Toxicology Reports 8 (2021) 282–294

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Systematic review of biomarker findings from clinical studies of electronic cigarettes and heated tobacco products

Yukio Akiyama [a,1,*], Neil Sherwood [b,1]

[a] Department of Environmental Management, University of Occupational and Environmental Health, Kitakyushu, Japan
[b] Neil Sherwood Consulting, Nyon, Switzerland

ARTICLE INFO

Edited by: DR. A.M Tsatsaka

Keywords:
Clinical study
Electronic cigarette
Heated tobacco products
Biomarkers of tobacco smoke exposure (BOE)
Biomarkers of biological effect (BOBE)

ABSTRACT

Background: Worldwide adoption of electronic cigarettes (e-cigarettes) and heated tobacco products (HTPs) has increased exponentially over the past decade. These products have been proposed as non-combustible alternatives to traditional tobacco products such as cigarettes and may thus reduce the negative health consequences associated with tobacco smoke. However, the overall health impact and safety of using these products remains unclear. This review seeks to provide an updated 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.
Methods: A systematic review was conducted through major databases (Medline/PubMed, Scopus, EMBASE) searching for articles directly comparing biomarker levels in humans using e-cigarettes or HTPs and those using combustible cigarettes. We included peer reviewed articles with comparative or longitudinal design and extracted key information for our purpose (type of population, demographics, biomarkers measurements, and health effects). An initial qualitative analysis was performed followed by a summary of findings.
Results: A total of 44 studies were included from initial citations. The vast majority of the literature reported reductions in levels of biomarkers of tobacco smoke exposure (BOE), especially nicotine, MHBMA, 3-HPMA, S-PMA, 1−OHP and NNAL, when using e-cigarettes and HTPs compared to combustible cigarettes. There was a slight tendency toward a larger reduction in these biomarkers levels with the use of e-cigarettes, although direct comparisons between e-cigarettes and HTPs were lacking. There was also a trend toward positive changes in levels of biomarkers of biological effect (BOBE) with the use of e-cigarettes and HTPs.
Conclusions: A comparison of levels of biomarkers of tobacco-related exposure collected in clinical studies revealed that the use of e-cigarettes and HTPs could lead to a significant reduction in exposure to harmful substances compared to combusted cigarettes. In tandem, the health status of e-cigarettes and HTP users, indexed by levels of biomarkers of biological effect showed potential for improvement compared to smoking. However, larger and longer-term population-based studies are needed to further clarify these findings.

1. Introduction

Non-combustible forms of tobacco use, such as electronic cigarettes (e-cigarettes) and heated tobacco products (HTPs) have been emerging and gaining attention in several countries. These products have been proposed as potentially less-risky alternatives to traditional combusted tobacco products such as cigarettes on the basis of reported improvements in levels of biomarkers of tobacco smoke exposure and biological effect, but the long term health impact of these products is still unknown [1]. Because of their worldwide propagation but unclear safety [2],

Abbreviations: BAT, British American Tobacco; BOBE, biomarkers of biological effect; BOE, biomarkers of tobacco smoke exposure; CHTP, Carbon-Heated Tobacco Product; E-cigarettes, electronic cigarettes; EHCSS, Electrically Heated Cigarette Smoking System; EVPs, electronic vapor products; FV, Fontem Ventures; HC, heated cigarette; HTPs, heated tobacco products; JT, Japan Tobacco; mTHS, Menthol Tobacco Heating System; NOS scale, The Newcastle-Ottawa Scale; NSPS, nicotine-salt pod system; NTV, Novel Tobacco vapor products; PMI, Philip Morris International; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RAI, Reynolds American Inc; RCT, randomized controlled trial; RJR, R.J. Reynolds Tobacco Company; RJRVC, R.J. Reynolds Vapor Company; RTP, reduced-toxicant-prototype cigarette; THP, tobacco heating product; THS, Tobacco Heating System; UCS, Uncontrolled smoking conditions; WHO, World Health Organization.

* Corresponding author at: 3-3-41-1202 Orio, Yahatanishi-ku, Kitakyushu, 807-0825, Japan.
E-mail address: y.akiyama55@gmail.com (Y. Akiyama).

[1] Two authors contributed equally to the study.

https://doi.org/10.1016/j.toxrep.2021.01.014
Received 20 October 2020; Received in revised form 21 January 2021; Accepted 21 January 2021
Available online 27 January 2021

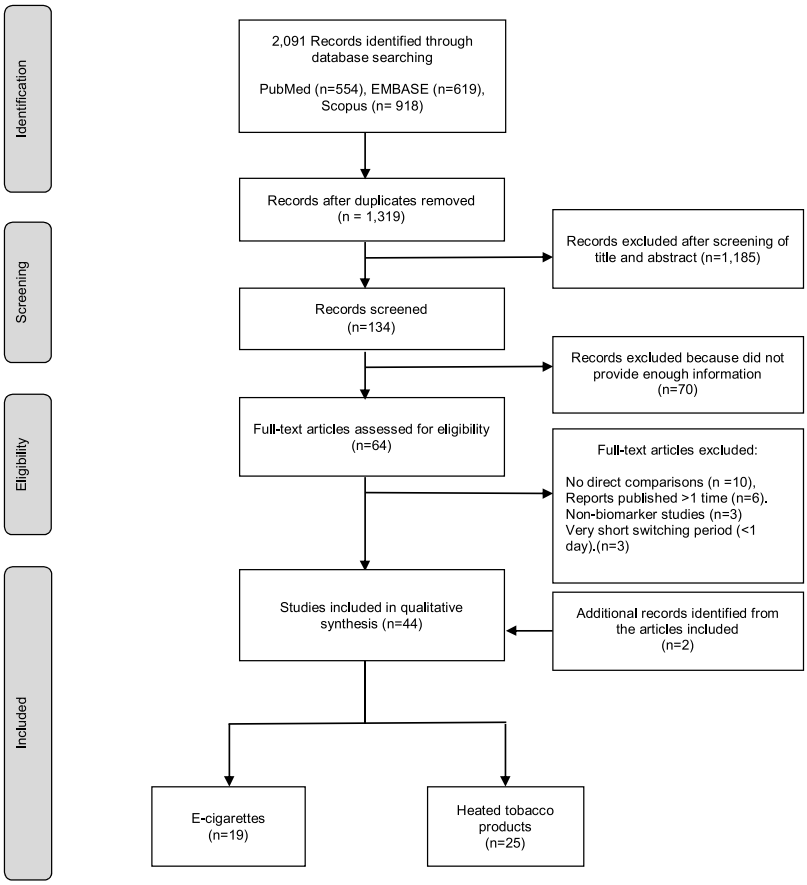

Fig. 1. PRISMA flow chart of the selection of studies.

healthcare authorities have raised various opinions as to the potential health consequences associated with their use and some international institutions have cautioned the need to continuously survey potential adverse events [3]. For example, the World Health Organization (WHO) has aimed to evaluate the health-risks of e-cigarettes [4] and HTPs [5], and proposed strategies to balance their benefits and risks [4,5]. However, to date there has not been any agreement between international healthcare authorities which could expedite a general consensus [1].

Although there are a few epidemiological studies underway examining the long-term impact of e-cigarettes and HTPs on disease endpoints, there are many short-term clinical studies of biomarkers of tobacco smoke exposure (BOE) and biological effect (BOBE) and some systematic literature reviews which have summarized such study results [6,7], including a meta-analysis of BOEs [8] found during the use of HTPs. However, these reviews and meta-analyses have considered the results of either e-cigarettes or HTPs separately, and did not consistently address the results of clinical studies on biomarkers of biological effect (BOBE) that many consider to lie on the pathway to smoking-related diseases.

In the light of this heterogenous evidence, and to examine suggestions that e-cigarettes and HTPs can serve as less-risky alternatives to conventional tobacco products, we aimed to survey and summarize differences in both BOE and BOBE during use of either e-cigarettes or HTPs compared to the use of conventional tobacco products such as cigarettes.

2. Methods

This is a systematic review conducted in accordance with recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [9].

2.1. Search strategy and selection criteria

We conducted a systematic review of the literature within three main electronic databases (Medline/PubMed, Scopus, EMBASE) to identify all articles comparing biomarkers between human beings exposed to e-cigarettes / HTPs and smoking. Literature search was conducted using the electronic search strategy: [(“e-cigarette” OR “electronic cigarette” OR “e-vapor”) AND ("biomarker" OR "trial")] OR [("heated tobacco" OR "heat not burn" OR "heat-not-burn" OR "tobacco heating" OR "IQOS" OR "Ploom" OR "glo" OR "novel tobacco ") AND ("biomarker" OR "trial")] from inception until April 15 of 2020 and was restricted to peer reviewed articles published in English. The search strategy was translated in accordance to the other database Boolean operators. We also searched cross-references to complement the evidence given in this review. The main types of studies included were randomized trials, case-control studies, and cohort studies. Design of the studies could be either comparative (e-cigarettes/HTPs users, smokers, non-smokers/past smokers) or longitudinal with a switch from smoking to e-cigarettes or HTPs. Publications were excluded if they were conducted *in vitro* or *in vivo*, written in languages other than English or not peer reviewed.

2.2. Data extraction

The title and abstract were screened by two reviewers independently to confirm the inclusion criteria. The full text of the selected articles was retrieved, and each reference list was screened to identify additional publications on this topic. Any discrepancies in the selected studies were solved by a third reviewer. Selected articles were stratified into two groups: (1) studies comparing biomarkers of exposure between e-cigarettes/HTPs and conventional smoking, (2) studies comparing

Table 1
Studies included in the review.

Authors, year of publication [Reference]	Affiliation	Study location	Study design	Product Name (Reference product)	Intervention period
HTPs RCT studies on biomarker of exposure (Table 2)					
Ludicke et al., 2016 [12]	PMI	Poland	RCT	CHTP (Cigarette)	5 days
Haziza et al., 2016 [13]	PMI	Japan	RCT	THS 2.2 (Cigarette)	5 days
Haziza et al., 2017 [14]	PMI	Poland	RCT	THS 2.2 (Cigarette)	5 days
Ludicke et al., 2017 [15]	PMI	Poland	RCT	THS 2.1 (Cigarette)	5 days
Ludicke et al., 2018b [16]	PMI	Japan	RCT	mTHS (menthol Cigarette)	5 days
	PMI	Japan	RCT	mTHS (menthol Cigarette)	90 days
Haziza et al., 2020a [17]	PMI	U.S.A.	RCT	mTHS (menthol Cigarette)	5 days
	PMI	U.S.A.	RCT	mTHS (menthol Cigarette)	90 days
Yuki et al., 2018 [18]	JT	Japan	RCT	NTV (Cigarette)	5 days
Tricker et al., 2012c [19]	PMI	Japan	RCT	EHCSS-K6m (menthol Cigarette)	6 days
Gale et al., 2019 [20]	BAT	Japan	RCT	glo™/THP1.0 (Cigarette)	6–7 days
	BAT	Japan	RCT	menthol glo™/THP1.0 (menthol Cigarette)	6–7 days
	BAT	Japan	RCT	iQOS/THS (Cigarette)	6–7 days
Roethig et al., 2007 [65]	PM USA	–	RCT	EHCSS -UCS (Cigarette)	8 days
Frost-Pineda et al., 2008a [66]	PM USA	–	RCT	EHCSS (Cigarette)	8 days
Roethig et al., 2005 [21]	PM USA	U.S.A.	RCT	EHCSS1 (Cigarette)	8 days
	PM USA	U.S.A.	RCT	EHCSS2 (Cigarette)	8 days
Tricker et al., 2012b [22]	PMI	Japan	RCT	EHCSS-K3 (Cigarette)	8 days
	PMI	Japan	RCT	EHCSS-K6 (Cigarette)	8 days
Martin Leroy et al., 2012 [23]	PMI	Poland	RCT	EHCSS-K6 (Cigarette)	8 days
Tricker et al., 2012d [24]	PMI	UK	RCT	EHCSS-K3 (Cigarette)	8 days
	PMI	UK	RCT	EHCSS-K6 (Cigarette)	8 days
Tricker et al., 2012a [25]	PMI	Korea	RCT	EHCSS-K3 (Cigarette)	8 days
Sakaguchi et al., 2014 [26]	JT	Japan	RCT	HC (Cigarette)	28 days
Frost-Pineda et al., 2008b [67]	PM USA	–	RCT	EHCSS (Cigarette)	12 weeks
Ludicke et al., 2019 [27]	PMI	U.S.A.	RCT	THS 2.2 (Cigarette)	3 months
	PMI	U.S.A.	RCT	THS 2.2 (Cigarette)	6 months
Shepperd et al., 2015 [28]	BAT	Germany	RCT	RTP (Cigarette)	6 months
Ogden et al., 2015a [29]	RAI, RJR	U.S.A.	RCT	Eclipse (Cigarette)	24 weeks
Roethig et al., 2008 [68]	PM USA	–	RCT	EHCSS (Cigarette)	postbaseline (<12 months)
E-cigarettes RCT studies on biomarker of exposure (Table 3)					
O'Connell et al., 2016 [30]	Fontem Ventures	U.S.A.	RCT	blu (Cigarette)	5 days
Round et al., 2019 [31]	RJR VC	U.S.A.	RCT	Vuse Solo (Cigarette)	5 days
	RJR VC	U.S.A.	RCT	menthol Vuse Solo (menthol Cigarette)	5 days
Jay et al., 2020 [32]	JUUL Labs	U.S.A.	RCT	JUUL NSPS (Cigarette)	5 days
Goniewicz et al., 2017 [33]	Department of Health Behavior, Roswell Park Cancer Institute	Poland	RCT	M201 Mild (Cigarette)	2 weeks
McRobbie et al., 2015 [34]	Tobacco Dependence Research Unit & UK Centre for Tobacco andAlcohol Studies,Wolfson Institute	UK	RCT	Green Smoke EC (Cigarette)	4 weeks
Pulvers et al., 2018 [35]	Department of Psychology, California State University San Marcos	U.S.A.	RCT	e-Go C (Cigarette)	4 weeks
Hatsukami et al., 2019 [36]	Department of Psychiatry, University of Minnesota	U.S.A.	RCT	Vuse Solo Blu cigarettes Fin (Cigarette)	8 weeks
Cravo et al., 2016 [37]	Fontem Ventures	UK	RCT	EVP (Cigarette)	12 weeks
Walele et al., 2018 [38]	Fontem Ventures	U.S.A.	RCT	Puritane™ (Cigarette)	24 months
E-cigarettes cross sectional studies on biomarker of exposure (Table 4)					
Shahab et al., 2017 [39]	Department of Epidemiology and Public Health, University College London	UK	Cross Sectional	E-cigarettes (Cigarette)	–
Goniewicz et al., 2018 [40]	Department of Health Behavior, Roswell Park Comprehensive Cancer Center	U.S.A.	Cross Sectional (PATH)	E-cigarettes (Cigarette)	–
Oliveri et al., 2020 [41]	Altria	U.S.A.	Cross Sectional	EVP (Cigarette)	–
Ye et al., 2020 [42]	Eastman Institute for Oral Health, University of Rochester Medical Center	U.S.A.	Cross Sectional	Electronic cigarettes (Cigarette)	–
Lorkiewicz et al., 2019 [43]	American Heart Association	U.S.A.	Cross Sectional	Electronic cigarettes (Cigarette)	–
Bustamante et al., 2018 [44]	Division of Environmental Health Sciences, University of Minnesota	U.S.A.	Cross Sectional	Electronic cigarettes	–
Ghosh et al., 2019 [45]	Marsico Lung Institute	U.S.A.	Cross Sectional	E-cigarettes (Cigarette)	–
HTPs and E-cigarettes RCT studies on biomarker of effect (Table 5)					
Martin Leroy et al., 2012 [23]	PMI	Poland	RCT	EHCSS-K6 (Cigarette)	35 days
Ludicke et al., 2018a [46]	PMI	Japan	RCT	mTHS (menthol Cigarette)	90 days
Haziza et al., 2020b [47]	PMI	U.S.A.	RCT	mTHS 2.2 (methol Cigarette)	3 months

(continued on next page)

Table 1 (*continued*)

Authors, year of publication [Reference]	Affiliation	Study location	Study design	Product Name (Reference product)	Intervention period
Ludicke et al., 2019 [27]	PMI	U.S.A.	RCT	THS 2.2 (Cigarette)	3 months
	PMI	U.S.A.	RCT	THS 2.2 (Cigarette)	6 months
Shepperd et al., 2015 [28]	BAT	Germany	RCT	RTP (Cigarette)	6 months
Ogden et al., 2015b [48]	RAI, RJR	U.S.A.	RCT	Eclipse (Cigarette)	24 weeks
Roethig et al., 2008 [68]	PM USA	–	RCT	EHCSS (Cigarette)	postbaseline (<12 months)
D'Ruiz et al., 2017 [49]	Fontem Ventures	U.S.A.	RCT	blu (Cigarette)	5 days
Cravo et al., 2016 [37]	Fontem Ventures	UK	RCT	EVP (Cigarette)	12 weeks
E-cigarettes cross sectional studies on biomarker of effect (Table 6)					
Song MA et al., 2020 [50]	Comprehensive Cancer Center, The Ohio State University and James Cancer Hospital	U.S.A.	Cross Sectional	E-cigarettes (Cigarette)	–
Ye et al., 2020 [42]	Eastman Institute for Oral Health, University of Rochester Medical Center	U.S.A.	Cross Sectional	Electronic cigarettes (Cigarette)	–
Oliveri et al., 2020 [41]	Altria	U.S.A.	Cross Sectional	EVP (Cigarette)	–
Ghosh et al., 2019 [45]	Marsico Lung Institute	U.S.A.	Cross Sectional	E-cigarettes (Cigarette)	–
Tsai et al., 2019 [51]	Ohio State Wexner Medical Center	U.S.A.	Cross Sectional	E-cigarettes (Cigarette)	–

biomarkers of biological effect between e-cigarettes/HTPs and conventional smoking. We extracted clinical information such as the study design, demographic characteristics, and type of biomarker. Lastly, the sample size and the levels of biomarkers were obtained for each study.

2.3. Study assessment

The methodological quality was assessed using the Cochrane bias components (used for randomized trials) also known as six domains (selection, performance, detection, attrition, reporting, and other) each one sum 2 point if low risk, 1 point if unclear risk or 0 if high risk [10]. The Newcastle-Ottawa Scale (NOS) was used for observational studies [11], which is a scale that ranges from 0 to 8 and considers the following aspects: representativeness of the exposed cases/cohort, selection of non-exposed group, exposure ascertainment, outcome not present at baseline, comparability between groups, outcome assessment, follow-up long enough, non-response rate [11]. Those studies with score ≥ 3 were considered of moderate quality.

3. Results

3.1. Literature search results

Initially the literature search yielded 2091 citations, of which 1319 studies remained after 772 duplicates were removed. An additional 1185 articles were removed based on a title or abstract that was not relevant according to the inclusion criteria. Subsequent full-text screening resulted in exclusion of another 70 articles, leaving us with a total of 64 articles. Cross-reference checking did not reveal any additional articles missed by the search strategy. Of the 44 publications that met the inclusion and exclusion criteria for data extraction and final analyses (Fig. 1) [12–51,65–68], 25 articles for HTPs [12–29,46–48,65–68], and 19 for e-cigarettes were identified [30–45,49,13–51]. With some overlap, 38 articles for biomarkers of exposure and 14 for biomarkers of biological effect were identified. 12 publications were identified as independent studies, and 32 manufacturer-funded studies. Table 1 summarizes the characteristics of the studies included in this systematic review.

3.2. Study assessment

Overall the quality of the studies was moderate/good. All trials included in this systematic review had a moderate/high methodological quality according to the Cochrane tool which considered five domains for assessing the risk of bias. The cross-sectional studies included in this review had mostly moderate methodological quality according to the NOS scale (median 5, interquartile range 4–6) which considered eight domains explained previously.

3.3. Biomarkers of exposure (BOE)

Supplementary Table 1 shows the list of biomarkers of exposure and corresponding constituents. For HTPs, there were 30 trials comparing BOE profiles with combustible cigarettes, with a median intervention period of 8 days (range from 5 days to 12 months). The most common studied BOEs were COHb, MHBMA, 4-ABP, 3-HPMA, S-PMA, o-Toluidine, NEQ and 1−OHP. The levels of all of these biomarkers were significantly reduced after switching from a conventional cigarette to HTPs, and on average the reductions in the levels of biomarkers exceeded half of the baseline values. All trials showed reductions in most of the measured biomarkers. In some studies nicotine and cotinine biomarker concentrations increased (when the data was available) whereas in others they decreased. It is possible that differences between products in their nicotine content and release, and/or changes to user behaviour on switching to HTPs may account for these divergent results. Table 2a and 2b provides more details and BOE comparisons of the studies on HTPs.

For e-cigarettes, a total of 10 trials were included comparing BOE profiles between e-cigarettes and combustible cigarettes. The median follow-up period was 2 weeks (range from 5 days to 12 weeks). Carbon monoxide, MHBMA, CEMA, 3-HPMA, S-PMA, HMPMA, NEQ, NNAL and NNN were the most frequently studied BOEs. The levels of all these biomarkers were consistently reduced from their baseline value. In some studies nicotine and cotinine biomarker concentrations increased (when the data was available) whereas in others they decreased. It is possible that differences between products in their nicotine content and release, and/or changes to user behaviour on switching to HTPs may account for these divergent results. Table 3 shows more details and biomarker comparisons of the studies on e-cigarettes. 7 cross sectional studies also demonstrated a consistent and significant decrease in some BOEs (CEMA, GAMA, HEMA, 2MHA, NNAL) as shown in Table 4. In one study [43] the 1,3-butadiene metabolite MHBMA2 showed an increase of 1200 %, while all other related metabolites (DHBMA, MHBMA1, and MHBMA3) decreased in the same study. It was unclear why only MHBMA2 increased so significantly. The authors of the original study did not discuss this result in detail and it appears no data were collected which could help validate this finding, such as 1,3-butadiene levels in the mainstream e-cigarette aerosol.

3.4. Biomarkers of biological effect (BOBE)

Supplementary Table 2 shows the list of biomarkers of effect and corresponding effects. Regarding BOBE, the results show that levels found during the use of both e-cigarettes and HTPs were generally

Table 2a
HTPs RCT studies on biomarker of exposure, % change from baseline[a].

References	[12]	[13]	[14]	[15]	[16]	[17]	[18]	[19]	[20]	[20]	[20]	[65]	[66]	[21]	[21]
Affiliation	PMI	PMI	PMI	PMI	PMI	PMI	JT	PMI	BAT	BAT	BAT	PM USA	PM USA	PMI	PMI
Study location	PL	JP	PL	PL	JP	US	JP	JP	JP	JP	JP	–	–	US	US
Product Name	CHTP	THS 2.2	THS 2.2	THS 2.1	mTHS	mTHS	NTV	EHCSS-	glo/THP	mglo/THP	iQOS/THS	EHCSS-	EHCSS	EHCSS1	EHCSS2
(Reference product)	(Cig)	(Cig)	(Cig)	(Cig)	(mCig)	(mCig)	(Cig)	K6m (mCig)	1.0 (Cig)	1.0 (mCig)	(Cig)	UCS (Cig)	(Cig)	(Cig)	(Cig)
End of the study	5 d	5 d	5 d	5 d	5 d	5 d	5 d	6 d	6–7 d	6–7 d	6–7 d	8 d	8 d	8 d	8 d
p	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*<.001*	*< .001*	*< .001*	*< .001*	*< .001*	*nd*	*nd*	*nd*
CO	nd	nd	nd	nd	nd	nd	−85.08	nd	−87.25	−89.62	−85.33	nd	nd	−79	−80
COHb	−59.7	−51.13	−76.20	−75.79	−51.46	−64.41	nd	−57.0	nd	nd	nd	−86	−66.3	−92	−93
MHBMA	−87.6	−66.41	−84.98	−86.71	−87.50	−92.02	−89.68	ns	−91.32	−89.47	−84.30	nd	−63.8	nd	nd
DHBMA	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
3-ABP	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4-ABP	−74.8	−74.08	−82.12	−57.11	−78.88	−83.64	−86.56	−40.8	−80.57	−81.89	−78.26	nd	−59.8	nd	nd
HBMA	nd	nd	nd	nd	nd	nd	−72.65	nd	nd	nd	nd	nd	nd	nd	nd
CEMA	nd	−79.42	−86.10	−85.62	−83.49	−84.12	−87.21	nd	−89.23	−87.80	−87.17	nd	nd	nd	nd
3-HPMA	−70.6	−47.33	−49.68	−66.89	−54.35	−60.63	−53.00	−27.9	−52.95	−48.74	−37.42	−48	−40.1	nd	nd
AAMA	nd	nd	nd	nd	nd	nd	nd	ns	−31.48	−33.12	−43.79	nd	nd	nd	nd
GAMA	nd	nd	nd	nd	nd	nd	nd	nd	−22.91	−20.49	−27.82	nd	nd	nd	nd
2-cyanoethylvaline Hb Adduct	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
HEMA	nd	−50.99	−60.71	nd	−64.48	−69.06	−74.10	nd	−56.46	−60.71	−59.62	nd	nd	nd	nd
S-PMA	−82.2	−77.24	−92.03	−90.59	−88.82	−91.15	−89.51	−83.4	−89.13	−92.48	−89.78	−85	nd	nd	nd
TMA	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
3-OH-B[a]P	nd	−64.76	−71.43	nd	−75.25	nd	−61.65	nd	nd	nd	nd	nd	nd	nd	nd
3-HMPMA	nd	nd	nd	nd	−58.51	nd	nd	−58.6	nd	nd	nd	nd	nd	nd	nd
HMPMA	nd	−60.61	−80.58	nd	nd	−67.98	nd	nd	−78.81	−80.92	−76.13	nd	−52.8	nd	nd
o-Toluidine	−50.4	−44.23	−50.96	−30.88	−59.71	−56.99	−71.87	−53.3	−48.78	−63.14	−49.23	nd	−15.8	nd	nd
S-BMA	nd	−20.57	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	−76.7	nd	nd
1-NA	nd	−93.12	−94.16	nd	−94.89	−95.90	−93.94	nd	nd	nd	nd	nd	nd	nd	nd
2-NA	−79.7	−75.84	−85.39	−87.13	−87.28	−87.96	−90.70	ns	−90.63	−90.19	−89.94	nd	−66.1	nd	nd
NEQ	19.1	16.94	22.95	−1.59	7.88	−10.37	−46.23	−49.2	−24.72	−38.10	−7.56	−43	−46.4	−71	−67
NICT	nd	22.47	35.98	−16.50	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cotinine	23.9	16.14	11.94	−10.11	nd	nd	nd	−46.7	nd	nd	nd	−50	nd	nd	nd
NIC-P	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B[a]P	nd	nd	nd	nd	nd	−75.27	nd	nd	nd	nd	nd	nd	nd	nd	nd
1-NAP	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
2-NAP	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total OH Naphthalene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
1-OHP	−46.8	−58.57	−60.17	−63.01	−69.89	−55.73	−10.54	−67.7	−64.23	−73.49	−78.78	−72	−62.7	nd	nd
NNAL	−44.7	−48.04	−53.98	−64.34	−55.74	−61.97	−62.67	−55.2	−35.06	−36.98	−53.90	−60	−65.5	nd	nd
NAB	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
NAT	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
NNN	nd	−59.81	−69.75	−85.26	−73.03	−86.89	−89.22	nd	−49.35	−51.89	−88.38	nd	nd	nd	nd
Urine mutagenicity	−89.4	nd	nd	nd	nd	nd	nd	−80.99	nd	nd	nd	−68	−61.3	−53	−66

Cig, cigarette; d, days; DE, Germany; JP, Japan; KR, Republic of Korea; mCig, menthol cigarette; m, months; nd, no data; ns, not significant; PL, Poland; UK, United Kingdom; US, United States of America; w, weeks;
[a] Calculated in tow ways. 1) Calculated by averaging the rate of change from baseline in individual subjects. [12–14,19,21,22,24,25,65–68]. 2) Calculate by using the mean (arithmetic mean, geometric mean, LS mean) or median of each marker at baseline and last day. [15–18,20,23,26,27].

Table 2b
HTPs RCT studies on biomarker of exposure, % change from baseline[a].

References	[22]	[22]	[23]	[24]	[24]	[25]	[26]	[67]	[17]	[16]	[27]	[27]	[28]	[29]	[68]
Affiliation	PMI	PMI	PMI	PMI	PMI	PMI	JT	PM USA	PMI	PMI	PMI	PMI	BAT	RAI, RJR	PM USA
Study location	JP	JP	PL	UK	UK	KR	JP	–	US	JP	US	US	DE	US	–
Product Name (Reference product)	EHCSS-K3 (Cig)	EHCSS-K6 (Cig)	EHCSS-K6 (Cig)	EHCSS-K3 (Cig)	EHCSS-K6 (Cig)	EHCSS-K3 (Cig)	HC (Cig)	EHCSS (Cig)	mTHS (mCig)	mTHS (mCig)	THS 2.2 (Cig)	THS 2.2 (Cig)	RTP (Cig)	Eclipse (Cig)	EHCSS (Cig)
End of the study	8 d	8 d	8 d	8 d	8 d	8 d	28 d	12 w	90 d	90 d	3 m	6 m	6 m	24 w	postbaseline (<12 m)
p	*<.001*	*<.001*	*<.001*	*<.05*	*<.05*	*<.05*	*<.05*	*nd*	*nd*	*nd*	*nd*	*nd*	*< .001*	*nd*	*nd*
CO	nd	nd	ns	nd	nd	nd	nd	nd	nd	nd	−26.08	−21.30	−19.2	nd	nd
COHb	−56.2	−53.7	−54.76	−60.4	−70.1	−74.2	7.59	−23	−59.01	−41.87	−23.80	−21.54	nd	nd	−80
MHBMA	−49.5	−55.3	−64.47	−54.4	−53.8	−32.4	−51.30	nd	−81.74	−78.31	−32.43	−28.93	−30.5	−56	nd
DHBMA	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	8	nd
3-ABP	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	−30.6	−56	nd
4-ABP	−53.4	−48.6	−63.02	nd	nd	−1.5	−68.55	nd	−67.10	−77.81	nd	nd	−16.7	−64	−43
HBMA	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
CEMA	nd	nd	nd	nd	nd	nd	nd	nd	−84.68	−89.49	−37.12	−37.72	−57.4	nd	nd
3-HPMA	−23.1	−24.2	−22.72	−41.2	−35.5	ns	−37.14	−25	−57.54	−42.11	−23.42	−19.81	−33.9	20	−35
AAMA	−34.7	−27.8	nd	nd	nd	−15.00	nd	nd	nd	nd	nd	nd	nd	−38	nd
GAMA	nd	nd	nd	nd	nd	ns	nd	nd	nd	nd	nd	nd	nd	−18	nd
2-cyanoethylvaline Hb Adduct	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	−39.3	nd	nd
HEMA	nd	nd	nd	nd	nd	nd	nd	nd	−61.50	−45.64	nd	nd	nd	nd	nd
S-PMA	−71.0	−75.6	ns	−83.1	−79.4	−40.1	−40.09	−48.6	−78.77	−86.25	nd	nd	nd	−51	nd
TMA	nd	nd	nd	nd	nd	nd	−44.31	nd	nd	nd	nd	nd	nd	nd	nd
3-OH-B[a]P	nd	nd	nd	nd	nd	nd	nd	nd	nd	−64.14	−19.25	−19.87	nd	nd	nd
3-HMPMA	−38.3	−41.2	nd	−54.8	−52.8	ns	nd	nd	nd	−48.57	−25.40	−21.22	nd	nd	nd
HMPMA	nd	nd	nd	nd	nd	nd	−56.48	nd	−66.38	nd	nd	nd	−73.7	−34	nd
o-Toluidine	−73.0	−68.4	−47.42	−66.2	−61.7	−61.8	nd	nd	−51.98	−46.68	nd	nd	ns	−36	nd
S-BMA	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
1-NA	nd	nd	nd	nd	nd	nd	nd	nd	−84.81	−94.22	nd	nd	nd	nd	nd
2-NA	ns	ns	−65.62	nd	nd	−29.1	nd	nd	−82.32	−84.89	nd	nd	ns	−66	nd
NEQ	−54.7	−39.4	−23.12	−60.9	−43.8	−40.3	−58.52	−33.2	−14.32	19.96	−5.90	−9.62	25.5	−14	−18
NICT	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cotinine	−60.3	−43.7	nd	−53.9	−36.5	−44.7	nd	nd	nd	nd	nd	nd	nd	nd	−16
NIC-P	−56.1	−42.9	nd	nd	nd	−20.0	nd	nd	nd	nd	nd	nd	nd	nd	nd
B[a]P	nd	nd	nd	nd	nd	nd	nd	nd	−61.02	nd	nd	nd	nd	nd	nd
1-NAP	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	−12	nd
2-NAP	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	−40	nd
Total OH Naphthalene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	54.7	nd	nd
1-OHP	−66.7	−66.7	−69.80	−64.0	−63.2	−38.2	−41.83	17.5	−26.51	−44.49	−15.17	−15.86	−29.5	25	−53
NNAL	−52.6	−51.5	2.74	−60.1	−55.2	−50.5	−53.35	−62.6	−69.40	−72.87	−31.73	−36.53	−39.4	−39	−73
NAB	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	−43.1	nd	nd
NAT	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	−27.9	nd	nd
NNN	nd	nd	nd	nd	nd	nd	nd	nd	−87.94	−68.53	−35.37	−35.99	−64.6	nd	nd
Urine mutagenicity	−31.0	−41.5	nd	−66.9	−67.8	−31.8	−46.97	nd	nd	nd	nd	nd	nd	−37	−81

Cig, cigarette; d, days; DE, Germany; JP, Japan; KR, Republic of Korea; mCig, menthol cigarette; m, months; nd, no data; ns, not significant; PL, Poland; UK, United Kingdom; US, United States of America; w, weeks;
[a] Calculated in tow ways. 1) Calculated by averaging the rate of change from baseline in individual subjects. [12–14,19,21,22,24,25,65–68]. 2) Calculate by using the mean (arithmetic mean, geometric mean, LS mean) or median of each marker at baseline and last day. [15–18,20,23,26,27].

Table 3
E-cigarettes RCT studies on biomarker of exposure, % change from baseline[a].

References	[30]	[31]	[31]	[32]	[33]	[34]	[35]	[36]	[37]	[38]
Affiliation	FV	RJR VC	RJR VC	JUUL Labs	independent	independent	independent	independent	FV	FV
Study location	US	US	US	US	PL	UK	US	US	UK	US
Product Name (Reference product)	blu (Cig)	Vuse Solo (Cig)	mVuse Solo (mCig)	JUUL NSPS (Cig) Pooled 4 flavours	M201 Mild (Cig)	Green Smoke EC (Cig)	e-Go C (Cig)	Vuse Solo Blu cig Fin (Cig)	EVP (Cig)	Puritane™ (Cig)
End of the study	5 d	5 d	5 d	5 d	2 w	4 w	4 w	8 w	12 w	24 m
p	*<.001*	*<.05*	*<.05*	*nd*	*≤.001*	*<.001*	*<.01*	*<.01*	*nd*	*nd*
CO	−89.33	nd	nd	nd	ns	−80	−37.46	−57	nd	nd
COHb	nd	−75.3	−77.1	−72.8	nd	nd	nd	nd	nd	nd
MHBMA	−93.87	−55.5	−56.0	−96.3	−84.30	nd	nd	nd	nd	nd
3-ABP	nd	−74.0	−78.6	nd	nd	nd	nd	nd	nd	nd
4-ABP	nd	−63.5	−73.0	nd	nd	nd	nd	nd	nd	nd
CEMA	−84.79	−85.9	−85.6	nd	nd	nd	nd	−66	nd	nd
3-HPMA	−85.91	−70.5	−71.0	−88.7	−47.49	−79	ns	−47	−29.1	−30.48
Acrylamide equivalents	nd	−50.0	−54.3	nd	nd	nd	nd	nd	nd	nd
CNEMA	nd	nd	nd	nd	−75.94	nd	−51.59	nd	nd	nd
HEMA	nd	−62.3	−53.9	nd	−63.36	nd	ns	nd	nd	nd
AAMA	nd	nd	nd	nd	ns	nd	ns	ns	nd	nd
S-PMA	−95.23	−89.7	−89.0	−94.7	−79.92	nd	nd	nd	−35.1	−36.50
PMA	nd	nd	nd	nd	nd	nd	−16.90	nd	nd	nd
3-OH-B[a]P	nd	−63.8	−70.0	nd	nd	nd	nd	nd	nd	nd
HMPMA	−86.38	−77.5	−77.2	nd	nd	nd	nd	−47	nd	nd
HPMMA	nd	nd	nd	nd	−65.96	nd	ns	nd	nd	nd
o-Toluidine	nd	−57.6	−55.7	nd	nd	nd	nd	nd	nd	nd
2HPMA	nd	nd	nd	nd	−46.66	nd	ns	nd	nd	nd
1-NA	nd	−95.5	−95.0	nd	nd	nd	nd	nd	nd	nd
2-NA	nd	−90.4	−91.9	nd	nd	nd	nd	nd	nd	nd
NEQ	ns	−38.3	−37.8	nd	ns	nd	nd	nd	−25.3	−0.08
NICT	nd	nd	nd	nd	ns	nd	nd	nd	nd	nd
Cotinine	nd	−32.0	−32.2	nd	ns	ns	ns	nd	nd	nd
HCTT	nd	nd	nd	nd	ns	nd	nd	nd	nd	nd
COXT	nd	nd	nd	nd	ns	nd	nd	nd	nd	nd
NOXT	nd	nd	nd	nd	ns	nd	nd	nd	nd	nd
NCCT	nd	nd	nd	nd	ns	nd	nd	nd	nd	nd
NNCT	nd	nd	nd	nd	ns	nd	nd	nd	nd	nd
NIC-P	nd	−40.1	−36.0	nd	nd	nd	nd	nd	nd	nd
Naphthalene equivalents	nd	−83.6	−70.1	nd	nd	nd	nd	nd	nd	nd
1-NAP	nd	nd	nd	nd	ns	nd	nd	nd	nd	nd
2-NAP	nd	nd	nd	nd	ns	nd	nd	nd	nd	nd
1-Hydroxypyrene	−70.47	−63.5	−67.2	nd	ns	nd	nd	nd	nd	nd
NNAL	−59.23	−58.7	−55.0	−68.4	−56.88	nd	−45.64	−53	−30.9	−29.17
NAB	nd	−89.5	−86.5	nd	nd	nd	nd	nd	nd	nd
NAT	nd	−98.7	−97.9	nd	nd	nd	nd	nd	nd	nd
NNN	−93.54	−87.4	−91.8	−96.3	nd	nd	nd	nd	nd	nd
Urine mutagenicity	nd	−88.1	−90.0	nd	nd	nd	nd	nd	nd	nd
PG	nd	nd	nd	nd	nd	nd	nd	nd	119.2	464.17

Cig, cigarette; d, days; DE, Germany; JP, Japan; KR, Republic of Korea; mCig, menthol cigarette; m, months; nd, no data; ns, not significant; PL, Poland; UK, United Kingdom; US, United States of America; w, weeks;

[a] Calculated in three ways. 1) Calculated by averaging the rate of change from baseline in individual subjects. [31,34,36,37]. 2) Calculated by determining the median in the rate of change from baseline in individual subjects. [30,32,33,35,38]. Calculate by using the mean (arithmetic mean, geometric mean, LS mean) or median of each marker at baseline and last day.

moved in a direction believed to be consistent with improved health outcomes (Tables 5, 6). 10 trials and 5 cross sectional studies assessed the effects of BOBE changes, with a follow up period ranging from 5 days to 12 months. Those studies measured a total of 90 BOBEs in blood, urine or saliva, including markers related to clinical laboratory test (13 markers), inflammation/oxidative damage (52 markers), lipids (6 markers), hypercoagulable state (7 markers), growth factors (11 markers), and tissue injury and repair (1 marker).

The most consistent finding across the studies was the reduction in the levels of thromboxane (11-DTX-B2) by 10–30 % and white blood cells between 0–13 % from baseline. There were also some benefits in terms of lipid profile, showing an increase of HDL and reduction of LDL. Other BOBEs which showed reduction in multiple studies were FEV1% pred, Systolic blood pressure, Diastolic blood pressure, 812-iso-iPF2α-VI, 8-epi-PGF2α, sICAM1, CRP, Neutrophil count, OxLDL, Triglycerides, Fibrinogen and HgB (Table 5).

Additionally, 5 cross sectional studies favoured the use of e-cigarettes over combustible cigarettes, demonstrating better profiles for oxidative damage and growth factors (Table 6), which included a reduction in levels of 8-epi-PGF2α, sICAM1, 11-DTX-B2, macrophages and IL1ß. There was only one study that measured and recorded significant differences regarding growth factors [42]. (Table 6).

4. Discussion

This systematic review identified clinical studies which had examined biomarkers of tobacco smoke exposure (BOE) and biological effect (BOBE) during the use of e-cigarettes and HTPs, taken from major literature databases. The results provide elemental insights for a critical appraisal of e-cigarettes and HTPs as alternatives to combusted tobacco products such as cigarettes. Taken together, all findings suggest that BOE levels measured in users of e-cigarettes and HTPs show a significant

Table 4
E-cigarettes cross sectional studies on biomarker of exposure, % difference between cigarettes[a].

References	[39]	[40]	[41]	[42]	[43]	[44]	[45]
Affiliation	independent	independent	Altria	independent	independent	independent	independent
Study location	UK	US	US	US	US	US	US
Product Name (Reference product)	E-cig (Cig)	E-cig (Cig)	EVP (Cig)	E-cig (Cig)	E-cig (Cig)	E-cig (Cig)	E-cig (Cig)
p	*<.001*	*<.05*	*≤.001*	*nd*	*nd*	*nd*	*nd*
COHb	nd	nd	−46.34	nd	nd	nd	nd
BPMA	15.62	ns	nd	nd	−70.32	nd	nd
DHBM	nd	−27.93	nd	nd	nd	nd	nd
DHBMA	−22.89	nd	nd	nd	−5.94	nd	nd
MHB3	nd	−84.55	nd	nd	nd	nd	nd
MHBMA1	nd	nd	nd	nd	−100.00	nd	nd
MHBMA2	nd	nd	nd	nd	1200.00	nd	nd
MHBMA3	−85.10	nd	nd	nd	−52.44	nd	nd
TTCA	ns	ns	nd	nd	−93.34	nd	nd
Acetate	nd	nd	nd	nd	46.88	nd	nd
CEMA	−54.42	−60.22	nd	nd	−83.30	nd	nd
3-HPMA	−64.10	nd	−45.95	nd	−38.95	nd	nd
HPMA	nd	−72.47	nd	nd	nd	nd	nd
AAMA	−55.33	−58.90	nd	nd	61.37	nd	nd
GAMA	−45.94	−42.73	nd	nd	−85.68	nd	nd
AMCA	nd	−68.15	nd	nd	nd	nd	nd
CYHA	nd	−88.84	nd	nd	nd	nd	nd
CYMA	−97.15	−96.80	nd	nd	31.81	nd	nd
HEMA	−48.14	−60.78	nd	nd	−100.00	nd	nd
TMA	ns	nd	nd	nd	69.87	nd	nd
HPMM	nd	−81.23	nd	nd	nd	nd	nd
HPMMA	−70.66	nd	nd	nd	−22.95	nd	nd
ATCA	ns	nd	nd	nd	28.11	nd	nd
AMCC	−62.51	nd	nd	nd	−14.92	nd	nd
PGHA	ns	−40.47	nd	nd	49.81	nd	nd
Formate	nd	nd	nd	nd	96.62	nd	nd
IPM3	nd	−88.81	nd	nd	nd	nd	nd
HPM2	nd	−51.54	nd	nd	nd	nd	nd
2HPMA	−28.71	nd	nd	nd	−58.52	nd	nd
PHEMA	ns	nd	nd	nd	−50.00	nd	nd
MADA	−46.55	−50.41	nd	nd	4.95	nd	nd
S-BMA	nd	ns	nd	nd	−77.27	nd	nd
1,2DCVMA	nd	nd	nd	nd	−76.11	nd	nd
2,2DCVMA	nd	nd	nd	nd	−100.00	nd	nd
2MHA	−74.94	−71.88	nd	nd	−64.98	nd	nd
3MHA+ 4MHA	−80.71	−72.71	nd	nd	59.82	nd	nd
NEQ	ns	−92.83	ns	nd	nd	nd	nd
NICT	ns	−60.63	nd	nd	−96.40	57.53	−44.67
Cotinine	ns	−93.21	nd	26.37	111.94	7.69	−43.45
HCTT	ns	−92.85	nd	nd	−6.98	nd	nd
COXT	ns	−60.49	nd	nd	nd	nd	−43.23
NOXT	ns	−56.09	nd	nd	nd	nd	nd
NCCT	ns	−64.72	nd	nd	nd	nd	nd
NNCT	ns	−68.72	nd	nd	nd	−29.51	nd
1-NAP	nd	−86.04	nd	nd	nd	nd	nd
2-NAP	nd	−61.99	nd	nd	nd	nd	nd
1-Hydroxypyrene	nd	−46.86	nd	nd	nd	nd	nd
NNAL	−97.24	−97.59	−86.26	nd	nd	−98.01	nd
NAB	−82.65	−90.92	nd	nd	nd	nd	nd
NAT	−94.54	−95.93	nd	nd	nd	nd	nd
NNN	nd	−70.58	nd	nd	nd	−99.66	nd

Cig, cigarette; d, days; DE, Germany; JP, Japan; KR, Republic of Korea; mCig, menthol cigarette; m, months; nd, no data; ns, not significant; PL, Poland; UK, United Kingdom; US, United States of America; w, weeks;

[a] Calculate by using the mean (arithmetic mean, geometric mean, LS mean) of each marker on e-cigarette group and cigarette group.

reduction compared to a cigarette condition (or cigarette baseline). There is also some evidence to suggest that e-cigarette users are exposed to fewer harmful substances overall, and in lower concentrations, than users of HTPs.

We studied the majority of biomarkers of exposure associated with tobacco. There are numerous substances of concern and related biomarkers based on the list of priority toxicants proposed by the WHO Study Group on Tobacco Product Regulation. Most of them have been widely studied due to their potential link to smoking-related health risks [52–54]. Our biomarker findings imply that the majority of toxicants are emitted in lower amounts (if at all) from e-cigarettes and HTPs compared to combusted tobacco products such as cigarettes. This is consistent with the results of research on mutagenicity, which has been used as an indicator of the genetic mutagenic potential of substances present in human urine [55].

Relevant biomarker levels in users of e-cigarettes and HTPs were indicative of reduced exposure to butadiene, acrolein, benzene, toluidine, naphthylamine and methylnitrosamines. Most of these chemicals are considered carcinogens and hazardous for human health. For example, according to the United States Environmental Protection Agency, butadiene is a potent carcinogen that is also derived from motor vehicle exhaust and is known to increase the risk of cardiovascular diseases, leukemia and lung irritation [56]. Similarly, other authorities have also suggested that toxicants like acrolein or benzene may cause respiratory tract irritation as well as gastrointestinal mucosa hyperplasia.

Table 5
HTPs and E-cigarettes RCT studies on biomarker of effect, % change from baseline[a,b].

References	[23]	[46]	[47]	[27]	[27]	[28]	[48]	[68]	[49]	[37]
Affiliation	PMI	PMI	PMI	PMI	PMI	BAT	RAI, RJR	PM USA	FV	FV
Study location	PL	JP	US	US	US	DE	US	–	US	UK
Product type	HTPs	HTPs	HTPs	HTPs	HTPs	HTPs	HTPs	HTPs	e-cig	e-cig
Product Name (Reference product)	EHCSS-K6 (Cig)	mTHS (mCig)	mTHS 2.2 (mCig)	THS 2.2 (Cig)	THS 2.2 (Cig)	RTP (Cig)	Eclipse (Cig)	EHCSS (Cig)	blu (Cig)	EVP (Cig)
End of Study	35 d	90 d	3 m	3 m	6 m	6 m	24 w	postbaseline (12 m)	5 d	12 w
p	⩽ ***.001***	*nd*	*nd*	*nd*	*nd*	***<.001***	***<.05***	*nd*	***< .05***	*nd*
Clinical laboratory test										
FEV1%pred	nd	1.55	nd	−0.62	−1.46	nd	nd	nd	**6.0**	nd
FVC	nd	nd	nd	nd	nd	nd	nd	nd	**1.9**	nd
CEP	nd	nd	nd	nd	nd	nd	55	nd	nd	nd
HgBA1C	nd	0.00	nd	nd	nd	nd	**3**	nd	nd	nd
Homocysteine	2.75	11.35	9.27	nd	nd	nd	−1	nd	nd	nd
SCE	nd	nd	nd	nd	nd	nd	−3	nd	nd	nd
RBC count	−2.22	nd	nd	nd	nd	nd	nd	0.00	nd	nd
Glucose	nd	5.77	0.96	nd	nd	nd	nd	nd	nd	nd
Body weight	nd	0.51	nd	nd	nd	nd	nd	nd	nd	nd
Waist circumference	nd	−7.00	nd	nd	nd	nd	nd	nd	nd	nd
Systolic blood pressure	nd	−5.44	nd	nd	nd	nd	nd	nd	**−6.0**	nd
Diastolic blood pressure	nd	−6.26	nd	nd	nd	nd	nd	nd	**−5.7**	nd
Heat rate	nd	nd	nd	nd	nd	nd	nd	nd	**−7.2**	nd
Inflammation/Oxidative damage										
iPF2α-III	nd	nd	nd	nd	nd	nd	−8	nd	nd	nd
PGF2α	nd	nd	nd	nd	nd	nd	2	nd	nd	nd
2,3-dinor-iPF2α-III	nd	nd	nd	nd	nd	nd	3	nd	nd	nd
(±)5-iPF2α-VI	nd	nd	nd	nd	nd	nd	−11	nd	nd	nd
812-iso-iPF2α-III	nd	nd	nd	nd	nd	3.2	nd	nd	nd	nd
812-iso-iPF2α-VI	nd	nd	nd	nd	nd	−6.3	−2	nd	nd	nd
8-epi-PGF2α	−7.14	−3.73	2.98	−6.26	−10.08	nd	nd	7.19	nd	nd
sICAM1	nd	−15.47	−10.10	0.00	−0.76	**59.9**	**−11**	nd	nd	nd
WBC	−4.34	−6.10	nd	−3.10	−2.02	0.0	**−13**	−12.00	nd	−3.58
CRP	−21.42	20.00	3.63	nd	nd	−21.6	−14	−18.18	nd	nd
8-OHdG	nd	nd	nd	nd	nd	**−16.6**	nd	nd	nd	nd
11-DTX-B2	**−10.23**	−14.16	−31.13	−9.79	−13.68	**−19.2**	nd	−20.59	nd	nd
SOD activity to Hb ratio	nd	nd	nd	nd	nd	−13.0	nd	nd	nd	nd
GPx activity to Hb ratio	nd	nd	nd	nd	nd	**−12.3**	nd	nd	nd	nd
Glutathione reductase activity to Hb ratio	nd	nd	nd	nd	nd	**−79.8**	nd	nd	nd	nd
Catalase activity to Hb ratio	nd	nd	nd	nd	nd	8.8	nd	nd	nd	nd
Malondialdehyde to Hb ratio	nd	nd	nd	nd	nd	**171.0**	nd	nd	nd	nd
Ascorbic acid	nd	nd	nd	nd	nd	**−12.1**	nd	nd	nd	nd
Dehydroascorbic acid	nd	nd	nd	nd	nd	−8.5	nd	nd	nd	nd
Total antioxidant capacity	nd	nd	nd	nd	nd	**6.5**	nd	nd	nd	nd
MCP-1	nd	nd	nd	nd	nd	4.8	nd	nd	nd	nd
Neutrophil elastase	nd	nd	nd	nd	nd	**−57.1**	nd	nd	nd	nd
LTB4	nd	nd	nd	nd	nd	−37.9	nd	nd	nd	nd
Neutrophil count	−5.12	nd	nd	nd	nd	−2.3	nd	nd	nd	nd
Lymphocytes	−4.76	nd	nd	nd	nd	nd	nd	nd	nd	nd
Monocyte count	0.00	nd	nd	nd	nd	−3.1	nd	nd	nd	nd
Eosinophils	0.00	nd	nd	nd	nd	nd	nd	nd	nd	nd
Basophils	0.00	nd	nd	nd	nd	nd	nd	nd	nd	nd
cis-thymidine glycol	nd	nd	nd	nd	nd	−12.7	nd	nd	nd	nd
IL-6	0.00	nd	nd	nd	nd	nd	nd	nd	nd	nd
MPO	−2.01	nd	nd	nd	nd	nd	nd	nd	nd	nd
Lipids										
HDL	10.52	5.97	nd	0.73	0.73	8.0	0	10.81	nd	0.56
LDL	−4.91	−6.51	nd	nd	nd	2.1	−1	−0.88	nd	−1.69
HDL/LDL	nd	nd	nd	nd	nd	nd	2	nd	nd	nd
OxLDL	**60.76**	nd	nd	nd	nd	−3.7	−2	nd	nd	nd
Triglycerides	nd	−0.71	nd	nd	nd	−4.2	15	3.50	nd	nd
Total cholesterol	1.47	−3.24	nd	nd	nd	2.7	nd	nd	nd	nd
Hypercoaguable state										
Fibrinogen	6.06	−1.17	−5.94	nd	nd	−1.3	−1	−3.77	nd	nd
Platelets	0.90	nd	nd	nd	nd	nd	**−6**	nd	nd	nd
HCT	−2.81	nd	nd	nd	nd	nd	0	−1.66	nd	nd
HgB	−2.09	nd	nd	nd	nd	nd	1	−1.38	nd	−1.27
vWF	−11.11	nd	nd	nd	nd	nd	nd	−4.72	nd	nd
ADP-induced platelet aggregation: slope	0.86	nd	nd	nd	nd	nd	nd	nd	nd	nd
ADP-induced platelet aggregation: amplitude (%)	**1.28**	nd	nd	nd	nd	nd	nd	nd	nd	nd

Cig, cigarette; d, days; DE, Germany; JP, Japan; KR, Republic of Korea; mCig, menthol cigarette; m, months; nd, no data; ns, not significant; PL, Poland; UK, United Kingdom; US, United States of America; w, weeks;

[a] Calculated in two ways. 1) Calculated by averaging the rate of change from baseline in individual subjects. [49]. 2) Calculate by using the mean (arithmetic mean, geometric mean, LS mean) or median of each marker at baseline and last day. [23,27,28,37,46–48,68].
[b] Bold is statistically significant.

Table 6
E-cigarettes cross sectional studies on biomarker of effect, % difference between cigarettes[a,b].

References	[50]	[42]	[41]	[45]	[51]
Affiliation	independent	independent	Altria	independent	independent
Study location	US	US	US	US	US
Study design	Cross Sectional	Cross Sectional	Cross Sectional	Cross Sectional	Cross Sectional
Product type	E-cig	E-cig	E-cig	E-cig	E-cig
Product Name (Reference product)	E-cig (Cig)	E-cig (Cig)	EVP (Cig)	E-cig (Cig)	E-cig (Cig)
p	***<.05***	*nd*	***<.05***	*nd*	*nd*
Clinical laboratory test					
FEV1%pred	nd	nd	nd	−6.67	nd
FVC	nd	nd	nd	−16.91	nd
Inflammation/Oxidative damage					
8-epi-PGF2α	nd	nd	**−22.85**	nd	nd
sICAM1	nd	nd	**−15.72**	nd	nd
WBC	nd	nd	−8.69	nd	nd
11-DTX-B2	nd	nd	**−29.09**	nd	nd
Neutrophil count	−70.00	nd	nd	nd	−70.00
Lymphocytes	30.00	nd	nd	nd	30.00
Eosinophils	nd	nd	nd	42.50	nd
Macrophages	**−35.52**	nd	nd	−1.60	−35.52
Polymorphonuclear cells	nd	nd	nd	39.03	nd
Bronchial epithelial cells	nd	nd	nd	113.33	nd
Squamous epithelial cells	nd	nd	nd	15.00	nd
IL1ß	**−75.16**	−48.01	nd	nd	nd
IL2	12.90	nd	nd	nd	nd
IL4	0.00	nd	nd	nd	nd
IL6	−62.94	nd	nd	nd	nd
IL8	−25.33	nd	nd	nd	nd
IL10	0.00	nd	nd	nd	nd
IL13	16.91	nd	nd	nd	nd
IL 12p70	8.33	nd	nd	nd	nd
IFNγ	13.84	nd	nd	nd	nd
TNFα	−5.76	nd	nd	nd	nd
MPO	nd	−42.52	nd	nd	nd
PGE2	nd	−41.53	nd	nd	nd
EN-RAGE	nd	−31.38	nd	nd	nd
RAGE	nd	−69.91	nd	nd	nd
MMP-9	nd	−20.81	nd	nd	nd
S100A8	nd	3.86	nd	nd	nd
S100A9	nd	17.47	nd	nd	nd
Galectin-3	nd	−4.73	nd	nd	nd
Uteroglobin/CC-10	nd	−72.44	nd	nd	nd
Lipids					
HDL	nd	nd	2.47	nd	nd
Growth factors (pg/mg protein)					
BDNF	nd	−84.91	nd	nd	nd
Basic EGF	nd	−67.89	nd	nd	nd
β NGF	nd	−69.28	nd	nd	nd
SCF	nd	−95.15	nd	nd	nd
BMP-2	nd	−88.36	nd	nd	nd
HGF	nd	−39.59	nd	nd	nd
PDGF-AA	nd	−62.79	nd	nd	nd
TGF-α	nd	−33.99	nd	nd	nd
EGF	nd	−53.37	nd	nd	nd
PlGF	nd	−89.52	nd	nd	nd
VEGF	nd	−49.95	nd	nd	nd
Tissue injury and repair					
Serpine1/PAI-1	nd	−21.21	nd	nd	nd

Cig, cigarette; d, days; DE, Germany; JP, Japan; KR, Republic of Korea; mCig, menthol cigarette; m, months; nd, no data; ns, not significant; PL, Poland; UK, United Kingdom; US, United States of America; w, weeks;
[a] Calculate by using the mean (arithmetic mean, geometric mean, LS mean) of each marker on e-cigarette group and cigarette group.
[b] Bold is statistically significant.

Globally it is understood that smoke-related diseases are consequences of pathophysiological processes that involve oxidative stress and chronic inflammation [69]. It is therefore hypothesized that a favorable change in BOBEs, comprising variables related to lipid metabolism, endothelial function, inflammation, oxidative stress, platelet activation, and pulmonary function, could potentially contribute to improved health outcomes. In particular, some of the BOBE which showed significant level changes in this review (sICAM-1, WBC, 11-DHTXB2 and 8-epi-PGF2α) have been reported as associated with smoking-related diseases such as CVD [57–63]. However, this is still a fertile area of research with some topics that need to be clarified such as the real health benefits that may results from the conversion to

e-cigarettes/HTPs. Of note, it has also recently been reported that HTPs showed reductions in quantitative risk estimates [70] and an absence of significant in vitro toxicological activity [71] compared to conventional cigarettes.

Despite these promising findings, the scientific literature about e-cigarettes and HTPs is diverse and specific consensus is lacking. In this review, a few biomarkers were not shown to be consistently changed, such as the sICAM1 [28], CRP [46,47], WBC [28], OxLDL [23], which could create difficulties in interpretation. Consequently some public health authorities have supported the use of e-cigarettes or HTPs only as a bridge to smoking cessation and warn about possible health effects, particularly among youth and young adults [64]. More importantly it is still unknown whether e-cigarettes or HTPs have long-term effectiveness in reducing exposure to toxins compared to smoking combusted tobacco. Consequently, for the longer-term, little is known about the health effects of the use of e-cigarettes and HTPs, as relevant scientific evidence is currently not sufficient.

The results of our review suggest no major or consistent differences between e-cigarettes and HTPs. Levels of selected BOEs were similar in both groups, with similar reduction rates after switching from combusted tobacco. Regarding those biomarkers with a long half-life, only one cross sectional study showed higher reduction rates when participants were switched from conventional tobacco products to e-cigarettes for a prolonged period. This suggests that such effects are time sensitive and further studies with longer interventions and follow up periods are needed.

This systematic review is subject to some limitations. First, most clinical studies were manufacturer-funded studies, which could lead to publication bias. Second, since studies on BOBEs may require longer intervention periods, the number of reports was limited without the necessary follow up time to show changes in biological functions. Third, while the BOBEs employed in these studies may reflect processes on the pathway to smoking-related disease, their predictive and discriminative power has yet to be established so further studies such as long-term epidemiological studies are needed to show their relevance to tobacco related disease and the impact of HTP or e-cigarette use.

We conclude that the current evidence supports the use of non-combustible smoking alternatives such as e-cigarettes and HTPs, which on the evidence presented in this review have been shown to improve levels of both BOEs and BOBEs. Although this may suggest plausible effects on the incidence of smoke-related disease, confirmatory data is not yet available, so this remains a fertile research area in the coming years.

Funding

This work was supported by Japan Tobacco Inc.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

Editorial support, in the form of medical writing, assembling tables and creating high-resolution images based on authors' detailed directions, collating author comments, copyediting, fact checking, and referencing, was provided by Editage, Cactus Communications

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.toxrep.2021.01.014.

References

[1] O. Rom, A. Pecorelli, G. Valacchi, A.Z. Reznick, Are e-cigarettes a safe and good alternative to cigarette smoking? Ann. N. Y. Acad. Sci. 2015 (1340) (2015) 65–74, https://doi.org/10.1111/nyas.12609.
[2] J.E. Gotts, S.E. Jordt, R. McConnell, R. Tarran, What are the respiratory effects of e-cigarettes? BMJ. 2019 (366) (2019) l5275, https://doi.org/10.1136/bmj.l5275.
[3] M. Unger, D.W. Unger, E-cigarettes/electronic nicotine delivery systems: a word of caution on health and new product development, J. Thorac. Dis. 10 (Suppl 22) (2018) S2588–S2592, https://doi.org/10.21037/jtd.2018.07.99, 2018.
[4] WHO Regional Office for Europe, Electronic Nicotine and Non-nicotine Delivery Systems: a Brief, 2020. https://www.euro.who.int/_data/assets/pdf_file/0009/443673/Electronic-nicotine-and-non-nicotine-delivery-systems-brief-eng.pdf.
[5] WHO Regional Office for Europe, Heated Tobacco Products: a Brief, 2020. https://www.euro.who.int/_data/assets/pdf_file/0008/443663/Heated-tobacco-products-brief-eng.pdf.
[6] E. Simonavicius, A. McNeill, L. Shahab, L.S. Brose, Heat-not-burn tobacco products: a systematic literature review, Tob. Control 28 (5) (2019) 582–594, https://doi.org/10.1136/tobaccocontrol-2018-054419, 2019.
[7] M. Jankowski, G.M. Brożek, J. Lawson, S. Skoczyński, P. Majek, J.E. Zejda, New ideas, old problems? Heated tobacco products - a systematic review, Int. J. Occup. Med. Environ. Health 32 (5) (2019) 595–634, https://doi.org/10.13075/ijomeh.1896.01433, 2019.
[8] A. Drovandi, S. Salem, D. Barker, D. Booth, T. Kairuz, Human biomarker exposure from cigarettes versus novel heat-not-Burn devices: a systematic review and meta-analysis, Nicotine Tob. Res. 22 (7) (2020) 1077–1085, https://doi.org/10.1093/ntr/ntz200, 2019.
[9] A. Liberati, D.G. Altman, J. Tetzlaff, C. Mulrow, P.C. Gøtzsche, J.P. Ioannidis, M. Clarke, P.J. Devereaux, J. Kleijnen, D. Moher, The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration, BMJ 21 (July339) (2009), https://doi.org/10.1136/bmj.b2700 b27002009.
[10] J.P. Higgins, D.G. Altman, P.C. Gøtzsche, P. Jüni, D. Moher, A.D. Oxman, J. Savovic, K.F. Schulz, L. Weeks, J.A. Sterne, Cochrane Bias Methods Group, Cochrane Statistical Methods Group, The Cochrane Collaboration's tool for assessing risk of bias in randomised trials, BMJ 2011 (343) (2011) d5928, https://doi.org/10.1136/bmj.d5928.
[11] J. Peterson, V. Welch, M. Losos, P. Tugwell, The Newcastle-ottawa Scale (NOS) for Assessing the Quality of Nonrandomised Studies in Meta-analyses, Ottawa Hospital Research Institute, Ottawa, 2011. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.
[12] F. Lüdicke, C. Haziza, R. Weitkunat, J. Magnette, Evaluation of biomarkers of exposure in smokers switching to a carbon-heated tobacco product: a controlled, randomized, open-label 5-Day exposure study, Nicotine Tob. Res. 18 (7) (2016) 1606–1613, https://doi.org/10.1093/ntr/ntw022, 2016.
[13] C. Haziza, G. de La Bourdonnaye, S. Merlet, M. Benzimra, J. Ancerewicz, A. Donelli, G. Baker, P. Picavet, F. Lüdicke, 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, Regul. Toxicol. Pharmacol. 2016 (81) (2016) 489–499, https://doi.org/10.1016/j.yrtph.2016.09.014.
[14] C. Haziza, G. de La Bourdonnaye, D. Skiada, J. Ancerewicz, G. Baker, P. Picavet, F. Lüdicke, Biomarker of exposure level data set in smokers switching from conventional cigarettes to Tobacco Heating System 2.2, continuing smoking or abstaining from smoking for 5 days, Data Brief 2017 (10) (2017) 283–293, https://doi.org/10.1016/j.dib.2016.11.047.
[15] F. Lüdicke, G. Baker, J. Magnette, P. Picavet, R. Weitkunat, Reduced exposure to harmful and potentially harmful smoke constituents with the tobacco heating system 2.1, Nicotine Tob. Res. 19 (2) (2017) 168–175, https://doi.org/10.1093/ntr/ntw164, 2017.
[16] F. Lüdicke, P. Picavet, G. Baker, C. Haziza, V. Poux, N. Lama, R. Weitkunat, 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. 20 (2) (2018) 161–172, https://doi.org/10.1093/ntr/ntw287, 2018.
[17] C. Haziza, G. de La Bourdonnaye, A. Donelli, V. Poux, D. Skiada, R. Weitkunat, G. Baker, P. Picavet, F. Lüdicke, 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. 22 (4) (2020) 539–548, https://doi.org/10.1093/ntr/ntz013, 2020.
[18] D. Yuki, Y. Takeshige, K. Nakaya, Y. Futamura, 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) (2018) 127–134, https://doi.org/10.1016/j.yrtph.2018.05.001.
[19] A.R. Tricker, S. Kanada, K. Takada, C. Martin Leroy, D. Lindner, M.K. Schorp, R. Dempsey, Reduced exposure evaluation of an Electrically Heated Cigarette Smoking System. Part 6: 6-Day randomized clinical trial of a menthol cigarette in Japan, Regul. Toxicol. Pharmacol. 2018 (96) (2012) 127–134, https://doi.org/10.1016/j.yrtph.2012.08.007.
[20] N. Gale, M. McEwan, A.C. Eldridge, I.M. Fearon, N. Sherwood, E. Bowen, S. McDermott, E. Holmes, A. Hedge, S. Hossack, L. Wakenshaw, J. Glew, O. M. Camacho, G. Errington, J. McAughey, J. Murphy, C. Liu, C.J. Proctor, Changes

in biomarkers of exposure on switching from a conventional cigarette to tobacco heating products: a randomized, controlled study in healthy japanese subjects, Nicotine Tob. Res. 21 (9) (2019) 1220–1227, https://doi.org/10.1093/ntr/nty104, 2019.
[21] H.J. Roethig, R.D. Kinser, R.W. Lau, R.A. Walk, N. Wang, Short-term exposure evaluation of adult smokers switching from conventional to first-generation electrically heated cigarettes during controlled smoking, J. Clin. Pharmacol. 45 (2) (2005) 133–145, https://doi.org/10.1177/0091270004271253, 2005.
[22] A.R. Tricker, S. Kanada, K. Takada, C.M. Leroy, D. Lindner, M.K. Schorp, R. Dempsey, Reduced exposure evaluation of an Electrically Heated Cigarette Smoking System. Part 5: 8-Day randomized clinical trial in Japan, Regul. Toxicol. Pharmacol. 64 (2 Suppl) (2012) 54–63, https://doi.org/10.1016/j.yrtph.2012.08.003, 2012.
[23] C. Martin Leroy, K. Jarus-Dziedzic, J. Ancerewicz, D. Lindner, A. Kulesza, J. Magnette, 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. 64 (2 Suppl) (2012) 74–84, https://doi.org/10.1016/j.yrtph.2012.08.006, 2012.
[24] A.R. Tricker, A.J. Stewart, C.M. Leroy, D. Lindner, M.K. Schorp, R. Dempsey, Reduced exposure evaluation of an Electrically Heated Cigarette Smoking System. Part 3: eight-day randomized clinical trial in the UK, Regul. Toxicol. Pharmacol. 64 (2 Suppl) (2012) 35–44, https://doi.org/10.1016/j.yrtph.2012.08.010, 2012.
[25] A.R. Tricker, I.J. Jang, C. Martin Leroy, D. Lindner, R. Dempsey, Reduced exposure evaluation of an Electrically Heated Cigarette Smoking System. Part 4: Eight-day randomized clinical trial in Korea, Regul. Toxicol. Pharmacol. 64 (2 Suppl) (2012) 45–53, https://doi.org/10.1016/j.yrtph.2012.08.013, 2012.
[26] C. Sakaguchi, A. Kakehi, N. Minami, A. Kikuchi, Y. Futamura, Exposure evaluation of adult male Japanese smokers switched to a heated cigarette in a controlled clinical setting, Regul. Toxicol. Pharmacol. 69 (3) (2014) 338–347, https://doi.org/10.1016/j.yrtph.2014.04.016, 2014.
[27] F. Lüdicke, S.M. Ansari, N. Lama, N. Blanc, M. Bosilkovska, A. Donelli, P. Picavet, G. Baker, C. Haziza, M. Peitsch, R. Weitkunat, Effects of switching to a heat-not-Burn tobacco product on biologically relevant biomarkers to assess a candidate modified risk tobacco product: a randomized trial, Cancer Epidemiol. Biomarkers Prev. 28 (11) (2019) 1934–1943, https://doi.org/10.1158/1055-9965.EPI-18-0915, 2019.
[28] C.J. Shepperd, N. Newland, A. Eldridge, L. Haswell, F. Lowe, E. Papadopoulou, O. Camacho, C.J. Proctor, D. Graff, I. Meyer, Changes in levels of biomarkers of exposure and biological effect in a controlled study of smokers switched from conventional cigarettes to reduced-toxicant-prototype cigarettes, Regul. Toxicol. Pharmacol. 72 (2) (2015) 273–291, https://doi.org/10.1016/j.yrtph.2015.04.016, 2015.
[29] M.W. Ogden, K.M. Marano, B.A. Jones, W.T. Morgan, M.F. Stiles, Switching from usual brand cigarettes to a tobacco-heating cigarette or snus: part 2. Biomarkers of exposure, Biomarkers 20 (6-7) (2015) 391–403, https://doi.org/10.3109/1354750X.2015.1094134, 2015.
[30] G. O'Connell, D.W. Graff, C.D. D'Ruiz, Reductions in biomarkers of exposure (BoE) to harmful or potentially harmful constituents (HPHCs) following partial or complete substitution of cigarettes with electronic cigarettes in adult smokers, Toxicol. Mech. Methods 26 (6) (2016) 443–454, https://doi.org/10.1080/15376516.2016.1196282, 2016.
[31] E.K. Round, P. Chen, A.K. Taylor, E. Schmidt, Biomarkers of tobacco exposure decrease after smokers switch to an E-Cigarette or nicotine gum, Nicotine Tob. Res. 21 (9) (2019) 1239–1247, https://doi.org/10.1093/ntr/nty140, 2019.
[32] J. Jay, E.L. Pfaunmiller, N.J. Huang, G. Cohen, D.W. Graff, 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. 22 (8) (2020) 1285–1293, https://doi.org/10.1093/ntr/ntz206, 2020.
[33] M.L. Goniewicz, M. Gawron, D.M. Smith, M. Peng, P. Jacob 3rd, N.L. Benowitz, Exposure to nicotine and selected toxicants in cigarette smokers who switched to electronic cigarettes: a longitudinal within-subjects observational study, Nicotine Tob. Res. 19 (2) (2017) 160–167, https://doi.org/10.1093/ntr/ntw160, 2017.
[34] H. McRobbie, A. Phillips, M.L. Goniewicz, K.M. Smith, O. Knight-West, D. Przulj, P. Hajek, Effects of switching to electronic cigarettes with and without concurrent smoking on exposure to nicotine, carbon monoxide, and acrolein, Cancer Prev. Res. Phila. (Phila) 8 (9) (2015) 873–878, https://doi.org/10.1158/1940-6207.CAPR-15-0058, 2015.
[35] K. Pulvers, A.S. Emami, N.L. Nollen, D.R. Romero, D.R. Strong, N.L. Benowitz, J. S. Ahluwalia, Tobacco consumption and toxicant exposure of cigarette smokers using electronic cigarettes, Nicotine Tob. Res. 20 (2) (2018) 206–214, https://doi.org/10.1093/ntr/ntw333, 2018.
[36] D.K. Hatsukami, E. Meier, B.R. Lindgren, A. Anderson, S.A. Reisinger, K.J. Norton, L. Strayer, J.A. Jensen, L. Dick, S.E. Murphy, S.G. Carmella, M.K. Tang, M. Chen, S. S. Hecht, R.J. O'connor, P.G. Shields, A randomized clinical trial examining the effects of instructions for electronic cigarette use on smoking-related behaviors and biomarkers of exposure, Nicotine Tob. Res. 22 (9) (2020) 1524–1532, https://doi.org/10.1093/ntr/ntz233, 2020.
[37] A.S. Cravo, J. Bush, G. Sharma, R. Savioz, C. Martin, S. Craige, T. Walele, A randomised, parallel group study to evaluate the safety profile of an electronic vapour product over 12 weeks, Regul. Toxicol. Pharmacol. 81 (Suppl 1) (2016) S1–S14, https://doi.org/10.1016/j.yrtph.2016.10.003, 2016.
[38] T. Walele, J. Bush, A. Koch, R. Savioz, C. Martin, G. O'Connell, Evaluation of the safety profile of an electronic vapour product used for two years by smokers in a real-life setting, Regul. Toxicol. Pharmacol. 2018 (92) (2018) 226–238, https://doi.org/10.1016/j.yrtph.2017.12.010.
[39] L. Shahab, M.L. Goniewicz, B.C. Blount, J. Brown, A. McNeill, K.U. Alwis, J. Feng, L. Wang, R. West, Nicotine, carcinogen, and toxin exposure in long-term E-Cigarette and nicotine replacement therapy users: a cross-sectional study, Ann. Intern. Med. 166 (6) (2017) 390–400, https://doi.org/10.7326/M16-1107, 2017.
[40] M.L. Goniewicz, D.M. Smith, K.C. Edwards, B.C. Blount, K.L. Caldwell, J. Feng, L. Wang, C. Christensen, B. Ambrose, N. Borek, D. van Bemmel, K. Konkel, G. Erives, C.A. Stanton, E. Lambert, H.L. Kimmel, D. Hatsukami, S.S. Hecht, R. S. Niaura, M. Travers, et al., Comparison of nicotine and toxicant exposure in users of electronic cigarettes and combustible cigarettes, JAMA Netw Open. 1 (8) (2018) e185937, https://doi.org/10.1001/jamanetworkopen.2018.5937, 2018.
[41] D. Oliveri, Q. Liang, M. Sarkar, Real-world evidence of differences in biomarkers of exposure to select harmful and potentially harmful constituents and biomarkers of potential harm between adult e-vapor users and adult cigarette smokers, Nicotine Tob. Res. 22 (7) (2020) 1114–1122, https://doi.org/10.1093/ntr/ntz185, 2020.
[42] D. Ye, S. Gajendra, G. Lawyer, N. Jadeja, D. Pishey, S. Pathagunti, J. Lyons, P. Veazie, G. Watson, S. McIntosh, I. Rahman, Inflammatory biomarkers and growth factors in saliva and gingival crevicular fluid of e-cigarette users, cigarette smokers, and dual smokers: A pilot study. [published online ahead of print, 2020 Feb 12], J. Periodontol. (2020), https://doi.org/10.1002/JPER.19-0457, 2020; 10.1002/JPER.19-0457.
[43] P. Lorkiewicz, D.W. Riggs, R.J. Keith, D.J. Conklin, Z. Xie, S. Sutaria, B. Lynch, S. Srivastava, A. Bhatnagar, Comparison of urinary biomarkers of exposure in humans using electronic cigarettes, combustible cigarettes, and smokeless tobacco, Nicotine Tob. Res. 21 (9) (2019) 1228–1238, https://doi.org/10.1093/ntr/nty089, 2019.
[44] G. Bustamante, B. Ma, G. Yakovlev, K. Yershova, C. Le, J. Jensen, D.K. Hatsukami, I. Stepanov, Presence of the carcinogen N'-Nitrosonornicotine in saliva of E-cigarette users, Chem. Res. Toxicol. 31 (8) (2018) 731–738, https://doi.org/10.1021/acs.chemrestox.8b00089, 2018.
[45] A. Ghosh, R.D. Coakley, A.J. Ghio, M.S. Muhlebach, C.R. Esther Jr, N.E. Alexis, R. Tarran, Chronic E-Cigarette use increases neutrophil elastase and matrix metalloprotease levels in the lung, Am. J. Respir. Crit. Care Med. 200 (11) (2019) 1392–1401, https://doi.org/10.1164/rccm.201903-0615OC, 2019.
[46] F. Lüdicke, P. Picavet, G. Baker, C. Haziza, V. Poux, N. Lama, R. Weitkunat, Effects of switching to the menthol tobacco heating system 2.2, smoking abstinence, or continued cigarette smoking on clinically relevant risk markers: a randomized, controlled, open-label, multicenter study in sequential confinement and ambulatory settings (part 2), Nicotine Tob. Res. 20 (2) (2018) 173–182, https://doi.org/10.1093/ntr/ntx028, 2018.
[47] C. Haziza, G. de La Bourdonnaye, A. Donelli, D. Skiada, V. Poux, R. Weitkunat, G. Baker, P. Picavet, F. Lüdicke, Favorable changes in biomarkers of potential harm to reduce the adverse health effects of smoking in smokers switching to the menthol tobacco heating system 2.2 for 3 months (Part 2), Nicotine Tob. Res. 22 (4) (2020) 549–559, https://doi.org/10.1093/ntr/ntz084, 2020.
[48] M.W. Ogden, K.M. Marano, B.A. Jones, W.T. Morgan, M.F. Stiles, Switching from usual brand cigarettes to a tobacco-heating cigarette or snus: part 3. Biomarkers of biological effect, Biomarkers. 20 (6-7) (2015) 404–410, https://doi.org/10.3109/1354750X.2015.1094135, 2015.
[49] C.D. D'Ruiz, G. O'Connell, D.W. Graff, X.S. Yan, Measurement of cardiovascular and pulmonary function endpoints and other physiological effects following partial or complete substitution of cigarettes with electronic cigarettes in adult smokers, Regul. Toxicol. Pharmacol. 2017 (87) (2017) 36–53, https://doi.org/10.1016/j.yrtph.2017.05.002.
[50] M.A. S Song, J.L. Freudenheim, T.M. Brasky, E.A. Mathe, J.P. McElroy, Q. A. Nickerson, S.A. Reisinger, D.J. Smiraglia, D.Y. Weng, K.L. Ying, M.D. Wewers, P. G. Shields, Biomarkers of exposure and effect in the lungs of smokers, nonsmokers, and electronic cigarette users, Cancer Epidemiol. Biomarkers Prev. 29 (2) (2020) 443–451, https://doi.org/10.1158/1055-9965.EPI-19-1245, 2020.
[51] M. Tsai, M.A. Song, C. McAndrew, T.M. Brasky, J.L. Freudenheim, E. Mathé, J. McElroy, S.A. Reisinger, P.G. Shields, M.D. Wewers, Electronic versus combustible cigarette effects on inflammasome component release into human lung, Am. J. Respir. Crit. Care Med. 199 (7) (2019) 922–925, https://doi.org/10.1164/rccm.201808-1467LE, 2019.
[52] G. Scherer, Biomonitoring of inhaled complex mixtures–ambient air, diesel exhaust and cigarette smoke, Exp. Toxicol. Pathol. 57 (Suppl 1) (2005) 75–110, https://doi.org/10.1016/j.etp.2005.05.007, 2005.
[53] E.O. Gregg, E. Minet, M. McEwan, Urinary biomarkers of smokers' exposure to tobacco smoke constituents in tobacco products assessment: a fit for purpose approach, Biomarkers. 18 (6) (2013) 467–486, https://doi.org/10.3109/1354750X.2013.821523, 2013.
[54] G. Scherer, Suitability of biomarkers of biological effects (BOBEs) for assessing the likelihood of reducing the tobacco related disease risk by new and innovative tobacco products: a literature review, Regul. Toxicol. Pharmacol. 94 (2018) 203–233, https://doi.org/10.1016/j.yrtph.2018.02.002.
[55] S.D. Varella, R.A. Rampazo, E.A. Varanda, Urinary mutagenicity in chemical laboratory workers exposed to solvents, J. Occup. Health 50 (5) (2008) 415–422, https://doi.org/10.1539/joh.l7151, 2008.
[56] U.S. Environmental Protection Agency. 1,3-Butadiene. https://www.epa.gov/sites/production/files/2016-08/documents/13-butadiene.pdf.
[57] D.K. Arnett, R.S. Blumenthal, M.A. Albert, A.B. Buroker, Z.D. Goldberger, E. J. Hahn, C.D. Himmelfarb, A. Khera, D. Lloyd-Jones, J.W. McEvoy, E.D. Michos, M. D. Miedema, D. Muñoz, S.C. Smith Jr, S.S. Virani, K.A. Williams Sr, J. Yeboah, B. Ziaeian, 2019 ACC/AHA guideline on the primary prevention of cardiovascular disease: executive summary: a report of the american college of Cardiology/American heart association task force on clinical practice guidelines, J. Am. Coll.

Cardiol. 74 (10) (2019) 1376–1414, https://doi.org/10.1016/j.jacc.2019.03.009, 2019.
[58] D.J. Gordon, J.L. Probstfield, R.J. Garrison, J.D. Neaton, W.P. Castelli, J.D. Knoke, D.R. Jacobs Jr., S. Bangdiwala, H.A. Tyroler, High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies, Circulation. 79 (1) (1989) 8–15, https://doi.org/10.1161/01.cir.79.1.8, 1989.
[59] P.M. Ridker, C.H. Hennekens, B. Roitman-Johnson, M.J. Stampfer, J. Allen, Plasma concentration of soluble intercellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men, Lancet. 351 (9096) (1998) 88–92, https://doi.org/10.1016/S0140-6736(97)09032-6, 1998.
[60] I. Malik, J. Danesh, P. Whincup, V. Bhatia, O. Papacosta, M. Walker, L. Lennon, A. Thomson, D. Haskard, Soluble adhesion molecules and prediction of coronary heart disease: a prospective study and meta-analysis, Lancet. 358 (9286) (2001) 971–976, https://doi.org/10.1016/S0140-6736(01)06104-9, 2001.
[61] M. Madjid, I. Awan, J.T. Willerson, S.W. Casscells, Leukocyte count and coronary heart disease: implications for risk assessment, J. Am. Coll. Cardiol. 44 (10) (2004) 1945–1956, https://doi.org/10.1016/j.jacc.2004.07.056, 2004.
[62] J. Nowak, J.J. Murray, J.A. Oates, G.A. FitzGerald, Biochemical evidence of a chronic abnormality in platelet and vascular function in healthy individuals who smoke cigarettes, Circulation. 76 (1) (1987) 6–14, https://doi.org/10.1161/01.cir.76.1.6, 1987.
[63] E. Schwedhelm, A. Bartling, H. Lenzen, D. Tsikas, R. Maas, J. Brümmer, F. M. Gutzki, J. Berger, J.C. Frölich, R.H. Böger, Urinary 8-iso-prostaglandin F2alpha as a risk marker in patients with coronary heart disease: a matched case-control study, Circulation. 109 (7) (2004) 843–848, https://doi.org/10.1161/01.CIR.0000116761.93647.30, 2004.
[64] V.H. Murthy, E-cigarette use among youth and young adults: a major public health concern, JAMA Pediatr. 171 (3) (2017) 209–210, https://doi.org/10.1001/jamapediatrics.2016.4662, 2017.
[65] H.J. Roethig, B.K. Zedler, R.D. Kinser, S. Feng, B.L. Nelson, Q. Liang, Short-term clinical exposure evaluation of a second-generation electrically heated cigarette smoking system, J. Clin. Pharmacol. 47 (4) (2007) 518–530, https://doi.org/10.1177/0091270006297686, 2007.
[66] K. Frost-Pineda, B.K. Zedler, D. Oliveri, S. Feng, Q. Liang, H.J. Roethig, Short-term clinical exposure evaluation of a third-generation electrically heated cigarette smoking system (EHCSS) in adult smokers, Regul. Toxicol. Pharmacol. 52 (2) (2008) 104–110, https://doi.org/10.1016/j.yrtph.2008.05.016, 2008.
[67] K. Frost-Pineda, B.K. Zedler, D. Oliveri, Q. Liang, S. Feng, H.J. Roethig, 12-week clinical exposure evaluation of a third-generation electrically heated cigarette smoking system (EHCSS) in adult smokers, Regul. Toxicol. Pharmacol. 52 (2) (2008) 111–117, https://doi.org/10.1016/j.yrtph.2008.05.015, 2008.
[68] H.J. Roethig, S. Feng, Q. Liang, J. Liu, W.A. Rees, B.K. Zedler, A 12-month, randomized, controlled study to evaluate exposure and cardiovascular risk factors in adult smokers switching from conventional cigarettes to a second-generation electrically heated cigarette smoking system, J. Clin. Pharmacol. 48 (5) (2008) 580–591, https://doi.org/10.1177/0091270008315316, 2008.
[69] National Center for Chronic Disease Prevention and Health Promotion (US) Office on Smoking and Health, The Health Consequences of Smoking—50 Years of Progress: A Report of the Surgeon General. Centers for Disease Control and Prevention (US), 2014. https://www.ncbi.nlm.nih.gov/books/NBK179276/.
[70] C. Hirn, Y. Kanemaru, T. Stedeford, T. Paschke, I. Baskerville-Abraham, Comparative and cumulative quantitative risk assessments on a novel heated tobacco product versus the 3R4F reference cigarette, Toxicol. Rep. 7 (2020) 1502–1513, https://doi.org/10.1016/j.toxrep.2020.10.019.
[71] D. Thorne, J. Whitwell, J. Clements, P. Walker, D. Breheny, M. Gaca, The genotoxicological assessment of a tobacco heating product relative to cigarette smoke using the in vitro micronucleus assay, Toxicol. Rep. 7 (2020) 1010–1019, https://doi.org/10.1016/j.toxrep.2020.08.013.