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

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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-nitrosonornicotine [-0.95 (95% CI -1.49 to -0.41), Z=3.46, p=0.00005], 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),

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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-phenylmercuric 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-nitrosonornicotine (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:

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1. Carboxyhemoglobin (CoHB)
2. 4-aminobiphenyl (4-ABP)
3. 3-hydroxypropylmercapturic acid (3-HPMA)
4. N-nitrosonornicotine (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-hydroxypropylmercapturic acid (3-HPMA) - a mercapturic acid metabolite of acrolein, which measures exposure to volatile organic compounds of tobacco smoke

8. N-nitrosonornicotine (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 | Inclusion | Exclusion | Intervention | Outcome | Methods |
|--------------|------------|-----------|-----------|--------------|---------|---------|
| Haziza²⁵² (2019) | 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-hydroxypropylmercaptapenic acid (3-HPMA), N-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), Benzo[a]pyrene | Randomized, three-arm parallel group, controlled clinical study |
| Leroy²³ (2012) | 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-hydroxypropylmercaptapenic acid (3-HPMA), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), Total Nicotine Equivalents (TNeq), | Randomized, open-label, controlled study |

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| Ludicke (2018) N=160 | Japanese male and female smokers aged 23-65 years with a BMI of 18.5-32 kg/m² 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-hydroxypropylmercapturic acid (3-HPMA), N-nitrosornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)- 1-butanol (NNAL) | Three-arm, parallel-group randomized controlled study |
| Miura (2015) | 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/m² 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 | N=78 | 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-hydroxypropylmercapturic acid (3-HPMA), 4-[(methylnitrosamino)-1-(3-pyridyl)]-1-butanol (NNAL) | Permuted-block, randomized (for smokers), controlled, forced-switching, open-label, parallel group design |

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| 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 | Subjects | Treatment Group | Carboxyhemoglobin (CoHB), 4-aminobiphenyl (4-ABP), 3-hydroxypropylmercapturic acid (3-HPMA), N-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), Benzo[a]pyrene, Benzene | Controlled, semi-randomized, open-label, parallel group, residential, 4-sites design |
|-----------|----------|-----------------|-----------------------------------------------------------------|------------------------------------------------------------------|
| Sakaguchi (2014) | 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 | 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 experience of hand-rolled cigarette, cigarillo, cigar, pipe, smokeless tobacco or chewing tobacco within 4 weeks before screening, or who donate 400 ml or more of blood within 12 weeks before screening (including blood donation) etc. | Carboxyhemoglobin (CoHB), 4-aminobiphenyl (4-ABP), 3-hydroxypropylmercapturic acid (3-HPMA), N-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), Benzo[a]pyrene, Benzene | Controlled, semi-randomized, open-label, parallel group, residential, 4-sites design |

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| Shepperd et al. (2013) | Healthy adult 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 day; 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 PPIs). | 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-hydroxypropylmercapturic 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 have no ongoing 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.

3) “GROUP 6”: Nonsmoking group -Provided background levels of BoE conventional cigarette (CC1)

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| Tricker\(^8\) (2012) | Adult male and female Korean smokers (aged 20-50) with acceptable health conditions who smoked 10-30 conventional light-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 | Have a urine cotinine level of <10 ng/mL. | 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) | Carboxyhemoglobin (CoHB), 4-aminobiphenyl (4-ABP), 3-hydroxypropylmercapturic acid (3-HPMA), 4-(methyleneiminosamino)-1-(3-pyridyl)-1-butanol (NNAL), Total Nicotine Equivalents (TNeq) | Randomized, controlled, open-label parallel-group, single-center study |

Pregnant or lactating women; Existence of a clinically significant disease, clinically relevant abnormal findings on physical examination, medical history, and clinical laboratory results, alcohol or drug abuse, and a positive test for human immunodeficiency virus (HIV) or hepatitis; subjects using a nicotine-containing product other than cigarettes within 3 months prior to admission to the clinic.
| Yuki (2018) N=60 | 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 | Screening, or having <1.5% COHb saturation (suggestive of being a non-smoker) | No Smoking Group (N=16) | Treatment Group: Novel tobacco vapor product Control Group: Conventional cigarettes Smoking Abstinence Group | 4-aminobiphenyl (4-ABP), 3-hydroxypropylmercapturic 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 |
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.

![Consolidated funnel plots for the assessment of publication bias](image)

**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-nitrosonornicotine (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.
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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-aminoapththalene (2-AN), 3-cyanoethylmercapturic acid (CEMA), 3-hydroxypropylmercapturic acid (3-HPMA), 4-aminobiphenyl (4-ABP), 4-(methylnitrosamo)-1-(3-pyridyl)-1-butanol (NNAL), carboxyhemoglobin (COHb), monohydroxybutenyl-mercapturic acid (MHBMA), n-nitrosonornicotine, o-toluidine, (0-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.

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**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.

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