

Wastewater-Based Epidemiology (WBE): Potential Application for Assessing the Use of Conventional and New Generation Tobacco and Nicotine Products *

by

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SUMMARY

Wastewater-based epidemiology (WBE) is a recent methodology for assessing the exposure to and (intentional) use of all kinds of chemicals in defined populations (communities) by analysis of suitable biomarkers in wastewater (WW) samples, which are commonly collected at the inlet of wastewater treatment plants (WWTPs) serving this population. WBE has been also applied to determine the use of life-style products containing caffeine, alcohol and nicotine on a global basis. This paper outlines the methodological principles of WBE and presents the outcome of a systematic review on WBE studies that utilize cotinine as biomarker in WW samples to assess tobacco/nicotine product use prevalence. For the latter purpose, 60 studies conducted on four continents (no studies were performed in Africa) could be evaluated. Back-calculated WBE-derived mean nicotine consumptions were found to be in the range of 2000–3000 mg/d/1000 persons, with large variations. WBE-derived nicotine consumption data were mostly in fair agreement with the results of ‘classical’ methods such as surveys, (official) statistics, cigarette sales data and epidemiological studies. As reasons for deviations between WBE and classical methods, most frequently the use of illicit tobacco products, nicotine replacement therapy (NRT) products, electronic cigarettes (ECs),

reporting bias or outdated statistics were discussed by the study authors. Hitherto available long-term WBE studies covered time periods of 1–8 years showing mostly constant or decreasing time trends for nicotine consumption, depending on the country and time period investigated. The decrease is mostly interpreted as a consequence of lower smoking rates due to tobacco control activities. In a few studies, the minor tobacco alkaloid anabasine (AB) was measured in WW samples as a specific indicator for tobacco-containing products. The currently available results imply that AB in WW is decreasing faster over time than cotinine in WW does, suggesting that the use of tobacco products (mainly combustible cigarettes, CCs) is gradually replaced by tobacco-free nicotine products such as NRT products, ECs and others.

Taking up this approach, we conducted a Monte Carlo simulation (MCS) by which the time courses over 10 years of cotinine (indicator of the consumption of (any) nicotine product), AB (indicator of the consumption of (any) tobacco-product) and 2-cyanoethyl-mercaptopuric acid (2CyEMA, indicator of the exposure to the combustion product acrylonitrile, assumed to originate mainly from CC use) concentrations in WW samples collected at the inlet of a (hypothetical) WWTP serving a large city were modelled. The outcome of the MCSs for two simplified, but, in our view, not unrealistic scenarios revealed that time courses,

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in particular those of the WW concentration ratios AB/cotinine and 2CyEMA/cotinine, can provide useful information on the use habits for conventional (CCs and oral tobacco products) and smokeless nicotine products (ECs, HTPs, oral nicotine pouches, NRT products) in a population over time. These changes are the net result of transitions between user groups of the various nicotine/tobacco products and non-users and thus would allow to deduce the success (or failure) of tobacco harm reduction (THR) measures in a population.

From the presented evidence we conclude that WBE is a potential tool to monitor the progress of THR in populations, provided that suitable biomarkers are measured in WW samples collected over a time period of several years. [Contrib. Tob. Nicotine Res. 34 (2025) 59–83]

KEYWORDS

Wastewater-based epidemiology; new generation tobacco products; new generation nicotine products; combustible cigarettes; tobacco harm reduction; Monte Carlo simulation

ZUSAMMENFASSUNG

Die abwasserbasierte Epidemiologie (WBE) ist eine neuere Methode zur Bewertung der Exposition durch sowie des (bewussten) Gebrauchs von Chemikalien aller Art in bestimmten Bevölkerungsgruppen (Gemeinden) durch die Analyse geeigneter Biomarker in Abwasserproben, die üblicherweise am Eingang der Kläranlagen genommen werden, welche diesen Gemeinden dienen. Diese Methode wurde auch global angewandt, um den Konsum von koffein-, alkohol- und nikotinhalten Lifestyle-Produkten zu bestimmen. In dieser Arbeit werden die methodischen Grundsätze der WBE umrissen und die Ergebnisse einer systematischen Überprüfung von WBE-Studien vorgestellt, in denen Cotinin als Biomarker in Abwasserproben verwendet wird, um die Prävalenz des Konsums von Tabak- und Nikotinprodukten zu ermitteln. Für den letztgenannten Zweck konnten 60 Studien auf vier Kontinenten ausgewertet werden (in Afrika wurden keine Studien durchgeführt). Der rückgerechnete, von der WBE abgeleitete mittlere Nikotinkonsum lag im Bereich von 2000–3000 mg/d/1000 Personen, mit großen Schwankungen. Die von der WBE abgeleiteten Daten zum Nikotinkonsum stimmten größtenteils mit den Ergebnissen „klassischer“ Methoden wie Umfragen, (amtlichen) Statistiken, Zigarettenverkaufsdaten und epidemiologischen Studien überein. Als Gründe für Abweichungen der Ergebnisse zwischen WBE und klassischen Methoden wurden von den Autoren der Studien am häufigsten die Verwendung illegaler Tabakerzeugnisse, Nikotinersatztherapieprodukte (NRT), elektronische Zigaretten (ECs), Bias oder veraltete Statistiken genannt. Die bisher vorliegenden Langzeitstudien zur WBE, welche Zeiträume von 1–8 Jahren abdecken, zeigen je nach Land und untersuchtem Zeitraum meist konstante oder abnehmende Trends beim Nikotinkonsum. Der Rückgang wird meist als Folge der zurückgehenden Raucherzahlen aufgrund von Maßnahmen zur Eindämmung des Tabakkonsums interpretiert. In einigen wenigen Studien

wurde das Tabakalkaloid Anabasin (AB) in WW-Proben als spezifischer Indikator für tabakhaltige Produkte gemessen. Die derzeit vorliegenden Ergebnisse deuten darauf hin, dass AB im Abwasser im Laufe der Zeit schneller abnimmt, als Cotinin in WW, was darauf hindeutet, dass der Konsum von Tabakerzeugnissen (hauptsächlich von konventionellen Zigaretten, CCs) allmählich durch tabakfreie Nikotinprodukte wie NRT-Produkte, ECs und anderen ersetzt wird. Basierend auf diesem Ansatz haben wir eine Monte-Carlo-Simulation (MCS) durchgeführt, um die zeitliche Entwicklung der Konzentrationen von Cotinin (Indikator für den Konsum eines (beliebigen) Nikotinprodukts), AB (Indikator für den Konsum eines (beliebigen) Tabakprodukts) und 2-Cyanoethylmercaptursäure (2CyEMA, Indikator für die Exposition gegenüber dem Verbrennungsprodukt Acrylnitril, von dem angenommen wird, dass es hauptsächlich aus dem Konsum von Zigaretten stammt) in Abwasserproben, die am Eingang einer (hypothetischen) Kläranlage für eine Großstadt gesammelt wurden, über einen Zeitraum von 10 Jahren modelliert.

Die Ergebnisse der MCS für zwei vereinfachte, aber unseres Erachtens nicht unrealistische Szenarien zeigten, dass die Zeitverläufe, insbesondere die der Konzentrationsverhältnisse AB/Cotinin und 2CyEMA/Cotinin im Abwasser, nützliche Informationen über die Verwendungsgewohnheiten von CC liefern können. Diese Veränderungen sind das Ergebnis von Wechseln zwischen Gruppen von Nutzern der verschiedenen Nikotin-/Tabakprodukte und Nichtnutzern und würden somit Rückschlüsse auf den Erfolg (oder Misserfolg) von Maßnahmen zur Verringerung der schädlichen Auswirkungen des Tabakkonsums (THR) in einer Bevölkerung ermöglichen.

Aus den vorliegenden Erkenntnissen schließen wir, dass die WBE ein potenzielles Instrument zur Überwachung des Fortschritts der Tabakentwöhnung in der Bevölkerung ist, vorausgesetzt, dass geeignete Biomarker in Abwasserproben gemessen werden, die über einen Zeitraum von mehreren Jahren gesammelt wurden. [Contrib. Tob. Nicotine Res. 34 (2025) 59–83]

RESUME

L'épidémiologie basée sur les eaux usées (EBE) est une méthodologie récente qui permet d'évaluer l'exposition et l'utilisation (intentionnelle) de toutes sortes de produits chimiques dans des populations définies (communautés) par l'analyse de biomarqueurs appropriés dans des échantillons d'eaux usées, qui sont généralement collectés à l'entrée des stations d'épuration des eaux usées (STEP) desservant cette population. L'EBE a également été appliquée pour déterminer l'utilisation de produits de style de vie contenant de la caféine, de l'alcool et de la nicotine à l'échelle mondiale. Cet article expose les principes méthodologiques de l'EBE et présente les résultats d'un examen systématique des études de l'EBE qui utilisent la cotinine comme biomarqueur dans les échantillons d'eaux usées pour évaluer la prévalence de la consommation de produits à base de tabac ou de nicotine. À cette fin, 60 études menées sur quatre continents (aucune étude n'a été réalisée en Afrique) ont pu être évaluées. Les consommations moyennes de nicotine calculées à rebours par l'EMOH se

situent entre 2000 et 3000 mg/j/1000 personnes, avec d'importantes variations. Les données sur la consommation de nicotine dérivées de l'EMOH concordent généralement assez bien avec les résultats des méthodes « classiques » telles que les enquêtes, les statistiques (officielles), les données sur les ventes de cigarettes et les études épidémiologiques. Pour expliquer les écarts entre l'EMB et les méthodes classiques, les auteurs de l'étude ont le plus souvent évoqué l'utilisation de produits du tabac illicites, de produits de thérapie de remplacement de la nicotine (TRN), de cigarettes électroniques (CE), les biais de déclaration ou les statistiques obsolètes. Les études à long terme de l'EMB disponibles jusqu'à présent couvraient des périodes de 1 à 8 ans et montraient des tendances temporelles généralement constantes ou décroissantes pour la consommation de nicotine, en fonction du pays et de la période étudiée. Cette diminution est généralement interprétée comme une conséquence de la baisse des taux de tabagisme due aux activités de lutte antitabac. Dans quelques études, l'anabasine (AB), alcaloïde mineur du tabac, a été mesurée dans des échantillons de WW en tant qu'indicateur spécifique des produits contenant du tabac. Les résultats actuellement disponibles indiquent que l'anabasine dans les échantillons de WW diminue plus rapidement que la cotinine dans les échantillons de WW, ce qui suggère que l'utilisation de produits du tabac (principalement les cigarettes combustibles, CC) est progressivement remplacée par des produits à base de nicotine sans tabac tels que les produits NRT, les EC et d'autres produits.

Reprenant cette approche, nous avons effectué une simulation de Monte Carlo (MCS) en modélisant l'évolution sur 10 ans des concentrations de cotinine (indicateur de la consommation de (tout) produit à base de nicotine), d'AB (indicateur de la consommation de (tout) produit à base de tabac) et d'acide 2-cyanoéthyl-mercaptopurique (2CyEMA, indicateur de l'exposition au produit de combustion acrylonitrile, supposé provenir principalement de l'utilisation de CC) dans des échantillons d'eaux usées prélevés à l'entrée d'une STEP (hypothétique) desservant une grande ville. Les résultats des MCS pour deux scénarios simplifiés, mais, à notre avis, non irréalistes, ont révélé que les évolutions temporelles, en particulier celles des rapports de concentration WW AB/cotinine et 2CyEMA/cotinine, peuvent fournir des informations utiles sur les habitudes d'utilisation des produits du tabac conventionnels (CC et oraux) et des produits à base de nicotine sans combustion (EC, HTP, sachets de nicotine oraux, produits NRT) au sein d'une population au fil du temps. Ces changements sont le résultat net des transitions entre les groupes d'utilisateurs des différents produits du tabac et de la nicotine et les non-utilisateurs et permettraient donc de déduire le succès (ou l'échec) des mesures de réduction des méfaits du tabac (RDT) dans une population.

Sur la base des éléments présentés, nous concluons que l'EBM est un outil potentiel pour suivre les progrès de la THR dans les populations, à condition que des biomarqueurs appropriés soient mesurés dans des échantillons de WW collectés sur une période de plusieurs années. [Contrib. Tob. Nicotine Res. 34 (2025) 59–83]

1. INTRODUCTION

Wastewater-based epidemiology (WBE) has been introduced in 2001 by DAUGHTON (1), who proposed the analysis of chemicals in municipal sewage as a tool for assessing the use and abuse of life-style products and illicit drugs as well as the exposure to environmental pollutants. Over the past 20 years, WBE found numerous applications including the use of illicit (2–4) and licit drugs (pharmaceuticals) (5–11), personal care and household products (12, 13), life-style products containing caffeine (14–21), alcohol (15, 16, 22–48) and nicotine (15, 16, 18, 20–23, 25, 27, 29–31, 33–36, 39, 42, 43, 47, 49–64), as well as the exposure to environmental pollutants (65–68). Furthermore, WBE turned out to be a suitable tool for the almost real-time assessment of the incidence and prevalence of human infections with bacteria and viruses such as SARS-CoV-2 (69–83), influenza virus (84), noro virus (85) and others (86). In particular, WBE has undergone dramatic advancement in the course of the COVID-19 pandemic (87, 88). There are also further, less widespread WBE approaches: In a systematic review (89), 64 proteins, including calprotectin, uromodulin, CRP (C-reactive protein) and CC16 (club cell-secretory protein 16) were named, which could serve as promising biomarkers of disease in WBE studies, owing to their relatively high concentrations in urine and stool. It is interesting to note that the biological effect marker for oxidative stress PGF2 α and the smoking-related exposure marker 3'-*trans*-hydroxycotinine (OH-Cot) in wastewater were reported to be strongly correlated (55). In another study with the 'stress markers' 8-hydroxydesoxyguanosine (8-OHdG), 3-hydroxypropyl-mercaptopuric acid (3-HPMA), di-, nitro-, chloro- and bromotyrosine in wastewater, no consistent results were obtained, either due to instability in wastewater reactors (3-HPMA), no detection (8-OHdG) or implausibly high levels (chloro-tyrosine) (90). The F2-isoprostanes (used as oxidative stress markers) PGF2 α , dnPGF2 α and PGE2 α were reported to be stable in WW (91, 92) and would, therefore, be potential effect markers for smoking.

The objective of this paper is to:

- (i) briefly describe the principles of WBE including the advantages and limitations compared to other methodologies, particularly for assessment of life-style product use such as alcohol, caffeine and tobacco/nicotine;
- (ii) present the results of a systematic review on the application of WBE to the use of tobacco/nicotine products;
- (iii) provide a perspective view on the potential application of WBE for monitoring the progress of tobacco harm reduction (THR) by differentiating the use of conventional tobacco products (in particular combustible cigarettes, CC) and new generation tobacco/nicotine products such as electronic cigarettes (ECs), heated tobacco products (HTPs) and oral nicotine pouches (ONPs);
- (iv) present results of Monte Carlo simulations (MCSs) for two scenarios of tobacco/nicotine product use in a (hypothetical) large city in order to demonstrate the capabilities and limitations of WBE for the intended purpose.

2. THE PRINCIPLES OF WASTEWATER-BASED EPIDEMIOLOGY (WBE)

2.1 Working steps in WBE application for use of life-style products

In the following, the major working steps necessary for applying WBE for the assessment of life-style product use, exemplarily shown for products containing alcohol, caffeine and nicotine are briefly described (8).

2.1.1 Biomarker (BM) selection

The selection of suitable BMs in wastewater (WW) is of fundamental importance for successful WBE applications. For instance, alcohol consumption can be best assessed with the minor metabolite ethyl sulfate (ES) in WW. Ethyl glucuronide (EG), despite occurring in higher amounts in urine than ES, was found to be unsuitable, because it is split in WW by fecal glucuronidases (23, 30, 45). ES degradation under WW sampling conditions was reported to be 8%/h (30). For application of ES in WBE, a urinary excretion rate (UER) of ~0.05% is usually assumed with a half-life of several hours (93, 94).

Of the 20 caffeine metabolites, paraxanthine (1,7-dimethyl-xanthine) was found to be suitable for the application of WBE to caffeine use (16, 17, 19, 95, 96). Stability was reported to be sufficient (17, 97) for paraxanthine, which is the major caffeine metabolite (representing 70–80% of which a main part (~50%) is excreted into urine resulting in a UER of 35–40%) (98, 99).

Cotinine (Cot) is a major metabolite of nicotine, absorbed by various routes upon use of tobacco and nicotine products (100). However, in many WBE studies, nicotine consumption is assessed by measuring 3'-trans-hydroxycotinine (OH-Cot) in addition to or instead of Cot (16, 18, 23, 25, 27, 36, 42, 43, 49, 50, 52–54, 56, 59, 62–64, 95, 96, 98, 101). For the purpose of differentiating the use of tobacco-containing (e.g., CC, oral tobacco) and tobacco-free nicotine (oral nicotine pouches (ONPs), nicotine replacement therapy (NRT) products), anabasine (AB) is used as a BM for tobacco exposure (52, 57, 59, 62–64, 101, 102). Cot shows the highest stability, whereas OH-Cot and AB proved sufficiently stable for WBE studies (23, 30, 62). An UER of about 30% can be assumed for Cot, comprising the free and conjugated forms (103, 104). This percentage considers that the glucuronides of cotinine and OH-Cot are not stable under WW sampling conditions and only free Cot is present in WW samples (49). Fecal β -glucuronidase is assumed to split these conjugates (50). Also the molar sum of Cot and OH-Cot in WW was used as BM for nicotine uptake. In this case an excretion rate of 74% was most frequently applied for back-calculations (50).

2.1.2 WW sample collection (time, place, type of WW samples)

WBE has the potential to provide spatial and temporal data on substance use and exposure (24, 36). Therefore, careful selection of time and place of WW sampling is important, particularly with regard to the study aim. Also, detailed information on the eligible wastewater treatment plants

(WWTPs) is necessary, in order to select the optimal sampling points and conditions (2). For studying the use of life-style products, it is of significance to deliberately select the year, season and month of WW sample collection (16, 105). Especially the choice of workdays or weekend days, holidays, days of special events (e.g., music festivals, etc.) are of importance (26).

As suitable sampling places, country, city, community, special areas (sub-sewersheds) or buildings (e.g., university campus (58)) can be considered, depending on the problem to be investigated.

Various types of WW samples can be collected. Most suitable are representative 24h-composite WW samples at the WWTP inlet (before any WW treatment takes place). Sampling can conveniently be conducted by means of auto-sampler devices (2, 106–108). Also passive sampling (using microporous polyethylene passive samplers) was found to be a promising, simple and cheap method for WW sample collection (109).

2.1.3 WW sample preparation and analysis

Analytical chemistry is regarded as the fundamental driver in the WBE field (108). Determinations of biomarkers (BM) in WW is very similar to that of BM analysis in urine with respect to sample preparation (liquid-liquid extraction (LLE), solid-phase extraction (SPE) or direct injection) and instrumentation (most frequently LC-MS/MS is used) (110–112). As mentioned above, due to fecal glucuronidase activity, BMs usually do not occur as glucuronides. Since concentrations of (for example) cotinine in WW compared to those in urine of smokers are much lower (by approximately a factor of 100 to 1000), analysis of WW samples is more challenging than that of urine from (exposed) people. Enantiomer-selective analysis can improve the specificity for human excretion data (3, 29, 96, 108). For special purposes, also untargeted analysis of WW samples can be of interest (96).

2.1.4 Data evaluation and interpretation

WW analysis accrues data in form of concentrations of BMs (usually of metabolites of the consumed compounds of interest, in addition possibly also of the markers for population size estimates). Back-calculation of these raw data to population-based consumption/exposure results would allow comparisons with other (geographical) places and time points. Information on the size of the wastewater catchment population (the population covered by the WWTP, where WW sampling took place) is crucial for WW-based results (113). General formulae for back-calculations are presented in the following (2, 3, 114):

Daily mass load of biomarker (DML, mg/d):

$$DML = C \times F \times SF$$

with:

C = Concentration of BM (mg/L);

F = Flow rate of WW (L/d);

SF = Stability correction factor

(to account for possible instabilities of the BM)

Drug consumption (DC, mg/d) of the total catchment population:

$$DC = \frac{DML \times MW_{par}}{UER \times MW_{met}} = DML \times CF$$

with:

UER = Urinary excretion rate (%),

MW_{par} = Molecular weight of parent chemical (mg/mmol)

MW_{met} = Molecular weight of metabolite (mg/mmol)

The term MW_{par}/MW_{met} × UER is frequently expressed as correction factor CF.

Population-normalized daily consumption (PNC, mg/d/1000 persons):

$$PNC = \frac{DC}{P_{norm}} \times 1000$$

with:

P_{norm} = population size used for normalization (e.g., the catchment population of the WWTP)

Units (of a product) consumed (UC, e.g., cig/d):

$$UC = \frac{DC}{AC}$$

with:

AC = Average content in product (e.g., mg nicotine/cig; more precisely would be to take the average amount of nicotine absorbed per cigarette)

Estimate for P_{norm} (using a suitable BM for estimating the population size):

$$P_{norm} = \frac{DML(PE)}{PML}$$

with:

PML = Personal daily excretion (mg/d/person);

DML (PE) = daily mass load of a BM used for estimating the population size, such as 5-HIAA (5-hydroxy-indol acetic acid), NH₄-N, etc. (113, 115–117).

The usual data sources for tobacco consumption in larger populations are for example tax excise clearances, sales data, and self-report surveys (53). WBE data can be a complementary source for assessing the consumption of nicotine, alcohol or caffeine containing products. Agreement in consumption data between WBE and the conventional information sources was frequently used to show the suitability of WBE for this purpose (16, 26, 37, 43, 48, 50). Deviations between WBE and conventional methodologies can have plausible reasons (e.g., under-reporting, contraband, launch of new products, etc.). This issue will be discussed in more detail for tobacco/nicotine products in the section “Comparison of WBE results with those of ‘classical’ methods”.

With respect to the market introduction of new generation tobacco/nicotine products (NGPs), temporal trends in WBE data could be of particular interest. As mentioned earlier in this section, use of anabasine as a tobacco-specific and cotinine as a nicotine-specific biomarker in WW samples would allow the differentiation of various products (e.g., CCs and ECs) and, as a consequence, the actual use of these products in the population. This topic will be discussed in more depth in the section “Results and discussion of the WBE Review”.

2.2 Advantages, disadvantages and limitations of WBE

WBE is an innovative approach that uses the analysis of WW to gather epidemiological information about a population’s exposure, health status and behavior. This method is based on the principle that many chemicals, which people consume or are exposed to are excreted in urine and feces, which ultimately end up in the sewage system (88, 118). The core concept of WBE involves collecting and analyzing wastewater (WW) samples at the inlet of WW treatment plants (WWTPs) or other strategic points in the sewage network. These samples contain a complex mixture of human metabolic products, including biomarkers that reflect various aspects of population health, lifestyle habits, and exposure to environmental stressors (118).

WBE offers several advantages over traditional epidemiological methods, including:

- Near real-time data: WBE can provide rapid insights into population health trends, allowing for timely interventions (87, 89). WBE can thus serve as early warning system in pandemic events such as Covid-19 but also in the use of new drugs (119–121) and life-style products such as caffeine, alcohol and tobacco/nicotine.
- Comprehensive coverage: WBE captures information from the entire population connected to the sewage system, including asymptomatic and unexposed individuals (87, 95). On the other hand, this circumstance leads to a ‘dilution’ of the biomarker concentrations in WW samples, which can lead to analytical challenges.
- Ethical aspects and data protection: The method does not identify specific individuals, avoiding ethical concerns related to personal data and data protection (67, 122, 123).
- Cost-effectiveness: WBE is generally less expensive than large-scale individual testing programs (16, 28, 124). It was estimated that there is a large cost difference per person of 0.58 \$ versus 127 \$ between WBE and conventional epidemiological studies, respectively (16).

Despite its potential, WBE also faces some obvious challenges, including:

- Identification of suitable markers to be measured in wastewater samples: In general, human metabolites of the compounds of interest are of higher priority than the parent compounds, thus limiting the contribution of sources other than human excretion to the measurable wastewater concentrations (11, 96). Suitability of the biomarker also requires knowledge of the human urinary and fecal excretion pharmacokinetics (particularly the urinary excretion rate, UER) of the chemical for a reliable back-calculation of the consumption/exposure in the covered population (47).

- Of key importance for the application of a biomarker in WBE is its stability under the wastewater collection conditions (125–128). Moderate degradation rates (< 50% in 12 h) need to be considered in the back-calculation process (30, 62, 97, 126, 127, 129, 130). BMs with higher degradation rates (> 50%/12 h) must be regarded as unsuitable for application in WBE.
- Potential *in situ* biotransformation by microorganism in WW must be considered for BMs to be applied in WBE (131). Also photochemical transformation of nicotine in WW to the metabolites cotinine (up to 3%) and OH-Cot (up to 5%) is discussed to be of relevance (132).
- Estimates of the population size covered by the collected WW samples (of relevance for the back-calculations): There are various possibilities to estimate the population size to be used to normalize analyte concentrations in WW samples (78, 104, 114, 133). These include hydrochemical parameters in WW (e.g., chemical oxygen demand (COD), biochemical oxygen demand (BOD)), utility consumption (e.g., drinking water, electricity), exogenous biomarkers (e.g., 4-pyridoxic acid, caffeine), endogenous biomarkers (e.g., creatinine, coprostanol (a cholesterol metabolite), 5-HIAA (5-hydroxyindol acetic acid, a serotonin metabolite), ammonium nitrogen excreted (NH₄-N), total phosphorous excreted (P), total nitrogen excreted (N), signaling records (e.g., mobile phone data). 5-HIAA, caffeine, and artificial sweeteners were reported to be potential candidates (78), whereas creatinine appears to be not sufficiently stable for this application (51, 78).
- Uncertainties of WBE data include variations contributed by population size estimates, flow rates (F) of inlet WW, stability of the selected biomarkers, sampling strategy, back-calculations (including UER of biomarkers), and analytical measurements (47, 54, 134, 135).

3. LITERATURE REVIEW OF WBE-DERIVED DATA ON TOBACCO AND NICOTINE PRODUCT USE

3.1 Methods

A systematic review was conducted according to the guidelines of PRISMA (Preferred Reporting Items for Systemic reviews and Meta-Analysis) (136).

The online literature databases PubMed, Web of Science (WoS) and Google Scholar were searched with the term (“wastewater-based epidemiology”) AND (“tobacco” OR “cigarettes” OR “nicotine”). The number of hits were 95, 93, 32 in the three online libraries, respectively. After removing 61 duplicates, 159 articles remained, of which the titles and abstracts were screened for meeting the inclusion and exclusion criteria.

Inclusion criteria were:

- Original WBE studies on use of tobacco products (combustible cigarettes (CCs), oral tobacco, any conventional tobacco products) and nicotine products (e-cigarettes (ECs), heated tobacco products (HTPs), tobacco-free oral nicotine pouches (ONPs), nicotine replacement therapy (NRT) products (nicotine gums, patches, inhalers)
- Using Cot and/or OH-Cot as WW biomarker (BM) for tobacco or nicotine product use

- Clear information on place (country, city, community) and time (year, months, work-/holiday, weekend, days with special events) and catchment population
- Provision of “Population-normalized nicotine consumption” (PNC, expressed as mg/d/1000 persons) or something equivalent
- Sufficient information on back-calculation (flow rate, population normalization, excretion rates for BMs used)
- 95% Confidence intervals (CI) for results (or something which can be converted to CI)

Exclusion criteria were:

- Reviews, editorials, commentaries
 - No typical WBE approach followed or only partly followed (see chapter on WBE working steps)
 - Studies with insufficient information on time, place and population covered by the WW samples analyzed
 - Not either Cot or OH-Cot used as BM
 - Back-calculation not traceable
 - No usable value for the variation of the results provided
- Application of these inclusion and exclusion criteria resulted in 60 articles for evaluation in the systematic review (Figure 1).

3.2 Results and discussion of the WBE review

In total 60 WBE studies, which used Cot and/or OH-Cot in WW to quantitate the use of tobacco and nicotine products in certain populations were identified ([Supplementary Table S1](#)). The studies were performed between 2009 and 2023 on all continents, except Africa. The investigations summarized in Table S1 served a variety of different purposes related to smoking and tobacco/nicotine product use, such as:

- Determine the population-normalized consumption (PNC) of nicotine by using tobacco and nicotine products in various populations and subpopulations
- Discover the long-term time trends (over years) of the habit (18, 25, 31, 53, 59, 63, 102, 137, 138)
- Find out the influence of special events (e.g., weekends (many studies in Table S1), seasonal effects (22, 33, 35, 56, 63, 139–142), holidays and major events (21, 39, 140), festivals (143), Ramadan (34), COVID-19 restrictions (29, 138, 141, 144–146)) on tobacco/nicotine product use
- Compare WBE-derived nicotine consumption data with those derived by ‘classical’ methods (surveys, sales and tax records, governmental and WHO statistics, epidemiological studies) (15, 16, 18, 27, 29, 31, 35, 36, 42, 43, 50, 53, 54, 56, 58, 59, 63, 64, 138, 140, 142–144, 147–150)
- Differentiate between tobacco-containing and tobacco-free nicotine products (e.g., by using anabasine (AB) or anatabine (AT) in WW in addition to Cot/OH-Cot) (57, 62–64, 66, 101, 102)
- Investigate biomarkers of biological effects (BOBEs, e.g., on oxidative stress) in order to assess the general health status of the covered population (55)
- Investigate methodological aspects of WBE such as stability of biomarkers (52, 57, 62), WW sampling methods (109), suitability of markers in WW for estimating the population size served by a WWTP (96, 115, 116, 139, 143, 144, 148, 149, 151–153)

In the following sections, the general outcome on the various aspects listed above are summarized.

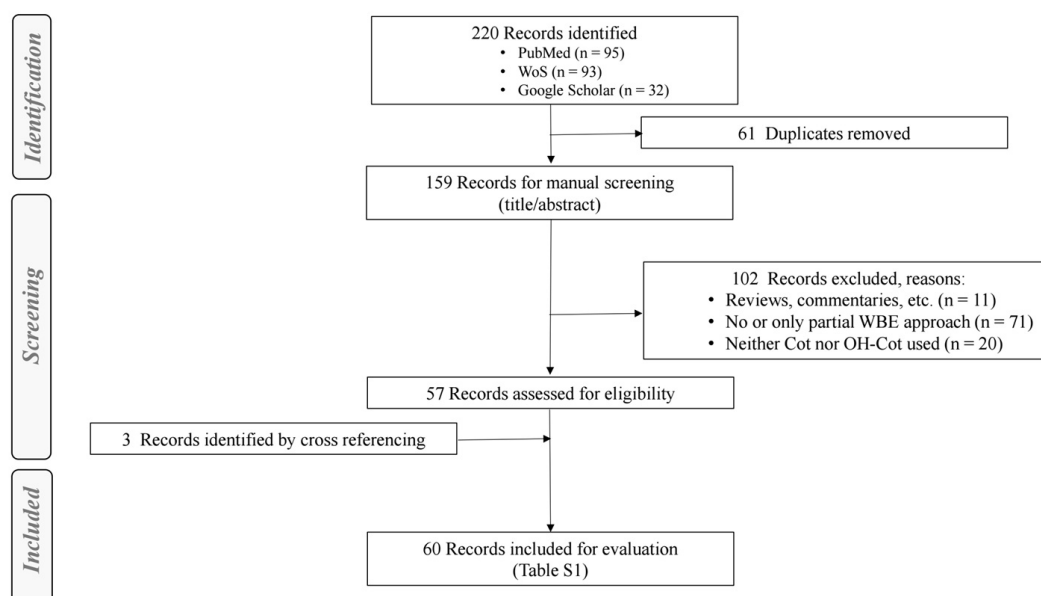


Figure 1. Flow chart on identification, exclusion and inclusion of studies considered in this systematic review on WBE studies of tobacco/nicotine product use according to the PRISMA guidelines (136).

3.2.1 Population-normalized consumption (PNC) of nicotine

An established biomarker in WW for the consumption of nicotine-containing products is cotinine (Cot), which was a major inclusion criterium for the studies in Table S1. In some studies, additionally 3'-*trans*-hydroxycotinine (OH-Cot) or the parent compound nicotine (Nic) was determined in WW samples. If possible, the Cot-based nicotine consumption data were evaluated. In some cases, only consumption data based on the molar sum (Cot + OH-Cot) were reported and used in this evaluation. Nic in WW was not included in our evaluation, due to the fact that also non-consumed but disposed nicotine (cigarette butts, ashes) can considerably contribute to the Nic measurable in WW (27, 96). If PNC values reported in the publication were not normalized to the general population (including all age groups), values were adjusted appropriately. Adjustments were also carried out, if the applied urinary excretion rates (UER) for Cot and Cot + OH-Cot substantially deviated from the established values of 30 and 74%, respectively (50). The evaluation in this section is based on WW data sets which can comprise results from WW samples of one sampling point (usually a WWTP) at different time-points or results from WW samples derived from different sampling points at one point in time.

Results by continent of PNC of nicotine (expressed in mg/d/1000 persons) are summarized in Table 1 and Figure 2. PNC data for nicotine were obtained from 170 data sets (Table 1) obtained from 60 studies in 4 continents (no data from Africa were available). Most studies were performed in Europe (N = 107 WW data sets) with investigations in 17 different countries (most frequently in Belgium (5 data sets), Italy (4) and Turkey (4), followed by Australia (10)/NZ (2), Asia (with 9 data sets from China) and America (USA (7) and Brazil (1)). The means for nicotine PNCs for the 4 continents were found to be in a relative narrow range of 2000–3000 mg/d/1000 p. However, the variability within continents, as can be deduced from the

min–max ranges, 95 %-CIs and SDs were large. This has to be presumed primarily as an indication of the diversity of the investigated populations, since data known to be influenced by factors such as COVID-19 restrictions, data from WWTPs serving a university campus, special events were excluded from this evaluation. By no means, the presented data are representative for a continent or a country, but should first of all show that WBE for use of nicotine-containing products has been successfully applied on a global basis with comparable results, which mainly depend on the population served by the WWTP from which the WW samples were derived. The following sections provide further support for this statement.

Table 1. Population-normalized consumption of nicotine (PNC, mg/d/1000 persons) based on Cot or Cot + OH-Cot levels in WW reported in 170 data sets from 60 publications.

	All	Europe	America	Asia	Australia/ New Zealand
N (data sets)	170	107	18	20	25
Mean	2769	2947	2543	2028	2763
SD	1866	1428	1112	1543	3480
Median	2394	2650	2673	1442	1800
5% Percentile	807	1227	189	564	571
95% Percentile	5633	5673	3940	4262	8588
Min	86	669	162	440	86
Max	17000	7056	4433	7034	17000

3.2.2 Long-term trends of nicotine product use assessed by WBE

In Table 2, results of WBE studies on nicotine consumption covering a time period of at least one year are summarized. Four of the nine studies were conducted in Australia (also reflecting the fact that a very active WBE research group is working in this country).

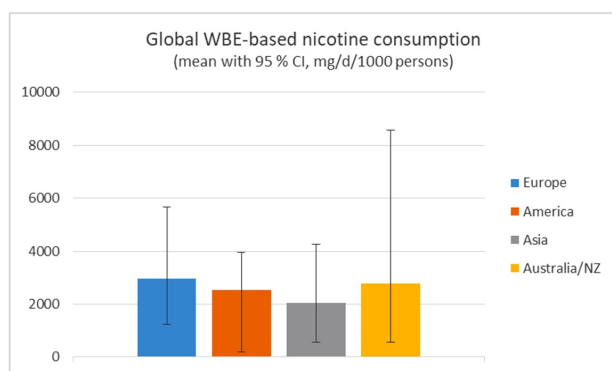


Figure 2. Mean WBE-derived nicotine consumption in 4 continents. Error bars represent the variation between 5% and 95% quantiles.

The other studies were performed in China (2), Italy (1), Spain (1) and Lithuania (1). The studies starting at about 2010 showed consistently a population-normalized decrease in nicotine consumption until about 2018 (18, 53, 59, 63, 102) or remained at a stable level (31). After about 2018, WW-derived nicotine consumption was stable or increased (25, 59, 138). As indicated in Table 2, the study authors attribute different reasons for the decrease. Mainly a decline in cigarette smoking as a result of tobacco control policies is mentioned. Use of NRT products and new generation tobacco/nicotine products (NGPs), particularly ECs, as a replacement source for nicotine in WW were suggested to play a role only after 2018 (25, 59, 138). The decrease in the consumption of tobacco-containing products (mainly CCs) over time could convincingly be demonstrated by the application of AB analysis in WW, which showed a time pattern different from Cot (59, 63). Time courses in WW-based nicotine consumption were predominantly in agreement with tobacco (cigarette) sales and other survey data. Deviations, however, were observed for the absolute consumption data, which were commonly higher in the WW-derived results. As possible reasons, the authors discussed use of illicit tobacco products, too low self-reported consumption, and use of tobacco-free nicotine products (particularly NRTs and ECs).

3.2.3 Influence of special events on WBE-derived nicotine consumption

In many WBE studies on nicotine/tobacco consumption, influence of weekends (Saturday, Sunday) compared to common workdays (Monday to Friday) were investigated (Table S1). The results predominantly show that nicotine consumption is hardly different between workdays and weekends (22, 25, 32, 33, 35, 38, 42, 43, 50, 63, 76, 116, 147, 150). Two studies (11, 16) reported significantly lower nicotine consumptions on weekends: one investigated a university campus, which also found lower alcohol but stable caffeine consumption on weekends (16); the other study mentioned work-related commuting as a reason for lower levels on weekends (11). In contrast to nicotine, more alcohol was found to be consumed on weekends in some studies (42, 43, 63, 139, 144).

Also events such as (religious) holidays, tourist seasons and

festivals were reported to have no major influence on WW-based nicotine consumption (21, 39, 140). Increased nicotine consumption was found during a rock festival (143). A significant decrease in WW Cot levels were reported during Ramadan, as was the case for ethyl sulfate (ES), a WW biomarker for alcohol consumption (34).

Several WBE reports deal with nicotine consumption during COVID-19 restrictions (29, 138, 141, 144–146). Three studies reported no or only minor impact of restrictions or lockdowns during the pandemic (29, 141, 144), two investigations found a decrease (145, 146). In one study (138) an increase in nicotine consumption was reported.

The findings presented in this section convincingly show that WBE data reflect the use of life-style and other products in almost real-time. Contrary to expectations, smoking is mostly not influenced by special events, in contrast to alcohol consumption.

3.2.4 Comparison of WBE results with those of 'classical' methods

Since WBE is a relatively young method for assessing changes in habits, exposures and health status in populations, for validation reasons many studies compare the WBE-derived results with those from 'classical' methods such as surveys, epidemiological studies, official statistics as well as sales and tax data (Table S1). The outcome of these comparisons can be of interest, not only when WBE and 'classical' approaches were in agreement, but also if deviations are consistent and have plausible and accountable reasons (e.g., see Table 2). Comparisons between WBE-derived and survey/sales data of three studies (59, 63, 138) are already presented in Table 2. Most of the studies investigating this aspect show a fair accordance between WBE and 'classical' methods in terms of daily cigarette consumption or percentage of smokers in the population (18, 27, 31, 36, 42, 50, 54, 58, 140, 142–144, 147–150). In a couple of studies WW cotinine-based tobacco (mostly cigarette) consumption was found to be higher than observed in surveys or deduced from sales data (15, 27, 35, 53, 64). The most frequently discussed reasons for this discrepancy were other nicotine source (other than cigarettes, such as nicotine replacement therapy (NRT) products, snus, new generation tobacco/nicotine products (NGPs)), use of illicit tobacco products or too low self-reports of the consumption. One study reported a better agreement of tobacco sales data with WW anabasine (AB)-based (only 5% higher) than WW cotinine-based consumptions (2–28)% higher) (64). A few investigations found lower WBE-derived consumption levels than those derived from surveys or sales data (16, 27, 56). The discussed reasons for these deviations were less obvious and mostly explained with demographic differences between the wastewater treatment plant (WWTP) catchment population and that used for the 'classical' methods.

In general, there are limitations in the comparisons of the results derived by WBE and classical methods. For instance, cigarette consumption was based on tax data in part of the studies or on retail sales data in other studies. A serious bias for consumption could result from products purchased abroad. This is of particular importance for border regions.

Table 2. Studies investigating long-term time trends for WBE-assessed tobacco/nicotine consumption.

Study	Country	Period (years)	Time trends in nicotine consumption observed	Comparison to sales/surveys	Interpretation by authors
MACKIE <i>et al.</i> 2019 (53)	Australia	2010–2017 (7 years)	- Decrease by 25% - ~3% decrease per year	- Similar decrease in sales, but at lower level (reasons, see right cell)	- Self-reports too low - Illicit tobacco use incompletely assessed - Other Nic sources (NRT, EC) unimportant
BADE <i>et al.</i> 2020 (102)	Australia	2015–2019 (4 years)	- Cot decreased by 3.3% - AB decreased by 30%	- None reported	- Reason for difference between change in Cot and AB: Increased use of NRT - EC played no role in Australia at that time
GARCIA-LOR <i>et al.</i> 2020 (18)	Italy	2013–2015 (2 years)	- Decrease in North and South Italy - Unchanged in Central Italy	- In fair agreement with survey data on cigarette consumption	- Success of tobacco control policies - Other Nic sources (NRT, EC, snus) considered to be insignificant
ZHENG <i>et al.</i> 2020 (63)	China	2012–2017 (5 years)	- Decrease of Cot in WW by 2.1 % per year - AB decreased by 3.0 % per year	- Tobacco tax and survey data: decreased by ~4%	- Faster decrease in tobacco than in nicotine consumption: other Nic sources play a role - Market share of Nic patches increased from 0.47 to 0.95%
GAO <i>et al.</i> 2020 (31)	China	2014–2016 (2 years)	- Cotinine relatively consistent over time	- In agreement with WHO statistics, surveys and sales data for CC	- NRT and EC market shares were negligible in China at that time
BOOGAERTS <i>et al.</i> 2021 (25)	Lithuania	2018–2019 (1 year)	- Stable in Lithuania (whole country) - Unexplained decrease of 25% in the city of Kaunas	- Cig sales over time also stable but by 35% lower (various reasons, see right cell)	- Outdated sales data (from 2016) - Other Nic sources play a role (NRT, EC, pipes) - Illegal tobacco products in circulation - Disposed cig butts (chemical conversion to Cot and OH-Cot)
CAMPO <i>et al.</i> 2023 (137)	Spain	2018–2020 (2 years)*	- Decrease by 65% (!) during the study period	* Contradictory statements for study period: 2014–2017 in the text	- Unfortunately, the authors gave no explanation or interpretation of their results
THAI <i>et al.</i> 2023 (138)	Australia	2017–2020 (3 years)	- Decrease 2017-2019: 5%/a (slope: -0.035) - Increase 2019-2020	- Stronger decrease in sales (slope: -0.12)	- Increase in NRT use 2017–2020 (estimated contribution to Nic consumption: < 10%) - EC use of less importance (prohibited in Australia)
WANG <i>et al.</i> 2024 (59)	Australia	2013–2021 (8 years)	- First (until 2018) decrease (-18%), then increase (+40%) - AB: First strong (-21%), then slight (-10%) decrease	- Tobacco sales show similar pattern to AB, but at lower consumption levels	- Illicit tobacco use suggested - Non-tobacco nicotine use (NRT, ECs, etc.) suggested to increase after 2018

3.2.5 Application of additional biomarkers in WW for characterizing the nicotine products used

The application of Cot and OH-Cot in WW can assess only the use of nicotine-containing products in populations, but not differentiate between tobacco-containing products such as combustible cigarettes (CCs), pipes, cigars, oral tobacco and tobacco-free nicotine products such as NRT products (nicotine gum, patch, inhaler), electronic cigarettes (ECs), oral nicotine patches. As mentioned earlier in this paper the differentiation between tobacco-containing and tobacco-free products is possible by measuring also anabasine (AB) and anatabine (AT) in WW. This has been done for a number of studies (52, 57, 59, 62–64, 101, 102), which are briefly summarized in Table S1 and Table 2. AB and AT concentrations in WW are about 500–1000-fold lower than those of Cot and OH-Cot. AB and AT were found to be less stable in WW reactors than Cot and OH-Cot (62). AB is preferred to AT as a biomarker in WW due to its lower variability in tobacco products (64, 102). Beyond that, AB showed similar per person load in pooled urine and WW

(adjusted appropriately), whereas AT load in WW was by 50% higher (62) than expected. The authors alleged AT occurrence in food (tomatoes, eggplants, peppers) as reason for the latter observation (62). In general, the AB levels in WW, particularly the AB/Cot ratios, were found to be a well-suited indicator for the use of tobacco products in contrast to Cot and OH-Cot alone, which reflect the overall nicotine consumption by use of any nicotine-containing product. Reported levels of AB and AT in cigarette tobacco are in the range of 100–200 and 900–1400 µg/g, respectively (154, 155). Tobacco contents of NAB (*N'*-nitrosoanabasine), NAT (*N'*-nitrosoanatabine) and NNK (4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone) were found to be in the ranges 15–23, 114–145 and 80–960 ng/g, respectively (156, 157). Additionally, the *N'*-nitrosamines NAB and NAT may be formed by nitrosation from the respective precursors AB and AT in wastewater under acidic conditions. The issue of tobacco (instead of human urine) as source of AB, AT, NAB, NAT and NNK in wastewater has not yet been investigated in detail and would require further research. The amounts of these compounds in tobacco

compared to those taken up and excreted in urine of users of combustible cigarettes (CC) and oral tobacco suggest that a potential contribution of tobacco to the WW levels of AB, AT, NAB and NAT cannot be ignored.

The tobacco-specific nitrosamines (TSNAs), more specifically their urinary metabolites NAB, NAT, NNN, NNK and NNAL in WW have been addressed in two studies (52, 101). In one study (52), all compounds except for NAT were found to be < LOD. In the other study (101), NNK exposure in four populations in Greece, Switzerland and Belgium were reported to range from 0.5 to 1.5 mg/d/1000 persons, which appears uncommonly high, particularly when considering the authors' explanation for this observation: third-hand (!) exposure to NNK.

Matter of particular interest would be the differentiation between the use of combustible tobacco products (above all CCs) and NGPs (mainly ECs, HTPs and ONPs), which are all non-combustible nicotine products. In a recent review of ours, it was shown that urinary metabolites of tobacco combustion chemicals such as acrolein (3-HPMA), crotonaldehyde (HMBMA), acrylonitrile (2CyEMA), acrylamide (AAMA or GAMA), 1,3-butadiene (MHBMA) and a number of others are suitable BMs to distinguish combustible from non-combustible tobacco/nicotine products (158). This has been confirmed in a controlled clinical study with users and non-users of these products (159, 160). In this context, a recent publication by KUMAR *et al.* (66) is of interest, which applied WBE to quantify the exposure to volatile organic chemicals (VOCs) such as acrolein, crotonaldehyde, acrylonitrile, acrylamide, 1,3-butadiene and several others in two U.S. communities differing in proximity to manufacturing facilities. As biomarkers in WW, predominantly mercapturic acids were used, mostly in agreement with those employed in the investigation mentioned above (159). Although the focus of the KUMAR *et al.* study (66) was exposure of the general population to VOCs emitted by factories, the application to smoking-derived VOCs appears possible and of supreme interest for differentiating the use of combustible and non-combustible tobacco/nicotine products. Of course, the suitability of the mercapturic acids of interest as biomarkers in WW, particularly their stability under WBE conditions, have to be validated prior to application for the intended purpose. A successful distinction between these two product groups by WBE methodology would allow to monitor the progress of tobacco harm reduction (THR) in populations by selecting suitable WWTPs for WW sample collection. This subject will be covered in more detail in the next section.

4. CAN THE PROGRESS OF TOBACCO HARM REDUCTION (THR) BE MONITORED BY WBE?

4.1 General considerations

Apart from smoking cessation and the prevention of adolescents to start with the habit, switching to risk-reduced (non-combustible) tobacco/nicotine products is a third way in tobacco harm reduction (THR), gaining increased acceptance in the health community (161–163), but still finds the WHO reluctant in actively integrating this part of THR in its tobacco control program (164). Be that as it

may, an objective, rapid and low-cost methodology for assessing the progress or failure of THR in selected populations over time would be of great value for public health decision makers. The facts presented in this paper so far suggest that WBE could be a potential tool for this purpose. In this section, a model assuming prevalences of tobacco/nicotine product use and cessation rates with subsequent Monte Carlo simulation (MCS) is applied for predicting concentrations of three biomarkers in WW (namely cotinine (Cot), anabasine (AB) and 2-cyanoethyl-mercapturic acid (2CyEMA, urinary metabolite of acrylonitrile) collected at the inlet of a hypothetical WWTP serving a (hypothetical) large city (1.5 million inhabitants). The results should further elucidate, whether and under which conditions WBE could be applied for monitoring the success or failure of THR.

4.2 Monte Carlo simulation (MCS): methodological approach

MCS is a computational technique that uses repeated random sampling to model the probability of different outcomes in systems influenced by uncertainty. MCS can help to predict potential scenarios by averaging multiple results from varying inputs. The method focusses on randomness to provide probabilistic forecasts (165, 166). The applied conditions and assumptions together with the underlying rationales for the MCSs simulations conducted are summarized in Table 3. Scenario 1 of the approach considers 7 groups in a hypothetical population of 1.5 million covered by a (hypothetical) WWTP:

- 1) non-users (NUs), not using any tobacco/nicotine product
- 2) smokers of combustible cigarettes (CCs)
- 3) users of e-cigarettes (ECs)
- 4) users of heated tobacco products (HTPs)
- 5) users of oral (tobacco-free) nicotine pouches (ONPs)
- 6) users of nicotine replacement therapy products (NRTs)
- 7) users of oral tobacco (OTs). In Scenario 2, an additional group of dual users (DUs) of CC and ECs is introduced.

In contrast to approaches which aim to predict the potential changes in health risks associated with the introduction of NGPs (167, 168), the WBE approach does not require the consideration of the tobacco/nicotine product use history in the various groups. Rather, it is sufficient to factor in the current number of subjects in each group at the different time points during the study period. For this purpose, only the net result of all possible transitions (switches to other tobacco/nicotine use habits) of each group has to be considered. For simplicity, it is assumed that the net changes in the groups proceed continuously over the investigation periods of 5 or 10 years. Furthermore, in this first approximation the 7 or 8 groups in the population are not differentiated by sex or age subgroups.

MCSs are conducted for the prediction of inlet WW concentrations of the three biomarkers cotinine (Cot, indicator of nicotine consumption derived from any of the tobacco/nicotine products mentioned above), anabasine (AB, indicator of the exposure to tobacco-containing products) and 2-cyanoethyl-mercapturic acid (2CyEMA, indicator for the exposure to acrylonitrile, a combustion

product mainly originating from the use of cigarettes). Following the WBE relationships presented in Section “Data evaluation and interpretation”, the WW biomarker concentrations (C_{BM}) in each year (t_n) of the 10-year simulation (e.g. from 2025 to 2034) can be estimated with Equation [1]:

$$C_{BM} \text{ (mass/L)} = \frac{\text{Pop}}{F} \times (\text{UER}_{BMCC} \times f_{CCtn} + \text{UER}_{BMEC} \times f_{ECtn} + \text{UER}_{BMHTP} \times f_{HTPtn} + \text{UER}_{BMONP} \times f_{ONPtn} + \text{UER}_{BMNRT} \times f_{NRTtn} + \text{UER}_{BMOOT} \times f_{OTtn} + \text{UER}_{BMDU} \times f_{DUtn} + \text{UER}_{BMNU} \times f_{NUtn}) \quad [1]$$

with:

Pop = the catchment population size of the WWTP (1.5 million people in our case)

F = the flow rate of the WWTP (L/d)

UER = the daily urinary excretion rate of a biomarker

f = the fraction of the user groups (CC, EC, HTP, ONP, NRT, OT, DU, NU) in the total population (Pop)

t_n = standing for the year (e.g. 2025 to 2034) of the simulation.

Further information on the input values and variables are provided in Table 3.

The ratios C_{AB}/C_{Cot} ($= R_{BM/Cot}$) and C_{2CyEMA}/C_{Cot} ($= R_{2CyEMA/Cot}$) were calculated by applying a MCS to Equation [2]:

$$R_{BM/Cot} \text{ (ng/}\mu\text{g)} = \frac{(\text{UER}_{BMCC} \times f_{CCtn} + \text{UER}_{BMEC} \times f_{ECtn} + \text{UER}_{BMHTP} \times f_{HTPtn} + \text{UER}_{BMONP} \times f_{ONPtn} + \text{UER}_{BMNRT} \times f_{NRTtn} + \text{UER}_{BMOOT} \times f_{OTtn} + \text{UER}_{BMDU} \times f_{DUtn} + \text{UER}_{BMNU} \times f_{NUtn})}{(\text{UER}_{CotCC} \times f_{CCtn} + \text{UER}_{CotEC} \times f_{ECtn} + \text{UER}_{CotHTP} \times f_{HTPtn} + \text{UER}_{CotONP} \times f_{ONPtn} + \text{UER}_{CotNRT} \times f_{NRTtn} + \text{UER}_{CotOT} \times f_{OTtn} + \text{UER}_{CotDU} \times f_{DUtn} + \text{UER}_{CotNU} \times f_{NUtn})} \quad [2]$$

Note that the ratios (R) are independent of Pop and F.

MCSs were conducted with Excel Software (Microsoft Professional Plus 2019) by applying the functions NORMINV and RAND to the variables Pop, F and UER in Equations 1 and 2. For each of the 10 years, 11,000 runs were performed, resulting in a total of 1.1 million single calculations for the concentrations of the three BMs and two ratios in the two scenarios.

4.3 Results and discussion of the conducted Monte Carlo simulations (MCSs)

The MCS results of the two scenarios for the WW concentrations of the three BMs are shown in Figure 3. The cotinine concentrations at the start (2025) of the MCSs for Scenario 1 and 2 were calculated to be about 4.1 and 4.7 $\mu\text{g/L}$ (Figure 3 top), which is in the same range as reported by other studies in Europe and Australia (2.3–3.9 $\mu\text{g/L}$) (52, 59). With the assumptions used, Cot concentrations are higher in Scenario 2 compared to Scenario 1. Differences between the two scenarios, however, are not significant at any point in time. The decrease over time is slightly more pronounced in Scenario 1 than 2. However, as can be deduced from Figure 3 (top), *intra*-scenario differences for cotinine between Year 1 (2025) and Year 10 (2034) do not reach statistical significance, neither in Scenario 1 nor in Scenario 2.

Simulations for WW concentrations of AB (Figure 3,

middle) and 2CyEMA (Figure 3, bottom) revealed results similar to those for Cot. The AB concentrations at the start of the MCSs for Scenario 1 and 2 were calculated to be 10.3 and 11.1 ng/L, in good agreement with studies in Europe and Australia (11.2–15.6 ng/L) (52, 59). There is only one study on 2CyEMA in WW reporting a concentration of 117 ng/L in a community in closer proximity to manufacturing facilities (66). This 2CyEMA level is very similar to the starting levels of our MCSs for Scenario 1 and 2 (115 and 127 ng/L, respectively, Figure 3 bottom). Rates of decrease over time for 2CyEMA are similar for the two scenarios. This has to be expected, since exposure to both AB and acrylonitrile (parent compound of 2CyEMA) is dominated by CC use. Overlaps of 95%-CIs indicate that there are neither *intra*-scenario nor *inter*-scenario statistically significant differences.

Outcome of the MCSs for the WW concentration ratios AB/Cot and 2CyEMA/Cot are shown in Figure 4. An evident difference to the results for the WW concentrations are the much lower 95%-CIs. For *intra*-scenario comparisons, the error bars suggest that for the AB/Cot ratio statistically significant differences can be expected as from Year 6 (2030) and for the 2CyEMA/Cot ratio as from Year 3 (2027). The difference between AB/Cot ratio of Scenario 1 and Scenario 2 becomes statistically significant as from Year 7 (2031). The corresponding time point for the 2CyEMA/Cot ratio is Year 4 (2028). These results demonstrate that the variability in the WW results can be significantly reduced by evaluating concentration ratios of biomarkers in the same WW samples, thus eliminating the contributions of population size (Pop) and flow rate (F) to the overall uncertainty of the WW results.

Taken together, the results of our MCSs clearly suggest that WW concentrations of biomarkers (and preferably suitable ratios of BM concentrations) have to be monitored over several years in order to draw profound conclusions with respect to success or failure of THR.

Apart from the variables Pop and F, further sources of variability in our approach are the urinary excretion rates (UER) of Cot, AB and 2CyEMA in the 7 (Scenario 1) or 8 (Scenario 2) tobacco/nicotine habit groups. Assumed uncertainties for the UER are currently substantial (up to 50%, Table 3), due to the fact that these values are based on rather small group sizes. A significant reduction in these uncertainties for the future is probable when more results from larger studies will become available. Detailed sensitivity analyses of our model, which could contribute to judge the suitability of WBE as a tool for the evaluation of THR measures, are in progress.

Answering the title question of this section: Yes, WBE has the potential to monitor the progress (either success or failure) of THR measures in populations. Preconditions for an efficient application of WBE in surveilling progress of THR are:

- the validation of the hitherto not fully established WW biomarkers AB, 2CyEMA and probable others
- application of WW analyses of the biomarkers found to be suitable over sufficiently long time spans (several years)
- extending the WW analyses to a sufficiently high number of WWTPs so that different populations are assessed, which should be representative for instance for a country.

Table 3. Conditions and assumptions applied in two scenarios of Monte Carlo Simulations (MCSs) for WW concentrations of cotinine (Cot), anabasine (AB) and 2-cyanoethyl mercapturic acid (2CyEMA) of a hypothetical WWTP serving a large city.

Input variables	Mean value (unit)	Uncertainty	Distribution	Assumptions and Rationale
Pop: Catchment population size of the WWTP	1500000	15%	Normal	- Stable over the study period of 10 years - In other studies normal distribution of Pop with uncertainties of 10 (20), 15 (36) and 20% (40) were assumed
F: Flow rate of WWTP	350,000,000 (L/d)	15%	Normal	- In other studies normal distribution of F with uncertainties of 10 (20, 166) and 20% (40) were assumed - Reported F values for comparable Pops were in the same range (166)
UER _{BM} : Daily urinary excretion rates of the BMs	In the rows below, the group- and BM-specific UER values are listed. These are derived from a controlled study with 10 subjects per group (159, 160, 169). As uncertainty, the relative standard errors (RSE) are provided. Specifics are indicated in the last column. Most distributions are between normal and lognormal. As a first approach, normal distributions are assumed. It is assumed that UERs are valid for steady-state conditions.			
UER _{CotCC}	5.736 (mg/d)	18%	Normal	UE _{Cot} values were obtained from reported nicotine equivalents (Nic+10) (160) assuming that Nic+10 represents 95% of the absorbed nicotine dose and that 32.3% are excreted as Cot (free + conjugated) (50)
UER _{CotEC}	2.309 (mg/d)	23%	Normal	
UER _{CotHTP}	4.240 (mg/d)	17%	Normal	
UER _{CotONP}	6.625 (mg/d)	18%	Normal	No values for UER _{CotONP} are available. It is assumed that the UER for ONP users is between those of CC and OT users.
UER _{CotNRT}	2.220 (mg/d)	31%	Normal	There were mainly nicotine gum users in the controlled study (160)
UER _{CotOT}	7.174 (mg/d)	19%	Normal	There were mainly snus users in the controlled study (160)
UER _{CotNU}	0.020 (mg/d)	47%	Normal	
UER _{ABCC}	14.6 (µg/d)	21%	Normal	UER _{AB} values were obtained from the published controlled study (169)
UER _{ABEC}	0.50 (µg/d)	25%	Normal	Outliers were excluded
UER _{ABHTP}	2.51 (µg/d)	22%	Normal	
UER _{ABONP}	0.38 (µg/d)	32%	Normal	No data for ONP were available, the UER _{AB} values for NRT users were applied
UER _{ABNRT}	0.38 (µg/d)	32%	Normal	
UER _{ABOT}	22.2 (µg/d)	50%	Normal	There were mainly snus users in the controlled study (160)
UER _{ABNU}	0.21 (mg/d)	12%	Normal	
UER _{2CyEMACC}	186.4 (µg/d)	18%	Normal	UER _{2CyEMA} values were obtained from the published controlled study (159)
UER _{2CyEMAEC}	2.52 (µg/d)	39%	Normal	
UER _{2CyEMAHTP}	14.00 (µg/d)	28%	Normal	
UER _{2CyEMAONP}	0.58 (µg/d)	6%	Normal	
UER _{2CyEMANRT}	0.58 (µg/d)	6%	Normal	Due to obvious outliers, UER _{2CyEMA} values of NU were used
UER _{2CyEMAOT}	0.58 (µg/d)	6%	Normal	
UER _{2CyEMANU}	0.58 (µg/d)	6%	Normal	
UER for DU (only in Scenario 2)	It is assumed that DUs (simultaneous use of CCs and ECs) exhibit the same daily nicotine uptake as CCs do and would have the same UER _{Cot} . For a 20 CPD (cigarettes per day) smoker this could be achieved by reducing the consumption to 10 CPD (= 50% reduction) and simultaneously carry out 25 EC sessions per day. UER _{ABDU} and UER _{2CyEMADU} were therefore reduced by 50% compared to the corresponding UER levels for CC-only users.			

Table 3. (Contd.)

Scenario 1: No dual use of CC and EC is assumed				
f_{CC} : Fraction of smokers of CC in the total Pop	Year 1 (Y1): 0.14 Y10: 0.05	A constant yearly net decrease of 0.01 is assumed	- Group fractions are related to the whole Pop (all age groups, both genders) - f_{CC} is based on a smoking prevalence of 16.5% in the population aged 15+ which represent 85% in Pop	
f_{EC} : Fraction of vapers (EC) in the total Pop	Y1: 0.014 Y10: 0.0464	A constant yearly net increase of 0.0036 is assumed	- No reliable prevalence data for EC use are available to date - f_{EC} is assumed to be 10% of that of smokers (CC)	
f_{HTP} : Fraction of HTP users in the total Pop	Y1: 0.007 Y10: 0.0232	A constant yearly net increase of 0.0018 is assumed	- No reliable prevalence data for HTP use are available to date - f_{HTP} is assumed to be 50% of that of vapers (EC) both in Year 1 and 10	
f_{ONP} : Fraction of ONP users in the total Pop	Y1: 0.0014 Y10: 0.0113	A constant yearly net increase of 0.0011 is assumed	- No reliable prevalence data for ONP use are available to date - f_{ONP} is assumed to be 10% of that of vapers (EC) in Year 1 and ~50% of that of HTP users in Year 10	
f_{NRT} : Fraction of NRT (any type) users in the total Pop	Y1:0.007 Y10: 0.007	A constant rate of NRT product use over the 10 study years is assumed	- No net change of NRT product use over the study period is assumed	
f_{OT} : Fraction of OT (any type) users in the total Pop	Y1: 0.007 Y10: 0.00133	A constant yearly decrease of 0.00063 is assumed	- f_{OT} is assumed to be similar to f_{NRT} in Year 1 - A steady decrease to 10% of the Year 1 use after 10 years is assumed, owing to the fact that a replacement product (ONP) is available	
f_{NU} : Fraction of non-users (NU) users in the total Pop	Y1: 0.8236 Y10: 0.8649	Fraction of NU arises as a result of subtracting all user groups from the total Pop		
Scenario 2: Dual use of CC and EC is assumed				
f_{CC} : Fraction of smokers of CC in the total Pop	Y1: 0.14 Y10: 0.054	A constant yearly net decrease of = 0.01 is assumed; from Year 7 to Year 10 an increase of 0.001/year is assumed (back-switchers from DU group)	See Scenario 1	
f_{EC} : Fraction of vapers (EC) in the total Pop	Y1: 0.015 Y10: 0.055	A constant yearly net increase of 0.004 is assumed; from Year 7 to Year 10 an additional increase of 0.001/year is assumed (DU staying with EC only)	- No reliable prevalence data for EC use are available to date - f_{EC} is assumed to be ~11% of that of smokers (CC) in Year 1	
f_{HTP} : Fraction of HTP users in the total Pop	Y1: 0.010 Y10: 0.037	A constant yearly net increase of 0.003 is assumed	- No reliable prevalence data for HTP use are available to date - A somewhat higher starting level and increase rate than in Scenario 1 is assumed	
f_{ONP} : Fraction of ONP users in the total Pop	Y1: 0.002 Y10: 0.02	A constant yearly net increase of 0.002 is assumed	- No reliable prevalence data for ONP use are available to date - A somewhat higher starting level and increase rate than in Scenario 1 is assumed	
f_{NRT} : Fraction of NRT (any type) users in the total Pop	Y1: 0.010 Y10: 0.0055	A constant decrease of 0.0005 of NRT product use over time is assumed	- A slightly higher starting level than in Scenario 1 is assumed - A slight decrease over 10 years is assumed due to the availability of alternatives (NGPs)	
f_{OT} : Fraction of OT (any type) users in the total Pop	Y1: 0.010 Y10: 0.0055	A constant yearly decrease of 0.0005 of OT use is assumed	- f_{OT} is assumed to be similar to f_{NRT} in Year 1 and 10 - A steady decrease is assumed, owing to the fact that a replacement product (ONP) is available	
f_{DU} : Fraction of dual users (DU) of CC and EC in the total Pop	Y1: 0.015 Y10: 0.012	A constant yearly increase of 0.001 until Year 7 followed by a net decrease of 0.002 until Year 10 is assumed	- F_{DU} is assumed to be similar to f_{EC} in Year 1 - The net decrease from Year 7 onwards is assumed, due to equal transitions to CC, EC and NU	
f_{NU} : Fraction of non-users (NU) users in the total Pop	Y1: 0.798 Y10: 0.813	Fraction of NU arises as a result of subtracting all user groups from the total Pop		

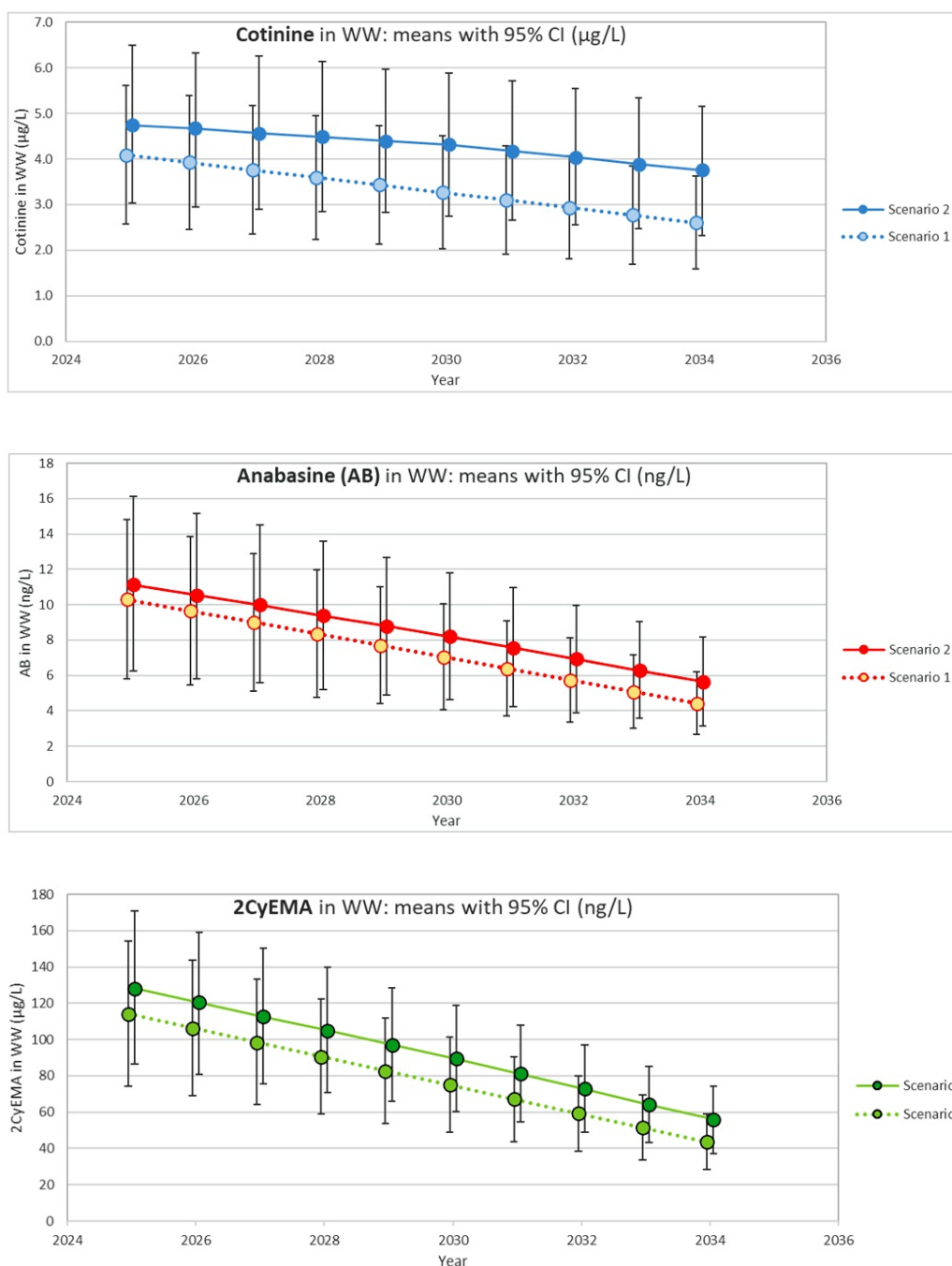


Figure 3. Monte Carlo simulations (MCSs) of Scenario 1 and 2 over 10 years for WW concentrations of cotinine (top), AB (middle) and 2CyEMA (bottom).

5. CONCLUSIONS AND OUTLOOK

There is ample evidence in the literature that WBE has great potential to deliver temporal and spatial information on the exposure to chemicals as well as use of pharmaceuticals, licit and illicit drugs as well as recreational compounds (caffeine, alcohol, nicotine) in defined populations (14, 170). WBE can thus complement, support or even partially replace the ‘classical’ methods for gaining this information such as surveys, (governmental) statistics, product sales data and (conventional) epidemiological studies.

The presented systematic literature review on the application of WBE to the use of tobacco and nicotine products provides convincing evidence that WBE is a suitable tool to quantitatively assess the nicotine consumption in defined populations (usually the catchment population of a WWTP), when Cotinine (Cot) or 3'-trans-Hydroxycotinine (OH-Cot) is used as a biomarker. WW measurements at one point in time (usually reflecting the time span of a day) provide information on the current exposure (or product use) of that particular day. Long-term WBE studies of defined populations lasting over years have shown changes in nicotine consumption which can be explained by changes

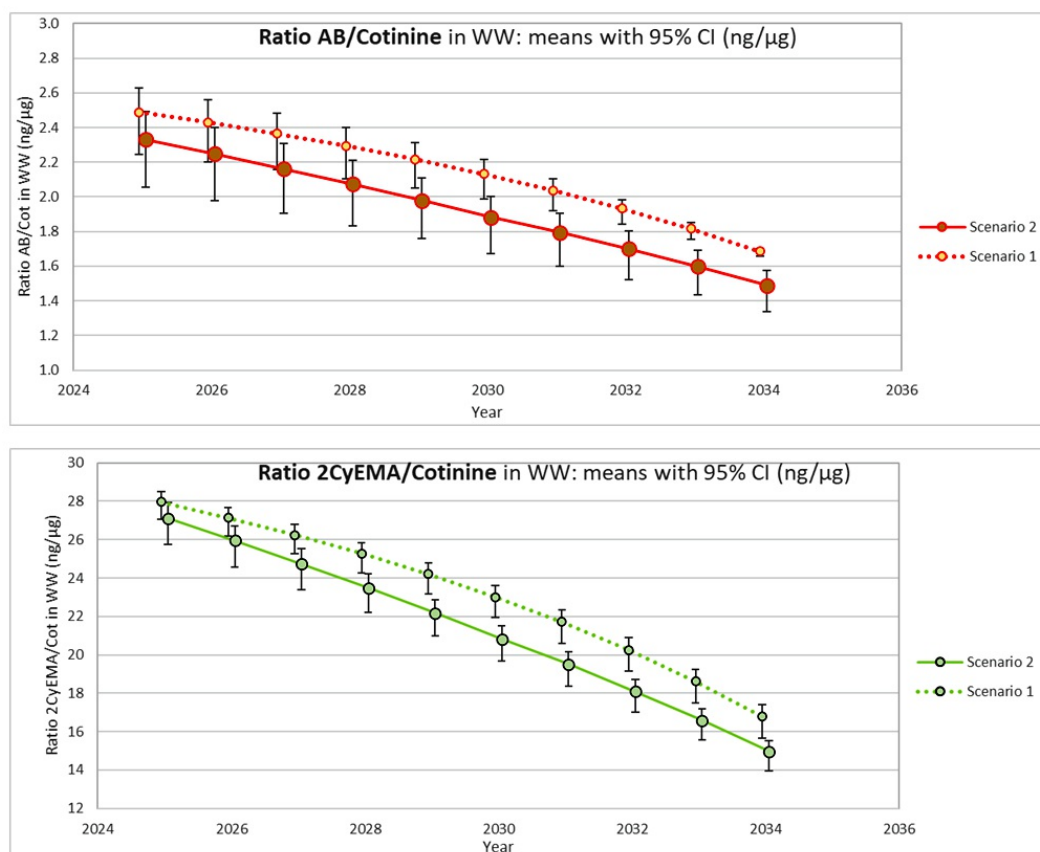


Figure 4. Monte Carlo simulations of Scenario 1 and 2 over 10 years for the WW concentration ratios AB/cotinine (top) and 2CyEMA/cotinine (bottom).

in the use of traditional tobacco products (mainly CCs), quitting rates, use of NRT products, and the advent of NGPs (e.g., ECs, HTPs). Observed changes, however, always reflect the net results of all transitions between the various groups in the population. A couple of studies have shown that by additionally measuring the tobacco-derived biomarker AB, assignment of product use responsible for the observed nicotine consumption can be improved (for review, see (14)). We hypothesize that further improvement would be possible by including biomarkers of tobacco combustion products such as acrylonitrile (BM: 2CyEMA) in the WW analysis. By this, the use of the most harmful tobacco product (CCs) could be assessed and a decrease of these biomarkers in WW over time would provide strong evidence that THR is in progress. Depending on the time course of other product use-related biomarkers (e.g., cotinine, PG), THR is caused by CC cessation, switching to a NGP (e.g., EC, HTP, OTP, others) or a combination of these. As a first step for proving this concept, we performed Monte Carlo simulations (MCSs) for the WW biomarkers Cot, AB and 2CyEMA. For a time period of 10 years, various assumptions for product use cessations and switching rates were made for a hypothetical population served by a (hypothetical) WWTP. Results of the MCSs clearly suggest that biomarker concentrations show appreciable variations at the single timepoints so that the analysis of time trends (spanning over several years) would be more

promising. Variation can be significantly reduced by using ratios of BM concentrations (e.g., AB/Cot, 2CyEMA/Cot). With the assumptions applied, significant differences within a population for the AB/Cot and 2CyEMA/Cot ratios can, according to our simulations, be expected to occur after already 3 years (Figure 4).

The suitability of cotinine in WW as an indicator for the use of nicotine products in a population is well established. With some restrictions, this also applies for AB in WW as an indicator for the exposure to tobacco-containing products (combustible and smokeless products) (57, 62–64, 66, 101, 102). Only limited information is available on the suitability of 2CyEMA in WW as a biomarker for exposure to acrylonitrile (66), an indicator for combustible tobacco products (in particular CCs). Further research is required to prove the suitability 2CyEMA and AB for the intended purpose. In particular, the stability of both biomarkers under WBE conditions has to be verified. The inclusion of additional biomarkers suitable for studying THR by WBE should be kept in mind. For instance, the use of other CC combustion markers such as 3-hydroxypropyl-mercaptopuric acid (3-HPMA, parent compound: acrolein) or 3-hydroxy-3-methylpropyl-mercaptopuric acid (HMPMA, parent compound: crotonaldehyde) could possibly support 2CyEMA as a biomarker for the use of combustible tobacco products. The application of 1,2-propylene glycol (PG) in WW could be suitable as a relatively specific biomarker for EC use, as

has been shown for urinary PG on an individual basis (171). Application of PG in WW as an indicator for EC use in a population would require an evaluation from scratch, including assessment of the stability under WBE conditions, selection of a suitable PG biomarker in WW (free PG, conjugate), determination of the urinary excretion rate (UER_{PG}) as well as estimating the influence of other sources for PG in WW. The effort, however, could be worthwhile, given the reasonable presumption that ECs will play a significant role in THR in the future. Finally, WBE-derived data similar to those presented as outcomes of the MCSs could provide useful evidence on the long-term impact of NGPs such as ECs, HTPs and ONPs on population risks, a major requirement for NGPs to be classified as modified risk tobacco/nicotine products (172, 173).

ABBREVIATIONS

2CyEMA	2-Cyanoethyl mercapturic acid (metabolite of acrolonitrile)
5-HIAA	5-Hydroxyindole acetic acid (a serotonin metabolite)
8-OH-dG	8-Hydroxy-2'-deoxyguanosine (a BM of oxidative stress)
AB	Anabasine
AT	Anatabine
BM	Biomarker
CC	Combustible cigarette
CI	95%-Confidence interval
Cot	Cotinine
DC	Drug consumption
DML	Daily mass load
DU	Person who simultaneously uses CCs and ECs (so called dual use)
EC	Electronic cigarette
ES	Ethyl sulfate (metabolite of ethanol)
F	Flow rate (influent of a WWPT in L/d or m ³ /d)
f	fraction (of Pop)
HTP	Heated tobacco product
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
LLE	Liquid-liquid extraction
MCS	Monte Carlo simulation
N	Nitrogen
Nic	Nicotine
NAB	N'-Nitrosoanabasine
NAT	N'-Nitrosoanatabine
NGP	New generation tobacco/nicotine product
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (metabolite of NNK)
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
NNN	N'-Nitrosornicotine
NNO	Nicotine-N-1'-oxide
NRT	Nicotine replacement therapy
ns	not (statistically) significant
NS	Persons not using cigarettes (mostly termed 'non-smokers' in the literature)
NU	Persons not using any tobacco/nicotine products
OH-Cot	<i>trans</i> -3'-hydroxycotinine

ONP	Oral nicotine pouch
OT	Oral tobacco (and user)
PG	1,2-Propylene glycol
PGF2 α	Prostaglandin F2 α
PNC	Population-normalized consumption (of a drug or chemical)
Pop	Population size (number of persons served by a WWTP)
SD	Standard deviation of the mean
SEM	Standard error of the mean
SPE	Solid-phase extraction
TSNA	Tobacco-specific nitrosamine
U	Urine
UC	Units consumed (e.g., cigarettes/d)
UER	Urinary excretion rate (percentage of intake dose excreted as metabolite in urine)
WBE	Wastewater-based epidemiology
WW	Wastewater
WWTP	Wastewater treatment plant

SUPPLEMENTARY INFORMATION

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