Cooperation Centre for Scientific Research Relative to Tobacco

CORESTA Guide N° 29

Best Practice in the Application of Biomarkers of Exposure as Compliance Measures in Long-Term and Epidemiological Studies of New Nicotine and Tobacco Products

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Biomarkers Sub-Group

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1. INTRODUCTION

Over the past decade, the tobacco landscape has changed drastically with several new emerging product categories available such as e-cigarettes (ECs), heated tobacco products (HTPs) and oral nicotine delivery products (nicotine pouches (NP)). The use of these products comes along with a drastically reduced exposure to most harmful constituents in contrast to tobacco smoke exposure. In order to assess the potential benefits for public health, long-term and large population-representative studies are needed. However, such settings come along with a potential lack in compliance when participants are categorized in one of the new nicotine/tobacco product categories by self-report. Suitable biomarkers of exposure (BoE) as compliance measures, so-called biomarkers of compliance, are needed for a robust risk assessment over a longer term and in uncontrolled settings. Such BoE shall be specific to the use of one nicotine/tobacco product category so that the self-reported use can be biochemically verified within the study. Historically, exhaled carbon monoxide (eCO) and cotinine (major metabolite of nicotine) have been used to verify tobacco use or abstinence. Now that smoking is becoming less abundant while new forms of tobacco and nicotine products are emerging, those two BoE are not sufficient to monitor product use compliance in most studies. Vapers, HTP users, smokeless tobacco (SLT) or NP consumers will all have low eCO levels in the range of non-smokers with elevated cotinine concentrations, and measurements of eCO and/or cotinine alone will not be able to distinguish their product use. Hence, a larger set of BoE is needed to verify product use compliance.

This guide discusses the suitability of BoE for measuring product compliance based on their abundance, specificity and half-life and suggests single BoE and BoE patterns to verify product use status for the most popular product categories (EC, HTP, NP) as well as combustible cigarettes (CC) and traditional, smokeless tobacco (SLT) like moist snuff based on the current scientific literature.

The guide may help researchers in the field in the design of new studies with a robust assessment of subjects' compliance in long-term, epidemiological and/or cross-sectional studies where the use behaviour of the subjects is not under rigorous control but mainly assessed by self-report.

2. FIELD OF APPLICATION

The guide provides an overview of the current research on potential compliance biomarkers, and gives recommendations for biomarkers of compliance in a clinical study assessing new nicotine/tobacco products. The guide will be updated based on new research which identifies biomarkers specific to the exposure of one or several nicotine product categories.

It is applicable for researchers conducting clinical studies with users of nicotine/tobacco products, especially for longitudinal, cross-sectional, and epidemiological studies.

3. BASIC CONSIDERATIONS FOR THE EVALUATION OF A BOE AS A COMPLIANCE MEASURE

The properties which need to be taken into account in the evaluation of the suitability of a BoE as a measure of compliance in terms of product use or abstinence are summarized in this section. A robust biomarker of compliance is ideally specific to the use of one nicotine/tobacco product.

If a BoE is present in users of several product categories at different concentrations (e.g., CC > HTP > EC) it can still be useful to discriminate those groups from one another and/or from non-users, especially as part of a pattern of several biomarkers of compliance. While BoE levels may be significantly different between groups, it is hard to discriminate the use behaviour on an individual level applying one single biomarker to which different product user groups are exposed, albeit at different concentrations. Especially, if the BoE has a short half-life of only few hours, individuals with a presumably high exposure might be misclassified as users of a product with generally less exposure if the time gap between sample collection and product use is too large. This limitation will be explained in Section 4.1 for eCO. Optimally, a biomarker of compliance is only detectable in one exposure group. At least, the concentrations should differ with high significance so that there is no pronounced overlap between different user groups on an individual level. Thresholds should be implemented based on data from large cohorts in order to properly validate the analyte as biomarker of compliance, wherever possible.

Elevated occupational exposure or dietary intake may occur for a biomarker of compliance. Hence, the subject's profession, as well as dietary habits should be documented in the study so that these sources can be checked as a cause for observed non-compliance.

Moreover, a long half-life of the BoE would be preferred in order to monitor the exposure over longer time periods of days or even weeks. This would allow investigators to have robust compliance monitoring especially in study designs where in-clinic visits may be several weeks apart.

Ideally, the excretion kinetics and the concentration levels in exposed and non-exposed populations of the specific BoE will have been investigated in detail so that thresholds can be set to detect current use and abstinence.

Finally, the biological matrix in which the BoE is measured shall be easily accessible.

E.g., in terms of cigarette smoking, a long-term biomarker for acrylonitrile exposure (*N*-(2-cyanoethyl)valine (CEVal)) was recently suggested. This biomarker is highly specific to tobacco smoke exposure and can be used to discriminate people who smoke CC from non-smokers, HTP users and even dual users [1].

4. CANDIDATES FOR BIOMARKERS OF COMPLIANCE

4.1 BoE for the verification of smoking and smoking abstinence

Tobacco smoke exposure from CC has been extensively investigated over the last 60 years. The toxicant exposure profile from smoking is well understood and numerous harmful and potentially harmful constituents (HPHC) can serve as suitable candidates to monitor the smoking status.

eCO and Cotinine

As discussed in the introduction, smoking status has been verified by means of eCO and/or cotinine in the past. eCO is elevated in people who smoke CC at 2-18 ppm in contrast to non-smokers with 1-4 ppm [2]. Historically, eCO cut-off concentrations between 7 and 10 ppm have been applied in clinical studies. The SRNT Subcommittee on Biochemical Verification report recommended cut-off concentrations for eCO between 8 and 10 ppm [3]. One limitation with this proposed biomarker is that it has a short half-life of around 4.6 ± 1.6 h in smoking subjects meaning that it can only detect very recent smoking [4]. Moreover, exhaled CO is rather insensitive and light smoking and/or plain inhalation may not be detected accurately [4, 5].

Cotinine as the major metabolite of nicotine is not specific to smoking as it can be detected by exposure to any nicotine containing product (including vaping, heated tobacco and oral products), making it obsolete in long-term switching trials for the verification of the smoking status. But it can still be used to distinguish nicotine consumers from non-users. Plasma cotinine of 3 ng/mL was defined to distinguish people who smoke CC from non-smokers based on data from the US National Health and Nutrition Examination Survey [6]. Urinary cotinine concentrations are, on average, five times higher compared to plasma [7]. Therefore, a level of 15 ng/mL in urine could be proposed as a cut-off to differentiate active smoking from passive smoking and non-smokers. Urinary cotinine thresholds at screening are mostly reported at 200 ng/mL which is the nominal cut-off concentration of the point-of-care strip test commonly used at screening. When measuring cotinine in plasma or urine by means of LC-MS/MS the lower thresholds shall be considered.

The use of a biomarker specific to smoking with longer half-life would be desirable in combination with biomarkers specific to the use of other nicotine products such as ECs or NPs to distinguish between use of different product categories.

Yet, due to the availability of low-cost, easy-to-use tests for eCO and urine cotinine (strip-test), their application during screening to receive a fast response regarding the participants' eligibility to be included into the study is still encouraged. For cotinine, saliva strip-test with higher sensitivity compared to urine is now available and, therefore, recommended [8].

Acrylonitrile Exposure Markers

Exposure to the combustion product acrylonitrile is highly specific to smoking. The urinary metabolite 2-cyanoethylmercapturic acid (2CyEMA) was recently suggested to distinguish smoking from other forms of nicotine and tobacco use [9]. Researchers from the US Center for Disease Control proposed a cut-off concentration of 7.32 ng/mL in urine to classify people who smoke CC [10].

The hemoglobin adduct formed from acrylonitrile (CEVal) has a longer half-life of around 30 days [1] compared to 8 hours for 2CyEMA [11] and can therefore detect past smoking over a longer time frame which is beneficial in long-term trials where subjects re-visit the clinic only after several weeks or months. Based on large clinical studies in smokers quitting or switching to an HTP, a cut-off concentration of 35 pmol/g globin was suggested to verify the smoking status [1].

Additional Potential Markers

Other BoE with significantly higher concentrations in users of CC compared to the other nicotine/tobacco product use categories include several volatile organic compounds (VOCs), aromatic amines (AAs), polyaromatic hydrocarbons (PAHs), and tobacco-specific nitrosamines (TSNAs) in urine. Scherer *et al*. recently conducted a review to identify BoE capable of distinguishing between various tobacco and nicotine product user groups [12]. There, urinary metabolites of *N*-nitrosoanabasine (NAB), acrolein (3HPMA), crotonaldehyde (3HMPMA), isoprene (IPMA3), benzene (PhMA), acrylonitrile (2CyEMA), 2-aminonaphthalene (2-AN), 4-aminobiphenyl (4-ABP) and naphthalene (1-hydroxynaphthalene (1-OH-Nap)) were suggested as possible biomarkers to distinguish smoking from non-smoking and the use of other products. Recent findings confirm this hypothesis for all BoE listed here. However, with regard to 3HPMA elevated levels were recently also found in HTP users albeit lower compared to smoking of CC [13].

Nicotine metabolites like cotinine can detect nicotine use in general as discussed previously. Minor alkaloids and TSNAs can be helpful to identify tobacco use. For instance, anabasine (AB), anatabine (AT), nicotelline, and NNAL have been used to detect tobacco use in terms of smoking or SLT use in urine [8, 14-17]. However, nicotelline has a very short half-life of 2-3 hours, followed by AB and AT with 10 - 16 hours. Hence, NNAL seems superior in that regard with an average half-life of 10 – 40 days [8] and can be used to differentiate CC consumption from EC or NP use.

4.2 BoE for the verification of vaping ECs

Propylene glycol (PG) in urine and plasma has been the only BoE reported in the literature so far to be significantly elevated in exclusive vapers compared to all other forms of nicotine/tobacco use or non-users [18, 19]. Elevated levels compared to non-users were only observed in vapers. PG in urine and plasma, respectively, can be considered for the verification of EC use. However, further studies in larger cohorts will be needed to prove its suitability and to define thresholds.

4.3 BoE for the verification of HTP use

In general, toxicant exposure is significantly reduced in HTP users compared to people who smoke CC [20]. However, a few reports suggest elevated BoE levels in HTP users compared to non-users, e.g., the urinary metabolites of acrylamide (AAMA/GAMA), 3HPMA (mercapturic acid of acrolein) and HMPMA (mercapturic acid of crotonaldehyde), as well as TSNAs in urine [13, 21, 22]. Hence, if these BoE had levels significantly different from both CC smokers and non-users they might be of interest for compliance monitoring. However, further research in larger cohorts will be needed to determine the interindividual variability for the different user groups and finally to distinguish appropriate cut-offs which are specific to HTP use. Moreover, confounding factors such as dietary and other environmental sources may add to the overall burden, which needs to be considered when applying these BoE to verify HTP use.

4.4 BoE for the verification of SLT use

SLT use comes with the exposure to tobacco-specific constituents like nicotine, minor alkaloids and TSNAs but without the constituents formed during combustion. Therefore, a combination of either TSNAs or minor alkaloids (AB/AT) and biomarkers of combustible exposure like 2-CyEMA have been recently applied to verify SLT use/abstinence in urine [14].

4.5 BoE for the verification of NP use

In the same study as mentioned in Section 0, NP use was verified by means of urinary cotinine to confirm nicotine exposure and the lack of the minor alkaloids AB/AT [14]. While this BoE pattern confirms abstinence from tobacco-derived nicotine consumption per se, it is insufficient to verify NP use, since this pattern may also be achieved in EC vapers or users of nicotine replacement therapies like nicotine inhalers or gums. Further research is needed to identify specific biomarkers of NP exposure, especially since AB/AT were detected in NP users in a cross-sectional study [14].

5. SUGGESTED PANEL OF BIOMARKERS FOR COMPLIANCE

Especially in terms of smoking, a large set of BoE can be applied to monitor compliance. In general, all combustion-derived biomarkers which were found to be significantly elevated in people who smoke can apply. BoE with long half-lives, high detection rate and specificity are preferred. Per the guidelines proposed herein, CEVal in blood is superior as a biomarker of smoking to all other compounds discussed in Section 0 in terms of sensitivity, specificity and long-term detectability. If CEVal cannot be measured, for instance, in studies where only urine is collected or due to other technical constraints, 2CyEMA is suggested. eCO is measured rapidly and cost-effectively and can be implemented for screening to get a rapid verification while CEVal and 2CyEMA are usually analyzed with a time delay of days to weeks after sample collection.

Verifying tobacco use, either from combustible or non-combustible products (CC, SLT, HTP), can be monitored by minor alkaloids and TSNAs in urine. In terms of TSNAs, CC and SLT consumers have higher levels as compared to HTP users. More data are needed to confirm the significant difference between CC/SLT versus HTP users. Adding further BoE which show altered concentrations for the different use groups can help to differentiate between these three groups.

PG was reported as a specific BoE to EC vaping, however this observation needs further verification. According to the current literature, PG in urine seems to be the best option to verify EC use.

In terms of NP use, no robust pattern of BoE can be proposed at the current stage. Detection of urinary cotinine in combination with the lack of minor alkaloids, TSNAs and PG in urine can be regarded as an indicator, however, this biomarker pattern would not detect NP use unequivocally.

Figure 1 illustrates a decision tree suggesting a panel of biomarkers of compliance in order to distinguish all product categories. The decision tree is intended to help identify suitable biomarkers of compliance for the intended study and can be modified depending on the study design and especially the user groups which need to be distinguished.

Examples are illustrated in:

- Figure 2: verification of CC and EC use
- Figure 3: verification of CC, SLT, and NP use
- Figure 4: verification of CC and HTP use.

The symbols "+" and "-" illustrate the detection of the biomarker of compliance above (positive; +) or below (negative; -) a pre-defined cut-off. The cut-off can be a fixed concentration or defined as quantifiable / detectable (below or above the lower limit of quantification; below or above the limit of detection).

For proposed biomarkers of compliance where more data is needed to verify its suitability, dashed lines are given in the decision tree. For instance, PG in urine has been shown to be strongly elevated in vapers of ECs in two studies. Yet, the results need to be confirmed with larger sample sizes to substantiate the validity of urinary PG as biomarker of compliance. The same applies to the proposed biomarkers of compliance to verify HTP use.

Where several BoE can be applied, their prioritization is ranked with a), b), and c).

(u): urine; (b): blood

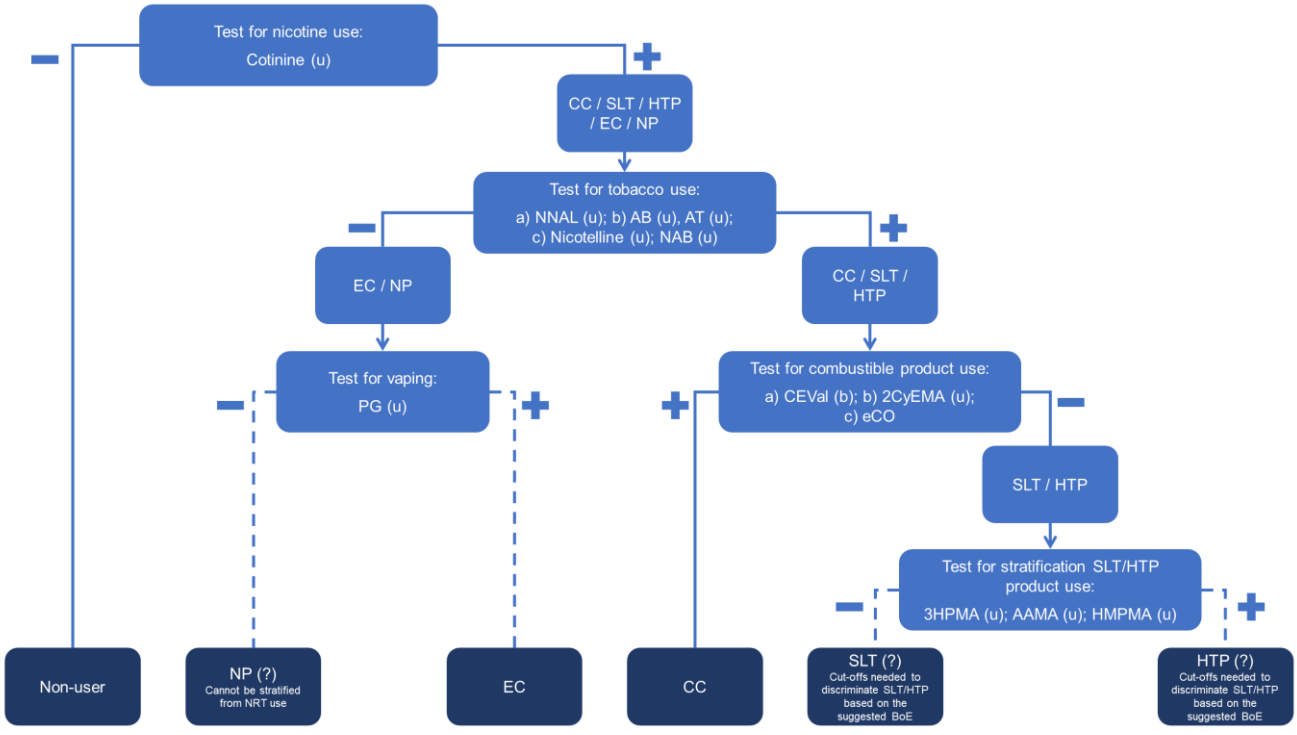

Figure 1: Decision tree with suggested panel of BoE to discriminate between the different product user groups (CC, EC, SLT, NP, HTP) and non-users. Dashed lines illustrate lower certainty due to insufficient data at present.

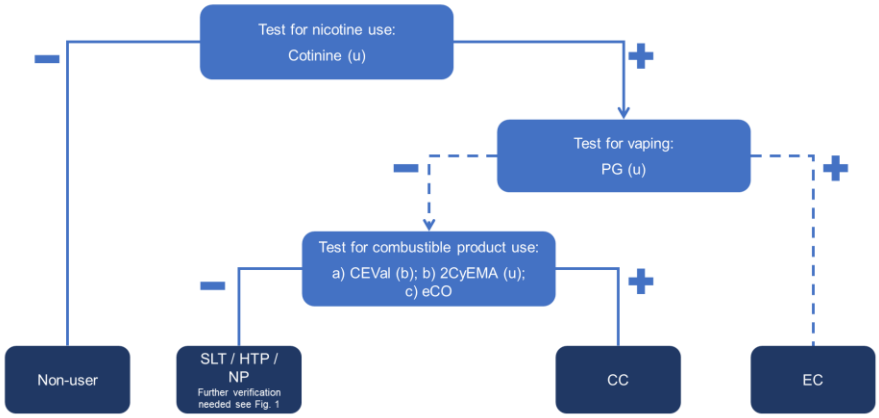

Figure 2: Decision tree for the verification of CC and EC use. Dashed lines illustrate lower certainty due to insufficient data at present.

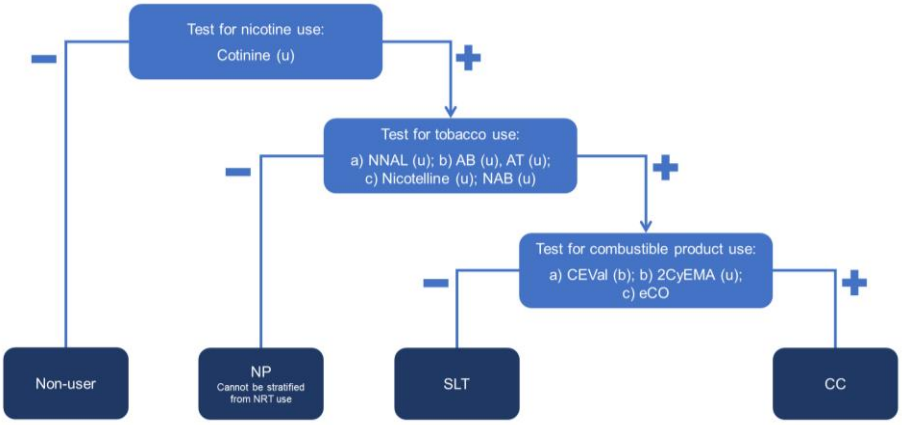

Figure 3: Decision tree for the verification of CC, SLT and NP use. Dashed lines illustrate lower certainty due to insufficient data at present.

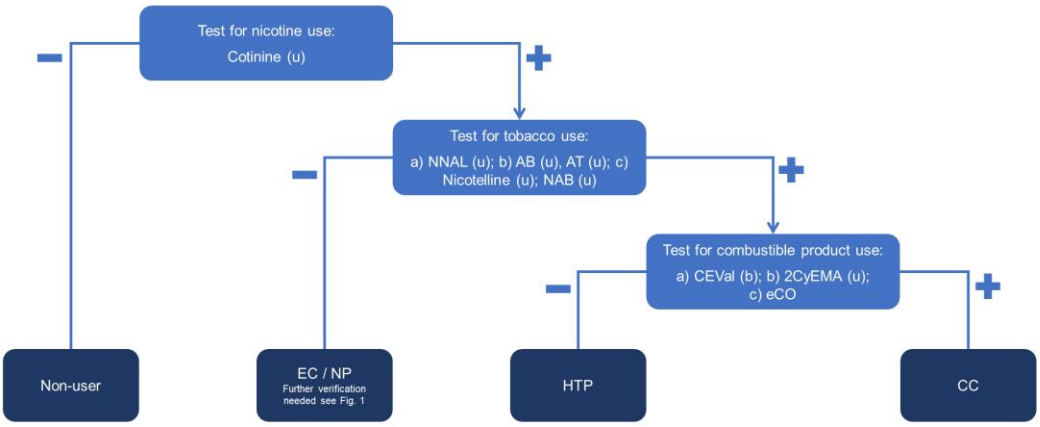

Figure 4: Decision tree for the verification of CC and HTP use. Dashed lines illustrate lower certainty due to insufficient data at present.

6. FUTURE DIRECTIONS

The identification of BoE specific to the use of one product category is challenging. Improvements in non-targeted analytical approaches such as chromatographic separation techniques coupled to high resolution mass spectrometry like gas chromatography coupled with time-of-flight mass spectrometry or liquid chromatography coupled with orbitrap high resolution mass spectrometry reveal new insights into the global exposure profile of the various product user groups. Group comparisons between people who smoke CC, non-nicotine users and other nicotine/tobacco product user groups can detect significant differences in the exposure profiles (exposome) and identify the corresponding compounds by means of high-resolution mass spectrometry. Moreover, promising candidates like PG in urine for compliance with EC vaping will need further investigations in larger cohorts to validate their specificity and sensitivity as biomarkers of compliance. Even for BoE for which cut-offs have been proposed, more research will be needed to substantiate the findings. For instance, the CEVal cut-offs were proposed based on few studies in European populations only and need verification in other populations. Finally, biomarker ratios (e.g., 2CyEMA/cotinine or 2CyEMA/NNAL) may increase the certainty instead of the absolute concentrations of a biomarker panel to distinguish between use groups in the future [15].

7. REVISION OF THE GUIDE

The BMK Sub-Group will review the literature with regard to new findings in terms of BoE capable of discriminating between the use groups on a regular basis and revise the guide accordingly.

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