Technical Report:

Literature Review of Biomarkers of Exposure Related to Traditional and Emerging Nicotine Products

SEPTEMBER 7, 2018

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Literature Review of Biomarkers of Exposure Related to Traditional and Emerging Nicotine Products

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The Foundation for a Smoke-Free World

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Executive Summary

ToxStrategies was contracted by the Foundation for a Smoke-Free World (the Foundation) to conduct a literature search and review of biomarkers of exposure related to traditional and emerging nicotine delivery products. This review was commissioned as part of the Foundation's strategy to support research that assesses the impacts of cessation and harm reduction products. The objective of the literature search and review was to characterize biomarkers of exposure related to traditional and emerging nicotine delivery products, and specifically, to characterize three particular aspects of the evidence base: (1) ability to discern product usage status (e.g., non-smoker vs. smoker, smokeless tobacco user vs. cigarette smoker, etc.), (2) potential confounding from other sources of exposure (e.g., environmental exposures, dietary exposures), and, (3) ease/invasiveness of sample collection.

A multidisciplinary team of scientists and information specialists from ToxStrategies developed a systematic map aimed at characterizing the landscape of publicly available, peer-reviewed data for biomarkers of exposure associated with nicotine delivery products. Following a protocol developed in the initial stages of the project, >6,000 articles were screened, which included 147 studies that characterized biomarkers of exposure associated with use of nicotine products other than, or in addition to, conventional cigarettes. A total of 134 unique biomarkers of exposure were reported across the evidence base. The majority of the exposure biomarker data were categorized into eight broad "biomarker groups" (**Figure 2**): amines, elements, carbon monoxide, polycyclic aromatic hydrocarbons (PAHs), tobacco alkaloids, tobacco-specific nitrosamines (TSNAs), and volatile organic compounds (VOCs), as well as "others" for those that did not fit into any of the other groups.

Key findings across the evidence base were as follows:

- While some studies identified biomarkers that can discern between types of products used (e.g., cigarette smokers vs. users of smokeless tobacco; electronic cigarette users vs. smokers of traditional cigarettes), no specific biomarkers of exposure were identified among the literature that consistently demonstrated the capability of discerning across product categories.

- Results varied by the products tested, the biomarkers evaluated, and the matrices in which the biomarker was assessed.

- Potential confounding from other sources of exposure is insufficiently addressed by the current evidence base.

- The ease of sampling (i.e. biological matrix collected) varied within the evidence base, with the majority of biomarkers measured in urine.

Collectively, the evidence demonstrates that there are some studies in which biomarkers were reported to discern between types of products used; however, no specific biomarker(s) of exposure consistently demonstrated the capability to discern across product categories. Additional research is needed to develop (or further refine) biomarkers that have the ability to discern both between tobacco use status and tobacco product types, are readily distinguishable from environmental or other confounding exposure, and can be evaluated with relatively non-invasive methods. Such research can

build upon the current evidence base, which provides important, but not conclusive, characterizations regarding one or more biomarkers that offer these features.

1 Background and Objective

The development, marketing, and use of alternative tobacco products (e.g., "reduced-risk products" (RRPs)—such as products that heat tobacco without burning it—is increasing. While many RRPs are designed to reduce exposure to harmful and potentially harmful constituents (HPHCs) and other chemicals associated with traditional tobacco product use, such products may also expose the user to novel chemicals and/or chemical mixtures, a scenario that has raised concerns regarding adverse effects potentially related to frequent and chronic use (IOM, 2012; Chang et al., 2017; Kaur et al., 2018). Demonstrative reports of the risk reduction potential of RRPs typically include measurement of biomarkers, which can inform exposure to specific potentially harmful constituents of RRPs (Peck et al., 2018). It is generally believed that a reduction in exposure to tobacco toxicants will lead to reduced risk of development of tobacco-related disease (Stratton et al., 2001). Biomarkers of exposure to nicotine products indicate that both contact and uptake have occurred, representing an appropriate metric for exposure assessment (Ogden et al., 2015). Further, specific biomarkers of exposure may be informative to variations in smoking behavior, such as puff volume and frequency, number of cigarettes smoked per day, etc. Related to this, the Group Smoking Prevention and Tobacco Control Act of 2009 directs the US FDA to issue regulations or guidance on these products based on "validated biomarkers, intermediate clinical endpoints, and other feasible outcome measures, as appropriate."
In response, there have been recent efforts to summarize the state of the science on exposure biomarkers related to nicotine delivery products (Chang et al., 2017; Schick et al., 2017). Both of these reviews are informative and provide useful digests on the subject. They describe a database that is extensive, and illustrate the considerable diversity of the biomarkers that have been investigated and used to characterize nicotine product use and exposure. It is evident that the innovation of RRPs and other emerging nicotine delivery products has not only greatly diversified the market, but also created new challenges for developing the means by which nicotine exposure is measured and monitored. The reviews identify gaps in the state of the science, including the lack of validated biomarkers that can reliably discern among the use of various combustible products (e.g., cigarettes, cigars, cigarillos, etc.), and the absence of biomarkers that have been validated to discern between non-combustible products. While many biomarkers have been measured extensively and researched for use in subjects who smoke conventional combustible cigarettes, further research on the reliability of biomarkers to identify exposure to various emerging nicotine delivery products is needed. Further, validation studies of candidate biomarkers of exposure to emerging products is also important and necessary. Notably missing from the current literature at this point is a systematic review of the available literature—that is, a review conducted using a transparent and reproducible protocol for searching, screening, and reviewing the relevant literature. The first step in advancing a strategy to fill in these biomarker gaps is to understand the landscape of the available literature.

ToxStrategies was contracted by the Foundation to conduct a comprehensive literature search and review of biomarkers of exposure and effect related to traditional and emerging nicotine delivery products. This review was commissioned as part of the Foundation's strategy to support research that assesses the impacts of cessation and of harm-reduction products.

Objective: conduct a literature search and review of biomarkers of exposure related to traditional and emerging nicotine delivery products, in an effort to characterize the available literature relative to:

   1   Ability to discern product usage status (e.g., non-smoker vs. smoker, smokeless tobacco user vs. cigarette smoker, etc.)

   2   Potential confounding from other sources of exposure (e.g., environmental exposures, dietary exposures)

   3   Ease/invasiveness of sample collection.

The objective of the literature search and review was to characterize biomarkers of exposure related to traditional and emerging nicotine delivery products, and specifically, to characterize three particular aspects of the evidence base: (1) ability to discern product usage status (e.g., non-smoker vs. smoker, smokeless tobacco user vs. cigarette smoker, etc.), (2) potential confounding from other sources of exposure (e.g., environmental exposures, dietary exposures), and (3) ease/invasiveness of sample collection.

In this technical report, details of the approach, findings, and synthesis of results are presented. In addition to the technical report, supplemental materials are available, as well as an online public summary.

2   Methods

The literature review was carried out using a "systematic map" approach. By definition, such an approach provides a structured, reproducible, and transparent process to describe the state of knowledge for a question or topic (James et al., 2016). Systematic maps allow for data visualization that can readily facilitate synthesis of data, as well as identification of data gaps. Specifically, such maps provide a tool to characterize evidence for each type of biomarker as it relates to specific nicotine delivery products. The project involved the following tasks:

      1. Problem formulation and protocol development

      2. Identification of studies (i.e., evidence base)

      3. Review of studies and production of systematic map

      4. Synthesis and overall assessment

      5. Reporting.

Tasks were carried out by a multidisciplinary project team that included subject-matter experts, information specialists, and systematic review experts (Attachment A). Various software tools, including DistillerSR and Microsoft Excel, were used to facilitate the systematic search, screening, extraction, and synthesis.

2.1 Problem Formulation and Protocol Development

Upon project initiation, a series of problem formulation tasks were conducted in an effort to ensure that the efforts would provide meaningful information that met stakeholder needs. To ensure adequate context around evaluation of the human data, this task included clarification and clear identification of populations of interest, tobacco products of interest, and exposure scenarios of interest, and a general characterization of relevant co-exposures and confounding variables. This series of exercises resulted in a targeted research question with contextual rationale. Subsequently, a succinct protocol was developed (Attachment A) documenting the literature search strategy *a priori*. As part of this task, literature search and extraction templates were iteratively developed via piloting exercises.

2.2 Identification of the Evidence Base

A literature search was conducted in the PubMed database[1] to identify published literature that included reports of biomarkers of exposure to both traditional and emerging nicotine delivery products. Search syntax was developed and validated by an information specialist. Key literature reviews were studied to support and inform the development of the search syntax. A search string that was relatively broad (versus narrowly focused on specific biomarkers), to ensure identification of all relevant articles, included key words for tobacco delivery products and for biomarkers. The finalized search string[2] was employed for a query of literature indexed within the PubMed database, which was executed on May 25th, 2018.

Following retrieval of all potentially relevant literature from PubMed, titles and abstracts were reviewed and marked for inclusion for further review, or were excluded because the article did not appear to fit the outlined inclusion criteria (Attachment A). Pilot screening included collaborative review of titles and abstracts, and dynamic updates to categorization and criteria in an iterative manner, based on discussions among team members from ToxStrategies and the Foundation. In brief, articles were excluded according to the following criteria:

- Article not available in English

- Not original research (i.e., review article, comment, editorial, etc.)

- *In vitro* data were the sole type of data reported

- Co-exposure to non-nicotine products was the primary focus of the research

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[1] Additional databases (Embase) were considered but not included. The volume of literature, timeline, and additional resources needed were considered collectively as part of feasibility determinations during problem formulation and protocol development. It was determined that the map would be based on evidence from PubMed Information, because that was expected to be sufficient for assessing the landscape of currently available information. Subsequent and confirmatory searching for investigations of specific biomarkers may be warranted for future evaluations.

[2] The literature search involved biomarkers of both exposure and effect in a concatenated form. Biomarkers of effects will be reported separately.

- Environmental exposure to tobacco smoke was the sole exposure source

- Animal studies

- Ecological-type studies

- Generally not relevant (examples include cases in which biomarkers of something unrelated to exposure to nicotine products were measured in a population of nicotine product users, biomarkers in tobacco plants, etc.)

Literature screening was conducted using DistillerSR (Evidence Partners, Ottawa, Canada). All included articles were categorized according to the nicotine delivery product(s) analyzed, as follows: conventional combustible cigarettes only, nicotine delivery products other than/in addition to conventional combustible cigarettes, or specific products unclear from the title and abstract.

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Categories of included studies:
1. Conventional combustible cigarettes only

2. Nicotine delivery products other than/in
   addition to conventional combustible
   cigarettes

3. Specific products unclear from the title and
   abstract.
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2.3   Review of Studies and Production of Systematic Map

For all articles that were marked as containing information regarding biomarkers of exposure and that included information on subjects using nicotine delivery products other than or in addition to conventional combustible cigarettes, the full text was obtained and reviewed. As was the case with title and abstract screening, pilot screening included collaborative review of several full-text articles, and extraction form questions and answers were updated based on discussions among team members from ToxStrategies and the Foundation. For studies that met inclusion criteria after full-text review, data were extracted at the study and individual biomarker levels. At the study level, the objective, study type, and design were recorded, as well as author conclusions in general. At the biomarker level (i.e., for each biomarker/matrix), the biomarker, the biological matrix sampled, the ability to discern product usage status, the ability to discern between various products, any additional exposure sources that may introduce potential confounding, and results/conclusions specific to the biomarker were all recorded for each individual biomarker evaluated in each study. All data extraction was conducted using the hierarchical data extraction tool in DistillerSR to enable singular entries for study information and multiple entries for biomarkers per study. Each biomarker/matrix combination is referred to as a data set herein; multiple data sets were often obtained for a single study.

All extracted data are those reported by the author. Evidence tables contain information directly from the publication (e.g., objectives/conclusion as reported by the author). The only exceptions concerned determination of discernment categories. All biomarker data were categorized according to two aspects of the ability to discern: (1) ability to discern nicotine product use status/level and (2) discernment of the usage between various products for biomarker data. If the authors reported this information, it was recorded. If the authors did not report such information directly, but provided information that allowed the analyst to make such a determination, the analysts made the determination. Separate fields were used to track whether this information was reported by the original authors or if it was determined by an analyst. For example, a biomarker for which

> **Categories of nicotine biomarker discernment:**
>
> 1. **Ability to discern nicotine product use status (e.g., user/non-user)**
>
> 2. **Ability to discern between nicotine products (e.g., combustible cigarette vs. smokeless tobacco user)**

the measurements were clearly significantly different between cigarette smokers and non-smokers, and for which this fact was clearly stated by the authors, would be marked as having the ability to discern between product use status. A biomarker that had significantly different levels in users of different products, regardless of the level of use, would be marked as having the ability to discern between products—for example, a biomarker that was much higher in chewing tobacco users compared to smokers or vice versa. In many studies, the reported data were not amenable to such determinations (either because of inadequate data reporting or because the study design was not appropriate for making such conclusions). For example, a study that compared chewing tobacco users to cigarette smokers but did not compare either user group to non-users would not be able to determine whether the biomarker was able to discern use status/level, but would be able to determine if the biomarker was able to discern between different products.

In the cases in which the articles did not provide enough information for the analyst to determine the ability of the biomarker to discern either usage status or products (or both), discernment ability was characterized as "unclear" or "not applicable" or "not addressed," as appropriate. It should be re-emphasized, however, that the objective of this effort was to characterize the landscape of the exposure biomarker literature for nicotine-delivery products, thus providing an overview of the literature for future researchers to explore. The vastness of the evidence base and the limited time frame meant that data extraction from the screened studies was conducted at a relatively basic level. For all articles that reported biomarker data for exposure to conventional combustible cigarettes only, or for which the product was not specified, biomarker information was extracted only from the title and abstract. Specifically, the biomarker and the biological matrix were recorded. Study information was not collected for the articles in these categories. Tabular summaries of the extracted data were compiled for summary statistics and identification of overall themes within the data set. Biomarker groups were assigned, and all individual biomarker measurements from the literature were associated with the appropriate biomarker groups (and in some cases, biomarker subgroups). Tabular summaries (i.e., evidence tables) were produced within Excel based on filters for the

various biomarker groups. Data visualizations were constructed for the overall evidence base and groups of biomarkers, based on extracted data. Tabular summaries and data visualizations (collectively referred to as the systematic map) were used as tools to facilitate synthesis and reporting.

2.4   Synthesis and Overall Assessment

The landscape of exposure biomarker literature was synthesized by characterizing both the overall body of evidence and the evidence for each biomarker group. The synthesis focuses on the results from the literature search and the overall utility of each biomarker group as it relates to the sensitivity for differentiating smoking status or tobacco product use (i.e., non-user of nicotine delivery products vs. cigarette smoker, nicotine replacement therapy [NRT] user, etc.), other potential sources of exposure (possible confounding), and ease/invasiveness of sample collection. However, as was determined at project initiation, no recommendations were to be made regarding the biomarker(s) with the greatest potential utility for assessing the impact of RRPs on tobacco product use patterns, or on the areas of future research that are likely to fill in the identified data gaps, because this overall effort is being conducted solely to characterize the biomarker landscape as it relates to the available information on the use of nicotine delivery products.

In this technical report, the syntheses for the biomarker groups each contains:

  a. General background on biomarker group
  b. Data characterization for biomarkers within the group
       i. Identification and frequency of reporting
       ii. Ability to discern nicotine product use status
       iii. Ability to discern between nicotine product types
       iv. Matrices evaluated
       v. Confounding/overall sources of biomarker
  c. Summary and conclusions for the biomarker group.

As part of characterizing the identity and frequency, comparisons are also made to studies that evaluated cigarettes only or were categorized as unknown products. The objective was to make simple comparisons between the study types (e.g., similar or different biomarkers used).

2.5   Reporting

In addition to this technical report, an online public summary of the data, evidence tables (Excel), and data visualizations (PowerPoint) are available.

3   Results

3.1   Characterization of the Overall Evidence Base

A flow diagram of the literature search and screening is provided in **Figure 1.** More than 6,000 articles were identified as a result of a comprehensive search in PubMed for articles that included terms related to biomarkers and terms related to nicotine delivery products. Screening of titles and abstracts, and subsequent full-text screenings resulted in identification of 147 studies that characterized biomarkers of exposure associated with the use of nicotine products other than, or in addition to, conventional cigarettes, and met all other inclusion criteria. A total of 232 studies were identified that measured biomarkers of exposure in users of conventional combustible cigarettes only, and 179 studies for which the specific nicotine products were not described in the title or abstract. Following categorization based on title and abstract screening, extraction of biomarker information from all studies in which the use of nicotine products other than, or in addition to, conventional cigarettes was conducted, as described in the Methods section. A total of 134 unique biomarkers of exposure were reported in the literature database for this category of articles, taking synonyms into account (i.e., the same compound referred to differently by study authors was counted as one unique biomarker). The majority of the exposure biomarker data were categorized into eight broad "biomarker groups" (**Figure 2**): amines, elements, carbon monoxide, PAHs, tobacco alkaloids, TSNAs, and VOCs, along with "others" for those that did not fit into any of the other seven groups. The following themes regarding these biomarker groups are summarized as follows:

- The most frequently reported biomarkers were tobacco alkaloids (206 data sets) followed by VOCs, PAHS, and TSNAs at 183, 130, and 117 data sets, respectively.

- Other exposure biomarker groups were grouped into amines (72 data sets) and elemental measures (34 data sets, primarily metals).

- Carbon monoxide (CO)—a common byproduct of combustion and component of smoke—was, by itself, a common exposure biomarker (69 data sets measured CO either as a gas in exhaled breath or in blood hemoglobin).

- A number of other less commonly reported biomarkers were grouped together into an "other" category, which includes dioxins & furans, glucuronides, hemoglobin adducts, phosphates, propylene glycol, and thiocyanate.

In addition to these eight biomarker groups, some studies included DNA adducts and DNA methylation. While such biomarkers were generally categorized as biomarkers of effect during title and abstract screening, cases in which the authors specifically reported these as biomarkers of exposure were also categorized as such. However, these biomarkers are not reviewed herein, although they do appear in the evidence tables assigned to the biomarker family "DNA adduct; Consider for biomarker of effect"

Six biological matrices were sampled for the measurement of biomarkers across all studies: blood/serum, expired breath, feces, hair, saliva, and urine (**Figure 2**). Urine was by far the most commonly collected biospecimen (653 of the total 877 exposure

biomarker data sets), with blood/serum/plasma being the next most common biological matrix studied (155 data sets). The remaining exposure biomarker data sets (cumulatively making up <10% of the database) were examined in expired air, saliva, hair, and feces, with the latter two matrices being reported in only a single study.
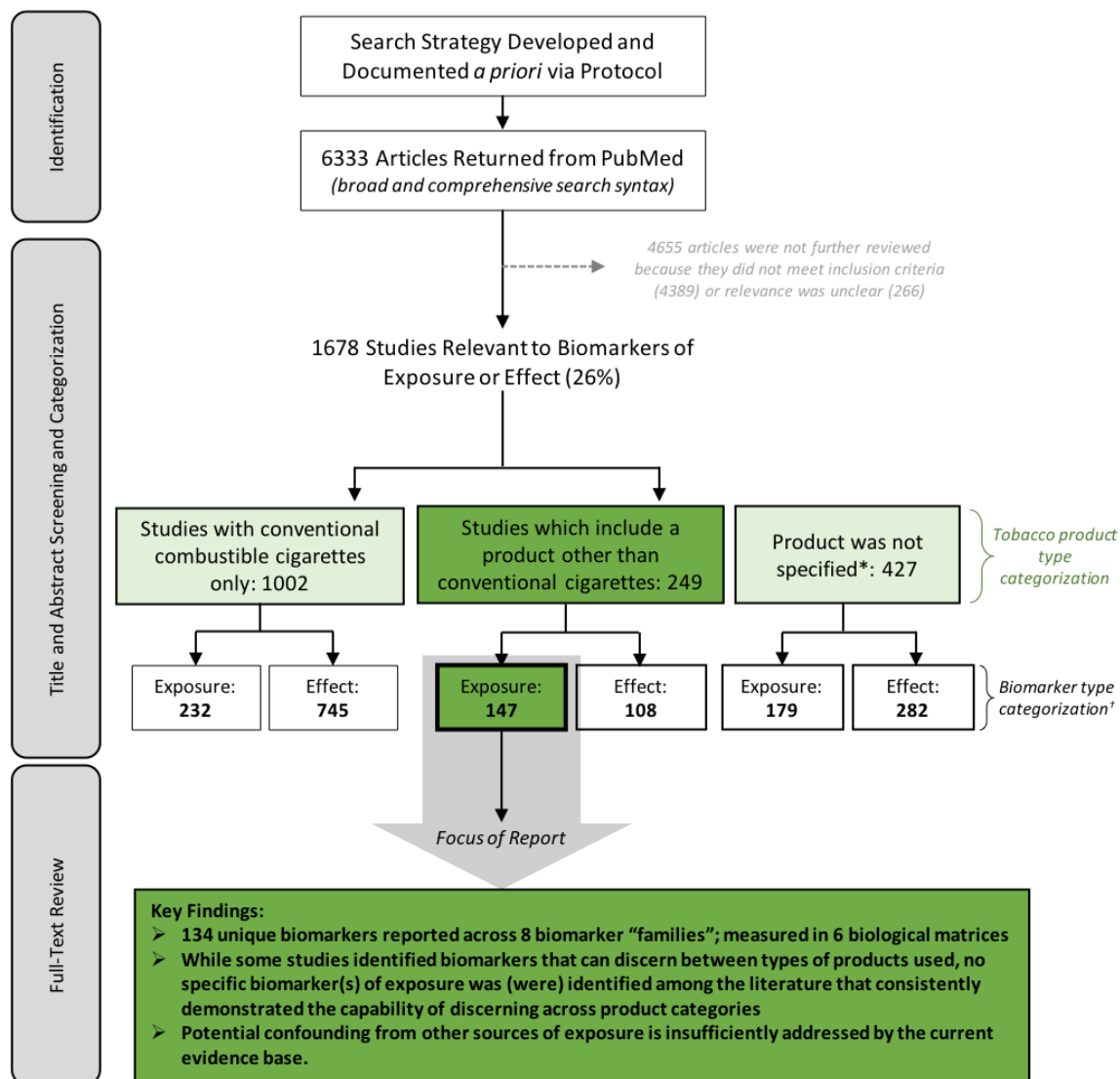
Figure 1.   Summary of literature search and key findings related to biomarkers of exposure.

The type of tobacco product was not clear in the abstract; most commonly, the authors referred to "smokers" or "tobacco users" and did not specify further.
† Some studies included more than one type of biomarker.
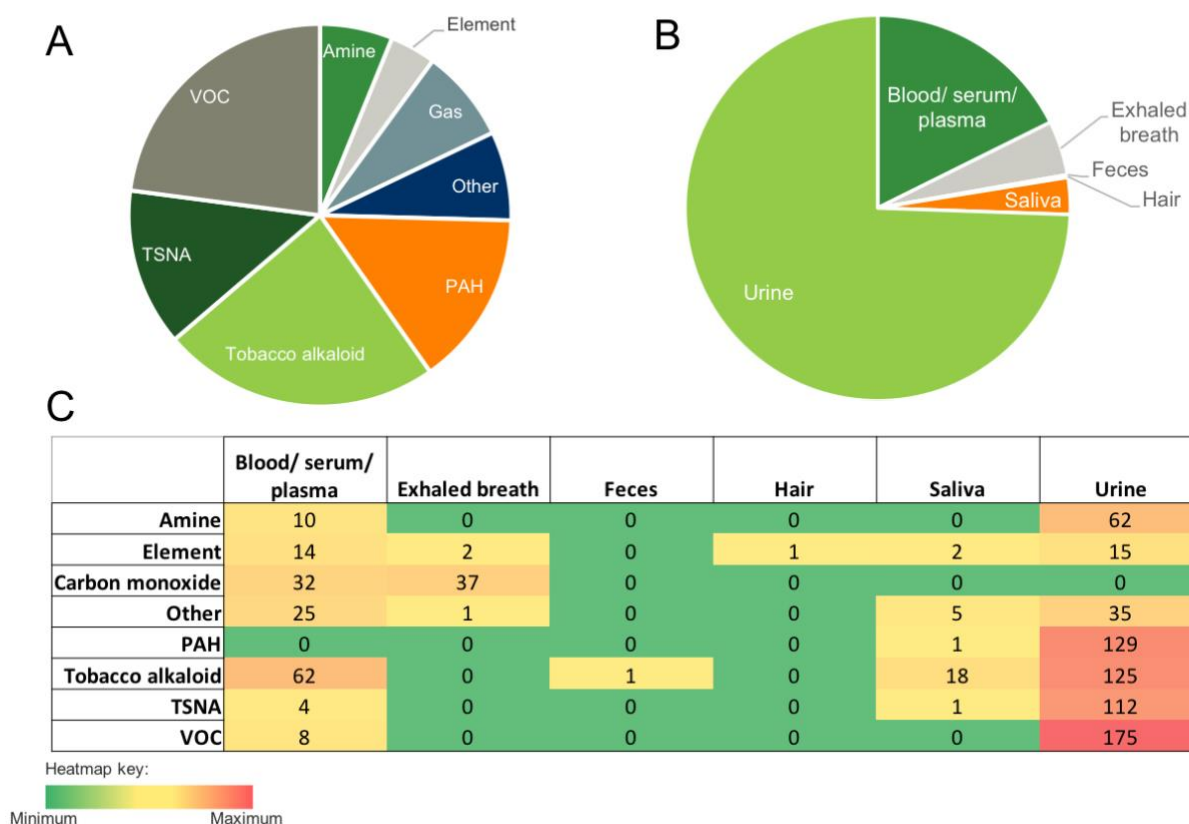
Figure 2. Overview of reporting according to biomarker groups and biological matrices

A. Pie chart depicting the spread of the number of biomarker measurements per biomarker group.
B. Pie chart depicting the spread of the number of biomarker measurements per biological matrix in which the biomarker was measured.
C. Heat map showing the intersections of all biomarkers measured according to the biomarker group and by biological matrix sampled.

The ability of each biomarker to discern the usage status/level of nicotine delivery products was recorded, as described in the Methods section. Nearly half (~48%) of all the exposure biomarker data sets for studies evaluating nicotine delivery products other than, or in addition to, conventional cigarettes were clearly reported to be different between non-users and users of the various nicotine products evaluated in this data set. In contrast, approximately 9% of the exposure biomarker data sets did not clearly differentiate between users and non-users, and no clear conclusion could be reached on user discernment for the remaining ~40% of the data sets, either because relevant groups were not included in the study, or because the data reported were not sufficient to answer this question.

The ability of each biomarker to discern between various nicotine delivery products was also recorded, as described in the Methods section. Approximately 58% of all the exposure biomarker data sets for studies evaluating nicotine delivery products other than, or in addition to, conventional cigarettes were clearly reported to be different between

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users of different products. and users of the various nicotine products evaluated in this data set. In contrast, approximately 17% of the exposure biomarker data sets did not clearly differentiate between users and non-users, and no clear conclusion could be made on user discernment for the remaining ~20% of the data sets, either because relevant groups were not included in the study, or because the data reported were not sufficient to answer this question.

The database for each biomarker group is described in further detail below, including descriptive statistics regarding the biological matrices collected, themes for individual biomarkers within the groups, ability to discern product use status and between product types, as well as any additional exposures that may confound estimates of nicotine product exposure. Further, the themes collected from the screening of titles and abstracts of articles that were categorized as evaluating users of conventional combustible cigarettes only, or for which the product(s) was(were) not specified, are also included for each biomarker group.

3.2 Summary of Exposure Biomarker Literature Based on Studies of Nicotine Delivery Products Other than Conventional Cigarettes

3.2.1 Volatile Organic Compounds

Volatile organic compounds (VOCs; Table 1) represent a large group of ubiquitous chemicals produced from numerous natural and anthropomorphic sources, including tobacco smoke. In general, human exposures to VOCs can occur through inhalation, ingestion, and dermal contact, because these compounds readily vaporize at room temperature and pressure. These compounds and their associated metabolites have been studied as biomarkers for their utility in characterizing VOC exposures experienced by cigarette smokers. As summarized below, the literature also includes studies that examined the utility of VOC biomarkers in users of nicotine delivery products other than conventional cigarettes. Across this narrower evidence base for biomarkers of exposure, and accounting for the different biomarkers used to characterize exposure to some of the parent compounds, the following 13 chemicals were categorized as VOCs: acrolein, acrylamide, acrylonitrile, benzene, 1,3-butadiene, crotonaldehyde, ethylbenzene, ethylene oxide, glycidamide, propylene oxide, toluene, styrene, and xylene.

- *Identification and Frequency of Reporting:* When combining their specific biomarkers (parent compound and associated metabolites), the most frequently analyzed VOCs were acrolein and benzene, followed by 1,3-butadiene (Table 1). Other common, though less frequently evaluated, VOCs in the current literature database include acrylonitrile, crotonaldehyde, and acrylamide.

  - *Comparison of biomarkers reported from cigarette-only studies:* Many of the same VOC biomarkers were also frequently identified in the cigarette-only studies. Two notable observations based on reviews of title and abstracts are: (1) *t,t*-muconic acid (VOC metabolite) and benzene were measured more frequently in studies evaluating only combustible cigarettes than in studies evaluating biomarkers for nicotine delivery products other than, or in addition to, conventional cigarettes; and (2) the cigarette-only literature also included an amino acid VOC adduct as a

potential exposure biomarker. A complete list of biomarkers identified in the titles and abstracts of cigarette-only studies is provided in Attachment B.

- The VOC biomarkers identified in the studies of unknown nicotine products are similar to those in the literature of nicotine delivery products other than, or in addition to, conventional cigarettes, covering mostly mercapturic acid metabolites and VOC parent compounds. There were no notable differences other than the inclusion frequency of VOC biomarkers in the unknown products subset of studies, which was far smaller than the latter database. The complete list of biomarkers identified in the titles and abstracts of unknown products studies can be found in Attachment C.

- *Ability to discern nicotine product use status*: In general, the studies in the database reported that biological measures of acrolein, benzene, and 1,3-butadiene exposure were used successfully as biomarkers to discern nicotine product use status. Approximately 56% of all biomarker data sets in the evidence base for these three VOCs reported an ability to discern nicotine product use (Table 1). Approximately 43% of the biomarker studies for these VOCs did not explicitly demonstrate or report on the utility of a biomarker to distinguish user discernment, either because they were not designed to address this user discernment (tagged by data extractors as "not addressed" or "NA") or because reported results that were not explicitly clear on nicotine product use discernment (tagged by data extractors as "not clear"). Relative to the VOC biomarker database as a whole, few VOC biomarker data sets reported negative discernment results (i.e., insignificant difference in biomarker measurements between users and non-users). For example, of the three most frequently analyzed VOCs in this database, only 2 of the 37 total 1,3-butadiene biomarker results (one each for MHBMA and DHBMA) indicated that the exposure biomarker measurements did not discern between user and non-user. Of the less frequently assessed VOCs, failure to discern between users and non-users was also reported in one or two data sets specific to crotonaldehyde, acrylonitrile, ethylene oxide, propylene oxide, toluene, and xylene biomarkers (Table 1).

- *Ability to discern between nicotine product types*: Relative to user discernment results, a greater portion (approximately 78%) of the literature database specific to acrolein, benzene, and 1,3-butadiene reported that their respective biomarkers discerned among the various nicotine delivery products evaluated. Of the most frequently analyzed VOCs in this database, only results for acrolein biomarkers indicated specific instances (three data sets for 3-HPMA) where the exposure biomarker measurements did not discern between use of different nicotine delivery products. As reported in Table 1, the inability to discern between nicotine product types was also evident in some data sets specific to crotonaldehyde, acrylamide, glycidamide, propylene oxide, toluene, and xylene biomarkers.

- *Matrices tested*: VOCs were evaluated primarily in urine samples as mercapturic acid metabolites, as opposed to parent VOCs, although there were some limited data sets that examined parent VOCs in either blood or urine samples (Table 1). Mercapturic acids are fairly specific to individual VOCs, forming from glutathione conjugates of the VOC via the actions of γ-glutamyltranspeptidase and dipeptidases and N-

acetyltransferase (Ding et al., 2009). These urinary metabolites are typically measured using some form of liquid chromatography/mass spectrometry; for example, the method published and used by the CDC for Laboratory Procedure Manual describes an Ultra Performance Liquid Chromatography coupled with electro-spray tandem mass spectrometry (UPLC-ESI/MSMS) protocol (Alwis et al., 2012; CDC, 2018). Several mercapturic acids are highly specific to their respective parent VOCs: 3-hydroxypropylmercapturic acid [3-HPMA] and 2-carboxyethylmercapturic acid [CEMA] are acrolein biomarkers; S-phenylmercapturic acid [SPMA] is a benzene metabolite, and monohydroxybutenyl mercapturic acids [HBMAs] are used to characterize 1,3-butadiene exposure.[3] Notable advantages of evaluating VOC exposure using urine mercapturic acid analysis include obtaining urine samples, which is a less invasive process than blood collection, and the fact that mercapturic acids are more stable in urine than their VOC counterparts (Ding et al., 2009; CDC, 2018).

- *Confounding and other sources of biomarkers*: There are well-known sources of exposure to these VOCs aside from tobacco use (e.g., environmental, occupational, dietary), and these represent potential confounders for the VOC exposure biomarkers included in this section. However, the studies on VOC biomarkers captured in the current evidence base generally did not address confounding from other sources of exposure. When potential confounders were addressed in studies, it was not in a quantitative or otherwise meaningful manner, but as a point in the discussion.

Tobacco smoke from conventional cigarettes is a major source of VOC exposure. The ubiquitous presence of environmental VOCs and the volatile nature of these compounds present important challenges for using biological matrices to characterize VOC exposures and highlight the importance of potential co-exposures and timing when designing sample collection protocols for study designs. The current VOC exposure biomarker literature on nicotine delivery products other than, or in addition to, conventional cigarettes includes several studies comparing VOC biomarker measurements (mostly represented as urine mercapturic acid metabolites) in cigarette smokers and users of other nicotine delivery products. This appears to have affected the user/non-user status vs. product type discernment findings: many more studies reported clear conclusions on biomarker product-type discernment than reported clear user/non-user status discernment data. Because VOC exposures are expected to be lower for users of nicotine delivery products that do not involve tobacco combustion, compared to those who use tobacco combustion products, the utility of VOC biomarkers for discerning between nicotine products that do not involve tobacco combustion is unclear from this high-level analysis, and warrants closer examination.

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[3]   *Note: The chemical names for certain mercapturic acids varied among studies. In most cases, synonyms were identified by cross-checking the references in the original literature or by using the chemical names and acronyms and synonyms presented in CDC's laboratory guidelines. In some cases, however, it was unclear whether a particular chemical name represented a synonym for another biomarker in the current database.*

Table 1.   Summary of VOC biomarker nicotine product use and product discernment data

Biomarker (acronym; parent compound of metabolite)†	Biomarker group	Biological matrix	Total No. of data sets	Nicotine Product User/Non-User Discernment††			Product Type Discernment		
				Could discern	Could not discern	N/A or unclear	Could discern	Could not discern	N/A or unclear
3-Hydroxypropyl mercapturic acid (3-HPMA; acrolein)	VOC metabolite, mercapturic acid	urine	35	18	0	17	24	3	8
S-Phenylmercapturic acid (SPMA; benzene)	VOC metabolite, mercapturic acid	urine	32	18(19)	0(1)	13	27	0	5
(1- or 2-)Monohydroxybutenyl mercapturic acid (MHBMA; 1,3-butadiene)	VOC metabolite, mercapturic acid	urine	25	14(15)	1(2)	9	20	0	5
3-Hydroxy-1-methylpropyl mercapturic Acid (HMPMA; crotonaldehyde)	VOC metabolite, mercapturic acid	urine	18	11(12)	1(2)	5	15	1	2
2-Cyanoethylmercapturic acid (CYMA; acrylonitrile)	VOC metabolite, mercapturic acid	urine	13	6(7)	0(1)	6	9	0	4
2-Hydroxyethylmercapturic acid (HEMA; acrylonitrile, ethylene oxide)	VOC metabolite, mercapturic acid	urine	13	5	1	7	9	0	4
Acrylamide mercapturic acid (AAMA; acrylamide)	VOC metabolite, mercapturic acid	urine	11	7	0	4	7	2	2
Glycidamide mercapturic acid (GAMA; glycidamide)	VOC metabolite, mercapturic acid	urine	6	5	0	1	3	2	1
1,2-Dihydroxybutyl-mercapturic acid (DHBMA; 1,3-butadiene)	VOC metabolite, mercapturic acid	urine	3	1	1	1	3	0	0
2-Hydroxypropyl-mercapturic acid (2-HPMA; propylene oxide)	VOC metabolite, mercapturic acid	urine	3	0	1	2	1	1	1
trans,trans-Muconic acid (benzene)	VOC metabolite, other	urine	3	2	0	1	3	0	0
Benzene	VOC parent	*all*	*2*	*1*	*0*	*1*	*2*	*0*	*0*
		blood/serum/plasma	1	1	0	0	1	0	0
		urine	1	0	0	1	1	0	0
Xylene	VOC parent	blood/serum/plasma	2	1	1	0	1	1	0

Biomarker (acronym; parent compound of metabolite)†	Biomarker group	Biological matrix	Total No. of data sets	Nicotine Product User/Non-User Discernment††			Product Type Discernment		
				Could discern	Could not discern	N/A or unclear	Could discern	Could not discern	N/A or unclear
Methylmercapturic acid (MMA)	VOC metabolite, mercapturic acid	urine	2	0	1	1	0	2	0
N-acetyl-S-(2-carboxyethyl)-l-cysteine (CEMA; acrolein)	VOC metabolite, mercapturic acid	urine	2	2	0	0	1	0	1
S-benzylmercapturic acid (SBMA; toluene)	VOC metabolite, mercapturic acid	urine	2	0	2	0	0	1	1
Acrylamide	VOC parent	blood/serum/plasma	1	1	0	0	1	0	0
Ethylbenzene	VOC parent	blood/serum/plasma	1	1	0	0	1	0	0
Hb adducts: Glycidamide	VOC, hemoglobin adduct	blood/serum/plasma	1	1	0	0	1	0	0
Styrene	VOC parent	blood/serum/plasma	1	1	0	0	1	0	0
Toluene	VOC parent	blood/serum/plasma	1	1	0	0	1	0	0
1,3-Butadiene	VOC parent	urine	1	0	0	1	1	0	0
4-Hydroxybutyl-2-mercapturic acid (4-HBMA; 1,3-butadiene)	VOC metabolite, mercapturic acid	urine	1	0	0	1	0	0	1
Acrolein	VOC parent	urine	1	0	0	1	1	0	0
CNEMA (undefined mercapturic acid)	VOC metabolite, mercapturic acid	urine	1	0	0	1	1	0	0
Mercapturic acid metabolites (unspecified)	VOC metabolite, mercapturic acid	urine	1	1	0	0	0	0	1
N-acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine (HPMMA, possible synonym of HMPMA; crotonaldehyde)	VOC metabolite, mercapturic acid	urine	1	0	0	1	0	1	0
Total			**183**	**97(101)**	**9(13)**	**73**	**133**	**14**	**36**

† Mercapturic acid synonyms were identified using CDC VOC Laboratory Manual Procedure document for 2013-2014 NHANES. Synonyms were not identified for all mercapturic acids, however, so some listed names may have additional synonyms also on the list above.

†† In some cases, biomarkers in a single study counted as both "can discern" and "cannot discern," because it could discern use for at least one product, but not another. The number in parentheses indicates the count with this dual-result study accounted for.

3.2.2   Polycyclic Aromatic Hydrocarbons

The polycyclic aromatic hydrocarbon (PAH; Table 2) group is another large group of ubiquitous compounds that have been examined as exposure biomarkers for users of nicotine delivery products. These compounds are primarily by-products of the incomplete combustion or pyrolysis of organic materials, and therefore originate from numerous natural and anthropomorphic sources (IARC, 2010). Tobacco smoke is a significant contributor of PAH exposure for smokers, whereas diet is the primary source of PAHs for those not exposed via tobacco smoke or occupationally. Human exposures to PAHs occur through inhalation, ingestion, and dermal contact. A summary of the PAH biomarker literature for studies on nicotine delivery products other than, or in addition to, conventional cigarettes is provided as follows:

- *Frequency:* The most frequently analyzed PAH was by far the pyrene metabolite 1-hydroxypyrene (Table 2). Other common though less frequently evaluated PAHs in the current literature database include various isoforms of hydroxyfluorene, hydroxyphenanthrene, and naphthols.

  - *The PAH biomarkers identified in the literature on nicotine delivery products other than, or in addition to, conventional cigarettes captured the same list of PAH biomarkers identified in the titles and abstracts of the cigarette-only studies (Attachment B), with 1-hydroxypyrene also the most frequently measured PAH biomarker in this literature.*

  - *The PAH biomarkers identified in the literature on nicotine delivery products other than, or in addition to, conventional cigarettes captured the same list of PAH biomarkers identified in the titles and abstracts of the unknown nicotine products studies. 1¬Hydroxypyrene represented the most frequently measured PAH biomarker in this literature (Attachment C).*

- *Ability to discern nicotine product use status*: In general, the studies in the database reported that biological measures of 1-hydroxypyrene exposure were not used as successfully to discern nicotine product use status relative to the most frequently evaluated VOCs. Approximately 35% of all data sets in the evidence base for these PAH and PAH metabolite biomarkers reported significant user discernment (Table 2). The majority of the studies that did not explicitly demonstrate or report on the utility of a PAH biomarker to distinguish user discernment either were not designed to address this parameter (tagged by data extractors as "not addressed" or "NA") or reported results that were not explicitly clear on nicotine product use discernment (tagged by data extractors as "not clear"). Approximately 25% of the 1-hydroxypyrene data sets from appropriately designed studies reported negative discernment results (i.e., insignificant difference in biomarker measurements between users and non-users). Of the less frequently assessed PAHs, failure to discern between users and non-users was reported in only one of the data sets for 1-naphthol and hydroxyphenanthrenes (generic listing, isoforms not specified) (Table 2).

- *Ability to discern between nicotine product types*: Relative to user discernment results, a greater portion of the literature database for PAH exposure biomarkers (approximately 50%) reported that the PAH biomarkers discerned between the various nicotine delivery products evaluated. This reflected a combination of a greater number

of PAH biomarker data sets demonstrating discernible differences between products used and also many fewer data sets for which discernment was either unclear or not addressed. Paradoxically, there were also many more data sets for which biomarkers could clearly not discern between products than could discern between use status (Table 2). This is best exemplified by 1-hydroxypyrene: 24 data sets could discern 1-hydroxypyrene levels between users of different nicotine delivery products, but 12 data sets demonstrated that 1-hydroxypyrene measurements failed to achieve such discernment.

- *Matrices tested*: PAH biomarkers were almost exclusively measured in urine samples, mostly as a hydroxylated metabolite of one of several PAH parent compounds (e.g., pyrene, fluorene, phenanthrene). The lone exception was a single saliva measurement of hydroxyfluorenes (unspecified isoforms) (Table 2). PAH biomarkers were typically analyzed using gas or liquid chromatography/tandem mass spectrometry (GC/MS/MS or LC/MS/MS) methods (Benowitz et al., 2007; CDC, 2013). These biomarkers are highly specific to their parent PAH compound.

- *Confounding/other sources of biomarker*: There are well-known sources of exposure to these PAHs aside from tobacco use (e.g., environmental and occupational combustion sources, dietary), and these represent potential confounders for the PAH exposure biomarkers included in this section. It is likely that these common sources played a role in the relatively low user and product discernment frequency in the literature reviewed in this effort.

  - Exposure to PAHs occurs through many common sources: automobile exhaust, fireplaces, grilling, occupational settings, etc. Tobacco smoke is a major source of exposure to PAHs for those who smoke or are in proximity to smoking. The biomarkers for PAH exposure are most commonly measured as hydroxylated pyrene, fluorene, phenanthrene, and/or naphthalene, with 1-hydroxypyrene the most frequently measured PAH biomarker in the current literature. In the current PAH biomarker data set, there is a considerable difference between the outcomes of the two discernment types: considerably fewer studies reported user status discernment results that were amenable to concluding one way or the other for PAH biomarkers, compared with the number of studies reporting results for discerning between product types. As with the VOC biomarker data sets, it appears that the studies that included PAH biomarkers were designed to evaluate product type discernment; that is, they included more than a single nicotine delivery product, reporting comparisons in biomarker measures between two or more products. It should be noted, however, that 1-hydroxypyrene is commonly included in biomarker batteries to help differentiate smokers from nonsmokers (Hecht, 2002), and there is some evidence to indicate that 1-hydroxypyrene can discern between smokers of conventional cigarettes and those who switch to electronic cigarettes (Roethig et al., 2008). A significant limitation to the utility of 1-hydroxypyrene and other PAHs is the lack of specificity to tobacco exposure (Chang et al., 2017), and the considerable portion of the current literature that is amenable to a conclusive characterization of user/non-user discernment indicates that this data set

requires closer evaluation to determine whether the available discernment data can be refined.

Table 2.   Summary of PAH biomarker nicotine product use and product discernment data

Biomarker (acronym; parent compound of metabolite)	Biomarker group	Biological matrix	Total No. of data sets	Nicotine Product User/Non-User Discernment†			Product Type Discernment		
				Could discern	Could not discern	N/A or unclear	Could discern	Could not discern	N/A or unclear
1-Hydroxypyrene (1-HOP; 1-OHP)	PAH, metabolite	urine	42	15(16)	5(6)	21	25	12	5
2-Hydroxyfluorene (2-FLU)	PAH, metabolite	urine	12	3	0	9	8	2	2
2-Naphthol (2-NAP)	PAH, metabolite	urine	12	4	0	8	7	2	3
1-Naphthol (1-NAP)	PAH, metabolite	urine	6	3	1	2	3	1	2
2-Hydroxyphenanthrene	PAH, metabolite	urine	6	2	0	4	3	2	1
3-Hydroxybenzo[a]pyrene (3-OHBaP)	PAH, metabolite	urine	6	3	0	3	4	0	2
1-Hydroxyphenanthrene	PAH, metabolite	urine	5	2	0	3	1	3	1
Hydroxyphenanthrenes	PAH, metabolite	urine	5	1	1	3	2	3	0
PAHs (unspecified)	PAH, unspecified	urine	5	1	0	4	0	4	1
3-Hydroxyfluorene	PAH, metabolite	urine	4	1	0	3	4	0	0
3-Hydroxyphenanthrene	PAH, metabolite	urine	4	2	0	2	3	0	1
4-Hydroxyphenanthrene	PAH, metabolite	urine	4	2	0	2	3	0	1
1-Hydroxyfluorene	PAH, metabolite	urine	3	0	0	3	3	0	0
9-Hydroxyphenanthrene (9-PHE)	PAH, metabolite	urine	3	1	0	2	1	0	2
1-/9-Hydroxyphenanthrene	PAH, metabolite	urine	2	1	0	1	1	1	0
3+4-Hydroxyphenanthrene	PAH, metabolite	urine	2	0	0	2	1	1	0
9-Hydroxyfluorene (9-FLU)	PAH, metabolite	urine	2	1	0	1	0	1	1
Hydroxyfluorenes	PAH, metabolite	*all*	*2*	*1*	*0*	*1*	*1*	*1*	*0*
		saliva	1	1	0	0	0	1	0
		urine	1	0	0	1	1	0	0
1-Hydroxypyrene glucuronide (1-OHP-gluc)	PAH, metabolite	urine	1	1	0	0	1	0	0
2-/3-Hydroxyphenanthrene	PAH, metabolite	urine	1	1	0	0	0	1	0
Naphthols (unspecified)	PAH, metabolite	urine	1	1	0	0	0	0	1

Biomarker (acronym; parent compound of metabolite)	Biomarker group	Biological matrix	Total No. of data sets	Nicotine Product User/Non-User Discernment†			Product Type Discernment		
				Could discern	Could not discern	N/A or unclear	Could discern	Could not discern	N/A or unclear
OH-PAH metabolites (sum)	PAH, metabolite	urine	1	1	0	0	1	0	0
Phenanthrene	PAH, parent	urine	1	0	0	1	0	1	0
Total			**130**	**47(48)**	**7(8)**	**75**	**72**	**35**	**23**

† In some cases, biomarkers in a single study counted as both "can discern" and "cannot discern," because it could discern use for at least one product, but not another. The number in parentheses indicates the count with this dual-result study accounted for.

3.2.3   Tobacco Alkaloids

Among the many active constituents of tobacco, tobacco alkaloids (Table 3) are a class of compounds represented by nicotine, the primary and most pharmacologically active of the alkaloids. Nicotine is the most abundant alkaloid in tobacco, accounting for up to as much as 96%–98% of the total alkaloid content in tobacco leaves; the remaining alkaloid constituents are the minor tobacco alkaloids, and include nornicotine, anabasine, anatabine, cotinine, and myosmine (Huang & Hsieh, 2007). Tobacco alkaloids and their metabolites are the most common group of biomarkers for monitoring nicotine product use, and most of the alkaloids are present to some degree in most nicotine products. Notable exceptions are anabasine nor anatabine, which are not present in nicotine replacement therapy (NRT), and thus have been examined as biomarkers of tobacco abstinence in NRT users (Jacob et al., 2002). Thus human exposures to tobacco alkaloids are specific to use of or exposure to tobacco products other than NRTs.

- *Frequency:* The most frequently analyzed tobacco alkaloid was cotinine, which is both a constituent alkaloid of tobacco and a nicotine metabolite. Nicotine and total nicotine equivalents (i.e., the combined measure of urinary nicotine, cotinine, and several nicotine and cotinine metabolites) were also common biomarkers measured in the literature on nicotine delivery products other than, or in addition to, conventional cigarettes (Table 3). The metabolites hydroxycotinine, cotinine N-oxide, and nicotine N-oxide were each studied less frequently than cotinine, as were the minor alkaloids anatabine, nornicotine, anabasine, and norcotinine.

  - *As with the evidence base on nicotine delivery products other than, or in addition to, conventional cigarettes, cotinine—followed by nicotine and total nicotine equivalents— were the most frequently studied tobacco alkaloid biomarkers in the titles and abstracts of the pertinent cigarette-only literature. Some of the minor alkaloids were not identified in this literature set (e.g., anabasine nor anatabine), but the cigarette-only evidence base included three alkaloid glucuronide metabolites (of cotinine, hydroxycotinine, and nicotine) that were not evaluated in the literature on nicotine delivery products other than, or in addition to, conventional cigarettes (Attachment A).*

  - *The tobacco alkaloid biomarkers identified in the literature on nicotine delivery products other than, or in addition to, conventional cigarettes, and conventional cigarettes only, were also captured in the titles and abstracts of the unknown nicotine products studies. Unlike these biomarker evidence bases, however, the titles and abstracts of the unknown nicotine products literature also included the minor alkaloid myosimine (Attachment B).*

- *Ability to discern nicotine product use status*: Taken together, the studies in the database reported that biological measures of cotinine, nicotine, and nicotine equivalents are sensitive as biomarkers to discern nicotine product use status. While only approximately 42% of all data sets in the evidence base for these tobacco alkaloids reported significant user discernment (Table 3), less than 5% of the biomarker data sets from appropriately designed studies reported negative discernment results (i.e., insignificant difference in biomarker measurements between users and non-users). These few negative user discernment results were indicated for urine cotinine (n=4 compared), saliva cotinine (n=1), and urine nicotine and nicotine equivalents (n=1 each). The user discernment status of tobacco alkaloids for most of the studies (approximately 50%) did not explicitly demonstrate or report on the utility of a biomarker to

distinguish user discernment either were not designed to address this parameter (tagged by data extractors as "not addressed" or "NA") or reported results that were not explicitly clear on the discernment of nicotine product usage status (tagged by data extractors as "not clear").

- *Ability to discern between nicotine product types*: Close to half of the tobacco alkaloid literature reported positive evidence supporting discernment between nicotine product types; however, a considerable portion of the literature (25%) also reported that tobacco alkaloid biomarkers could NOT discern between the various nicotine delivery products evaluated. Of the most frequently analyzed alkaloids in this database, cotinine, nicotine equivalents, and nicotine constituted a large portion of the negative nicotine product-type discernment results. As reported in Table 3, inability to discern between nicotine product types was also evident for the few data sets specific to norcotinine, cotinine-oxide, and nicotine-N-oxide biomarkers. Urine anatabine was the most successful of the less commonly investigated minor alkaloids at discerning between nicotine products.

- *Matrices tested*: Tobacco alkaloids were evaluated primarily in urine samples, with the parent compounds the most common biomarkers of analysis. Unlike the other common biomarker groups, however, both blood/serum/plasma and saliva were also common matrices for measuring nicotine, cotinine, and hydroxycotinine (Table 3). Multiple methods are described in the literature for the measurement of tobacco alkaloids in biological samples. Jacobs et al. (2002) analyzed nicotine and cotinine in urine using gas chromatography, and the minor alkaloids anabasine, anatabine, and nornicotine. More recently, von Weymarn et al. (2016) described a liquid chromatography-tandem mass spectrometry method for measuring the minor alkaloids nornicotine, anatabine, and anabasine in urine. For serum and saliva samples, the CDC's Laboratory Procedure Manual describes an isotope-dilution high-performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometric method (ID HPLC-APCI MS/MS) for measuring cotinine and trans-3'-hydroxycotinine (CDC, 2016). The metabolite biomarkers (e.g., hydroxycotinine, nicotine-N-oxide) are highly specific to their respective parent alkaloids. Urinalysis techniques are easier to implement than serum sample collection and analysis, and calculating total nicotine equivalents in this analysis accounts for the kinetic variance inherent within and between human populations.

- *Confounding/other sources of biomarker*: Because tobacco alkaloids are inherently specific to tobacco and tobacco products, the primary confounders are environmental tobacco smoke and use of more than one nicotine delivery product. Nicotine and cotinine are present in NRTs, although the minor alkaloids anatabine and anabasine are specific to tobacco and not detected in NRTs.
  - Exposure to tobacco smoke and/or the use of any tobacco products are the primary sources of exposure to tobacco alkaloids. These tobacco product constituents are commonly used as biomarkers of nicotine product use, and have been well characterized for this objective in the literature. The discernment analysis for the tobacco alkaloid data set demonstrated that the majority of the evidence base was not amenable to making conclusions about the ability of tobacco alkaloids to discern users of nicotine products from non-users. This contrasted drastically with the adequacy of the relevant literature to discern between product types, because only about a quarter of the evidence base was not amenable to making a definitive conclusion on this question for these biomarkers. As with the VOC and PAH biomarker data sets, it is likely that the difference in the results for tobacco alkaloid biomarkers between the two

discernment types (user/non-user vs. nicotine product types) is likely due to a greater number of data sets coming from studies in which alkaloid biomarkers were being evaluated specifically for the discernment between nicotine product types (vs. designed to discern nicotine product users vs. non-users). Additionally, the analysis of this data set also indicates that tobacco alkaloids are considerably less successful at discerning between tobacco product types relative to user/non-user discernment. (That is, while more alkaloid data sets were amenable for product type vs. user/non-user discernment, a large portion of these data sets demonstrated that the alkaloid biomarker under study could not discern between product types.) This reflects the fact that tobacco alkaloids are present in nearly all tobacco products, and therefore, most alkaloids (on their own) have less utility for discerning between nicotine products than overall user/non-user status. There are certain exceptions to this, however, as the minor alkaloids anatabine and anabasine are specific to tobacco products but absent from NRTs. Thus, anatabine and anabasine have been used successfully to monitor NRT users abstaining from other nicotine products (Jacob et al., 2002). Conversely, it is not clear how effective these minor alkaloids will be in discerning between tobacco combustion nicotine products and other nicotine products such as e-cigarettes, because they have been detected in the e-liquids used in e-cigarettes, as well as in smokeless tobacco (Chang et al., 2017). Very few data sets on the minor tobacco alkaloids were captured in the current literature search and review, making anatabine and anabasine, and perhaps other minor tobacco alkaloids, targets for further biomarker research.

Table 3.   Summary of tobacco alkaloid biomarker nicotine product use and product discernment data

Biomarker (acronym; parent compound of metabolite)	Biomarker group	Biological matrix	Total No. of data sets	Nicotine Product User/Non-User Discernment†			Product Type Discernment†		
				Could discern	Could not discern	N/A or unclear	Could discern	Could not discern	N/A or unclear
Cotinine	Tobacco alkaloid, nicotine metabolite	*all*	*103*	*48(49)*	*4(5)*	*50*	*46*	*27*	*30*
		blood/serum/plasma	36	18	0	18	22	8	6
		saliva	14	5	1	8	3	9	2
		urine	53	25(26)	3(1)	24	21	10	21
Nicotine equivalent	Tobacco alkaloid, nicotine metabolite	*all*	*44*	*17*	*1*	*26*	*27*	*9*	*8*
		feces	1	0	0	1	0	0	1
		urine	43	17	1	25	27	9	7
Nicotine	Tobacco alkaloid	*all*	*34*	*15*	*1*	*18*	*15*	*8*	*11*
		blood/serum/plasma	20	10	0	10	11	5	4
		saliva	2	0	0	2	0	2	0
		urine	12	5	1	6	4	1	7
Hydroxycotinine	Tobacco alkaloid, nicotine metabolite	*all*	*11*	*3*	*0*	*8*	*4*	*5*	*2*
		blood/serum/plasma	6	2	0	4	4	2	0
		saliva	2	0	0	2	0	1	1
		urine	3	1	0	2	0	2	1
Anatabine	Tobacco alkaloid	urine	4	1(1)	0(1)	2	3	0	1
Nornicotine	Tobacco alkaloid, nicotine metabolite	urine	4	1	0	3	1	1	2
Anabasine	Tobacco alkaloid	urine	2	0(1)	0(1)	1	0(1)	0(1)	1
Norcotinine	Tobacco alkaloid, nicotine metabolite	urine	2	1	0	1	0	1	1

Biomarker (acronym; parent compound of metabolite)	Biomarker group	Biological matrix	Total No. of data sets	Nicotine Product User/Non-User Discernment†			Product Type Discernment†		
				Could discern	Could not discern	N/A or unclear	Could discern	Could not discern	N/A or unclear
Cotinine N-oxide	Tobacco alkaloid, nicotine metabolite	urine	1	0	0	1	0	1	0
Nicotine N-oxide	Tobacco alkaloid, nicotine metabolite	urine	1	0	0	1	0	1	0
Total			**206**	**86(89)**	**6(9)**	**111**	**96(97)**	**53(54)**	**56**

† In some cases, biomarkers in a single study counted as both "can discern" and "cannot discern," because it could discern use for at least one product, but not another. The number in parentheses indicates the count with this dual-result study accounted for.

3.2.4   *Tobacco-Specific Nitrosamines*

Tobacco-specific nitrosamines (TSNAs; Table 4) are nitroso derivatives of tobacco alkaloids (Chang et al., 2017). The most prominent of the TSNAs are nicotine-derived nitrosamine ketone (4-methylnitrosamino-1-3-pyridyl-1-butanone; aka, NNK) and N-nitrosonornicotine (NNN); other TSNAs include N'-nitrosoanabasine (NAB), and N'-nitrosoanatabine (NAT). NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol) is a metabolite of NNK that is commonly used to characterize exposure to tobacco products. NNK and NNN are produced primarily during the tobacco curing process and, to some extent, during the combustion of tobacco; therefore, they are present in nearly all tobacco products and tobacco smoke (IARC, 2012). However, the levels of these compounds (and therefore exposures) vary among tobacco products; factors such as tobacco type, agricultural practices, and manufacturing processes are key determinants for TSNA levels in nicotine and tobacco products (Chang et al., 2017). As with the tobacco alkaloids, TSNAs are an important group of biomarkers, given their specificity to tobacco products.

- *Frequency:* The most frequently analyzed TSNA was NNAL, followed by NNN (Table 4). Other less frequently evaluated TSNAs in the current literature database include NAB, NAT, the NNK Hb adduct HBP, and NNK.

  - *As with the literature on nicotine delivery products other than, or in addition to, conventional cigarettes, NNAL was the most common TSNA biomarker identified in the cigarette-only literature. In addition, 4-hydroxy-4-(3-pyridyl)butanoic acid (hydroxy acid, a metabolite of NNN) and NNAL-glucuronide were identified in the titles and abstracts of the cigarette-only studies. While the Hb adduct, 4-hydroxy-l-(3-pyridyl)-1-butanone (HBP) was included in the literature on nicotine delivery products other than, or in addition to, conventional cigarettes, it was not identified in the cigarette-only biomarker literature (Attachment B).*

  - *Again, NNAL was the most frequently identified TSNA biomarker in the unknown-products literature. There were two TSNA metabolites examined as biomarkers in the titles and abstracts of the unknown-products literature [4-hydroxy-4-(3-pyridyl)butanoic acid (hydroxy acid, a metabolite of NNN) and 4-oxo-4-(3-pyridyl)butanoic acid (keto acid)] that were not included in the literature on nicotine delivery products other than, or in addition to, conventional cigarettes. Conversely, the two minor TSNAs (NAT and NAB) in the latter database were absent from the titles and abstracts of the unknown-products literature (Attachment C).*

- *Ability to discern nicotine product use status*: Approximately 42% of all data sets in the TSNA biomarker evidence base reported significant user discernment (Table 4). The majority of the studies (54%) did not explicitly demonstrate or report on the utility of a biomarker to distinguish user discernment (tagged by data extractors as "not addressed" or "NA" or did not report clear data that would support nicotine product use one way or the other). Very few TSNA biomarker data sets from appropriately designed studies reported negative discernment results (i.e., insignificant difference in biomarker measurements between users and non-users): two data sets each for urinary NNAL and urinary NNN (Table 4).

- *Ability to discern between nicotine product types*: Relative to user discernment results, a greater portion of the literature database for TSNA exposure biomarkers (approximately 62%) reported the ability to discern between the various nicotine delivery products evaluated. The

nearly 10% of the TSNA data sets that failed to discern product type were composed of urinary NNAL and NNN biomarkers (seven and three data sets, respectively).

- *Matrices tested*: The majority of the TSNA biomarker data were reported from urine samples, with very few TSNAs investigated in blood/serum/plasma and saliva. For measuring NNAL in urine, the CDC Laboratory Procedure Manual describes a liquid chromatography linked to tandem mass spectrometry (LC/MS/MS) method (CDC, 2009). This method includes measurements for "total" NNAL (where NNAL-13C6 is used as an internal standard in the urine samples and then hydrolyzed using β-glucuronidase) and "free" NNAL (conducted without prior enzymatic hydrolysis). To measure the hydroxy acid and keto acid (NNK metabolites), Hecht et al. (1999) summarize a liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry (LC-APCI-MS/MS) method. In addition, the presence of NNN, NAT, and NAB in urine samples has been analyzed by gas chromatography with nitrosamine-selective detection (GC-TEA), and confirmed using gas chromatography-tandem mass spectrometry (Stepanov and Hecht, 2005). In all, NNAL urinalysis is advantageous due to the non-invasive sampling method and the long half-life of NNAL in the body (Benowitz et al., 2009).

- *Confounding/other sources of biomarker*: As described in the name, TSNAs are specific to tobacco and tobacco products, and therefore, the primary confounders are environmental tobacco smoke and use of more than one nicotine delivery product. There is also some evidence that TSNAs form endogenously in users of oral NRTs, where the gastrointestinal tract may provide favorable conditions for converting nicotine to nornicotine to NNN (Stepanov et al., 2009). It is not clear whether this is a common phenomenon or limited to particular populations.

Tobacco smoke from conventional cigarettes is the primary source of TSNA exposure, but due to their specificity to tobacco, TSNA exposure is significant for users of non-combustion tobacco products, as well. Urinary NNAL is the most frequently used TSNA biomarker of exposure due to its specificity to tobacco products, its relatively long half-life in biological samples (10 to 45 days; Chang et al., 2017) and the non-invasive nature of sample collection. The inherent specificity to tobacco makes TSNAs such as NNAL useful exposure biomarkers for discerning between users and non-users. In the current analysis, this is largely supported by the user status discernment results in the studies amenable to determining the "can discern" vs. "cannot discern" distinction. Interestingly, there were a considerably greater number of tobacco alkaloid biomarker data sets that could discern between product types than user/non-user status. This is surprising, given the ubiquity of TSNAs across most nicotine delivery products on the market (NRTs being a notable exception). Differences in the tobacco manufacturing process can influence a user's overall exposure to TSNAs, although it is not clear that such differences are stark enough to affect a difference in the levels of TSNA biomarkers. The evidence base should be reviewed further to characterize the product type discernment findings.

Table 4.   Summary of TSNA biomarker nicotine product use and product discernment data

Biomarker (acronym; parent compound of metabolite)	Biomarker group	Biological matrix	Total No. of data sets	Nicotine Product User/Non-User Discernment†			Product Type Discernment		
				Could discern	Could not discern	N/A or unclear	Could discern	Could not discern	N/A or unclear
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)	TSNA, metabolite	*all*	*79*	*30(31)*	*2(3)*	*46*	*52*	*8*	*19*
		blood/serum/plasma	1	0	0	1	0	1	0
		urine	78	30(31)	2(3)	45	52	7	19
N-Nitrosonornicotine (NNN)	TSNA	urine	23	10(11)	2(3)	10	14	3	6
N-Nitrosoanabasine (NAB)	TSNA	urine	5	2	0	3	2	0	3
N'-Nitrosoanatabine (NAT)	TSNA	urine	5	3	0	2	1	0	4
Hb adduct: 4-Hydroxy-l-(3-pyridyl)-1-butanone (HBP)	TSNA, hemoglobin adduct	blood/serum/plasma	3	2	0	1	3	0	0
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)	TSNA	saliva	1	0	0	1	0	0	1
TSNA (unspecified)	TSNA	urine	1	1	0	0	1	0	0
Total			**117**	**48(50)**	**4(6)**	**63**	**73**	**11**	**33**

† In some cases, biomarkers in a single study counted as both "can discern" and "cannot discern," because it could discern use for at least one product, but not another. The number in parentheses indicates the count with this dual-result study accounted for.

3.2.5   Amines

Aromatic and heterocyclic amines (Table 5) are a diverse group of chemicals, many of which are constituents of tobacco smoke (Chang et al., 2017). The type of tobacco used in nicotine products, and nitrogen levels within the tobacco, are thought to be factors in the levels of aromatic amines in tobacco smoke (CDC, 2010). Amine derivatives of naphthalene and biphenyl are the most common aromatic amines used as tobacco biomarkers, and their utility as exposure biomarkers has extended, in some cases, to the evaluation of their respective hemoglobin adducts.

- *Frequency:* The most frequently analyzed amines were 4-aminobiphenyl (4-ABP), toluidine, and 2-naphthylamine (2-NA), followed by 1-naphthylamine (1-NA) and 3-aminobiphenyl (3-ABP) (Table 5). Other common though less frequently evaluated amines in the current literature database include metabolites of aniline, anisidine, and 2-aminobiphenyl.

    - *There were considerable differences in the amine biomarkers included in the cigarette-only literature compared with the literature on nicotine delivery products other than, or in addition to, conventional cigarettes. 1-Methyldecyclamine, ammonium, pyrrole, and a couple of pyridines and indoles were unique to the titles and abstracts of the cigarette-only literature. Conversely, several amine biomarkers (2-ABP, Hb adduct: 2-ethyl-aniline, Hb adduct: 2,4-dimethyl-aniline, Hb adduct: aniline, or Hb adduct: anisidine) that were evaluated in the literature on nicotine delivery products other than, or in addition to, conventional cigarettes were not identified in the cigarette-only database (Attachment B).*

    - *Only four amine biomarkers were identified in the titles and abstracts of the unknown-products literature: the common biomarker 4-ABP, two of its metabolites (glucuronide and N-acetyl derivative), and the Hb adduct: 2,6-dimethylaniline. Of these, only 4-ABP was also included in the other literature databases (Attachment C).*

- *Ability to discern nicotine product use status*: Approximately 61% of all amine biomarker data sets in the evidence base reported significant user discernment (Table 5). This contrasts with the PAH, tobacco alkaloid, and TSNA data sets, for which less than half their respective data sets reported explicit user discernment. Only one-third of the amine biomarker data sets did not explicitly demonstrate or report positive or negative user discernment (e.g., tagged by data extractors as "not addressed" or "NA" or "not clear"). Only three amine biomarker data sets reported negative discernment results (i.e., insignificant difference in biomarker measurements between users and non-users), and these were for the infrequently assessed hemoglobin adducts, 2-ethyl-aniline, aniline, and anisidine (Table 5).

- *Ability to discern between nicotine product types*: Approximately 74% of the amine biomarker literature database reported the ability to discern between the various nicotine delivery products evaluated. Only the infrequently evaluated hemoglobin adduct biomarkers for aniline and anisidine were unable to discern between use of different nicotine delivery products, while only approximately 22% of the amine biomarker data set were not amenable to a discernment conclusion one way or the other.

- *Matrices tested*: Amine biomarkers were analyzed primarily in urine samples; less common was the assessment of amine-specific hemoglobin adducts, which were mostly measured in

blood samples. Riedel et al. (2006) describe a gas chromatography/mass spectrometry method using negative-ion chemical ionization to analyze urine samples for toluidine, 4-ABP, and 2-ABP. More recently, Niu et al. (2018) described an updated analytical method involving gas chromatography-mass spectrometry/mass spectrometry (GC-MS/MS) for measuring 1-NA and 2-NA.

- *Confounding/other sources of biomarker*: Aromatic amines are present in color additives, paints, food colors, and leather and textile dyes and in the fumes from heating oils and fuels (CDC, 2010).

Tobacco smoke from conventional cigarettes contains both aromatic and heterocyclic amines. The latter group of amines were not studied as exposure biomarkers in the literature on nicotine delivery products other than, or in addition to, conventional cigarettes, but were identified as biomarkers in the cigarette-only and unknown-products database. The most frequently studied amines in the current database (4-ABP, 2-NA, and toluidine) appeared to discern both user/non-user status and product types. This conflicts with the review of Chang et al. (2017), who concluded that aromatic amines do not differentiate smokers and non-smokers very well. Thus, the utility of amine biomarkers for discerning user status and between nicotine product types is inconsistent based on this high-level analysis, and warrants closer examination.

Table 5.   Summary of amines biomarker nicotine product use and product discernment data

Biomarker(acronym; parent compound of metabolite)	Biomarker group	Biological matrix	Total No. of data sets	Nicotine Product User/Non-User Discernment			Product Type Discernment		
				Could discern	Could not discern	N/A or unclear	Could discern	Could not discern	N/A or unclear
2-Naphthylamine (2-NA)	Aromatic amines	urine	16	10	0	6	13	0	3
o-Toluidine	Aromatic amines	urine	16	10	0	6	12	0	4
4-Aminobiphenyl (4-ABP)	Aromatic amines	urine	15	9	0	6	12	0	3
Hb adduct: 4-Aminobiphenyl	Aromatic amines, hemoglobin adduct	*all*	*6*	*5*	*0*	*1*	*5*	*0*	*1*
		blood/serum/plasma	4	3	0	1	3	0	1
		urine	2	2	0	0	2	0	0
1-Naphthylamine (1-NA)	Aromatic amines	urine	5	3	0	2	3	0	2
3-Aminobiphenyl	Aromatic amines	urine	5	3	0	2	4	0	1
Hb adduct: Toluidine	Aromatic amines, hemoglobin adduct	*all*	*2*	*0*	*2*	*0*	*2*	*0*	*0*
		blood/serum/plasma	1	0	1	0	1	0	0
		urine	1	0	1	0	1	0	0
Hb adduct: 2-Ethyl-aniline	Aromatic amines, hemoglobin adduct	blood/serum/plasma	1	0	1	0	1	0	0
Hb adduct: 2,4-Dimethyl-aniline	Aromatic amines, hemoglobin adduct	blood/serum/plasma	1	1	0	0	1	0	0
Hb adduct: 3-Aminobiphenyl	Aromatic amines, hemoglobin adduct	blood/serum/plasma	1	1	0	0	0	0	1
Hb adduct: Aniline	Aromatic amines, hemoglobin adduct	blood/serum/plasma	1	0	1	0	0	1	0
Hb adduct: Anisidine	Aromatic amines, hemoglobin adduct	blood/serum/plasma	1	0	1	0	0	1	0
2-Aminobiphenyl	Aromatic amines	urine	1	0	0	1	1	0	0

Biomarker(acronym; parent compound of metabolite)	Biomarker group	Biological matrix	Total No. of data sets	Nicotine Product User/Non-User Discernment			Product Type Discernment		
				Could discern	Could not discern	N/A or unclear	Could discern	Could not discern	N/A or unclear
Aromatic amines (unspecified)	Aromatic amines	urine	1	1	0	0	0	0	1
Total			**72**	**43**	**5**	**24**	**54**	**2**	**16**

3.2.6   Elements

Elements (Table 6)—especially metals—are widespread in the environment, and exposures occur through common daily activities. However, tobacco can be a significant source of exposure to metals, because tobacco plants absorb metal ions from the soil, with cadmium and lead being the most common elements associated with tobacco use (Chang et al., 2017). The level of metal uptake can vary depending on a number of variables, including the fertilizers used (Schick et al., 2017). For the some of the emerging nicotine products, there is concern that metals from metal heating elements, wires, solder joints, and electrical connectors may be another source of metal exposures associated with the use of these products (Schick et al., 2017). The elements evaluated in the current literature on nicotine delivery products other than, or in addition to, conventional cigarettes are summarized below.

- *Frequency:* As a biomarker group, element biomarkers were not commonly evaluated in the literature on nicotine delivery products other than, or in addition to, conventional cigarettes (only 34 total element biomarker data sets). Of these, the most frequently analyzed was cadmium, followed by lead, chromium, and nickel (Table 6). Other less frequently evaluated elements in the current literature database include the metalloids arsenic and selenium, as well as mercury, beryllium, cobalt, and tin.

    - *Copper, iron, polonium, and uranium were identified as biomarkers in the titles and abstracts of the cigarette-only literature but not in the literature on nicotine delivery products other than, or in addition to, conventional cigarettes. On the other hand, several element biomarkers evaluated in the latter literature database were not included in the cigarette-only literature, including arsenic, beryllium, chromium, cobalt, nickel, selenium, and tin (Attachment B).*

    - *In addition to copper and iron, the unknown-products literature included aluminum and undefined trace metals as biomarkers not evaluated in the literature on nicotine delivery products other than, or in addition to, conventional cigarettes. Several element biomarkers that were included in the latter literature database were absent from the titles and abstracts of the unknown-products literature, including beryllium, chromium, cobalt, mercury, nickel, selenium, and tin (Attachment C).*

- *Ability to discern nicotine product use status*: As a group, elemental biomarkers were able to discern user status in approximately 65% of data sets (Table 6). Unlike most of the biomarker groups summarized in the prior sections, a relatively small portion of the elemental biomarker data sets (less than 10%) did not explicitly demonstrate or report on discernment status (i.e., tagged by data extractors as "not addressed" or "NA" or "not clear"). Another observation that distinguishes this biomarker group is that a relatively larger portion of the data sets (26%) reported negative discernment results (i.e., insignificant difference in biomarker measurements between users and non-users). The elements that demonstrated evidence of insensitivity to user discernment were chromium, nickel, arsenic, selenium, beryllium, cobalt, and tin (Table 6).

- *Ability to discern between nicotine product types*: On the whole, the evidence for the elemental biomarker to discern between nicotine product types was weak relative to the other biomarker groups described above. Only approximately 26% of the literature database for elemental biomarkers reported positive discernment between the nicotine products evaluated. The same portion of the elemental biomarker data sets reported lack of discernment between product

types, leaving nearly half the data sets that either did not explicitly address product type discernment or reported unclear results.

- *Matrices tested*: Most of the elemental biomarker data sets were based on chemical analysis of urine and blood samples; a few results came from saliva, hair, and breath samples. The analytical method used by the CDC Laboratory Procedure Manual for measuring most of these metals in urine is inductively coupled plasma mass spectrometry (ICP-MS), used with dynamic reaction cell technology (DRC; CDC, 2014). The method is used to quantify either arsenic, a 15-element panel (antimony, barium, beryllium, cadmium, cesium, cobalt, lead, manganese, molybdenum, platinum, strontium, thallium, tin, tungsten, and uranium), or any subgroup of these.

- *Confounding/other sources of biomarker*: Cadmium is a common food contaminant (Vacchi-Suzzi et al., 2015). Confounding lead exposures may occur in individuals who live in very old houses where lead paint may still be on the walls (Jones et al., 2013).

Tobacco smoke from conventional cigarettes is a recognized source of exposure to certain metals, the most commonly detected being cadmium and lead. Richter et al. (2009) found elevated levels of these two metals in smokers relative to nonsmokers in the 1999–2004 NHANES cohort. At the same time, they found several other metals (mercury, beryllium, cesium, cobalt, molybdenum, platinum, thallium, and tungsten) to be present at equal or lower levels in smokers vs. nonsmokers. This would seem to support the discernment results summarized for the current literature database, which demonstrated limited discernment for user/non-user status and between product type for metals other than cadmium and lead. While the potential utility of metals as exposure biomarkers for discerning user/non-user status is limited to these two metals, some of the emerging nicotine products contain multiple metal components that represent potential exposure sources during use, raising the possibility that a unique spectrum of metals in user biomatrices may be associated with the use of particular products (Schick et al., 2017). The current evidence base is relatively sparse compared with the biomarker groups described above; however, with the increased popularity of e-cigarettes and heat-not-burn nicotine products, elements and metals as potential biomarkers represent an area for further research.

Table 6.   Summary of elements biomarker nicotine product use and product discernment data

Biomarker(acronym; parent compound of metabolite)	Biomarker group	Biological matrix	Total No. of data sets	Nicotine Product User/Non-User Discernment†			Product Type Discernment		
				Could discern	Could not discern	N/A or unclear	Could discern	Could not discern	N/A or unclear
Cadmium	Element, heavy metal	*all*	*10*	*8*	*0*	*2*	*5*	*0*	*5*
		blood/serum/plasma	6	5	0	1	3	0	3
		hair	1	0	0	1	0	0	1
		urine	3	3	0	0	2	0	1
Lead	Element, heavy metal	*all*	*5*	*5*	*0*	*0*	*2*	*2*	*1*
		blood/serum/plasma	4	4	0	0	2	1	1
		urine	1	1	0	0	0	1	0
Chromium	Element, heavy metal	*all*	*4*	*1*	*3*	*0*	*1*	*0*	*3*
		exhaled breath	1	1	0	0	0	0	1
		saliva	1	0	1	0	0	0	1
		urine	2	0	2	0	1	0	1
Nickel	Element, heavy metal	*all*	*4*	*3*	*1*	*0*	*1*	*0*	*3*
		exhaled breath	1	1	0	0	0	0	1
		saliva	1	1	0	0	0	0	1
		urine	2	1	1	0	1	0	1
Arsenic	Element, metalloid	all	3	1	1	1	0	2	1
		blood/serum/plasma	1	1	0	0	0	0	1
		urine	2	0	1	1	0	2	0
Mercury	Element, heavy metal	all	3	3	0	0	0	2	1
		blood/serum/plasma	2	2	0	0	0	1	1
		urine	1	1	0	0	0	1	0
Selenium	Element, metalloid	all	2	1	1	0	0	1	1
		blood/serum/plasma	1	1	0	0	0	1	0
		urine	1	0	1	0	0	0	1

Biomarker(acronym; parent compound of metabolite)	Biomarker group	Biological matrix	Total No. of data sets	Nicotine Product User/Non-User Discernment†			Product Type Discernment		
				Could discern	Could not discern	N/A or unclear	Could discern	Could not discern	N/A or unclear
Beryllium	Element, heavy metal	urine	1	0	1	0	0	1	0
Cobalt	Element, heavy metal	urine	1	0	1	0	0	1	0
Tin	Element, metal	urine	1	0	1	0	0	0	1
Total			**34**	**22**	**9**	**3**	**9**	**9**	**16**

3.2.7   Carbon Monoxide

Carbon monoxide (CO; Table 7) is a product of incomplete combustion of organic materials (Chang et al., 2017). Carbon monoxide levels are measured both in blood (as carboxyhemoglobin, or HbCO) and in exhaled breath, and is a common biomarker for characterizing recent tobacco smoking activity. Carbon monoxide has a relatively brief half-life (2–6 hours), however, and is a common environmental constituent (e.g., automobile emissions, environmental tobacco smoke, etc.), so the utility of CO measured in exhaled breath is limited by poor sensitivity and specificity (Benowitz et al., 2009; Marrone et al., 2011). Cut-off values for CO levels in exhaled breath have been proposed by various research groups, but there is not a consensus on a level that optimally discriminates smokers from non-smokers. Marrone et al. (2011) have proposed an analytical cut-point for CO in exhaled breath of 6 ppm to distinguish smokers of conventional cigarettes from non-smokers; it is not clear, however, how well exhaled CO differentiates between smokers of conventional cigarettes and other inhalation-based nicotine delivery products (e.g., heat-not-burn, electronic cigarettes). The CO discernment data in the alternative nicotine products literature is summarized below.

- *Frequency:* Carbon monoxide (either in exhaled breath or blood HbCO) was a common biomarker of exposure in the literature on nicotine delivery products other than, or in addition to, conventional cigarettes (Table 7), appearing in over 70 data sets. There were no other variations of CO measurement identified in this literature.

  - *Carbon monoxide measures (both exhaled breath and blood HbCO) were identified in the titles and abstracts of the cigarette-only literature. There were fewer CO data sets in the cigarette-only literature, but of these, CO was more commonly measured in exhaled breath than blood (Attachment B).*

  - *Again, carbon monoxide in both exhaled breath and blood (HbCO) were identified in the titles and abstracts of the unknown nicotine products literature. There were fewer CO data sets than in the other nicotine product literature databases, but of these, CO was more commonly measured in exhaled breath than blood (Attachment C).*

- *Ability to discern nicotine product use status*: Both biological measures of CO were about equal in the percentage of data sets in which CO was identified as an exposure biomarker able to discern nicotine product use status (approximately half of the total data sets) (Table 7). Fewer than half of the studies that included CO as a biomarker explicitly demonstrated or reported on the utility (or lack thereof) of CO to distinguish user discernment. In those that did not, either the study was not designed to characterize the ability for CO to discern user status (tagged by data extractors as "not addressed" or "NA") or the reported results were not explicitly clear on nicotine product use discernment (tagged by data extractors as "not clear"). For each CO biomarker measure, only two studies clearly indicated that the CO biomarker was NOT able to discern between nicotine product users and non-users, with a third study of multiple nicotine products provided evidence that CO could discern user/non-user status for one of the products, but not the other (Table 7).

- *Ability to discern between nicotine product types*: A greater portion of the HbCO data sets provided evidence of nicotine product type discernment compared with CO measured in exhaled breath (75% vs 50% of data sets in which a discernment determination could be made one way or the other).

- *Matrices tested*: Carbon monoxide was measured in exhaled breath and blood (as carboxyhemoglobin) in the current literature database, with the former matrix more commonly examined for CO (Table 7). The analysis of exhaled CO levels is a non-invasive, relatively simple and inexpensive method that involves the use of a commercially available instrument that uses a catalytic electrode to detect the rate of CO conversion to carbon dioxide (CO_2) (SRNT, 2002). The quantification of HbCO in blood samples is performed using spectrophotometry (SRNT, 2002).

- *Confounding/other sources of biomarker*: Carbon monoxide is a common combustion by-product, and as such, there are many exposure sources outside of combustible tobacco products that can confound its utility as an exposure biomarker, including automobile emissions, environmental tobacco smoke, marijuana smoke, etc. (Schick et al., 2017).

Tobacco smoke from conventional cigarettes is a primary source of carbon monoxide exposure. Biological measures of carbon monoxide (exhaled breath or carboxyhemoglobin in blood) have been used for detecting recent and/or heavy smoking activity, and is a useful biomarker for distinguishing between recent users of combustion- and non-combustion-based nicotine products. Reviewing the results of the discernment outcomes from the current evidence base, the most significant differences between the user/non-user and product-type results for CO biomarkers in the literature on nicotine delivery products other than, or in addition to, conventional cigarettes is that (1) blood CO more frequently discerned product type than user/non-user status, and (2) blood CO more frequently discerned product type than when measured in exhaled breath. For the former observation, it is likely that the underlying studies included nicotine products of both combustible and non-combustible materials, a scenario for which CO as a biomarker would be expected to have some utility. The reasons for the latter observation are beyond the scope of this effort, but it suggests that the more invasive method for evaluating CO might be more sensitive for this parameter. However, the relatively short half-life of CO in the body and its ubiquity in the environment are limitations on the utility of CO as an exposure biomarker for nicotine products. The current database should be examined more closely to better characterize the utility of CO for discerning between nicotine products.

Table 7.   Summary of carbon monoxide biomarker nicotine product use and product discernment data

Biomarker (acronym; parent compound of metabolite)	Biomarker group	Biological matrix	Total No. of data sets	Nicotine Product User/Non-User Discernment†			Product Type Discernment		
				Could discern	Could not discern	N/A or unclear	Could discern	Could not discern	N/A or unclear
		all	*40*	*18(19)*	*2(3)*	*19*	*21*	*12*	*7*
		exhaled breath	37	17(18)	2(3)	17	19	11	7
Carbon monoxide	Carbon monoxide	blood/serum/plasma	3	1	0	2	2	1	0
Carboxyhemoglobin	Carbon monoxide, hemoglobin	blood/serum/plasma	29	15(16)	2(3)	11	22	2	5
Total			**69**	**33(35)**	**4(6)**	**30**	**43**	**14**	**12**

† In some cases, biomarkers in a single study counted as both "can discern" and "cannot discern," because it could discern use for at least one product, but not another. The number in parentheses indicates the count with this dual result study accounted for.

3.2.8 Other Exposure Biomarkers (Table 8)

The literature on nicotine delivery products other than, or in addition to, conventional cigarettes included a number of other exposure biomarkers that, by themselves, were minor constituents of the larger exposure biomarker database. As is illustrated in Table 8, they represent a fairly diverse set of chemicals or endpoints. Of these, the biomarker best associated with use of nicotine delivery products is thiocyanate, a metabolite of the tobacco smoke constituent cyanide. Others represent environmental contaminants and/or products of incomplete combustion (e.g., dioxins, furans, organic phosphates). At least two others are associated with nicotine product ingredients (i.e., propylene glycol and menthol glucuronide). These biomarkers are summarized below.

- *Frequency:* The most frequently analyzed biomarkers of exposure outside of the common groups and markers noted above were unspecified mutagens (mostly derived from the results of urine samples being tested in generic mutagenicity tests such as the AMES test), thiocyanate, a collection of dioxin and furan congeners, and organic phosphates (Table 8).

  - *The titles and abstracts of the cigarette-only study database identified several additional exposure biomarkers relative to the literature on nicotine delivery products other than, or in addition to, conventional cigarettes, including: 2,5-dimethylfuran (a known biomarker of cigarette use), indole and pyridine derivatives, ketones (2-butanone and 2-pentanone), ferritin expression, as well as an instance of using gene expression as a biomarker of exposure (Attachment B).*

  - *The titles and abstracts of the unknown-products database also identified a few additional exposure biomarkers relative to the literature on nicotine delivery products other than, or in addition to, conventional cigarettes: 2,5-dimethylfuran (a known biomarker of cigarette use), derivatives of thiobarbituric acid, a propionamide metabolite, flavor and fragrance aldehydes (hexanal and nonanal), as well as expression, and also general proteomics and the expression of particular proteins (Attachment C).*

- *Ability to discern nicotine product use status*: The collection of urine mutagenicity assay results indicates that over 75% of the results were able to discern user/non-user status, with a couple of negative discernment results. The results for the thiocyanate biomarker indicate it is able to discern user/non-user status, as 75% of the data sets reported positive user status discernment. The remaining thiocyanate data sets were not amenable for making a conclusion on user discernment one way or the other. As a group, dioxin and furan exposure biomarkers were unable to discern user/non-user status. The ability of the organic phosphate biomarkers to discern user status is uncertain—a positive or negative conclusion on discernment could not be determined for five of the eight data sets. Overall, the database for these biomarkers is fairly small, so the discernment results should be interpreted with caution (Table 8).

- *Ability to discern between nicotine product types*: Overall, the ability of the "other" biomarkers to discern between product types mirrored the results of the user status discernment. Urine mutagenicity discerned product type in over 80% of the relevant data sets. While fewer thiocyanate data sets (four of eight) were able to discern product type relative to user status, none of the data sets indicated that this exposure biomarker was not able to make this discernment (the remaining four were not amenable to a discernment conclusion for product type). Dioxin and furan exposure biomarkers were unable to discern between nicotine product types. The product type discernment data for the organic phosphate biomarkers were similar

to the user status discernment results, in that most of the data sets were not amenable to making a discernment conclusion one way or the other.

- *Matrices tested*: Several of the biomarkers were analyzed in blood samples, including most of the thiocyanate data sets and the dioxins/furans. The generic mutagenicity test used urine samples, as did the analytical methods for organic phosphates, thioesters, and propylene glycol.

- *Confounding/other sources of biomarker*: Most of the exposure biomarkers in this category are not specific to nicotine products, and are either common environmental contaminants or biological endpoints that are not specific to any particular exposure. However, the studies on these biomarkers captured in the current evidence base were limited and generally did not address confounding from other sources of exposure.

Other than urine mutagenicity, the discernment data on the biomarkers collected into this "other" category were extremely limited. Because cyanide is a by-product of the combustion of tobacco smoke, there is evidence that this is an exposure biomarker of some utility. Thiocyanate has a half-life of 7–14 days, but can also be formed through dietary exposures (Benowitz et al., 2009). The evidence also suggests that the urine mutagenicity assay may be useful as a generic biomarker as well, although it is not limited to detecting mutagens generated from nicotine delivery products. The data for the remaining exposure biomarkers in this category are severely limited and require additional research before a decision on exposure utility can be developed.

Table 8.   Summary of other exposure biomarker nicotine product use and product discernment data

Biomarker (acronym; parent compound of metabolite)	Biomarker group	Biological matrix	Total No. of data sets	Nicotine Product User/Non-User Discernment†			Product Type Discernment		
				Could discern	Could not discern	N/A or unclear	Could discern	Could not discern	N/A or unclear
Mutagens, unspecified	Other	urine	17	13	2	2	14	1	2
Thiocyanate	Cyanide metabolite	*all*	*8*	*6*	*0*	*2*	*4*	*0*	*4*
		blood/serum/plasma	6	5	0	1	4	0	2
		saliva	1	1	0	0	0	0	1
		urine	1	0	0	1	0	0	1
Thioethers	Other	urine	4	3	0	1	0	1	3
Propylene glycol	Diol	urine	2	1	0	1	1	0	1
1,2,3,4,5,6,7,8-Octachlorodibenzo-p-dioxin (OCDD)	Dioxins/furans	blood/serum/plasma	1	1	0	0	1	0	0
1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin (HpCDD)	Dioxins/furans	blood/serum/plasma	1	1	0	0	1	0	0
1,2,3,4,6,7,8,9-Octachlorodibenzofuran	Dioxins/furans	blood/serum/plasma	1	0	1	0	0	1	0
1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	Dioxins/furans	blood/serum/plasma	1	0	1	0	0	1	0
1,2,3,4,7,8-Hexachlorodibenzofuran (HxCDF)	Dioxins/furans	blood/serum/plasma	1	0	1	0	0	0	1
1,2,3,4,7,8,9-Heptachlorodibenzofuran	Dioxins/furans	blood/serum/plasma	1	0	1	0	0	1	0
1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin (HxCDD)	Dioxins/furans	blood/serum/plasma	1	0	1	0	1	0	0
1,2,3,6,7,8-Hexachlorodibenzofuran	Dioxins/furans	blood/serum/plasma	1	0	1	0	0	1	0
1,2,3,7,8-Pentachlorodibenzo-p-dioxin	Dioxins/furans	blood/serum/plasma	1	0	1	0	0	1	0
1,2,3,7,8-Pentachlorodibenzofuran	Dioxins/furans	blood/serum/plasma	1	0	1	0	0	1	0
1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	Dioxins/furans	blood/serum/plasma	1	0	1	0	0	1	0

Biomarker (acronym; parent compound of metabolite)	Biomarker group	Biological matrix	Total No. of data sets	Nicotine Product User/Non-User Discernment†			Product Type Discernment		
				Could discern	Could not discern	N/A or unclear	Could discern	Could not discern	N/A or unclear
1,2,3,7,8,9-Hexachlorodibenzofuran	Dioxins/furans	blood/serum/plasma	1	0	1	0	0	1	0
2,3,4,6,7,8-Hexachlorodibenzofuran	Dioxins/furans	blood/serum/plasma	1	0	1	0	0	1	0
2,3,4,7,8-Pentachlorodibenzofuran (PeCDF)	Dioxins/furans	blood/serum/plasma	1	0	1	0	0	1	0
2,3,7,8-Tetrachlorodibenzo-p-dioxin	Dioxins/furans	blood/serum/plasma	1	0	1	0	0	1	0
2,3,7,8-Tetrachlorodibenzofuran	Dioxins/furans	blood/serum/plasma	1	0	1	0	0	1	0
Cornulin	Other	blood/serum/plasma	1	1	0	0	0	0	1
Menthol glucuronide (MG)	Other	blood/serum/plasma	1	0	0	1	1	0	0
Methemoglobin	Other	blood/serum/plasma	1	0	1	0	0	0	1
Nitric oxide (NO)	Other	exhaled breath	1	0(1)	0(1)	0	1	0	0
Malondialdehyde	Other	saliva	1	1	0	0	1	0	0
pH	Other	saliva	1	0	1	0	0	0	1
Total sialic acid (TSA)	Other	saliva	1	1	0	0	1	0	0
Uric acid	Other	saliva	1	0	1	0	0	0	1
2,3,4,5-Tetrabromobenzoic acid (TBBA)	Other	urine	1	0	0	1	0	0	1
Bis(1-chloro-2-propyl) phosphate (BCPP)	Phosphate	urine	1	0	0	1	0	0	1
Bis(1,3-dichloro-2-propyl) phosphate (BDCPP)	Phosphate	urine	1	0	0	1	0	0	1
Bis(2-chloroethyl) phosphate (BCEP)	Phosphate	urine	1	1	0	0	0	1	0
Di-o-cresyl phosphate (DoCP)	Phosphate	urine	1	0	0	1	0	0	1
Di-p-cresyl phosphate (DpCP)	Phosphate	urine	1	0	0	1	0	0	1
Dibenzyl phosphate (DBzP)	Phosphate	urine	1	0	0	1	0	0	1
Dibutyl phosphate (DBUP)	Phosphate	urine	1	1	0	0	1	0	0

Biomarker (acronym; parent compound of metabolite)	Biomarker group	Biological matrix	Total No. of data sets	Nicotine Product User/Non-User Discernment†			Product Type Discernment		
				Could discern	Could not discern	N/A or unclear	Could discern	Could not discern	N/A or unclear
Diphenyl phosphate (DPhP)	Phosphate	urine	1	0	1	0	1	0	0
Glucuronides (non-specific)	Other	urine	1	1	0	0	0	0	1
NO2+NO3	Other	urine	1	1	0	0	0	0	1
Total			**66**	**32(33)**	**20(21)**	**13**	**28**	**15**	**23**

† In some cases, biomarkers in a single study counted as both "can discern" and "cannot discern," because it could discern use for at least one product, but not another. The number in parentheses indicates the count with this dual result study accounted for.

4 Synthesis and Conclusions

4.1.1 Key Findings

The systematic map described herein was developed to transparently characterize the available biomarker literature relative to (1) ability of biomarkers to discern product usage status (e.g., non-smoker vs. smoker, smokeless tobacco user vs. cigarette smoker, etc.); (2) potential confounding from other sources of exposure (e.g., environmental exposures, dietary exposures); and (3) ease/invasiveness of sample collection. When the literature search results (i.e., the evidence base) were considered collectively, the following key findings were observed relating to the state of the science regarding the ability of available biomarkers to gauge the exposure and use patterns:

- *While some studies identified biomarkers that can discern between types of products used (e.g., cigarette smokers vs. users of smokeless tobacco; electronic cigarette users vs. smokers of traditional cigarettes), no specific biomarkers of exposure were identified among the literature that consistently demonstrated the capability of discerning across product categories*. The inconsistency, complexity, and heterogeneity of the evidence base preclude the ability to readily make conclusions regarding the ability of available biomarkers to discern nicotine delivery product user status and/or between usage of various products. Some biomarkers were reported more frequently by the study authors as having the ability to better discern between nicotine delivery product use status or between various products than others (e.g., benzene, acrolein, 1,3-butadiene, VOCs, cotinine, total NNAL, NNN), but there were no particular biomarkers that appeared to be consistently effective at doing so (Figure 3).[4]

- *Results varied by the products tested, the biomarkers evaluated, and the matrices in which the biomarker was assessed*. Discrete conclusions regarding individual biomarkers are difficult, given the complexity and heterogeneity of study designs (e.g., controlled trials and observational studies, variations in time between product cessation or switch relative to biomarker evaluation, etc.) combined with the volume of data (or lack of data in many cases). In many instances, authors discussed the likelihood of using multiple biomarkers to characterize exposure. Additional research is needed to further evaluate the utility of available biomarkers and to develop more sensitive biomarkers.

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[4] Based on review of descriptive statistics resulting from the literature review and data as reported by the authors. The scope of work did not include critical appraisal of individual studies, nor did the scope include specific assessment or recommendations regarding biomarkers with the greatest utility.
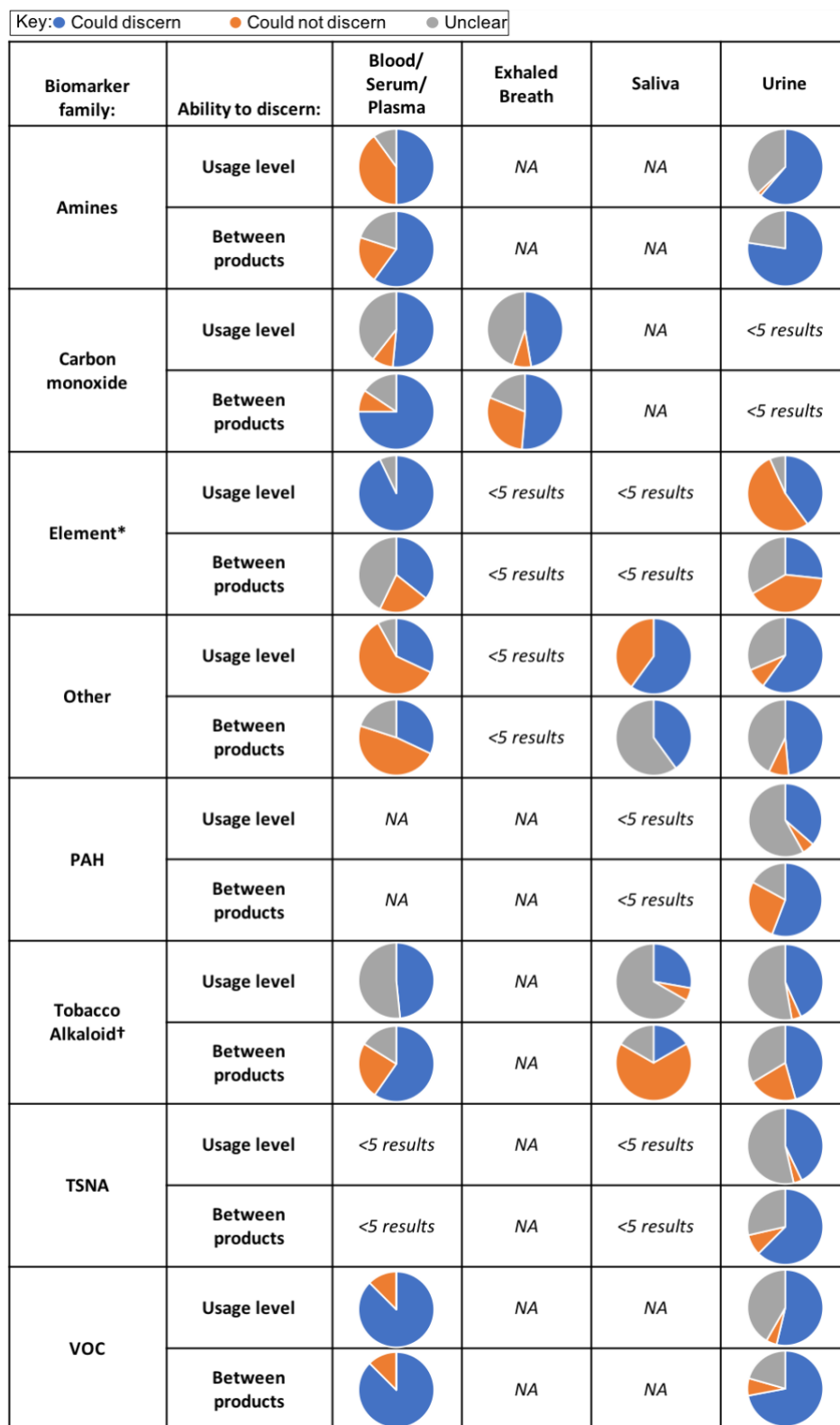
Figure 3. **Overview of biomarker results for articles that included nicotine delivery products other than, or in addition to, conventional combustible cigarettes. (See note below.)**

Pie charts demonstrate the distribution of the results for the ability to discern both usage level, organized by biomarker group, and biological matrix sampled. Pie charts are displayed only for those biological matrices / biomarker groups for which ≥5 results were reported. In addition to the biological matrices shown here, there was a single report of: *an element (cadmium) measured in hair, for which the ability to discern both usage level and between products was not clear, and †a tobacco alkaloid (nicotine equivalent) measured in feces, for which the ability to discern both usage level and between products was not clear.

- ***Potential confounding from other sources of exposure is insufficiently addressed by the current evidence base***: The majority of studies did not address this topic (those that did most often did not do so quantitatively); thus, so the literature review suggests that more studies are needed to investigate potential confounding from other sources as it relates to biomarkers of tobacco exposure or tobacco product use status. The number of biomarkers for which potential confounding exposure was reported is shown in Figure 4.

Potential confounding sources of exposure	Number of biomarker measurements
Authors indicate none exist	22
Automobile or diesel exhaust	8
Cannabis use	1
Diet	54
Endogenous processes	3
Environmental (not specified)	16
ETS	31
Incomplete combustion from heating systems	2
Methodological background	2
Medication: Nicotine-containing medicines noted	5
Not addressed	882
Occupational	16
Pesticides	4
Pollution	7
Occupational: genotoxic chemicals (general)	2
Tobacco products not surveyed	4

Figure 4.   Distribution of potential confounding sources of exposure across biomarkers of exposure and type of nicotine product across all biomarkers reported. Multiple sources may have been reported for a single biomarker measurement.

- ***The ease of sampling matrices varied within the evidence base***: Urine was the most common specimen collected for biomarker measurement; the only

biomarkers for which urine was not the most common matrix were metals, for which blood was most commonly collected. A smaller number of studies measured biomarkers in saliva, and expired breath was also used to measure carbon monoxide. Testing in saliva is gaining attention, although it also represents an area for additional research.

4.2   Contextual Considerations for Key Findings

The results of this review indicate that a considerable number of studies within the evidence base for each biomarker group provided insufficient evidence for user/non-user and product-type discernments to be made. This may have been partially due to a combination of factors, including the strict nature of the criteria applied herein for determining categorical assignments in the generation of the systematic map, as well as the combined complexity and heterogeneity of the underlying study designs that make up the evidence base. Because this effort was meant to systematically characterize the landscape across all biomarkers of exposure, the approach involved reliance on author report or readily available information in the study. Some of the studies in which insufficient evidence was provided for this effort may still contain important information unique to a given biomarker. Notably, the systematic map provided herein provides a tool for such specific investigations in the future.

It is also important to note that the quality of the studies was not assessed as part of the systematic map exercise herein. Critical appraisal of study validity is likely to be an important aspect of future investigations of specific exposure biomarkers. Related to this, the vast majority of the alternative nicotine product biomarker evidence base did not provide an assessment of potentially confounding exposures and other "outside" factors that could affect the biomarker results and author interpretation of these results. Those studies that did address the issue of confounding factors did so in a cursory manner, acknowledging the potential for confounding exposures but not exploring their potential in their study design. The lack of consideration for the role of confounding exposures associated with environmental exposures, in particular, is a clear limitation of the current evidence base.

One of the most prominent trends observed in the evidence base for biomarkers of exposure is that urinalysis is the predominant method of biomarker analysis— approximately three-fourths of the total biomarker data sets relied on urine sampling. This reflects one of the key issues in biomonitoring: invasiveness and ease of sampling. Urine collection is clearly advantageous over blood/serum/plasma sampling on these issues. In addition, investigators are able to obtain large volumes over multiple timepoints with short intervals, without discomfort to the participant (Strickland et al., 1996).

When compared with current biomarker literature reviews (Chang et al., 2017; Schick et al., 2017), it is evident that not all potential biomarkers of exposure were measured in the alternative nicotine product literature. For example, nicotelline is a tobacco alkaloid that was not captured in the evidence base on nicotine delivery products other than, or in addition to, conventional cigarettes. However, it was reported in studies evaluating only exposure via conventional cigarettes. This tobacco constituent holds promise as a potentially useful exposure biomarker for distinguishing between tobacco-smoke exposure and nicotine products that do not involve tobacco combustion. It is a stable and

low-volatility compound that is specific to particulate matter derived from tobacco smoke (Jacob et al., 2013; Chang et al., 2017). Other areas of research include the chemicals associated with e-liquids and e-cigarettes (e.g., propylene glycol, flavors and fragrance constituents, metals in the filament, etc.) One obstacle to using these as potential exposure biomarkers specific to the emerging nicotine products is that they are ubiquitous in the diet and consumer products, so innovative approaches will need to developed if their utility is to be realized (Chang et al., 2017; Schick et al., 2017).

4.3   Conclusions

This systematic map provides a structured, reproducible, transparent, and objective approach for characterizing the voluminous evidence base related to biomarkers of exposure associated with traditional and emerging nicotine products. The technical report and accompanying evidence tables provide a tool for synthesis and identification of data gaps, as well as a tool to facilitate future research for investigators seeking to further optimize exposure biomarkers.

Collectively, the evidence demonstrates that there are some studies in which biomarkers were reported to discern between types of products used, but no specific biomarker(s) of exposure consistently demonstrated the capability to discern across product categories. Additional research is needed to develop (or further refine) biomarkers that have the ability to discern both between tobacco use status and tobacco product types, are readily distinguishable from environmental or other confounding exposure, and can be evaluated with relatively non-invasive methods. Such research can build upon the current evidence base, which provides important, but not conclusive, characterizations regarding one or more biomarkers that have these features.

Conclusion: Additional research is needed to develop (or further refine) biomarkers that have the ability to discern both between tobacco use status and tobacco product types, are readily distinguishable from environmental or other confounding exposure, and can be evaluated in samples collected using relatively non-invasive methods. Such research can build upon the current evidence base, which provides important, but not conclusive, characterizations regarding one or more biomarkers that have these features.

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Systematic Map Protocol

Systematic Map Protocol for a Literature Review of Biomarkers of Exposure Related to Traditional and Emerging Nicotine Products

1. Review title

Literature Review of Biomarkers of Exposure Related to Traditional and Emerging Nicotine Products

2. Anticipated or actual start date

May 21, 2018

3. Anticipated completion date

August 2018

4. Review team members and their organizational affiliations

Seneca Fitch, ToxStrategies, Inc.
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Kara Franke, ToxStrategies, Inc.

5. Funding sources/sponsors

Foundation for a Smoke Free World

6. Review question(s)

The overall objective of this research is to develop a systematic map of potential biomarkers of exposure and effect (both clinical and pre-clinical) associated with tobacco and nicotine delivery products

7. Searches

The following search string was used to search the PubMed database, which returned a total of 6,333 articles when the search was executed on May 25th, 2018.

(nicotine[mh] OR nicotine OR tobacco[mh] OR "Tobacco smoking" OR tobacco[All Fields] OR "Tobacco, Smokeless"[mh] OR "Tobacco, Waterpipe"[mh] OR "tobacco smoke pollution"[mh] OR "tobacco smoke"[All Fields] OR "secondhand smoke" OR cigarette[All Fields] OR cigarettes[All Fields] OR "Electronic Nicotine Delivery Systems"[mh] OR "e-cigarette" OR "electronic cigarettes" OR Vaping[mh]) AND (biomarker* OR biomarkers[mh])

8. Conditions or domain being studied

Biomarkers of exposure to any tobacco or nicotine delivery products among users of such products. These will include chemical, biochemical, and or biomolecular measures, as well as possible cellular characteristics, associated with exposures from use of these products.

Biomarkers of potential health effects (both clinical and pre-clinical) related to such products, including (broadly) cancer, respiratory disease, cardiovascular disease, reproductive and developmental effects, and addiction/dependence. These will include physiological measures and biochemical and/or biomolecular measures of health effects/responses to exposures of tobacco or nicotine delivery product use. In addition, biomarkers used as tools to detect early stage diseases associated with tobacco product use (i.e., synthetic biomarkers) will be noted.

9. Participants/population

Populations: Tobacco and nicotine delivery product users and laboratory animals directly exposed in relevant study designs.

Include: General population and worker cohorts with known use or exposure to tobacco product use.

Include: Sensitive populations (pregnant women, children, individuals with potential genotype, non-healthy and/or patient populations) and post-mortem populations.

Exclude: Non-user populations exposed to secondary smoke/environmental tobacco smoke (ETS). *Note: Studies on ETS populations will be excluded from further review and synthesis (i.e., full text review, data extraction, systematic map integration), but tagged at the title and abstract screening level to define this segment of the literature for the purposes of institutional memory.*

10. Intervention(s), exposure(s)

Exposure: Use of tobacco and nicotine delivery products.

Include: Any such products, including nicotine replacement therapy products, combusted cigarettes, electronic cigarettes, heat-not-burn products (a.k.a. non-combusted tobacco products; e.g., iQOS, Ploom, glo), and oral tobacco products (e.g. chewing tobacco, moist snuff, snus, etc.).

Include: Studies in experimental animals for relevant routes of exposure; relevant routes of exposure include oral, inhalation (nose-only and whole-body exposure designs) and dermal.

Exclude: For humans, studies with documented co-exposures to chemicals other than those from non-tobacco/nicotine products (e.g., chemical occupational exposures; exposure to smoke/vapors such as wood smoke, marijuana, etc.). *Note: Studies with clear co-exposures in title and abstract will be excluded from further review and synthesis (i.e., full text review, data extraction, systematic map integration), but tagged at the title and abstract screening level to define this segment of the literature for the purposes of institutional memory.*

Exclude: For animals, studies with irrelevant routes of administration/exposure (e.g., intravenous, intraperitoneal).

11. Comparator(s)/control*

Comparator: Either unexposed population (including non-tobacco product users) or animal group, or traditional tobacco product users/exposure groups if study is designed to compare biomarkers between traditional tobacco product (e.g., cigarettes) and emerging nicotine delivery products (e.g., e-cigarettes) or between levels of exposure (e.g., light smoker vs. heavy smoker). For switch study designs, wherein tobacco users (commonly smokers of conventional cigarettes) switch between products, the comparator is the biomarker measurement reported at baseline (before the product switch).

12. Types of study to be included initially

Include: For humans, include epidemiology studies, clinical studies; animals, relevant route of exposure per product type (e.g., inhalation for cigarette or e-cig); any duration.

Exclude: For humans, studies that do not assess biomarkers at the individual level (e.g., ecological studies) and/or do not provide biomarker measures within a group or population (e.g., case series, case studies). *Note: Ecological-type epidemiology studies and case reports captured in the literature search will be excluded from further review and synthesis (i.e., full text review, data extraction, systematic map integration), but tagged at the title and abstract screening level to define this segment of the literature for the purposes of institutional memory.*

Exclude: In vitro studies (human and non-human). *Note: Any in vitro studies captured in the literature search will be excluded from further review and synthesis (i.e., full text review, data extraction, systematic map integration), but tagged at the title and abstract screening level to define this segment of the literature for the purposes of institutional memory.*

Exclude: Non-English language papers. *Note: Any papers not available in English that are captured in the literature search will be excluded from further review and synthesis (i.e., full text review, data extraction, systematic map integration), but tagged at the title and abstract screening level to define this segment of the literature for the purposes of institutional memory.*

Exclude: Review papers. *Note: Any papers that are themselves reviews of the literature and/or do not provide original biomarker data that are captured in the literature search will be excluded from further review and synthesis (i.e., full text review, data extraction, systematic map integration), but tagged at the title and abstract screening level to define this segment of the literature for the purposes of institutional memory.*

Exclude: Studies in which full text copies cannot be obtained following reasonable measures (online). Exception will be made if the abstract contains necessary information. *Note: This pertains to papers that are initially flagged as relevant and included further review during the title and abstract screening step, but for which full text copies are found to be unavailable.*

13. Primary outcome(s)*

Biomarkers of exposure: Any chemical, biochemical, or biomolecular endpoint measured in association with the use or exposure to a tobacco or nicotine delivery product.

Biomarker of effects (clinical and pre-clinical): FDA defines a biomarker of effect as an indicator of a change in biologic function in response to a chemical exposure[5]. Specific endpoints within five (5) broad categories will be defined via secondary literature: cancer, respiratory disease, cardiovascular disease, reproductive and developmental effects, and addiction/dependence. *Note: It is expected that additional outcomes (e.g., other CNS-related effects) will be identified during search and screen. Also, synthetic biomarkers used to detect early disease states will be included.*

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[5] Chang CM, Edwards SH, Arab A, Del Valle-Pinero AY, Yang L, Hatsukami DK. 2017. Biomarkers of tobacco exposure: Summary of an FDA-sponsored public workshop. Cancer Epidemiol Biomarkers Prev 26(3):291-302.

Summary of Biomarkers Used in Cigarette-Only Studies

Table B1.   Biomarkers identified in title and abstracts of cigarette-only studies

Biomarker (acronym; parent compound of metabolite)	Biomarker group	Total No. data sets	Biological Matrix													
			Urine	Blood/serum /plasma	Breast milk	Cervical mucus	Exhaled breath	Hair	Meconium	Nail (finger, toe)	Placenta	Saliva	Sperm	Tissue, lung	Tissue, tumor	Not stated
1-Methyldecyclamine	Amine	1					1									
1-Naphthylamine (1-NA)	Amine	1	1													
2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)	Amine	1	1													
2-Amino-9H-pyrido[2,3-b]indole (AαC)	Amine	2	2													
2-amino-9H-pyrido[2,3-b]indole-3-yl sulfate (AαC-3-OSO3H)	Amine	1	1													
2-Naphthylamine (2-NA)	Amine	2	1													1
3-Aminobiphenyl (3-ABP)	Amine	1	1													
4-Aminobiphenyl (4-ABP)	Amine	2	1													1
Ammonium	Amine	1					1									
Aromatic amines	Amine	1	1													
Hb adduct: 2-Amino-9H-pyrido[2,3-b]indole (AαC)	Amine, Hb adduct	1		1												
Hb adduct: 3-Aminobiphenyl	Amine, Hb adduct	1		1												
Hb adduct: 4-Aminobiphenyl	Amine, Hb adduct	8	1	6											1	
o-Toluidine	Amine	1														1

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Biomarker (acronym; parent compound of metabolite)	Biomarker group	Total No. data sets	Biological Matrix													
			Urine	Blood/serum /plasma	Breast milk	Cervical mucus	Exhaled breath	Hair	Meconium	Nail (finger, toe)	Placenta	Saliva	Sperm	Tissue, lung	Tissue, tumor	Not stated
Pyridine	Amine	1					1									
Pyrrole	Amine	1					1									
Carbon monoxide	Carbon monoxide	24					18									6
Carboxyhemoglobin	Carbon monoxide	6	1	5												
Copper	Element	1		1												
Iron	Element	1	1													
Lead	Element	2		1	1											
Lead 210 (210Pb)	Element	1	1													
Mercury	Element	2		1	1											
Polonium 210 (210Po)	Element	1	1													
Uranium	Element	1														1
Zinc	Element	1	1													
1-/9-Hydroxyphenanthrene	PAH	1	1													
1-Hydroxyfluorene	PAH	1	1													
1-Hydroxyphenanthrene	PAH	2	2													
1-Hydroxypyrene (1-HOP; 1-OHP)	PAH	2019	17													2
1-Naphthol (1-NAP)	PAH	3	3													
2-/3-Hydroxyphenanthrene	PAH	1	1													
2-Hydroxyfluorene (2-FLU)	PAH	2	2													

Biomarker (acronym; parent compound of metabolite)	Biomarker group	Total No. data sets	Biological Matrix													
			Urine	Blood/serum /plasma	Breast milk	Cervical mucus	Exhaled breath	Hair	Meconium	Nail (finger, toe)	Placenta	Saliva	Sperm	Tissue, lung	Tissue, tumor	Not stated
2-Hydroxyphenanthrene	PAH	2	2													
2-Naphthol (2-NAP)	PAH	4	4													
3-Hydroxybenzo[a]pyrene (3-OHBaP)	PAH	1	1													
3-Hydroxyfluorene	PAH	1	1													
3-Hydroxyphenanthrene	PAH	2	2													
4-Hydroxyphenanthrene	PAH	2	2													
Fluorene	PAH	1														1
Naphthalene	PAH	1														1
Phenanthrene	PAH	1														1
Phenanthrene metabolites	PAH	1	1													
Phenanthrene tetraol (PheT)	PAH	1	1													
Pyrene	PAH	3	1													2
Cotinine	Tobacco alkaloid	133	43	45		1		5	2	1	1	26	3			6
Cotinine glucuronide	Tobacco alkaloid	1	1													
Cotinine N-oxide	Tobacco alkaloid	1														1
Hydroxycotinine	Tobacco alkaloid	16	3	4				1	2			4				2
Hydroxycotinine glucuronide	Tobacco alkaloid	1	1													
Nicotine	Tobacco alkaloid	38	7	6		1		9	2	3		3				7
Nicotine equivalent	Tobacco alkaloid	13	11													2

Biomarker (acronym; parent compound of metabolite)	Biomarker group	Total No. data sets	Urine	Blood/serum /plasma	Breast milk	Cervical mucus	Exhaled breath	Hair	Meconium	Nail (finger, toe)	Placenta	Saliva	Sperm	Tissue, lung	Tissue, tumor	Not stated
																Biological Matrix
Nicotine N-oxide	Tobacco alkaloid	1						1								
Nicotine-N-glucuronide	Tobacco alkaloid	1	1													
Nornicotine	Tobacco alkaloid	3						1				2				
4-hydroxy-4-(3-pyridyl)butanoic acid (hydroxy acid)	TSNA	1	1													
NAB	TSNA	1	1													
NAT	TSNA	1	1													
NNAL	TSNA	36	29	1						1						5
NNAL-Gluc	TSNA	5	5													
NNK	TSNA	6	3													3
NNN	TSNA	1	1													
Hb adduct: Acrylamide	VOC, Hb adduct	1		1												
Hb adduct: Hydroxyethylvaline (HOEtVal)	VOC, Hb adduct	4		4												
(1- or 2-) Monohydroxybutenyl mercapturic acid (MHBMA)	VOC, mercapturic acid metabolite	5	4													1
1,2-Dihydroxybutyl mercapturic acids (DHBMA)	VOC, mercapturic acid metabolite	4	4													

Biomarker (acronym; parent compound of metabolite)	Biomarker group	Total No. data sets	Biological Matrix													
			Urine	Blood/serum /plasma	Breast milk	Cervical mucus	Exhaled breath	Hair	Meconium	Nail (finger, toe)	Placenta	Saliva	Sperm	Tissue, lung	Tissue, tumor	Not stated
2-carboxyl-l-methyl ethylmercapturic acid (CMEMA)	VOC, mercapturic acid metabolite	1	1													
2-Cyanoethylmercapturic acid (CYMA)	VOC, mercapturic acid metabolite	2	2													
2-Hydroxyethylmercapturic acid (HEMA)	VOC, mercapturic acid metabolite	2	2													
2-Hydroxypropyl mercapturic acid (2-HPMA)	VOC, mercapturic acid metabolite	1	1													
3-Hydroxy-1-methylpropyl Mercapturic Acid (HMPMA)	VOC, mercapturic acid metabolite	1	1													
3-Hydroxypropyl mercapturic acid (3-HPMA)	VOC, mercapturic acid metabolite	8	7													1
4-hydroxybutyl-2-mercapturic acid (HBMA)	VOC, mercapturic acid metabolite	1	1													
Acrylamide mercapturic acid (AAMA)	VOC, mercapturic acid metabolite	2	2													
Glycidamide mercapturic acid (GAMA)	VOC, mercapturic acid metabolite	2	2													
N-2-carbamoylethylvaline (AAVal)	VOC, amino acid adduct	1	1													

Biomarker (acronym; parent compound of metabolite)	Biomarker group	Total No. data sets	Urine	Blood/serum /plasma	Breast milk	Cervical mucus	Exhaled breath	Hair	Meconium	Nail (finger, toe)	Placenta	Saliva	Sperm	Tissue, lung	Tissue, tumor	Not stated
S-Phenylmercapturic acid (SPMA)	VOC, mercapturic acid metabolite	6	6													
Trihydroxybutyl mercapturic acid (THBMA)	VOC, mercapturic acid metabolite	1	1													
trans,trans-Muconic acid	VOC, metabolite	8	8													
1,3-Butadiene	VOC, parent	2					1									1
Acetonitrile	VOC, parent	2		1			1									
Acrolein	VOC, parent	2														2
Acrylonitrile	VOC, parent	1														1
Benzene	VOC, parent	12	6	2			2									2
Ethylbenzene	VOC, parent	3	2	1												
Styrene	VOC, parent	2		1												1
Toluene	VOC, parent	4	2	1			1									
Xylene	VOC, parent	4	2	1												1
2-Butanone	Other	1					1									
2-Methylfuran	Other	1					1									
2-Pentanone	Other	1					1									
2,5-Dimethyl hexane	Other	1					1									
2,5-Dimethylfuran	Other	6		3			3									

Biomarker (acronym; parent compound of metabolite)	Biomarker group	Total No. data sets	Biological Matrix													
			Urine	Blood/serum /plasma	Breast milk	Cervical mucus	Exhaled breath	Hair	Meconium	Nail (finger, toe)	Placenta	Saliva	Sperm	Tissue, lung	Tissue, tumor	Not stated
Calcium-containing particles	Other	1												1		
Dodecane	Other	1					1									
Ferritin	Other	1		1												
Gene signature (exposure prediction)	Other	1		1												
Glucuronides (non-specific)	Other	1		1												
HB adducts: Methyl-, hydroxyethyl-, cyanoethyl-	Other, Hb adduct	1		1												
Hb Ethylation	Other, Hb adduct	1		1												
Nitric oxide (NO)	Other	1					1									
O-cresol sulfate	Other	1		1												
pH	Other	1		1												
Thiocyanate	Other	7	2	5												
Unspecified biomarkers of cigarette smoke	Other	5	3	2												
Uric acid	Other	1		1												
Xenobiotic metabolites (not identified in abstract)	Other	1														1
Total		502	229	107	3	2	37	17	6	5	2	35	3	1	1	54

Summary of Biomarkers Used in Studies of Unknown Tobacco Products

Table C1. Biomarkers identified in title and abstracts of studies evaluating unstated nicotine delivery products[†]

Biomarker (acronym; parent compound of metabolite)	Biomarker group	Total No. data sets	Biological Matrix												
			Urine	Blood/serum/ plasma	Breast milk	Buccal sample	Cerebro-spinal fluid	Exhaled breath	Hair	Meconium	Nail (finger, toe)	Placenta	Saliva	Sweat	Not stated
4-ABP glucuronide	Amine	1	1												
4-Aminobiphenyl (4-ABP)	Amine	2	1	1											
Hb adduct: 2,6-Dimethylaniline (2,6-DMA)	Amine, Hb adduct	1		1											
Hb adduct: 4-Aminobiphenyl	Amine, Hb adduct	2		2											
N-acetyl-4-ABP	Amine	1	1												
Carbon monoxide	Carbon monoxide	10						9							1
Carboxyhemoglobin	Carbon monoxide	2		2											
Aluminum	Element	1						1							
Arsenic	Element	1	1												
Cadmium	Element	3		1				1				1			
Copper	Element	1						1							
Iron	Element	1						1							
lead	Element	1						1							
Trace elements	Element	1						1							
1-Hydroxyphenanthrene	PAH	1	1												
1-Hydroxypyrene (1-HOP; 1-OHP)	PAH	6	6												

Biomarker (acronym; parent compound of metabolite)	Biomarker group	Total No. data sets	Biological Matrix												
			Urine	Blood/serum/ plasma	Breast milk	Buccal sample	Cerebro- spinal fluid	Exhaled breath	Hair	Meconium	Nail (finger, toe)	Placenta	Saliva	Sweat	Not stated
1-Hydroxypyrene glucuronide (1-OHP-gluc)	PAH	2	2												
2-Hydroxyfluorene (2-FLU)	PAH	1	1												
2-Hydroxyphenanthrene	PAH	1	1												
2-Naphthol (2-NAP)	PAH	1	1												
3-Hydroxybenzo[a]pyrene (3-OHBaP)	PAH	2	2												
3-Hydroxyfluorene	PAH	1	1												
3-Hydroxyphenanthrene	PAH	1	1												
7,8,9,10-Tetrahydroxy-7,8,9,10-tetrahydro benzo(a)pyrene (7,8,9,10-OHBaP)	PAH	1	1												
9-Hydroxyfluorene (9-FLU)	PAH	1	1												
9-Hydroxyphenanthrene (9-PHE)	PAH	1	1												
Albumin adduct: benzo(a)pyrene	PAH, Albumin adduct	1	1												
Hb adduct: benzo(a)pyrene	PAH, Hb adduct	1	1												
Hb adduct: benzo(a)pyrene diol epoxide (BPDE-Hb)	PAH, Hb adduct	1	1												

Biomarker (acronym; parent compound of metabolite)	Biomarker group	Total No. data sets	Biological Matrix												
			Urine	Blood/serum/plasma	Breast milk	Buccal sample	Cerebro-spinal fluid	Exhaled breath	Hair	Meconium	Nail (finger, toe)	Placenta	Saliva	Sweat	Not stated
OH-PAH metabolites (sum)	PAH	1	1												
PAH metabolites	PAH	3	3												
PAHs	PAH	1	1												
Phenanthrene	PAH	1													1
Phenanthrene tetraol (PheT)	PAH	1	1												
3-Hydroxynorcotinine	Tobacco alkaloid	1	1												
Anabasine	Tobacco alkaloid	2	2												
Anatabine	Tobacco alkaloid	1	1												
Cotinine	Tobacco alkaloid	112	33	41			1		5	5	2		17		8
Cotinine glucuronide	Tobacco alkaloid	3	1	1											1
Cotinine N-oxide	Tobacco alkaloid	2	2												
Hydroxycotinine	Tobacco alkaloid	9	2	3						2			2		
Hydroxycotinine glucuronide	Tobacco alkaloid	2	1	1											
Myosmine	Tobacco alkaloid	3		1							1		1		

Biomarker (acronym; parent compound of metabolite)	Biomarker group	Total No. data sets	Biological Matrix												
			Urine	Blood/serum/ plasma	Breast milk	Buccal sample	Cerebro-spinal fluid	Exhaled breath	Hair	Meconium	Nail (finger, toe)	Placenta	Saliva	Sweat	Not stated
Nicotine	Tobacco alkaloid	27	6	2			1		8	3	3		4		
Nicotine equivalent	Tobacco alkaloid	2	2												
Nicotine N-oxide	Tobacco alkaloid	2	2												
Nicotine-N-glucuronide	Tobacco alkaloid	2	2												
Norcotinine	Tobacco alkaloid	2	1										1		
Nornicotine	Tobacco alkaloid	4	3								1				
4-Hydroxy-4-(3-pyridyl)butanoic acid (hydroxy acid)	TSNA	2	2												
4-oxo-4-(3-pyridyl)butanoic acid (keto acid)	TSNA	1	1												
Hb adduct: 4-hydroxy-l-(3-pyridyl)-1-butanone (HBP)	TSNA, Hb adduct	5		3		2									
NNAL	TSNA	17	11	3							2				1
NNAL-Gluc	TSNA	1	1												
NNK	TSNA	3	3												
NNN	TSNA	4	3												1
NNN-N-glucuronide	TSNA	1	1												

Biomarker (acronym; parent compound of metabolite)	Biomarker group	Total No. data sets	Biological Matrix												
			Urine	Blood/serum/ plasma	Breast milk	Buccal sample	Cerebro-spinal fluid	Exhaled breath	Hair	Meconium	Nail (finger, toe)	Placenta	Saliva	Sweat	Not stated
N-Methylenvaline	VOC, Amino acid adduct	1		1											
Hb adduct: acrylamide	VOC, Hb adduct	2		2											
HB adduct: 2-cyanoethylvaline	VOC, Hb adduct	1		1											
Hb adduct - carbamoylethylvaline	VOC, Hb adduct	1		1											
Hb adducts - glycidamide	VOC, Hb adduct	1		1											
(1- or 2-)Monohydroxy butenyl mercapturic acid (MHBMA)	VOC, mercapturic acid metabolite	2	2												
1,2-Dihydroxybutyl mercapturic acids (DHBMA)	VOC, mercapturic acid metabolite	2	2												
2-Cyanoethylmercapturic acid (CYMA)	VOC, mercapturic acid metabolite	1	1												
2-Hydroxyethylmercapturic acid (HEMA)	VOC, mercapturic acid metabolite	1	1												
2-Hydroxypropyl mercapturic acid (2-HPMA)	VOC, mercapturic acid metabolite	1	1												

Biomarker (acronym; parent compound of metabolite)	Biomarker group	Total No. data sets	Biological Matrix												
			Urine	Blood/serum/ plasma	Breast milk	Buccal sample	Cerebro- spinal fluid	Exhaled breath	Hair	Meconium	Nail (finger, toe)	Placenta	Saliva	Sweat	Not stated
2,3-Dihydroxypropyl mercapturic acid (DHPMA)	VOC, mercapturic acid metabolite	1	1												
3-Hydroxy-1-methylpropyl Mercapturic Acid (HMPMA)	VOC, mercapturic acid metabolite	1	1												
3-Hydroxypropyl mercapturic acid (3-HPMA)	VOC, mercapturic acid metabolite	3	3												
3,4-Dihydroxybutyl mercapturic acid (DHBMA)	VOC, mercapturic acid metabolite	1	1												
Mercapturic acid metabolites	VOC, mercapturic acid metabolite	1	1												
N-Acetyl-S-(4-hydroxy-2-methyl-2-buten-1-yl)-L-cysteine (IPMA3)	VOC, mercapturic acid metabolite	1	1												
N-Acetyl-S-(propionamide)-cysteine	VOC, mercapturic acid metabolite	1	1												

Biomarker (acronym; parent compound of metabolite)	Biomarker group	Total No. data sets	Biological Matrix												
			Urine	Blood/serum/plasma	Breast milk	Buccal sample	Cerebro-spinal fluid	Exhaled breath	Hair	Meconium	Nail (finger, toe)	Placenta	Saliva	Sweat	Not stated
S-Phenylmercapturic acid (SPMA)	VOC, mercapturic acid metabolite	1	1												
trans,trans-Muconic acid	VOC, metabolite	1	1												
Methylhippuric acid isomers (MHAs)	VOC, metabolites	1	1												
VOC metabolites	VOC, metabolites, unspecified	1	1												
1,3-Butadiene	VOC, parent	1	1												
Acetonitrile	VOC, parent	2						2							
Acrolein	VOC, parent	1	1												
Benzene	VOC, parent	4	1	1				2							
Crotonaldehyde	VOC, parent	1	1												
Ethylbenzene	VOC, parent	1		1											
Styrene	VOC, parent	1		1											
Toluene	VOC, parent	2		1				1							
Xylene	VOC, parent	2		2											
VOCs	VOC, unspecified	2						2							
1,3-Dibutyl-2-thiobarbituric acid (DBTB)	Other	1	1												

Biomarker (acronym; parent compound of metabolite)	Biomarker group	Total No. data sets	Biological Matrix												
			Urine	Blood/serum/ plasma	Breast milk	Buccal sample	Cerebro-spinal fluid	Exhaled breath	Hair	Meconium	Nail (finger, toe)	Placenta	Saliva	Sweat	Not stated
1,3-Diethyl-2-thiobarbituric acid (DETB)	Other	1	1												
2-Amino-2-thiazoline-4-carboxylic acid (ATCA)	Other	3	1	1									1		
2,3-Dihydroxy-propionamide (OH-PA)	Other	1	1												
2,5-Dimethylfuran	Other	1						1							
Ethylene oxide	Other	1	1												
Furan derivatives	Other	2	1					1							
Glucuronides (non-specific)	Other	1									1				
Hexanal	Other	1						1							
mRNA	Other	3		2								1			
Mutagens, unspecified	Other	2	2												
Nitric oxide (NO)	Other	1						1							
Nonanal	Other	1						1							
Protein - pancreatic alpha amylase	Other	1	1												
Protein - proepidermal growth factor	Other	1	1												
Protein - prostatic acid phosphatase	Other	1	1												
Protein - protein 4.1	Other	1	1												

Biomarker (acronym; parent compound of metabolite)	Biomarker group	Total No. data sets	Biological Matrix												
			Urine	Blood/serum/plasma	Breast milk	Buccal sample	Cerebro-spinal fluid	Exhaled breath	Hair	Meconium	Nail (finger, toe)	Placenta	Saliva	Sweat	Not stated
Proteomic profile	Other	2	1	1											
Thiocyanate	Other	17	2	5	1				1	1		1	5	1	
Thioethers	Other	1	1												
Total		351	153	83	1	2	2	28	14	13	8	3	31	1	13

† Most study titles and/or abstracts referenced "smokers", "tobacco smoke", or used some similar description that indicated use of a tobacco product. It is likely many of these studies evaluated biomarkers in smokers of conventional cigarettes, but this was not explicitly stated in the title or abstract. The papers will need to be reviewed to identify nicotine delivery products.