Comparative and cumulative quantitative risk assessments on a novel heated tobacco product versus the 3R4F reference cigarette

Carole Hirn 1, Yuki Kanemaru 1, Todd Stedeford 2, Thilo Paschke, Irene Baskerville-Abraham *

Scientific and Regulatory Affairs, JT International SA, Geneva, Switzerland

ARTICLE INFO

Keywords:
Quantitative risk assessment
Heated tobacco product
Harmful and potentially harmful constituents

ABSTRACT

Novel tobacco products that heat rather than burn tobacco (heated tobacco products or HTPs) have been shown to produce lower levels of harmful and potentially harmful constituents than conventional combusted cigarettes. The present study uses a quantitative risk assessment approach to compare non-cancer and cancer risk estimates for emissions generated by an HTP with smoke from a reference cigarette (3R4F). Fifty-four analytes were evaluated from the HTP aerosol and the 3R4F cigarette smoke. Emissions were generated using the ISO and the Health Canada Intense smoking regimes. The measured values were extrapolated to define a conservative exposure assumption for per day use and lifetime use based on an estimated maximum usage level of 400 puffs per day i.e., approximately 8 HTP tobacco capsules or 40 combustible cigarettes. Non-cancer and cancer risk estimates were calculated using these exposure assumptions for individual and per health outcome domains based on toxicological reference values derived by regulatory and/or public health agencies. The results of this assessment showed a reduction of non-cancer and cancer risk estimates by more than 90 % for the HTP versus the 3R4F cigarette, regardless of the smoking regime.

1. Introduction

After the publication of two landmark articles indicating a dose-response relationship between smoking and lung cancer in 1950 [1,2], significant efforts were made for the chemical characterization of cigarette smoke, identification of toxic smoke constituents considered most relevant for smoking related diseases and reduction of these smoke constituents in cigarette smoke. Between 1954 and 1993, the efforts to reduce toxic constituents in smoke and smoking related health risks resulted in a stepwise reduction of tar and nicotine yields in cigarette smoke from US cigarettes by almost 70 % [3]. Although the introduction of lower yield cigarettes did not reduce the prevalence of smoking related diseases proportionally to the reduction in smoke constituents, the U.S. National Cancer Institute concluded that overall the available epidemiological studies suggest that there is evidence for a lower risk of lung cancer among populations of smokers who use lower yield products [4]. More than 8000 constituents have been identified in tobacco smoke [5] and several research groups have compiled lists with prioritized toxic smoke constituents [6–8].

Although the role of single, particular smoke constituents in the etiology of smoking related diseases is still not clear [9], the monitoring of toxic smoke constituents has been suggested for the characterization of cigarettes by the scientific community and adopted by regulatory bodies [10–13]. Recently, Heated Tobacco Products (HTPs) have gained popularity among smokers [14,15]. HTPs are heating and not combusting the tobacco, which results in a substantial reduction of carcinogens and other toxicants in their aerosol when compared to cigarette smoke [16,17]. Likewise, the genotoxicity and cytotoxicity of HTPs is significantly reduced when compared to conventional cigarettes, suggesting that these product categories have significant potential to reduce cancer and other smoking related diseases [17–19].

In 2019, the U.S. Food and Drug Administration (FDA) stated that the comparison of harmful and potentially harmful constituents (HPHCs) between two tobacco products is critical in determining whether those two products present similar risks or whether one of the two products presents greater risk [20]. Additionally, it was proposed to use toxicity reference values (TRVs) to evaluate inhalation exposure to constituents.

https://doi.org/10.1016/j.toxrep.2020.10.019
Received 20 April 2020; Received in revised form 9 October 2020; Accepted 23 October 2020
Available online 2 November 2020
2214-7500 © 2020 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license
in tobacco smoke or aerosols [21]. Hence, quantitative risk assessment (QRA) is seen as useful in a comparative assessment of two tobacco products. As a proof of concept, Marano et al. proposed a way of assessing the potential impact of some defined risks by modeling the QRA after the methodology for the assessment of constituent mixtures at superfund sites [22], as presented in the U.S. Environmental Protection Agency’s Risk Assessment Guidance for Superfund (RAGS) [23]. This approach requires measuring yields of machine generated smoke and aerosol constituents using a standardized smoking regime to calculate non-cancer risk using a hazard quotient (HQ) and the cancer risk using Excess Life Time Cancer Risk (ELCR) for each constituent, allowing a risk comparison. The HQs and ELCRs of single constituents are then added to determine the hazard index (HI) and the sum of the ELCRs of a mixture for a given toxicological hazard. The HI of mixtures, such as smoke from different cigarettes or aerosol from HTVs or e-cigarettes, can then be used to compare the theoretical toxicological hazard(s) emerging from various mixtures. A comprehensive review of the use of HIs for the theoretical evaluation of cigarette smoke toxicity has been published by Haussmann [24].

Japan Tobacco Inc. has developed a novel HTP that eliminates combustion through indirectly heating a tobacco blend. The product uses a hybrid technology to create a tobacco-enriched aerosol, by heating a non-nicotine containing liquid, which passes through a capsule containing granulated tobacco. In doing so, the tobacco is heated at around 30 °C and no combustion occurs throughout the process. A recently published study showed that biologically active smoke constituents are substantially reduced in the aerosol generated from the HTP compared to a reference cigarette and that the aerosol displayed reduced genotoxic or cytotoxic response in in vitro assays [17]. The work described herein aims to further investigate the potential reduction in toxicological risk associated with the use of this HTP when compared with cigarettes by applying QRA principles. For this purpose, we calculated the individual and per health domain-cumulative toxicological risk for 54 constituents measured in the aerosol of the HTP and in smoke from a Kentucky reference cigarette (i.e., 3R4F). The objective of this study was to establish the use of QRA as a tool for a comparative toxicological assessment of emissions from an HTP relative to emissions from a conventional cigarette.

2. Materials and methods

2.1. Chemical analysis

The constituents measured from the aerosol of the HTP and from the smoke of the Kentucky reference cigarette 3R4F were selected based on the following regulatory lists of major constituents and toxic compounds (Supplemental Table I):

- The abbreviated list of HPHCs required for reporting by the FDA [12].
- The constituents recommended for measurement in the FDA’s Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems (ENDS) [25].
- The Health Canada Tobacco Reporting Regulations (SOR/2000-273) [11].
- The “Study on the development of a EU common reporting format for submission of data on ingredients contained in tobacco and related products, and disclosure of the collected data to the public” [26] and
- The non-exhaustive list of priority contents and emissions of tobacco products identified by the WHO Study Group on Tobacco Product Regulation [27].

The 54 analytes evaluated in this assessment were measured by Labstat International ULC, a third-party contract research organization. In those studies, Labstat reported analyte yields in aerosol and smoke based on five independent measurements per HTP capsule or per cigarette using two different smoking regimes. Smoke and aerosol were generated by machine smoking using the ISO (i.e., 35 ml puff volume, 2 s duration, 60 s puff interval) and the Health Canada Intense (HCI) (i.e., 55 ml puff volume, 2 s duration, 30 s puff interval) smoking regimes. For the HTP, the total puff number was 85 puffs for ISO regime and 70 puffs for HCI regime per capsule. For cigarettes, the total puff number was 7.8–8.7 puffs for ISO regime and 9.6–11.6 puffs for HCI regime. In the assessments performed herein, the mean yield values reported by Labstat were used for calculating the exposure concentrations.

2.2. Human health toxicity values

The EPA hierarchy for using human health TRVs in superfund risk assessments was applied to select values when more than one value for the same chemical was available from different organizations [28]. Briefly, EPA established a tiered approach for selecting TRVs based on the following:

- Tier 1 – EPA Integrated Risk Information System (IRIS) values for reference concentrations (RfCs) and Inhalation Unit Risks (IURs)
- Tier 2 – EPA Provisional Peer Reviewed Toxicity Values (PPRTV) for provisional RfCs and IURs
- Tier 3 – Other toxicity values, including the California Environmental Protection Agency (CalEPA) Recommended Exposure Levels (RELs), IURs, and Inhalation Slope Factors (ISFs), the Agency for Toxic Substances and Disease Registry Minimal Risk Levels, or the Texas Commission on Environmental Quality (TCEQ) Effects Screening Levels (ESLs).

This tiered approach was used unless a more recent assessment was available. For non-cancer effects, EPA RfCs, CalEPA chronic RELs, or TCEQ long-term ESLs were used. For cancer risk estimates, EPA IURs, CalEPA IURs, or TCEQ IURs were used. When cancer risk estimates were reported as ISFs, these values were converted to IURs.

When no TRV was available for a specific analyte, either an analog approach or a threshold of toxicological concern (TTC) approach was used which was based on the European Medicines Agency (EMA) recommended level for genotoxic impurities (i.e., 1.5 μg/day) [29].

2.3. Exposure concentrations

Exposure concentrations (ECs) were estimated for assessing non-cancer and cancer risks using the following equation, where the EC is based on the analyte concentration in emission that is time-weighted to account for the duration of exposure and the activity patterns particular for smoking and vaping [30].

\[ \text{EC} = \frac{(CA \times ET \times EF \times ED)}{AT} \]

Where EC (μg/m³) = exposure concentration, CA (μg/m³) = analyte concentration in air, ET (h/day) = exposure time, EF (days/year) = exposure frequency, ED (years) = exposure duration, and AT (ED in years × 365 days/year × 24 h/day) = averaging time

The analyte concentration in air (CA) was estimated based on the level of the substance measured in specified puff count (per collection, i.e., 85 puffs for the HTP and 7.8–8.7 puffs for the 3R4F under the ISO regime, and 70 puffs for the HTP or 9.6–11.6 puffs for the 3R4F under the HCI regime) and the volume of air exchanged for 400 puffs. When substances were at the limit of quantification (LOQ) or the limit of detection (LOD), these values were substituted with constant values for the LOQ as (LOD + LOQ)/2 and for the LOD as half the LOD. For example, the measured quantities of 1-aminonaphthalene for the HTP were below the LOD of <0.082 ng in aerosol under the HCI regime. These values were converted to a “μg/m³” level based on the air exchange for the HCI regime and the specified puff counts, i.e., 70 puffs. Example calculations using the HCI regime are shown below:
Quantity in 400 puffs = 0.082 ng/2 × 1 ng/1,000 μg × 400 puffs/70 puffs = 0.000234 μg
CA = 0.000234 μg / (55 mL/puff × 400 puffs × 1E-06 m³ / 1 mL) = 0.0106 μg/m³

No corrections were made for absorption or for mouth spill.

The exposure time (ET) was calculated based on the puffing regime used for measuring the analytes (i.e., ISO and HCI [2 s duration]) per collection adjusted to 400 puff counts. Example calculations using the HCI regime are shown below:

ET (h/day) = 2 s/puff × 400 puffs × 0.00028 h/l s = 0.224 h/day

The puff time was used instead of smoking session time (e.g., ISO = 2 s puff and 60 s interval between puffs; HCl = 2 s puff and 30 s interval between puffs) because of the absence of side-stream aerosol from the HTP versus a combusted cigarette [31]. It should be noted that this is a conservative approach as it increases, rather than decreases, the calculated risks for the HTP versus the cigarette.

The exposure frequency (EF) was assumed to be daily (i.e., 365 days per year) for an exposure duration (ED) of 54 years based on an assumed initiation at 18 years of age and a global life expectancy of 72 years [32].

Of note, the estimated ECs do not account for intermittent exposures and dilution that would occur under intended usage conditions. This was considered an acceptable omission given that it will overestimate the potential risks.

2.4. Risk assessment

2.4.1. Non-cancer risks

Non-cancer risks were quantified with the HQ approach, which is based on the following equation:

HQ = EC (μg/m³) / TRV (μg/m³)

The percent change in HQ between the HTP and 3R4F (i.e., % to 3R4F) was calculated as follows:

% to 3R4F = HQHTP / HQ3R4F × 100

2.4.2. Cancer risks

Cancer risks were calculated for the analytes that are listed by FDA as a carcinogen on the established list of HPHCs and on the subsequent list of proposed additions to the established list of HPHCs in tobacco products and tobacco smoke [33,34].

ELCRs in a population of 10,000,000 were calculated using the following equation [30]:

ELCR = IUR (μg/m³)⁻¹ × EC (μg/m³) × 1,000,000

FDA identified mercury as a carcinogen on the established HPHC list [33]; however, for this assessment, mercury was not included for quantifying cancer risks as there is no agency consensus on the carcinogenicity of this substance. For example, in the European Union, the harmonized classification for mercury does not include carcinogenicity for the hazard class and category codes under Annex VI of Regulation (EC) No 1272/2008 [35]. Further, the International Agency for Research on Cancer (IARC) evaluated mercury and inorganic mercury compounds and concluded that they were “not classifiable as to its carcinogenicity to humans” (i.e., Group 3) [36]. Finally, EPA classified elemental mercury as “not classifiable as to human carcinogenicity” (i.e., Class D) [37].

2.4.3. Cumulative risks

Cumulative risks were assessed based on the FDA guidance for comparing and evaluating HPHCs in two tobacco products and applying the cumulative risk approaches for non-cancer and cancer QRAs established by the EPA [20,30]. The established HPHC list includes constituents that are linked to five different health outcome domains, including: addictive compounds (ADs), cardiovascular toxicants (CTs), respiratory toxicants (RTs), reproductive or developmental toxicants (RDTs), and carcinogens (CAs) [33,34].

The FDA guidance recommends separate approaches for assessing non-cancer and cancer outcomes. For example, if an HPHC is identified as an RT, it cannot be offset by a decrease in an HPHC that is not an RT [20]. Therefore, cumulative non-cancer health risks were assessed within the health outcome domains stated above using the HI approach, as shown below:

HIADs, CTs, RTs, or RDTs = ΣHQADs, CTi, RTi, orRDTi

Where: HIADs, CTs, RTs, or RDTs = the cumulative non-cancer HI for a specific health outcome domain for the sum of the HQs for that domain; and HQADs, CTi, RTi, or RDTi = the non-cancer HQ for a specific health outcome domain for the ith toxicant.

The HI approach assumes that the magnitude of the adverse effects will be proportional to the sum of the HQs. A composite value of 1.0 is typically used as the benchmark for determining whether the HI presents a potential health concern (i.e., HI > 1.0) from the cumulative exposures and was applied herein.

For carcinogens, the FDA guidance recommends considering these endpoints as equivalent [20]. For example, if an HPHC increases the risk of liver cancer, this increase may be offset by a decrease in an HPHC that increases the risk of lung cancer. Therefore, the cumulative cancer risk, regardless of site, was calculated by determining the incremental increase in the probability of an individual developing cancer over a lifetime, as follows:

ELCRcumulative = ΣELCRi

Where: ELCRcumulative = the total excess lifetime cancer risk expressed as the number of excess cancers in a population of 10,000,000; and ΣELCRi = the excess lifetime cancer risk for the ith toxicant expressed as the number of excess cancers in a population of 10,000,000.

3. Results

3.1. Aerosol chemistry

Aerosol chemistry analyses were conducted with the HTP and 3R4F reference cigarettes. Fifty-four HPHCs were selected for evaluation and measured by using the established procedures for the ISO and HCI smoking regimes. As illustrated in Fig. 1, qualitative and quantitative differences were observed for the major constituents of the HTP aerosol versus the 3R4F reference cigarette smoke. More than 70 % of the HTP aerosol particulate matter consisted of propylene glycol (PG) and vegetable glycerol (VG), whereas the 3R4F reference cigarette smoke consisted primarily of “other”s.

The analyte yield was measured per consumable unit (collection), that is, one tobacco capsule for HTP and one 3R4F reference cigarette. The total number of collected puffs was dependent on the smoking regime used. Under the ISO smoking regime, 85 puffs were collected from the HTP capsule, and 7.8–8.7 puffs were collected from the 3R4F reference cigarette. Under the HCI regime, 70 puffs were collected from the HTP capsule, and 9.6–11.6 puffs were collected from the 3R4F reference cigarette. A summary of the analyte yields for the HTP aerosol and the 3R4F reference cigarette smoke is provided in Table 1.

As shown in Table 1, five analytes were quantified from the HTP aerosol under the ISO smoking regime, and six analytes were quantified under the HCI smoking regime. Under both smoking regimes, the common quantifiable analytes for the HTP aerosol included: ammonia, PG, VG and nicotine. Formaldehyde was quantified from the HTP aerosol only under the ISO smoking regime, whereas acetaldehyde and acetone were quantified from the HTP aerosol only under the HCI...
Fig. 1. Major Constituents in the HTP Aerosol (left panel) and the 3R4F Reference Cigarette Smoke (right panel) collected onto the Cambridge filter pads under the ISO (upper panel) or the HCI (lower panel) smoking regimes.
Further, 40 analytes were below the LOD under both smoking regimes. The emission values per tobacco capsule for heated tobacco product and per cigarette for 3R4F are presented as mean ± standard deviation. These results are consistent with the findings of Counts et al. [38], who reported that greater smoke constituent yields were typically obtained with the HCl smoking regime. In addition, several analytes were detected but not quantified under the ISO (four analytes) and HCl (two analytes) smoking regimes, whereas several other analytes were below the LODs under the ISO (five analytes) and HCl (four analytes) smoking regimes.

The aerosol chemistry analysis results were extrapolated to estimate ECs for the individual analytes, based on the previously stated assumptions and the following considerations. When an analyte was not quantified or detected from both the HTP aerosol and the 3R4F reference cigarette smoke, the analyte was excluded from the risk analysis. Therefore, eight analytes were excluded from further analysis under the ISO smoking regime (i.e., eugenol, lead, chromium, nickel, tin, menthol, smoking regime. In addition, multiple analytes were detected but not quantified from the HTP aerosol including nine analytes under the ISO smoking regime and eight analytes under the HCl smoking regime. Further, 40 analytes were below the LOD under both smoking regimes. In comparison, 45 and 48 analytes were quantified from the 3R4F reference cigarette smoke under the ISO and HCl smoking regimes, respectively. These results are consistent with the findings of Counts et al. [38], who reported that greater smoke constituent yields were typically obtained with the HCl smoking regime. In addition, several analytes were detected but not quantified under the ISO (four analytes) and HCl (two analytes) smoking regimes, whereas several other analytes were below the LODs under the ISO (five analytes) and HCl (four analytes) smoking regimes.

The aerosol chemistry analysis results were extrapolated to estimate ECs for the individual analytes, based on the previously stated assumptions and the following considerations. When an analyte was not quantified or detected from both the HTP aerosol and the 3R4F reference cigarette smoke, the analyte was excluded from the risk analysis. Therefore, eight analytes were excluded from further analysis under the ISO smoking regime (i.e., eugenol, lead, chromium, nickel, tin, menthol,
diethylene glycol, and resorcinol), and six analytes were excluded under the HCI smoking regime (i.e., eugenol, chromium, nickel, tin, menthol, and diethylene glycol). In total, 46 and 48 analytes were selected for quantitative risk assessment under the ISO and HCI smoking regimes, respectively.

3.2. Human health TRVs

The human health TRVs used for the non-cancer and cancer risk assessments are summarized by Hirn et al. [65]. If a human health TRV was not identified, tentative values based on LOQ and/or LOD of the analysis methods were derived for comparative purposes.

3.3. HQs & HIs – non-cancer risks

Non-cancer risks for individual analytes were assessed using the HQ approach. A benchmark of 1.0 was used for determining whether the estimated exposures to the individual analytes would translate into potential risks of concern. If an HQ for an individual analyte was below 1.0, it was considered to represent a negligible risk of concern. If an HQ for an individual analyte was equal to or greater than 1.0, it was considered to represent a potential risk of concern. Non-cancer cumulative risks were assessed for all analytes using the HI approach per their respective health outcome domains. As with the HQs, a benchmark of 1.0 served as the threshold for identifying potential risks of concern for cumulative exposures. The individual and cumulative non-cancer risk values are described by Hirn et al. [65].

3.3.1. Non-cancer risks for individual analytes

3.3.1.1. Under ISO smoking regime. Non-cancer risk assessments were performed on 46 analytes. Thirty-seven HQs for the individual analytes obtained from the HTP aerosol were less than 1.0 in the HTP aerosol, and nine HQs for individual analytes were greater than 1.0 in the HTP aerosol; however, six of these values were calculated for analytes that were below their LOD or LOQ. The remaining three quantified values were for nicotine, PG, and VG. In comparison, most of the HQs for the individual analytes from the 3R4F reference cigarette smoke were greater than 1.0. Only six HQs for the individual analytes were less than 1.0.

A comparison of the HQs for the individual analytes obtained from the HTP aerosol versus the 3R4F reference cigarette smoke was conducted. The percent reduction was calculated for the individual analytes using the corresponding HQs for the individual analytes from the 3R4F reference cigarette smoke. Each of these values, reported as “% to 3R4F”, is shown in Fig. 2.

Thirty-seven HQs for the individual analytes obtained from the HTP aerosol were reduced by more than 99%; seven HQs were reduced in a range of 99% to 78%; and the HQs for PG and VG were increased for the HTP aerosol compared to the 3R4F reference cigarette smoke (Fig. 2).

3.3.1.2. Under HCI smoking regime. Non-cancer risk assessments were performed on 48 analytes from the HCI smoking regime. Thirty-nine HQs for the individual analytes were less than 1.0. Nine HQs for individual analytes were greater than 1.0 in the HTP aerosol; however, six of these values were calculated for analytes that were below their LOD or LOQ. The remaining three quantified values were for nicotine, PG, and VG. In comparison, most of the HQs for the individual analytes from the 3R4F reference cigarette smoke were greater than 1.0. Only seven HQs for the individual analytes were less than 1.0.

A comparison of the HQs for the individual analytes obtained from the HTP aerosol versus the 3R4F reference cigarette smoke was conducted. The percent reduction was calculated for the individual analytes using the corresponding HQs for the individual analytes from the 3R4F reference cigarette smoke. Each of these values, reported as “% to 3R4F”, is shown in Fig. 3.

Thirty-eight HQs for the individual analytes obtained from the HTP aerosol were reduced by more than 99%; eight HQs were reduced in a range of 99-90%; and the HQs for PG and VG were increased for the HTP aerosol compared to the 3R4F reference cigarette smoke (Fig. 3).

3.3.2. Cumulative non-cancer risks for all analytes

Cumulative non-cancer risks (HIs) were calculated based on the health outcome domains designated for each of the analytes on the established HPHC list [33, 34] (Fig. 4). The HQs of the individual analytes per health outcome domain were summed. When no health outcome domain or hazard classification was available, the toxicity was described as “other”.

3.3.2.1. Under ISO smoking regime. Twenty-three analytes were RTs, and of these, chromium and nickel met the exclusion criteria. In the RT health outcome domain, fourteen HQs for the individual analytes obtained from the HTP aerosol were greater than 1.0. Six HQs for the individual analytes were less than 1.0 under the ISO smoking regime.

A comparison of the HQs for the individual analytes obtained from the HTP aerosol versus the 3R4F reference cigarette smoke was conducted. The percent reduction was calculated for the individual analytes using the corresponding HQs for the individual analytes from the 3R4F reference cigarette smoke. Each of these values, reported as “% to 3R4F”, is shown in Fig. 2.

Thirty-seven HQs for the individual analytes obtained from the HTP aerosol were reduced by more than 99%; seven HQs were reduced in a range of 99% to 78%; and the HQs for PG and VG were increased for the HTP aerosol compared to the 3R4F reference cigarette smoke (Fig. 2).

A comparison of the HQs for the individual analytes obtained from the HTP aerosol versus the 3R4F reference cigarette smoke was conducted. The percent reduction was calculated for the individual analytes using the corresponding HQs for the individual analytes from the 3R4F reference cigarette smoke. Each of these values, reported as “% to 3R4F”, is shown in Fig. 3.

Thirty-eight HQs for the individual analytes obtained from the HTP aerosol were reduced by more than 99%; eight HQs were reduced in a range of 99-90%; and the HQs for PG and VG were increased for the HTP aerosol compared to the 3R4F reference cigarette smoke (Fig. 3).

3.3.2.1. Under ISO smoking regime. Twenty-three analytes were RTs, and of these, chromium and nickel met the exclusion criteria. In the RT health outcome domain, fourteen HQs for the individual analytes obtained from the HTP aerosol were less than 1.0, whereas seven HQs were...

![Fig. 2. Comparison of HQs for individual analytes measured from the HTP aerosol and from 3R4F cigarette smoke expressed as percent reduction from the 3R4F reference cigarette smoke under the ISO smoking regime.](image-url)
greater than 1.0. Out of the seven analytes, five were measured below their LOD or LOQ, the remaining two quantified values were for PG and VG. In contrast, only two HQs for the individual analytes obtained from the 3R4F reference cigarette smoke were less than 1.0, and all analytes were quantified, with the exception of PG.

The cumulative non-cancer risk for the RT health outcome domain was reduced by more than 99 % for the HTP aerosol compared with the 3R4F reference cigarette smoke (Fig. 4). For the HTP aerosol, the major contributor to the HI was from PG, whereas for the 3R4F reference cigarette smoke, the major contributor was from 2,3-butanedione.

Eleven analytes were identified as RDTs on the established HPHC list [33,34], and of these, lead and chromium met the previously stated exclusion criteria.

In the RDT health outcome domain, seven HQs for the individual analytes obtained from the HTP aerosol were less than 1.0. Two analytes (nicotine and ethylene glycol) had an HQ greater than 1.0; however, ethylene glycol was measured at its LOQ. In contrast, only two HQs for the individual analytes obtained from the 3R4F reference cigarette smoke were less than 1.0; seven HQs for the individual analytes were greater than 1.0. The yields for all analytes were quantified from the 3R4F reference cigarette smoke.

The cumulative non-cancer risk for the RDT health outcome domain for the HTP aerosol was reduced by more than 91 % when compared with the analytes obtained from the 3R4F reference cigarette smoke. The major contributor for the HIs for the HTP aerosol and the 3R4F reference cigarette was nicotine, although the HQ for nicotine was reduced by more than 91 % in the HTP aerosol compared with the 3R4F reference cigarette smoke.

The cumulative non-cancer risk in the CT health outcome domain for the HTP aerosol was reduced by more than 99.9 % when compared with the cumulative non-cancer risks from the 3R4F reference cigarette smoke (Fig. 4). Acrolein was the major contributor to the HIs in the HTP aerosol and the 3R4F reference cigarette smoke, although the HQ for acrolein was reduced by more than 99.9 % in the HTP aerosol compared with the 3R4F reference cigarette smoke.

Eleven analytes were identified as RDTs on the established HPHC list [33,34], and of these, lead and chromium met the previously stated exclusion criteria.

In the RDT health outcome domain, seven HQs for the individual analytes obtained from the HTP aerosol were less than 1.0. Two analytes (nicotine and ethylene glycol) had an HQ greater than 1.0; however, ethylene glycol was measured at its LOQ. In contrast, only two HQs for the individual analytes obtained from the 3R4F reference cigarette smoke were less than 1.0; seven HQs for the individual analytes were greater than 1.0. The yields for all analytes were quantified from the 3R4F reference cigarette smoke.

The cumulative non-cancer risk for the RDT health outcome domain for the analytes obtained from the HTP aerosol was reduced by more than 91 % when compared with the analytes obtained from the 3R4F reference cigarette smoke (Fig. 4). The major contributor for the HIs for the HTP aerosol and the 3R4F reference cigarette was nicotine, although the HQ for nicotine was reduced by more than 91 % in the HTP aerosol compared with the 3R4F reference cigarette smoke.

Three analytes were identified as ADs on the established HPHC list [33,34]. None of these analytes met the previously stated exclusion criteria, so each of these was assessed for their potential cumulative non-cancer risk.

In the AD health outcome domain, two HQs for individual analytes (anabasine and acetaldehyde) obtained from the HTP aerosol were less than 1.0, and one analyte (nicotine) had an HQ greater than 1.0. For the 3R4F reference cigarette smoke, no individual analytes had an HQ less than 1.0, and all analytes were quantified.

The cumulative non-cancer risk for the AD health outcome domain for the analytes obtained from the HTP aerosol was reduced by more than 92 % when compared with the analytes obtained from the 3R4F reference cigarette smoke (Fig. 4). The major contributor for the HIs of the HTP aerosol and the 3R4F reference cigarette smoke was nicotine, although the HQ for nicotine was reduced by more than 91 % in HTP aerosol when compared with the 3R4F reference cigarette smoke.
Thirteen analytes were categorized as “other” toxicants, and of these, five analytes met the previously stated exclusion criteria.

In the “other” category, seven HQs for individual analytes obtained from the HTP aerosol were less than 1.0. Only nitrogen oxides had an HQ greater than 1.0; however, the HQ was calculated from the LOQ. In comparison, one HQ for an individual analyte obtained from the 3R4F reference cigarette smoke was less than 1.0. Seven HQs for individual analytes obtained from the 3R4F reference cigarette smoke were greater than 1.0, and all of the analytes were based on quantified values.

The cumulative non-cancer risk for the “other” category for the analytes obtained from the HTP aerosol was reduced by more than 99.9% when compared with the 3R4F reference cigarette smoke (Fig. 4). The major contributor for the HIs of the HTP aerosol and the 3R4F reference cigarette smoke was nitrogen oxides. The individual HQ value for this analyte was reduced by 99.9% in the HTP aerosol when compared with the 3R4F reference cigarette smoke.

3.3.2.2. Under HCI smoking regime. Twenty-three analytes were RTs, and of these, chromium and nickel met the exclusion criteria. In the RT health outcome domain, fourteen HQs for the individual analytes obtained from the HTP aerosol were less than 1.0, whereas seven HQs were greater than 1.0. Out of the seven analytes, five were measured below their LOD or LOQ, the remaining two quantified values were for PG and VG. In contrast, only two HQs for the individual analytes obtained from the 3R4F reference cigarette smoke were less than 1.0, and all analytes were quantified.

The cumulative non-cancer risk for the RT health outcome domain was reduced by more than 99% for the HTP aerosols compared with the 3R4F reference cigarette smoke (Fig. 4). For the HTP aerosol, the major contributor to the HI was from PG, whereas for the 3R4F reference cigarette smoke, the major contributor was from 2,3-butanedione.

Seven of the analytes were identified as CTs on the established HPHC list [33,34]. In the CT health outcome domain, five analytes obtained from the HTP aerosol had an HQ less than 1.0. Two analytes (hydrogen cyanide and acrolein) had an HQ greater than 1.0; however, both HQs were calculated from analytes at their LOD. In contrast, no HQs for the individual analytes obtained from the 3R4F reference cigarette smoke were less than 1.0, and all analytes were quantified.

The cumulative non-cancer risk in the CT health outcome domain for the HTP aerosol was reduced by more than 99.9% when compared with the cumulative non-cancer risks from the 3R4F reference cigarette smoke (Fig. 4). Acrolein was the major contributor to the HI in the HTP aerosol and the 3R4F reference cigarette smoke, although the HQ for acrolein was reduced by more than 99.9% in the HTP aerosol compared with the 3R4F reference cigarette smoke.

Eleven analytes were identified as RTDs on the established HPHC list [33,34], and of these chromium met the exclusion criteria.

In the RDT health outcome domain, eight HQs of individual analytes obtained from the HTP aerosol were less than 1.0 under the HCI smoking regime. Two analytes (nicotine and ethylene glycol) had an HQ greater than 1.0 under both smoking regimes; however, ethylene glycol was measured at its LOQ. In contrast, only two HQs for the individual analytes obtained from the 3R4F reference cigarette smoke were less than 1.0; eight HQs for the individual analytes were greater than 1.0. The yields for all analytes were quantified from the 3R4F reference cigarette smoke.

The cumulative non-cancer risk for the RDT health outcome domain for the analytes obtained from the HTP aerosol was reduced by more than 94% when compared with the analytes obtained from the 3R4F reference cigarette smoke (Fig. 4). The major contributor for the HIs for the HTP aerosol and the 3R4F reference cigarette was nicotine, although the HQ for nicotine was reduced by more than 94% from the HTP aerosol compared to the 3R4F reference cigarette smoke.

Three analytes were identified as ADs on the established HPHC list [33,34]. None of these analytes met the previously stated exclusion criteria, so each of these was assessed for their potential cumulative non-cancer risk.

In the AD health outcome domain, two HQs for individual analytes (anabasine and acetaldehyde) obtained from the HTP aerosol were less than 1.0, and one analyte (nicotine) had an HQ greater than 1.0. For the 3R4F reference cigarette smoke, no individual analytes had an HQ less than 1.0, and all analytes were quantified.

The cumulative non-cancer risk for the AD health outcome domain for the analytes obtained from the HTP aerosol was reduced by more than 94% when compared with the analytes obtained from the 3R4F reference cigarette smoke (Fig. 4). The major contributor for the HIs of the HTP aerosol and the 3R4F reference cigarette smoke was nicotine, although the HQ for nicotine was reduced by more than 94% in HTP aerosol when compared with the 3R4F reference cigarette smoke.

Thirteen analytes were categorized as “other” toxicants, and of these, four analytes met the exclusion criteria under the HCI smoking regime.

In the “other” category, eight HQs for individual analytes obtained from the HTP aerosol were less than 1.0. Nitrogen oxides had an HQ greater than 1.0; however, the HQ was calculated from an LOQ. In contrast, two HQs were less than 1.0. Seven HQs for individual analytes obtained from the 3R4F reference cigarette smoke were greater than 1.0, and all of the analytes were based on quantified values.

The cumulative non-cancer risk for the “other” category for the analytes obtained from the HTP aerosol was reduced by more than 99.9% when compared with the 3R4F reference cigarette smoke (Fig. 4). The major contributor for the HIs of the HTP aerosol and the 3R4F reference cigarette smoke was nitrogen oxides. The individual HQ value for this analyte was reduced by 99.9% in the HTP aerosol when compared with the 3R4F reference cigarette smoke.

Collectively, under both smoking regimes, the cumulative non-cancer risk for all measured analytes obtained from the HTP aerosol was reduced by more than 99% when compared with the 3R4F reference cigarette smoke (Fig. 4).

3.4. ELCR – cancer risks

Cancer risks for individual analytes were assessed using the ELCR approach for CAs [30]. Twenty-five of the selected analytes were identified as CAs on the established HPHC list [33,34]; mercury was excluded as described in the Materials and Methods, and two of the analytes (i.e., chromium and nickel) met the previously stated exclusion criteria under both smoking regimes. Additionally, lead met the exclusion criteria under the ISO smoking regime. A benchmark of 1 excess cancer in a population of 1 million (i.e., $1 \times 10^{-6}$) was used for determining whether the estimated exposures to the individual analytes presented potential risk concerns. Cumulative cancer risks were evaluated by summing the individual ELCRs. The individual and cumulative cancer risk values are described in Hirn et al. (2020).

3.4.1. ELCR for individual analytes

The ELCRs for six of the individual analytes obtained from the HTP aerosol met the $1 \times 10^{-6}$ benchmark under the ISO smoking regime, and the ELCRs for eight of the analytes met this benchmark under the HCI smoking regime. Conversely, all of the ELCRs for the individual analytes obtained from the 3R4F reference cigarette smoke exceeded the benchmark under both smoking regimes.

The ELCRs for the individual analytes obtained from the HTP aerosol were reduced by greater than 96% under both smoking regimes when comparing to the 3R4F reference cigarette smoke (Fig. 5).

3.4.2. ELCRs for all analytes

Cumulative cancer risks were calculated by summing individual ELCRs. Under both smoking regimes, the cumulative cancer risk for the analytes obtained from the HTP aerosol were reduced by more than 99.9% when compared with the 3R4F reference cigarette smoke (Fig. 5).
Fig. 5. ELCRs comparison for individual analytes detected in HTP aerosol and 3R4F reference cigarette and \( \Sigma \)ELCR expressed as a percent reduction of 3R4F smoke the under ISO (open circle) and HCI (closed circle) smoking regimes.

4. Discussion

Mixture toxicology and the determination of potential risks from exposures to mixtures have presented challenges to regulatory agencies since their inception. In 1988, the U.S. EPA published guidance titled the “Risk Assessment Guidance for Superfund (RAGS)”, which has since become one of the most utilized approaches for estimating risks from individual substances and mixtures [23]. The RAGS and their predecessor documents were initially developed as guidance for EPA and state employees conducting baseline risk assessments at hazardous waste sites. The RAGS include approaches for assessing risks via HQs/HIs for non-carcinogenic substances and via ELCRs/ΣELCRs for carcinogens. However, since the passage of the U.S. Family Smoking Prevention and Tobacco Control Act in 2009 [39], the FDA Center for Tobacco Products (CTP) has applied the approaches from the RAGS for assessing potential risks from HPHCs in tobacco products. For example, on January 23, 2017, FDA published a proposed rule titled “Tobacco Product Standard for N-Nitrosornornicotine Level in Smokeless Tobacco Products”, which used the ELCR approach for establishing the standard [40]. More recently, Marano et al. proposed using the approaches from the RAGS for performing QRAs to inform substantial equivalence determinations for different tobacco products [22].

The evaluations performed in this study are an extension of our initial evaluation that, to our knowledge, provide the first example of applying the approaches from the RAGS with estimating the comparative risks from a commercialized HTP to the 3R4F reference cigarette [41,42]. For the evaluated analytes, the potential non-cancer and cancer risks from the HTP are significantly reduced in comparison to the 3R4F reference cigarette. For each of the non-cancer health domains, all measured analytes were significantly reduced compared to the 3R4F reference cigarette. For each of the non-cancer health domains, all measured analytes were significantly reduced compared to the 3R4F reference cigarette under both smoking regimes. Under the ISO and HCI smoking regimes, only PG and VG were significantly increased above the levels measured under both smoking regimes for the 3R4F reference cigarette. However, these increases were expected given their use as carriers for the aerosol in the HTP. Though the HQs for PG and VG were increased by more than 100 % compared to the 3R4F reference cigarette, these exceedances were not interpreted as potentially unreasonable risks, given the low heating temperature of the HTP and the absence of combustion. It should be noted that the reduced risk comparison calculated herein to combustible cigarettes does not mean “no risk” or “safe”, since the HTP aerosol contained analytes that are included within the health outcome domains on the FDA’s established list of HPHCs. However, it is important to characterize the identified risks, particularly for those that were based on LODs/LOQs. Since the HQs/HIs calculated from the LOD/LOQ values contributed to the individual and cumulative risk estimates, better analytical methods would be needed to characterize whether these risks warrant further investigation (e.g., acrolein, ethylene glycol, hydrogen cyanide, and nitrogen oxides). For analytes with calculated HQs using quantified aerosol emissions a 78–99.9 % reduction under the RT (e.g., acetaldehyde, acetone, ammonia, and formaldehyde), RDT (e.g., Nicotine) health outcome domains were found. For the CA health domain, all measured HPHC analytes were significantly reduced compared to the 3R4F reference cigarette. Under the ISO smoking regime, the individual ELCR for arsenic was approximately 3 % of the 3R4F reference cigarette with the remaining ELCR values for the HPHCs being less than 1 % of the 3R4F reference cigarette. Under the HCI smoking regime, the individual ELCRs for lead and arsenic were approximately 1–2 % of the 3R4F reference cigarette. All the remaining ELCR values for the HTP were less than 1 % of the 3R4F reference cigarette. While the de minimis cut-off of 1 excess cancer in a population of 1000,000 was selected in this evaluation, the FDA has used a benchmark of one excess cancer in a population of 10,000 (i.e., \( 1 \times 10^{-4} \)) [40]. If the \( 1 \times 10^{-4} \) cutoff is applied, all of the individual ELCRs for the HTP are below this benchmark under both smoking regimes, whereas 21 of the individual ELCRs for the 3R4F reference cigarette exceeded this benchmark under the ISO and HCI smoking regimes.

Though the above results are suggestive of a considerable (over 90 %) reduction in non-cancer and cancer risks between the HTP and the 3R4F reference cigarette, these findings are theoretical and not based on empirical data for control and exposed populations. In comparison, the causal link between combustible cigarettes and adverse health outcomes, including cancer is well established through human data. It is unclear how much of a reduction in the levels of HPHCs generated by a non-combustible tobacco product would translate to a reduction in adverse health outcomes, including cancer [43–45]. There is, however, evidence from in vitro and in vivo systems that reducing the levels of HPHCs reduces the biological activity of aerosols generated by non-combustible tobacco products. For example, Takahashi et al. assessed the mutagenic potential of the total particulate matter (TPM) from the aerosol for the HTP assessed herein [17]. The study authors tested the TPM in the Ames assay using five tester strains (i.e., TA98, TA100, TA1535, TA1537 and TA102) with and without metabolic activation (i.e., +/− S9) and exposed to concentrations of up to 5000 μg/plate. The TPM from a combustible cigarette was used as the comparator (up to 500 μg/plate). No mutagenicity was observed in the tester strains exposed to the TPM from the HTP, whereas statistically significant increases in the number of revertant colonies were reported in three of the five tester strains (i.e., TA98, +/−S9; 100, +S9; and 1537, +/−S9) for the cigarette. Takahashi et al. also evaluated the genotoxicity of the TPM from the HTP applied up to 1000 μg/mL versus a comparator cigarette applied up to 200 μg/mL using an in vitro micronucleus assay, +/−S9 for three hours and -S9 for 24 h. No genotoxicity was observed with TPM from the HTP under any of the assay conditions, whereas TPM from the cigarette exhibited statistically significant positive results under all assay conditions. Additionally, when compared to 3R4F
reference cigarettes in a murine model for chronic obstructive pulmonary disease, no histopathological or morphometric changes were found in the HTP and filtered air exposed groups in a 6 month-inhalation study, while lung inflammation and emphysema-like changes were detected in the group exposed to 3R4F cigarettes [46]. A recent observational study analyzed biomarkers of exposure and biomarkers of potential harm among the HTP users, cigarette smokers, and never smokers under real-world conditions in Japan and found a significant reduction in biomarkers of exposure and biomarkers of potential harm in the HTP users in comparison to cigarette smokers. Moreover, no significant differences for some biomarkers of potential harm were found between the HTP users and never smokers (manuscript in preparation). A similar finding was reported during another study comparing biomarkers of exposure in people switching from conventional cigarette to heated tobacco products [47,64].

These findings are consistent with the reduced biological activity and reductions in some biomarkers of effect for the aerosols from non-combusted tobacco products where the tobacco is heated, but not burned [48–54]. Further, the available repeated inhalation toxicity data evaluating heated tobacco products is limited but does allow some inferences to be made about the tumorigenicity of these products in comparison to combustible cigarettes, as discussed below.

Werley et al. performed a 26-week dermal initiation-promotion carcinogenicity study in SENCAR mice exposed to the condensate from an HTP (peak operating temperature approximately 500 °C) or to the condensate from a 2R4F reference cigarette [48]. The study authors reported that the mice exposed to the HTP had delayed time to tumor onset, lower incidences of tumors, reduced multiplicity of tumors, and lower proportions of malignant tumors in comparison to the mice exposed to the condensate from the 2R4F reference cigarette. More recently, the FDA CTP provided its evaluation of an unpublished carcinogenicity study in A/J mice exposed via inhalation to three concentrations of aerosols from an HTP or one concentration of a reference cigarette [55]. The FDA CTP stated the following about the study: “Preliminary data indicate that after 10 months of exposure, neoplastic lesions (e.g., bronchioalveolar adenoma) were found in the lungs of female mice exposed to reference cigarette smoke and the heated tobacco product aerosols.” Based on the summarized data, the incidence of these lesions was approximately 25 % in the controls and 58 % in the reference cigarette group. Whereas for the HTP, the incidences were 9 % in the low concentration group, 55 % in the mid concentration group, and 15 % in the high concentration group. Collectively, the in vivo carcinogenicity data suggest that aerosols from HTPs have lower biological activity than the comparator reference cigarettes.

Interestingly, the findings reported by Werley et al. and summarized by the FDA CTP are comparable to the estimated carcinogenic risk findings reported herein, that is, our findings did not identify the absence of any risks, rather they suggest a substantial reduction in the potential cancer risks in comparison to the 3R4F reference cigarette. Our findings are also comparable to the carcinogenic risk assessments performed by Stephens, Rodrigo et al., and Slob et al. [56–58]. Stephens concluded that HTPs had a lower mean lifetime cancer risk than combustible cigarettes, based on an evaluation of 15 carcinogens reported in the emissions from 44 different products. Rodrigo et al. found that mean lifetime cancer risk estimates were lower for 21 carcinogens in HTPs compared to 13 carcinogens in combustible cigarettes. The authors also reported comparable reductions in non-cancer risks. Slob et al. reported that the mean age at cumulative exposure in smokeless cigarette users was 10- to 25-fold lower with an HTP compared to a conventional cigarette. Therefore, the applicability of the approaches from the RAGs appear to have merit with assessing the potential risks of other and novel tobacco products through tobacco product risk comparison with combustible tobacco products. Further, there are several strengths with the approaches used herein, including that they intended to err on the side of conservatism. For example, we provided quantitative non-cancer and cancer risk estimates for all analytes, including those that were at or below the LOD and/or LOQ. Therefore, even compounds that were not detected were included in the cumulative risk estimates. This is an important consideration as Stephens found that metals, even in very low concentrations, played a potentially major role in the cancer unit risk values. Second, we did not assess the contributory risk of side stream smoke when calculating the non-cancer and cancer risks of the 3R4F reference cigarette. This approach was intended to provide more conservative risk estimates, thereby increasing the estimated risks of the HTP in comparison to the 3R4F reference cigarette. Finally, we utilized the reference concentrations and inhalation unit risk values derived by public health and regulatory agencies for 48 of the 54 analytes assessed. These values were chosen because they were derived by government officials and have undergone extensive peer review.

Notwithstanding these strengths, there are some limitations that warrant discussion. To begin with, the QRA performed in this study was conducted on a subset of analytes; therefore, the estimated risks do not account for the potential contribution from other compounds that may be present in the HTP aerosol. Furthermore, when an analyte was not quantified or detected from both the HTP aerosol and the 3R4F reference cigarette smoke, the analyte was excluded from the risk analysis. Thus, to allow a more complete picture there are some analytes (i.e., chromium, nickel, diethylene glycol, etc.) for which analytical methods need to be improved or other risk analyses should be performed. As noted previously, the smoke from combustible cigarettes contains more than 8000 compounds; however, comprehensive vapor chemistry analyses including non-targeted analyses on heated tobacco products and the HTP evaluated have not been included as part of this study. While our analysis focused on analytes selected by several regulatory bodies for monitoring of cigarette smoke and ENDS emissions, additional analytes may be present in the aerosols of heated tobacco products that are not routinely evaluated for or found in combustible cigarettes. For instance, the current analysis is based solely on the use of previously described non-cancer and cancer health domain outcomes in comparison to combustible cigarettes. Therefore, if one is seeking to gain more insight as to the product category itself, additional assessments may be needed to determine whether other non-apical endpoints such as oxidative stress equilibrium, platelet activation, and endothelial dysfunction are more relevant for hazard identification for the analytes that may be present in the aerosols from HTPs [59–62]. Second, we utilized analyte measurements from aerosols generated by machines under the ISO and HCl smoking regimes, which may not account for the variability in the smoking topography of individuals that transition from combustible cigarettes to HTPs.

For future analyses, our methodology could be used for performing comparisons of other potentially Reduced Risk Products with combustible cigarettes or even within various product categories such as HTPs or e-cigarettes. QRA could also be used as a powerful tool in Product Development to perform an early assessment of potential risks posed by newly developed prototypes and to identify individual aerosol constituents that may pose the greatest contribution to these risks.

In conclusion, we performed QRA assessments on an HTP versus the 3R4F reference cigarette using aerosol chemistry data and applying the approaches set forth in the EPA RAGS. Our findings suggest that the non-cancer and cancer risks posed by the HTP are substantially and significantly lower than the comparator cigarette, for the analytes evaluated. Although this type of assessment does not preclude the need for empirical data, such as toxicity and human biomarkers studies, it does provide a foundation of comparative research and development efforts on non-combustible tobacco products by helping to identify specific analytes for removal that would otherwise present the greatest contribution to the potential risks of a product.

CRediT authorship contribution statement

Carole Hirm: Conceptualization, Methodology, Validation, Formal analysis, Writing - original draft. Yuki Kanemaru: Investigation,
Formal analysis, Data curation, Visualization, Writing - original draft. Todd Stedeford: Conceptualization, Methodology, Writing - review & editing. Thilo Paschke: Supervision, Writing - review & editing. Irene Baskerville-Abram: Conceptualization, Resources, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was supported by the Japan Tobacco Group of Companies.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:10.1016/j.toxrep.2020.10.019.

References

[1] E.L. Wynder, E.A. Graham, Tobacco smoking as a possible etiologic factor in bronchogenic carcinoma, a study of 684 proven cases, J. Am. Med. Assoc. 143 (1950) 329–336, https://doi.org/10.1001/jama.1950.02910390001001.
[2] R. Doll, A.B. Hill, Smoking and carcinoma of the lung: preliminary report, Br. Med. J. 2 (1950) 739–748, https://doi.org/10.1136/bmj.2.4672.739.
[3] D. Hoffmann, M.V. Djordjevic, I. Hoffmann, The changing cigarette, Prev. Med. 26 (1997) 427–434, https://doi.org/10.1016/S0091-7435(97)00183-5.
[4] U.S. Department of Health and Human Services, National Institutes of Health, National Cancer Institute, Risks Associated with Smoking Cigarettes with Low Machine-Measured Yields of Tar and Nicotine, Available at, 2001, https://cancercalc.org/cancercontrol/brtp/brtp/monographs/13/m13_complete.pdf.
[5] A. Rodgman, T.A. Perfetti, The Chemical Components of Tobacco and Tobacco Smoke, 2nd edition, 2013, https://doi.org/10.1201/b13973. Available at.
[6] D. Hoffmann, I. Hoffmann, Letters to the Editor-Tobacco smoke components, Beiträge zur Tabakforschung International/Contributions to Tobacco Research 18 (1998) 49–52, https://doi.org/10.2478/v10070-003-0068-y.
[7] J. L. Jones, Chemical analysis and in vitro toxicological evaluation of aerosol from a novel tobacco vapor product: a comparison with cigarette smoke, Regul. Toxicol. Pharmacol. 92 (2018) 94–103, https://doi.org/10.1016/j.yrtph.2017.11.005.
[8] F.J. Teitel, T.J. Meisger-Vellet, E. Roemer, G. Patikan, Toxicological evaluation of an electrically heated cigarette. Part 3: genotoxicity and cytotoxicity of mainstream smoke, J. Appl. Toxicol. 23 (2003) 341–348, https://doi.org/10.1002/jat.925.
[9] Committees on Toxicity, Carcinogenicity and Mutagenicity of Chemicals in Food, Consumer Products and the Environment, Toxicological Evaluation of Novel Heat-not-burn Tobacco Products – Non-technical Summary, Available at, 2017, https://cfsan.fda.gov/~dms/heat-nbtob-smoke-overview.pdf.
[10] U.S. Food and Drug Administration, Harmful and Potentially Harmful Constituent (HPHC) Comparison and Evaluation Procedure for Comparing Two Tobacco Products in the Substance Equivalence Reports, Memorandum, Available at, 2019, https://cfsan.fda.gov/~dms/hp-hpce-setting.pdf.
[11] U.S. Food and Drug Administration, Use of Reference Values in the Toxicological Evaluation of Inhaled Tobacco Products, Memorandum, Available at, 2019, https://www.fda.gov/media/124686/download.
[12] K.M. Marano, C. Liu, W. Fuller, P.R. Gentle, Quantitative risk assessment of tobacco products: a potentially useful component of substantial equivalence evaluations, Regul. Toxicol. Pharmacol. 95 (2018) 371–384, https://doi.org/10.1016/j.yrtph.2018.03.026.
[13] U.S. Environmental Protection Agency, Risk Assessment Guidance for Superfund, Volume I, Human Health Evaluation Manual (Part A), Interim Final, EPA/540/1-89/002, Available at, 1989, https://www.epa.gov/production/files/files/2015/09/documents/rags/a.pdf.
[14] H.-J. Haussmann, Use of hazard indices for a theoretical evaluation of cigarette smoke composition, Chem. Res. Toxicol. 25 (2012) 794–810, https://doi.org/10.1021/tx200565w.
[15] U.S. Food and Drug Administration, Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems, Guidance for Industry, Available at, 2019, https://www.fda.gov/media/127853/download.
[16] European Regulatory Science on Tobacco Consortium, Study on the Development of a EU Common Reporting Format for Submission of Data on Ingredients Constituent in Tobacco Products and Related Products, and Description of the Chemical Data to the Public, Final Report, Available at, 2015, https://ec.europa.eu/health/sites/health/files/tobacco/docs/reportingformat_dataingredients_en.pdf.
[17] World Health Organization, Report on the scientific basis of tobacco product regulation: Fifth report of a WHO study group, WHO Technical Report Series No. 989, 2015, Available at, https://apps.who.int/iris/bitstream/handle/10665/161151/9789241208992.pdf?sequence=1.
[18] U.S. Environmental Protection Agency, Human Health Toxicity Values in Superfund Risk Assessments, Memorandum, Available at, 2003, https://www.epa.gov/production/files/files/2015/11/documents/bhmememo.pdf.
[19] European Medicines Agency, ICH Guideline M7R1 on Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, Step 5, Committee for Human Medicinal Products, EMA/CHMP/I/83812/2013, Available at, 2015, https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-m7r1-assessment-control-dna-reactive-mutagenic-impurities-pharmaceuticals-limits_en.pdf.
[20] U.S. Environmental Protection Agency, Risk Assessment Guidance for Superfund, Volume I: Human Health Evaluation Manual, (Part F, Supplemental Guidance for Inhalation Risk Assessment), EPA-540-R-07-002, OSWER 9285.7-82, Available at, 2002, https://www.epa.gov/production/files/files/2015/09/documents/part_f_00901_final.pdf.
[21] H. Ichitubo, M. Kottaki, Indoor air quality (IAQ) evaluation of a Novel Tobacco Vapor (NTV) product, Regul. Toxicol. Pharmacol. 92 (2018) 278–294, https://doi.org/10.1016/j.yrtph.2017.10.016.
[22] World Health Organization, World Health Statistics 2018: Monitoring Health for the SDGs, Sustainable Development Goals, Available at, 2018, https://apps.who.int/iris/bitstream/handle/10665/272596/9789241565585-eng.pdf?ua=1.
[23] U.S. Food and Drug Administration, Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke; Established List, Federal Register, 77, 2012, Available at, https://www.govinfo.gov/content/pkg/FR-2012-04-03/pdf/2012-10727.pdf.
[24] U.S. Food and Drug Administration, Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke; Established List; Proposed Additions; Request for Comments, Federal Register, 84, 2019, Available at, https://www.govinfo.gov/content/pkg/FR-2019-08-05/pdf/2019-16658.pdf.
[25] European Chemicals Agency, Mercury, CASRN 7439-97-5, EC/List No. 231-106-7, Index Number: 080-001-00-0, Summary of Classification and Labelling, Harmonised Classification – Annex VI of Regulation (EC) No 1272/2008 (CLP Regulation), C&I Inventory, 2020. Available at, https://echa.europa.eu/materials-information-on-chemicals/cl-inventory-database/-/discli/details/15915 (accessed April 6, 2020).
[26] International Agency for Research on Cancer, Beryllium, Cadmium, Mercury, and Exposure in the Glass Manufacturing Industry, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 58, 1993, Available at, https://publications.iarc.fr/Book-And-Report-Series/IARC-Monographs-On-The-Evaluation-of-Carcinogenic-Hazards-To-Humans/Beryllium-Cadmium-Mercury-and-Exposures-In-The-Glass-Manufacturing-Industry.
[27] U.S. Environmental Protection Agency, Mercury, elemental; CASRN 7439-97-6, Carcinogenicity Assessment (Last Revised 05/01/1995), Chemical Assessment Summary, Integrated Risk Information System, Available at, 1995, https://cfpub.epa.gov/ncea/inventories/display.cfm?inner=crs&crs=0005856.
[28] M.E. Counts, M.J. Morton, S.W. Laffoon, R.H. Cox, P.J. Lipowicz, Smoke composition and predicting relationships for international commercial cigarettes
smoked with three machine-smoking conditions, Regul. Toxicol. Pharmacol. 41 (2005) 185–227, https://doi.org/10.1016/j.yrtph.2004.12.002.

[39] Family smoking prevention and tobacco control act, Pub. L. No.111-31, 123 Stat. 1776, 2009. Available at, https://www.govinfo.gov/content/pkg/PLAW-111p ubl31/pdf/PLAW-111publ31.pdf.

[40] U.S. Food and Drug Administration, Tobacco Product Standard for N-Nitrosornornicotine Level in Finished Smokeless Tobacco Products, Federal Register, 82, 2017. Available at, https://www.govinfo.gov/content/pkg/FR-2017- 01-23/pdf/2017-01030.pdf.

[41] C. Hirn, T. Steedford, I. Baskerville-Abraham, Comparative quantitative risk assessments on a novel heated tobacco product versus the 384F reference cigarette (poster), Society of Toxicology 57th Annual Meeting (2018). Available at, https://www.jt-science.com/sites/default/files/2018-p4-jt.pdf.

[42] C. Hirn, T. Steedford, I. Baskerville-Abraham, Cumulative quantitative risk assessments on a novel heated tobacco product versus the 384F reference cigarette (poster), Toxicology and Risk Assessment Conference (2018). Available at, https://www.jt-science.com/sites/default/files/2018-p5-jt.pdf.

[43] N. Mallock, L. Boss, R. Burk, M. Danziger, T. Welsch, H. Hahn, L. Trieu, J. Hahn, E. Pieper, F. Henkel-Stephani, C. Hatzler, A. Luch, Levels of selected analytes in the emissions of ‘heat not burn’ tobacco products that are relevant to assess human health risks, Arch. Toxicol. 92 (2018) 2145–2149, https://doi.org/10.1007/s00204-018-2215-y.

[44] K.E. Farsalinos, N. Yannovits, T. Sarri, V. Voudris, K. Poulas, S.J. Leischow, D. Breheny, J. Adamson, D. Azzopardi, A. Baxter, E. Bishop, T. Carr, I. Crooks, M.S. Werley, S.A. Freelin, S.E. Wrenn, B. Gerstenberg, E. Roemer, H. Schramke, D. Thorne, D. Breheny, C. Proctor, M. Gaca, Assessment of novel tobacco heating products: a case study on heated tobacco versus cigarettes, Risk Anal. 40 (2020) 1355–1366, https://doi.org/10.1111/risa.13482.

[45] G. Rodrigo, G. Jaccard, D. Tafin Djoko, A. Korneliou, M. Esposito, M. Belushkin, Carbonyl emissions from a novel heated tobacco product (IQOS): comparison with an e-cigarette and a tobacco cigarette, Addiction 113 (2018) 2099–2106, https://doi.org/10.1111/add.14365.

[46] M. van der Toorn, K. Koshibu, W.K. Schlage, S. Majeed, P. Pospisil, J. Hoeng, M. Aerts, V. Cammisotto, V. Valenti, F. Coluzzi, E. Cavarretta, A. Carrizzo, F. Prati, R. Carnevale, G. Frati, Acute effects of heat-not-burn, electronic vaping, and traditional tobacco combustion cigarettes: the sapienza university of rome-vascular atherosclerosis assessment of proatherosclerotic effects of smoking (SUR - VAPES) 2 randomized trial, J. Am. Heart Assoc. 8 (2019), e010455, https://doi.org/10.1161/JAHA.118.010455.

[47] D. Thorne, R. Leverette, D. Breheny, J. Clements, B. Bombick, M. Gaca, Genotoxicity evaluation of tobacco and nicotine delivery products: part one. Mouse lymphoma assay, Food Chem. Toxicol. 132 (2019), 110584, https://doi.org/10.1016/j.fct.2019.110584.

[48] D. Thorne, J. Whitwell, J. Clements, P. Walker, D. Breheny, M. Gaca, The genotoxicological assessment of a tobacco heating product relative to cigarette smoke using the in vitro micronucleus assay, Toxicol. Rep. 7 (2020) 1010–1019, https://doi.org/10.1016/j.toxrep.2020.08.013.

[49] F. Lüdicke, S.M. Anarsi, N. Lama, N. Blanc, M. Bosilkovska, A. Donelli, P. Picavet, G. Baker, C. Hazzia, M. Peitsch, R. Weitkunat, Effects of switching to a heat-not-Burn tobacco product on biologically relevant biomarkers to assess a candidate modified risk tobacco product: a randomized trial, Cancer Epidemiol. Biomarkers Prev. 28 (2019) 1934–1943, https://doi.org/10.1158/1055-9965.EPI-18-0915.

[50] U.S. Food and Drug Administration, Center for Tobacco Products, Evidence Related to the Health Risk of IQOS Use, Evaluation of Nonclinical Studies, Available at, 2018, https://www.fda.gov/media/110748/download.

[51] W.E. Stephens, Comparing the cancer potencies of emissions from vapourised nicotine products including e-cigarettes with those of tobacco smoke, Tob. Control 27 (2018) 10–17, https://doi.org/10.1136/tobaccocontrol-2017-058080.

[52] W. Siob, L.G. Soeteman-Hernandez, W. Bil, Y.C.M. Staal, W.E. Stephens, R. Tallouh, A method for comparing the impact on carcinogenicity of tobacco products: a case study on heated tobacco versus cigarettes, Risk Anal. 40 (2020) 1355–1366, https://doi.org/10.1111/risa.13482.

[53] R. Talhout, A method for comparing the impact on carcinogenicity of tobacco products that are relevant to assess human health risks, Arch. Toxicol. 92 (2018) 2145–2149, https://doi.org/10.1007/s00204-018-2215-y.