



A Systematic Review and Meta-Analysis on Human Biomarkers of Exposure from Heated Tobacco Products Compared to Conventional Cigarettes among Adult Smokers

Joan Dymphna P Reaño^{1,2*}, Marie Barrientos-Regala^{1,2}, Reginald P Arimado¹, Rafael R Castillo^{1,2}

¹CardioMetabolic Research Unit (CaMeRU), FAME Leaders Academy, Makati City, Philippines

²Section of Adult Cardiology, Department of Internal Medicine, Manila Doctors Hospital, Manila, Philippines

***Corresponding Author:** Joan Dymphna P Reaño, MD, CardioMetabolic Research Unit (CaMeRU), FAME Leaders Academy, Makati City, Philippines and Section of Adult Cardiology, Department of Internal Medicine, Manila Doctors Hospital, Manila, Philippines; Email: jreano@fla.ph; dp.reano@gmail.com

Received Date: 14-07-2022; **Accepted Date:** 05-08-2022; **Published Date:** 12-08-2022

Copyright © 2022 by Reaño JDP, et al. All rights reserved under CC BY-NC-ND. This is an open access article distributed under the terms of the Creative Commons Attribution License, which provides freedom to read, share, copy and redistribution of material in any of the medium, provided with the original author and source are credited.

Abstract

Introduction: The health effects of Heated Tobacco Products (HTP), which are non-combustible alternatives to Conventional Cigarettes (CC), may be assessed by the measurement of Biomarkers of Exposure (BoE).

Methods: This meta-analysis aims to compare the BoE levels between HTP and CC. Systematic computerized search was performed using Pubmed, Google Scholar, and Cochrane database. The studies included randomized controlled trials that evaluated the effectiveness of HTP compared to CC in reducing human BoE. Two reviewers independently appraised each study. Any disparity in assessment was settled by an independent adjudicator.

Results: Data from the trials included in this study (N=1,193) showed that there was a significant difference in the levels of BoEs between HTP and CC. The levels of carboxyhemoglobin [-2.0 (95% CI -3.08 to -0.92), Z=3.64, p=0.00001], 4-aminobiphenyl [-1.67 (95% CI -2.36 to -0.99), Z=4.80, p=0.00001], 3-hydroxypropylmercaptapuric acid [-1.09 (95% CI -1.54 to -0.63), Z=4.70, p=0.00001], N-nitrosornicotine [-0.95 (95% CI -1.49 to -0.41), Z=3.46, p=0.0005], 4-(methylnitrosamino)-1-(3-pyridyl)- 1-butanol [-1.30 (95% CI -1.72 to -0.89), Z=6.18, p=0.00001], Total Nicotine Equivalents [-0.85 (95% CI -1.36 to -0.34),

Reaño JDP | Volume 3, Issue 2 (2022) | JCMR-3(2)-058 | Review Article

Citation: Reaño JDP, et al. A Systematic Review and Meta-Analysis on Human Biomarkers of Exposure from Heated Tobacco Products Compared to Conventional Cigarettes among Adult Smokers. Jour Clin Med Res. 2022;3(2):1-27.

DOI: <http://dx.doi.org/10.46889/JCMR.2022.3204>

Z=3.24, p=0.001], Benzo[a]pyrene [-1.70 (95% CI -2.08 to -1.33), Z=8.92, p=0.00001], and S-phenylmercapturic acid [-1.18 (95% CI -1.36 to -0.99), Z=12.36, p= 0.0001] among the participants were significantly reduced in those who used HTP as compared to those who smoked CC.

Conclusion: The study showed that the use of HTP could significantly reduce exposure to harmful substances compared to CC using BoEs that are assessed to be most suitable and practical for tobacco product regulation.

Keywords

Heated Tobacco Products; Cigarettes; Biomarkers of Exposure; Smokers

Epidemiology / Burden of Disease

Tobacco use is a single preventable risk factor for developing non-communicable diseases leading to increased incidence of premature deaths in many populations worldwide [1]. The World Health Organization projects that by year 2025, more than one billion people would continue to smoke [1]. The combustion of tobacco leads to the formation of about 1% of over 7000 chemical substances associated with smoking-related diseases such as lung cancer, cardiovascular diseases, and emphysema [2].

Due to the increasing evidence on smoking-related health risks resulting from exposure to toxic compounds from the combustion of Conventional Cigarette (CC), Heated Tobacco Products (HTP) were developed. Current data suggest that HTP has the potential to be a reduced risk product for public health compared to CC [3-12]. HTP has been proposed as an alternative to CC to achieve Tobacco Harm Reduction (THR) [3,13-16]. THR is a strategy to minimize harm from combustion and to decrease morbidity and mortality without necessitating complete elimination of tobacco and nicotine use [17]. Nevertheless, THR recognizes that complete tobacco abstinence is still the ideal target which should be aimed at in all smokers; but if it could not really be attained for various reasons despite best efforts, alternative or complementary ways to reduce harm among tobacco users should be considered. The underlying concept of THR in tobacco control is that the damage or harm caused by tobacco consumption should be at least reduced when it cannot be totally prevented [12,18-20].

HTPs are non-combustible alternatives to CCs which could potentially reduce the negative health consequences associated with tobacco smoke [21]. HTP is also known as “Modified Risk Tobacco Product” (MRTP), which is defined as “any tobacco product that is sold or distributed for use to reduce harm or the risk of tobacco-related disease associated with

commercially marketed tobacco products” [22]. Regulatory laws require manufacturers that an MRTP should significantly reduce harm and risk of tobacco-related diseases and that it should benefit the population as a whole [22-24]. This regulatory step requires studies that measure the exposure or health effects in biological systems of such products. These measurements make use of substances or chemical constituents generally referred to as biomarkers.

Review of Related Literature

Studies investigating the general effect of HTPs on health and safety are emerging. A systematic review by Akiyama, et al. provided a summary of available evidence on changes to levels of tobacco-related biomarkers to aid the overall assessment of the consequences of using e-cigarettes and HTPs [21]. The study showed that the use of MRTPs could significantly reduce exposure to harmful substances compared to CC using Biomarkers of Exposure (BoE) as a parameter but with limitation of needing larger and longer-term population-based studies [25].

A meta-analysis by Drovandi, et al., compared BoE levels in humans using HTP versus CC. The analysis assessed 10 Randomized Controlled Trials (RCTs) from 2014 to 2019 [26]. The study showed significant reductions in BoE levels in those using HTPs compared to CCSs but also noted limitations such as relatively small number of studies, limited BoE ranges assessed, and tobacco industry involvement [26].

There are numerous tobacco exposure biomarkers that can be measured but it may not always be practical to measure all available BoEs; hence the United States (US) Food and Drug Administration (FDA) identified the most applicable biomarkers for regulatory use. According to the FDA findings, the commonly-used biomarkers belonging to the different chemical classes and biomarkers in development are the following: nicotine and tobacco alkaloids, Carbon monoxide (CO), Tobacco-Specific Nitrosamines (TSNAs), Polycyclic Aromatic Hydrocarbons (PAHs), Volatile Organic Compounds (VOCs), aromatic amines and heterocyclic amines, and metals [27].

Belonging to the nicotine class of biomarkers are the Total Nicotine Equivalents (TNeq), which is considered the gold standard for daily nicotine intake. It accommodates factors that influence nicotine metabolism and exhibits strong correlation with several tobacco exposure biomarkers [28,29]. Another class of biomarkers are the CO biomarkers, which can be measured in the blood as Carboxyhemoglobin (CoHb). Since exhaled CO and CoHb are closely related, CoHb can serve as proxy for CO [30]. Meanwhile, belonging to the TSNAs are N-nitrosornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)- 1-butanol (NNAL), which are the most widely studied TSNA biomarkers [9]. Additionally, NNAL correlates with other tobacco-specific biomarkers such as TNeq [31,32]. Biomarkers of PAH uptake, on the other hand, includes benzo[a]pyrene, which is an effective measure of PAH uptake and metabolic

activation [33-35]. Like benzo[a]pyrene, benzene is among the established list of Harmful and Potentially Harmful Constituents (HPHCs) in tobacco products and are both associated with cancer [36]. S-phenylmercapturic Acid (SPMA) is a BoE corresponding to benzene. Furthermore, exposure to tobacco smoke VOCs is most commonly measured as urinary mercapturic acids, which include 3-hydroxypropylmercapturic acid (3-HPMA). It is mercapturic acid metabolite of acrolein, another identified HPHC, which is four times higher in smokers compared to non-smokers [37]. Lastly, aromatic amines and heterocyclic amines are combustion products in the particulate phase of tobacco smoke, which include 4-aminobiphenyl (4-ABP). The hemoglobin adducts of 4-ABP are significantly elevated in smokers versus non-smokers [38-41]. Aside from being the most applicable biomarkers for regulatory use, the abovementioned biomarkers were also among the most commonly studied BoEs by systematic reviews comparing BoEs in HTP versus CC.

Implication and Importance

HTP is proposed as a less harmful alternative to CC in terms of BoE among other parameters. Although there are already a number of studies comparing the relative safety of HTPs versus CCs, they are still limited by small sample size and short duration. Since larger and more independent studies about HTPs are recommended, this meta-analysis aims to update the existing literature comparing BoE levels in HTPs versus traditional tobacco cigarette. Furthermore, it aims to focus only on the common BoEs, falling under the abovementioned different classes of BoEs, assessed to be most suitable and practical for tobacco product regulation.

Research Question

Among adult smokers, how effective are Heated Tobacco Products (HTPs) compared to conventional Combustible Cigarettes (CCs) in reducing human Biomarkers of Exposure (BoE)?

Objectives

A. General Objective

To compare the BoE levels between HTP and CC

B. Specific Objectives

To compare the BoE between HTP and CC using the following specific parameters:

Reaño JDP | Volume 3, Issue 2 (2022) | JCMR-3(2)-058 | Review Article

Citation: Reaño JDP, et al. A Systematic Review and Meta-Analysis on Human Biomarkers of Exposure from Heated Tobacco Products Compared to Conventional Cigarettes among Adult Smokers. *Jour Clin Med Res.* 2022;3(2):1-27.

DOI: <http://dx.doi.org/10.46889/JCMR.2022.3204>

1. Carboxyhemoglobin (CoHB)
2. 4-aminobiphenyl (4-ABP)
3. 3-hydroxypropylmercaptapuric acid (3-HPMA)
4. N-nitrosornicotine (NNN)
5. 4-(methylnitrosamino)-1-(3-pyridyl)- 1-butanol (NNAL)
6. Total Nicotine Equivalents (TNeq)
7. Benzo[a]pyrene (3-OH-BaP)
8. S-phenylmercapturic Acid (SPMA)

Methodology

A. Criteria for considering studies for this review

The studies included Randomized Controlled Trials (RCTs) that evaluated the effectiveness of HTP compared to CC in reducing human BoE. A study was included if any of the outcomes assessed were: CoHB, 4-ABP, 3-HPMA, NNN, NNAL, TNeq, benzopyrene, SPMA.

B. Definition of Terms:

1. Heated Tobacco Product (HTP) - refers to a tobacco product heated at a lower temperature than a conventional cigarette. HTP is heated (at ~350 °C) by an electrically powered element or carbon instead of being combusted (at ~800 °C).
2. Conventional Cigarette (CC) smoking - refers to patients who are actively smoking tobacco combustion cigarettes at the time of study
3. Tobacco Harm Reduction (THR) - a strategy aimed at lowering the risk of harm in individuals using conventional tobacco products by switching to non-combustible tobacco products
4. Biomarkers of Exposure (BoE) - the chemical, its metabolite, or the product of an interaction between a chemical and some target molecule or cell that is measured in a compartment in an organism that captures actual human exposure to tobacco products
5. Carboxyhemoglobin (CoHB) - a stable complex of carbon monoxide (CO) that forms in red blood cells when CO, a product of incomplete combustion of organic products in tobacco smoke, enters the body

6. 4-aminobiphenyl (4-ABP) - a combustion product, specifically an aromatic amine, which is a major environmental carcinogen
7. 3-hydroxypropylmercaptapuric acid (3-HPMA) - a mercapturic acid metabolite of acrolein, which measures exposure to volatile organic compounds of tobacco smoke
8. N-nitrosornicotine (NNN) - a BoE that belongs to tobacco-specific nitrosamines (TSNAs), which are N-nitroso-derivatives of pyridine-alkaloids (e.g., nicotine, nornicotine) and are present in tobacco and cigarette smoke
9. 4-(methylnitrosamino)-1-(3-pyridyl)- 1-butanol (NNAL) - an advantageous BoE belonging to the TSNAs because it has a relatively longer half-life (10-45 days) and completely tobacco-specific
10. Total Nicotine Equivalents (TNeq) - the gold standard for daily nicotine intake which is the sum of urinary nicotine, cotinine, and several metabolites in the nicotine metabolic profile
11. Benzo[a]pyrene (3-OH-BaP) - a BoE of uptake and activation of Polycyclic Aromatic Hydrocarbons (PAH), which are chemicals formed by the combination of incomplete combustion and pyrolysis of organic matter, including tobacco, fossil fuels, and wood
12. S-phenylmercapturic acid (SPMA) -a specific and sensitive BoE of benzene, which is a volatile organic compound correlated with daily smoking levels

C. Search methods for identification of studies

Systematic computerized search (Appendix A) was performed using the Pubmed, Google Scholar, and Cochrane database. The last search was run on November 10,2021. MESH and free text of the following main key terms were used: “randomized controlled trials”, “clinical trial”, “meta-analysis”, “systematic review”, “heat-not-burn cigarettes”, “heated tobacco products”, “modified risk tobacco product”, “risk reduction product”, “reduced risk product”, “standard cigarette/tobacco smoking”, “traditional cigarette/ tobacco smoking”, “conventional cigarette/tobacco smoking”, “smokers”.

D. Data Collection and Analysis

Selection of Studies: The initial search yielded a total of 21 studies. Through review of reference lists of relevant articles, 6 more additional studies were assessed for eligibility. Among these, 18 were relevant studies, which were assessed for eligibility. Of the 18 relevant studies, 1 was excluded due to different intervention; 1 was excluded due to different outcomes of interest and 2 of the studies are still ongoing [42-45]. The remaining 14 studies were eligible

but 6 of them had incomplete data on the outcomes of interest (Appendix B) [46-51]. Fig. 1 shows the 8 RCTs included in the meta-analysis [52-59].

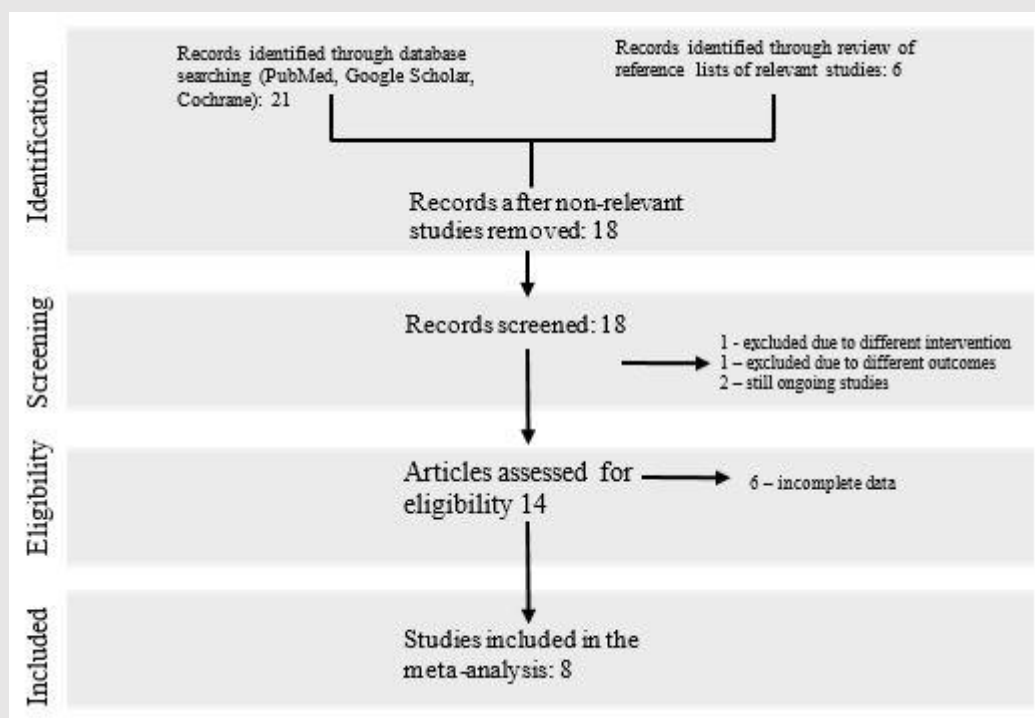


Figure 1: Search strategy for identification of studies.

Description of Studies

Table 1 describes the eight randomized controlled trials involving a total of 1,193 participants who met the inclusion criteria [52-59].

Author (Year)	Population		Intervention	Outcome	Methods
	Inclusion	Exclusion			
Haziza ⁵² (2019) <i>N=160</i>	Healthy male and female U.S. smokers, 22+ years of age	Subjects with safety-relevant diseases or with a history of alcohol and/or drug abuse, as well as pregnant or breastfeeding women	Treatment Group: Menthol THS 2.2 Control Group: Menthol CCs of the subjects' preferred commercially available brands Smoking Abstinence Group	Carboxyhemoglobin (CoHB), 4-aminobiphenyl (4-ABP), 3-hydroxypropylmercaptapuric acid (3-HPMA), N-nitrosornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)- 1-butanol (NNAL), Benzo[a]pyrene	Randomized, three-arm parallel group, controlled clinical study
Leroy ⁵³ (2012) <i>N=316</i>	Caucasian smokers aged 30-60 years with acceptable health conditions. Subjects were current smokers of commercially available, non-men- tholated conventional cigarettes with a	Subjects with clinically relevant abnormal findings based on the screening assessments were excluded. Pregnant or lactating women and women of child-bearing potential who were not using an	Treatment Group: EHCSS series-K cigarette (EHCSS-K6 Group) Control Group: Conventional cigarettes	Carboxyhemoglobin (CoHB), 4-aminobiphenyl (4-ABP), 3-hydroxypropylmercaptapuric acid (3-HPMA), 4-(methylnitrosamino)-1-(3-pyridyl)- 1-butanol (NNAL), Total Nicotine Equivalents (TNeq),	Randomized, open-label, controlled study

	3-10 mg tar yield with a smoking history of at least 10 years prior to screening.	acceptable method of contraception were also excluded.			
Ludicke ⁵⁴ (2018) <i>N=160</i>	Japanese male and female smokers aged 23-65 years with a BMI of 18.5-32 kg/m ² who smoked ≥ 10 commercially available mCCs per day (self-reported) in the last 4 weeks (maximum yield of 1 mg nicotine per cigarette) and if they reported to have smoked mCCs for ≥ 3 years.	Smokers of non-menthol CCs were not eligible for this study to avoid a change in smoking patterns which is likely to result from switching a current smoker of non-menthol CCs to a menthol product	Treatment Group: mTHS (2.62 mg/stick menthol, 1.21 mg/stick nicotine and 3.94 mg/stick of glycerin used as aerosol former, obtained under Health Canada Intense (HCI) smoking regimen, maximum heating temperature 350°C) Control Group:	Carboxyhemoglobin (CoHB), 4-aminobiphenyl (4-ABP), 3-hydroxypropylmercaptapuric acid (3-HPMA), N-nitrosornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)	Three-arm, parallel-group randomized controlled study

			mCCs of the participant's preferred commercially available brand		
Miura ⁵⁵ (2015) <i>N</i> =78	Japanese healthy males, 60 smokers and 20 non-smokers, aged 21-49 years and having a body mass index (BMI) in the range of 18.5-25.0 kg/m ² who had smoked a 1 mg ISO tar conventional cigarette without regard to menthol or non-menthol with a daily consumption of at least 20 cigarettes for at least 1 year prior	Not mentioned (Data requested via email from corresponding author)	Treatment Group: Non-combustion inhaler type of tobacco product (NCIT) Control Group: 1- mg ISO tar conventional cigarette	Carboxyhemoglobin (CoHB), 4-aminobiphenyl (4-ABP), 3-hydroxypropylmercaptapuric acid (3-HPMA), 4-(methylnitrosamino)-1-(3-pyridyl)- 1-butanol (NNAL)	Permuted-block, randomized (for smokers), controlled, forced-switching, open-label, parallel group design

to screening and their serum cotinine levels had exceeded 14 ng/ml at the screening Eligible non-smokers had no experience of routinely smoking the conventional cigarette and had not used any tobacco products including the conventional cigarette for at least 1 year before screening Non-smokers with their serum cotinine levels lower than 14 ng/ml at the				
---	--	--	--	--

	screening were enrolled.				
Sakaguchi ⁵⁶ (2014) <i>N=70</i>	Healthy Japanese male smokers aged 21-49 years who smoked ≥ 20 CC per day for ≥ 1 year and same brand for ≥ 8 weeks preceding screening	1. Subjects who have regular use experience of hand-rolled cigarette, cigarillo, cigar, pipe, smokeless tobacco or chewing tobacco 2. Subjects who donate 200 ml or more of blood within 4 weeks before screening, or who donate 400 ml or more of blood within 12 weeks before screening (including blood donation) etc.	Treatment Group: The test cigarette, HC, was a 190 prototype of a heated tobacco product prepared by Japan Tobacco 191 Inc. Control Group: CC10, was a commercially available cigarette brand in Japan with the values of 10 mg tar and 0.8 mg nicotine printed on the package	Carboxyhemoglobin (CoHB), 4-aminobiphenyl (4-ABP), 3-hydroxypropylmercaptapuric acid (3-HPMA), N-nitrosornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), Benzo[a]pyrene, Benzene	Controlled, semi-randomized, open-label, parallel group, residential, 4-sites design

Shepperd ⁵⁷ (2013) <i>N</i> =277	Healthy adult (>21 years) smokers and non-smokers of either sex and any ethnic origin who lived in or around Hamburg, Germany. Eligibility was assessed by the principal investigator on the basis of the following criteria: regular smokers of either 6-7 mg or 1-2 mg ISO tar yield cigarettes; to have typically self-reported cigarette consumption of between 6 and 30 cigarettes per	A clinically relevant health condition or abnormal findings on physical examination; participation in a clinical trial within 90 days of day 1; donation or loss of P400 mL blood in the past 90 days; acute illness requiring treatment within the previous 4 weeks; use of nicotine or tobacco products other than filtered cigarettes; any history of drug or alcohol abuse; use of bronchodilators within the previous 12 months; use of systemic medication (except	1) Treatment group: “GROUP 2”: 6- mg RTP Group (Switch from CC6 to TSS6) “GROUP 4”: Switch from CC1 to TSS1 RTP “GROUP 5”: Switch from CC1 to BT1 RTP 2) Control Group: “GROUP 1”: 6 mg- conventional cigarette group (CC6) GROUP 3: 1 mg-	4-aminobiphenyl (4-ABP), 3- hydroxypropylmercaptapuric acid (3-HPMA), N- nitrosonornicotine (NNN), 4-(methylnitrosamino)-1- (3-pyridyl)-1-butanol (NNAL)	Single- blinded randomised controlled study with occasional clinical confinement
---	---	---	--	---	---

	day; to have been a smoker for >3 years; to have smoked their current brand for >6 months (where the current brand was typical of the German market in terms of format (“king size”), blend style (American blended, non-mentholated) and filter type (plain cellulose acetate)); and to be willing to switch to a novel product. Eligible non-smokers were required to have not smoked for >5 years and	hormonal contraception or hormone-replacement therapy) within the previous 14 days; employment in the tobacco, journalism, public relations, market research or advertising industries; a positive urine pregnancy test, use of non-reliable contraceptive methods or lactation in women of childbearing age; smokers were excluded if they self-reported or were observed to be non-inhalers.	conventional cigarette (CC1) 3) <u>“GROUP 6”</u>: Nonsmoking group -Provided background levels of BoE		
--	--	---	--	--	--

	have a urine cotinine level of <10 ng/mL.				
Tricker ⁵⁸ (2012) <i>N</i> =72	Adult male and female Korean smokers (aged 20-50) with acceptable health conditions who smoked 10-30 conventional lit- end non-menthol cigarettes (1.0- 3.0 mg tar) per day and the Lark1 (1.0 mg tar, 0.1 mg nicotine, and 1.5 mg CO) as their exclusive brand for at least 2 weeks prior to admission to the clinic	Pregnant or lactating women; Existence of a clinically significant disease, clinically relevant abnormal findings on physical examination, medical history, and clinical lab- oratory results, alcohol or drug abuse, and a positive test for hu- man immunodeficiency virus (HIV) or hepatitis; subjects using a nicotine- containing product other than cigarettes within 3 months prior to	Treatment Group: (N=28) Electrically Heated Cigarette Smoking System (EHCSS) and EHCSS-K3 cigarette (3 mg tar, 0.2 mg nicotine, and 0.6 mg CO) Control Group: (N=28) A leading commercial conventional cigarette (CC) brand (Lark One) in Korea	Carboxyhemoglobin (CoHB), 4-aminobiphenyl (4-ABP), 3- hydroxypropylmercaptapu- ric acid (3-HPMA), 4- (methylnitrosamino)-1-(3- pyridyl)- 1-butanol (NNAL), Total Nicotine Equivalents (TNeq)	Randomized, controlled, open-label parallel- group, single-center study

		screening, or having <1.5% COHb saturation (suggestive of being a non-smoker)	No Smoking Group (N=16)		
Yuki ⁵⁹ (2018) <i>N=60</i>	Healthy male and female Japanese adult smokers aged 21-65 years were eligible to participate if they smoked an average of 11 or more manufactured cigarettes per day at screening, had smoked for at least 12 months before entering the trial and had a positive result for a urinary cotinine test	Pregnant or breast-feeding females were excluded from this study. Males having a body mass index (BMI) of less than 18.5 or greater than 27.7 kg/m ² and females having a BMI of less than 16.8 or greater than 26.1 kg/m ² were excluded. Participants were also excluded if they had used any pre- scription drugs, over-the-counter medications	Treatment Group: Novel tobacco vapor product Control Group: Conventional cigarettes Smoking Abstinence Group	4-aminobiphenyl (4-ABP), 3-hydroxypropylmercaptapuric acid (3-HPMA), N-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)- 1-butanol (NNAL), Total Nicotine Equivalents (TNeq)	Controlled, randomized, 3-arm parallel, single-center study

		(including smoking-cessation medications) or dietary supplements within 2 weeks prior to the investigational period; or if they had used any tobacco products other than commercial cigarettes (i.e., hand-rolled cigarette, cigarillo, cigars, pipes, snuff tobacco, chewing tobacco, etc.) within 1 week before screening.			
--	--	--	--	--	--

Table 1: Characteristics of Included Studies (Randomized Controlled Trials).

E. Assessment of risk of bias of included trials

Two reviewers (JR, MR) independently extracted the data of interests using a standardized data collection form and individually appraised each study. Any disparity in assessment was settled by an independent adjudicator. The reviewers discussed the quality of included studies, outcomes to be collected, and risks of bias. Funnel plots in Fig. 2 illustrate the presence of

selection, performance, attrition, detection, and reporting or publication bias using the quality scale for meta-analytic review, which is the Cochrane Collaboration Tool for Risk of Bias.

F. Data analysis

Review Manager 5.3 was used to analyze the data. Analysis of dichotomous data was done using risk ratio, 95% confidence interval, and Mantel-Haenszel method with fixed effects model. Heterogeneity between trials was tested using a standard Chi-square test and I² statistics. The p-value of <0.10 was considered to be statistically significant and I² of 50% was considered as high heterogeneity.

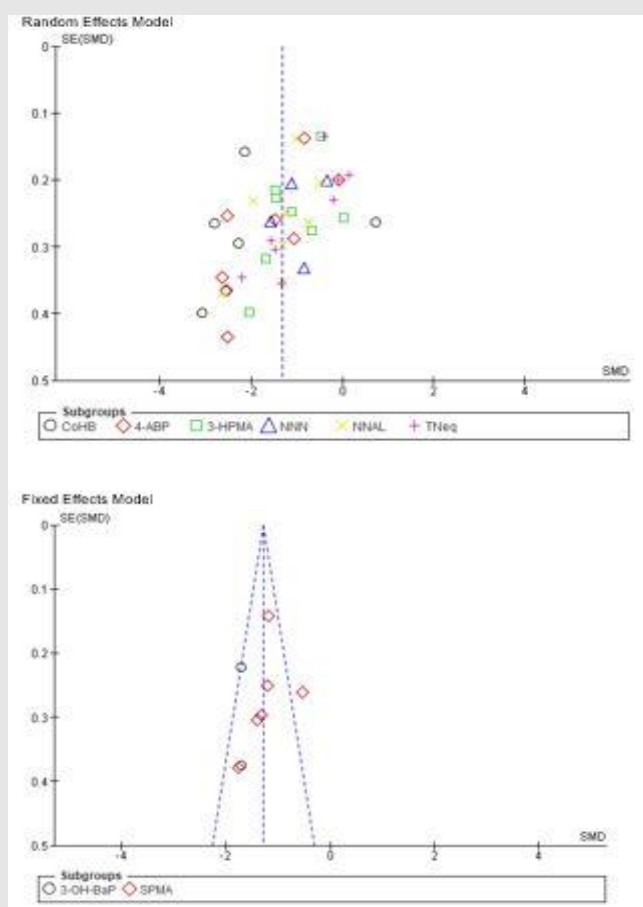


Figure 2: Consolidated funnel plots for the assessment of publication bias.

Results

Effects of intervention on outcomes of interest

Fig. 3 illustrates the effects of intervention on the outcomes of interest.

A. Carboxyhemoglobin (CoHb)

In the analysis of CoHb, the Random Effects Model was used since the resulting p-value of 0.00001 and 96% I² suggested that heterogeneity among studies exist. The pooled analysis of CoHb showed that the mean difference between HTP and CC of -2.0 (95% CI -3.08 to -0.92), with Z value of 3.64 and p-value of 0.00001, was significant. This was further supported by the location of the diamond marker in the Forest plot wherein it was not intersecting the zero axis. Additionally, the diamond marker was at the side of HTP; hence, favoring HTP as results showed that CoHB is significantly lower in HTP than CC.

B. 4-aminobiphenyl (4-ABP)

The resulting p-value of 0.00001 and 93% I² suggest that heterogeneity exist; hence, the Random Effects model was used. The pooled analysis of 4-ABP showed that the mean difference between HTP and CC of -1.67 (95% CI -2.36 to -0.99), with Z value of 4.80 and p-value of 0.00001, was significant. The diamond marker in the Forest plot did not intersect the 0 axis, further illustrating that the mean difference was significant. Additionally, the diamond marker was at the side of HTP, favoring HTP as results showed that 4-ABP is significantly lower in HTP than CC.

C. 3-hydroxypropylmercaptapuric acid (3-HPMA)

The resulting p-value of 0.00001 and 87% I² suggest that heterogeneity exist; hence, the Random Effects model was used. The pooled analysis of 3-HPMA showed that the mean difference between HTP and CC of -1.09 (95% CI -1.54 to -0.63), with Z value of 4.70 and p-value of 0.00001, was significant. The diamond marker in the Forest plot did not intersect the 0 axis, further illustrating that the mean difference was significant. Additionally, the diamond marker was at the side of HTP, favoring HTP as results showed that 3-HPMA is significantly lower in HTP than CC.

D. N-nitrosornicotine (NNN)

The resulting p-value of 0.001 and 81% I² suggest that heterogeneity exist; hence, the Random Effects model was used. The pooled analysis of NNN showed that the mean difference between HTP and CC of -0.95 (95% CI -1.49 to -0.41), with Z value of 3.46 and p-value of 0.0005, was significant. The diamond marker in the Forest plot did not intersect the 0 axis, further illustrating that the mean difference was significant. Additionally, the diamond marker was at the side of HTP, favoring HTP as results showed that NNN is significantly lower in HTP than CC.

E. 4-(methylnitrosamino)-1-(3-pyridyl)- 1-butanol (NNAL)

The resulting p-value of 0.00001 and 83% I² suggest that heterogeneity exist; hence, the Random Effects model was used. The pooled analysis of NNAL showed that the mean

difference between HTP and CC of -1.30 (95% CI -1.72 to -0.89), with Z value of 6.18 and p-value of 0.00001, was significant. The diamond marker in the Forest plot did not intersect the 0 axis, further illustrating that the mean difference was significant. Additionally, the diamond marker was at the side of HTP, favoring HTP as results showed that NNAL is significantly lower in HTP than CC.

F. Total Nicotine Equivalents (TNeq)

The resulting p-value of 0.00001 and 90% I² suggest that heterogeneity exist; hence, the Random Effects model was used. The pooled analysis of TNeq showed that the mean difference between HTP and CC of -0.85 (95% CI -1.36 to -0.34), with Z value of 3.24 and p-value of 0.001, was significant. The diamond marker in the Forest plot did not intersect the 0 axis, further illustrating that the mean difference was significant. Additionally, the diamond marker was at the side of HTP, favoring HTP as results showed that TNeq is significantly lower in HTP than CC.

G. Benzo[a]pyrene (3-OH-BaP)

In the analysis of 3-OH-BaP, the Fixed Effects Model was used since the resulting p-value of 0.98 and 0% I² suggest that there was no significant heterogeneity among the included studies. The pooled analysis of 3-OH-BaP showed that the mean difference between HTP and CC of -1.70 (95% CI -2.08 to -1.33), with Z value of 8.92 and p-value of 0.00001, was significant. The diamond marker in the Forest plot did not intersect the 0 axis, further illustrating that the mean difference was significant. Additionally, the diamond marker was at the side of HTP, favoring HTP as results showed that 3-OH-BaP is significantly lower in HTP than CC.

H. S-phenylmercapturic acid (SPMA)

In the analysis of SPMA, the Fixed Effects Model was used since the resulting p-value of 0.10 and 45% I² suggest that there was no significant heterogeneity among the included studies. The pooled analysis of SPMA showed that the mean difference between HTP and CC of -1.18 (95% CI -1.36 to -0.99), with Z value of 12.36 and p-value of 0.00001, was significant. The diamond marker in the Forest plot did not intersect the 0 axis, further illustrating that the mean difference was significant. Additionally, the diamond marker was at the side of HTP, favoring HTP as results showed that SPMA is significantly lower in HTP than CC.

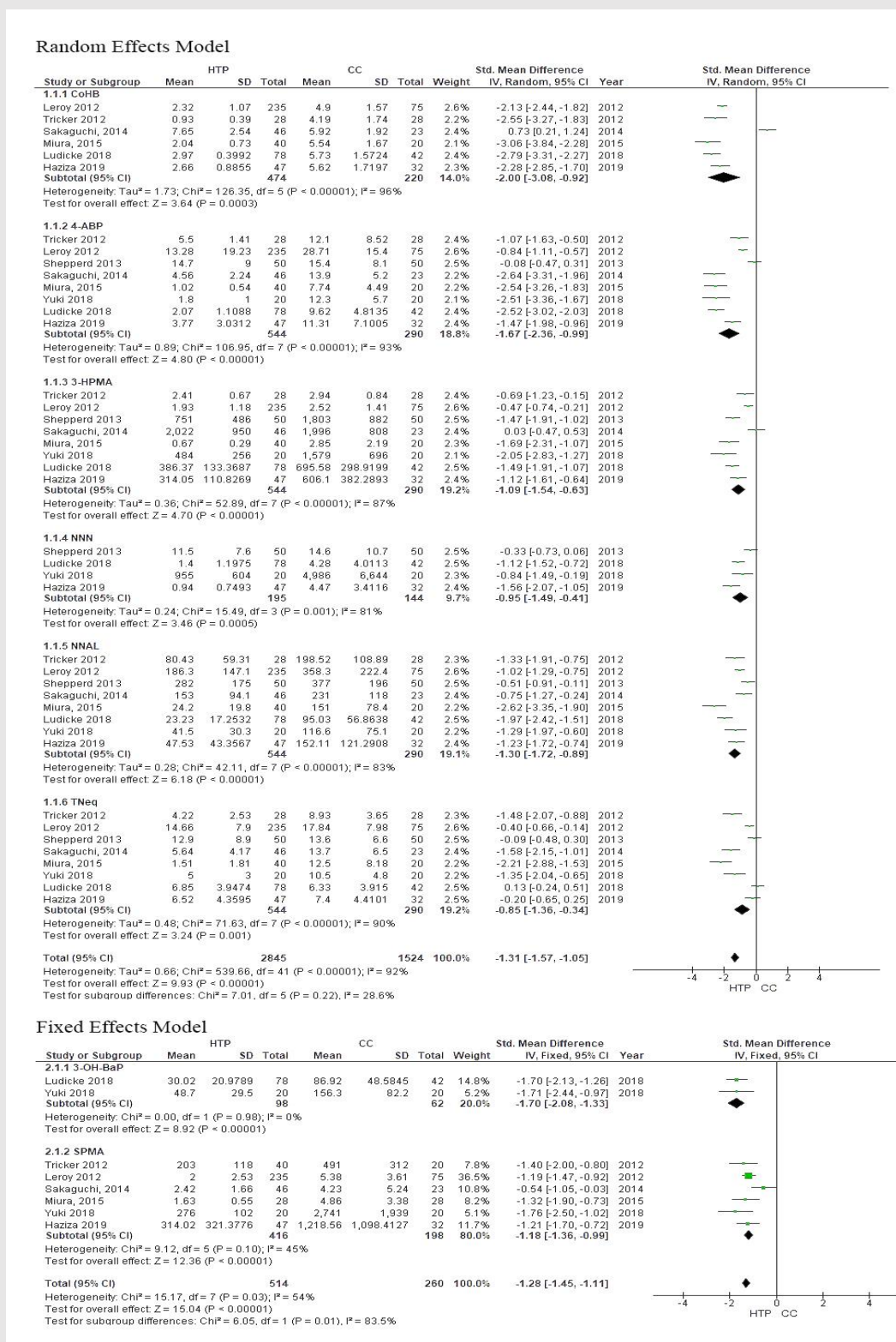


Figure 3: Comparison between HTP and CC with the outcomes of different biomarkers using random and fixed effect models.

Discussion

Meta-analysis of data from the 8 trials included in this study showed that there was a significant difference in the levels of BoEs between HTP and CC. Specifically, the levels of CoHb, 4-ABP, 3-HPMA, NNN, NNAL, TNeq, 3-OH-BaP, and SPMA in the participants were significantly reduced in those who used HTP as compared to those who smoked CC.

In the analysis of 6 out of 8 outcomes (CoHb, 4-ABP, 3-HPMA, NNN, NNAL, TNeq), it was noted that significant heterogeneity existed among the included studies. The possible sources of heterogeneity may be the differences in sample size, age range, race of subjects, study duration, and units of measurement among the studies. Nevertheless, in the analysis of 2 out of 8 outcomes (3-OH-BAP and SPMA), there were no issues of heterogeneity.

The systematic review done by Akiyama, et al., compared the biomarker levels in subjects exposed to electronic cigarettes (e-cigarettes) and HTPs [21]. The main type of studies included in their review were RCTs, case control studies, and cohort studies and the design of the studies was either comparative or longitudinal. They studied majority of the BoEs associated with tobacco. The results of their study suggested no major differences between e-cigarettes and HTPs as the reduction of BoE levels were similar after switching from CC [21].

On the other hand, this study focused only on comparing BoEs in HTPs versus CC. This objective was similar to that of Drovandi, et al., [26]. Their study included 10 RCTs and they limited the BoEs assessed to only those that were reported in at least 8 of the 10 included studies. There were 12 BoEs that met the criteria, which were as follows: 1-hydroxypyrene (1-OHP), 2-aminoaphthalene (2-AN), 3-cyanoethylmercapturic acid (CEMA), 3-hydroxypropylmercapturic acid (3-HPMA), 4-aminobiphenyl (4-ABP), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), carboxyhemoglobin (COHb), monohydroxybutenyl-mercapturic acid (MHBMA), n-nitrosoornicotine, o-toluidine, (O-tol), s-phenylmercapturic acid (S-PMA), and total nicotine equivalents (TNeq). Their study showed that these BoEs were significantly reduced in participants who used HTPs versus those who used CC [26].

Similar to Drovandi, et al., the studies included in our review were all RCTs [26]. However, during our review, there were other RCTs that were not yet included in their study. Two studies done in 2012, one study in 2013, and one study in 2015 were the additional eligible studies included in our systematic review [53,55-58]. In terms of the selection of BoEs to be analyzed, our study selected only those BoEs grouped under the chemical classes that were identified by the US FDA to be the most applicable for regulatory use; hence, we narrowed down our outcomes of interest to the 8 particular BoEs.

The BoEs analyzed in this study may be the most practical or applicable measures of harm that may be used for regulation of use of tobacco products. In some countries, there is still a paucity

of data as to which BoEs are being measured to regulate tobacco products including MRTPs. This study may be a reasonable starting point for HTP regulation by assessing a practical set of BoEs.

Overall, this study may be supportive of the current evolving science involving non-combustible cigarette alternatives. Since this study showed lower levels of BoE in HTP compared to CC, HTP may be considered as a promising intervention to reduce the risk of harm in tobacco smokers, especially in recalcitrant smokers.

Limitations of the Study

Since HTPs are relatively new MRTPs, existing literature comparing them to CCs are limited. The sample sizes of the studies included in this meta-analysis are relatively small but the pooled total of 1,193 subjects may be sufficient enough to draw reasonable conclusions. Long-term studies are also limited at the time of study. It is also important to note that most data may include only the available international data, mostly performed in countries where HTPs are already available and approved by regulatory authorities. The present study includes only published studies. There may be a need to search for other unpublished studies.

Conclusion

The study showed that the use of HTP could significantly reduce exposure to harmful substances compared to CCs using as parameters BoEs that are assessed to be more suitable and practical for tobacco product regulation. The measurement of specific biomarkers (CoHb, 4-ABP, 3-HPMA, NNN, NNAL, TNeq, 3-OH-BaP, and SPMA) may aid in the evaluation of safety and regulation of various tobacco products and their alternatives.

Acknowledgement

The authors would like to thank God, their family, and friends for their support and encouragement in making this meta-analysis possible.

Conflict of Interest

RRC: member of advisory board or speakers' pool of Servier, Boehringer Ingelheim, Menarini, LRI-Therapharma, Sanofi, UAP Pharma, Unilab and trustee/council member of International

Society of Hypertension, which has pharmaceutical and medical device companies as corporate members; the rest declare no conflict of interest.

References

1. WHO global report on trends in prevalence of tobacco use 2000-2025, third Edition Geneva: World Health Organization. 2019.
2. Başaran R, Güven NM, Eke BC. An Overview of iQOS® as a New Heat-Not-Burn Tobacco Product and Its Potential Effects on Human Health and the Environment. *Turk J Pharm Sci.* 2019;16(3):371-4.
3. Mallock N, Pieper E, Hutzler C. Heated tobacco products: a review of current knowledge and initial assessments. *Front Public Health.* 2019;7:287.
4. Centers for Disease Control and Prevention (US); National Center for Chronic Disease Prevention and Health Promotion (US); Office on Smoking and Health (US). How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease: A Report of the Surgeon General. Atlanta (GA): Centers for Disease Control and Prevention (US); 2010. 3, Chemistry and Toxicology of Cigarette Smoke and Biomarkers of Exposure and Harm. Last accessed on: August 05, 2022 <https://www.ncbi.nlm.nih.gov/books/NBK53014/>
5. Richard R. Baker, Smoke generation inside a burning cigarette: Modifying combustion to develop cigarettes that may be less hazardous to health. *Progress in Energy and Combustion Sci.* 2006;32(6):373-85.
6. Baker RR. Product formation mechanisms inside a burning cigarette. *Prog Energy Combust Sci.* 1981;7:135-53.
7. Baker RR. Smoke chemistry. In: Davis DL, Nielsen MT, editors. Tobacco production, chemistry and technology. Oxford, UK: Blackwell Science. 1999;12:398-439.
8. Muramatsu M. An approach to modelling a burning cigarette. *Beitr Tabakforsch Int.* 2005;21:286-93.
9. Glantz SA. Heated tobacco products: the example of IQOS. *Tobacco Control.* 2018;27:s1-s6.
10. Newland N, Lowe FJ, Camacho OM. Evaluating the effects of switching from cigarette smoking to using a heated tobacco product on health effect indicators in healthy subjects: study protocol for a randomized controlled trial. *Intern Emerg Med.* 2019;14:885-98.
11. Polosa R, Rodu B, Caponnetto P. A fresh look at tobacco harm reduction: the case for the electronic cigarette. *Harm Reduct J.* 2013;4(10):19.
12. Rodu B. The scientific foundation for tobacco harm reduction, 2006-2011. *Harm Reduct J.* 2011;8:19.
13. Schaller JP, Keller D, Poget L. Evaluation of the tobacco heating system 2.2. Part 2: chemical composition, genotoxicity, cytotoxicity, and physical properties of the aerosol. *Regul Toxicol Pharmacol.* 2016;81(Suppl 2):S27-47.
14. Forster M, Fiebelkorn S, Yurteri C. Assessment of novel tobacco heating product THP1.0. Part 3: comprehensive chemical characterisation of harmful and potentially harmful aerosol emissions. *Regul Toxicol Pharmacol.* 2018;93:14-33.
15. Caponnetto P, Maglia M, Prosperini G. Carbon monoxide levels after inhalation from new generation heated tobacco products. *Respir Res.* 2018;19(1):164.
16. Jaccard G, Tabin Djoko D, Moennikes O. Comparative assessment of HPHC yields in the tobacco heating system THS2.2 and commercial cigarettes. *Regul Toxicol Pharmacol.* 2017;90:1.
17. Hatsukami DK, Carroll DM. Tobacco harm reduction: Past history, current controversies and a proposed approach for the future. *Prev Med.* 2020;140:106099.

18. Kenneth E Warner. How to think-not feel-about tobacco harm reduction. *Nicotine and Tobacco Res.* 2019;21(4):1299-309.
19. Amos A, Arnott D, Aveyard P, et al. Nicotine without smoke: Tobacco harm reduction. Royal College of Physicians, 2016. Last accessed on: August 05, 2022
<https://www.rcplondon.ac.uk/projects/outputs/nicotine-without-smoke-tobacco-harm-reduction>
20. O'Leary R, Polosa R. Tobacco harm reduction in the 21st century. *Drugs and Alcohol Today.* 2020;20(3):219-34.
21. Akiyama Y, Sherwood N. Systematic review of biomarker findings from clinical studies of electronic cigarettes and heated tobacco products. *Toxicol Rep.* 2021;8:282-94
22. Mattes W, Yang X, Orr MS, et al. Biomarkers of tobacco smoke exposure. *Adv Clin Chem.* 2014;67:1-45.
23. Lempert LK, Glantz SA. Heated tobacco product regulation under US law and the FCTC. *Tobacco Control* 2018;27:s118-25.
24. Ashley D, Backinger C, van Bommel D. Tobacco regulatory science: research to inform regulatory action at the food and drug administration's center for tobacco products. *Nicotine and Tobacco Research,* 2014;16(8):1045-49.
25. Bernert JT, Jain RB, Pirkle JL. Urinary tobacco-specific nitrosamines and 4-aminobiphenyl hemoglobin adducts measured in smokers of either regular or light cigarettes. *Nicotine Tob Res.* 2005;7:729-38
26. Drovandi A, Salem S, Barker D. Human biomarker exposure from cigarettes versus novel heat-not-burn devices: a systematic review and meta-analysis. *Nicotine Tob Res.* 2020;22(7):1077-85.
27. Chang C, Edwards S, Arab A. Biomarkers of tobacco exposure: summary of an fda- sponsored public workshop. *Cancer Epidemiol Biomarkers Prev.* 2017;26(3):291-302.
28. Benowitz NL, Dains KM, Dempsey D. Racial differences in the relationship between number of cigarettes smoked and nicotine and carcinogen exposure. *Nicotine Tob Res.* 2011;13:772-83.
29. St HG, Goniewicz ML, Dempsey D. Exposure and kinetics of Polycyclic Aromatic Hydrocarbons (PAHs) in cigarette smokers. *Chem Res Toxicol.* 2012;25:952-64.
30. Jarvis MJ, Russell MA, Saloojee Y. Expired air carbon monoxide: a simple breath test of tobacco smoke intake. *Br Med J.* 1980;281:484-5.
31. Yuan JM, Gao YT, Wang R. Urinary levels of volatile organic acarcinogen and toxicant biomarkers in relation to lung cancer development in smokers. *Carcinogenesis.* 2012;33:804-9.
32. Park SL, Carmella SG, Ming X. Variation in levels of the lung carcinogen NNAL and its glucuronides in the urine of cigarette smokers from five ethnic groups with differing risks for lung cancer. *Cancer Epidemiol Biomarkers Prev.* 2015;24:561-9.
33. Hecht SS, Carmella SG, Le KA. 4-(methylnitrosamino)-1- (3-pyridyl)-1-butanol and its glucuronides in the urine of infants exposed to environmental tobacco smoke. *Cancer Epidemiol Biomarkers Prev.* 2006; 15:988-92.
34. Hecht SS, Chen M, Yagi H. r-1,t-2,3,c-4-Tetrahydroxy-1,2,3,4- tetrahydrophenanthrene in human urine: a potential biomarker for assessing polycyclic aromatic hydrocarbon metabolic activation. *Cancer Epidemiol Biomarkers Prev.* 2003;12:1501-8.
35. Hecht SS, Yuan JM, Hatsukami D. Applying tobacco carcinogen and toxicant biomarkers in product regulation and cancer prevention. *Chem Res Toxicol.* 2010;23:1001-8.
36. Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke: Established List. 2012. Content current as of 10/07/2019. Last accessed on: August 05, 2022
<https://www.fda.gov/tobacco-products/rules-regulations-and-guidance/harmful-and-potentially-harmful-constituents-tobacco-products-and-tobacco-smoke-established-list>
37. Alwis KU, Blount BC, Britt AS. Simultaneous analysis of 28 urinary VOC metabolites using ultra high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC-ESI/MSMS). *Anal Chim Acta.* 2012;750:152-60.

38. Turesky RJ, Le ML. Metabolism and biomarkers of heterocyclic aromatic amines in molecular epidemiology studies: lessons learned from aromatic amines. *Chem Res Toxicol*. 2011;24:1169-214.
39. Castela JE, Yuan JM, Skipper PL. Gender- and smoking-related bladder cancer risk. *J Natl Cancer Inst*. 2001;93:538-45.
40. Bernert JT, Jain RB, Pirkle JL. Urinary tobacco-specific nitrosamines and 4-aminobiphenyl hemoglobin adducts measured in smokers of either regular or light cigarettes. *Nicotine Tob Res*. 2005;7:729-38.
41. Gan J, Skipper PL, Gago-Dominguez M. Alkylaniline- hemoglobin adducts and risk of non-smoking-related bladder cancer. *J Natl Cancer Inst*. 2004;96:1425-31.
42. Jay J, Pfaumiller EL, Huang NJ. Five-day changes in biomarkers of exposure among adult smokers after completely switching from combustible cigarettes to a nicotine-salt pod system. *Nicotine Tob Res*. 2020;22(8):1285-93.
43. Peruzzi M, Cavarretta E, Frati G. Comparative Indoor Pollution from Glo, Iqos, and Juul, Using Traditional Combustion Cigarettes as Benchmark: Evidence from the Randomized SUR-VAPES AIR Trial. *Int J Environ Res Public Health*. 2020;17(17):6029.
44. Sibul FT Burkhardt A Kachhadia. Identification of biomarkers specific to five different nicotine product user groups: Study protocol of a controlled clinical trial. *Contemporary Clinical Trials Communications*. 2021;22:100794.
45. Newland N, Lowe FJ, Camacho OM. Evaluating the effects of switching from cigarette smoking to using a heated tobacco product on health effect indicators in healthy subjects: study protocol for a randomized controlled trial. *Intern Emerg Med*. 2019;14:885-98.
46. Haziza C, G de La Bourdonnaye, S Merlet. Assessment of the reduction in levels of exposure to harmful and potentially harmful constituents in Japanese subjects using a novel tobacco heating system compared with conventional cigarettes and smoking abstinence: A randomized controlled study in confinement. *Regulatory Toxicology and Pharmacology*.
47. Haziza C, G de La Bourdonnaye, D Skiada. Evaluation of the Tobacco Heating System 2.2. Part 8: 5-Day randomized reduced exposure clinical study in Poland. *Regulatory Toxicology and Pharmacology*. 2016;81:S139eS150.
48. Yingst JM, Veldheer S, Hrabovsky S, Nichols TT, Wilson SJ, Foulds J. Factors associated with electronic cigarette users' device preferences and transition from first generation to advanced generation devices. *Nicotine and Tobacco Research*. 2015;17(10):1242-6.
49. Lüdicke F, Baker G, Magnette J, Picavet P, Weitkunat R. Reduced exposure to harmful and potentially harmful smoke constituents with the tobacco heating system 2.1. *Nicotine & Tobacco Research*. 2017;19(2):168-75.
50. Gale, N, M McEwan, A Eldridge. Changes in biomarkers of exposure on switching from a conventional cigarette to tobacco heating products: a randomized, controlled study in healthy Japanese Subjects. *Nicotine and Tobacco Research*. 2018;1-8.
51. Lüdicke F, Ansari SM, Lama N, Blanc N, Bosilkovska M, Donelli A, et al. 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 effects of switching to a heat-not-burn tobacco product. *Cancer Epidemiology, Biomarkers and Prevention*. 2019;28(11):1934-43.
52. Haziza C, de La Bourdonnaye G, Donelli A. Reduction in exposure to selected harmful and potentially harmful constituents approaching those observed upon smoking abstinence in smokers switching to the menthol tobacco heating system 2.2 for 3 months (part 1). *Nicotine Tob Res*. 2020;22(4):539-48.
53. Leroy, M, Jarus-Dziedzic K, Ancerewicz J. Reduced exposure evaluation of an Electrically Heated Cigarette Smoking System. Part 7: A one-month, randomized, ambulatory, controlled clinical study in Poland. *Regul Toxicol Pharmacol*. 2012;64(2):S74-84.
54. Lüdicke F, Picavet P, Baker G. Effects of switching to the tobacco heating system 2.2 menthol, smoking abstinence, or continued cigarette smoking on biomarkers of exposure: a randomized, controlled, open-label,

- multicenter study in sequential confinement and ambulatory settings (Part 1). *Nicotine Tob Res.* 2018;20(2):161-72.
55. Miura N, Yuki D, Minami N. A study to investigate changes in the levels of biomarkers of exposure to selected cigarette smoke constituents in Japanese adult male smokers who switched to a non-combustion inhaler type of tobacco product. *Regul Toxicol Pharmacol.* 2015;71(3):498-506.
 56. Sakaguchi C, Kakehi A, Minami N. Exposure evaluation of adult male Japanese smokers switched to a heated cigarette in a controlled clinical setting. *Regul Toxicol Pharmacol.* 2014;69(3):338-47.
 57. Shepperd CJ, Newland N, Eldridge A. A single-blinded, single-centre, controlled study in healthy adult smokers to identify the effects of a reduced toxicant prototype cigarette on biomarkers of exposure and of biological effect versus commercial cigarettes. *BMC Public Health.* 2013;13:690
 58. Tricker AR, Jang IJ, Martin Leroy C. Reduced exposure evaluation of an Electrically Heated Cigarette Smoking System. Part 4: Eight-day randomized clinical trial in Korea. *Regul Toxicol Pharmacol.* 2012;64(2 Suppl):S45-53.
 59. Yuki D, Takeshige Y, Nakaya K. Assessment of the exposure to harmful and potentially harmful constituents in healthy Japanese smokers using a novel tobacco vapor product compared with conventional cigarettes and smoking abstinence. *Regul Toxicol Pharmacol.* 2018;96:127-34.