Harm Reduction Journal

METHODOLOGY

Open Access

Estimating lung cancer risk from e-cigarettes and heated tobacco products: applications of a tool based on biomarkers of exposure and of potential harm

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Abstract

Background Reliable epidemiological data are limited on the lung cancer risk of groups using e-cigarettes (ECIGs) and groups using heated tobacco products (HTPs).

Aim We describe a methodology to estimate the lung cancer risk of these groups according to their levels of biomarkers of exposure (BOEs) and of potential harm (BOPHs).

Methods Using 28 search terms for BOEs and 82 for BOPHs we sought publications reporting biomarker-specific data from North America and Europe comparing individuals who smoke cigarettes and individuals who use other established products (ETPs; cigars, pipes, smokeless tobacco (ST) and/or snuff/snus). Publications were selected using defined inclusion/exclusion criteria. Additionally using lung cancer relative risk (RR) estimates for users of specific ETPs derived from recent meta-analyses of epidemiological studies in these regions, we derived a regression model predicting the lung cancer RR by level of each specific biomarker. Separately for groups using ECIGs and using HTPs the lung cancer risk was then estimated by combining RR estimates for selected biomarkers. Our primary estimates only considered biomarkers statistically significantly ($p < 0.01$) related to lung cancer risk which showed no significant ($p < 0.01$) misfit to the RR of 1.0 for non-users—those with no use of ETPs, ECIGs or HTPs.

Results Based on 38 available publications, we extracted biomarker-specific data for ETPs for 56 BOEs within 21 of the 28 search terms considered and for 54 BOPHs within 29 of the 82. The regression slope fitted to the lung cancer risk was significant ($p < 0.01$) for 22 BOEs and six BOPHs. However, the predicted RR for non-users significantly ($p < 0.01$) differed from 1.0 for 16 of these biomarkers. We estimated the lung cancer RR for using ECIGs, derived from 30 estimates for 10 biomarkers, as 1.88 (95% CI 1.60–2.22), the excess risk (ER = RR − 1) being 6.8% of that for smokers of cigarettes. The RR estimate varied little in most sensitivity analyses conducted, but increased markedly after removing the restriction to significant model fit.

We estimated the lung cancer RR for using HTPs, combining estimates for four BOEs, as 1.44 (0.41–5.08), the ER being 3.4% of that for smokers of cigarettes.

Note that P.N. Lee Statistics and Computing Ltd. has now closed.

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Conclusions Despite some methodological limitations, our approach estimates risk when reliable epidemiological data are unavailable. Using the biomarkers considered here, the model indicates that the lung cancer risk for individuals using ECIGs is much lower than for smokers of cigarettes, and suggests that the risk for those using HTPs is also low. Research using additional data could add precision to these findings.

Keywords Lung cancer, E-cigarettes, Heated tobacco products, Biomarkers, Smoking, Modelling

Background

E-cigarettes (ECIGs) were developed in the early 2000's as a new tobacco product with a potentially reduced disease risk compared to cigarettes, and were first marketed in the USA in 2007 [1–3]. Since then, the prevalence of ECIG use has increased dramatically, though few current users in 2025 will have used them for much more than 10 years. Heated tobacco products (HTPs) were not introduced until about 10 years ago, and most current users in 2025 will have used them for less than 5 years. Particularly for ECIGs, and especially for ischaemic heart disease and stroke where the decline following quitting in the smoking-associated relative risk (RR) is relatively rapid [4, 5], useful epidemiological data could have been collected from a suitably designed large prospective study. This would compare risk for those smoking cigarettes at baseline who (a) subsequently continued to smoke cigarettes, (b) at differing times during follow-up, switched partly or wholly to the new product or (c) quit completely. However, no such study has been conducted, and good epidemiological data on risk, especially for lung cancer and chronic obstructive lung disease, where the decline in the RR on quitting is much slower [6, 7], may not be available for some time after 2025.

Given the paucity of epidemiological data, a critical population health question that remains unanswered is whether or not the use of ECIGs and HTPs is, in fact, safer than for smoking of cigarettes, and how to quantify the extent of the risk reduction. An early paper [3], which compared the risk of 12 nicotine-containing products, estimated that use of ECIGs caused only 5% of the harm of smoking of cigarettes. However, this was based on the opinions of experts using what was described as a "multi-criteria decision analysis approach", with a noted limitation of the study being "the lack of hard evidence for the harms of most products on most of the criteria". A number of other reviews, such as those of ECIGs [8–10], of HTPs [11, 12], or both [13–15], have used data from a range of biomarkers of exposure (BOEs) and of biomarkers of potential harm (BOPHs) to give insight into whether ECIGs and HTPs might in fact reduce disease risk, but generally do not reach any quantitative conclusions specifically for lung cancer. Some [12–15] only compare risk from use of ECIGs or HTPs with risk from smoking of cigarettes, and do not consider how biomarker levels and lung cancer risk varies between users of various established tobacco products (ETPs) other than cigarettes, including cigars, pipes, smokeless tobacco (ST) and snuff/snus.

Here, we apply a new approach to try to estimate quantitatively the excess lung cancer risk for users of ECIGs and HTPs relative to that for smokers of cigarettes. The applications of our approach combine data on the risk of lung cancer among individuals who smoke/smoked cigarettes and of users of the various ETPs, expressed relative to that of individuals who have never used tobacco products, and data on the levels of a range of BOEs and BOPHs measured in groups never using tobacco, using the ETPs, and using ECIGs and/or HTPs. For each biomarker considered, we attempt to use the available data for groups who have never used tobacco products and that for users of the different ETPs to fit a regression model relating the reduction in biomarker level, compared to that for those smoking cigarettes, to the corresponding reduction in excess RR (ER = RR-1). Then, for those biomarkers with a good fit to the model (showing a statistically significant ($p < 0.01$) relationship to lung cancer risk and no significant ($p < 0.01$) misfit to the RR of 1.0 for non-users of any product), we use the model to estimate the ER for ECIGs and for HTPs based on their levels of that biomarker. These estimates are then combined over a range of biomarkers taking into account the differing uncertainties of the individual ER estimates for the biomarkers considered.

Below we describe the methodology used to develop the equations used to predict the excess lung cancer RR for each biomarker, and to combine the estimates over a range of biomarkers showing a good model fit. We then use the methodology to predict the excess lung cancer RR based on data from publications which give levels of different sets of biomarkers for users of ECIGs and/or HTPs as well as for smokers of cigarettes.

We also make available to those interested both the database we have collected for the work described herein, as well as the methodological applications reported here. These applications also allow the user to extend our estimates given additional biomarker data, and also to derive RR estimates relating to ECIG and HTP use for diseases other than lung cancer, given suitable input data on RRs related to use of the various ETPs.

Methods

RRs for ETPs

A recent meta-analysis [16] provides separate estimates of the lung cancer RR for individuals smoking cigarettes, cigars and pipes based on epidemiological studies conducted in the US and Europe, and published in English in 2010–2020. For cigarettes, it estimated the RRs for smokers relative to individuals who have never smoked as 15.15 (95% CI 12.77–17.96) for North America and as 12.30 (9.77–15.49) for Europe, and the work described here is based on a combined estimate of 13.86 (11.32–16.96). For cigars and pipes, where there are far fewer data, the cited RRs of 2.73 (2.36–3.15) for cigar smoking and of 4.93 (1.97–12.32) for pipe smoking are used, each RR being estimated relative to individuals never using the product. Estimates of 1.59 (1.06–2.39) for individuals using ST and of 0.80 (0.40–1.30) for snuff/snus use are taken from another recent meta-analysis [17], based on publications in 1990–2020.

Identifying a candidate list of biomarkers for study

We identified (see Tables 1 and 2) BOEs and BOPHs based on recent publications [8, 11, 13, 15, 18–22] using 28 search terms for BOEs and 82 for BOPHs. We ignore biomarkers directly related to nicotine exposure, it being well known that nicotine levels in those smoking cigarettes and those using snus are quite similar despite their very different lung cancer risks [16, 17]. We also do not include carbon monoxide as, according to Braznell et al. (2024) [11], there is no association with lung cancer risk, or chemicals related to propylene glycol and glycol, as

Table 1 Search results for BOE data for ETPs

	Search terms used	Hits from search	Possibly relevant from abstract	Studies providing data	Numbers of specific biomarkers considered
1	Acrylamide	7	1	5	5
2	Acrylonitrile	15	6	5	3
3	Aminobiphenyl	6	3	3	1
4	Anabasine	22	4	7	2
5	Anatabine	15	5	7	2
6	Benzene	40	3	3	3
7	Bromopropane	0	0	0	0
8	Butadiene	18	3	2	2
9	Carbon disulfide	0	0	1	1
10	Cyanide	15	1	0	0
11	Dimethylformamide	1	1	1	1
12	Fluorene	6	1	5	4
13	Furan	10	0	1	3
14	Hydrogen cyanide	8	0	0	0
15	Isoprene	6	1	1	1
16	Mercapturic acid	9	3	5	5[1]
17	Naphthalene	30	1	7	3
18	Naphthylamine	3	0	0	0
19	Nitrosodimethylamine	9	0	0	0
20	NNAL	54	25	14	2
21	NNK	112	17	1	1
22	NNN	103	9	0	0
23	Phenanthrene	21	1	4	7
24	Pyrene	77	6	1	1
25	Styrene	25	1	2	3
26	Toluene	27	0	5	3
27	Trichloroethylene	3	0	0	0
28	Xylene	15	1	2	3
	Total	657	93	20	56[1]

[1] Excluding CEMA, which is the same chemical as CYMA considered in search term 2

Table 2 Search results for BOPH data for ETPs

	Search terms used	Hits from search	Possibly relevant from abstract	Studies providing data	Number of specific biomarkers considered
1	Adhesion molecule	22	3	2	1
2	Albumin	34	2	0	0
3	Antioxidant capacity	14	0	0	0
4	Ascorbic acid	36	3	0	0
5	Augmentation index	8	0	0	0
6	Basophil	11	0	0	0
7	Binding protein	221	2	0	0
8	Bleeding on probing	30	1	1	1
9	Blood pressure	324	38	5	4
10	Body weight	252	39	16	5
11	Bone morphogenetic protein	1	0	0	0
12	Catalase	35	0	0	0
13	Cholesterol	117	30	5	6
14	Circulating endothelial precursor cell	1	0	0	0
15	Clinical attachment level	32	1	0	0
16	Club cell protein	1	0	0	0
17	C-reactive protein	14	6	2	1
18	Dehydrothromboxane	2	0	0	0
19	Deoxyguanosine	17	0	1	2
20	Diffusion capacity	16	1	0	0
21	Endothelin	3	0	0	0
22	Eosinophil	50	1	0	0
23	Epithelial cell	267	1	0	0
24	FEV1	30	4	1	1
25	Fibrinogen	28	10	5	1
26	Functional residual capacity	5	0	0	0
27	Galectin	2	0	0	0
28	Gingival inflammation index	25	5	0	0
29	Glucose	119	20	0	0
30	Glutathione	80	1	1	2
31	Glycation	16	0	0	0
32	Growth factor	224	4	0	0
33	Haematocrit/hematocrit	20	5	1	1
34	Haemoglobin/hemoglobin	113	21	1	1
35	Heart rate	210	10	1	1
36	Homocysteine	2	1	2	1
37	Hydrogen peroxide	29	0	0	0
38	Inflammatory cell	92	2	0	0
39	Interferon	15	1	0	0
40	Interleukin	88	4	2	1
41	Isoprostane	16	4	3	5
42	Leukotriene	4	1	1	1
43	Ligand	54	0	0	0
44	Lung capacity	60	4	0	0
45	Lymphocyte	102	5	0	0
46	Macrophage	46	0	0	0
47	Malondialdehyde	40	1	0	0
48	Matrix metallopeptidase	10	1	0	0

Table 2 (continued)

	Search terms used	Hits from search	Possibly relevant from abstract	Studies providing data	Number of specific biomarkers considered
49	Microbiological status	22	0	0	0
50	Monocyte	18	2	0	0
51	Myeloperoxidase	36	1	0	0
52	Neurotrophic factor	10	0	0	0
53	Neutrophil	36	1	0	0
54	Nitric oxide	51	2	0	0
55	Nitrotyrosine	3	0	1	2
56	Nonenal	2	0	0	0
57	Oxygen saturation	15	1	0	0
58	Periodontal pocket depth	31	5	2	2
59	Plaque	110	19	2	2
60	Plasminogen activator inhibitor	5	1	1	2
61	Platelet	64	3	2	3
62	Polymorphonuclear cell	23	1	0	0
63	Prostaglandin	27	2	1	1
64	Protein carbonyl	8	0	0	0
65	Pulse wave velocity	25	0	0	0
66	Red blood cell count	8	0	0	0
67	Residual volume	15	1	0	0
68	Selectin	7	0	0	0
69	Serpine	4	1	0	0
70	Sister chromatid exchange	24	1	1	1
71	Squalene	1	0	0	0
72	Stem cell factor	9	0	0	0
73	Superoxide dismutase activity	31	0	0	0
74	Thymidine glycol	0	0	0	0
75	Tooth mobility	5	3	0	0
76	Triglyceride	64	19	3	1
77	Tumor necrosis factor	40	2	0	0
78	Uteroglobin	0	0	0	0
79	Vitamin E	17	4	1	1
80	Von Willebrand factor	43	0	1	1
81	Waist circumference	33	7	2	2
82	White blood cell count	16	4	1	1
	Total	3,741	312	25	54

these predominately result from use of ECIGs and not from use of ETPs.

Initial searches—for biomarker data for ETPs

For each search term listed in Tables 1 and 2, we carried out Medline searches for papers including (in their title or abstract) the search term and also one or more references to pipe, cigar, ST, snus or snuff, it being clear that studies only investigating cigarettes or smoking (unidentified) are useless for comparing biomarker levels between the products of interest. Thus, for example for BOE search term 1 (acrylamide), we used the following query:

(Acrylamide) AND ("tobacco, smokeless" [Mesh Terms] OR "Pipe Smoking" [Mesh Terms] OR "cigar" [All Fields] OR "snus" [All Fields] OR "pipe" [All Fields] OR "snuff" [All Fields] OR "smokeless" [All Fields])

Examination of the output from the initial searches

For each search with non-zero hits, PNL first examined the search output, his conclusions then being checked by KJC. This output consists, for each paper, of the full

reference, the title, the authors and the abstract. From this information, we selected papers appearing likely to provide relevant data. We used the following exclusion criteria to reject papers:

- no relevant results for any selected biomarker;
- results only available for a single ETP (results for water pipes being ignored);
- no results specifically for smokers of cigarettes (for example. the paper referred to smoking but did not clarify if this was smoking of cigarettes or of any product);
- results only available for heavy (> 10 per day) smokers of cigarettes;
- the paper was nothing to do with tobacco;
- described only a study on cells or animals;
- described only a study on diseased patients;
- described only a study on a population with specific exposures;
- concerned a study not in North America or Europe;
- considered marker levels measured only in the product and not in the individual;
- described results of a trial in which users were tested after use of a range of products; or
- was a review, rather than a study reporting biomarker levels.

Examination of the papers on ETPs identified as possibly relevant

Each paper identified from the title and abstract as possibly having relevant data was then obtained and examined, initially by PNL and then by KJC. For each paper which they agreed had no relevant data, a reason for exclusion was given, the remaining papers then being passed on for possible data entry. The papers passed on were then examined in more detail, with further exclusions and reasons for exclusion agreed by PNL and KJC.

Entry of biomarker data for ETPs

For each paper meeting our inclusion criteria, we entered the following data: reference ID, study location (country), year of publication, sex(es) for which data were available, and for each biomarker with data available, the biomarker search number and name, biomarker short and long name, data source in the paper (for example the Table or Figure number), matrix (for example urine), units, and means (geometric or not). For smoking of cigarettes, the data we recorded for each biomarker included the number of subjects for which data were available, the definition of use, and the mean level and either the standard deviation or the 95% CI. We entered similar information, where available, for non-users of tobacco, for each other ETP, and for ECIGs and HTPs. We also recorded details of biomarker data not entered and why, and any relevant comments.

Predicting the lung cancer RR based on levels of a given biomarker

For each biomarker, we used the available data from each study on biomarker levels for cigarettes, other ETPs and non-users, and the data on lung cancer RRs by ETP to derive a formula predicting the lung cancer RR relative to non-users (and its standard error) corresponding to any given biomarker level.

The model used was:

$$\ln\left(\frac{RR_C}{RR_p}\right) = \beta.\ln\left(\frac{BioM_C}{BioM_P}\right) + \varepsilon$$

where RR_C is the RR for cigarette smoking,

RR_P is the RR for product P (cigars, pipe, ST or snuff/snus),

$BioM_C$ is the mean value of the biomarker for individuals smoking cigarettes,

$BioM_P$ is the mean value of the biomarker for individuals using product P,

β is the estimate of the slope relating the log RR ratios to the log biomarker ratios, and

ε is the error term, assumed to be an independent and identically distributed normal variable with mean 0 and variance σ^2.

It should be noted that we used:

1) ratios of the biomarkers, so that the units used for the measurement of the biomarker would be irrelevant;
2) a model with no intercept so that when the product was set to be cigarettes it would be expected that the log RR ratio would be zero;
3) RRs associated with use of the different products taken from the literature as described above in the section "RRs for ETPs"; and
4) a model fitted using weighted estimates of the log ratios of the biomarker means. First, we calculated the standard error for each biomarker mean from the data available. Next, we used the approximation $V[\log(X)] \approx E[X]^{-2}V[X]$ to estimate the variance of the log biomarker mean. Finally we set the weighting to be the inverse of the sum of variances for the two log biomarker means forming the ratio.

Combining predictions for a set of biomarkers

For the purposes of combining predictions for a set of biomarkers we mainly restricted attention to "good" biomarkers showing a significant slope ($p < 0.01$) in the

fitted model (suggesting a real relationship between the biomarker level and the RRs), and where the model did not show a significant ($p < 0.01$) misfit to the RR of 1.0 for non-users.

We then derived the predicted values of log RR as a weighted average of the individual predictions, using the inverse of the square of the SEs as the weighting. This procedure also returned the standard error of the predicted value, a t-test on whether the log RR was 0 (equivalent to no excess RR), and lower and upper CIs. Finally, we obtained estimates of the RR with lower and upper 95% CIs by taking e to the power of the log values, and using an approximate standard error estimated from the difference between the high and low 95% CIs divided by 2*Normal Inverse (0.975).

We then used this methodology to produce combined RR estimates for ECIGs and for HTPs and to check the validity of the modelling for non-users.

Sensitivity analyses

We carried out five sets of sensitivity analyses, the first four used in relation to the combined ECIG and HTP estimates and all five being used in relation to the combined estimate for non-users.

Sensitivity analysis 1 restricted attention to biomarkers where there were either at least four, or at least six, separate estimates of the ratio of the biomarker values for use of another product (cigars, pipes, ST, or snuff/snus) compared to that for smoking cigarettes.

Sensitivity analysis 2 restricted attention to biomarkers where the fitted β value was statistically significant at least at $p < 0.05$ or $p < 0.001$, or did not restrict attention at all on the significance of the fitted β value.

Sensitivity analysis 3 restricted attention to data from studies with "well-defined groups", where the definition of the smoking groups was clearly exclusive; so that those classified as currently using one of the five ETPs (cigarettes, cigars, pipes, ST, snuff/snus) were not currently using any of the others.

Sensitivity analysis 4 varied the RR for snuff/snus from the value of 0.8 reported above in the section "RRs for ETPs" to the value of 1.00 as it might be considered unlikely that use of snuff/snus actually reduces the risk of lung cancer.

Sensitivity analysis 5 gave separate estimates based either on BOEs or on BOPHs.

Note that while sensitivity analyses 1, 2 and 5 use the fitted regression coefficients from the main model, sensitivity analyses 3 and 4 use alternative regression coefficients, either those fitted to the data from the studies with "well-defined groups" or those using the alternative RR estimate for individuals using snuff/snus.

Further searches—for biomarker data for ECIGs and HTPs

For each broad search term with relevant data identified for ETPs, we carried out further Medline searches for publications that included (in their title or abstract) the search term and one or more references to ECIGs or HTPs. Thus, for example for BOE search term 1 (acrylamide), the search used the following query:

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(Acrylamide) AND ((((((((((("Vaping"[Mesh]) OR (vaping)) OR (ECig)) OR (E-cig)) OR (Ecigarette)) OR (E-cigarette)) OR (Vape)) OR (vapes)) OR (ENDS)) OR ("electronic nicotine delivery system")) OR (electronic nicotine delivery system)) OR ("Electronic Nicotine Delivery Systems"[Mesh])) OR ((((((((((Heated tobacco product) OR (heated tobacco products)) OR (HTP)) OR ("heat not burn")) OR (tobacco heating system)) OR ("tobacco heating system")) OR (electrically heated cigarette smoking system)) OR ("electrically heated cigarette smoking system")) OR (carbon heated tobacco product)) OR ("carbon heated tobacco product")) OR (carbon-heated tobacco product)) OR ("carbon-heated tobacco product")))
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Examination of the output from the further searches

For each search with non-zero hits, KJC examined the output first, and PNL then checked it, this output, for each paper, consisting of the full reference, title, authors and abstract. From this information, papers appearing likely to provide relevant data were selected. A paper was excluded if:

- it contained no relevant results for any specific biomarker where regression equations had been derived above;
- it provided no results for individuals smoking cigarettes;
- no results were available for individuals using either ECIGs or HTPs; or
- for any other reason listed above under "Examination of the output from the initial searches".

Examination of the papers on ECIGs and HTPs and entering the data

The procedures were as described above in the corresponding sections for ETPs.

Estimating the lung cancer RR for use of ECIGs and HTPs and conducting sensitivity analyses

The available data for ECIGs and NTPs were then used to estimate the lung cancer RR and carry out sensitivity analyses using the regression models developed from the ETP data as described above.

Results

Initial examination of the searches for biomarker data for ETPs

As shown in Tables 1 and 2 there were 657 hits from the 28 searches for data on BOEs, and 3,741 hits from the 82 searches for data on BOPHs. Initially PNL identified 442 papers as possibly relevant from examination of the abstracts, but on checking by KJC this reduced to 405. 183 of these were duplicates identified in multiple searches, leaving 222 papers to be obtained.

Initial examination of the papers on ETPs

Among the 222 papers, full texts for two studies [23, 24] could not be accessed, and initial examination of the 220 obtained suggested that 74 could be considered for data entry, the excluded papers being listed in Additional File 1 Table 1, with reasons for exclusion given, the most common reason being that the paper contained no relevant results.

Data entry from the initial searches

As shown in Additional File 1 Table 2, we excluded about half the papers considered after initial examination, common reasons for exclusion being that smoking was undefined or included multiple products. Finally, as shown in Table 3, data were entered from 38 of the papers identified and also from two additional papers [20, 21] obtained from other sources which provided data for ECIGs and HTPs. As can be seen in Table 3, all the papers provided biomarker results for groups smoking cigarettes, with all but five providing results for non-users of tobacco. Each paper provided results for at least one ETP of interest, with eight giving results for cigars, four for pipes, 18 for ST and 14 for snus or snuff. One paper identified in the searches [19] provided data for ECIGs and HTPs, as well as for cigarettes, ST and non-use. As shown in Tables 1 and 2, relevant biomarker data proved to be available for 56 BOEs, falling within 21 of the 28 groups considered, and for 54 BOPHs, falling within 29 of the 82 groups considered. The data recorded for each study and each biomarker are given in Additional File 2.

Main regression equations for each biomarker fitted to the ETP data

Additional File 2 also gives the results of the regression equations for each biomarker. Tables 4 (BOE) and 5 (BOPH), with the summary for each biomarker giving its search number, the biomarker name, the number of observations on which the regression analysis was based, and the fitted slope, together with its standard error, t value and p value, and finally the maximum significance for the non-users. For each table the results are shown in increasing order of the p value, so the biomarkers with the strongest relationship between the biomarker level and the RR appear first. Note that results for the BOE CYMA, identified under search term 2, and the BOE CEMA, identified under search term 16, were combined as CYMA under search term 2 as CYMA and CEMA are alternative abbreviations for the same chemical, and the majority of the results were found under search term 2. Note also that results for one particular BOPH, nitrotyrosine (search term 55), do not appear in Table 5 as the means were the same in each tobacco group for which data were available, so a slope could not be fitted.

As shown in Table 4, 22 of the 56 BOEs considered have a fitted slope significant at $p < 0.01$, including five of the seven markers for phenanthrene and all three of the markers for naphthalene. Further restricting attention to those that did not show a significant ($p < 0.01$) misfit from an RR of 1.0 for non-users, leaves the nine BOEs underlined in Table 4:—two markers of acrylamide (AAMA and GAMA), two of acrylonitrile (CYMA and HEMA), two of phenanthrene (2/3-OH-Phe and 4-OH-Phe), and one each of anabasine (NAB), anatabine (NAT) and toluene (Toluene).

As seen in Table 5, six of the 53 BOPHs have a fitted slope significant at $p < 0.01$, each relating to a different search term. Three of these showed no significant ($p < 0.01$) misfit for non-user:—markers of adhesion molecule (SICAM-1), interleukin (IL-6) and isoprostane (8,12-iso-iPF2α-VI).

Further details of the regressions are given in Additional File 3, which presents separate plots for each biomarker studied. In the plots the x-axis is the ratio of the mean biomarker value for those smoking cigarettes to that for those using the other ETP, while the yaxis is the lung cancer RR (relative to never smokers) for those smoking cigarettes relative to those using the other ETP. The scales for the axes are both in logarithmic form as the regression is based on the logs of those values. The plots show the fitted regression line and show the individual points to which the line is fitted, with different colours distinguishing the different other products (pipe, snuff, cigar, ST and none). On the regression line the default point for those smoking cigarettes is marked as a black circle.

Figures 1 and 2 give examples of the plots. The first is for the acrylonitrile BOE CYMA where the mean values for those using cigarettes, cigars, ST and never tobacco reduce in correspondence with the lung cancer RRs for the products and the regression slope is highly significant ($p = 0.0000$). The second is for the BOE anabasine where

Table 3 Papers accepted for data entry from initial searches

Reference	Number of biomarker groups considered from searches			Whether data available for users of specific products							
	BOE	BOPH	Total	Cigarette	None	Cigar	Pipe	ST	Snus/snuff	ECigs	HTPs
Andersen et al. (2022) [38]	3		3	Yes	Yes			Yes		Yes[a]	
Benowitz et al. (2018) [39]	1		1	Yes				Yes			
Byhamre et al. (2023) [40]		1	1	Yes	Yes				Yes		
Campbell et al. (2015) [41]	6	1	7	Yes	Yes				Yes		
Chang et al. (2019) [28]	17		17	Yes	Yes	Yes					
Chang et al. (2021) [42]		5	5	Yes	Yes			Yes			
Chen et al. (2014) [43]	1	1	2	Yes	Yes	Yes					
Dai et al. (2023) [44]	5	1	6	Yes		Yes					
Eliasson et al. (1991) [30]		7	7	Yes	Yes				Yes		
Eliasson et al. (1995) [45]		7	7	Yes	Yes				Yes		
Eliasson et al. (2004) [46]		2	2	Yes	Yes				Yes		
England et al. (2003) [47]		1	1	Yes	Yes				Yes		
Giraud et al. (1995) [48]		1	1	Yes	Yes			Yes			
Giraud et al. (1995) [29]		2	2	Yes	Yes			Yes			
Hecht et al. (1991) [49]	1		1	Yes	Yes				Yes		
Jacob et al. (1993) [50]	2		2	Yes				Yes	Yes		
Jacob et al. (1999) [51]	2		2	Yes		Yes	Yes	Yes			
Krall et al. (1999) [52]		3	3	Yes	Yes	Yes	Yes				
Lange et al. (1990) [53]		1	1	Yes	Yes	Yes	Yes				
Marano et al. (2015) [54]		1	1	Yes	Yes			Yes			
Mushtaq et al. (2023) [55]		3	3	Yes	Yes			Yes			
Naufal et al. (2011) [56]	10	1	11	Yes	Yes			Yes			
Prasad et al. (2016) [31]	10	11	21	Yes	Yes				Yes		
Rezk-Hanna et al. (2022) [57]	2	5	7	Yes	Yes			Yes		Yes[a]	Yes[a]
Rodu et al. (2004) [58]		1	1	Yes	Yes				Yes		
Rostron et al. (2015) [59]	2	1	3	Yes	Yes			Yes			
Scherer et al. (2022) [18]	3		3	Yes	Yes				Yes	Yes[a]	Yes[a]
Scherer et al. (2022) [19]	3		3	Yes	Yes			Yes		Yes	Yes
Scherer et al. (2022) [20][b]	4		4	Yes	Yes			Yes		Yes	Yes
Scherer et al. (2023) [21][b]	4		4	Yes	Yes			Yes		Yes	Yes
Sharma et al. (2024) [60]		3	3	Yes	Yes	Yes					
Söderström et al. (2021) [61]		3	3	Yes	Yes				Yes		
Wei et al. (2016) [62]	2		2	Yes	Yes			Yes			
Wennmalm et al. (1991) [63]		3	3	Yes	Yes				Yes		
Wickholm et al. (2004) [64]		2	2	Yes	Yes				Yes		
Wulf et al. (1983) [65]		1	1	Yes	Yes	Yes	Yes				
Xia et al. (2021) [66]	3		3	Yes	Yes			Yes			
Zhu et al. (2013) [67]	1		1	Yes				Yes			
Total				38	33	8	4	18	14	6	5

[a] Data not included in illustrative examples

[b] Not in original search

Table 4 Summary of fitted regression slopes for BOEs shown in increasing order of p value

Search number (see Table 1)	Biomarker name	N[1]	Fitted slope	Std.Error	t[2]	p	Signif[3]
2 (16)[4]	CYMA	9	0.55	0.03	18.99	0.0000	0.0287
12	3-OH-Flu	7	1.08	0.06	16.93	0.0000	0.0008 + +
16	MHBMA	6	1.43	0.08	18.24	0.0000	0.0000 + + +
17	2-naphthol	8	1.89	0.15	12.73	0.0000	0.0000 + + +
26	o-Tol	6	1.8	0.08	21.77	0.0000	0.0000 + + +
1	AAMA	7	2.82	0.29	9.83	0.0001	0.012
8	DHBMA	4	6.36	0.24	26.88	0.0001	0.0014 + +
12	2-OH-Flu	6	1.4	0.11	12.73	0.0001	0.0000 + + +
16	3-HPMA	6	1.3	0.11	11.54	0.0001	0.0093
17	2-AN	6	0.99	0.09	10.93	0.0001	0.0000 + + +
23	1-OH-Phe	6	4.73	0.5	9.38	0.0002	0.0000 + + +
1	GAMA	4	3.4	0.18	19.11	0.0003	0.2193
17	1-naphthol	9	1.15	0.19	6.03	0.0003	0.0000 + + +
16	S-PMA	4	1.56	0.09	18.25	0.0004	0.0002 –-
4	NAB	8	0.84	0.15	5.77	0.0007	0.1396
2	HEMA	4	2.14	0.17	12.77	0.001	0.1813
23	3-OH-Phe	4	3.49	0.28	12.67	0.0011	0.0000 + + +
23	2/3-OH-Phe	4	2.71	0.26	10.53	0.0018	0.4838
5	NAT	8	0.61	0.13	4.8	0.002	0.1686
23	4-OH-Phe	4	3.34	0.34	9.86	0.0022	0.0889
23	2-OH-Phe	4	4.56	0.48	9.48	0.0025	0.0000 + + +
26	Toluene	2	2.14	0.02	93.2	0.0068	0.3521
1	Acrylamide Hb adducts	2	2.78	0.05	54.85	0.0116	0.3837
16	HMPMA	2	1.65	0.05	34.27	0.0186	0.1726
1	Glycidamide Hb adducts	2	3.42	0.11	30.58	0.0208	0.3677
1	GAMA2	2	3.44	0.18	19.28	0.033	0.2132
28	m/p-xylene	2	5.21	0.31	17.03	0.0373	0.2474
6	Ethylbenzene	2	3.68	0.25	14.54	0.0437	0.3239
8	MHBMA3	2	1.29	0.09	13.8	0.0461	0.4798
15	IPMA3	2	1.01	0.07	13.48	0.0472	0.4827
23	9-OH-Phe	2	1.32	0.1	13.24	0.048	0.1723
25	Styrene	2	2.94	0.25	11.67	0.0544	0.2545
6	Benzene	2	1.65	0.15	11.19	0.0568	0.0477
28	MHA34	2	1.59	0.15	10.94	0.058	0.48
11	AMCA	2	1.56	0.14	10.87	0.0584	0.475
28	MHA2	2	1.57	0.16	9.83	0.0646	0.4857
23	1/9-OH-Phe	2	1.6	0.17	9.16	0.0692	0.1888
21	NNK	2	− 1.17	0.14	− 8.5	0.0745	0.0000 + + +
25	MADA	2	3.03	0.37	8.29	0.0765	0.4795
12	2/3/9-OH-Flu	2	1.14	0.15	7.65	0.0827	0.1088
16	2-HPMA	2	2.9	0.39	7.38	0.0858	0.4737
2	CYHA	2	0.95	0.13	7.32	0.0864	0.496
25	PGA	2	3.83	0.54	7.08	0.0893	0.4821
24	3-OH-Bap	2	1.69	0.24	7.05	0.0897	0.2348
13	PeDCF	2	− 10.98	1.67	− 6.58	0.0961	0.2846
12	9-OH-Flu	2	4.07	0.92	4.42	0.1417	0.4885
13	HxCDF	2	− 18	4.88	− 3.69	0.1684	0.2049
4	Anabasine	13	0.39	0.3	1.33	0.2098	0.0308
9	TTCA	2	23.46	14.96	1.57	0.3614	0.1437

Table 4 (continued)

Search number (see Table 1)	Biomarker name	N[1]	Fitted slope	Std.Error	t[2]	p	Signif[3]
3	3-ABP	6	0.24	0.34	0.7	0.5136	0.0066
5	Anatabine	13	0.19	0.31	0.62	0.5493	0.0243
6	PMAC	4	1.12	1.77	0.63	0.5726	0.0000 + + +
20	NNAL	21	0.11	0.24	0.45	0.6574	0.0000 + + +
13	HpCDF	2	−8.79	15.94	−0.55	0.6792	0.0000 + + +
26	BMA	2	7.94	17.52	0.45	0.729	0.0827
20	HPB	2	−0.55	1.76	−0.32	0.8055	0.035

Underlined biomarkers are those used in the predictions showing no significant ($p < 0.01$) misfit to the RR for non-users

[1] Number of paired means for cigarettes and other products on which the estimate is based

[2] Student t-value for slope

[3] Greatest significance for non-users (+ + +, –- $p < 0.001$, + +, – $p < 0.01$)

[4] Seven paired means were identified for CYMA under search number 2 and two were identified for CEMA under search number 16. As CYMA and CEMA are alternative abbreviations for the same chemical, the results have been combined as CYMA for the purposes of estimating the slope and standard error

the fit to the model is poor, because levels for users of ST and snuff are higher than for smokers of cigarettes, despite the much higher lung cancer risk for smokers of cigarettes.

Initial examination of the searches for biomarker data for ECIGs and HTPs

As shown in Tables 6 and 7 there were 1,451 hits from the 21 searches for data on BOE, and 5,440 hits from the 29 searches for data on BOPH. Initially PNL identified 209 papers as possibly relevant from examination of the abstracts, but on checking by KJC this reduced to 182, 84 of which were duplicates identified in multiple searches, leaving 98 papers to be obtained.

Initial examination of the papers on ECIGs and HTPs

Initial examination of the papers suggested that 50 could be considered for data entry, the excluded papers being listed in Additional File 4 Table 1, with reasons for exclusion given. This additional file also cites seven exclusions of comments on these papers not considered elsewhere.

Data entry from the further searches

As shown in Additional File 4 Table 2, 21 papers considered after initial examination were excluded for various reasons. Finally, as shown in Table 8, data were available for ECIG and/or HTP users from 29 papers, including some considered in our earlier searches. Each paper provided biomarker results for those smoking cigarettes, with 24 providing results for non-users of tobacco, 27 for ECIGs and 7 for HTPs. The actual data recorded for each study and for each biomarker are given in Additional File 5.

Estimated lung cancer RRs for ECIGs

As shown in Table 9, the estimated RR (95% CI) for ECIG use, based on 30 individual estimates for 10 different biomarkers, is 1.88 (1.60–2.22), with the estimate very similar to this based on the 23 estimates for BOHs (1.88, 1.55–2.28) or on the 7 estimates for BOPHs (1.89, 1.23–2.91). The 29 estimates vary between 0.18 and 3.21, each indicating a much lower RR than the combined estimate of 13.86 for smoking of cigarettes. Indeed, the estimated excess risk (ER) of 0.88 for use of ECIGs is only about 6.8% of the ER of 12.86 for smoking of cigarettes.

Sensitivity analyses for ECIGs

Table 10 summarizes the results of the sensitivity analyses. While most results indicate little variation in the overall estimate for those using ECIGs, the estimates are substantially increased if the restriction to biomarkers with a slope that is significant at $p < 0.01$ is lifted. Indeed, with no restriction, the estimate is as high as 13.03 (95% CI 11.91–14.25). This estimate is based partly on five extremely high individual RR estimates; 340.62 for the BOPH marker deoxyguanosine, derived from data for one study [25], 155.44, 87.46 and 66.92 for the BOPH markers haematocrit, platelets and haemoglobin, based on data for another study [26] and 52.99 for a marker of the BOE carbon disulfide from data for a third study [27]. The implausibility of these five estimates, and the need to insist on the model fitting the data very well, is emphasised by various considerations.

First, as can be seen in Additional file 5, all these five estimates were derived using data only from a single study showing relatively small differences between biomarker levels for smokers of cigarettes, non-users and users of either cigars [28], chewing tobacco [29] or snuff [30, 31]. Second, when the fitted models were

Table 5 Summary of fitted regression slopes for BOPHs shown in increasing order of p value

Search number (see Table 2)	Biomarker name	N[1]	Fitted slope	Std.Error	t[2]	p	Signif[3]
1	sICAM-1	4	11.14	0.72	15.51	0.0006	0.1718
13	LDL-C	4	26.97	2.3	11.74	0.0013	0.0000 + + +
10	Obese %	8	−7.12	1.47	−4.86	0.0018	0.0000 + + +
25	Fibrinogen	10	27.26	6.28	4.34	0.0019	0.0000 + + +
40	IL-6	4	10.95	1.06	10.34	0.0019	0.3229
41	8,12-iso-iPF2α -VI	2	10.02	0.14	72.75	0.0088	0.1057
60	PAI-1	2	17.12	0.28	62.1	0.0102	0.2017
63	8-iso-PGF2α	2	6.46	0.12	52.54	0.0121	0.259
42	Leukotriene E4	2	4.06	0.12	33.85	0.0188	0.0127
13	HDL-C	6	−35.38	11.18	−3.17	0.0249	0.0000 + + +
55	Dinitrotyrosine	2	38.31	1.56	24.62	0.0258	0.1493
34	Hb	2	37.27	1.52	24.6	0.0259	0.1518
10	Overweight %	4	−13.97	3.66	−3.82	0.0316	0.0027 + +
59	PI 2.0 + %	2	8.12	0.42	19.14	0.0332	0.471
58	PPD	3	7.18	1.37	5.24	0.0345	0.3217
41	8-isoprostane	3	4.8	1.04	4.62	0.0437	0.1616
58	PD 5 + %	2	2.81	0.21	13.64	0.0466	0.4723
13	VLDL-C	2	16.15	1.2	13.49	0.0471	0.0000 –-
17	hs-CRP	4	10.39	3.31	3.14	0.0516	0.1115
80	VWF	2	27.72	2.72	10.18	0.0624	0.1796
9	SBP	6	−76.23	32.36	−2.36	0.0651	0.0020 + +
41	iPF2α-III	2	3.98	0.43	9.16	0.0692	0.1593
9	HyperT %	2	−8.39	0.97	−8.64	0.0734	0.3027
76	Triglycerides	6	4.43	2.06	2.15	0.084	0.0000 + + +
19	8-OH-2-deoxyg	2	24.24	3.84	6.31	0.1001	0.22
81	WHR	2	106.49	17.1	6.23	0.1014	0.1784
41	2,3-dinor-iPF2α-III	2	10.64	1.75	6.08	0.1037	0.0535
79	VIT E	2	−4.06	0.67	−6.04	0.1044	0.1928
33	Hematocrit	2	69.95	16.4	4.27	0.1466	0.3091
82	WBCC	2	7.4	1.76	4.2	0.1489	0.2418
36	Homocysteine	4	14.46	7.58	1.91	0.1527	0.0000 + + +
30	Glut-ox	2	−32.01	7.83	−4.09	0.1528	0.1707
61	11-dehydro-TXB2	2	11.41	3.47	3.29	0.1879	0.0126
13	High-C %	2	−11.73	3.65	−3.21	0.192	0.5
81	WCC	2	225.05	78.09	2.88	0.2126	0.0572
61	2,3-dinor-TXB2	2	3.31	1.17	2.83	0.2162	0.0024 + +
13	Total-C	6	17.61	15.3	1.15	0.3019	0.1924
60	tPA	2	−24.87	14.87	−1.67	0.3431	0.0013 + +
70	SCE	3	13.18	11.62	1.13	0.3745	0.2203
59	Plaque	3	−4.86	5.23	−0.93	0.4512	0.1621
9	DBP	4	55.93	74.97	0.75	0.5098	0.0000 + + +
30	Glut-red	2	−21.37	26.54	−0.81	0.5684	0.0000 + + +
10	BMI high %	2	−9.12	11.87	−0.77	0.5828	0.0098
61	PC	2	32.53	43.92	0.74	0.5941	0.4574
8	GB	3	16.38	26.81	0.61	0.6034	0.0000 + + +
24	FEV1 decline	5	1.86	3.62	0.51	0.6346	0.0051
41	iPF2α-VI	2	3.41	5.66	0.6	0.6544	0.4189
10	BMI	16	−4.84	21.3	−0.23	0.8233	0.0000 + + +
13	Chol high %	2	−6.55	25.83	−0.25	0.8419	NA

Table 5 (continued)

Search number (see Table 2)	Biomarker name	N[1]	Fitted slope	Std.Error	t[2]	p	Signif[3]
10	BWT	4	−13.39	76.46	−0.18	0.8721	0.0000+++
19	8-OH-guanosine	2	10.98	62.2	0.18	0.8887	0.1211
9	BP high %	2	4.67	29.76	0.16	0.9008	0.0299
35	Max HR	2	−12.53	259.39	−0.05	0.9693	0.1789

Underlined biomarkers are those used in the predictions showing no significant ($p < 0.01$) misfit to the RR for non-users

[1] Number of paired means for cigarettes and other products on which the estimate is based

[2] Student t-value for slope [3] Greatest significance for non-users (+++, -- $p < 0.001$, ++, – $p < 0.01$)

[3] Greatest significance for non-users (+++, -- $p < 0.001$, ++, – $p < 0.01$)

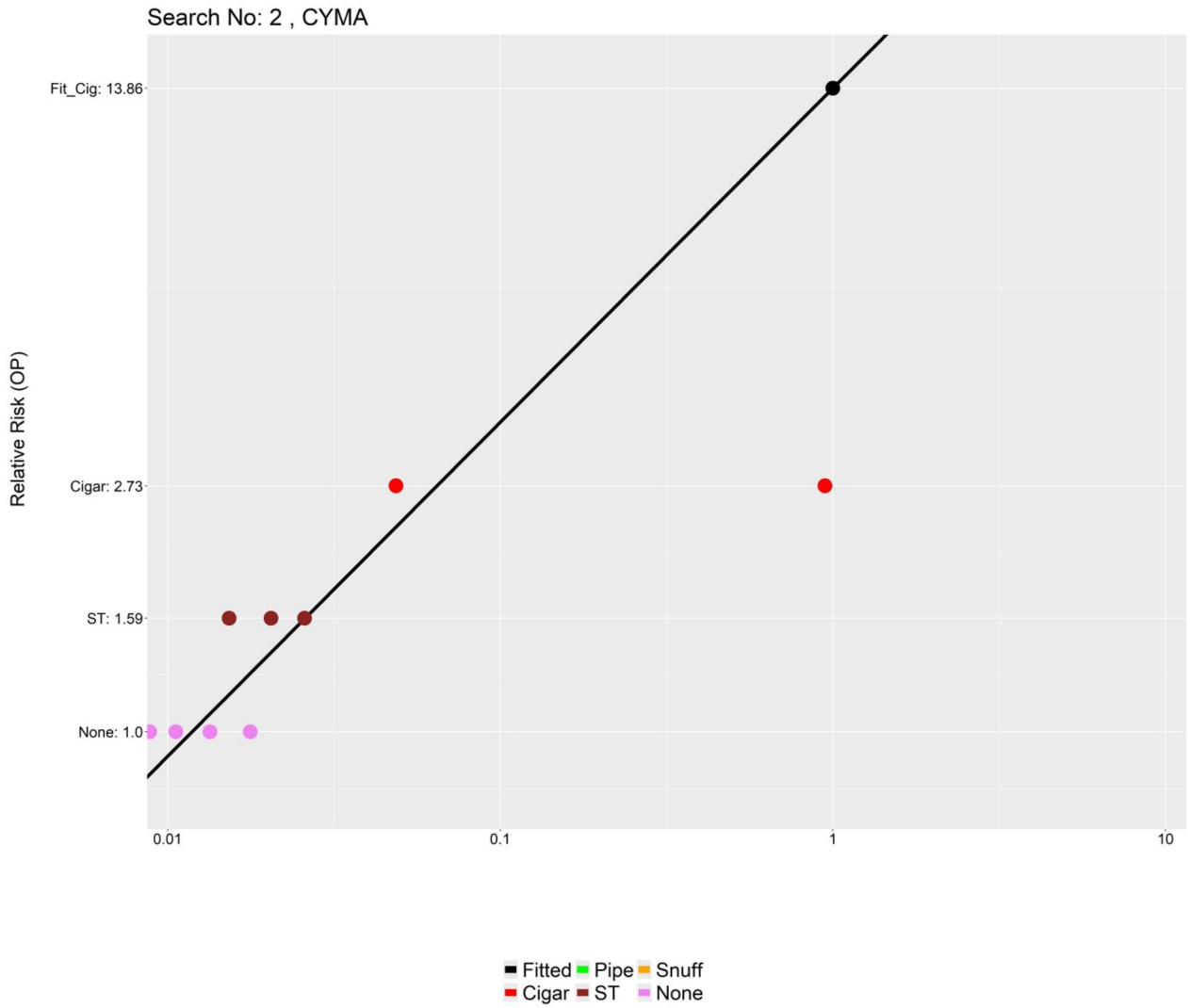

Fig. 1 Plot for biomarker CYMA

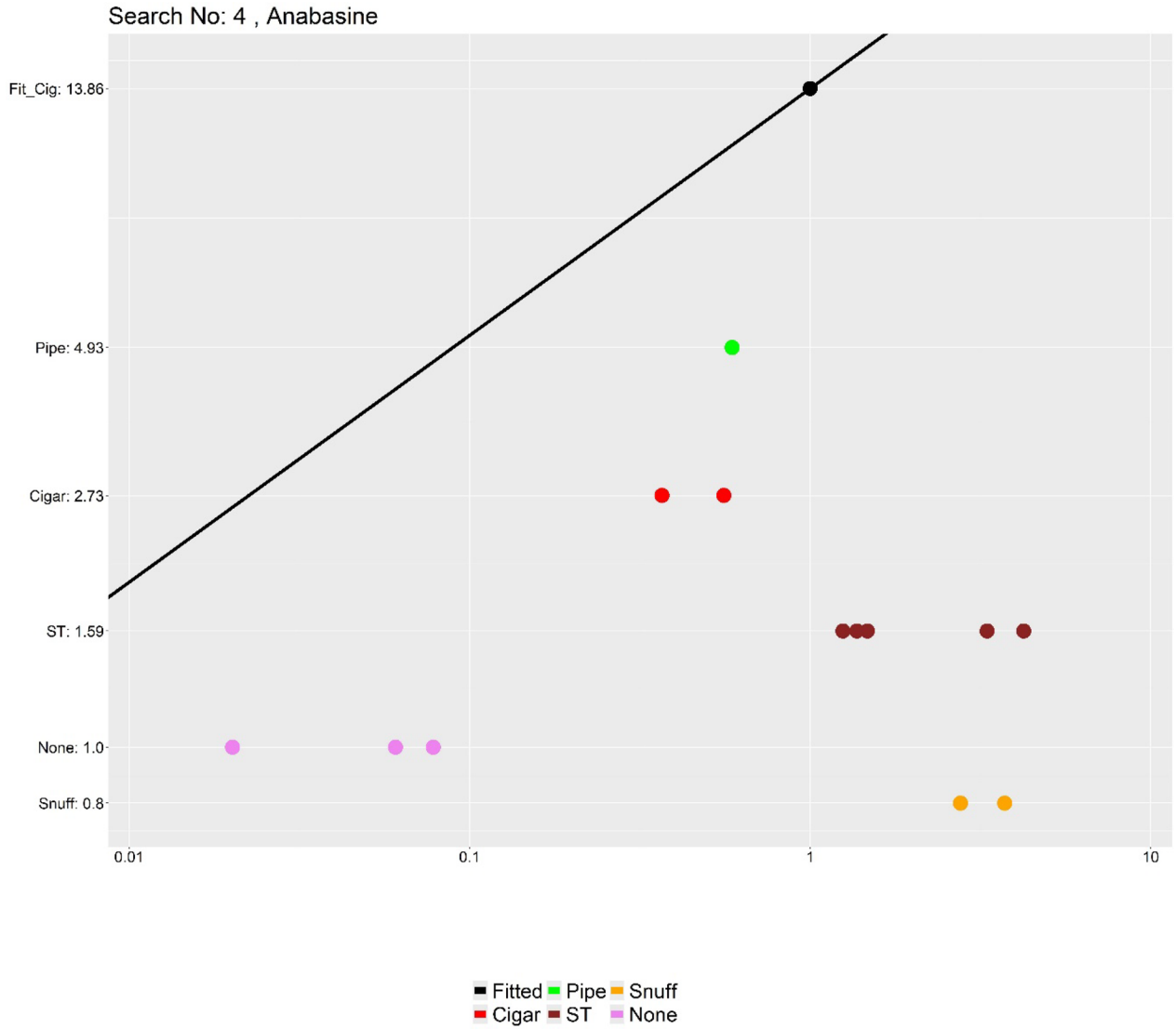

Fig. 2 Plot for Biomarker Anabasine

used to predict RRs for non-users based on the ratio of biomarker levels for non-users to those smoking cigarettes from the ECIG studies providing data for these five biomarkers, the 95% CI for the predicted RR for non-users never included 1.0 and in many cases was similar to or greater than that for those smoking cigarettes. For example non-user RR estimates were 24.39 (5.47–108.78) from data for one study of platelets [26], 18.87 (8.34–42.66) from another study of platelets [32], 13.86 (13.86–13.86) from a study of haemoglobin [33] and 10.03 (6.69–15.03) from a study of deoxyguanosine [34]. Interestingly, when there was no restriction to biomarkers based on the significance of their fitted slope, but there was a restriction to using data for studies with "well-defined groups" the ECIG RR estimate of 12.89 (11.73–14.16) dropped dramatically to 2.06 (1.74–2.43), mainly because the first four of the five very high RR estimates cited above occurred in studies where the study was not classified as having "well-defined groups" as the definition of the smoking groups was not clearly exclusive.

Table 6 Search results for BOE data for use of ECIGs and HTPs

	Search terms used	Hits from search	Possibly relevant from abstract	Studies providing data	Numbers of specific biomarkers considered
1	Acrylamide	99	7	3	2
2	Acrylonitrile	28	10	3 (+1)	3
3	Aminobiphenyl	6	2	1	1
4	Anabasine	16	2	4 (+1)	2
5	Anatabine	11	2	4 (+1)	2
6	Benzene	150	6	2 (+1)	1
8	Butadiene	40	5	2 (+1)	2
9	Carbon disulfide	12	0	2	1
11	Dimethylformamide	17	3	2	1
12	Fluorene	19	1	2 (+1)	2 (+1)
13	Furan	87	2	0	0
15	Isoprene	18	2	2	1
16	Mercapturic acid	41	7	2 (+1)	2 (+1)
17	Naphthalene	372	3	4 (+1)	3
20	NNAL	53	19	6 (+1)	1
21	NNK	33	2	0	0
23	Phenanthrene	134	1	2 (+1)	2 (+4)
24	Pyrene	129	2	0 (+1)	0 (+1)
25	Styrene	86	4	2	2
26	Toluene	83	2	3	2
28	Xylene	17	2	2	2
	Total	1,451	84	8 (+3)	32 (+7)

N.B. Search terms are numbered as in Table 1. Bracketed numbers relate to the three studies [19–21] providing data on ECIGs or HTPs found in our initial searches

Estimated lung cancer RRs for HTPs

As shown in Table 11 the estimated lung cancer RR for use of HTPs was 1.44 (95% CI 0.41–5.08). This estimate is based only on data identified in our initial searches, as no additional studies were found in our further searches that included biomarkers satisfying the requirements for inclusion in the combined estimates. Two studies [35, 36] not considered in our initial searches did provide data for blood pressure, body weight and cholesterol, while one study [37] provided data for blood pressure and body weight, and one [18] data for biomarkers of aminobiphenyl, naphthalene and toluene, but none of these biomarkers were considered adequate predictors to be considered in a combined analysis.

Availability of data and software

Statistical analyses were carried out using R Version 4.2.2 (2022-10-31) for linear modelling, Available on request are the data files we have used, and an R program which enables users to enter biomarker data from their own studies and estimate the lung cancer RR of the product(s) considered. This is also available as a Shiny App at https://roelee.shinyapps.io/Biomarkers/

Discussion

We have described a method to estimate the lung cancer risk for those using new tobacco products such as ECIGs and HTPs, based on North American and European data on biomarker levels for those smoking cigarettes and those using other ETPs, combined with recent estimates of the lung cancer RR of these products relative to individuals who do not use tobacco products. When combining data from multiple biomarkers, we have restricted attention to biomarkers which significantly ($p < 0.01$) fit the regression model we used, and which did not significantly ($p < 0.01$) misfit the estimate of 1.0 for non-users.

Table 7 Search results for BOPH data for use of ECIGs and HTPs

	Search terms used	Hits from search	Possibly relevant from abstract	Studies providing data	Number of specific biomarkers considered
1	Adhesion molecule	398	5	4	1
8	Bleeding on probing	37	3	0	0
9	Blood pressure	751	13	6	2
10	Body weight	764	12	11	4
13	Cholesterol	266	7	6	4
17	C-reactive protein	58	6	4	1
19	Deoxyguanosine	68	3	1	1
24	FEV1	27	3	0	0
25	Fibrinogen	132	3	3	1
30	Glutathione	328	1	1	2
33	Haematocrit/hematocrit	32	1	1	1
34	Haemoglobin/hemoglobin	198	3	3	1
35	Heart rate	430	7	0	0
36	Homocysteine	35	0	0	0
40	Interleukin	410	4	3	1
41	Isoprostane	17	4	4	1
42	Leukotriene	18	0	0	0
55	Nitrotyrosine	5	0	0	0
58	Periodontal pocket depth	11	1	0	0
59	Plaque	440	3	0	0
60	Plasminogen activator inhibitor	11	0	0	0
61	Platelet	424	6	2	1
63	Prostaglandin	137	5	1	1
70	Sister chromatid exchange	68	0	0	0
76	Triglyceride	102	3	3	1
79	Vitamin E	164	2	1	1
80	Von Willebrand factor	43	0	0	0
81	Waist circumference	14	0	0	0
82	White blood cell count	52	3	4	1
	Total	5,440	98	20	25

N.B. Search terms are numbered as in Table 2

Limitations

Below, we discuss some limitations of the work we have described. It should be made clear that many are not limitations of the methodology itself, but of our application of it, and could be overcome by additional research using our methods.

One limitation is the list of biomarkers considered, which some may regard as incomplete. The set of data considered could also be extended, perhaps by searching on detailed biomarker names as well as the broad search terms we considered (as listed in Tables 1 and 2). We did not consider variation in biomarker levels or lung cancer risk by sex, age, exposure to other hazards (such as those resulting from environmental and occupational exposure, including second-hand smoke and radon gas) or sub-types of tobacco product (for example, menthol versus non-menthol cigarettes, small versus large cigars, or different types of ST). We only considered risk of lung cancer. However, given up-to-date RR estimates for other common smoking-related diseases and data for additional biomarkers possibly related to these diseases, our software can readily be used to generate predicted RRs for ECIGs and HTPs.

Levels of some biomarkers we considered might result partly from exposures nothing to do with cigarettes, other ETPs, ECIGs or HTPs. In principle this

Table 8 Papers accepted for data entry from further searches

Reference	Number of biomarker groups considered from searches			Whether data available for use of specific products			
	BOE	BOPH	Total	Cigarettes	None	ECigarettes	HTPs
Andersen et al. (2022) [38][a]	3	0	3	Yes	Yes	Yes	
Anic et al. (2022) [27]	27	0	27	Yes		Yes	
Badea et al. (2019) [32]	0	11	11	Yes	Yes	Yes	
Caliri et al. (2020) [68]	0	1	1	Yes	Yes	Yes	
Carroll et al. (2018) [69]	1	0	1	Yes		Yes	
Christensen et al. (2021) [70]	0	5	5	Yes		Yes	
Cook et al. (2023) [71]	0	1	1	Yes	Yes	Yes	
Fetterman et al. (2020) [72]	0	2	2	Yes	Yes	Yes	
Goniewicz et al. (2018) [34]	27	0	27	Yes	Yes	Yes	
Gupta et al. (2021) [73]	0	7	7	Yes	Yes	Yes	
Hickman et al. (2022) [74]	0	1	1	Yes	Yes	Yes	
Loffredo et al. (2021) [35]	0	4	4	Yes	Yes		Yes
Majek et al. (2023) [37]	0	3	3	Yes	Yes	Yes	Yes
Majid et al. (2021) [75]	0	5	5	Yes	Yes	Yes	
Metzen et al. (2021) [76]	0	1	1	Yes	Yes	Yes	
Oliveri et al. (2020) [77]	2	4	6	Yes		Yes	
Perez et al. (2021) [78]	4	5	9	Yes	Yes	Yes	
Podzolkov et al. (2020) [79]	0	5	5	Yes	Yes	Yes	
Russo et al. (2018) [80]	0	1	1	Yes		Yes	
Sakamaki-Ching et al. (2020) [25]	0	2	2	Yes	Yes	Yes	
Scherer et al. (2022) [18][a]	3	0	3	Yes	Yes	Yes	Yes
Scherer et al. (2022) [19][a]	5	0	5	Yes	Yes	Yes	Yes
Scherer et al. (2022) [20][a]	9	0	9	Yes	Yes	Yes	Yes
Scherer et al. (2023) [21][a]	4	0	4	Yes	Yes	Yes	Yes
Schirone et al. (2022) [36]	0	4	4	Yes	Yes		Yes
Stokes et al. (2021) [81]	0	5	5	Yes	Yes	Yes	
Tattersall et al. (2023) [33]	0	6	6	Yes	Yes	Yes	
Wang et al. (2022) [26]	0	4	4	Yes	Yes	Yes	
Xia et al. (2021) [66]	3	0	3	Yes	Yes	Yes	
Total				29	24	27	7

[a] Considered in initial searches

does not matter, as if the data available for the biomarker for users of ETPs and non-users adequately fits the regression model, it can still be used to estimate risk in users of new tobacco products. Perhaps more of a concern is where the set of biomarkers used to estimate risk includes multiple correlated biomarkers of the same exposure. Here analyses could be run limiting attention to at most one biomarker for any given exposure. We did not attempt such an approach, but the results in Table 9 clearly show that analyses based on any subset of the biomarkers we considered would still have predicted a lung cancer risk in ECIG users much lower than that for smokers of cigarettes.

An important limitation of our approach is the failure to include biomarkers for chemicals present in ECIGs or HTPs that, in the ETPs we considered, are either not present, are present in very small amounts, or which have not been measured. Our method will not, however, help if use of ECIGs or HTPs happens to cause a disease not known to be related to smoking.

Further considerations

Our combined lung cancer RR estimates of 1.88 (95% CI 1.66–2.22) for ECIG use and 1.44 (95% CI 0.41–5.08) for HTP use clearly suggest that the RR for use of these two products is substantially lower than that

Table 9 Estimated RRs for use of ECIGs for individual studies and biomarkers and combined

Biomarker				
Class	**Group**	**Marker**	**Study**	**RR (95% CI)**
BOE	Acrylamide	AAMA	Anic et al. (2022) [27]	1.21 (0.75–1.97)
			Goniewicz et al. (2018) [34]	1.13 (0.69–1.87)
			Perez et al. (2021) [78]	1.33 (0.83–2.12)
		GAMA	Anic et al. (2022) [27]	0.96 (0.73–1.26)
			Goniewicz et al. (2018) [34]	2.08 (1.71–2.53)
	Acrylonitrile	CYMA	Anic et al. (2022) [27]	3.13 (2.68–3.65)
			Goniewicz et al. (2018) [34]	2.12 (1.74 -2.57)
			Perez et al. (2021) [78]	2.36 (1.97–2.84)
			Andersen et al. (2022) [38]	1.72 (1.39–2.14)
		HEMA	Anic et al. (2022) [27]	1.13 (0.77–1.66)
			Goniewicz et al. (2018) [34]	1.86 (1.37–2.54)
			Scherer et al. (2023) [21]	0.45 (0.26–0.76)
	Anabasine	NAB	Anic et al. (2022) [27]	1.60 (0.77–3.33)
			Goniewicz et al. (2018) [34]	1.85 (0.94–3.67)
			Scherer et al. (2022) [19]	1.03 (0.43–2.50)
			Xia et al. (2021) [66]	1.67 (0.81–3.42)
	Anatabine	NAT	Anic et al. (2022) [27]	1.59 (0.66–3.86)
			Goniewicz et al. (2018) [34]	1.94 (0.87–4.33)
			Scherer et al. (2022) [19]	0.41 (0.10–1.72)
			Xia et al. (2021) [66]	1.67 (0.70–3.97)
	Phenanthrene	2/3-OH-Phe	Anic et al. (2022) [27]	1.37 (0.89–2.11)
			Goniewicz et al. (2018) [34]	1.26 (0.80–1.96)
		4-OH-Phe	Scherer et al. (2022) [20]	0.18 (0.07–0.42)
BOPH	Adhesion molecule	sICAM-1	Christensen et al. (2021) [70]	1.26 (0.93–1.71)
			Oliveri et al. (2020) [77]	1.45 (1.09–1.93)
			Perez et al. (2021) [78]	1.53 (1.16–2.02)
			Stokes et al. (2021) [81]	2.65 (2.15–3.27)
	Interleukin	IL-6	Christensen et al. (2021) [70]	0.39 (0.20–0.77)
			Perez et al. (2021) [78]	1.65 (1.11–2.48)
			Stokes et al. (2021) [81]	3.21 (2.44–4.24)
BOE		All		1.88 (1.55–2.28)
BOPH		All		1.89 (1.23–2.91)
All		All		1.88 (1.60–2.22)

for smoking of cigarettes, though the data for HTPs are very limited, with the RR having very wide CI. Our analyses for ECIGs clearly indicate that attention should be restricted to those biomarkers showing an adequate fit to the model.

Until reliable epidemiological results become available, future research could usefully extend our results by including further data for those biomarkers we used in our estimates, taking account of additional biomarkers thought relevant, and considering other smoking-related diseases. The fact that our database and software are available to carry out further analyses should facilitate this future research. We emphasise, however, that our methodology will not help if using ECIGs or HTPs causes some disease not known to be related to smoking.

Table 10 Estimated RRs for use of ECIGs. Results of sensitivity analyses

Sensitivity analysis	Description	N	Estimated RR (95% CI)
-	Original estimate	30	1.88 (1.60–2.22)
1	At least four ratio estimates	30	1.88 (1.60–2.32)
	At least six ratio estimates	15	2.18 (1.83–2.61)
2	Restricting attention to biomarkers with any slope	86	13.03 (11.91–14.25)
	Restricting attention to biomarkers with slope $p < 0.05$	43	11.63 (8.79–15.40)
	Restricting attention to biomarkers with slope $p < 0.001$	17	2.00 (1.67–2.39)
3	Restricting attention to studies with well-defined groups	22	1.98 (1.46–2.70)
	(a) and to biomarkers with any slope	72	2.06 (1.74–2.23)
	(b) and to biomarkers with slope $p < 0.05$	35	1.92 (1.54–2.39)
	(c) and to biomarkers with slope $p < 0.001$	4	2.07 (0.80–5.36)
4	Varying the RR for snuff/snus from 0.80 to 1.00	27	1.92 (1.61–2.29)

Table 11 Estimated RRs for use of HTPs for individual studies and biomarkers and combined

Biomarker				
Class	Group	Marker	Study	RR (95% CI)
BOE	Acrylonitrile	HEMA	Scherer et al. (2023) [21]	1.07 (0.73–1.59)
	Anabasine	NAB	Scherer et al. (2022) [19]	2.49 (1.39–4.47)
	Anatabine	NAT	Scherer et al. (2022) [19]	3.49 (1.98–6.13)
	Phenanthrene	4-OH-Phe	Scherer et al. (2022) [20]	0.43 (0.22–0.86)
All		All		1.44 (0.41–5.08)

Conclusions

We describe a methodology to derive estimates of lung cancer risk for users of new tobacco products such as ECIGs and HTPs, from biomarker data for these products. Our methodology has limitations, but seems useful for estimating disease risk in the absence of epidemiological data. Applying it, and limiting attention to biomarkers satisfactorily fitting the model, indicates that the lung cancer risk from using ECIGs is much less than that from smoking cigarettes. Limited data also suggests that the risk from using HTPs is relatively low. Future research can extend these results based on data for additional biomarkers and smoking-related diseases.

Abbreviations

BOE Biomarker of exposure
BOPH Biomarker of potential harm
CI Confidence interval
ECIG E-cigarettes
ER Excess risk
ETP Established tobacco product
HTP Heated tobacco product
RR Relative risk
ST Smokeless tobacco

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12954-025-01188-x.

Additional file 1 Details of the papers excluded in the initial searches and the reasons for exclusion, both based on the first look at the papers and at the data entry stage.

Additional file 2 The data recorded for each study and for each biomarker from the initial searches.

Additional file 3 Presents plots for each biomarker based on the data obtained from the initial searches.

Additional file 4 Details of the papers excluded in the further searches and the reasons for exclusion, both based on the first look at the papers and at the data entry stage.

Additional file 5 The data recorded for each study and for each biomarker from the further searches.

Acknowledgements
We thank Japan Tobacco International S.A. for financial support. We also thank Yvonne Cooper for secretarial assistance and for obtaining the required references, Jan Hamling for assistance in checking the text, and John Hamling for creating the plots and the Shiny App.

Author contributions
Lee PN planned the study; Literature searches and data entry were carried out by Coombs KJ and by Lee PN; Statistical analyses were carried out by Fry JS and checked by Lee PN; Lee PN drafted the text, which was checked by Coombs KJ and Hamling JS.

Funding
Japan Tobacco International S.A. supported the time spent by the authors in the publication of this paper.

Availability of data and materials
See final results section of paper "Availability of data and software".

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 16 September 2024 Accepted: 5 March 2025
Published online: 30 March 2025

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