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Intake and Uptake of Chemicals Upon Use of Various Tobacco/Nicotine Products: Can Users be Differentiated by Single or Combinations of Biomarkers?*

by

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SUMMARY

With increasing use of new generation tobacco/nicotine products (TNPs) instead or in addition to conventional cigarettes (CCs), the question arises, whether the user of these new TNPs and CCs can be distinguished on the basis of their exposure in terms of intake and uptake of specific chemicals. For this purpose, the exposure to chemicals for users of 5 product types including CCs, HNB (heat-not-burn products), electronic cigarettes (ECs), oral tobacco products (OT, with the focus on snus), nicotine replacement therapy (NRT) products (only nicotine gum (NG) was considered in this study) was evaluated on the basis of published data. For both intake and biomarker-based uptake, 4 relative exposure levels with the assigned values from 1 (lowest exposure) to 4 (highest exposure) were defined resulting in exposure level patterns. Unique single-biomarker-based exposure levels were found for smokers (11 chemicals), vapers (1 chemical) and non-users (NU, 1 chemical). A few selected biomarkers (usually with relative levels of 3–4) were found to be sufficient for unequivocal differentiation of one user/NU group from the other 5 groups. The impact of dual-product use is also briefly discussed. [Contrib. Tob. Nicotine Res. 30 (2021) 167–198]

KEYWORDS

Conventional cigarettes; e-cigarettes; heat-not-burn products; oral tobacco; nicotine gum; relative exposure levels; intake; uptake; biomarkers; dual-product use

ZUSAMMENFASSUNG

Mit der zunehmenden Verwendung von Tabak/Nikotin-Produkten (TNPs) der neuen Generation statt oder zusätzlich zum Konsum von konventionellen Zigaretten (CCs), ergibt sich die Frage, ob die Konsumenten dieser Produkte aufgrund ihrer Exposition und Absorption von spezifischen Chemikalien unterschieden werden können. Zur Beantwortung dieser Frage wurde auf der Basis von publizierten Daten die Aufnahme von Substanzen bei Verwendern von 5 Nikotin/Tabak-Produktkategorien, nämlich, CC, HNB (‚heat-not-burn' Produkte), EC (elektronische Zigaretten), OT (orale Tabakproducte, mit dem Fokus auf Snus), NG (Nikotinkaugummi als Vertreter von therapeutischen Nikotinersatzprodukten, NRT) untersucht. Die Expositionsdosis (‚intake') als auch die biomarker-basierte Aufnahmemenge (‚uptake') wurde in 4 relative Expositionsklassen von 1 (niedrigste) bis 4 (höchste) Exposition eingeteilt, so dass sich für jede Konsumentengruppe typische Expositionsmuster für die untersuchten Chemikalien ergaben. Für Raucher wurden 11 Einzelsubstanzen, für EC-Konsumenten und NU (Nichtkonsumenten von TNPs) jeweils eine Substanz identifiziert, die eine eindeutige Differenzierung von allen anderen 5 Untersuchungsgruppen zuließ. Des Weiteren wurde gefunden, dass eine begrenzte Zahl von ausgewählten Biomarkern (mit relativen Leveln von üblicherweise 3–4) ausreicht, um eine Konsumentengruppe von den restlichen zu unterscheiden. Der Einfluss des Gebrauchs von zwei Produkten (‚dual use') nebeneinander wird ebenfalls kurz diskutiert. [Contrib. Tob. Nicotine Res. 30 (2021) 167–198]

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RESUME

Au vu de l'utilisation croissante de la nouvelle génération de produits à base de tabac et de nicotine venant remplacer ou compléter les cigarettes conventionnelles, se pose la question de la possibilité de distinguer l'utilisateur de ces nouveaux produits du fumeur de cigarettes conventionnelles sur la base de leur exposition en termes d'absorption et d'imprégnation de certaines substances chimiques spécifiques. A cette fin, l'exposition aux substances chimiques d'utilisateurs de cinq types de produits, parmi lesquels les cigarettes conventionnelles, les produits de tabac chauffé sans combustion, les cigarettes électroniques, les produits de tabac à usage oral (et plus particulièrement le tabac à priser humide) et les produits de thérapie de remplacement de la nicotine (TRN - seules les gommes à la nicotine furent incluses dans cette étude) fut évaluée sur la base de données publiées. Tant au niveau de l'absorption que de l'imprégnation basée sur les biomarqueurs, quatre niveaux d'exposition relative avec des valeurs assignées allant de 1 (exposition la plus faible) à 4 (exposition la plus élevée) furent délimités, faisant émerger des modèles d'exposition. Des niveaux d'exposition distincts, basés sur un seul biomarqueur, furent relevés pour les fumeurs (11 substances), les vapoteurs (1 substance) et les non-utilisateurs (1 substance). Une poignée de biomarqueurs sélectionnés (habituellement associés à des niveaux relatifs de 3–4) s'avéra suffisante pour une différenciation sans équivoque d'un groupe d'utilisateurs/non-utilisateurs des cinq autres groupes. L'incidence de l'utilisation combinée de deux produits est également brièvement évoquée. [Contrib. Tob. Nicotine Res. 30 (2021) 167–198]

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ABBREVIATIONS

1-OH-Nap	1-Napththol (biomarker of naphthalene)
1-OH-Phe	1-Hydroxyphenanthrene (biomarker of phenanthrene)
1-OH-Pyr	1-Hydroxypyrene (biomarker of pyrene)
2-OH-Flu	2-Hydroxyfluorene (biomarker of fluorene)
2-NA	2-Naphthylamine
2-HPMA	2-Hydroxypropyl-mercapturic acid (biomarker of propylene oxide)
2-OH-B*a*P	3-HydroxyBenzo[*a*]pyrene (biomarker of Benzo[*a*]pyrene)
3-HPMA	3-Hydroxypropyl-mercapturic acid (biomarker of acrolein)
4-ABP	4-Aminobiphenyl
AA	Acetaldehyde
AAMA	*N*-acetyl-S-(2-carbamoylethyl)-l-cysteine (biomarker of acrylamide)
AB	Anabasine
AC	Acetoin
ACR	Acrolein
AM	Acrylamide
AN	Acrylonitrile
AT	Anatabine
B*a*P	Benzo[*a*]pyrene
BD	1,3-Butadiene
Be	Benzene
BoEx	Biomarker of exposure
CC	Conventional cigarette
CDC	(US) Centers for Disease Control and Prevention
CEMA	Cyanoethyl-mercapturic acid (biomarker of acrylonitrile)
CI	Confidence interval
CO	Carbon monoxide
COex	Carbon monoxide in exhaled air (biomarker of carbon monoxide)
COHb	Carboxyhemoglobin (biomarker of carbon monoxide)
Cot	Cotinine (biomarker of nicotine)
CPD	Cigarettes smoked per day
CRO	Crotonaldehyde
EC	Electronic cigarette
EO	Ethylene oxide
EPA	(US) Environmental Protection Agency
FA	Formaldehyde
Flu	Fluorene
Fu	Furan
G	Glycerol
Gly	Glycidol
GO	Glyoxal
HCN	Hydrogen cyanide
HEMA	Hydroxyethyl-mercapturic acid (biomarker of ethylene oxide)
HMPMA	Hydroxymethylpropyl-mercapturic acid (biomarker of crotonaldehyde)
HNB	Heat-not-burn
I	Intake
IARC	International Agency for Research on Cancer
IPMA3	*N*-acetyl-S-(4-hydroxy-2-methyl-2-buten-1-yl)-L-cysteine (biomarker of isoprene)
Iso	Isoprene
LOD	Limit of detection
LOQ	(lower) Limit of quantification
MC	Monte Carlo
MGO	Methylglyoxal
MHBMA	Monohydroxybutenyl-mercapturic acid (biomarker of 1,3-butadiene)
MSp	Mouth spill
N	Nicotine
NAB	*N*-Nitrosoanabasine
Nap	Naphthalene
NAS	National Academy of Sciences
NAT	*N*-Nitrosoanatabine
NG	Nicotine gum

Nequ	Nicotine equivalents (biomarker of nicotine)
NHANES	National Health and Nutrition Examination Survey
NN	Nornicotine
NNAL	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (biomarker of NNK)
NNK	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone
NNN	*N*-Nitrosonornicotine
NRT	Nicotine replacement therapy
NU	Non-user(s)
OT	Oral tobacco
o-Tol	Ortho-Toluidine
P	Plasma
PA	Propionaldehyde
PAH	Polycyclic aromatic hydrocarbon
PG	1,2-Propylene glycol
Phe	Phenanthrene
PHEMA	Phenylhydroxyethyl-mercapturic acid (biomarker of styrene)
PO	Propylene oxide
Pyr	Pyrene
R	(respiratory) Retention
Sal	Saliva
SCN	Thiocyanate (biomarker of hydrogen cyanide)
SD	Standard deviation
SLT	Smokeless tobacco
SPMA	*S*-Phenyl-mercapturic acid (biomarker of benzene)
Sty	Styrene
TNPs	Tobacco and nicotine (tobacco/nicotine) products
TSNA	Tobacco-specific nitrosamine
U	Urine
WHO	World Health Organization
Y	Yield

INTRODUCTION

Smoking is the single greatest preventable cause of death and disability in the world today. The exposure to toxicants from the mainstream smoke of cigarettes is causally related to the induction of many diseases (1, 2). The smoke of conventional cigarettes (CC) contains thousands of chemicals in amounts of sub-ng/cig to g/cig (range of about 10 orders of magnitude). One of the earliest reviews on the composition of tobacco and tobacco smoke was that by STEDMAN (3) and the book by WYNDER and HOFFMANN (4). STEDMAN (3) stated that the number of chemicals in tobacco smoke has increased from about 400 to 1200 in the decade since 1959. In a recent book of RODGMAN and PERFETTI (5), more than 8400 chemicals were named as tobacco smoke constituents. The large number of constituents in smoke of CC requires reduction by suitable criteria for the sake of reasonable regulations. Most frequently, toxicity and hazardousness of the chemicals are used as selection criteria. Examples of those 'reduced' lists are the "HOFFMANN LIST" containing 44 smoke constituents (6), a list of 9 chemicals mandated to be reduced in cigarette smoke established by a WHO working group in 2008 (7) or a list of 39 toxicants proposed by another WHO working group in 2015 (8), as well as the FDA list of 18 and a more extended list of 93 toxicants in smoke of CC (9). For the purpose of this study, the specificity of a chemical released by the product is the center of attention. In addition, the quantitative pattern of constituents could be characteristic of a product and be used for differentiation of its users as well. A global study of machine yields of mainstream smoke constituents of 48 cigarette brands has been reported by COUNTS *et al.* (10). The constituents analyzed mostly resembled those in the so-called "HOFFMANN LIST".

Given the huge number and the significant amounts of toxicants released by the process of conventional tobacco smoking, the cigarette industry put in great efforts to reduce the levels of toxic components in the smoke released from the product by introducing various technologies in the cigarette design, including smoke filtration, paper porosity, filter ventilation or expanded tobacco (11). However, reductions in the overall toxicity and consequently, in the risk for the consumer were of limited success, due to the fact that toxicants are mostly generated during the combustion process at temperatures of 600–900 °C (12). Smokeless tobacco (SLT) products such as chewing tobacco, snuff and snus avoid the problem of generation of toxicants during combustion and are thus less hazardous for the consumer (13, 14).

However, in most countries and populations use of SLT products is less popular than smoking cigarettes. With the advent of a new generation of tobacco and nicotine products, which generate an inhalable vapor or aerosol by not combusting but heating a suitable matrix at temperatures below 350 °C, smokers, not willing or able to quit the habit, appear to have available a less harmful alternative to cigarettes (15). The new generation of non-combustible and inhalable tobacco and nicotine products comprises mainly the two categories of heat-not-burn tobacco products (HNB) and electronic cigarettes (ECs). Also hybrids of both vapor generation systems are available today (16).

As mentioned above, the smoke of CCs has been extensively characterized in terms of its chemical composition. Comprehen-

sive data are also available on the daily consumption and use pattern of CCs (1, 2, 17–20). An objective measure for the exposure to nicotine and toxicants by using the various nicotine and tobacco products are biomarkers of exposure (BoEx) analyzed in body fluids. For cigarette smokers and non-smokers, a large number of BoEx data sets for various toxicants are available (e.g., (21–24)).

For HNB and their users, less data than for CCs on the release of toxicants (25–30), pattern of use (31–34) and biomarker levels upon use (33–39) are on-hand. In a recently published comprehensive untargeted screening study, a total of 529 chemical constituents, excluding water, glycerin, and nicotine, were found to be present in the aerosol of a HNB product at concentrations ≥ 100 ng/stick (40). In total, HNB-related data, however, appear to be sufficient for estimating the exposure doses for the most important constituents of this product category.

The same applies for ECs. The release of toxicants into the vapor has been studied in quite a number of recently published investigations (41–47). Biomarkers in body fluids of vapers have been extensively investigated, although the number of toxicants studied is limited (48–56) and most likely will stay low and never reach that in CCs. The pattern of use of ECs in terms of daily consumption is also well documented (49, 54, 56–66).

Oral tobacco (OT) has also been investigated intensively in terms of toxicant content (67–69), pattern of use (39, 70–73) and biomarker levels in OT users (54, 72–77).

Products for nicotine replacement therapy (NRT) such as nicotine patch, inhaler and gum are much simpler in terms of their composition as compared to the products discussed above. Basically, NRT products release nicotine and possibly some nicotine contaminants such as tobacco-specific nitrosamines (TSNAs) and some minor alkaloids (e.g., anabasine or anatabine). For nicotine gum (NG), which is used as representative NRT product for comparison to the other nicotine products in this review, only the nicotine content (54, 78) and that of TSNA (79) was analyzed. The use pattern of nicotine gum in smoking cessation studies has been reported (54, 80–82). Since nicotine gum users were included as a comparator or control group in some switching studies, biomarker data for this NRT group are available for a range of toxicants (54, 83, 84).

The purpose of this study is to compare the intake of chemicals in users of the following tobacco and nicotine products:

- Conventional cigarettes (CC)
- Heat-not-burn products (HNB)
- Electronic cigarettes (ECs)
- Oral tobacco products (OT, preferably snus)
- Nicotine replacement therapy (NRT) products (preferably nicotine gum; NG)

Furthermore, biomarker levels of nicotine and toxicants in users of the above products as well as in non-users (NU) were compared in order to support and extend the estimated intake data. As a result, typical patterns of exposure levels to chemicals (both in terms of intake and biomarker-based uptake) for users of each product and non-users will be available for the discrimination of the 6 study groups. We believe that unequivocal identification of product use (either as single, dual or multiple product use) is of eminent importance for risk assessment of the users of the new-generation tobacco/nicotine products. Self-reports on product use might not have the required reliability. The biochemical verification of the tobacco use and abstinence status (85) certainly represents a step in this direction, but might not be sufficient.

The objective of this evaluation, therefore, is to identify single or patterns of biomarker levels, which allow the assignment of the most probable use of one, two or none of the 5 tobacco/nicotine products included in this review. For this purpose, published data on chemicals released from the 5 products upon intended use and an average daily use pattern were employed to estimate the daily product-related intake. Furthermore, published data on corresponding biomarker levels (indicating the absorption and therefore systemic uptake of these chemicals) in users of these products as well as in non-users were also evaluated. The intake and uptake data formed the basis for establishing the 4-level exposure system for each chemical, resulting in biomarker-based exposure level patterns characteristic for each of the 6 user/non-user groups (CC, HNB, EC, OT, NG, NU). With this approach, product use could be assigned with high probability by means of only a few suitable biomarkers.

METHODS

This review is based on published data which are extracted from publicly available databases such as MEDLINE, BIOSIS and Chemical Abstracts. Included are articles published in peer reviewed journals which provide quantitative data on the content of nicotine, toxicants and other chemicals which are released by the 5 tobacco/nicotine product types upon intended use. Also considered are articles, which provide quantitative data on biomarkers of exposure (BoEx) in users of the products of interest as well as in non-users. Not included in this review were data on metals (neither contents in products nor biomarkers). The main reason for omitting metals was the fact that release of metals, particularly in ECs, is highly product-dependent and thus does not substantially contribute to the intended purpose. The origin of data for the intake of chemicals by NU is described in the section "*Exposure prediction for NU*".

Selection of chemicals to be included in the evaluation

The first and essential step for the intended evaluation was the selection of chemicals to be included. The strategy for establishing a suitable list of constituents released by the products of interest comprised the following criteria and considerations.

Nicotine- and tobacco-derived compounds

Nicotine, nornicotine (NN), anabasine (AB), anatabine (AT), *N*-nitrosonornicotine (NNN), nicotine-derived nitrosoketone (NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone), *N*-nitrosoanabasine (NAB), *N*-nitrosoanatabine (NAT) are tobacco-specific compounds. All tobacco and nicotine products can potentially contain these chemicals. Except for nicotine (wanted ingredient), all other substances are unwanted contaminants. Therefore, these chemicals are not unique for any of the products of interest, but their levels in the products and also the levels in the users (indicated by suitable BoEx) could be different. Of course, the listed chemicals can be used for discrimination between tobacco/nicotine product users and non-users, provided that the product-related intake significantly exceeds the background exposure.

Major ingredients (matrix) of the products to be compared

The tobacco-based products (CC, HNB, OT) contain, self-evidently, tobacco as the major matrix of the product. ECs contain propylene glycol (PG) and glycerol (G) in various ratios as matrix compounds in the e-liquid for aerosol formation. Although PG and G are ubiquitously used as food additives and G is also a prevailing endogenously occurring metabolite (major component of lipids), both chemicals (or their metabolites) might be used as BoEx for EC vapors under certain conditions (e.g., when applying stable isotope-labeled PG and G in e-liquids (56)). Furthermore, PG and G may be thermally decomposed to a number of aldehydes, ketones and epoxides such as formaldehyde (FA), acetaldehyde (AA), propionaldehyde (PA), acrolein (ACR), crotonaldehyde (CRO), glyoxal (GO), methylglyoxal (MGO), propylene oxide (PO) and glycidol (Gly) (46, 86). Matrix components of the nicotine gum (NG) are not expected to be absorbed in appreciable amounts by NG users.

Combustion-derived compounds

Of the products under study, CCs are the only products which apply (incomplete) combustion of organic materials during use as intended at temperatures between 600 and 900 °C (12), leading to combustion products such as carbon dioxide (CO_2), carbon monoxide (CO), ammonia (NH_3), hydrogen cyanide (HCN), benzene, 1,3-butadiene (BD), carbonyls, isoprene (Iso), acrylonitrile (AN), acrylamide (AM), polycyclic aromatic hydrocarbons (PAHs), *N*-heterocyclic PAHs, aromatic amines, and many others (5).

Therefore, in general, the BoEx of the combustion products would be suitable for discriminating CC users (smokers) from the other product users and the non-users, provided that there is no measurable interference from other sources (e.g., food, ambient air, endogenous formation). There are some compounds such as carbonyls (formaldehyde, acetaldehyde, acetone, acrolein, crotonaldehyde) and epoxides (propylene oxide, glycidol) which can be also formed when using e-cigarettes and possibly also HNBs (46, 87). Furthermore, there is evidence that acrylamide may be also formed by HNBs (25, 29), even though in lower amounts, which has to be considered when smokers and HNB users are to be discriminated on the basis of a BoEx for acrylamide.

The chemicals to be included in the evaluation not least depend on the availability of reported data for the 5 product types dealt with in this study. The basis for this is formed by some fundamental investigations of the products of interest (25–30, 33, 45, 67, 69, 75, 78, 88–90).

Metals and flavoring agents were not included in this evaluation because their occurrence in the products of interest are highly brand-specific and, therefore, would require a separate evaluation.

Estimation of the intake of chemicals by using the 5 product categories

For the estimation of the daily intake of chemicals by using the 5 tobacco/nicotine product types of interest, first the units of use for these products were defined. While it is obvious for CCs (1 cigarette), HNBs (1 stick) and NGs (1 piece), it had to be set for ECs (10 puffs) and OTs (1 g). These settings were commonly used in the scientific literature and turned out to be of practical use during the course of this study (for reference, see Table 1). A second predefinition pertained the daily use pattern (daily consumption in unit/day). For this purpose, suitable published data were evaluated. The outcome is presented in Table 1. It has to be emphasized that the general aim in this study was to come up with average consumption (and consequently intake and uptake) data and to avoid extremes in any direction. This principle did not only apply to product consumption data, but was also followed for release data for the chemicals from the products as well as reported biomarker levels.

For estimating the daily intake of the various chemicals by common use of the 5 nicotine products, ranges of the variables for the contents in the products, daily consumption (Table 1) and actual release of the chemical to the user (as defined below) were applied. In case that sufficient independent data sets for these variables were available (at least 3), the 25^{th}–75^{th} percentile was used as range for the intake estimations. When only 2 values were available, the min – max range was used. If only one value was available, a range of ± 50% of this value was applied for the estimation of the intake. If the content of a chemical was reported to be below the limit of quantification (LOQ) or limit of detection (LOD), 0.5 of the LOQ or LOD value was used. Daily intake calculations using these ranges were performed by applying Monte Carlo (MC) simulations using the random number generator of Microsoft Excel. For each calculation (one product, one chemical), 10,000 runs were conducted. Increasing the number of runs to 50,000 or 100,000 did not lead to a significant change of the intake estimates. The MC approach using the ranges of the variables instead of using only the means or medians provides also measures of the variation of the estimates. From the 10,000 runs of the MC simulation, means, standard deviations of the means, 95% confidence intervals (CIs), 10^{th}, 25^{th}, 50^{th}, 75^{th} and 90^{th} percentiles were calculated.

Individual puffing topographies for the inhalable products (CC, HNB, EC), which were found to be dependent on product characteristics, in particular the nicotine content in the smoke/vapor were not taken into account in this evaluation (19, 20, 91).

Intake estimates for smokers (CC users)

The calculation of the daily amount of a smoke constituent taken in by smoking conventional cigarettes (CC) was based on the approach described by LOGUE *et al.* (62):

$$I_{cc} = Y \times CPD \times (1 - MSp) \times R \quad [1]$$

with:

I_{cc} = Intake (mass/d) by smoking conventional cigarettes (CC)

Y = Yield (mass/cig); ranges used for calculation are shown in Table 2

CPD = Cigarettes smoked per day (cig/d); ranges used for calculation are shown in Table 1

MSp = Mouth spill (fraction of smoke immediately released from the mouth, not taken into the lung); range used for calculation: 20–40% (see below)

R = Respiratory retention (fraction of smoke constituent retained in the lung, not exhaled immediately after inhalation); range used for calculation: 93–99% (see below)

The mean mouth spill (MSp) is reported to amount to 30% with a range of 20–40% (62). For estimating the intake of all smoke constituents an MSp range of 20–40% was applied.

For the respiratory retention (R), a range of 93–99% (mean: 96%) is reported (62, 92). In an evaluation of the literature, BAKER and DIXON (93) came up with an estimated retention of 60 to 80% of

the mainstream smoke particulate matter and retention for nicotine, carbon monoxide, nitric oxide, and aldehydes amounting to 90–100, 55–65, 100, and approximately 90%, respectively, during cigarette smoke inhalation. MOLDOVEANU *et al.* (94) measured retentions of 8 different aldehydes of > 95% for cigarettes with "tar" levels of 5.0, 10.6 and 16.2 mg. In this evaluation, for estimating the intake by smoking cigarettes, a range for R of 93–99% was used. With the ranges described above as well as in Tables 1 and 2, the MC simulation for estimating the intake was calculated with the following equation (the daily nicotine intake by smoking is shown as an example):

$$I_{cc\,nic} = Y \times CPD \times (1 - MSp) \times R \quad [1a]$$

with the numbers for nicotine intake by smoking (CC) inserted:

$$I_{cc\,nic} = [0.59–1.67] \times [14.3–16.6] \times (1–[0.20–0.40]) \times [0.93–0.99]$$

By applying a random number generator, the calculation is performed with discrete values within each range in each of the 10,000 runs.

Intake estimates for HNB users

A similar approach as for smoking cigarettes (CC) was used for estimation of the daily intake of chemicals by using HNB products:

$$I_{hnb} = Y \times SPD \times (1 - MSp) \times R \quad [2]$$

with:

I_{hnb} = Intake (mass/d) by using HNB products
Y = Yield (mass/stick); ranges used for calculation are shown in Table 2
SPD = Sticks consumed per day (stick/d); ranges used for calculation are shown in Table 1
MSp = Mouth spill (fraction of smoke immediately released from the mouth, not taken into the lung); range used for calculation: 20–40%
R = Respiratory retention (fraction of smoke constituent retained in the lung, not exhaled immediately after inhalation); range used for calculation: 93–99%

The ranges for MSp (20–40%) and R (93–99%) used for the intake estimation from HNB products by MC simulation were assumed to be identical with those applied for CCs.

Intake estimates for vapers (EC users)

The estimation of the daily intake of chemicals by vaping (use of ECs) was performed in analogy to the other two inhalable products (CC and HNB):

$$I_{ec} = Y \times SePD \times (1 - MSp) \times R \quad [3]$$

with:

I_{ec} = Intake (mass/d) by vaping ECs
Y = Yield (mass/10 puffs); ranges used for calculation are shown in Table 2
SePD = Sessions run per day (each session comprise 10 puffs); ranges used for calculation are shown in Table 1
MSp = Mouth spill (fraction of smoke immediately released from the mouth, not taken into the lung); range used for calculation: 20–40%
R = Respiratory retention (fraction of smoke constituent retained in the lung, not exhaled immediately after inhalation); range used for calculation: 93–99%

As a unit of use, 10 puffs from the EC was defined. One vaping session (= 10 puffs) was assumed to be roughly equivalent to smoking one cigarette or using one HNB product (stick).

The averages and ranges for daily consumption of ECs (SePD: sessions with 10 puffs each per day) were calculated on the basis of 11 reports in the literature (compiled in Table 1). In some reports the consumption is provided as g liquid consumed per day. This unit is converted to number of puffs and sessions per day by using the following relation observed in a controlled study: 10 sessions/d with 10 puffs each resulted in an e-liquid consumption of 1.26–1.56 g/d (56). In cases where the daily consumption was provided as volume of e-liquid (mL), an e-liquid matrix of PG/G of 50/50 (vol/vol) with a density of 1.11485 g/mL was assumed.

For estimating the intake of all vapor constituents by means of the MC simulation, a consumption range of 11.6–21.25 sessions/d (25^{th}–75^{th} percentile) was used (Table 1).

The ranges for MSp (20–40%) and R (93–99%) used for the intake estimation from ECs by MC simulation were identical with those applied for CCs and HNB products.

Intake estimates for OT users

The estimation of the daily intake of chemicals by using OT (in particular snus) was performed by applying the following relationship:

$$I_{ot} = CCOT \times PEXOT \times DCOT \quad [4]$$

with:

I_{ot} = Intake (mass/d) of a chemical by using an OT product
CCOT = Content of a chemical in OT (mass/g); ranges used for calculation are shown in Table 2
PEXOT = Percentage extracted from OT; range used for calculation: 20–35%; see below
DCOT = Daily consumption of OT (g/d); range used for calculation: see Table 1

As a unit of use, 1 g of OT was defined. For estimating the intake of OT constituents by means of the MC simulation, a consumption range of 1.98–3.05 g/d (25^{th}–75^{th} percentile) was used (Table 1). Much higher daily consumption rates of pouched and loose snus were found in a telephone survey in Sweden (95). On average, about 12 g/d of pouched and about 30 g/d of loose snus were reportedly consumed. The results for nicotine intake and uptake appears to indicate, however, that our (lower) estimate is not unrealistic. According to DIGARD *et al.* (78), the extraction efficiency for a chemical from OT under realistic use conditions was assumed to range from 0.2–0.35.

Intake estimates for NG users

The estimation of the daily intake of chemicals by using NRT products (in particular NG) was performed by applying the following relationship:

$$I_{ng} = CCNG \times PEXNG \times DCNG \quad [5]$$

with:

I_{ng} = Intake (mass/d) of a chemical by using nicotine gum (NG)
CCNG = Content of a chemical in NG (mass/g); range used for calculation; see Table 2
PEXNG = Percentage extracted from NG; range used for calculation: 42–63%; see below
DCNG = Daily consumption of NG (pieces/d); see Table 1

As a unit of use, 1 piece of NG was defined. The averages and ranges for daily consumption of NG (DCNG, in pieces per day) were calculated on the basis of 9 average values reported in the

Table 1. Averages and ranges for the daily consumption of the five nicotine products [a].

Product	Unit of use	N[b]	Daily consumption (units/d)							References
			Mean	SD	Median	Min	Max	25th Perc.	75th Perc.	
CC	1 Cig.	19	15.95	1.95	16.0	12.4	18.8	14.3	16.6	(BERGEN *et al.*, 2015 (161); CZOLI *et al.*, 2019 (48); GALE *et al.*, 2019 (33); HAZIZA *et al.*, 2016 (34); KRAUTTER and BORGERDING, 2014 (71); ROUND *et al.*, 2019 (54); US DEPARTMENT OF HEALTH AND HUMAN SERVICES, 2014 (2))
HNB	1 Stick	4	16.83	1.26	16.9	15.5	18	15.9	17.85	(GALE *et al.*, 2019 (33); HAZIZA *et al.*, 2016 (34))
EC	10 Puffs	11	12.59	7.38	11.6	2.2	25	7.15	21.25	(ETTER, 2014 (57); ETTER and BULLEN, 2011 (58); FARSALINOS *et al.*, 2015 (59); FARSALINOS *et al.*, 2014 (87); HAMMETT *et al.*, 2017 (60); LANDMESSER *et al.*, 2019 (56); LEAVENS *et al.*, 2019 (61); NATIONAL ACADEMIES OF SCIENCES (NAS), 2018 (63); O'CONNELL *et al.*, 2016 (53); ROUND *et al.*, 2019 (54); WAGENER *et al.*, 2017 (66))
OT (Snus)	1 g	8	2.62	0.98	2.53	1.20	4.24	1.98	3.05	(JITNARIN *et al.*, 2014 (70); KRAUTTER and BORGERDING, 2014 (71); KRAUTTER *et al.*, 2014 (72); OGDEN *et al.*, 2015 (39); SARKAR *et al.*, 2010 (73); WANG *et al.*, 2016 (162))
NRT (NG)	1 Piece	9	6.35	2.30	5.15	3.2	9.45	5.00	8.50	(ETTER *et al.*, 2009 (80); ROUND *et al.*, 2019 (54); SHIFFMAN *et al.*, 2003 (81); XIAO *et al.*, 2014 (82))

[a] For intake calculations, preferably the reported medians were used. If only means or geometric means were reported, then these values were used. In case of reported ranges, the mean of the range was used. Consumption data were not differentiated for gender.
[b] N represents the number of data sets for consumption used (note that more than one data set can be reported in one referenced article).

literature (compiled in Table 1). For estimating the intake of NG constituents by means of the MC simulation, a consumption range of 5.0–8.5 pieces/d (25th–75th percentile) was used (Table 1).
Considering the data provided by DIGARD *et al.* (78), LUNELL and LUNELL (96) and BENOWITZ *et al.* (97), the extraction efficiency for a chemical from NG under realistic use conditions was assumed to range from 0.42–0.63.

Exposure predictions for non-users (NU)

The CompTox Chemicals Dashboard is a freely accessible online database created and maintained by the U.S. ENVIRONMENTAL PROTECTION AGENCY (EPA) (98). The database provides access to multiple types of data including physicochemical properties, environmental fate and transport, exposure, usage, *in vivo* toxicity, and *in vitro* bioassay. For the purpose of this study, "Exposure Predictions" for the chemicals of interest were used. "Exposure Predictions" were derived from a publication of WAMBAUGH *et al.* (99), who studied exposure rates for 106 chemicals that could be determined from urine samples collected during various National Health and Nutrition Examination Survey (NHANES) studies. Based on various influencing factors, exposure rates (expressed as mg/kg bodyweight/day) were extrapolated for almost 8000 chemicals. Medians and 95% confidence intervals were reported (99). According to the authors, "Median Exposure Predictions" mean that one can be 50% confident that the exposure for the chemical is below the median. "Exposure Predictions" from the EPA Chemical Dashboard were converted, in order to fit into the purpose of this study, namely, median values for adults (aged 20–65 years) were calculated for a bodyweight range of 60–90 kg. The derived exposure ranges (unit: mass/d) were used as daily intakes for non-users (NU). For a number of chemicals, the EPA Chemical Dashboard-derived exposure predictions significantly deviate from the evaluations in this study. These cases are discussed individually. Reasons for carried out corrections are provided in the text.

Evaluation of biomarker-based uptake data

An objective measure for the exposure to chemicals by using the 5 tobacco/nicotine products of interest are biomarkers of exposure (BoEx) determined in body fluids (100). For cigarette smokers and non-smokers a large number of BoEx data for various chemicals are available (e.g., (21–24)). Considerably less biomarker data are available for users of HNB products (33–39), ECs (48–56) , OT products (54, 72–77) and NGs (54, 83, 84). Many of the biomarker-based uptake data for users of the new generation tobacco/nicotine products originate from experimental switching studies, where cigarette smokers were asked to switch from CCs to one of the new products or to quit smoking during the course of the study. Possible problems implicated with this approach are discussed when appropriate.
As indicated earlier, this study aims at extracting average exposure data (both for intake and uptake) for the various product user groups. Therefore, wherever possible, reported median levels for user groups or, alternatively, means and geometric means were used for calculation. In general, gender, age and daily product consumption was not considered separately in the evaluation.
Cotinine concentrations reported for saliva were transformed to cotinine plasma concentrations by dividing the saliva level by 1.227 (101). Reported CO levels in exhaled breath (COex) were transformed to carboxyhemoglobin (COHb) levels by using the relationship COHb (%) = 0.6 + 0.3 × COex (ppm) (102). All

urinary biomarker levels were converted to a daily excretion rate (mass/d) by applying the following assumptions: average daily excretion volume for a mixed population of male and female adults is 1.25 L/d; average daily excretion rate of creatinine for a mixed population of males and females is 1.25 g/d.

RESULTS AND DISCUSSION

The intention of this review was to compile and evaluate data from the literature, which allow to estimate the average exposure to chemicals in 6 groups of healthy adults. The 6 groups of interest were: (i) smokers of conventional cigarettes (CC), (ii) users of heat-not-burn products (HNB), (iii) users of e-cigarettes (EC, vapers), (iv) users of oral tobacco (OT), (v) users of nicotine gum (NG, as a nicotine replacement therapy (NRT) product), and (vi) non-users (NU) of any of these or any other tobacco/nicotine products. The final aim of this endeavor was to differentiate members of these user groups from each other and from NU on the basis of either single or combinations of biomarker levels in their body fluids such as urine, plasma, saliva or exhaled breath. In addition, it was examined, whether the compiled data would also allow the identification of dual or even multiple users of the tobacco/nicotine products of interest.

In order to achieve the described aims, the selection of data from the literature and its evaluation followed some principle guidelines, including: (i) whenever possible, medians of reported data sets for the products and user groups of interest were used for evaluation. The reason is that the final results should be valid for average users/non-users and not for any extremes, which, of course, were frequently to be found in the published literature. This applies for both product properties and user habits. (ii) For a similar reason (namely to come up with generalizable results), particular traits within a product group such as flavors or release of metals (these are particularly variable between ECs) were not included in this review. According to FOWLES *et al.* (103), the large range of metals within and across e-cigarette brands indicate the need for improvements in product design, enforced product safety regulations and manufacturing quality control. These authors assume that the implementation of such measures could reduce metal exposure in e-cigarette users. (iii) No presets in terms of size, country of origin, type of study (experimental, clinical, field, epidemiological) were made. The same applied for the gender, race or ethnicity of investigated subjects. This had primarily practical reasons (for many chemicals only very limited data were available, which would not allow meaningful breakdown into subcategories). However, it was also observed that the differences between subcategories were usually small and would not essentially bias the results. (iv) As a general rule for the evaluation, data from various sources were checked for consistency and technical/biological plausibility. Through this, a couple of errors (e.g., in reported units) could be identified and corrected. When no plausible explanation for significant deviation was identified, the questionable data were corrected satisfying plausibility or excluded from the evaluation. Cases of implausible exposure levels for certain chemicals in various user groups or NU and the corrections made are compiled in Table S41.

The Results section comprises firstly the medians of the estimated daily intake and uptake exposure doses for 38 chemicals released by the 5 types of tobacco/nicotine products of interest. The chemicals were categorized as tobacco/nicotine-derived, PG-/G-derived and combustion-derived compounds. Secondly, a 4-grade-system of relative exposure levels is introduced for directly comparing the intake and uptake exposure doses. This system is then used to establish a biomarker-based exposure level matrix for the 6 user/non-user groups (CC, HNB, EC, OT, NG, NU) and 29 chemicals for which sufficient biomarker data were available (for 9 of the 38 chemicals no suitable biomarker data sets were available). Thirdly, applications of this matrix are then presented for differentiation of the 6 user/non-user groups based on either levels of single or combinations of biomarkers. The influence of dual- or multiple-product use on the established system is also briefly described. Finally, limitations and possibilities for improvements of the system are discussed.

Intake and uptake of chemicals by product use

A summary of the estimated median intake and biomarker-based uptake values is shown in Table 2. Detailed statistics for all intakes of chemicals and biomarker levels as well as references of all published data used for the evaluation are presented in Tables S1 to S38 of Supplemental File 1. Graphical comparison of intakes and biomarker-based uptakes for the 6 groups are shown in Figures S1 to S38 of Supplemental File 1.

It is evident from the Table 2 that there are quite a number of data gaps for particular products or user groups (indicated in Table 2 as "nda" (= no data available)). Intake and uptake can be compared on a relative basis and thus allow the validation of the agreement (or disagreement) between both parameters. There is one chemical, namely nicotine (Figure 1), for which an absolute comparison between intake and uptake is possible, due to the fact that the biomarker (urinary nicotine equivalents) represents close to 100% of the amount taken in by using the product (104). In most cases, a satisfying agreement between the product patterns for intake and uptake is observed. Major discrepancies are discussed in the sections of the respective chemicals. Frequent reasons for discrepancies are high background levels for the biomarker due to other sources, insufficient (too short) washout periods or incomplete compliance in product switching or cessation studies or simply very low numbers of data sets (in some cases only one) available for evaluation.

In Table 2, ranges (25^{th}–75^{th} percentiles, if possible) for the release of 38 chemicals from the 5 product types as well as medians of the daily intake estimates and corresponding biomarker levels from the literature are shown. For 9 out of 38 chemicals selected for evaluation, no biomarkers were available because either no suitable biomarker has as yet been identified or the available data did not allow reasonable comparisons. In total, results for 30 biomarkers are presented (for nicotine, 2 biomarkers were found to be suitable).

The chemicals were grouped into 3 groups as follows:

- Tobacco/nicotine-derived compounds: nicotine (N), nornicotine (NN), anatabine (AT), anabasine (AB), tobacco-specific nitrosamines (NNK, NNN, NAT, NAB).
- Compounds potentially derived from the e-liquid matrix: propylene glycol (PG), glycerol (G), glycidol (Gly), ethylene oxide (EO, although EO has not been identified as decomposition product of PG or G, it is assigned to this group of chemically similar epoxides for sake of better comparison), propylene oxide (PO), formaldehyde (FA), acetaldehyde (AA), acrolein (ACR), crotonaldehyde (CRO), propionaldehyde (PA), acetoin (AC), glyoxal (GO) and methylglyoxal (MGO). Note that except for PG and G all chemicals are also derived from combustion of organic material.

- Combustion-derived compounds: carbon monoxide (CO), 1,3-butadiene (BD), isoprene (Iso), furan (Fu), benzene (Be), styrene (Sty), acrylonitrile (AN), acrylamide (AM), hydrogen cyanide (HCN), *o*-toluidine (*o*-Tol), 2-naphthylamine (2-NA), 4-aminobiphenyl (4-ABP), naphthalene (Nap), fluorene (Flu), phenanthrene (Phe), pyrene (Pyr), benzo[*a*]pyrene (B*a*P).

A reasonable comparison of the large amount of data extracted from many different published reports required adjustments on the basis of a series of assumptions. This is mentioned at the corresponding passages in the text. An overview of all assumptions made, classified into four groups, is provided in Table S41 in Supplemental File 1.

Nicotine-/tobacco-derived chemicals

Estimates for the daily intake in non-users from the EPA Chemical Dashboard (98, 99) were only available for nicotine and NNK. Because of missing product release data for NN, AT and AB, no daily intakes could be estimated for NG users. No biomarker data are available for NN, since NN is a metabolite of nicotine as well. Users of HNB products lack biomarker data for the minor tobacco alkaloids AT and AB as well as the corresponding nitrosamines (NAT, NAB).

It is of interest to note that daily nicotine intake by using the various products was estimated to be in a relative narrow range of 7–12 mg/d. This range is confirmed by biomarker data for nicotine equivalents (Nequ, 7–14 mg/d). Due to the fact that the reported urinary Nequ data already cover 90–95% of the intake dose (and were computationally adjusted to 100% excreted amount), calculated intake and measured uptake can be directly compared (see Figure 1). The other established biomarker for nicotine uptake, plasma cotinine, is also in good agreement (on a relative basis) with the nicotine intake estimates and urinary Nequ (Figure 1). Non-users (NU) were found to excrete a median of 0.37 mg nicotine equivalents/d (Table 2), which is clearly at variance (4 orders of magnitude higher) with the data from the EPA database (98, 99). An explanation for this discrepancy could be that the biomarker data of NU in this review were partly derived from switching studies, in which usually smokers (CC) were asked to quit at least for the duration of the study and subjects might not be fully compliant and/or study duration was too short to ensure complete excretion of nicotine and its metabolites (105).

As predicted, NN intakes were observed to be highest in users of the tobacco containing products (CC, OT). However, only very few data are available for CC, HNB, EC and OT. No data were at hand for nicotine gums (NGs). More data for NN release by the 4 new generation products (in particular HNB) in order to perform profound intake estimates for the use of these products is required. This is of particular importance since NN is a proven precursor for the human carcinogen NNN (106).

It is noticeable that the estimated intake of AT by vaping is relatively high with a large range of variation. This is primarily due to some very high AT levels in e-liquids reported by Etter *et al.* (107) and Lisko *et al.* (108). These high levels of AT intake by vaping are not confirmed by the corresponding biomarker data (Table 2). On the other hand, urinary AT levels reported for OT users were the highest for all groups investigated, whereas the estimated daily intake for OT users was lower than that for smokers (CC) and vapers (EC). When interpreting these results, it has to be taken into consideration that the values presented here are based on relative few data sets.

Urinary AB excretions were observed to be highest in CC and OT users (Table 2). Estimated daily intake of AB for OT users, however, was almost 3-times lower compared to CC smokers. Biomarker levels (urinary AB excretion) were about the same in EC, NG users and non-users (NU) and significantly lower (> 20-fold) than in smokers (CC) and OT users. Again, when interpreting these results, it has to be considered that the values presented here are based on relatively few data sets.

Average intake of NNK for smokers (CC) was estimated to be more than 20-fold higher than in HNB product users, vapers (EC) and users of NGs. Intake of NNK in smokers was estimated to be only about 5-fold higher than in OT product users. Smokers excreted on average 158-fold higher amounts of NNAL than non-users. OT product users showed the highest urinary NNAL levels (1.6-times higher than in smokers). NNAL levels in users of HNB, ECs and NGs were only 2- to 13-fold lower than in smokers (CC), which is much less than expected from the daily NNK intakes and cannot be explained by a high background level caused by other (diffuse) NNK sources. The very low urinary NNAL levels in non-users (NU) clearly speak against this possibility. The most probable explanation for the relatively high NNAL levels in the users of the new generation products (HNB, ECs, NG) is that the time periods since switching from CC to the new products were, at least in some studies, too short for the relatively long elimination half-life of 10–45 d for NNAL (109–111). Most of the NNAL data for the new generation products were derived from CC-switching studies with too short time-periods to ensure complete wash-out of the smoking-related NNAL. On the other hand, only NNAL data of long-term non-users (NU) were included in the evaluation.

The predicted exposure to NNK of adults in the US is stated to be 8.5–12.7 ng/d (98, 99), which is in the same order of magnitude as the estimated intake of NNK by vaping (9.5 ng/d) and use of NGs (3.4 ng/d). Furthermore, using HNB products may lead to an appreciable higher NNK exposure (79.2 ng/d) than the predicted exposure of US adults. There appears to be some discrepancy between the results for the NNK daily intake and urinary NNAL excretion in smokers (CC) and OT users. The ratio (CC:OT) of the NNK intake was found to be about 5, whereas the corresponding ratio for the NNAL excretion is about 0.6. Two aspects have to be taken into account when these ratios are compared: (i) the difference in the urinary NNAL levels between the two groups is statistically not significant, (ii) the group of OT users evaluated in this review for their NNAL concentrations in urine was relatively heterogeneous and may contain a higher percentage of users of 'older' OT products, whereas the NNK intake estimates is based predominantly on 'modern' OT products.

Average intake of NNN for smokers (CC) is estimated to be 293- and 244-fold higher than in vapers (EC) and NG users, respectively. Differences in NNN intake between smokers and HNB product users (9.6-fold) and OT users (1.6-fold) are much smaller.

In terms of the BoEx for NNN (urinary NNN excretion), smokers were found to excrete about twice the amount of NNN compared to OT users, whereas the ratio between smokers and the other groups is about 5–8. Urinary NNN levels in smokers were significantly different from all groups except from OT users. On the other hand, urinary NNN levels between all groups except for smokers are not statistically different. This means that NNN levels in non-users (NU) were (statistically) similar to those of HNB, EC, OT and NG users. This, particularly with respect to OT users, somewhat surprising results can be explained

by the fact that OT users showed the highest 95%-confidence interval (3.2–27.1 ng/d) for NNN excretion, most probably because this group is relatively heterogeneous and may contain a higher percentage of users of 'older' OT products with still elevated TSNA levels. Furthermore, since many data in this evaluation originate from switching, it cannot be excluded that some subjects in these studies were non-compliant. Taken together, the obtained results provide evidence that there is appreciable exposure to NNN in OT users, which is strongly dependent on the product used. Furthermore, there is limited evidence that using HNB products is associated with some, although significantly lower exposure to NNN compared to using CCs and OT products. Additional data on the release of NNN from the new generation products (in particular HNB and ECs) are required to draw more solid conclusions. Furthermore, there are only limited data available on the endogenous formation of NNN from the precursor nornicotine (NN) in the user groups of interest (112–115), a fact that certainly requires further investigations.

Average intake of NAT by smokers (CC) and OT users was observed to be in the same range, whereas that of HNB, EC and NG users was at least 10-fold lower. Urinary NAT excretion in these groups confirm the relations of the estimated daily NAT intakes. No NAT biomarker data were, however, available for HNB product users. Urinary NAT levels between smokers and OT users were not significantly different, but both groups showed significantly higher NAT concentrations in urine than vapers (EC), NG users and non-users (NU). Urinary NAT levels between users of ECs, NGs and NU were found to be not significantly different. Taken together, the obtained results provide evidence that there is appreciable exposure to NAT in OT users, which is strongly dependent on the product used. Furthermore, there is limited evidence that using HNB products is associated with some exposure to NAT, which is about 10 times lower than in smokers. This finding requires confirmation by additional data for NAT release from HNB products and biomarker results for users of this product.

Average intake of NAB for smokers (CC) and OT users was about in the same range (2.8 times higher in smokers), whereas that of HNB, EC and NG users was 6-, 217- and 128-fold lower, respectively. Urinary NAB excretion in smokers (CC) is 16-, 10- and 45-fold higher than in users of ECs, NGs and non-users (NU), respectively. The ratio for the urinary NAB levels between smokers and OT users is about 3. In general, the ratios between the groups observed for the biomarker (NAB in urine) confirm those for the estimated intakes, although they were one order of magnitude lower, which most likely reflects a background exposure to NAB in the general population. No NAB biomarker data were available for HNB product users. Urinary NAB levels in smokers were significantly higher than in the other groups with data available (EC, OT, NG and NU). Urinary NAB levels in OT users were significantly higher than in users of ECs and NGs as well as non-users (NU). Taken together, the obtained results provide evidence that there is appreciable exposure to NAB in OT users, which is strongly dependent on the product used. Furthermore, there is limited evidence that using HNB products is associated with some exposure to NAB, which is about 6 times lower than in smokers. This finding again requires confirmation by additional data for NAB release from HNB products and biomarker results for users of this product.

PG-/G-derived chemicals

Thirteen chemicals were classified into this group: propylene glycol (PG), glycerol (G), glycidol (Gly), ethylene oxide (EO), propylene oxide (PO), formaldehyde (FA), acetaldehyde (AA), acrolein (ACR), crotonaldehyde (CRO), propionaldehyde (PA), acetoin (AC), glyoxal (GO), methylglyoxal (MGO). Recently, UCHIYAMA *et al.* (86) determined thermal decomposition products generated from PG and G, including the chemicals listed above, except for EO, CRO and AC but including in addition acetol. The reason for our inclusion of CRO in this group of chemicals is the assumption that CRO may be formed from acetaldehyde by aldol condensation. In this review, AC was included in this group, because AC is frequently reported as a constituent of EC aerosol, although most probably originating from flavoring compounds (116). It has to be noted that EO has not been reported to be formed from PG or G, but was included in this group due to the chemical similarity to the other epoxides (PO, Gly).

Median values for daily intake and corresponding biomarker levels are compiled in Table 2.

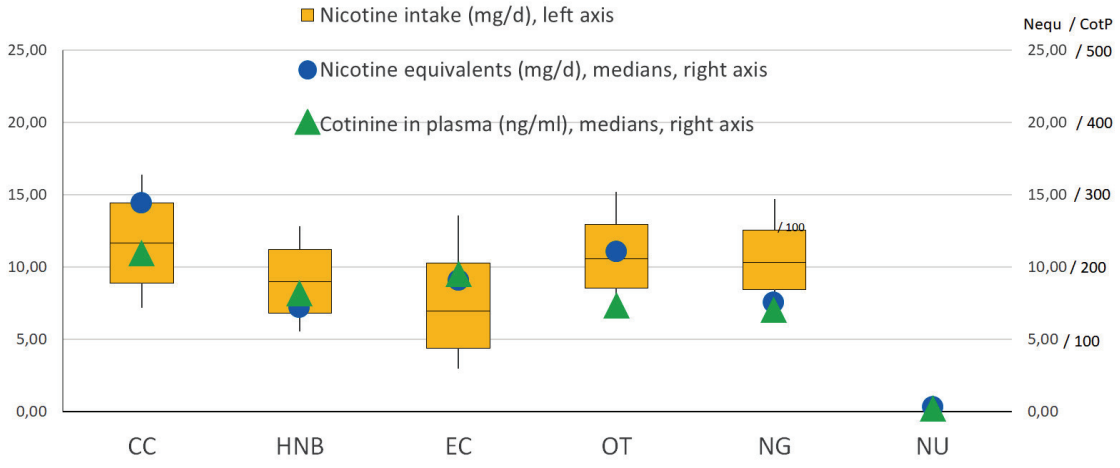


Figure 1. Box plots for the estimated daily nicotine intake (mg/d) (whiskers represent the 10th–90th percentiles) in users of CC, HNB, EC, OT and NG products. The filled blue circles represent medians of the urinary nicotine equivalents, while the green triangles show the plasma cotinine levels in users of these products and non-users (NU). Note that the right y-axis has two different scales.

Table 2. Release of 38 chemicals by the products of interest as well as medians of the estimated daily intakes and corresponding biomarker levels in the 6 user/non-user groups. The **bold** numbers in parentheses indicate the relative exposure levels (**1** to **4**). *Italic* blue numbers in parentheses indicate assumption-based changes (made as described in the text). Crossed out values were found to be implausible, a reasonable exposure level assigned instead. Also these numbers are presented in *italic* blue.

Chemical (CAS No)		CC	HNB	EC	OT	NG	NU [a]
Nicotine-/tobacco-derived chemicals							
N (54-11-5)	Content / unit	0.59–1.67 mg/cig	0.42–1.19 mg/stick	0.24–1.38 mg/10 puffs	10.7–21.0 mg/g	2.0–4.0 mg/piece	–
	Intake (mg/d)	11.7 (**4**)	9.01 (**4**)	6.99 (**3**)	10.58 (**4**)	10.35 (**4**)	0.05 (**1**)
	Nequ (mg/d)	14.4 (**4**)	7.19 (**3**)	9.1 (**3**)	11.09 (**4**)	7.58 (**3**)	0.37 (**1**)
	Cot P (ng/mL)	215.7 (**4**)	161.0 (**4**)	187.1 (**4**)	143.1 (**3**)	137.5 (**3**)	0.58 (**1**)
NN (494-97-3)	Content per unit	19.98–21.49 µg/cig	0.02–0.05 µg/stick	0.55–3.13 µg/10 puffs	140–173 µg/g	nda [b]	–
	Intake (µg/d)	215.2 (**4**)	0.4 (**1**)	15.93 (**1**)	106.18 (**3**)	nda (*1*)	nda (*1*)
	Uptake	*No specific biomarker for nornicotine available*					
AT (581-49-7)	Content per unit	7.00–13.45 µg/cig	1.16–1.27 µg/stick	0.81–34.47 µg/10 puffs	65.0–84.0 µg/g	nda	–
	Intake (µg/d)	105.57 (**3**)	13.71 (**1**)	152.75 (**4**)	50.23 (**2**)	nda (*1*)	nda (*1*)
	AT U (µg/d)	16.28 (**3**)	nda (*1*)	1.01 (**1**)	40 (**4**)	0.38 (**1**)	0.77 (**1**)
AB (13078-04-1)	Content per unit	1.12–3.31 µg/cig	0.21–0.62 µg/stick	0.0–0.55 µg/10 puffs	8.50–17.0 µg/g	nda	–
	Intake (µg/d)	22.65 (**4**)	4.59 (**2**)	2.33 (**2**)	8.52 (**2**)	nda (*1*)	nda (*1*)
	AB U (µg/d)	15.5 (**4**)	nda (*1*)	1.34 (**1**)	20 (**4**)	1.12 (**1**)	0.76 (**1**)
NNK (64091-91-4)	Content per unit	86.9–254 ng/cig	6.7–7.3 ng/stick	0.6–1.5 ng/10 puffs	200–870 ng/g	0.5–1.5 ng/piece	–
	Intake (ng/d)	1740.7 (**4**)	79.2 (**1**)	9.5 (**1**)	352.3 (**2**)	3.4 (**1**)	10.6 (**1**)
	NNAL U (ng/d)	347.9 (**3**)	104.5 [c] (**2**)	26.6 (**1**)	539.8 (**4**)	176.7 [c] (**2**)	2.2 (**1**)
NNN (16543-55-8)	Content per unit	96.4–270 ng/cig	10.5–24.0 ng/stick	0.4–1.0 ng/10 puffs	900–2650 ng/g	1.5–3.0 ng/piece	–
	Intake (ng/d)	1876.9 (**4**)	194.5 (**2**)	6.4 (**1**)	1183.2 (**3**)	7.7 (**1**)	nda (*1*)
	NNN U (ng/d)	17.9 (**4**)	3.6 (**2**)	2.7 (**2**)	8.7 (**3**)	2.9 (**2**)	2.2 (**2**)
NAT (71267-22-6)	Content per unit	141–268 ng/cig	14.0–20.5 ng/stick	0.4–1.2 ng/10 puffs	710–2350 ng/g	0.5–1.5 ng/piece	-
	Intake (ng/d)	2111 (**4**)	193.2 (**1**)	7.1 (**1**)	1006 (**3**)	3.4 (**1**)	nda (*1*)
	NAT U (ng/d)	275.3 (**4**)	nda (*1*)	4.4 (**1**)	163.3 (**3**)	3.7 (**1**)	2.9 (**1**)
NAB (37620-20-5)	Content per unit	18–24 ng/cig	1.9–4.7 ng/stick	0.1–0.2 ng/10 puffs	50–180 ng/g	0.5–0.8 ng/piece	-
	Intake (ng/d)	216.8 (**4**)	37.2 (**2**)	1 (**1**)	77.1 (**2**)	1.7 (**1**)	nda (*1*)
	NAB U (ng/d)	58.8 (**4**)	nda (*1*)	3.8 (**1**)	19.9 (**2**)	6.1 (**2**)	1.3 (**1**)

[a] Intake for non-users (NU) were taken from the 'Predicted Exposure' data published in the EPA Chemical Dashboard. Data in this table represent medians for 20–65 year old persons with an assumed bodyweight of 60–90 kg (the mean of the range was used).
[b] nda = no data available
[c] Levels are partly derived from switching studies and most probably too high, due to the long half-life of NNAL (10–45 d) in relation to the study duration.

Table 2. cont.

Chemical (CAS No)		CC	HNB	EC	OT	NG	NU [a]
PG-/G-derived chemicals							
PG (57-55-6)	Content per unit	0.03-0.60 mg/cig	0.39-0.63 mg/stick	49.2-69.9 mg/10 puffs	5.5-16.5 mg/g	nda	-
	Intake (mg/d)	3.2 (**1**)	5.8 (**1**)	555.3 (**4**)	7.3 (**1**)	nda (***1***)	0.005 (**1**)
	PG U (mg/d)	6.85 (**3**)	nda (***1***)	14.93 (**4**)	nda (***1***)	nda (***1***)	nda (***1***)
G (56-81-5)	Content per unit	0.93-2.27 mg/cig	1.83-3.44 mg/stick	15.30-67.52 mg/10 puffs	0.58-8.56 mg/g	nda	-
	Intake (mg/d)	16.42 (**1**)	29.55 (**1**)	360.75 (**4**)	3 (**1**)	nda (***1***)	0.003 (**1**)
	Uptake	*No suitable biomarker for PG available*					
Gly (556-52-5)	Content per unit	3.0-6.0 µg/cig	22-66 µg/stick	37.6-59.9 µg/10 puffs	-	-	-
	Intake (µg/d)	46.26 (**1**)	492.61 (**4**)	454.91 (**4**)	nda (***1***)	nda (***1***)	0.005 (**1**)
	Uptake	*No sufficient biomarker data for Gly available*					
EO (75-21-8)	Content per unit	14.5-21.8 µg/cig	0.02-0.20 µg/stick	nda	nda	nda	-
	Intake (µg/d)	186.6 (**4**)	1.23 (**1**)	nda (***1***)	nda (***1***)	nda (***1***)	nda (***1***)
	HEMA (µg/d)	5.23 (**4**)	2.53 (**3**)	1.8 (**2**)	~~7.19~~ (***2***)	~~7.9~~ (***2***)	1.194 (**2**)
PO (75-56-9)	Content per unit	1.22-2.14 µg/cig	0.008-0.148 µg/stick	0.622-0.992 µg/10 puffs	nda	nda	-
	Intake (µg/d)	17.27 (**4**)	0.88 (**1**)	7.53 (**3**)	nda (***1***)	nda (***1***)	nda (***1***)
	2-HPMA (µg/d)	72.69 (**4**)	nda (***1***)	~~33.31~~ (***2***)	~~164.61~~ (***2***)	~~46.75~~ (***2***)	~~37.99~~ (***2***)
FA (50-00-0)	Content per unit	40.5-74.0 µg/cig	4.35-16.5 µg/stick	1.60-2.02 µg/10 puffs	0.32-4.5 µg/g	nda	-
	Intake (µg/d)	588.93 (**4**)	89.35 (**2**)	16.86 (**1**)	1.02 (**1**)	nda (***1***)	0.08 (**1**)
	Uptake	*No biomarker data for FA available*					
AA (75-07-0)	Content per unit	1044-1766 µg/cig	168-212 µg/stick	0.77-2.30 µg/10 puffs	1.40-27.4 µg/g	nda	-
	Intake (µg/d)	14470 (**4**)	2141 (**2**)	16.5 (**1**)	9.6 (**1**)	nda (***1***)	0.2 (**1**)
	Uptake	*No biomarker data for AA available*					
ACR (107-02-8)	Content per unit	75-155 µg/cig	5.3-10.3 µg/stick	0.5-2.1 µg/10 puffs	nda	nda	-
	Intake (µg/d)	1176.4 (**4**)	87.6 (**1**)	11 (**1**)	nda (***1***)	nda (***1***)	0.007 (**1**)
	3-HPMA (µg/d)	1604.3 (**4**)	603.9 (**2**)	555.4 (**2**)	466.5 (**2**)	512.5 (**2**)	340.5 (**2**)
CRO (4170-30-3)	Content per unit	33.7-48.7 µg/cig	1.1-2.9 µg/stick	0.5-1.5 µg/10 puffs	0.2-2.4 µg/g	nda	-
	Intake (µg/d)	424.3 (4)	22.4 (**1**)	8.4 (**1**)	0.9 (**1**)	nda (***1***)	0.07 (**1**)
	HMPMA (µg/d)	708.5 (**4**)	76.7 (**2**)	251.1 (**2**)	130.6 (**2**)	128.9 (**2**)	227.5 (**2**)
PA (123-38-6)	Content per unit	111-125 µg/cig	8.8-13.3 µg/stick	0.2-0.5 µg/10 puffs	nda	nda	-
	Intake (µg/d)	1217.8 (**4**)	124.3 (**2**)	3.0 (**1**)	nda (***1***)	nda (***1***)	1.9 (**1**)
	Uptake	*No biomarker data for PA available*					
AC (513-86-0)	Content per unit	2.8-6.2 µg/cig	2.9-8.7 µg/stick	0.008-0.023 µg/10 puffs	nda	nda	-
	Intake (µg/d)	46.42 (**4**)	65.19 (**4**)	0.13 (**1**)	nda (***1***)	nda (***1***)	0.0002 (**1**)
	Uptake	*No biomarker data for AC available*					
GO (107-22-2)	Content per unit	8.8-12.3 µg/cig	0.03-0.1 µg/stick	0.24-0.72 µg/10 puffs	nda	nda	-
	Intake (µg/d)	108.86 (**4**)	0.71 (**1**)	4.24 (**1**)	nda (***1***)	nda (***1***)	0.02 (**1**)
	Uptake	*No biomarker data for GO available*					
MGO (78-98-8)	Content per unit	24.2–31.2 µg/cig	13.2–39.6 µg/stick	0.8–1.2 µg/10 puffs	nda	nda	-
	Intake (µg/d)	285.4 (**4**)	297.3 (**4**)	9.2 (**1**)	nda (***1***)	nda (***1***)	nda (***1***)
	Uptake	*No biomarker data for MGO available*					

[a] Intake for non-users (NU) were taken from the 'Predicted Exposure' data published in the EPA Chemical Dashboard. Data in this table represent medians for 20–65 year old persons with an assumed bodyweight of 60–90 kg (the mean of the range was used).
[b] nda = no data available
[c] Levels are partly derived from switching studies and most probably too high, due to the long half-life of NNAL (10–45 d) in relation to the study duration.

Table 2. cont.

Chemical (CAS No)		CC	HNB	EC	OT	NG	NU [a]
Combustion-derived chemicals							
CO (630-08-0)	Content per unit	22.7-32.0 mg/cig	0.30-0.53 mg/stick	0.25-0.75 mg/10 puffs	nda	nda	-
	Intake (mg/d)	281.7 (**4**)	4.66 (**1**)	4.42 (**1**)	nda (***1***)	nda (***1***)	nda (***1***)
	COHb (%)	5.62 (**4**)	1.62 (**2**)	1.52 (**2**)	1.05 (**2**)	1.35 (**2**)	1.35 (**2**)
BD (106-99-0)	Content per unit	67-100 µg/cig	0.21-0.40 µg/stick	0.25-0.75 µg/10 puffs	nda	nda	-
	Intake (µg/d)	863.24 (**4**)	3.43 (**1**)	4.48 (**1**)	nda (***1***)	nda (***1***)	nda (***1***)
	MHBMA (µg/d)	3.54 (**4**)	0.108 (**1**)	0.381 (**2**)	0.707 (**2**)	~~2.25~~ (***2***)	0.438 (**2**)
Iso (78-79-5)	Content per unit	794.9-869.0 µg/cig	1.96-2.69 µg/stick	1.25-3.75 µg/10 puffs	nda	nda	-
	Intake (µg/d)	8607 (**4**)	26.91 (**1**)	22.1 (**1**)	nda (***1***)	nda (***1***)	0.29 (**1**)
	IPMA3 (µg/d)	43.94 (**4**)	nda (***1***)	4.684 (**2**)	nda (***1***)	nda (***1***)	3.555 (**1**)
Fu (110-00-9)	Content per unit	49.3-56.3 µg/cig	0.58-1.74 µg/stick	-	nda	nda	-
	Intake (µg/d)	565.12 (**4**)	12.94 (**1**)	nda (***1***)	nda (***1***)	nda (***1***)	0.002 (**1**)
	Fu B (ng/mL)	0.06 (**4**)	nda (***1***)	nda (***1***)	nda (***1***)	nda (***1***)	0.013 (**2**)
Be (71-43-2)	Content per unit	62.6-85.2 µg/cig	0.54-0.63 µg/stick	0.25-0.75 µg/10 puffs	nda	nda	-
	Intake (µg/d)	762.57 (**4**)	6.82 (**1**)	4.45 (**1**)	nda (***1***)	nda (***1***)	0.52 (**1**)
	SPMA (µg/d)	1.36 (**4**)	0.195 (**2**)	0.375 (**2**)	0.523 (**2**)	0.5 (**2**)	0.4 (**2**)
Sty (100-42-5)	Content per unit	13.7-24.4 µg/cig	0.47-1.05 µg/stick	nda	nda	nda	-
	Intake (µg/d)	195.6 (**4**)	8.52 (**1**)	nda (***1***)	nda (***1***)	nda (***1***)	6.6 (**1**)
	PHEMA (µg/d)	1.22 (**4**)	nda (***2***)	~~0.6~~ (***2***)	nda (***2***)	~~0.94~~ (***2***)	~~0.656~~ (***2***)
AN (107-13-1)	Content per unit	15.8–24.5 µg/cig	0.16–0.26 µg/stick	0.131–0.38 µg/10 puffs	nda	nda	-
	Intake (µg/d)	208.5 (**4**)	2.4 (**1**)	2.2 (**1**)	nda (***1***)	nda (***1***)	0.17 (**1**)
	CEMA (µg/d)	162.6 (**4**)	17.2 (**2**)	36.6 (**2**)	37.7 (**2**)	29 (**2**)	1.8 (**1**)
AM (79-06-1)	Content per unit	4.4–4.8 µg/cig	1.04–1.73 µg/stick	nda	nda	nda	-
	Intake (µg/d)	47.4 (**4**)	15.6 (**2**)	nda (***1***)	nda (***1***)	nda (***1***)	0.002 (**1**)
	AAMA (µg/d)	164.9 (**4**)	88.8 (**3**)	53.3 (**2**)	~~146.8~~ (***2***)	42 (**2**)	66.1 (**3**)
HCN (74-90-8)	Content per unit	327–356 µg/cig	1.7–3.5 µg/stick	nda	nda	nda	-
	Intake (µg/d)	3529 (**4**)	29.5 (**1**)	nda (***1***)	nda (***1***)	nda (***1***)	nda (***1***)
	SCN P (µg/d)	123.8 (**4**)	nda (***3***)	nda (***3***)	80.6 (**3**)	nda (***3***)	75 (**3**)
o-Tol (95-53-4)	Content per unit	62–84 ng/cig	0.5–1.0 ng/stick	nda	nda	nda	-
	Intake (ng/d)	750.8 (**4**)	8.0 (**1**)	nda (***1***)	nda (***1***)	nda (***1***)	280 (**2**)
	o-Tol U (ng/d)	201 (**4**)	56.7 (**2**)	100.1(**3**)	114 (**3**)	96.9 (**3**)	63.5 (**2**)
2-NA (91-59-8)	Content per unit	10–14 ng/cig	0.02–0.05 ng/stick	0.25–0.75 ng/10 puffs	nda	nda	-
	Intake (ng/d)	121.7 (**4**)	0.4 (**1**)	4.4 (**1**)	nda (***1***)	nda (***1***)	~~116.5~~ (***1***)
	2-NA U (ng/d)	27.6 (**4**)	1.8 (**1**)	2.5 (**1**)	3.3 (**2**)	2.6 (**1**)	3.5 (**2**)
4-ABP (92-67-1)	Content per unit	2.0–2.8 ng/cig	0.003–0.02 ng/stick	0.01–0.04 ng/10 puffs	nda	nda	-
	Intake (ng/d)	25.1 (**4**)	0.1 (1)	0.2 (**1**)	nda (***1***)	nda (***1***)	~~117.75~~ (***1***)
	4-ABP U (ng/d)	20.9 (**4**)	2.3 (**2**)	7 (**2**)	3.8 (**2**)	6.9 (**2**)	2.4 (**2**)
Nap (91-20-3)	Content per unit	0.9–1.2 µg/cig	0.002–0.004 µg/stick	nda	nda	nda	-
	Intake (µg/d)	10.86 (**4**)	0.03 (**1**)	nda (***1***)	nda (***1***)	nda (***1***)	4.33 (**2**)
	1-OH-Nap (µg/d)	13.28 (**4**)	nda (***1***)	1.94 (**2**)	2.59 (**2**)	nda (***1***)	1.98 (**2**)

[a] Intake for non-users (NU) were taken from the 'Predicted Exposure' data published in the EPA Chemical Dashboard. Data in this table represent medians for 20–65 year old persons with an assumed bodyweight of 60–90 kg (the mean of the range was used).
[b] nda = no data available
[c] Levels are partly derived from switching studies and most probably too high, due to the long half-life of NNAL (10–45 d) in relation to the study duration.

Table 2. cont.

Chemical (CAS No)		CC	HNB	EC	OT	NG	NU [a]
Combustion-derived chemicals							
Flu (86-73-7)	Content per unit	237–337 ng/cig	1.5–5.4 ng/stick	nda	nda	nda	-
	Intake (µg/d)	2.91 (**4**)	0.04 (**1**)	nda (***1***)	nda (***1***)	nda (***1***)	0.006 (**1**)
	2-OH-Flu (µg/d)	1.94 (**4**)	nda (***1***)	1.05 (**3**)	0.61 (**2**)	1.05 (**3**)	0.58 (**2**)
Phe (85-01-8)	Content per unit	159–292 ng/cig	1.0–2.0 ng/stick	nda	nda	nda	-
	Intake (ng/d)	2323 (**4**)	16.7 (**1**)	nda (***1***)	nda (***1***)	nda (***1***)	76.5 (**1**)
	1-OH-Phe (ng/d)	221.9 (**4**)	nda (***1***)	133.8 (**3**)	152.2 (**3**)	nda (***1***)	122.9 (**3**)
Pyr (129-00-0)	Content per unit	79–89 ng/cig	2–7 ng/stick	nda	nda	nda	-
	Intake (ng/d)	871.7 (**4**)	51.1 (**1**)	nda (***1***)	nda (***1***)	nda (***1***)	114.8 (**2**)
	1-OH-Pyr (ng/d)	305 (**4**)	70 (**1**)	~~185~~ (***2***)	~~360~~ (***2***)	~~268~~ (***2***)	139 (**2**)
BaP (50-32-8)	Content per unit	13–16 ng/cig	0.2–0.8 ng/stick	0.25–0.75 ng/10 puffs	1.4–60.4 ng/g	nda	-
	Intake (ng/d)	149.1 (**4**)	5.5 (**1**)	4.4 (**1**)	20.4 (**2**)	nda (***1***)	76.5 (**3**)
	3-OH-BaP (ng/d)	203.1 (**4**)	36.9 (**2**)	~~87.4~~ (***2***)	78.9 (**2**)	~~119~~ (***2***)	61.9 (**2**)

[a] Intake for non-users (NU) were taken from the 'Predicted Exposure' data published in the EPA Chemical Dashboard. Data in this table represent medians for 20–65 year old persons with an assumed bodyweight of 60–90 kg (the mean of the range was used).
[b] nda = no data available
[c] Levels are partly derived from switching studies and most probably too high, due to the long half-life of NNAL (10–45 d) in relation to the study duration.

No intake amounts for this group of compounds could be calculated for NG users due to lack of data. For OT users, daily intakes for PG, G, FA, AA and CRO were available. EPA Chemical Dashboard-derived predicted exposures (as a substitute for non-users, NU) were available for all chemicals except for EO and PO. For the other user groups (CC, HNB, EC), almost complete intake data were available (EO intake data for vapers were lacking for the reason mentioned above). Biomarker data for the PG/G-derived chemicals are mostly incomplete for all groups (Table 2). Biomarker-based uptake data could be provided for EO (biomarker: HEMA), PO (2-HPMA), ACR (3-HPMA) and CRO (HMPMA). Data for PG uptake were available only for smokers (CC) and vapers (EC) from a study applying stable isotope-labeled precursors (56).

As expected, daily intake of PG and corresponding biomarker levels were by far highest in EC users (Table 2). The estimated daily intake levels in vapers were about 2 orders of magnitude higher than in users of CC, HNB and OT products. No data were available for the release of PG from NGs. The release of PG from NGs, however, can be assumed to be negligible. Predicted exposure provided in the EPA Dashboard is 3 orders of magnitude lower than the daily intakes for CC, HNB and OT users estimated in this review. Unfortunately, no PG biomarker data for non-users (NU) were available so that the predicted exposure provided in the EPA Dashboard cannot be verified. Comparison between estimated daily intakes and urinary biomarker levels is limited by the fact that biomarker data were available for smokers and vapers only.

As with PG, daily amounts of tobacco/nicotine product-related G intakes were by far highest in EC users. Since G is an endogenous compound and also used in a large number of foodstuffs, cosmetics and other consumer products, the G-related biomarker levels would by no means be specific for the habit of vaping. Studies with labeled G in e-liquid revealed no detectable labeled G in plasma and urine (most likely due to the high background levels of G in body fluids) (56).

The daily intake of Gly was estimated to be about similar in HNB product and EC users and significantly higher than in CC smokers. Unfortunately, these estimates are based on very few data only. Additionally, no biomarker data are as yet available, in order to either prove or disprove these findings. No data were available for Gly release of OT products and NGs. The occurrence and release of significant amounts of Gly in the latter two products is, however, unlikely.

Daily intake of EO in smokers (users of CC) was calculated to be about 150 times higher than in users of HNB products. It can be assumed that intake of EO by using ECs, OT and NG (for which no release data were available) is similar to that of using HNB products, e.g., EO intake through these products is probably negligible. The BoEx for EO (HEMA) was found to be higher in smokers compared to vapers (EC), users of HNB and non-users (NU). Quite unexpectedly, the HEMA levels in OT and NG users were higher than those in smokers. However, it has to be taken into account that the medians were calculated on the basis of relatively few studies and that the variance is high in all groups. The 95%-confidence intervals imply that HEMA levels in HNB users are significantly different only from smokers and users of NGs. HEMA excretions rates in all other groups (including NU) are not significantly different from each other. It has to be taken into account that urinary HEMA levels are mainly derived from the endogenous formation of the precursor ethylene (117).

Based on the estimated medians, daily intake of PO in smokers (CC) was about 20 times higher than in users of HNB products, but only 2.3 times higher than in vapers (ECs). These data have to be interpreted with caution, since release of PO from ECs

have only been reported by one working group (46). It can be assumed that intake of PO by using OT and NGs is probably negligible. The BoEx to PO (2-HPMA) was significantly higher in smokers compared to HNB users, vapers (EC), users of NGs and non-users (NU). Unexpectedly, OT users show the highest 2-HPMA levels (significantly higher than all other groups, Table 2). However, 2-HPMA levels are based on only one investigation (118).

Average intake of FA by smoking (CC) was estimated to be 7-, 35- and 577-fold higher than in users of HNB products, ECs and OT, respectively. No biomarker data for the exposure to FA in the groups of interest were as yet available. The results for the product-related intake of FA suggests that FA exposure from HNB has to be kept in mind for this product class. FA exposure in vapors appears to be possible, but requires further investigations. Exposure prediction for US adults (98, 99) amounts to about 0.06–0.09 µg/d, which is more than 2 orders of magnitude lower than the lowest estimate in this review (1 µg/d for OT users). The reason for this large difference is unclear. However, it appears obvious that the estimated exposure, irrespective of the exposure source, is by far lower than the generally observed background level of FA in body fluids, which most likely originate from endogenous formation (119).

Average intake of AA by smokers (CC) was estimated to be 7-, 900- and 1500-fold higher than in users of HNB products, ECs and OT, respectively. No biomarker data for the exposure to AA in the groups of interest were as yet available. The results for the product-related intake of AA suggests that AA exposure from HNB products has to be considered in the harm/risk evaluation for this product class. AA exposure in vapers and OT users requires further studies. Exposure prediction to AA for US adults (98, 99) amounts to about 0.16–0.24 µg/d, which is almost 50-fold lower than the lowest estimate for AA intake in this review (9.6 µg/d for OT users). The reason for this large difference is unclear. More data are necessary for a solid estimate of the exposure to AA by using the new generation products (in particular HNB products and ECs). In addition, a well-founded estimate of the background burden of AA in non-users is required for valid risk evaluation.

Intake data for ACR were limited to CC, HNB and ECs, whereas ACR-related biomarker data were available for all groups, including non-users (NU). Intake of ACR in smokers (CC) was calculated to be 13- and 107-fold higher than in users of HNB products and ECs, respectively. On the other hand, 3-HPMA levels in smokers were 2.7-, 2.9-, 3.4-, 3.1-, and 4.7-fold higher than in users of HNB, EC, OT, NG and in NU, respectively. The large differences in ratios based on intake and biomarker levels indicate a significant contribution of other sources (including endogenous lipid peroxidation (120)) to urinary 3-HPMA excretion. Urinary 3-HPMA levels in smokers were significantly higher compared to all other groups, whereas differences between HNB products, ECs, OT, NG users and NU were not statistically significant. The observed release of ACR from HNB and ECs requires further investigation for a more solid estimation of the intake and the associated risk. The exposure prediction derived from the EPA database (98, 99) amounts to about 7 ng/d for an average US adult. This is about 3 orders of magnitude lower than the estimate for vapers in this review (11 µg/d). Given the observation that urinary excretion of 3-HPMA is not significantly different between all user groups (except for smokers) as well as non-users (NU), it can be deduced that the EPA estimate is unrealistically low in terms of the background burden (exogenous and endogenous exposure) to ACR.

Intake of CRO in smokers (CC) was estimated to be about 19-, 50-, and 470-fold higher than in users of HNB, ECs, and OT products, respectively. On the other hand, HMPMA levels in smokers were 9.2-, 2.8-, 5.4-, 5.5-, and 3.1-fold higher than in users of HNB, ECs, OT, NG and non-users, respectively. The large differences in the group ratios based on intake and biomarker levels indicate a significant contribution of other sources to urinary HMPMA excretion. HMPMA excretion in smokers was significantly higher compared to all other groups, whereas biomarker levels in users of HNB products was significantly lower than in vapers (ECs) and non-users (NU). Also somewhat unexpected is the elevated median HMPMA level in vapers compared to the users of the other 'new' products (HNB, OT, NG). These findings cannot be explained by the release of CRO during vaping and might be a chance finding, fostered by the low number of investigations, particularly with the tobacco/nicotine products other than cigarettes. The exposure prediction from the EPA database (0.05–0.08 µg/d) is again much lower than the lowest estimate in this review (OT users: 0.9 µg/d).

Average intake of PA for smokers (CC) was calculated to be 64- and 400-fold higher than in users of HNB products and ECs, respectively. Only one investigation on the release of PA by ECs was available (45). Therefore, the results for this product type must be regarded as preliminary at best. No biomarker data for the exposure to PA in the groups of interest were available. In the EPA database (98, 99), exposure to PA is predicted to be in the range of 1.5–2.3 µg/d, which is close to the estimated exposure by vaping (3 µg/d). However, as mentioned, the available data are too few for any firm conclusions.

Average intake of AC for users of HNB products was estimated to be about 1.4-fold higher than in smokers of CCs. Furthermore, according to the available data on acetoin, HNB use was found to be implicated with an about 500-fold higher daily AC intake than vaping (ECs). However, it has to be taken into account that only limited data are available for the intake estimations. In addition, the identification of suitable BoEx for assessing the exposure to AC would be required to generate reliable exposure data for AC. The exposure prediction from the EPA Chemical Dashboard is < 0.3 ng AC/d for an average adult, which is far below the achievable detection level for the release of AC from the products of interest. This predicted exposure level is almost 3 orders of magnitude lower than the lowest intake estimate for AC in this review (ECs: 0.13 µg/d).

Average intake of GO for smokers (CC) was calculated to be 150-, and 26-fold higher than in users of HNB products and vapers (EC), respectively. Vapers were estimated to take in 6-times higher amounts of GO than HNB product users. However, it has to be taken into account that only limited data were available for the intake estimations. Again, the identification of suitable BoEx for assessing the exposure to GO would be needed to generate reliable exposure data for GO. The exposure prediction from the EPA Chemical Dashboard is about 0.02 µg/d for an average adult, which is more than 1 and 2 orders of magnitude lower than the intake related to vaping (4.24 µg/d) and using HNB products (0.71 µg), respectively.

Median intake of MGO by smokers (CC) and HNB product users was found to be about similar and about 30-fold higher than in vapers (EC). Again, only limited data were available for the intake calculations. In particular, only one investigation on

the release of MGO from HNB products was available (25). In addition, the application of existing BoEx for assessing the exposure to MGO (121–125) would be helpful to generate reliable exposure data for MGO. However, it is as yet unclear, whether the product use-related intake can measurably contribute to the already existing endogenous burden with MGO during endogenous glycolysis (126). No data on the exposure prediction for MGO from the EPA Chemical Dashboard are available.

Combustion-derived chemicals

Seventeen chemicals were classified into this group: carbon monoxide (CO), 1,3-butadiene (BD), isoprene (Iso), furan (Fu), benzene (Be), styrene (Sty), acrylonitrile (AN), acrylamide (AM), hydrogen cyanide (HCN), *o*-toluidine (*o*-Tol), 2-naphthylamine (2-NA), 4-aminobiphenyl (4-ABP), naphthalene (Nap), fluorene (Flu), phenanthrene (Phe), pyrene (Pyr), benzo[*a*]pyrene (B*a*P). Median values for daily intake estimates and corresponding biomarker levels are shown in Table 2.

For CC and HNB users, amounts of intake for all 17 chemicals could be calculated. No data for combustion chemicals for OT products and NGs were available, which, in general, is plausible for these products. Incomplete intake data sets were at hand for vapers (ECs) and non-users (NU). Complete biomarker data sets for typical combustion products are available for smokers (CC) and non-users (NU), whereas for the users of the other products (HNB, EC, OT, NG) data are partly missing (Table 2).

Estimated median daily intake of CO by using tobacco/nicotine products was found to be significantly elevated in smokers (CC), whereas the CO intake by vaping (ECs) and using HNB products was negligible. No data were available for the release of CO from OT products and NGs. However, no appreciable intake of CO is expected from the use of these products. COHb levels in the 6 groups of interest are in line with the CO intake estimates: smokers exhibited significantly elevated COHb levels (median: 5.62%) compared to all other groups, including the NU group (range of medians: 1.05–1.62%). It is well known that there is an endogenously caused background for COHb of about 1% (102).

The calculated daily intake of BD was highest in smokers, whereas the product-related intake in users of HNB and ECs was almost 200- to 300-fold lower. No BD exposure data from the EPA Chemical Dashboard is available. The BoEx for BD (MHBMA) was significantly higher in smokers compared to all other user groups, which were on the same levels as the non-users (NU). An exception appeared to be the group of NG users. However, the MHBMA data for this group is based on only two data sets obtained from one study (54).

Median daily intake of Iso was by far the highest in smokers, whereas the product-related intake in HNB and EC users is almost 300- to 400-fold lower. The BoEx for Iso (IPMA3) was about 10-fold higher in smokers compared to vapers (EC) and non-users (NU). It can be assumed that a similar ratio would exist between smokers and users of HNB, OT and NG. The discrepant ratios in the estimated intakes of Iso between smokers and the other groups (300–400) and the corresponding biomarker levels (~ 10) is most likely due to the ubiquitous exposure to and the endogenous formation of Iso (127).

Exposure prediction for Iso in the EPA database gives a range of 0.23–0.35 µg/d. This is about 100-fold lower than the lowest estimate in the review (vapers: 22.1 µg/d). In light of the observation that vapers and non-users exhibited about the same excretion rates for IPMA3, it appears unlikely that the use of ECs and HNB products can measurably increase the BoEx to Iso.

Furan intake data were limited to CC and HNB products, whereas Fu-related biomarker data were available for smokers (CC) and non-smokers (NU). Intake of Fu was estimated to be more than 40 times higher by smoking (CC) than be using HNB products. BoEx levels of Fu were found to be 4.6 times higher in smokers than in non-users. The difference in these ratios (almost 10-fold) suggests that significant contribution from other sources to the Fu exposure (e.g., food, coffee) has to be assumed. Interpretation of the results for the reported furan data is of course impaired by the lack of data for furan release from the products of interest as well as for furan biomarker data for the various user groups and non-users.

The exposure prediction to Fu in the EPA Chemical Dashboard amounts to 1.6–2.4 ng/d for US adults (91, 92). The lowest intake estimate for Fu in this review is 12.9 µg/d (HNB product users). The intake estimates and the biomarker results most probably suggest that the corresponding EPA prediction is by far too low.

Intake data for benzene (Be) were limited to CC, HNB products and ECs, whereas Be-related biomarker data were available for all six groups. Intake of Be for the non-combusted, non-heated products (OT, NG) is rather unlikely. Intake of Be is approximately 100–200-fold higher in smokers (CC) than in users of HNB products or ECs. SPMA levels in all groups (including NU) excluding smokers were about similar and approximately 3–7-fold lower than in smokers. The difference between the urinary SPMA levels in smokers and all other groups was significant. SPMA levels in users of HNB products were significantly lower than in users of ECs, OT, NGs and also in NU. This observation is difficult to explain and might be biased by the fact that most values originate from switching studies with some possible non-compliance. The EPA database-derived exposure prediction to Be amounts to about 0.5 µg/d, which is in the same range as those estimated in this study for users of ECs, OT products and NGs and about 2–3 times lower than for smokers. This can be regarded as good agreement between EPA database-derived exposure prediction and values from this evaluation.

Intake data for styrene (Sty) were limited to CC and HNB products, whereas Sty-related biomarker data are available for all groups, except for HNB and OT users. Intake of Sty for the non-combusted, non-heated products (OT, NG) is rather unlikely, but might be possible for vapers. As yet, however, no data on Sty in the aerosol of ECs have been reported. Intake of Sty was calculated to be 23-fold higher in smokers (CC) than in users of HNB products. On the other hand, PHEMA (phenyl-2-hydroxyethylmercapturic acid) levels in smokers were 1.3–2-fold higher than in groups with marginal or none tobacco/nicotine-product-related Sty exposure (EC, NG users, NU). Again, this suggests a significant contribution of other sources to urinary PHEMA excretion. The difference in PHEMA excretion between smokers and NU was statistically not significant. In general, available data for both intake estimates and biomarker levels were too limited to draw any solid conclusions. Exposure to Sty prediction derived from the EPA data base for US adults (5.3–7.9 µg/d) is close to the intake estimate for HNB product users (8.8 µg/d).

Average intake of acrylonitrile (AN) for smokers (CC) was estimated to be 87- and 95-fold higher than in HNB product

users and vapers (EC), respectively. Urinary CEMA (cyanoethyl-mercapturic acid, BoEx for AN) excretion in smokers, on the other hand, was between 4- and 10-fold higher than in users of the other 5 tobacco/nicotine products (HNB, EC, OT, NG) and 90-times higher than in non-users (NU). CEMA levels in NU were not significantly lower than in users of HNB and OT products as well as NGs, but significantly lower than in users of ECs. These findings suggest that vaping might moderately, but significantly contribute to the uptake of AN. This observation requires verification by additional studies. Note that as yet only one investigation on the release of AN from ECs is available (42). The exposure prediction from the EPA Chemical Dashboard is about 0.1–0.2 µg AN/d for an average adult, which is approximately 1 order of magnitude lower than the intake related to vaping (2.2 µg/d) and using HNB products (2.4 µg/d). In the underlying publication of WAMBAUGH *et al.* (99), it was stated that no CEMA measurements in NHANES were available for the prediction. However, in more recent NHANES studies (21), CEMA data were available. The CEMA levels reported in the NHANES studies were in good agreement with the values determined in this review.

No data for the release of acrylamide (AM) from ECs, OT and NG products were available, however, it can be assumed that the release of AM from OT and NG is negligible. Formation of AM in ECs cannot be excluded and requires further investigation. Median intake of AM for smokers (CC) was estimated to be about 3-fold higher than in HNB users. Urinary AAMA (BoEx of AM) excretion in smokers was found to be between 2- and 3-fold higher than in users of HNB products, ECs and NGs. Differences between AAMA levels of smokers and users of HNB products, ECs and NG were significant. Somewhat unexpectedly, AAMA levels in OT users were about similar to that in smokers. A possible explanation could be the non-compliance of OT users, who were experimentally switched from CC smoking to OT use. The exposure prediction from the EPA Chemical Dashboard is about 1–2 ng AM/d for an average US adult, which is approximately 3 orders of magnitude lower than the intake related to using HNB products. Our evaluation of the published AAMA data showed no significant difference between HNB product users and non-users (NU), which is in disagreement with the exposure prediction for AM (98, 99). The exposure prediction is also in plain disagreement with the estimate for the food-related intake of AM amounting to 0.4 µg/kg bw/d (for adults with 60–90 kg bodyweight, the intake range would be 24–36 µg/d) (128). It thus appears that the EPA database-derived exposure prediction to AM is by far too low.

No data for the release of HCN from ECs, OT and NG products were available. It can, however, be assumed that the release of HCN from ECs, OT products and NGs is negligible. Average intake of HCN for smokers was estimated to be about 120-fold higher than in HNB users. Similar ratios are to be expected between smoking and the other products (EC, OT, NG). The BoEx for HCN, thiocyanate (SCN) in plasma, was 1.5- and 1.7-fold higher in smokers than in OT users and NU, respectively. The difference in plasma SCN concentrations between smokers and the other two groups (OT, NU) was statistically significant. These results further suggest that there are high background levels of SCN in plasma, probably due to the food-related intake of HCN as well as the microbial formation of SCN in the body (102).

Average intake of *o*-Tol for smokers (CC) was estimated to be 94-fold higher than in HNB product users. Similar ratios are to be expected between smoking and the use of the other products (EC, OT, NG). The BoEx for *o*-Tol (*o*-Tol in urine) was 1.8–3.5-fold higher in smokers than in the other groups, including NU. The difference in urinary *o*-Tol excretion between smokers and the other groups (HNB, EC, OT, NG, NU) was statistically significant. The large difference in the ratios for the estimated intakes of *o*-Tol and the corresponding biomarker levels suggest that there is an additional high exposure from not well defined other sources (presumably ambient air and food). The predicted exposure to *o*-Tol of adults in the US amounts to 230–340 ng/d (98, 99) and is about half of that estimated for smokers in this study. The estimated additional intake of 8 ng *o*-Tol/d by using HNB products (and probably also the other tobacco/nicotine products) can be regarded as negligible.

No data for the release of 2-amino-naphthalene (2-NA) from OT and NG products were available. It can, however, be assumed that the release of 2-NA from OT products and NGs is negligible. Average intake of 2-NA for smokers was estimated to be more than 2 and 1 order of magnitude higher than in HNB product users and vapers (EC), respectively. Similar ratios are to be expected between smoking and the other products for which no release data exist (OT, NG). The BoEx to 2-NA, 2-NA in urine, was found to be 8–15-fold higher in smokers than in the other groups, including NU. The difference in urinary 2-NA excretion between smokers and the other groups (HNB, EC, OT, NG, NU) was statistically significant. There was no statistically significant difference in the biomarker levels between the groups other than cigarette smokers. The biomarker data for 2-NA further suggest that less well defined sources other than use of any of the products studied may play a significant role for the daily intake of 2-NA (presumably ambient air and food). The predicted exposure to 2-NA of adults in the US amounts to 93 – 140 ng/d (98, 99) and is about in the same range as 2-NA intake by smoking and much higher than by using HNB products (0.4 ng/d) or vaping (4.4 ng/d) (Table 2). Again, it must be taken into account that the estimates for the daily intake of 2-NA by using the new generation products are based on very few data.

Median intake of 4-aminobiphenyl (4-ABP) for smokers was estimated to be 250- and 126-fold higher than in HNB product users and vapers (EC), respectively. Similar ratios are to be expected between smoking (CC) and use of the other products (OT, NG). The BoEx to 4-ABP, 4-ABP in urine, was 3–9-fold higher in smokers than in the other groups, including NU. The difference in urinary 4-ABP excretion between smokers and the other groups (HNB, EC, OT, NG, NU) was statistically significant. Non-users (NU) showed significantly lower urinary 4-ABP levels than users of ECs and NGs. Users of HNB products had significantly lower 4-ABP biomarker levels than users of ECs, OT products and NGs. The biomarker data for 4-ABP further suggest that not well defined (diffuse) sources other than use of any of the products studied may play a significant role for the daily intake of 4-ABP (presumably ambient air and food). This assumption, however, is not well-founded in the reported data. The predicted exposure to 4-ABP of adults in the US is reported to be 94–141 ng/d (98, 99), which is about 4-times higher than the estimated intake for smokers (25 ng/d) and about 3 orders of magnitude higher than the 4-ABP intake estimated for users of HNB products or ECs (0.1–0.2 ng/d).

Particularly the discrepancy of the predicted exposure for average adults to that of smokers remains unexplained. Again, it must be taken into account that the estimates for the daily intake of 4-ABP by using the new generation products are based on very few data.

For naphthalene (Nap), no data for the release from ECs, OT products and NGs were available. Average intake of Nap for smokers (CC) was estimated to be more than 300-fold higher than by using HNB products. It is highly likely that release of Nap from ECs, OT and NGs is in the same range as for HNB products and, therefore, intake of Nap by using these products can most likely be neglected. Urinary excretion of 1-OH-Nap in smokers was found to be 5- to 7-fold higher than in users of ECs and OT as well as in NU. The difference in 1-OH-Nap excretion between smokers and the three other groups (EC, OT and NU) was statistically significant. Exposure prediction from the EPA database amounts to 3.5–5.2 µg/d for an average adult, which is about half of the estimate for the daily intake by smoking (10.9 µg/d). In comparison to this, the estimated intake of Nap by using HNB products amounts to 0.03 µg/d, which is more than 100-fold lower and can be regarded as negligible. The same can be assumed for the other new generation products evaluated in this review (EC, OT, NG). These mostly preliminary observations have to be confirmed by further product- and user-related data.

No data for the release of fluorene (Flu) from ECs, OT and NGs were available. Average estimated intake of Flu for smokers (CC) was 73-fold higher than by using HNB products. It is highly likely that release of Flu from ECs, OT and NGs is in the same range as for HNB products and, therefore, intake of Flu by using these products can probably be neglected. Urinary excretion of 2-OH-Flu in smokers was calculated to be 1.8- to 3.3-fold higher than in users of ECs, OT and NG as well as in NU. The difference in 2-OH-Flu excretion between smokers and the four other groups (EC, OT, NG and NU) was statistically significant, whereas differences in this parameter between these four groups were not significantly different. Exposure prediction from the EPA database for Flu amounts to 5–7 ng/d for an average adult, which is more than 400-fold lower than that estimated for smoking (2910 ng/d) and 6–8-fold lower than the intake by using HNB products (40 ng/d). Since the urinary 2-OH-Flu level for users of EC, OT and NG (probably also of HNB products) was observed not to be significantly different from that of NU, it has to be assumed that the exposure prediction for Flu in the EPA database is much too low. Again, the data base for the presented estimations and evaluations is again very limited. The more or less preliminary observations would have to be confirmed by further product- and user-related data on Flu exposure.

Also no data for the release of phenanthrene (Phe) from ECs, OT and NGs were available. Average estimated intake of Phe for smokers was 139-fold higher than by using HNB products. It is highly likely that release of Phe from ECs, OT and NGs is in the same range as observed for HNB products and, therefore, intake of Phe by using these products can probably be neglected. Urinary excretion of 1-OH-Phe in smokers (CC) was calculated to be 1.6- to 2.1-fold higher than in users of ECs, OT and NG as well as in NU. The difference in 1-OH-Phe excretion between smokers and the three other groups (EC, OT, NU) was statistically significant, whereas the biomarker levels of these three groups were very similar and not significantly different.

Exposure prediction from the EPA database for Phe amounts to 61–92 ng/d for an average adult, which is more than 25-fold lower than that estimated for smoking (2322 ng/d) and 4–6-fold higher than the intake estimated for users of HNB products (16.7 ng/d). Since the urinary 1-OH-Phe levels for users of EC and OT products (and most likely also for HNB products and NGs) were observed not to be significantly different from that of NU, it is suggested that the exposure prediction for Phe in the EPA database can be regarded to be in rough agreement with the intake estimates performed in this review. The described, mostly preliminary observations have to be confirmed by further product- and user-related data on Phe exposure for more solid conclusions.

Median estimated intake of pyrene (Pyr) for smokers (CC) was found to be 17-fold higher than for users of HNB products. It is highly likely that release of Pyr from ECs, OT and NGs is in the same range as for HNB products and, therefore, intake of Pyr by using these products is hardly of relevance. Urinary excretion of 1-OH-Pyr in smokers (CC) was calculated to be 1.1- to 4.4-fold higher than in users of HNB products, ECs, and NGs as well as in NU. Only the differences in biomarker levels between smokers and HNB product users and non-users were statistically significant. OT users were found to excrete, on average, 20% higher amounts of 1-OH-Pyr than smokers (CC). This difference is not significant. The high biomarker levels for OT users was unexpected and could be due to non-compliance of the subjects, since 6 of 7 data sets originate from switching studies (CC to OT). Exposure prediction from the EPA database for Pyr amounts to 92–138 ng/d for an average adult (98, 99), which is more than 6-fold lower than that estimated for smoking (872 ng/d) and 2–3-fold higher than the intake estimated for users of HNB products (51 ng/d). Since the urinary 1-OH-Pyr levels for users of HNB, ECs, OT and NGs were observed not to be significantly different from that of non-users (NU), it is suggested that the exposure prediction for Pyr in the EPA database can be regarded to be in agreement with the intake estimates performed in this review. Again, the reported, mostly preliminary observations have to be confirmed by further product- and user-related data on Pyr exposure.

For benzo[*a*]pyrene (B*a*P), no release data for NGs were available. Average estimated intake of B*a*P for smokers was 27-, 34- and 7.3-fold higher than by using HNB products, ECs and OT, respectively. It is highly likely that release of B*a*P from NGs is in the same range as for HNB products and ECs. The observed release of B*a*P from OT products is clearly bimodal: snus (13 products) were reported to have levels < 5 ng/g, whereas moist snuff (9 products) were found to have levels > 30 ng/g. Urinary excretion of 3-OH-B*a*P in smokers was calculated to be 1.7- to 5.5-fold higher than in users of HNB products, ECs, OT and NGs as well as in NU. All differences in urinary 3-OH-B*a*P excretion between smokers (CC) and the other groups were significant, whereas the differences among the new product users and non-users (HNB, ECs, OT, NG, NU) were not significant. Exposure prediction from the EPA database for B*a*P amounts to 61–92 ng/d for average adults (98, 99), which is half of that estimated for smoking (149 ng/d), but 11-, 14- and 3-fold higher than the intake estimated for users of HNB products (5.5 ng/d), vapers (4.4 ng/d) and OT users (20.4 ng/d). These data suggest that the use of the new generation of tobacco/nicotine products leads to no appreciably additional intake of B*a*P.

Note on chemical intake data for non-users (NU)

The estimation of the daily intake of the chemicals of interest by non-users (NU) proved difficult due to the poor availability of suitable data. Performing intake estimations similar to the product-related intakes would have gone beyond the scope of this review. It was, therefore, decided to use the "predicted exposure" data accessible in the web-based EPA Chemical Dashboard (98).

It is interesting to note that "predicted exposures" for the 4 PAHs with biomarker data were in fairly good agreement with the intake values estimated for the other user groups. However, for many chemicals "predicted exposure" was orders of magnitude lower than expected from the evaluated data in this review. This applies for example to N, PG, FA, ACR, AC, GO, AM. On the other hand, "predicted exposure" for 4-ABP in NU was the highest of all 6 groups (even higher than in smokers). No plausible explanation can be provided for these large discrepancies. When assigning relative exposure levels (Table 2), these discrepancies were eliminated by changing the questionable exposure values to more plausible values. It should, however, also be noted that the "predicted exposure" data from the EPA Chemical Dashboard have no direct impact on the relative exposure levels applied for group differentiation in this review, since this was based on biomarker levels only.

Other sources of exposure and carcinogenic properties of selected chemicals

Other possible sources for exposure to a particular chemical (including endogenous formation) are of importance with regard to the biomarker levels observed for the 6 study groups. In some cases, e.g., glycerol (G), formaldehyde (FA), acetaldehyde (AA), exogenous and endogenous sources can completely prevent the application of suitable biomarkers for assessing the uptake by using the 5 tobacco/nicotine products of interest. Except for the nicotine- and tobacco-derived chemicals (N, NN, AT, AB, NNK, NNN, NAT, NAB), all other chemicals dealt with in this review feature other sources of exposure (other than the 5 products of interest) which can more or less impair the suitability of the corresponding biomarker. This particularly pertains for biomarkers related to the exposure to PG, Gly, Iso, EO, AM, HCN, *o*-Tol, PAHs. For these chemicals, it is difficult to even differentiate the highest ranking product users (usually smokers (CC), vapers (EC) in case of PG) from the other groups, including non-users (NU).

Another important feature of the selected chemicals for this review are their genotoxic and carcinogenic properties. Of the 38 compounds, 10 (BD, EO, Be, FA, *o*-Tol, 2-NA, 4-ABP, NNK, NNN, B*a*P) have been classified as human carcinogens (IARC Class 1). Toxicological aspects were not the major focus of this review. However, exposure to chemicals classified as human carcinogens is of particular importance when comparing the tobacco/nicotine products in this discourse. As will be shown in the next chapter ("*Relative exposure levels*"), the new generation of tobacco/nicotine products (HNB, EC, NG and with restraints OT) rank in the lowest (1) and second lowest (2) relative exposure levels, very similar to the non-users (NU) with respect to the exposure to genotoxic chemicals. An exception being B*a*P, for which the uptake relative exposure levels for all groups (except smokers) varied between 1 and 3.

Relative exposure levels

Further steps of this evaluation were (i) the comparison between estimated intake and biomarker-based uptake data and (ii) the distinction between the 6 groups (5 user groups of tobacco/nicotine products and non-users). For this end, the exposure levels (by now expressed as daily intake or biomarker level in plasma and daily urinary excretion of the biomarkers) were categorized into 4 relative exposure levels.

Definition of relative exposure levels and agreement between intake and uptake estimates

The intake and (biomarker-based) uptake values as shown in Table 2 for the 38 chemicals and 6 consumer groups were first 'normalized' to the highest median value of a chemical for the groups (most frequently the CC group) yielding values between 0 and 1. These relative values were then assigned to four exposure levels (1, 2, 3, 4, with 1 representing the lowest and 4 the highest exposure level). Class boundaries were defined as follows: Level 1: < 0.100, Level 2: 0.100–0.399, Level 3: 0.400–0.699, Level 4: ≥0.700. Relative exposure levels for both intake and uptake are shown in Table 2 (bold numbers in parentheses). The boundaries of the absolute intake and uptake levels calculated with the ranges shown above for all 38 chemicals studied and the 5 TNP users and non-users are provided in Table S39 in Supplemental File 1. Assuming that an exposure level difference (intake – uptake) of within +/− 1 is tolerable, then almost 90% of the intake/uptake classification for the groups CC, HNB, EC, and NG were in acceptable agreement, whereas for OT (< 67%) users and NU (< 78%) this was not the case. As mentioned earlier, the group of OT products and users evaluated in this study was rather heterogeneous (consisting of new and old generation OT products). As also referred to previously, intake data of chemicals for NU were frequently diverting from expectations and probably not very reliable. Since relative exposure level differences between intake and uptake of > |1| in all likelihood point out to a problem in either intake or uptake values, these classifications were corrected according to highest plausibility (see chapter "Intake and uptake of chemicals by product use").

For the purpose of fairly meaningful comparisons within the frame of this evaluation, it was decided to fill most of these gaps with reasonable assumptions. Assumptions were made according to the following rules:

1. In cases of missing release data of a chemical for a product, an evidence-based assumption was made, e.g., it was assumed that typical combustion chemicals are not released from non-combustion products (HNB, EC, OT, NG) in appreciable amounts (therefore, usually exposure Level "1" was assigned in these cases; example: Nap for EC, OT, NG).
2. In cases of missing release data for nicotine-/tobacco-derived chemicals, it was assumed that in the new generation products (HNB, EC, NG) the minor alkaloids (NN, AB, AT) and the TSNA (NNK, NNN, NAT, NAB) were released in only very low amounts (exposure Level "1" was assigned; example: NN, AT, AB release from NG).
3. In case of missing biomarker data, the corresponding exposure level of the intake was assigned. If the intake was not available, a plausible exposure level (plausible in comparison to the users of other products as well as to NU) for the uptake was assigned. If no suitable biomarker data for a

chemical were available, no exposure level was assigned (e.g., for NN, FA, AA, AC, GO, MGO).

4. Some completely implausible outcomes in Table 2 were replaced as follows (also discussed in previous chapters): AT intake by vaping: Exposure Level 4 changed to 1; AM uptake by OT use: Level 4 was changed to 2; 2-NA and 4-ABP intake by NU: Level 4 was changed to 1; Pyr uptake in OT and NG users: Class 4 was changed to 2. HEMA (biomarker of EO) relative levels of 4 for OT and NG users were implausible and, therefore, changed to Level 2. The same is true for 2-HPMA (biomarker for PO) for OT and NG users. The changed relative exposure levels are shown in bold, italic numbers in Table 2.

The assumption-based changes and corrections in the assigned exposure levels (Table 2) lead to a relative consistent picture for exposure levels of the nicotine-/tobacco- and the combustion-derived chemicals. Somewhat inconsistent is the PG-/G-derived group of chemicals. In particular, the uptake levels for EO and PO for the OT and NG users are difficult to explain. However, it must be taken into account that particularly for EO endogenous formation can play a role.

For the combustion-derived chemicals, of course smokers always are ranging in the highest exposure level (Level 4), both for intake and uptake. It is interesting to note that the other groups (including NU) were estimated to range in the lowest daily intake level (Level 1), while the biomarker-derived uptake for these groups is most frequently distributed between the exposure levels 2 and 3. This observation is in most cases due to the fact that for the chemicals in this group, many other sources such as food, ambient air, traffic exhausts, etc. significantly contribute to the overall exposure in the general population.

Relatively large differences in terms of exposure levels are to be seen between smokers (CC) and all other groups, whereas differences were found to be rather small between the 5 groups of novel product users (HNB, EC, OT, NG) and NU. The following rank order with decreasing exposures could be deduced from Table 2:

CC >> OT > EC ≈ HNB > NG ≈ NU

Of course, this ranking is dependent on the set of chemicals evaluated in this review. However, there is good reason to believe that the set of chemicals used is representative for what is presently available and would not significantly change when the set of chemicals is enlarged with increasing knowledge about the products of interest.

How well do new data sets fit into the established relative exposure level system?

A possible suitability check can be done by testing the conformity of recently published data, which have not been used for calculating the intake and uptake for chemicals in the 6 consumer groups (mainly because they were not yet available when the relative exposure level system was developed). In total, 17 studies on either one or several of the consumer groups of interest were tested for conformity, 9 studies on the release of chemicals from tobacco/nicotine products (86, 129–136) and 8 studies on biomarker levels (137–144). For testing the exposure levels for daily intake, 140 comparisons could be performed, comprising tests for 21 different chemicals and 3 products (26 tests with CCs, 12 with HNB products and 114 with ECs). About 90% of the relative exposure level differences were within the range of +/− 1 with an absolute mean difference of 0.44. The 8 biomarker studies checked for fitting into the relative uptake levels covered 4 groups (CC: 24, EC: 18, OT: 7 and NU: 19 tests) with 17 different biomarkers allowing in total 69 tests. Of these, 65 tests (94%) were within the +/− 1 level range. Absolute mean difference (originally developed *minus* new uptake exposure level) amounted to 0.47. Thus, the conformity tests show that the developed exposure levels are in good agreement with new (independent) data sets for both intake and uptake. Level deviations were most frequently (> 90%) in the range of +/− 1. Higher deviations could be in almost all cases explained by some peculiarities in the experimental study design of the new data sets. Comparisons are shown in detail in Table S39 (Supplemental File 1).

Differentiation of consumer groups

The major intention of the present study was to establish tools, which would allow the differentiation of the 6 groups differing in their use of 5 tobacco/nicotine products on the basis of certain biomarker levels. Right from the start of writing this review, it was obvious that, in contrast to reports in the literature (145), there are no product-specific constituents (and consequently no biomarkers) which are unique to one of the studied tobacco/nicotine products, thus allowing the qualitative differentiation between user groups. Hence, distinction would be possible only on quantitative differences. It is also obvious that the group of NU can simply be distinguished from the other groups by their very low level of nicotine and nicotine metabolites in body fluids. Furthermore, it seems natural that smokers of conventional (combustible) cigarettes (CC) can be distinguished from the other groups, due to their elevated levels of typical combustion products (and/or their metabolites) in their body fluids. From the data presented here, it is evident that discrimination between the other four groups (HNB, EC, OT, NG) is much more challenging. In this chapter, approaches for the differentiation of the groups of interest are demonstrated and discussed. Basically, two ways for achieving this goal are shown, namely, (i) differentiation on the basis of single biomarker levels and (ii) differentiation on pattern of relative biomarker levels. In the third section of this chapter, implications of dual- and multi-product use are also discussed.

Differentiation on the basis of single biomarker levels

The result of the analysis for uniqueness of biomarker levels in the 6 groups of interest is shown in Table 3. For the purpose of this analysis, two levels of uniqueness were defined: (i) "unique", meaning that there is at least a difference of 2 relative exposure levels between the group identified and the other groups (a difference of 2 exposure levels is equivalent to about a factor of ~ 4 in absolute biomarker levels); (ii) "possibly unique", meaning that there is a difference of 1 relative exposure level (~ 2-fold difference in absolute biomarker levels) between the group identified and the other groups.

Smokers can be uniquely distinguished from all other groups, if the level of at least one of 12 biomarkers (NAB, 3-HPMA, HMPMA, COHb, IPMA3, Fu, SPMA, CEMA, 2-NA, 4-ABP 1-OH-Nap) exceeds a certain level (specified in Table 3). This is possible for only two other groups: vapers (EC), if urinary PG is above a certain level, and NU, if Nequ in urine or cotinine in plasma is below a defined level (Table 3). Smokers (CC) also have the highest number of biomarker levels classified as "possibly unique" (N = 8). OT users were classified as "possibly unique" for the highest urinary NNAL levels.

Table 3. Uniqueness of single biomarker levels for particular product users or non-users.

Users	"Unique" (at least 2 classes or ~4-fold difference expected to all other groups and either highest or lowest rank among the 6 groups)		"Possibly unique" (at least1 class or 2-fold difference expected and either highest or lowest rank among the 6 groups	
	Biomarker [a]	Level	Biomarker [a]	Level
CC	NAB (u)	> 41 ng/d	NNN (u)	> 12.5 ng/d
	3-HPMA (u)	> 1123 µg/d	NAT (u)	> 193 ng/d
	HMPMA (u)	> 496 µg/d	MHBMA (u)	> 2.5 µg/d
	COHb (b)	> 3.9%	SCN (p, u, sa)	> 87 µM
	IPMA3 (u)	> 30.8 µg/d	*o*-Tol (u)	> 141 ng/d
	Fu (b)	> 0.04 ng/d	2-OH-Flu (u)	> 1.36 µg/d
	SPMA (u)	> 0.95 µg/d	1-OH-Phe	> 155 ng/d
	CEMA (u)	> 113.8 µg/d	3-OH-BaP	> 142 pg/d
	2-NA (u)	> 19.3 ng/d		
	4-ABP (u)	> 14.6 ng/d		
	1-OH-Nap (u)	> 9.3 µg/d		
HNB	none		none	
EC	PG (u)	> 4.4 mg/d		
OT	none		NNAL (u)	> 378 ng/d
NG	none		none	
NU	Nequ (u)	< 0.037 mg/d	CEMA (u)	< 16.1 µg/d
	Cotinine (p)	< 21.4 ng/mL		

[a] Abbreviations in parentheses: u = urine, p = plasma, sa = saliva

Table 4. Complete biomarker exposure level-derived patterns for the 6 groups of single product users (or non-users) as well as 3 dual user groups.

Group	Pattern (based on 30 biomarker levels in the order as presented in Table 2), pattern for dual users calculated as described in the text
CC	443434443344444444444444444444
HNB	341122121212221112223321211122
EC	341112114222222212222322222222
OT	434443321222222112222322222222
NG	331122121222222112222321212122
NU	111112111222222122213322222222
CC/HNB	442333332333333333334433333333
CC/EC	442323334333333333333433333333
CC/OT	444444432333333333333433333333

As noted previously, this may be highly dependent on the type of OT product used. NU were "possibly unique" for the lowest levels of urinary CEMA. No "unique" or "possibly unique" biomarker levels could be identified for HNB and NG users.

Differentiation on the basis of patterns of biomarker levels

Given the limitations experienced with the use of single biomarker levels for unambiguously distinguishing the various user groups, it might be more promising to apply the levels of two or more biomarkers (in other words, patterns of biomarkers) for this purpose. This approach is described in more detail in this section.

The most extensive product user- (or non-user-) related pattern is that containing the 30 biomarker-based relative exposure levels in the fixed order as used in Table 2 resulting in the patterns shown in Table 4. These complete patterns are, most likely, unique for each user group. However, realistic applications will probably never comprise biomarker data for all of these 29 chemicals (and 30 corresponding biomarkers). Much more relevant are data for only a few biomarkers in subjects with unknown using habits of the products of interest.

Pattern recognition can be easily performed by entering the relative exposure levels for the measured biomarkers and replacing all other numbers with question marks ("?"). Comparing this 'unknown' pattern with those in Table 4 by using a simple spreadsheet (e.g., Excel) which would show none, one or several hits. An issue could be that the exposure levels for the 6 groups and 30 biomarkers as established in Tables 2 and 4 are frequently not exactly met. Rather, it has to be expected (and the Section "How well do new data set fit" has shown this) that a tolerance of +/− 1 exposure level is realistic. For this purpose, the relative exposure levels for biomarker were extended as follows: Level 1 comprises the levels 1 and 2; Level 2 comprises levels 1, 2 and 3, Level 3 comprises levels 2, 3 and 4; Level 4 comprises levels 3 and 4 (going above 4 is not necessary, since Level 4 has no upper limit of the absolute exposure level).

Both approaches (with and without a tolerance in the established biomarker-based exposure levels) have been implemented in an Excel sheet algorithm (see Supplemental File 2). While the non-tolerance approach is straight forward, the tolerance approach was realized by running more than 16000 cycles with the measured relative exposure levels of the 'unknown' user randomly assigned the values defined above. This number of cycles would allow to run the pattern analysis with +/– 1-level tolerance with values of up to about 10 biomarkers (resulting in approximately $2^{10} – 3^{10} = 1024 – 59049$ patterns).

Two examples should clarify the described product use identification by pattern recognition.

Example 1: Analysis of bio-samples from a subject showed the following results (already converted to biomarker relative exposure levels): Cot (p): 4, COHb: 2, resulting in the following pattern: ?4????????????2???????????????

Precise pattern recognition suggested that this pattern is compatible with either HNB or EC use, whereas the pattern recognition algorithm with a +/–1 tolerance revealed that the use of HNB, EC, OT and NG would be compatible with this pattern. This is an interesting result, since it leads directly to the challenge of differentiating between the 4 new generation products. Inspection of the relative exposure levels in Table 2 suggests by additionally evaluating urinary PG, EC use (vaping) could be unequivocally assigned, if the PG level falls into exposure Level 4 or 3 (the latter only with the level tolerance approach). Similarly, if the urinary levels for AT or AB fall into the exposure Levels 4 or 3, there would be clear evidence for use of OT products (again only when applying the level tolerance approach). On the other hand, there is no obvious third biomarker in this example, which would allow the unequivocal assignment of using HNB or NG.

Example 2: Analysis of bio-samples from a subject showed the following results (already converted to biomarker relative exposure levels): Nequ: 4, AB: 2, AAMA: 4, resulting in the following pattern: 4??2????????????????4?????????

The algorithm for precise comparison (no tolerance) results in no specific product use for this pattern, whereas the procedure for comparison with +/– 1 class tolerance came to the result that the unknown pattern would be compatible with the use of HNB products.

These examples demonstrate that (i) the biomarker-based uptake relative exposure levels established in Table 2 by all means requires a tolerance of +/– 1 level, (ii) the levels of three biomarkers (if selected properly) could be sufficient to unequivocally identify one of the 6 groups (single product users or non-users) and (iii) the differentiation of users of the 4 new generation products could be more challenging. In particular, distinguishing HNB product and NG users is difficult, since no unique uptake of chemicals is known for these products such as PG is for ECs and AT and AB is for OT (at least for the products considered in this evaluation).

Dual- and multi-product users

An even bigger challenge is the possible occurrence and unequivocal identification of dual- or multiple-product users. A theoretical approach for solving this problem by the use of combined patterns is briefly demonstrated in this section. As long as CCs are one product in dual usage, the identification is relatively straight forward, due to the existence of "unique" biomarkers (or more precisely "unique" biomarker levels) for smokers (Table 3). However, the disentanglement of dual (or multiple) use of new generation products (HNB, EC, OT, NG) could become too complex and possibly difficult to resolve. Completely different approaches for resolving this problem might have to be developed for this task.

From the previous section, it is quite clear that identification of single product users can already be complex. Identification of dual or multiple product users is, of course, even more challenging. A number of issues may contribute and increase this complexity:

1. Generally, the use frequency of the two or more products is mostly not known (partition between the use of the two products is probably not 1:1, as is assumed below for the sake of simplicity).
2. Dual or multiple product use is not necessarily additive (meaning that biomarker levels would be the sum of single product uses). In particular, use patterns can be influenced by pre-existing nicotine levels in blood and thus inducing compensational effects on the product use pattern (daily consumption, topography, etc.) (19, 20).
3. Most frequently, dual or multiple product use includes smoking (CC). Given the exceptional position of CC use in terms of relative exposure levels (mostly 4 or 3) for many biomarkers would blur the differences between product users.

A good chance to identify dual or multiple product use exists when there are "unique" biomarkers for each of the products used. According to Tables 2 and 3, this would be possible only for dual users of CCs and ECs.

A very formal (theoretical) approach could be the following: Assuming that there is dual use of CC together with one of the product categories HNB, EC or OT at about a 1:1 ratio. With the considerations provided above, biomarker relative exposure levels can be calculated by dividing the sum of each biomarker relative level for both products by 2 and rounding up to the next integer, resulting in the patterns shown in Table 4.

With the addition of these dual user groups, the results of the two examples presented in the previous section would have to be amended as follows:

Example 1: All three dual-user groups are compatible with the given biomarker levels (plasma cotinine = Level 4, COHb = Level 2). When adding urinary PG Level 4, dual users of CC/EC are additionally identified to be compatible with this pattern, whereas PG Level 3 would be consistent with single use of ECs and dual use of the three combinations (CC/HNB, CC/EC, CC/OT).

When AT Level 4 is added, the pattern would be compatible with OT only or dual CC/OT use, while AT Level 3 would be consistent with OT only or all three combinations of dual use. Integrating AB Level 3 or 4 would also identify OT or the three combinations of dual use as possible product habits.

Example 2: In addition to HNB product users, the dual users of CC/HNB and CC/EC also would fulfill the requirements of this pattern.

The simplistic examples provided above clearly demonstrate that dual use of products may significantly increase the complexity of the process of product use identification.

Limitations and possibilities for improvements

The described assessment of the exposure in terms of intake and uptake of chemicals by using 5 different tobacco/nicotine products and in non-users of these products suffers from a

number of limitations and weaknesses. Probably the strongest limitations are missing data for release of a number of chemicals from some products as well as the complete lacking of biomarker data for a total of 9 chemicals (Table 2). Another severe problem is the disparity of number of studies and data sets available for the various evaluation steps. For some purposes (e.g., release of a particular chemical from a particular product) only one data set was at hand, while for the same chemical but another product, plenty of studies or data sets could be used. This, undoubtedly could lead to uncontrollable biases in the evaluation. In general, more complete and abundant data were published for smokers (CC) than for the users of the new generation products (HNB, EC, OT, NG). This applies for the release of chemicals from the products, biomarker concentrations in the user groups as well as product usage patterns (Table 1, necessary for estimating the daily intake). Since non-smokers were frequently used for comparison to smokers in biomarker-based studies, uptake data for the NU group in this review are relatively complete. This, however, is not the case for the daily intake of chemicals in this group. Relying on the "predicted exposure" values published on the EPA Chemical Dashboard (98, 99) turned out to be problematic for a number of chemicals.

Another general issue is certainly the fact that in contrast to CCs, the new generation products show more variability in product design, product-use patterns and, as a consequence, exposure of their users. It is a matter of fact that design and technology of ECs changed over the past 10 or so years with measurable impact on the behavior and the exposure of the vapers (59, 66, 146–155). A similar, possibly even more severe problem, was encountered with OT products. Use of 'older' OT products is probably associated with higher exposures to TSNA than that of 'modern' OT products. A bias in this product user group in the mentioned direction can, therefore, not be excluded.

Another systematic bias in the evaluation of the biomarker-based exposure might result from the fact that many data were derived from so-called switching studies. In these studies, usually smokers (CC) were asked to switch from CCs to new generation products (HNB, EC, OT, NG) or stop smoking altogether (data for quitters were used as NU levels for quite a number of biomarkers in this evaluation). Bias can be generated by two circumstances: (i) subjects might not be completely compliant during the course of the study, this is a particular danger in (uncontrolled) field studies; (ii) the necessary washout period for the biomarker was too short so that there is the danger of carry-over to the samples which should reflect the use of the new products. In both cases, too high exposure levels would have been reported for the corresponding groups. A warning is given in the previous sections, if there is suspicion that this could have occurred. In obvious cases, strongly biased biomarker data were not used or corrected for the further evaluations.

There is warranted hope that the described limitations, weaknesses and biases in the evaluated data of this study will be significantly reduced with the advancement of new knowledge and data in this research field. There is a continuous flow of published new data suitable for refining the intake and uptake estimates necessary for a solid identification of the various consumer groups. In addition, new biomarkers with higher specificity for the various user groups of interest might be discovered in future studies. It is not unlikely that new biomarkers (both biomarkers of exposure (BoEx) and biomarkers of biological effects) will be identified by untargeted (132) rather than targeted (141) analytical studies. The targeted and untargeted analysis of samples from a controlled study with 6 consumer groups (as dealt with in this review) and different biological matrices (blood, urine, saliva, exhaled breath, exhaled breath condensate, and sputum) is in progress in our laboratory (156).

Finally, it should be mentioned that the presented approach for biomarker-based identification of user groups completely relies on acute biomarkers of exposure, which reflect the product use patterns of the last hours up to a few days. Long-term biomarkers of exposure such as hemoglobin adducts would cover the product use pattern of the last weeks to months. Cyanoethylvaline hemoglobin adducts were shown to have a high specificity for use of CCs (157). This long-term biomarker would be particularly useful to discover past non-compliance or dual use with CCs (158). On the other hand, hemoglobin adducts from TSNA unexpectedly proved to be rather unspecific for exposure in (159) and would be, therefore, less suitable as a long-term biomarker. It can be expected that biomarkers of biological effects, which most frequently reflect mid- to long-term exposures to chemicals (160), or more probably pattern of these type of biomarkers, could be helpful for differentiating the user groups of interest.

CONCLUSIONS

Differentiation of users of various tobacco/nicotine products such as CC, HNB, EC, OT, NG is essential for a solid risk evaluation of these products. An objective differentiation of users and non-users (NU) of these products appear to be possible on the basis of body fluid levels of either single or pattern of suitable biomarkers of exposure. In order to establish a solid basis for the described purpose, product-related daily intakes of 38 chemicals were estimated by using published data on chemicals released from the products and common product use patterns. Intake estimates were compared on a relative basis to available corresponding biomarker levels of the uptake of 6 groups of interest. In general, an acceptable agreement between the intake and uptake was observed, with some exceptions, which were corrected conform with the highest plausibility. Intake (available for 38 chemicals) and uptake (available for 30 biomarkers, representing 29 chemicals) were divided into 4 relative exposure levels (1 to 4 with 1 representing the lowest and 4 the highest exposure). Smokers (CC) could be unequivocally distinguished (with at least 2 exposure levels difference) from all other groups on the basis of 11 single biomarkers (10 of which were related to tobacco combustion chemicals). This was also possible for vapers (EC) with one biomarker (PG in urine) and for non-users (NU) with exposure Class 1 of 2 biomarkers (urinary nicotine equivalents and cotinine in plasma or saliva). Unique differentiation by means of single biomarkers was not possible for users of HNB, OT and NG products. As was shown by a few examples, consumer group differentiation was also achieved by making use of biomarker-based exposure level patterns. It turned out that applying 3 selected biomarkers can result in an unequivocal identification of one particular consumer group. Not unexpectedly, distinguishing the "new generation" tobacco/nicotine products (HNB, EC, OT, NG) from each other represents a major challenge based on the current literature. In particular, HNB and NG users were found to be difficult to distinguish, since, other than vapers (PG in

urine) or OT users (AT or AB in urine), consumers of these products do not exhibit elevated biomarker levels specific for HNB or NG. An even bigger challenge is the circumstance that dual- or multi-product use cannot be excluded. By applying a simple additive approach in dual users (CC and HNB or EC or OT), it was shown that the complexity of the pattern recognition system rapidly increased, thus preventing the unambiguous identification of user groups.

The presented biomarker-based relative exposure level system has a number of limitations, weaknesses and uncertainties, most of which are related to the lack or incompleteness of published data, particularly for the new generation products. The majority of the biomarkers available in literature were associated with tobacco combustion and thus were found to be increased only in smokers (users of CC). It appears highly likely that these flaws can, at least partly, be attenuated with the availability of more data on product- and operating temperature-related release of chemicals, product-use patterns and biomarkers data of larger consumer groups. Furthermore, it is expected that the application of newly developed untargeted analytical platforms to body fluids of the various consumer groups will result in the discovery of new biomarkers of exposure (BoEx) and of biological effect, allowing a more specific discrimination of single as well as dual- or even multi-product users. The integration of product-specific long-term biomarkers would significantly extend the possibilities for resolving the described issues.

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SUPPLEMENTAL MATERIAL

Supplemental File 2 is an inter-active Excel template. The interested reader is welcome to request the file from gerhard.scherer@abf-lab.com.

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