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Review article

Carcinogenic biomarkers of exposure in the urine of heated tobacco product users associated with bladder cancer: A systematic review

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Abstract

To identify biomarkers of exposure present in Heated Tobacco Products (HTPs) users' urine which are associated with bladder cancer and to compare quantitative biomarker levels to those seen in combustible cigarette users. A systematic literature review was conducted in December 2020 with no date limits. Relevant studies that reported quantitative urinary biomarker of exposure in HTP users were included. Biomarkers and their parent compounds were classified by carcinogenicity according to the International Agency for Research on Cancer Monographs and were cross-referenced with the Collaborative on Health and the Environment Toxicant and Disease Database to determine associations with bladder cancer. Our literature search identified 561 articles and 30 clinical trial reports. 11 studies met inclusion criteria. These studies identified 29 biomarkers of exposure present in HTP users' urine, which reflect exposure to 21 unique parent compounds. Of these parent compounds, 14 are carcinogens and 10 have a known link to bladder cancer. HTP users' biomarkers of exposure were present at lower levels than combustible cigarette users but higher than never-smokers. Biomarkers of exposure to bladder carcinogens are present in the urine of HTP users. While levels of these biomarkers appear to be lower than combustible cigarette users, chronic urothelial exposure to bladder carcinogens is concerning and degree of bladder cancer risk remains unknown. Further long-term study is needed to elucidate the bladder cancer risk of HTP use.

Keywords: Heated tobacco product; Bladder cancer; Carcinogens; Biomarkers of exposure; Urine

1. Introduction

Cigarette smoking is the most significant modifiable risk factor for the development of bladder cancer. Smokers are at least 3 times as likely to develop bladder cancer as non-smokers and 50% of bladder cancer cases are attributable to current or prior smoking [1]. Multiple carcinogens present

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in tobacco smoke have been causally linked to the development of bladder cancer including polycyclic aromatic hydrocarbons (PAHs), aromatic amines, tobacco-specific nitrosamines, and various volatile organic compounds. While ongoing research continues to elucidate these relationships, it appears that exposure to cigarette smoke is related to the development of bladder cancer in a dose-dependent fashion, with higher intensity of smoking leading to a greater risk of bladder cancer [1].

A variety of novel tobacco products have been introduced in the past decade, including e-cigarettes and heated tobacco products, which are purported to be a safer alternative to cigarette smoking, but whose long-term safety profile remains unknown. Previous studies have shown that carcinogens linked to bladder cancer are present in the urine of e-cigarette users [2], and that their toxicant and carcinogen profile is distinct from that of cigarette smokers. Heated tobacco products (HTPs), also known as heat-not-burn tobacco products, are electric devices or cigarette-like products that heat a puck of tobacco, producing an aerosol and gases that are inhaled by the user. In contrast to conventional cigarettes, HTPs do not directly burn the tobacco but rather heat it via an external source, thereby limiting the temperature the tobacco reaches [3]. Many of the harmful compounds present in cigarette smoke are byproducts of the combustion process, so it has been proposed that HTPs are a safer alternative to combustible cigarette smoking. Internationally, these products are used by up to 11% of adults [4], and the US Food and Drug Administration recently approved 1 such product (IQOS) as a "modified risk tobacco product" from Philip Morris International (PMI). Because these devices heat tobacco, they have the potential to expose users to many of the same risks as cigarette smokers, including a potential increased risk of developing bladder cancer.

HTPs expose users to similar levels of nicotine as conventional cigarettes, and many of the same carcinogens and toxicants previously identified in cigarette smoke have been identified in the emissions produced by HTPs. Some of these carcinogens have known associations with bladder cancer, and therefore the possibility is raised that, like conventional cigarette smoking, use of HTPs may expose users to an increased risk of developing bladder cancer. The aims of this systematic review are threefold: to identify biomarkers of exposure present in HTP users' urine, to determine which biomarkers, if any, are associated with bladder cancer, and to compare quantitative biomarker levels to those seen in combustible cigarette users. We also hope that this review will give practicing urologists useful evidence for discussing the potential risks of these products with their patients.

2. Methods

2.1. Evidence acquisition

We conducted a systematic literature review following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [5]. Our literature search sought to identify articles discussing bladder cancer-related toxicant and carcinogen exposures in heated tobacco product users as measured by biomarkers of exposure in their urine. Our search was conducted in December 2020. The protocol for this systematic review was registered in PROSPERO (Prospective Register of Systematic Reviews, CRD42021227973) on January 4, 2021, and accepted for registration on February 17, 2021.

2.1.1. Search strategy

The literature searches were performed by a medical librarian and co-author (EM) in the PubMed, Embase, Scopus, Cochrane CENTRAL, and clinicaltrials.gov for English language articles with no date limits. We searched keywords such as "heated tobacco products," "heat not burn," "heatsticks," names of specific Heat-Not-Burn devices, or a combination of heat and smoking related terms in conjunction with terms relating to toxicant exposure such as biomarkers of exposure, carcinogens, or pollutants. The complete PubMed search is shown in the Supplemental Table.

2.1.2. Eligibility criteria

Studies focusing on the urinary biomarkers of exposure of HTPs users were included. Only studies that included quantitative urinary biomarker levels were included. Only publications in English language were included in analysis, non-English articles, review articles, and case reports were excluded. Additional exclusion criteria included studies on second hand HTP aerosol exposure, non-urine based biomarkers or metabolites, studies with dual-use of HTPs and combustible cigarettes, animal studies, and non-in vivo studies.

2.1.3. Data extraction

The review process was conducted using the Covidence systematic review software package [6]. Titles and abstracts were screened by 2 authors (CS, AJ) who determined the final selection of included studies based on full text evaluation. Each study that was reviewed received 2 votes based on the a priori inclusion/exclusion criteria. Disagreements were highlighted and decided by consensus with the input of a third author (MB). Data extraction was performed by the same 2 authors, and data were then reviewed by all co-authors. For each study that that met inclusion criteria, 1 co-author extracted pertinent information regarding the study populations, urine biomarkers, comparators, outcomes, settings, and designs, and a second co-author reviewed data for completeness and accuracy. Disagreements were resolved by group discussion.

2.1.4. Quality appraisal

Methodological quality was assessed using the Version 2 of the Cochrane Collaboration Risk-of-Bias tool (ROB-2) [7]. For each study, the ROB-2 tool assesses 5 domains of potential methodological bias: the randomization process, deviations for intended interventions, missing outcome data, measurement of the outcome, and selection of the reported result, as well as an overall bias assessment. Based on the methods of the studies, each domain was assessed using multiple ROB-2 questions per domain, and were rated as "low risk of bias," "some concerns," or "high risk of bias." Initial bias assessments were conducted by 1 author (CS) and were reviewed and verified by a second author (MB).

2.2. Evidence synthesis

Findings for each included study were summarized in tabular and narrative format. Each identified parent compound and urinary biomarker was classified according to the International Agency for Research on Cancer (IARC) Monographs on the Evaluation of Carcinogenic Risks to Humans (Group 1, Carcinogenic to humans; Group 2A, Probably carcinogenic to humans; Group 2B, Possibly carcinogenic to humans; Group 3, Not classifiable as to its carcinogenicity to humans; or Group 4, Probably not carcinogenic to humans) [8]. These compounds were then cross referenced using the Collaborative on Health and the Environment, Toxicant and Disease Database [9] to determine a link to bladder cancer, grouped by strength of evidence (strong evidence, good evidence, limited evidence), similar to prior studies [2,10].

To determine whether a meta-analysis could be appropriately conducted, clinical and methodological heterogeneity were assessed among included studies. However, given the heterogeneity in reporting methods, variability in study designs, and variations in units of urinary biomarkers quantitative levels, the data extracted were deemed suitable for a systematic review but not for conducting a meta-analysis.

2.2.1. Study selection

We screened 561 articles and 30 clinical trial reports in Covidence after duplicates were excluded from the original 1,198 results (Fig. 1). 560 records were excluded during Title/Abstract screening and we obtained full text for 31 articles. Ultimately, 11 records were included in the study after removing 20 records at the full text level due to study type or incorrect or incomplete outcome data.

2.2.2. Study sources

Of the 11 studies included in our review, 10 were randomized, controlled trials [11−20], and 1 was a controlled crossover study [21]. All studies included users of combustible cigarettes as a control group, 1 included non-smoker controls [21], and 1 included a never-smoker control group [19]. Five studies observed participants for short period of confinement (5 days) [12,14−17], 2 for an extended ambulatory period (6−24 weeks) [19,21], and 4 included a period of confinement followed by an ambulatory period (5 days confinement, 85−86 days ambulatory) [11,13,18,20]. During confinement periods, subjects were physically present in a study center, and their diet, activities, and tobacco use were tightly controlled. Ambulatory periods involved subjects using their specified products in their typical life circumstances, returning to the study center periodically for evaluation and urine collection. Two studies were multicenter [13,19] and 9 were single-center [11,12,14−18,20,21], 5 took place in Poland [11,15−17,20], 3 in Japan [12,14,18], and 3 in the United States [13,19,21]. Together, the studies included 1375 participants, 650 of whom were part of an HTP arm. Five studies investigated the use of a carbon HTP [11,17,19−21], while 6 studied electric devices [12−16,18]. Three included menthol HTP groups [12,13,18], and 1 allowed participants to use either menthol or non-menthol products [19]. All of the studies presented here were directly funded by and conducted by employees of the tobacco industry with 8 produced by Philip Morris International [11,13−18,20], 2 by R. J. Reynolds Tobacco [19,21], and 1 by British American Tobacco [12], or their respective subsidiaries.

2.2.3. Urinary biomarkers

We assessed the presence and level of biomarkers of exposure in the urine of HTP users and cross referenced those biomarkers with known bladder carcinogens. Biomarkers of exposure are compounds that are either toxic themselves or are the urinary metabolites of a toxic parent compound. Our present focus is primarily on biomarkers of exposure for urinary bladder carcinogens, which are a particularly useful source of information when considering bladder cancer because their presence indicates direct urothelial exposure to the related carcinogens as the urine passed through the bladder. Urine is also easy and painless to obtain and methods to quantify urinary biomarkers are well-established. We divided the biomarkers of exposure identified in this review into biomarkers for: (1) nicotine and its metabolites, (2) nicotine-specific nitrosamines, (3) PAHs and amines, and (4) volatile organic compounds.

3. Results

3.1. Biomarkers present in the Urine of HTP users

A total of 29 biomarkers of exposure were reported in the studies reflecting exposure to 21 parent compounds (Table 1). Several parent compounds were associated with more than 1 biomarker of exposure across the studies. Nine of these parent compounds are IARC group 1 carcinogens (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, N-nitrosonornicotine, 2-aminonaphthalene, 4-Aminobiphenyl,

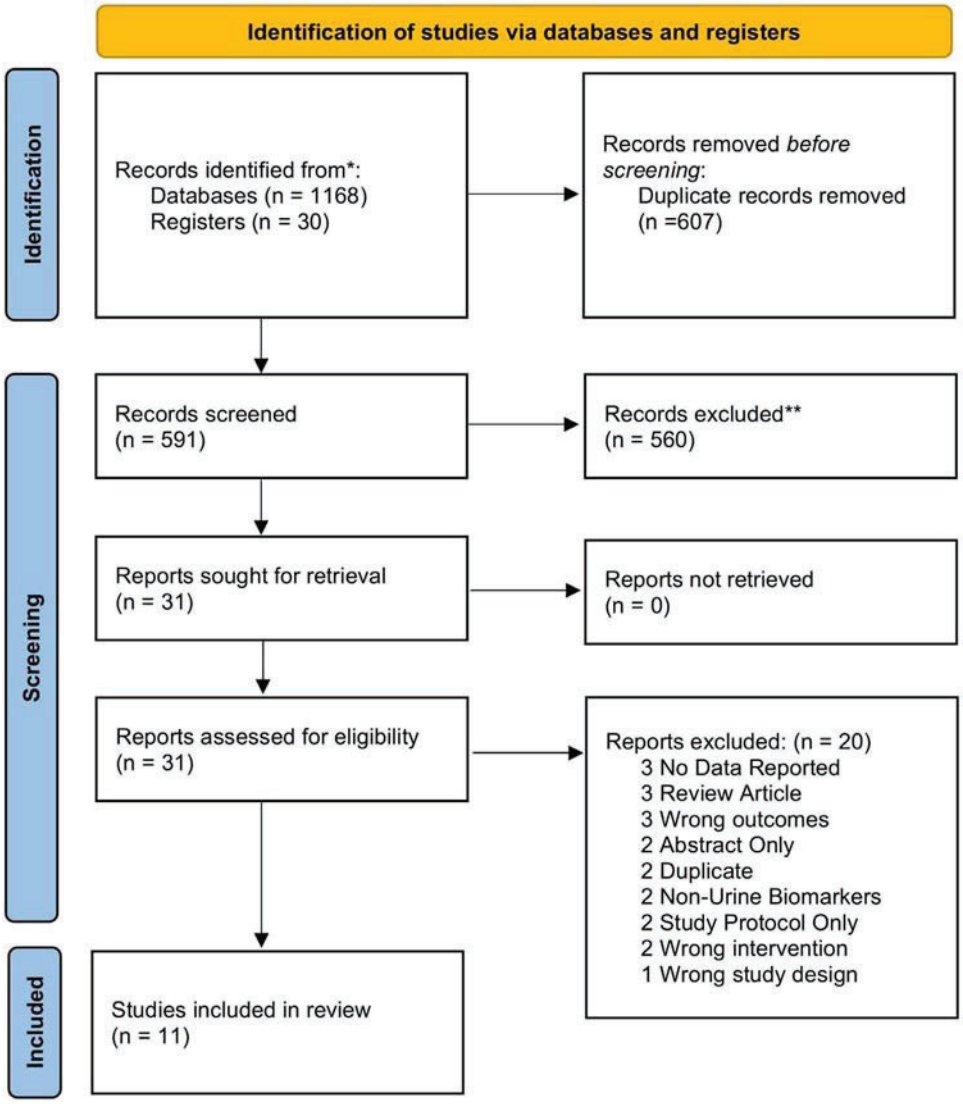

Fig. 1. PRISMA diagram.

benzo[a]pyrene, 1,3-butadiene, benzene, ethylene oxide and o-toluidine), 2 are in group 2A (Acrolein and Acrylamide), 3 are in group 2B (Naphthalene, Acrylonitrile, Crotonaldehyde), and 5 are in group 3. Two parent compounds that were not listed in the IARC monographs were also identified (nicotine and 3-Aminobiphenyl).

3.2. Associations between urinary biomarkers and bladder cancer

We compared the urinary biomarkers we identified and their parent compounds to the Collaborative on Health and the Environment (CHE), Toxicant and Disease Database [9] to determine the strength of any known association with bladder cancer (Table 1). According to the CHE database, 8 of the identified parent compounds have a "strong" association with bladder cancer: 2-aminonaphthalene, 4-aminobiphenyl, benzo[a]pyrene, fluorene, naphthalene, phenanthrene, pyrene, and o-toluidine. Two compounds, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and N-nitrosonornicotine, have a "limited" association with bladder cancer, and 2, 1,3-butadiene and Acrylamide, have a "strong" association with cancer not otherwise specified. 6 parent compounds did not have a known association with bladder cancer as reported by CHE, and 3 were not listed in their database. All the biomarkers of exposure we identified were present in the urine of both HTP users and combustible cigarette smokers.

3.3. Quantitative levels of biomarkers present in the urine of HTP users

We limited the present review to studies that presented quantitative urinary biomarker levels, which led to the exclusion of several studies that only reported changes from baseline levels. Among those who presented quantitative levels, biomarkers were reported either as total amount

Table 1
Biomarkers of Exposure, Associated Parent Compounds, IARC Carcinogens, and CHE Association with Bladder Cancer

Chemical Class	Biomarker	Abbreviation	Parent Compound	IARC Carcinogen Classification	CHE Association with Bladder Cancer	PC CAS
Nicotine						
	Cotinine	Cotinine	Nicotine	*Not Listed*	Not Associated	
	Nicotine	Nicotine	Nicotine	*Not Listed*	Not Associated	
	Nicotine Equivalents	Neq	Nicotine	*Not Listed*	Not Associated	
Tobacco-specific nitrosamines						
	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol	Total NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone	1	Limited[a]	64091-91-4
	N-nitrosonornicotine	Total NNN	N-nitrosonornicotine	1	Limited[a]	16543-55-8
Polycyclic aromatic hydrocarbons/amines						
	1-aminonaphtalene	1-NA	1-aminonaphthalene	3	*Not Listed*	134-32-7
	2-aminonaphthalene	2-NA	2-aminonaphthalene	1	Strong	**91-59-8**
	4-aminobiphenyl	4-ABP	4-Aminobiphenyl	1	Strong	**92-67-1**
	3-Aminobiphenyl	3-ABP	3-Aminobiphenyl	*Not Listed*	*Not Listed*	2243-47-2
	3-hydroxy(a)benzopyrene	3-OH-B[a]P	benzo[a]pyrene	1	Strong	**50-32-8**
	benzo[a]pyrene	B[a]P	benzo[a]pyrene	1	Strong	**50-32-8**
	Hydroxyfluorene	2-OHF	fluorene	3	Strong[b]	86-73-7
	1-Naphthol	1-Naphthol	Naphthalene	2B	Strong[b]	91-20-3
	2-Naphthol	2-Naphthol	Naphthalene	2B	Strong[b]	91-20-3
	1-/9-Hydroxyphenanthrene	1-/9-OHPh	Phenanthrene	3	Strong[b]	
	2-/3-Hydroxyphenanthrene	2-/3-OHPh	Phenanthrene	3	Strong[b]	
	1-hydroxypyrene	1-OHP	Pyrene	3	Strong[b]	129-00-0
Volatile organic compounds						
	1,2-Dihydroxybutylmercapturic acids	DHBMA	1,3-butadiene	1	Strong for Cancer, NOS	106-99-0
	monohydroxybutenyl mercapturic acid	MHBMA	1,3-butadiene	1	Strong for Cancer, NOS	106-99-0
	3-Hydroxypropylmercapturic acid	3-HPMA	Acrolein	2A	Not Associated	107-02-08
	N-Acetyl-S-(2-carbamoylethyl)cysteine	AAMA	Acrylamide	2A	Strong for Cancer, NOS	79-06-1
	N-acetyl-S-(2-hydroxy-2-carbamoylethyl)cysteine	GAMA	Acrylamide	2A	Strong for Cancer, NOS	79-06-1
	2-cyanoethylmercapturic acid	CEMA	Acrylonitrile	2B	Not Associated	107-13-1
	S-phenylmercapturic acid	S-PMA	Benzene	1	Not Associated	71-43-2
	3-hydroxy-1-methylpropylmercapturic acid	3-HMPMA	Crotonaldehyde	2B	*Not Listed*	4170-30-3
	Hydroxyl-1-methylpropyl-mercapturic acid	HMPMA	Crotonaldehyde	2B	*Not Listed*	4170-30-3
	2-hydroxyethylmercapturic acid	HEMA	Ethylene oxide	1	Not Associated	75-21-8
	o-toluidine	o-tol	o-toluidine	1	Strong	**95-53-4**
	S-benzylmercapturic acid	S-BMA	Toluene	3	Not Associated	108-88-3

Bold: linked to cancer.

IARC = International Agency for Research on Cancer. Classifications taken from Monographs on the Evaluation of Carcinogenic Risks to Humans (Group 1, Carcinogenic to humans; Group 2A, Probably carcinogenic to humans, Group 2B, Possibly carcinogenic to humans; Group 3, Not classifiable as to its carcinogenicity to humans; Group 4, Probably not carcinogenic to humans).

CHE = Collaborative on Health and the Environment Toxicant and Disease Database.

IARC classification and CHE link to bladder cancer are recorded for the parent compounds.

Link to bladder cancer classified by strength of evidence (strong evidence, good evidence, limited evidence).

[a] Reflects CHE's "limited" association between nitrosamines and bladder cancer.

[b] Reflects CHE's "strong" association between PAHs and bladder cancer.

Table 2
Urinary Biomarkers of Exposure in HTP and Combustible Cigarette Users (Ratio to Urinary Creatinine)

	Tran 2020		Lüdicke 2017		Lüdicke 2018				Haziza 2017		Haziza 2016		Haziza 2020				Bosilkovska 2020			
Measurement Parameters																				
Timing of Urine Collection (Days Since Randomization)	5		5		5		90		5		5		5		90		5		90	
Setting	Confinement		Confinement		Confinement		Ambulatory		Confinement		Confinement		Confinement		Ambulatory		Confinement		Ambulatory	
Tobacco Product	Carbon HTP	CC	Electric HTP	CC	Electric HTP	CC	Electric HTP	CC	Electric HTP	CC	Electric HTP	CC	Electric HTP	CC	Electric HTP	CC	Carbon HTP	CC	Carbon HTP	CC
Nicotine																				
Cotinine (ng/mL)									239.99 (211.3; 272.58)	219.73 (190.21; 253.83)	161 (131.19; 197.57)	164.3 (130.93; 206.17)								
Nicotine (ng/mL)									20.74 (17.46; 24.62)	19.01 (16.52; 21.87)	19.13 (15.6; 23.46)	21.34 (18.56; 24.55)								
Neq (mg/g creat)	11.56 (9.6; 13.93)	10.82 (9.47; 12.35)	11.12 (8.96; 13.8)	13.47 (11.5; 15.77)	6.16 (5.55; 6.83)	5.22 (4.35; 6.27)	6.85 (5.96; 7.88)	6.33 (5.11; 7.84)	10.6 (9.34; 12.04)	9.76 (8.54; 11.15)	5.44 (4.61; 6.41)	5.52 (4.58; 6.66)	6.74 (5.76; 7.89)	8.55 (7.18; 10.18)	6.52 (5.24; 8.11)	7.4 (5.81; 9.43)	10.63 (9.25; 12.23)	8.78 (6.88; 11.23)	8.53 (7.31; 9.96)	8.53 (6.59; 11.04)
Tobacco-specific nitrosamines																				
Total NNAL (pg/mg creat)	74.7 (59.9; 93.17)	117.82 (91.61; 151.53)	55.9 (36.95; 84.56)	186.8 (138.51; 251.91)	37.9 (32.29; 44.48)	85.94 (70.93; 104.13)	23.23 (19.34; 27.91)	95.03 (77.31; 116.82)	49.65 (42.47; 58.05)	107.04 (85.9; 133.37)	37.77 (31.43; 45.38)	76.55 (59.76; 98.04)	57.04 (46.17; 70.48)	120.29 (82.09; 176.26)	47.53 (34.8; 64.91)	152.11 (108.38; 213.47)	63.3 (51.3; 78)	134.5 (91.7; 197.3)	39.7 (29.3; 53.7)	169.7 (117.5; 245)
Total NNN (pg/mg creat)	2.13 (1.73; 2.63)	6.12 (4.87; 7.7)	0.806 (0.61; 1.06)	6.45 (4.76; 8.73)	1.2 (0.97; 1.49)	4.1 (2.94; 5.73)	1.4 (1.13; 1.73)	4.28 (3.03; 6.05)	1.55 (1.17; 2.05)	5.99 (4.94; 7.26)	1.31 (1.06; 1.61)	4.64 (3.51; 6.12)	0.9 (0.71; 1.13)	6.14 (4.42; 8.53)	0.94 (0.72; 1.23)	4.47 (3.24; 6.17)	1.96 (1.57; 2.45)	5.86 (3.74; 9.18)	1.49 (1.15; 1.95)	6.42 (4.24; 9.71)
Polycyclic aromatic hydrocarbons/amines																				
1-NA (pg/mg creat)	3.44 (2.82; 4.2)	115.76 (100.49; 133.35)			3.14 (2.85; 3.46)	53.27 (45.86; 61.89)	3.55 (2.96; 4.26)	55.34 (46.21; 66.26)	3.3 (2.89; 3.78)	89.37 (77.81; 102.64)	2.47 (2.23; 2.72)	57.08 (48.55; 67.11)	2.51 (2.18; 2.89)	63.05 (51.83; 76.71)	9.64 (7.31; 12.72)	59.69 (47.34; 75.24)	3.76 (3.21; 4.41)	69.92 (46.35; 105.5)	7.48 (5.77; 9.69)	101.1 (80.01; 127.9)
2-NA (pg/mg creat)	3.15 (2.7; 3.69)	29.37 (25.36; 34.02)	10.4 (8.26; 13.09)	99.72 (81.94; 121.37)	1.97 (1.8; 2.15)	14.23 (12.18; 16.62)	2.34 (2.11; 2.59)	14.84 (12.63; 17.44)	2.96 (2.67; 3.28)	25.32 (22.27; 28.79)	2.33 (2.1; 2.59)	13.38 (10.93; 16.37)	2.1 (1.85; 2.39)	16.28 (13.08; 20.26)	3.21 (2.57; 4)	17.29 (13.62; 21.95)	3.01 (2.69; 3.36)	24.16 (18.96; 30.77)	3.73 (3.14; 4.42)	25.73 (20.44; 32.38)
4-ABP (pg/mg creat)	3.71 (3.28; 4.18)	15.91 (13.79; 18.34)	9.88 (8.01; 12.18)	24.67 (18.74; 32.48)	1.97 (1.76; 2.21)	9.5 (8.15; 11.07)	2.07 (1.82; 2.36)	9.62 (8.12; 11.39)	1.9 (1.7; 2.12)	12.58 (11.03; 14.34)	1.53 (1.37; 1.7)	8.57 (7.11; 10.34)	1.76 (1.5; 2.08)	9.63 (7.76; 11.95)	3.77 (2.88; 4.93)	11.31 (8.75; 14.61)	1.95 (1.72; 2.21)	13.06 (10.14; 16.82)	4.17 (3.51; 4.94)	17.43 (13.43; 22.63)
3-OH-B[a]P (fg/mg creat)	33.23 (27.37; 40.35)	116.77 (95.09; 143.4)			20.72 (18.61; 23.07)	75.1 (62.6; 90.08)	30.02 (25.29; 35.65)	86.92 (71.78; 105.27)	37.07 (33.25; 41.32)	130.29 (110.17; 154.07)	29.52 (26.01; 33.5)	96.42 (80.55; 115.41)					38.5 (33.5; 44.3)	107.6 (81.9; 141.3)	58.6 (46.6; 73.7)	136.7 (106.9; 174.7)
B[a]P (fg/mg creat)													33.44 (29.29; 38.16)	107.09 (82.19; 139.54)	61.27 (48.6; 77.25)	116.04 (88.25; 152.58)				
1-OHP (pg/mg creat)	106.33 (93.18; 121.33)	199.12 (171.73; 230.88)	85.81 (73.65; 99.96)	187.84 (155.69; 226.62)	46.36 (41.68; 51.55)	122.9 (104.71; 144.26)	85.47 (76.64; 95.33)	167.38 (146.23; 191.58)	81.22 (74.82; 88.16)	182.85 (161.24; 207.37)	73.02 (65.19; 81.79)	149.62 (132.68; 168.72)	64.87 (57.55; 73.12)	135.14 (111.12; 164.35)	117.77 (98.44; 140.89)	163.8 (132.71; 202.16)	114.5 (103.5; 126.7)	185.2 (152.1; 225.6)	166.2 (143.4; 192.5)	242.3 (203.2; 289.1)
Volatile organic compounds																				
MHBMA (pg/mg creat)	339.73 (301.82; 382.42)	1840.61 (1275.38; 2656.32)	352 (260; 470)	3233 (2310; 4510)	81.71 (75.52; 88.41)	622.58 (454.6; 852.64)	141.74 (120.62; 166.57)	785.27 (576.82; 1069.04)	192.93 (174.9; 212.83)	2399.4 (1884.6; 3054.83)	107.39 (97.24; 118.6)	450.19 (300.07; 675.42)	113.01 (99.03; 128.96)	760.36 (457.27; 1264.34)	260.98 (205.28; 331.79)	1040.71 (677.79; 1597.94)	283 (255; 314)	1988 (1401; 2821)	420 (365; 483)	2552 (1802; 3612)
3-HPMA (ng/mg creat)	494.7 (417.53; 586.12)	1187.97 (1026.63; 1374.65)	327.31 (288.4; 371.46)	1227.45 (1023.62; 1471.86)	304.68 (284.63; 326.14)	591.33 (507.72; 688.69)	386.37 (356.3; 418.97)	695.58 (602.43; 803.13)	402.26 (366.55; 441.45)	931.01 (825.73; 1049.72)	311.08 (279.59; 346.12)	599.67 (511.7; 702.76)	283.88 (259.8; 310.2)	655.19 (530.57; 809.08)	314.05 (281.51; 350.34)	606.1 (468.27; 784.48)	612.2 (545.9; 686.4)	1036 (855.1; 1255)	378.2 (334.6; 427.6)	966 (786.4; 1187)
CEMA (ng/mg creat)	20.78 (16.66; 25.92)	143.18 (122.59; 167.22)	18.15 (13.5; 24.38)[a]	149.8 (121.83; 184.19)[a]	12.43 (11.12; 13.9)	68.17 (56.39; 82.4)	7.91 (6.74; 9.29)	83.98 (69.17; 101.95)	13.18 (11.37; 15.27)	99.48 (85.79; 115.35)	10.61 (9.17; 12.29)	54.19 (43.47; 67.55)	14.39 (12.3; 16.83)	88.4 (71.73; 108.94)	14.12 (10.16; 19.61)	87.35 (66.11; 115.41)	15 (12.6; 17.9)	95.2 (69.7; 130)	10.5 (7.74; 14.3)	124.1 (93; 165.8)
S-PMA (pg/mg creat)	361.48 (289.26; 451.74)	2898.46 (2172.62; 3866.79)	300 (210; 420)	4490 (3250; 6210)	118.36 (107.37; 130.48)	1096.47 (805.13; 1493.22)	145.58 (121.67; 174.18)	1157.25 (848.59; 1578.17)	164.45 (144.45; 187.22)	2922.81 (2362.8; 3615.54)	143.77 (126.08; 163.93)	850.02 (620.4; 1164.63)	133.64 (111.94; 159.55)	1062.05 (685.84; 1644.62)	314.02 (219.66; 448.93)	1218.56 (822.54; 1805.25)	380 (325; 444)	2493 (1749; 3553)	467 (365; 597)	2652 (1853; 3795)
3-HMPMA (ng/mg creat)					124.47 (115.36; 134.3)	286.8 (251.37; 327.21)	154.3 (137.07; 173.7)	299.41 (260.62; 343.97)	86.65 (80.31; 93.49)	376.78 (329.54; 430.8)										
HMPMA (ng/mg creat)	125.75 (110.46; 143.16)	484.18 (420.56; 557.41)									59.51 (53.4; 66.3)	157.83 (128.07; 194.51)	95.35 (82.67; 109.98)	270.99 (216.76; 338.79)	105.12 (86.68; 127.47)	215.2 (167.61; 276.3)	78.8 (71.5; 86.8)	231.4 (183.4; 292)	99.1 (88.7; 110.7)	247.6 (198.9; 308.3)
HEMA (pg/mg creat)	1146.84 (913.06; 1440.48)	3094.7 (2477.69; 3865.35)			1137.96 (995.5; 1300.81)	2235.37 (1742.88; 2867.03)	1741.53 (1510.19; 2008.3)	3739.46 (2858.39; 4892.12)	1342.4 (1140.44; 1580.12)	4504 (3506.73; 5784.88)	997.76 (866.57; 1148.82)	2099.41 (1614.3; 2730.24)	1145.34 (951.15; 1379.18)	2903.31 (2163.19; 3896.66)	1481.32 (1193.81; 1838.07)	3265.62 (2275.3; 4686.97)	1382 (1175; 1625)	3140 (2456; 4014)	1555 (1306; 1851)	3817 (2890; 5042)
o-tol (pg/mg creat)	49.26 (43.59; 55.67)	175.4 (156.97; 195.99)	157.82 (129.47; 192.38)	284.16 (247.83; 325.8)	51.64 (45.52; 58.59)	127.28 (103.27; 156.88)	68.35 (53.91; 86.67)	125.64 (96.13; 164.2)	51.15 (46.1; 56.75)	121.16 (105.07; 139.71)	50.4 (44.64; 56.91)	98.18 (82.69; 116.57)	42.2 (37.79; 47.13)	92.68 (77.8; 110.39)	47.53 (39; 57.92)	107.39 (84.14; 137.07)	59.7 (50.8; 70.3)	125.8 (99.5; 159.2)	56.6 (46.5; 69)	159.7 (128.4; 198.7)
S-BMA (pg/mg creat)											2098.09 (1833.19; 2401.26)	2354.17 (1968.1; 2816.02)								

Mean (95% CI).
See Table 1 for abbreviations.
[a] Listed as ng/g in paper, but it appears they intended ng/mg.

Table 3
Urinary Biomarkers of Exposure in HTP and Combustible Cigarette Users (Amount Excreted/24 hours)

Ogden 2015		Lüdicke 2016		Gale 2019					Dooloittle 1989 - Group 1			Dooloittle 1989 - Group 2		
84	168	5/6		7					Chronic (Baseline)	5–6[e]	12–14[e]	Chronic (Baseline)	5–6[e]	12–14[e]
Ambulatory	Ambulatory	Confinement		Confinement					Ambulatory			Ambulatory		
Carbon HTP	Carbon HTP	Carbon HTP	CC	Electric HTP[b]	Electric HTP[c]	Electric HTP[d]	CC	Menthol CC	CC	Carbon HTP		CC	Carbon HTP	
		319.8 (109.7)[a]	289.8 (76.4)[a]						2804 (1698)	2049 (1421)	2337 (1397)	2914 (2049)	1619 (1243)	2070 (1556)
24.5 (19.7; 29.3)	24 (19.4; 28.6)	19.1 (7.5)	17.2 (5)	6.15	5.75	7.58	8.33	9.77						
									2531 (1916)	1611 (1373)	1917 (1691)	2900 (2003)	1404 (1274)	1910 (2048)
502 (403; 600)	485 (398; 572)	145.3 (166.1)	279 (148)	128.63	149.38	80.35	197.85	167.02						
				5.85	5.57	1.06	15.36	9.62						
14.9 (11.4; 18.5)	15.5 (12.5; 18.4)	6.4 (8.9)	34 (16.3)	1.74	1.92	1.72	17.8	17.65						
12 (8.82; 15.3)	11.2 (9.08; 13.4)	3.4 (1.6)	21.4 (11.5)	2.45	2.31	2.25	10.86	10.44						
4.78 (3.68; 5.87)	6.07 (4.7; 7.45)													
2.52 (2.02; 3.02)	2.31 (1.9; 2.73)													
34.6 (25.4; 43.8)	41.9 (26.5; 57.3)													
19 (14.9; 23.2)	17.8 (15; 20.5)													
0.69 (0.58; 0.81)	0.7 (0.6; 0.8)													
0.56 (0.47; 0.64)	0.59 (0.5; 0.67)													
865 (536; 1195)	663 (553; 773)	247.5 (113)	434.8 (162.1)	75.58	63.46	50.18	172.86	195.19						
836 (716; 956)	840 (726; 955)													
3.23 (2.12; 4.33)	3.76 (2.7; 4.81)	0.8 (0.3)	7.9 (4.4)	49.87	98.4	118.38	0.77064	1.01018						
2.97 (2.35; 3.59)	2.8 (2.26; 3.34)	0.5 (0.2)	1.9 (0.8)	0.568	0.656	0.639	1.448	1.422						
232 (196; 267)	239 (203; 275)			91.75	88.82	65.76	111.65	114.96						
38.3 (31.7; 45)	43.4 (35.2; 51.7)			15.68	15.36	13.75	17.24	16.4						
				17.84	21.03	16.54	159.04	165.62						
		1.0 (0.4)	6.4 (3.1)				2.25	2.81						
6.34 (5.25; 7.43)	6.44 (5.45; 7.42)			79	73.23	79.63	0.3855	0.36245						
				2.46	2.84	2.6	5.08	7.13						
183 (156; 209)	196 (167; 224)	114 (144.9)	232.1 (77.6)	58.52	39.39	54.81	153.21	119.04						
6.36 (4.45; 8.26)	6.82 (5.05; 8.58)			0.2	0.2	0.19								

Mean (95% CI) or Mean (SD).
See Table 1 for abbreviations.
[a] Reported in ng/mL.
[b] Non-menthol "glo" HTP.
[c] Menthol "glo" HTP.
[d] "IQOS" HTP.
[e] Days since starting HTP.

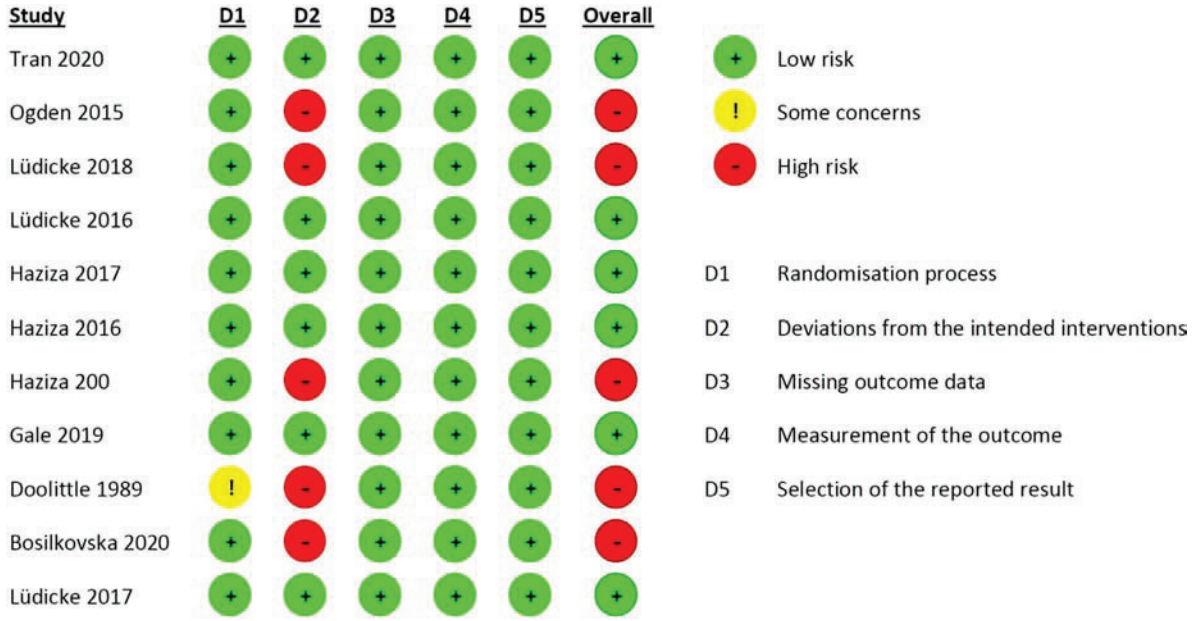

Fig. 2. Cochrane collaboration risk-of-bias.

of the biomarker excreted in 24 hours (e.g., ng/24 hours), or as biomarker level as a ratio to excreted creatinine (e.g., pg/mg Cr). These 2 data types are tabulated separately (Tables 2 and 3).

Users of HTPs were generally reported to have statistically significantly lower biomarkers of exposure (BoE) levels compared to the combustible cigarette arms of their respective studies, though levels of nicotine, as measured by nicotine equivalents, were statistically similar between HTP users and combustible cigarette users within each study (Fig. 3). The mean levels of NNAL reported in HTP users were 36.6% to 76.6% lower than those seen in their CC smoking counterparts, NNN was 42.1% to 93.1% lower, 2-NA was 81.2% to 90.3% lower, 4-ABP was 60.0% to 85.1% lower, 3-OH-B[a]P was 57.1% to 72.4% lower, B[a]P was 47.2% to 68.8% lower, 1-OHP was 28.1% to 71.0% lower, and o-tol was 44.5% to 71.9% lower. S-BMA [14], NNAL [20], and 1-OHP [13] were lower in HTP users than cigarette smokers by a statistically insignificant margin in 1 study each, though they were statistically significantly lower in all the other studies in which they were measured. No urinary biomarkers of exposure for known bladder carcinogens were shown to be higher in HTP users than in combustible cigarette users in any of the studies we reviewed.

In contrast to all other studies here reviewed, Ogden et al. [19] did not include a standard reference combustible cigarette smoking arm to their study. However, after switching to the "Eclipse" carbon HTP, no statistically significant differences were observed in 7 of 19 of subjects' reported biomarkers compared to their baseline levels (all subjects were combustible cigarette smokers prior to the study). These biomarkers included 1-Naphthol, 1-OHP, and 2-/3-OHPh, which are biomarkers for known bladder carcinogens.

A single study [19] included an arm of never-smokers. Compared to the never smoker arm, the HTP users in that study had higher levels of 17 of the 19 measured urinary biomarkers of exposure after 24 weeks of use. Nine of the biomarkers they measured have known associations with bladder cancer, and they were present at 1.9 to 13.0 times the levels seen in the never-smoking group.

3.4. Study quality and bias

Risk of methodological bias, as assessed by the ROB-2 tool, was variable between studies, and is shown in Fig. 2. One study [21] did not describe their randomization process and noted that the subjects work in the same office. Five studies [11,13,18,19,21] had high risk of bias in the "deviations from the intended interventions" domain, due to non-adherence during ambulatory periods of HTP use and their choice to only report per-protocol biomarker levels. Importantly, however, the use of combustible cigarettes in the HTP arms of these studies would likely tend to increase the level of the urinary biomarkers they report. None of these studies reported an analysis of the relationship of adherence to urinary biomarker levels. Other domains of methodological bias were deemed to be low risk across all studies.

While the ROB-2 tool assesses for methodological bias, it does not incorporate an assessment of conflicts of interest. All of the studies we review here have a risk of bias arising from conflicts of interest as the authors in each of the studies were employees of the companies that produce the products they were studying, and those companies also provided study funding.

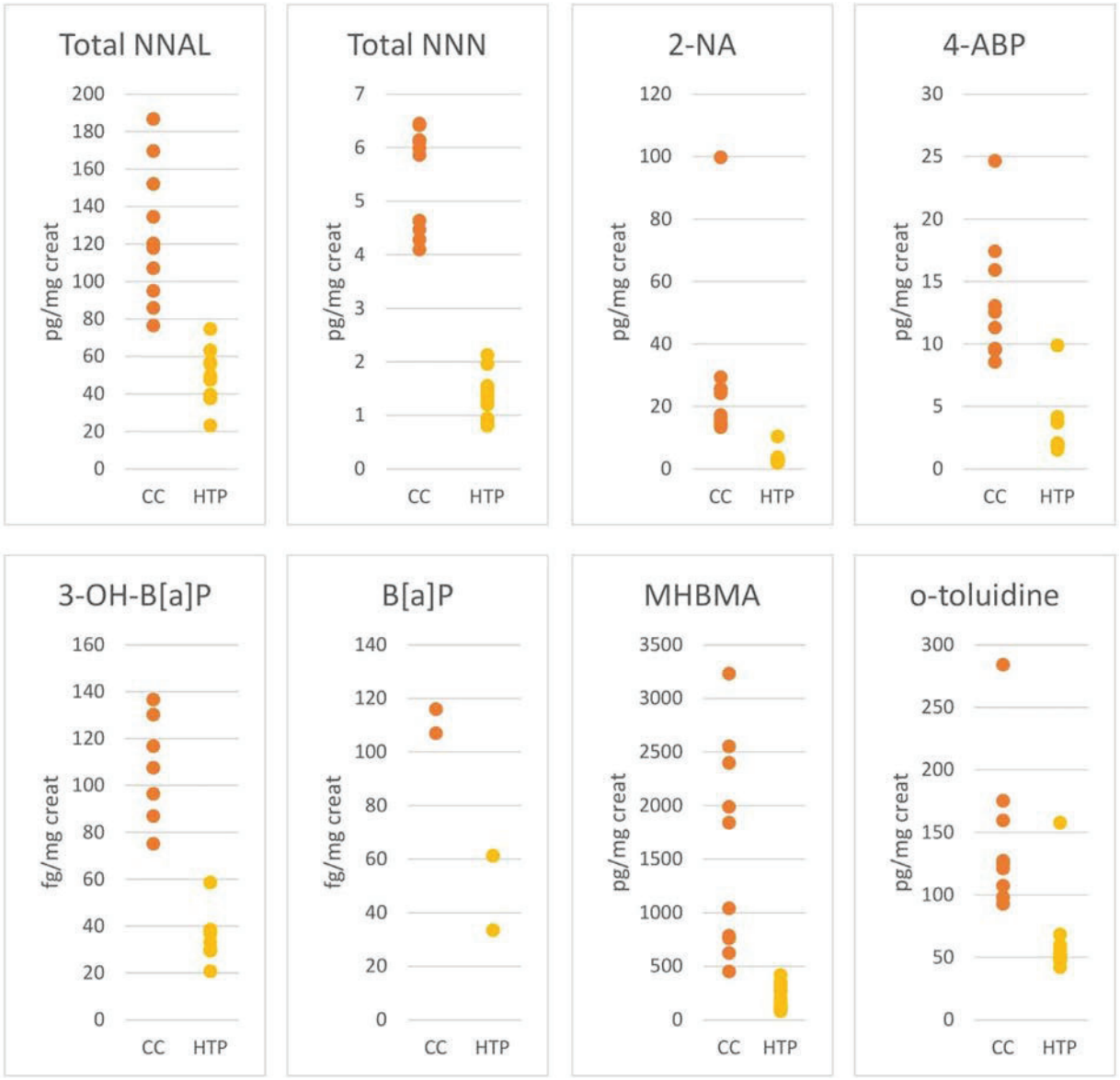

Fig. 3. Biomarkers of exposure to known bladder carcinogens in HTP users vs. combustible cigarette users. CC = Combustible Cigarette users (shown in orange); HTP = Heated Tobacco Product users (shown in yellow) (color version of figure is available online).

See Table 1 for biomarker abbreviations.

4. Discussion

The primary aim of this study was to identify BoE that are present in the urine of HTP users. We were able to identify 29 BoE demonstrating HTP users' exposure to 21 parent compounds, of which 14 are carcinogenic (IARC groups 1, 2A and 2B). These toxicants are all strongly associated with conventional cigarette use, though may be present in small quantities in non-smokers due to environmental sources. Many of the studies in the present review attempted to control environmental exposures, diet, and protocol compliance by conducting all or part of the investigation in a confinement setting. All the ambulatory studies in this review that reported biomarkers of carcinogenic compounds used per-protocol data analysis to control for any use of combustible cigarettes by subjects in the HTP arms of their studies.

Variations in user behavior could have an impact on BoE levels among HTP users. For example, in subjects using carbon HTPs, puffing topography affects the temperature of the tobacco with faster puffing leading to higher tobacco temperatures and likely causing an increase in toxicant exposure. Electric HTPs, in contrast, control the temperature of the tobacco independently of user puffing behavior, and should therefore theoretically have a more stable temperature profile and a more predictable toxicant exposure profile. None of the studies we reviewed controlled for puffing topography.

The presence of BoE to carcinogenic compounds in HTP users' urine indicates the possibility that these devices may

increase users' risk of bladder cancer. However, no long-term studies have yet been conducted to definitively establish what level of exposure correlates to what level of cancer risk over years to decades. These products have not been in widespread use long enough for such data to be available. The FDA's 2020 decision to approve marketing of PMI's "IQOS" HTP as a "reduced exposure" product but not a "reduced risk" product reflects this reality. They remark that "available evidence is insufficient to demonstrate that the product, as actually used by consumers, will significantly reduce harm and risk to individual users and benefit the health of the population as a whole" [22]. It is difficult, when assessing carcinogen exposures, to determine a level that is "safe" as the presence of any amount of a carcinogen entails a risk of cancer.

It should also be noted that the 29 BoE reported in this review do not reflect an exhaustive list of the toxicants to which HTP users are exposed. The studies reviewed here focused on comparing HTP users and cigarette smokers, and therefore reported levels of toxicants that were present in both groups. However, given that the composition of HTP emissions differs from cigarette smoke, it is to be expected that their toxicant profile will also be unique. The FDA noted this in their review of PMI's "IQOS" MRTP application. They reveal that, while the harmful and potentially harmful constituents (HPHCs) related to tobacco use were generally lower in IQOS aerosol compared to cigarette smoke, there were 80 non-HPHC compounds present in the IQOS aerosol that are either not present in the smoke of a reference cigarette, or are present at higher levels in the IQOS aerosol [22].

4.1. Biomarkers associated with bladder cancer

The second aim of this review was to determine which of the biomarkers we identified reflect exposure to compounds that have been linked to bladder cancer. We identified 8 compounds with a "strong" and 2 with a "limited" association with the development of bladder cancer. The concentration of these carcinogenic compounds in the urine and the period of direct urothelial exposure to this urine prior to micturition provide a causative mechanism for bladder carcinogenesis. It is also possible that there may be other compounds not identified in this review to which HTP users are exposed that could have associations with bladder cancer. Especially considering the strong relationship between combustible cigarette use and bladder cancer [1,10], further longitudinal research is needed to determine if HTP users are also at an increased risk of developing bladder cancer.

4.2. Biomarkers in HTP users compared to combustible cigarette users

The third aim of this review was to compare biomarker levels between HTP users and combustible cigarette users, where appropriate. Given the heterogeneity in chemical assays utilized, data types reported, and differences in study design, direct comparisons were limited. Additionally, there was a high variability in the analytical method employed to determine the levels of urinary biomarkers, which were not standardized across studies. This may contribute to the variability in the levels of the detected biomarkers and measurement bias between studies. However, some general patterns were observed in the data.

With the few exceptions noted in the Results section above, levels of biomarkers for carcinogenic compounds were lower by a statistically significant margin in HTP users compared to CC users within each study. There was some overlap in the ranges of biomarkers reported in CC users and HTP users when comparing across studies, suggesting that the levels of BOE in HTP users are of concern, although this may be due to the heterogeneity of the study methods rather than true reflection of relative toxicant levels.

It seems probable that higher levels of carcinogens, reflected by higher BoE levels, would correlate with a larger potential risk of developing bladder cancer. However, while a dose-response relationship between cigarette smoking and bladder cancer has been described [1], we were unable to identify any studies establishing such a relationship for individual carcinogens and bladder cancer. It also remains unknown if there is a minimum toxic dose or "safe" level for these toxicants, and it seems likely that bladder cancer risk will be a factor of both the carcinogen level and the length of the exposure.

It is not clear how representative the data we reviewed here are of real-world HTP use. This can be seen in the biomarker levels reported by Lüdicke et al., whose study did not meet inclusion criteria for our review [23]. All ambulatory studies that met inclusion criteria used per-protocol statistical analysis, removing data from analysis for study participants who used significant amounts of combustible cigarettes during the HTP study period. Conversely, the large, ambulatory study reported by Lüdicke et al. recorded biomarkers from their "predominant HTP use" arm as long as the subjects used at least 70% HTP during the study period. Subjects within this arm used an average of 16.5 ± 8.9 HTP tobacco sticks and 2.0 ± 2.4 cigarettes and were reported to have mean biomarkers levels at 6 months that ranged from 48.6% (CEMA) to 16.2% (1-OHP) lower than the combustible cigarette arm of the study—much more modest differences than those seen in the studies we reviewed here. In the "dual use" arm of the study, where subjects used similar numbers of HTP sticks and cigarettes, there was no significant difference in any of the urinary biomarkers measured at 6 months when compared to exclusive CC users. This study may be representative of the urinary biomarkers one would expect to be present in the typical HTP user, who is likely to use at least a small amount of combustible tobacco in addition to their HTP use.

4.3. Implications for urology practice and directions for future research

Urologists should be aware that HTPs are likely to grow in popularity among our patients in the coming years, and they should therefore become comfortable discussing the urologic impact of this type of tobacco use. It is reasonable to counsel patients considering HTP use that they will be exposed to known bladder carcinogens as well as compounds whose biological effects have not yet been clearly determined. Patients should also be counseled that, while exposure to many carcinogens appears to be lower in HTP users compared to combustible cigarette smokers, it is too soon to determine how they compare in terms of overall risk, including risk of bladder cancer. As always, the urologist should promote abstaining from tobacco use altogether rather than switching to alternative products [24,25].

Further study is needed to determine how the long-term use of these HTPs affects bladder cancer risk, as well as how using these products may change the efficacy of and risks associated with bladder cancer treatment. For example, previous research has demonstrated that active smokers have significantly higher mortality, cancer-specific mortality, and cancer recurrence after radical cystectomy [26] and smoking is also adversely associated with pathological response to neoadjuvant chemotherapy [27,28] — it remains to be seen if such effects may extend to HTP use. Further research should also focus on identifying toxicants that may be unique to HTP use and associations that they may have with bladder carcinogenesis, as well as clarifying how the level of bladder carcinogens in HTP users compares to never-smokers. It will be important that future research into these products be conducted by parties without conflicts of interest, as all the studies that this review identified were conducted by the same companies that sell these products.

5. Conclusion

Carcinogenic biomarkers are present in the urine of heated tobacco product (HTP) users, including several compounds that have strong associations with bladder cancer. The long-term implications of this exposure have not yet been determined, but the presence of these carcinogens in the urine raises the possibility of an association between heated tobacco product use and the development of bladder cancer. The level of carcinogen exposure in HTP users appears to be lower than combustible cigarette smokers, but significantly higher than never-smokers. Longitudinal studies of HTP users are therefore needed to assess the clinical consequences of their carcinogen exposure and the presence of carcinogens warrants a cautious approach to these products.

Conflict of interest

None of the authors have any conflicts of interest of disclosures.

Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.urolonc.2021.11.018.

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