quantification; LWRL, low working range limit; LOD, limit of detection; na, not applicable; nm, not measured; PM, particulate matter; SD, standard deviation; THS, Tobacco Heating System 2.2; UFP, ultrafine particles; [2] Mean values reported for the Store environment for replicates 2–4; average values per day for each simulated environment are summarized in Supplementary Materials, Table S2; [3] Standard deviation of mean values for the replicate sessions; [4] LOD of 4 μg/m$^{3.}$

A very slight rise in CO_2 levels (Table 2; Supplementary Tables S7–S9) was observed in THS 2.2 sessions relative to the background values. This increase was attributed to intensified breathing and was similar to those reported in other studies [4,6]. More specifically, a 10% increase at 0.5 h^{-1}, 5.7% at 2.4 h^{-1}, and 6.8% at 4.3 h^{-1} were seen, indicating an overall slight influence of ventilation rate on the global CO_2 concentrations.

While mean NH_3 (Table 2; Supplementary Table S7–S9; Figure 7) levels showed an increase above the background level during THS 2.2 use at 0.5 h^{-1}, the values remained within background variations at 2.4 h^{-1} and showed a negligible increase at 4.3 h^{-1}. The results at 0.5 h^{-1} can probably be attributed to the presence of an artefact caused by purging during the break. The artefact possibly resulted from the introduction of outdoor NH_3 after the ventilation rates were increased (Supplementary Tables S7–S9; Figure 7). With regard to O_3 (Table 2; Supplementary Tables S7–S9), it can be mentioned that the values remained below the LOD in all sessions and ventilation conditions, except during the break sessions in replicates 2–4 at 2.4 h^{-1}, which were the only sessions conducted during summer.

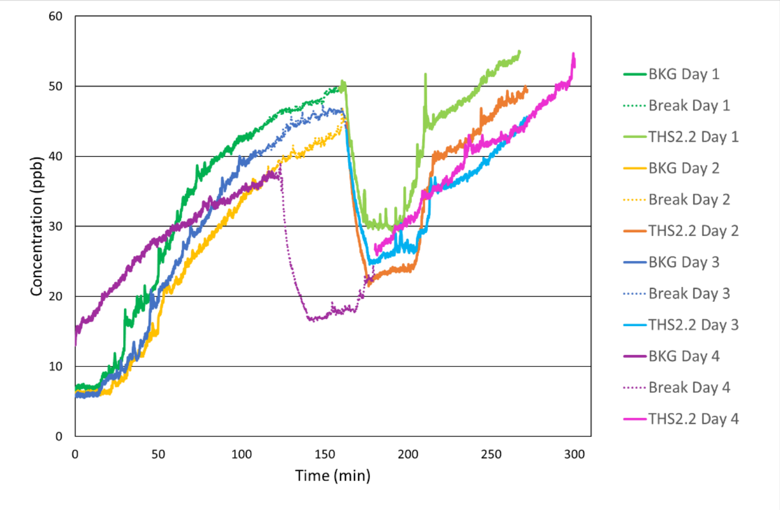

Figure 7. Comparison of NH_3 traces (ppb) during the background (BKG), break, and THS 2.2 sessions (THS) under the simulated Residential category III environment (0.5 h^{-1}).

The average indoor concentrations of PM_1 and $PM_{2.5}$ (Table 2; Supplementary Table S2) were below the LOD in all background sessions in the three simulated environments. Interestingly, average indoor concentrations below the LLOQ were detected in some THS 2.2 sessions at 0.5 h^{-1} and in a single session at 2.4 h^{-1}, while the average concentrations were below the LOD for the rest of the sessions at 2.4 h^{-1} and 4.3 h^{-1} (Table 2; Supplementary Table S2). Indeed, a pattern was observed in the online trace for PM_1 attributable to the use of a certain number of tobacco sticks. However, the peak maximum rarely exceeded the LLOQ of the method (Figure 8).

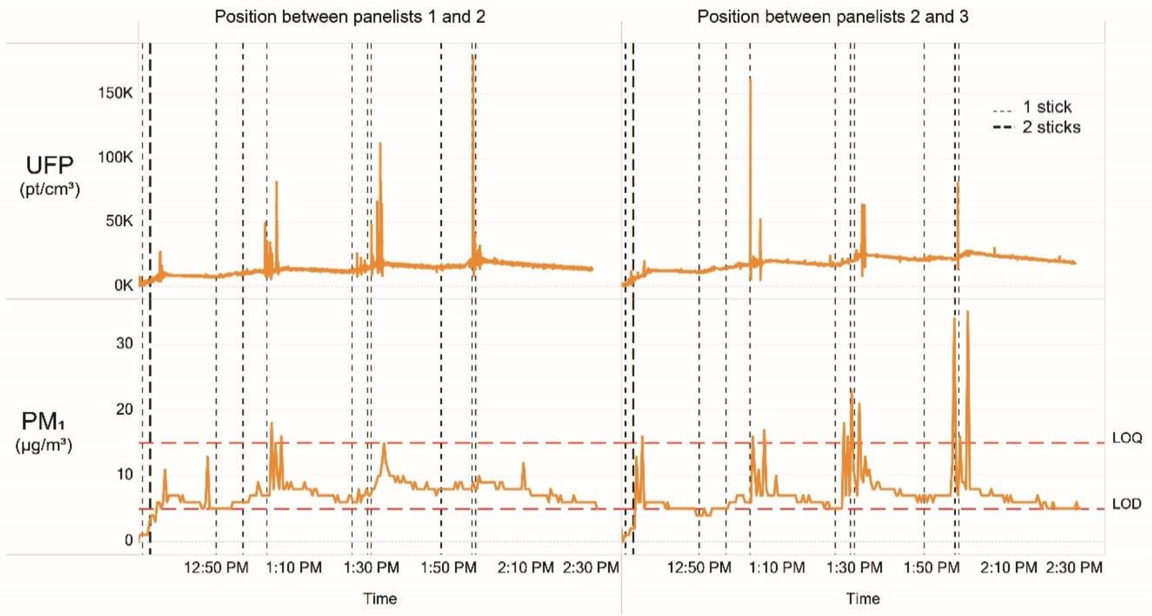

Figure 8. Comparison of airborne UFP and PM_1 (µg/m^3) traces in a typical session with THS 2.2 under the Residential category III simulated environment (0.5 h^{-1}) against the time for consumption of the tobacco sticks (reprinted with permission from [17]). Note: for the positions of the panelists, refer to Supplementary Materials, Figure S2.

With regard to UFP (Table 2), the levels of this analyte increased above the background levels in THS 2.2 sessions in all simulations. Similar to the PM_1 and $PM_{2.5}$ findings, the online trace of UFP showed a pattern attributable to the use of a certain number of tobacco sticks (Figure 8), with the overall mean particle count decreasing with increasing ventilation rate (Table 2). It is interesting to note that, in this instance, the average particle size was 70–125 nm (Table 2).

3.3. Results of Screening of the Gas–Vapor and Particulate Phases of THS 2.2 Environmental Aerosol

The TVOC method is a suitable screening method for obtaining qualitative and quantitative information on compounds eluting between and including hexane (C_6) and hexadecane (C_{16}) in a non-polar column [54]. In general, compounds with boiling points in the range of 50–290 °C are characterized by this method [55]. This would encompass a comprehensive list of compounds in the gas–vapor phase of the environmental aerosol of THS 2.2 in cases where they are emitted indoors.

Compounds identified in the C_6–C_{16} range by the TVOC method in the present study are listed in Supplementary Table S10 and illustrated in Figure 9, and quantitative data on a list of selected VOCs are summarized in Supplementary Tables S11–S13. Analysis of the TVOC data revealed the presence of a number of non-specific compounds. These compounds were present both in the background and THS 2.2 sessions. For instance, at 0.5 h^{-1} (Residential category III environment), typical indoor contaminants, such as benzene, benzaldehyde, benzyl alcohol, nonanal, and methyl cyclosiloxanes (dodecamethylcyclohexasiloxane, and tetradecamethylcycloheptasiloxane), were identified in all or almost all replicates (Supplementary Table S10; Figure 9). In addition, two other typical indoor contaminants (toluene and decanal) were present in some replicates (Supplementary Table S10). Interestingly, when the ventilation rate was increased to 2.4 h^{-1} (Store) or 4.3 h^{-1} (Restaurant), the concentrations of these VOCs decreased, leading to simplification of the TVOC composition (Supplementary Table S10; Figure 9). Equally interestingly, benzaldehyde was systematically detected in all samples, while the other VOCs only appeared sporadically in some samples (Supplementary Table S10).

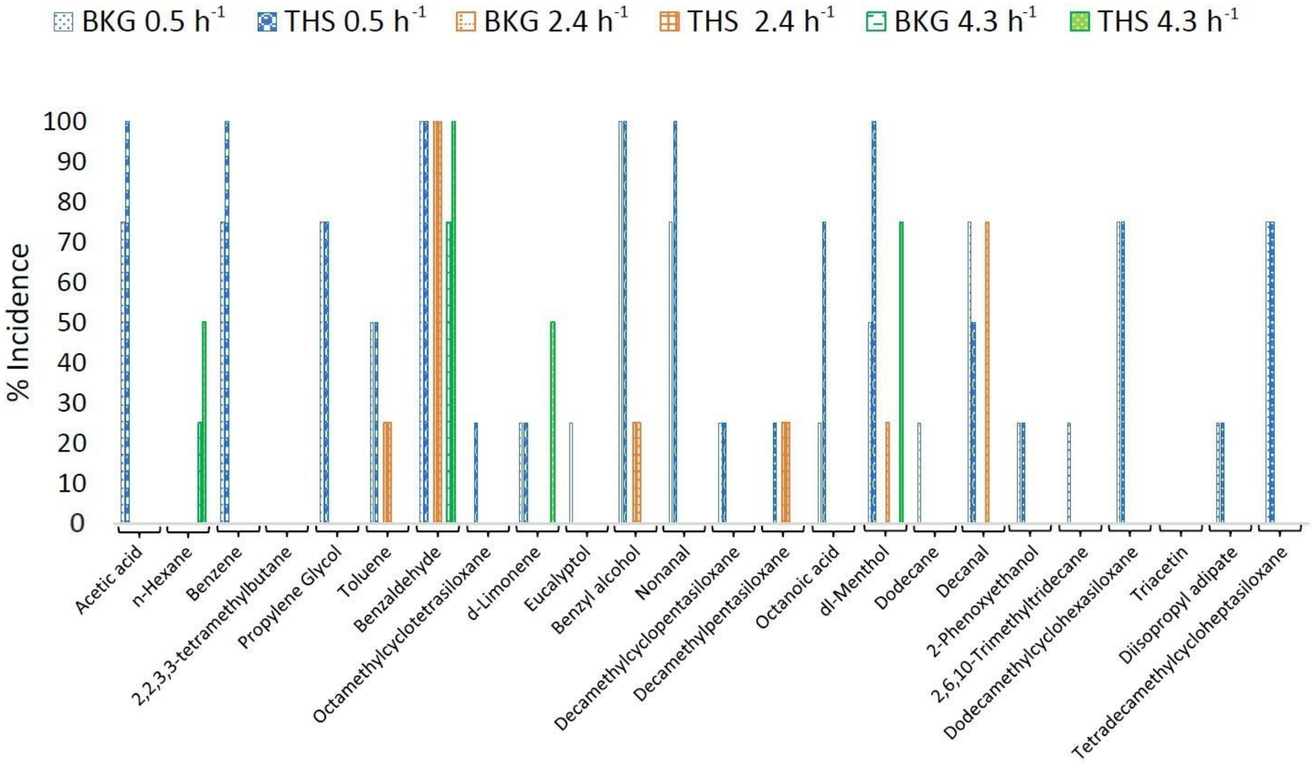

Figure 9. Comparison of the incidence of airborne compounds in the gas–vapor phase (C_6–C_{16} window in the TVOC method) under the Residential category III (0.5 h^{-1}), Store (2.4 h^{-1}), and Restaurant (4.3 h^{-1}) environmental conditions.

A second group of compounds were those that were product-related, considered as such because they fulfilled the following requirements: they were present in most THS 2.2 sessions, generally absent in the background sessions, and identified in the mainstream aerosol of THS 2.2 [53]. Such compounds were propylene glycol, used as solvent for the flavor mixture in the preparation of tobacco sticks (approximately 600 µg/stick in mainstream aerosol), and menthol, a flavor used in the Turquoise tobacco sticks (approximately 2.5 mg/stick in mainstream aerosol). Both compounds were observed in indoor air at 0.5 h^{-1} (Residential category III environment). However, it is important to note that these compounds were not uniquely observed in the THS 2.2 sessions, but were also detected, albeit in lower concentrations, in some background sessions at 0.5 h^{-1} (Supplementary Table S10). Acetic acid only partially fulfills the requirements for being considered a product-related compound. The reason for this is that, although this compound was detected in the mainstream aerosol of THS 2.2 (approximately 1000 µg/stick in mainstream aerosol) [53] and was present in most THS 2.2 sessions at 0.5 h^{-1}, it was also present in all but one background sessions at 0.5 h^{-1} at comparable concentrations (Supplementary Tables S10–S11). When the ventilation rate was increased to 2.4 h^{-1} (store environment) or 4.3 h^{-1} (Restaurant environment), the concentrations of propylene glycol and acetic acid decreased below the reporting limits of the method, while menthol was quantifiable in most of the samples at 4.3 h^{-1} (Supplementary Tables S10–S13). As mentioned above, the change in the design with the insertion of glass panels at 2.4 h^{-1} led to a notable decrease in menthol levels, i.e., a higher decrease than that observed at 4.3 h^{-1} (Supplementary Tables S10–S13).

Importantly, at 0.5 h^{-1}, a few compounds appeared randomly either in both sessions of a replicate on a specific day (octanoic acid, 2-phenoxyethanol, and diisopropyl adipate) or in a single background or THS 2.2 session. This could be attributed to either normal VOC variability or contamination. It should be mentioned that none of these compounds was quantifiable at 2.4 h^{-1} or 4.3 h^{-1} (Supplementary Table S10).

Selected compounds in the C_6–C_{16} trace were quantified, and the results are presented in Supplementary Tables S11–S13. In terms of percentage, the major compounds present in all background and THS 2.2 samples were benzaldehyde, benzyl alcohol, and siloxane derivatives, accounting for 37–100% of the TVOC value at 0.5 h^{-1} (Supplementary Table S11), 67–100% at 2.4 h^{-1} (Supplementary Table S12), and 100% at 4.3 h^{-1} (Supplementary Table S13). In addition, at 0.5 h^{-1}, acetic acid was quantified in all but one of the background sessions, while it was present in all of the THS 2.2 sessions at slightly higher concentrations. At 0.5 h^{-1}, propylene glycol levels in the THS 2.2 sessions were consistently higher than the background levels. All these compounds were below the LLOQ or LOD at 2.4 h^{-1} and 4.3 h^{-1} (Supplementary Tables S11–S13).

Concerning the minor compounds related to THS 2.2 mainstream aerosol [53], furfural, glycidol, 3-chloro-1,2-propandiol and 2-chloro-1,2-propandiol were present at levels below the quantification limit of 2 $\mu g/m^3$ in all background and THS 2.2 sessions in all ventilation conditions, whereas acetol and 2-furanmethanol were not detected. Notably, the insertion of glass panels for replicates 2–4 at 2.4 h^{-1} led to a significant decrease in the levels of furfural, which was, in fact, reported for these sessions as not detected.

Ethylbenzene, *o*-/*m*-/*p*-xylene, styrene, *p*-dichlorobenzene, and tetradecane levels were below the reporting limits in all samples in all ventilation conditions.

In addition to the investigation of the gas–vapor phase of the environmental aerosol of THS 2.2, the organic residue in the PM fraction was evaluated by targeted screening for 36 constituents. The results are summarized in Supplementary Table S6. Anatabine, α-tocopherol, and *N*-octanoylnornicotine levels in the THS 2.2 session increased relative to the background, while nicotine and cotinine were quantified at similar levels in all samples, including those of never-used filters. In addition, α-tocopherol was also quantified in lower concentrations in never-used filters.

Overall, increasing the ventilation from 0.5 h^{-1} to 4.3 h^{-1} led to a significant decrease in trace organic residue levels (Table 1; Supplementary Table S11–S13).

3.4. Results for MLE

The tobacco sticks used during the study were recovered for subsequent mouthpiece filter analysis to determine the user's MLE to nicotine [32]. The panelists' puffing regimen based on the MLE data is illustrated in Figure 10. The MLE calculated for all sticks and brands was 1.26 ± 0.069 mg nicotine/stick (mean ± 95% CI), which was close to, but significantly lower (p = 0.014) than, the mean nicotine yield (mean ± 95% CI, 1.37 ± 0.047 mg nicotine/stick) delivered by the same THS 2.2 products upon machine puffing in accordance with the standard HCI regimen. Therefore, these MLE results serve to support the fact that the sticks were actually and properly used by the panelists.

The estimated loss of nicotine (sum of nicotine levels retained by the panelists and those adsorbed on surfaces, Table 3) was computed for each session on the basis of the inert airborne nicotine levels (model) derived from the MLE values reported in Supplementary Table S15. The estimated loss of nicotine was in the range of 98.6–99.3% at 0.5 h^{-1}, 94.6–98.6% at 2.4 h^{-1}, and 93.9–96.4% at 4.3 h^{-1} (data without background subtraction).

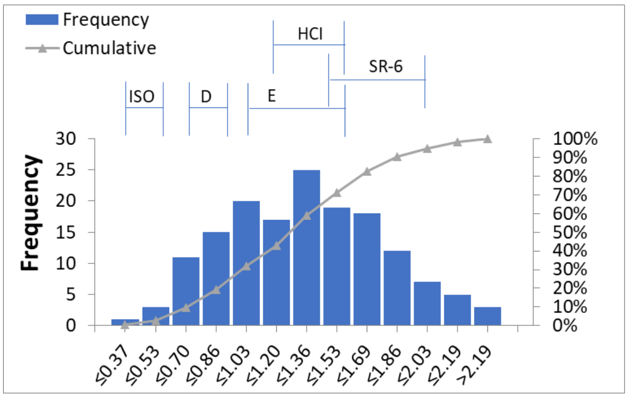

Figure 10. Histogram of MLE (mg nicotine/stick) distribution measured over all brands. H bars represent the range (min–max) covered by machine puffing of the tobacco sticks under the respective regimens described in Supplementary Materials, Table S14.

Table 3. Estimated loss of nicotine (sum of nicotine levels retained by the panelists and those adsorbed on surfaces) during individual THS 2.2 sessions, based on measured airborne nicotine concentrations (with and without background subtraction) and the inert airborne nicotine model (calculated from the measured MLE values).

ACH [1]	Session	Mean	Session	Mean	Mean, BKG-Subtracted	Inert Airborne Nicotine Model	Loss	Loss, BKG-Corrected
(h^{-1})		($\mu g/m^3$)		($\mu g/m^3$)	($\mu g/m^3$)	($\mu g/m^3$)	(%)	(%)
0.5	Background Rep 1	0.426	THS Rep 1	1.49	1.06	113.83	98.7	99.1
0.5	Background Rep 2	0.303	THS Rep 2	1.36	1.06	99.45	98.6	98.9
0.5	Background Rep 3	0.291	THS Rep 3	0.888	0.597	72.07	98.8	99.2
0.5	Background Rep 4	0.249	THS Rep 4	0.633	0.633	89.12	99.3	99.3
2.4	Background Rep 1	0.249	THS Rep 1	2.20	2.2	40.59	94.6	94.6
2.4	Background Rep 2	0.243	THS Rep 2	0.550	0.55	39.06	98.6	98.6
2.4	Background Rep 3	0.243	THS Rep 3	0.792	0.792	34.45	97.7	97.7
2.4	Background Rep 4	0.243	THS Rep 4	0.636	0.636	26.36	97.6	97.6
4.3	Background Rep 1	0.249	THS Rep 1	0.900	0.9	24.76	96.4	96.4
4.3	Background Rep 2	0.249	THS Rep 2	1.05	1.05	24.31	95.7	95.7
4.3	Background Rep 3	0.249	THS Rep 3	1.57	1.57	28.56	94.5	94.5
4.3	Background Rep 4	0.249	THS Rep 4	1.90	1.90	31.29	93.9	93.9

[1] Abbreviations: ACH, air changes/h; BKG, background; MLE, mouth-level exposure; THS, Tobacco Heating System 2.2; Rep, replicate.

4. Discussion

4.1. Simulated Environments

The most important parameters in designing experiments for simulating typical indoor environments are the ventilation rate, number of occupants, and rate of product use. These parameters are described in international norms, such as the European ventilation performance standard EN 15251:2007 [29] and US ventilation standard [30,31]. As illustrated in Supplementary Table S1, the calculated ventilation rates for some environments might be very similar, such as those for the restaurant and residential conditions. However, for some other environments, the proposed ventilation rates and design occupancies might vary.

Concerning ventilation rates, a small number of studies measured the levels of airborne constituents in enclosed environments with switched-off ventilation [7,10,21,23]. In one particular study, somewhat surprisingly, one of the experiments even included a miniscule room (1.4 m^3) with switched-off ventilation [10]. The reason for these experimental setups might have been linked to ensuring high concentrations of the measured airborne constituents to fit method sensitivity requirements or possibly to generate an exceedingly polluted indoor environment. In our opinion, it is questionable if such environments with very poor respect of hygiene norms are representative of real-life environments and, in particular, public environments.

The number of tobacco products consumed in an indoor environment is currently not specified explicitly in any norm. Previously, ventilation for smoking lounges—considered as specific indoor environments where all occupants are smokers—was calculated on the basis of a smoking rate of three cigarettes/person/hour or, for so-called “heavy-smoker lounges”, six cigarettes/person/hour [56]. Additional ventilation for cigarette smoking is calculated in EN 15251, which takes into account the following variables: design occupancy, 20% smokers, and 1.2 cigarettes/h [29]. An example for the calculated rate of consumption for 2-h experiments in the IAQ room in accordance with EN 15251 [29] is given in Supplementary Table S1.

Finally, taking all these considerations into account, we selected three characteristic simulated environments representing two public environments (Restaurant and Store) and a residential environment (Residential category III) (Supplementary Table S1). The Residential category III environment is a reference environment and corresponds to the ventilation given for the European reference room [57]. By convention, ventilation at 0.5 h^{-1} is considered to represent normal indoor air conditions and is used for comparison of indoor emissions [57]. The calculated air changes per hour for the restaurant environment match European and US ventilation norms [29,30]. Furthermore, to have a better understanding of the relationship between airborne constituents and ventilation rates, we selected a simulated environment with an intermediate ventilation between the residential category III and restaurant environments, i.e., the store environment. This environment corresponds to the “Department store” environment described in the European norm [29]. However, it should be noted that it has a greater ventilation rate than that described in the US norm [30] (Supplementary Table S1). The design occupancy and number of sticks were fixed for the three simulated environments (Supplementary Table S1). Thus, the residential category III simulation was representative of high-load conditions (low ventilation combined with a high rate of consumption), while the other two simulated environments—although both were above the minimum rate corresponding to 20% users and 1.2 sticks/h—did not entirely fulfil the requirements for a high-load environment.

The position of the panelists was defined to be at 1 m from the sampling traps in front of the panelists. This distance corresponds to the so-called “personal distance” used for interaction among good friends or family members [58].

4.2. Characterization of the Environmental Aerosol of THS 2.2

Consistent with the results of our previous studies [18,19], the data of this assessment clearly demonstrated that indoor use of THS 2.2 led to an increase—relative to the background levels—in the concentrations of acetaldehyde, glycerin, and nicotine, which are the three major compounds present in THS 2.2 mainstream aerosol [36]. Furthermore, these results are in agreement with the data from other researchers on nicotine [4,10,24] and acetaldehyde [4]. The presence of airborne nicotine and, to a lesser extent, glycerin in indoor environments is likely to be due to the use of nicotine- and/or tobacco-containing products, even if neither of these compounds is unique to such products. The absence of airborne nicotine in a previous study that involved high THS 2.2 use in a location with no ventilation [7] might be explained by the low method sensitivity in that study. In contrast to nicotine and glycerin, acetaldehyde is a common indoor pollutant, emitted by multiple indoor sources, including common everyday life activities [40]. Therefore, during THS 2.2 use in real-life environments, acetaldehyde levels might remain in the range of background variations because of the presence of confounding pollution sources, as reported in studies in cars [24] and a night club [23]. Accordingly, an increase in acetaldehyde concentrations above the background during THS 2.2 use would probably be monitored in environmentally controlled rooms with filtered air, but not in some real-life settings.

Similar to the outcome in a study that used mentholated tobacco sticks, we observed an increase in TVOC levels attributable to the indoor use of THS 2.2 [17]. This increase was mainly related to emission of menthol and, in some cases, a combination of menthol and limonene. However, as shown in a previous study, in cases where non-mentholated tobacco sticks were used, only low increases in TVOC levels above the background were measured, although the differences were not statistically significant [18].

Investigation of gas–vapor phase compounds eluting between C_6–C_{16} [54] indicated that two THS 2.2-related compounds (acetic acid and propylene glycol), together with three background-related compounds (benzaldehyde, benzyl alcohol, and siloxane derivative), contributed to some extent to the increase in TVOC levels during THS 2.2 sessions at 0.5 h^{-1} (Supplementary Tables S11–S13). An increase in the ventilation rate to 4.3 h^{-1} led to decreased levels of the above-mentioned airborne compounds along with a related drop in the TVOC values (Table 1; Supplementary Tables S11–S13). With regard to the gas–vapor phase components of THS 2.2 environmental aerosol, it is important to highlight that the levels of minor compounds detected in sidestream emissions of THS 2.2 in experiments in a small exposure chamber (acetol, 2-furanmethanol, furfural, glycidol [5]) as well as the trace compound 3-chloro-1,2-propandiol (previously reported in THS 2.2 mainstream aerosol [53]) were all below the LLOQ [54] of the method in all ventilation conditions (Supplementary Table S11–S13).

Some studies have reported an increase in UFP particle number concentrations during indoor use of THS 2.2 [2,4,11,12,16,23,24], and the present investigation confirmed this result. However, it is important to underline the following: at distances of 1.1–1.8 m between the panelists and measurement equipment (Supplementary Table S5) and with simultaneous analysis of the online traces in relation to the consumption patterns of tobacco sticks, one tobacco stick was notably not registered in the online trace of the UFP sensors, whereas two or three sticks used simultaneously or within a very short time lapse were easily detected (Figure 8). In agreement with these results, recent studies showed that the use of one stick of THS 2.2 led to only slightly elevated concentrations of UFP that lasted for a short time in sharp contrast to the other evaluated sources, such as burning of candles and incense sticks or smoking of cigarettes [16,59]. Meisutovic-Akhtarieva et al. evaluated the particle number concentrations in the range of 10–420 nm during indoor use of THS 2.2 in a series of experiments with varying numbers of parallel users, distances to the bystanders, ventilation rates, and relative humidity values [4]. In these experiments, a distinct saw-tooth pattern in the particle concentrations during THS 2.2 consumption was apparent at a distance of 0.5 m while progressively longer distances resulted in lower intensity or absence of peaks [4]. Furthermore, the particle number concentrations were significantly higher with five and three parallel users compared to one user [4]. Overall, the present UFP results are in line with those of other studies [2,4,11,12,16,24]. Indeed, the range of particle counts/cm^3 in the present study and above-mentioned studies is similar, even though the absolute values vary to a certain extent because of differences in experimental settings (volume, ventilation, distances between users and measurement equipment, etc.). Furthermore, as previously described [2,4,11,12,16,24], the temporary increase in the UFP levels in the present study was followed by a rapid decay in the particle number concentrations, leading to a distinct saw-tooth pattern in the online trace (Figure 8). As detailed by Meišutovič et al. [4], all these observations are explained by the high volatility of the liquid droplets generated during THS 2.2 use and their fast dispersion in enclosed spaces. Other investigations have reached a similar conclusion [11,12,16].

Likewise, all size-segregated channels of the light-scattering DustTrak monitor were activated and gave similar responses within the typical method uncertainties following the simultaneous use of two or three tobacco sticks (Figure 8). These measurements indicated that PM with an aerodynamic diameter <1 µm was generated during THS 2.2 use. However, the mean PM_1 values (as well as $PM_{2.5}$ values) during the indoor use of THS 2.2 were below the LLOQ of the method, even if several peaks were recorded in the online trace with similar temporal patterns as those for UFP (Figure 8).

The increase in the mean particle number concentrations of UFP accompanied by an absence of change in the mean PM_1 and $PM_{2.5}$ levels during indoor use of THS 2.2 coincided with the outcomes of some studies [16,24]. Previous studies have reported similar concentration ranges for PM_1 [2] and $PM_{2.5}$ [2,4] as those in the current study.

However, some studies have also reported somewhat higher concentrations for PM [6,14], and one study has even reported values as extreme as a median of 338 $\mu g/m^3$ for 12 puffs of THS 2.2 [22]. Similar broad-range variations in the mean concentrations of PM have been noted in indoor air quality studies on e-vapor products [17]. Such variability can be partially explained by the different experimental setups used. Nevertheless, in this context, it is important to emphasize that sensors for the real-time measurement of PM as well as all other sensors must be validated as is required for classical offline methods. Only through validation is it possible to establish the proper LOD and LLOQ of the methods for a specific matrix or the appropriate calibration factors, as discussed in detail for the real-time monitoring of suspended PM by DustTrak [42]. Thus, without having these methodological details, it is difficult to understand the reason for the discrepancies noted in the reporting of PM concentrations between this and some of the above-mentioned studies.

All in all, despite the differences in reported PM values inherent to the different experimental setups used—and which may have also resulted from some other factors—the plots of the online PM traces are very similar among the studies. Indeed, all experiments have shown peaks of PM attributable to puffing, with sharp increases in the particle concentrations and rapid decreases when THS 2.2 use was stopped.

During this study, the UVPM and FPM measurements indicated a slight increase (versus the background) in the organic residue in the PM fraction of the THS 2.2 environmental aerosol at 0.5 h^{-1} (Table 1). Even if these increases were within the range of method uncertainties (below the LWRL), considering the concerns raised in some publications [2,5], we deemed an investigation appropriate. Furthermore, it was important to understand the composition of the liquid droplets emitted into the environment during THS 2.2 use. Thus, it is altogether plausible to consider glycerin as one such constituent on the basis of its boiling point and the systematic increase in its levels in these (Figure 3) and previous experiments [18]. Our data revealed the presence of very low levels (<0.5 $\mu g/m^3$) of anatabine, *N*-octanoylnornicotine, and α-tocopherol in the organic residue of PM collected for UVPM and FPM measurements (2 $\mu g/m^3$ is considered as the threshold of toxicological concern for unknown or insufficiently characterized substances [54,60]) (Supplementary Table S6). Each of the three compounds has a chromophore, which might explain the slight increase in UVPM during THS 2.2 use. In addition, α-tocopherol is a fluorescent compound [61], which accounts for the very slight increase in FPM during the THS 2.2 sessions in this study.

With regard to the slight rise (considered as such as it falls within the range of method uncertainty; LLOQ < x < LWRL) in the indoor concentrations of acrolein above the background in THS 2.2 sessions at 0.5 h^{-1} (Table 1), no further investigations could be performed to verify the results with the current experimental setup. Indeed, acrolein is a minor airborne carbonyl which is generally analyzed with the classical method for carbonyl quantification by using a 2,4-dinitrophenylhydrazine cartridge. As has been well documented in the literature, this trapping method causes artifact formation and is generally considered semiquantitative [62]. Accordingly, the literature shows contradictory data on acrolein measurement, and serious issues have been observed in interlaboratory comparisons [62]. Our previous experiments with machine puffing for THS 2.2 had produced variable results for acrolein (Supplementary Figure S4). Therefore, the present results must be interpreted with caution, in particular, considering the very low concentration range measured (THS 2.2, 0.09–0.13 $\mu g/m^3$, <LWRL; Supplementary Table S2).

The indoor concentrations of gases are strongly influenced by their outdoor concentrations. Thus, as noted during a previous study [18], the CO, NO, and NO_x concentrations decreased during the afternoon sessions, and, accordingly, their levels were lower during the THS 2.2 use sessions (Supplementary Tables S7–S9). Interestingly, at first glance, it seemed that the NH_3 concentrations increased relative to the background during THS 2.2 use in low-ventilation conditions (0.5 h^{-1}) (Supplementary Table S7). However, the rise in NH_3 levels during the break (Supplementary Table S7) indicated that, very probably, this observation was related to the purge, which caused outdoor NH_3 to be pumped into the

IAQ room. This was unexpected and merits further investigation to understand the actual influence of THS 2.2 use on the indoor concentrations of NH_3.

The vast majority of the other constituents measured in this study remained below the reporting limits or at background levels. This category included 25 constituents quantified with ISO 17025-accredited methods (Tables 1 and 2), 24 constituents in the gas–vapor phase (C_6–C_{16} window; Supplementary Tables S11–S13), and 33 constituents in the particulate phase (Supplementary Table S6). In addition to these findings, the results of some independent studies have shown no increase in metal [2] or polycyclic aromatic hydrocarbon [2,7,21] emissions during indoor use of THS 2.2. All these findings serve to confirm that the level of indoor emissions during THS 2.2 use is low.

Interestingly, increasing the ventilation rate from 0.5 h^{-1} to 4.3 h^{-1} led to a decline in the indoor concentrations of acetaldehyde (Figure 2), TVOCs (Figure 4), UFP (Table 2), and airborne glycerin (Figure 3), albeit to a very slight extent in this latter case. In contrast, the levels of airborne nicotine were not at all influenced by the ventilation rate (Figure 1). However, the change in the design with the insertion of glass panels to separate the panelists brought about a substantial drop in airborne nicotine levels (Figure 1; Table 1; Supplementary Table S2). Furthermore, the presence of glass panels resulted in a decrease in airborne menthol concentrations and probably also furfural concentrations (Table 1; Supplementary Table S12). These findings are intriguing and warrant further investigation to explore whether introducing indoor materials that adsorb the few airborne constituents present in indoor environments during THS 2.2 use may be, together with the use of ventilation, a reliable means of controlling the levels of indoor contamination.

Finally, it should be highlighted that none of the constituents attributable to indoor use of THS 2.2 exceeded the exposure limits defined by cognizant authorities (Supplementary Table S16) [63–69]. In this context, it is important to emphasize that guideline exposure limits for TVOCs exist in some countries. For example, the Federal Environment Agency of Germany defines TVOC concentrations <300 $\mu g/m^3$ as hygienically harmless and those in the range of 300–1000 $\mu g/m^3$ as having no effect if individual substances do not exceed guideline levels [69]. Monocyclic terpenes such as menthol have chronic exposure levels of 1000 $\mu g/m^3$, which is well above those measured in the present experiments with mentholated tobacco sticks [69].

4.3. Comparison of Environmental Emissions between THS 2.2 and Other Sources

The scientific community recognizes that indoor emissions of THS 2.2 and electrically heated tobacco products in general are substantially lower than those of cigarettes and other combustion-based tobacco products [2–16,22,59]. Generally, it is accepted that THS 2.2 and similar products do not have persistent effects on indoor pollution relative to cigarettes [4,6,8,11,12,16,23,59]. Yet, many researchers working in the field of indoor air quality consider that only those products that do not expose potential bystanders to any kind of emission could be suitable for indoor use. This would directly exclude all products that generate aerosols and could theoretically be extended to many consumer goods if we consider the levels of airborne constituents they emit [17,40,70]. Understandably, concerns have been raised about the nonregulated use of THS 2.2 and similar products in confined environments with low ventilation, particularly with regard to the possible exposure of sensitive populations [5,10,24,71]. Furthermore, it is difficult to reach an agreement on what would be considered as low or negligible levels [18,19] compared with what would not be considered as such [2,5,24]. It is also interesting to note that several researchers are regarding the topic in a broader context and are debating the actual levels of particle or gaseous pollution attributable to these products in comparison to the overall pollution that exists in crowded real-life environments [15,23,59,72].

Figure 11 summarizes the data of the current and previous studies on THS 2.2 [17,18] in comparison to those from cigarettes [18], incense [17], and some common everyday-life activities [40]. These data show that indoor THS 2.2 emissions are substantially lower than those of the products and activities used for comparative purposes. These results are in line with the conclusion of a review which compares the environmental aerosol of THS 2.2 with the pollutant levels in public and residential environments [15]. The authors of this review concluded that, when compared with indoor THS 2.2 use, incense and mosquito coils emit higher levels of aldehydes, VOCs, and $PM_{2.5}$ [15]. Accordingly, persons in proximity to such non-nicotine combustion products might be exposed to greater levels of pollutants than would persons standing near a user of THS 2.2 or other similar products [15]. Furthermore, real-life public and transport environments have equivalent or even significantly higher levels of formaldehyde, acetaldehyde, benzene, and toluene when compared with the concentrations measured during THS 2.2 use [15]. The data in Figure 11 are in agreement with those in a study which showed that the particle numbers, mass concentrations, and acetaldehyde levels during the operation of a nightclub exceed those measured during THS 2.2 use [23]. In addition, Hirano and Takei concluded that the lifetime cancer risk resulting from exposure to environmental aerosol of THS 2.2 is at a tolerable level under common indoor conditions, in contrast to the increased risk resulting from exposure to environmental tobacco smoke [72].

	2016	**2020**	**2020**	2018	**2020**	2018	2018					
ACH	0.5	**0.5**	**2.4**	4.3	**4.3**	4.3	4.3	0.5	0.5	0.5	0.5	0.5
No persons	3	**4**	**4**	4	**4**	16	16	0	3	3	3	3
No products	12	**12**	**12**	8	**12**	8	39	1	12			
Mentholated products		*	*		*	*	*					
	THS 2.2	**THS 2.2**	**THS 2.2**	THS 2.2	**THS 2.2**	THS 2.2	THS 2.2	Incense	Cigarettes	Wine	Toiletries	Raclette + meat
UVPM-THBP								13.9	92.9	nm	nm	nm
FPM-scopoletin								3.62	20.4	nm	nm	nm
3-Ethenylpyridine								nm	10.3	nm	nm	nm
Nicotine	1.15	**1.09**	**0.66**	0.66	**1.36**	1.14	3.73	nm	49.8	nm	nm	nm
Acetaldehyde	3.44	**3.69**	**1.22**	0.75	**1.00**	0.90	4.56	22.2	122	27.6	3.69	58.5
Acrolein								5.31	12.4			15.4
Crotonaldehyde								0.86	3.57			2.06
Formaldehyde								29.0	58.9	1.60	2.29	21.6
Acrylonitrile								0.68	5.28			
Benzene								75.2	14.2			
1,3-Butadiene								11.6	17.6			
Isoprene								9.05	164	2.54		1.71
Toluene								11.6	25.2			
TVOC		**22.5**	**9.96**		**4.11**	6.39	44.5	323	445	51.1	1165	627
Catechol	nm			nm		nm	nm	nm	nm	nm	nm	nm
Hydroquinone	nm			nm		nm	nm	nm	nm	nm	nm	nm
Glycerin	13.3	**20.1**	**13.6**	8.59	**15.8**	7.38	32.1	nm	10.0	nm	nm	nm
Propylene glycol								nm	64.2	nm	nm	nm
NNK								nm	8.9E-03			
NNN								nm	1.5E-03			
PM_1								nm	687			441
$PM_{2.5}$								nm	688			452
UFP	nm	**15778**	**6912**	nm	**5520**	nm	nm	180702	77502	nm	nm	nm
CO								1497	2820			83.7
CO_2	nm			nm		nm	nm	nm	nm	nm	nm	nm
NH_3	nm							nm	nm	nm	nm	nm
NO								13.7	71.8			
NO_x								21.0	76.2			
O_3	nm			nm		nm	nm	nm	nm	nm	nm	nm
	(difference in units)							Combustion (difference in units)		Daily living (difference in units)		

Figure 11. Summary of the background-subtracted mean concentrations of the airborne constituents (µg/m^3) in environmental aerosol of THS 2.2 under simulated Residential category III (0.5 h^{-1}), Store (2.4 h^{-1}), and Restaurant (4.3 h^{-1}) environmental conditions (values in bold) compared with corresponding values from previous studies; *: use of mentholated products; Abbreviations: ACH, air changes/h; FPM, fluorescent particulate matter; nm, not measured; NNK, nicotine-derived nitrosamine ketone; NNN, *N*-nitrosonornicotine; PM, particulate matter; TVOC, total volatile organic compounds; UFP, ultrafine particles; UVPM-THBP, ultra-violet particulate matter-2,2′,4,4′-tetrahydroxybenzophenone. 2016 and cigarette reproduced with permission from [18]; 2018 and incense reproduced with permission from [17], the data on UFP levels in cigarette (12 cigarettes, 6 cigarettes/h) and incense stick (1 incense stick and 3 candles) emissions were acquired during a separate study; wine, toiletries, and raclette and meat reproduced with permission from [40]. Note: The TVOC concentrations from incense stick and cigarette emissions are in the range (300–1000 µg/m^3) defined by the German Federal Environment Agency as hygienically safe if no guideline value is exceeded [69]. As the exposure limits for benzene (a constituent eluting in the C_6–C_{16} range of the TVOC) from incense stick and cigarette emissions are exceeded, the TVOC levels during the use of these products are considered to be not safe (indicated in red). The TVOC concentrations during the preparation and consumption of raclette and meat are in the range of 300–1000 µg/m^3, while those during the use of toiletries are between 1000–3000 µg/m^3 (hygienically suspicious). Yet, only if the levels of individual substances exceed the guideline values shall the values for these activities be indicated in red.

A recent study investigated the biomarkers of exposure in bystanders during high-rate use of THS 2.2 in a real-life setting [73]. The evaluation showed that nonsmokers passively exposed to THS 2.2 aerosol do not have increased nicotine, NNN, or NNK exposure relative to non-exposed nonsmokers [73]. These data are consistent with the low levels of emissions measured during THS 2.2 use.

Finally, a recent review of current evidence on heated tobacco products suggested that, relative to cigarettes, these products might have reduced-risk potential for chronic diseases related to smoking and second-hand cigarette smoke exposure and that this potential should be confirmed by further studies [9].

5. Conclusions

We performed a comprehensive evaluation of the environmental aerosol of THS 2.2 in comparison to the background under three ventilation conditions representative of simulated residential category III (0.5 h^{-1}), store (2.4 h^{-1}), and restaurant (4.3 h^{-1}) environments. We determined the concentrations of a comprehensive list of 31 airborne constituents as well as the concentrations of 30 gas–vapor phase and 36 particulate phase constituents. This huge increase in the number and type of constituents evaluated has substantially broadened the scientific knowledge and understanding of the impact of THS 2.2 usage; it provides both a wider and deeper perspective of the topic, which will be of value to policy makers and help contribute to making informed decisions. Furthermore, the sheer volume of data accumulated for THS 2.2 environmental aerosol means that these data could serve as a reference for the electrically heated tobacco product category in future indoor air quality studies.

Most importantly, the current evaluation of environmental aerosols of THS 2.2 demonstrated low levels of contamination of the indoor environment irrespective of the ventilation rate applied. This includes contamination not only from cigarette smoking-related markers such as 3-ethenylpyridine, but also characteristic air contaminants such as formaldehyde, benzene, toluene, CO, NO, and NO_x. Indoor use of THS 2.2 causes a systematic increase, relative to the background, in the concentrations of UFP, airborne nicotine, acetaldehyde, glycerin, and, in cases where mentholated products are used, menthol, with a corresponding increase in TVOC values. Nicotine, acetaldehyde, glycerin, and TVOCs were measured in the low $\mu g/m^3$ range, and these results corroborate well with those of previous studies. At no time did the levels of these compounds surpass the exposure limits defined by cognizant authorities in any of the evaluated ventilation conditions. The indoor concentrations of acetaldehyde, TVOCs, and UFP decreased with increasing ventilation rates, while the levels of airborne glycerin were only slightly influenced and those of nicotine not at all. Notably, the introduction of additional surfaces in the environmentally controlled room (glass panels separating the panelists) led to a substantial decrease in the indoor concentrations of nicotine and probably some VOCs, such as menthol. These findings merit further investigation that may contribute to defining a proper strategy for reducing residual indoor emissions from heated tobacco products.

To summarize, the data presented here demonstrate that the levels of airborne constituents measured during THS 2.2 use in environments with adequate ventilation are substantially below threshold limits set forth in air quality guidelines established by cognizant authorities. Furthermore, to contextualize the actual impact of THS 2.2 and similar products indoors, it is crucial to consider the airborne pollutant levels in current real-life environments. Lastly, it is to be remembered that THS 2.2 and similar products should be used with caution and courtesy around other adults and only where local regulations permit such use [17]. Furthermore, as a general precaution, users should refrain from consuming such products in the presence of pregnant women or children [17].

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/atmos12080989/s1.

Author Contributions: Conceptualization, M.I.M. and C.G.G.-G.; writing—original draft preparation, M.I.M.; writing—method descriptions, preparation of tables and figures, C.C., D.C., M.I.M., S.K., L.P. and S.S.S.; statistical processing, S.K.; writing—review and editing, all. All authors have read and agreed to the published version of the manuscript.

Funding: Philip Morris International is the sole source of funding and sponsor of this research.

Institutional Review Board Statement: Ethical review and approval were waived for this study. Indoor air quality studies do not enter under the provisions of the Human Research Act [74,75]. Collection of air samples in a room where four subjects were present at the same time had no adverse effect whatsoever on these persons.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data are summarized in Supplementary Materials. Refer to Table S2: Descriptive statistics.

Acknowledgments: We would like to express our sincere appreciation to the following persons for their valuable support in this study: Sara Garcia Moreno for operating the exposure chamber and performing the sample collection, Noel Bielik, Josée Polier-Calame, Kelly Crespo, Anne Jaquier, Maria Gomez Lueso, Carole Medan, Chantal Pitton, Mathieu Schaller, and Vladimir Zaugg for performing analyses and results verification, Anneke Glabasnia and Michel Rotach for supervising these activities, Michel Rotach for compiling the results, Anne Guenin-Clottu for coordinating the panelists' activities, Julia Carroll and Sindhoora Bhargavi Gopala Reddy for editing the manuscript, Serge Maeder for supporting the study, and the Work Life Experience Department of R&D Philip Morris International for their continuous support.

Conflicts of Interest: All authors were Philip Morris International employees at the time the study was conducted.

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