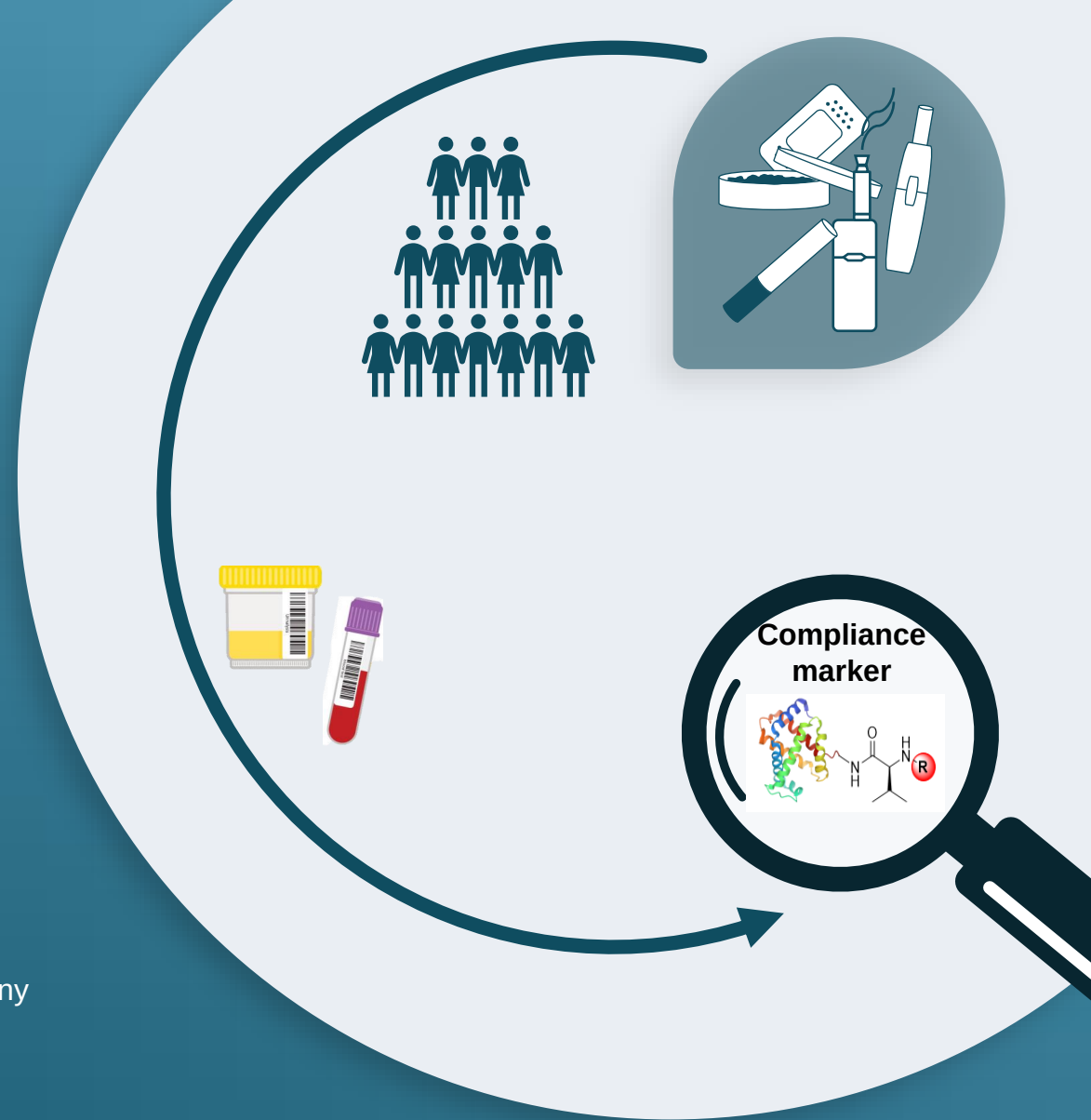


Application of biomarkers of exposure as compliance measures in long-term and epidemiological studies of new nicotine and tobacco products

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Introduction (1)

- A variety of **new tobacco and nicotine products...**
 - ... were introduced over the past decade
 - ... gained a lot attention as potentially reduced risk products in tobacco harm reduction
 - ... are discussed as cessation tools
- **Long-term (epidemiological) data** are needed to evaluate health risks and harm reduction potential for smokers after switching
- **Lack of product-specific biomarkers of exposure (BoE) for THR**

REQUIREMENT

Identification of product use-specific BoE



Risk evaluation



Compliance

Introduction (2)

- Ideally, a **robust biomarker of compliance** is **specific** to the use of **one nicotine/tobacco product**
- Biochemical verification of **self-reported use patterns**
- If precursors of BoE are present in several products categories at distinctly different concentrations it can still be useful if ...
 - ... the BoE exhibits significant differences between groups with
 - ... no pronounced overlap
- Half-lives should be considered and sampling time points standardized

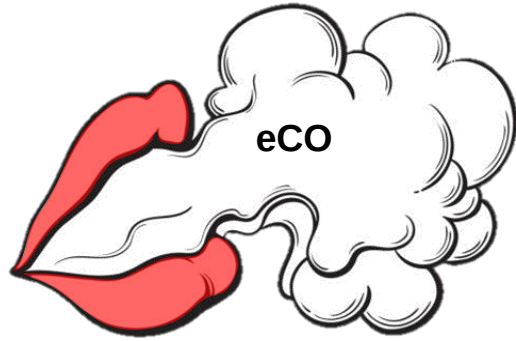
GENERAL CONSIDERATIONS

- Confounding factors such as occupational exposure or dietary intake may occur for a biomarker of compliance.
- Subject's profession, as well as dietary habits should be documented in the study so that these sources can be controlled for.
- Prolonged half-lives of a biomarker of compliance preferred to monitor chronic exposure over days, weeks or even months
- Non-invasively and easy accessible biological matrix for biomarker measurement

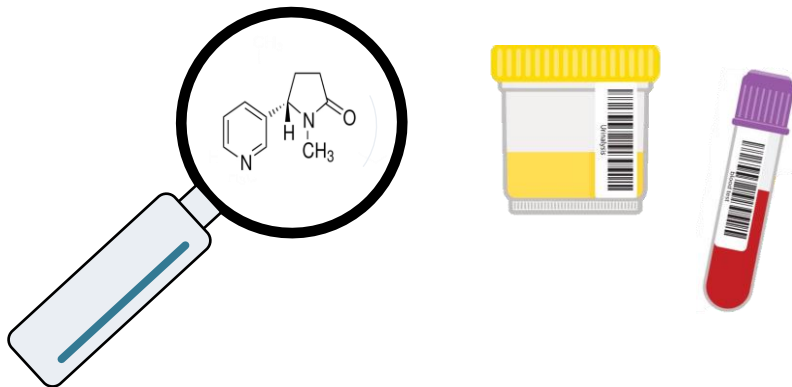
**ESTABLISH THRESHOLDS SPECIFIC
TO NICOTINE/TOBACCO USER
GROUPS**

Candidates for Biomarkers of Compliance (1)

- Exhaled carbon monoxide (eCO)



- Cotinine

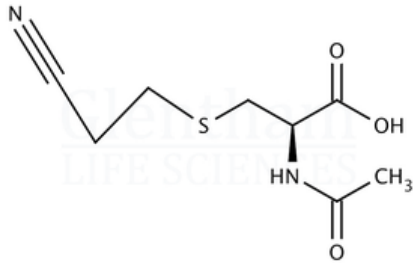


- Smoking status can be verified by eCO
 - eCO elevated in CC smokers 2 - 18 ppm compared to
 - Non-smokers 1-4 ppm
- The SRNT Subcommittee on Biochemical Verification Report recommended cut-off concentrations for eCO between smoker and non-smoker of **8 - 10 ppm**
- Limitations
 - Short half-life of about 5h; only very recent smoking can be detected
 - Insensitive and light smoking and/or plain inhalation may not be detected accurately
- Cotinine as the major metabolite of nicotine is not specific to smoking. Can only distinguish users of any nicotine product from non-users
- Cut-offs
 - Plasma cotinine 3 ng/mL (NHANES)
 - Urinary cotinine 200 ng/mL for strip tests
 - Urinary cotinine 15 ng/mL to distinguish active and passive smoking (sensitive methods such as LC-MS/MS)
- eCO and cotinine
 - Fast readout, applied at screening visits
 - Time- and cost-efficient

Candidates for Biomarkers of Compliance (2)

Acrylonitrile exposure

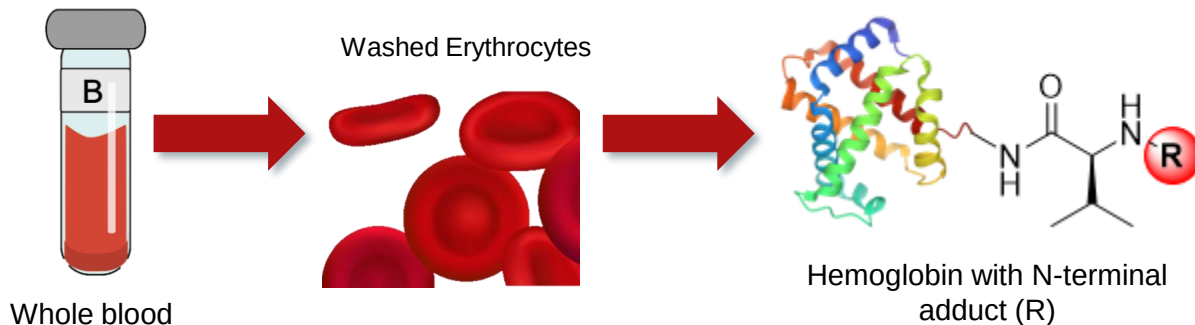
- Biomarker in urine



2-Cyanoethyl mercapturic acid (2CyEMA)



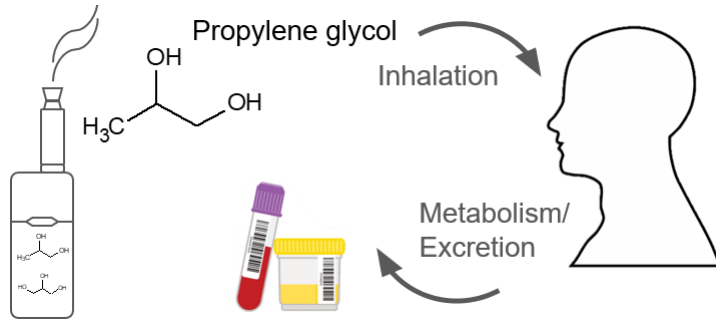
- Cyanoethyl valine hemoglobin adduct



- Exposure to the combustion product acrylonitrile is highly specific to smoking
- 2CyEMA in urine is the ideal candidate to discriminate between combustion and non-combustion products
- The half-life of CyEMA is 8 h
- The US Centers for Disease Control (CDC) proposed a cut-off of 7.32 ng/mL to classify people as smokers of CC
- The hemoglobin adduct formed from acrylonitrile (CeVal) has a half-life of 60 days (life-time of human erythrocytes: 120 d)
- CeVal is capable to detect past smoking over a longer time-frame compared to CyEMA, which is of importance for long-term trials under non-confined conditions
- A large clinical study with smokers switching to an HTP proposed a cut-off level of 35 pmol/g globin to verify the smoking status

Candidates for Biomarkers of Compliance (3)

- Biomarkers for the verification of vaping ECs



- Biomarkers for the verification of HTP use
 - Mercapturic acids (Acrylamide, Acrolein, Crotonaldehyde)
 - TSNAs (total NNAL, total NAB)

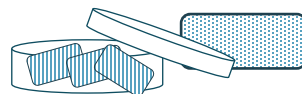
- Biomarkers for the verification of SLT use

- Minor tobacco alkaloids (AB, AT)
- TSNAs (total NNAL, total NAB)



- Biomarkers for the verification of NP use

- Nicotine metabolites (cotinine)



- EC biomarkers

- Propylene glycol (PG) in urine and plasma is the only BoE specific to vaping
- Other sources (food, cosmetic) for PG exist

- HTP biomarkers

- Toxicant exposure significantly reduced in HTP users
- Elevated levels of acrylamide, acrolein, crotonaldehyde and TSNAs reported

- SLT biomarkers

- SLT use comes with the exposure to tobacco-specific constituents but without the constituents formed during combustion
- Combination of either TSNAs or minor alkaloids (AB/AT) and biomarkers of combustible exposure promising biomarker candidates

- NP biomarkers

- Exposure to only nicotine, lack of tobacco-specific as well as combustion markers

Decision tree

CC / SLT /
HTP / EC / NP

How to differentiate between nicotine / tobacco user groups?

The decision tree is intended to help identify suitable biomarkers of compliance

⇒ Modification required depending on the study design and groups to be distinguished



Decision tree

Test for Nic use:
Cot (U)



CC / SLT /
HTP / EC / NP



NU



Introduction

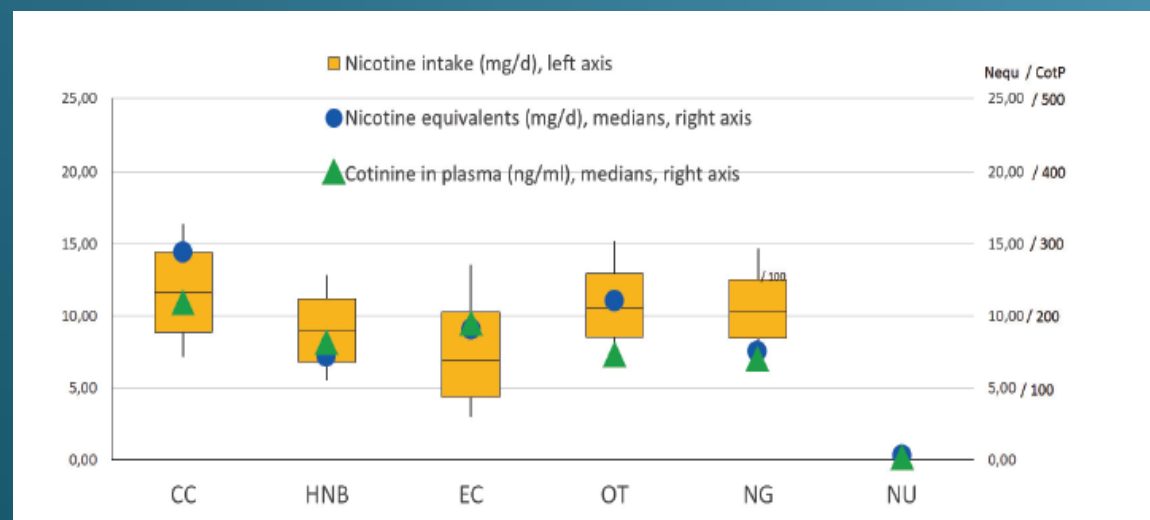
Biomarker
Candidates

Decision Tree

Conclusion

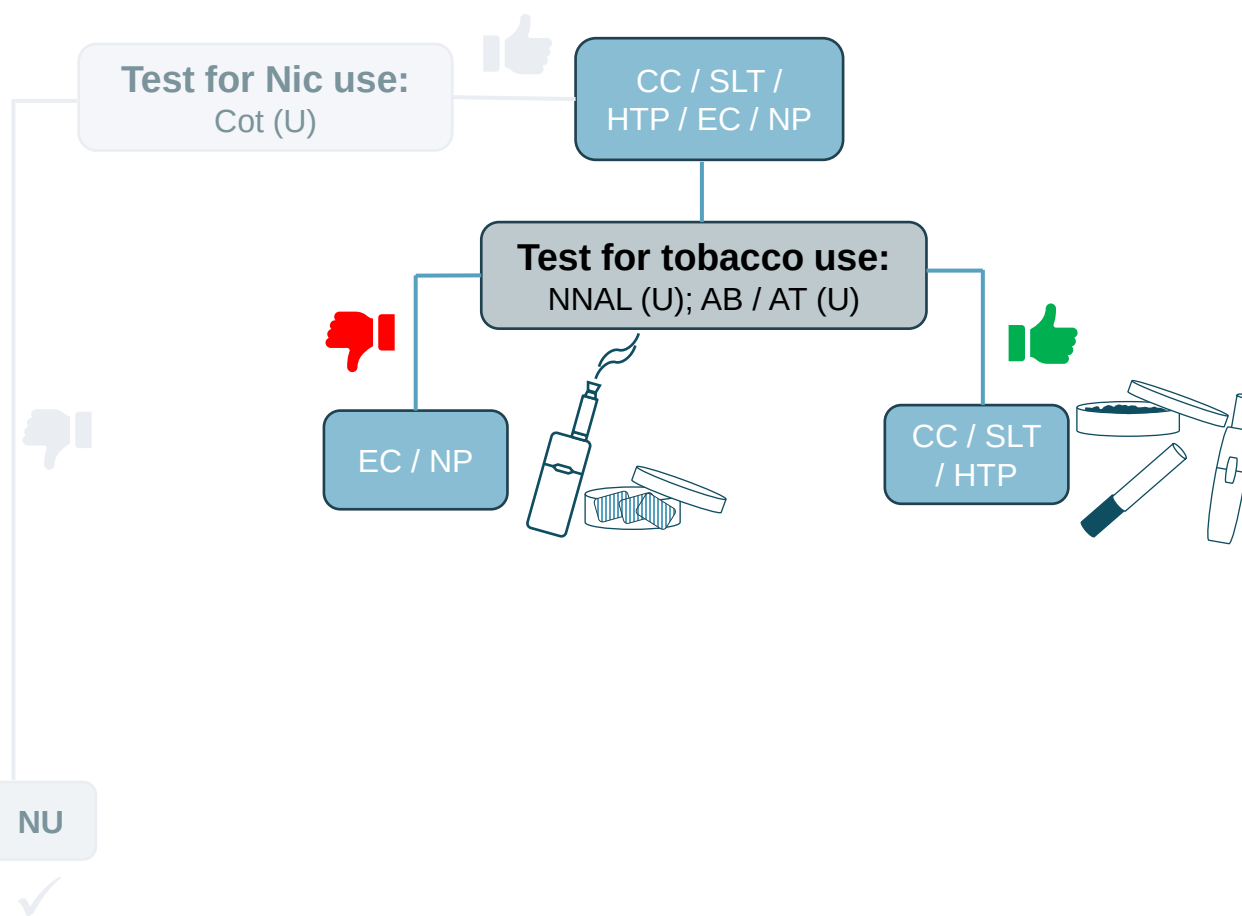
Nicotine uptake by different nicotine/tobacco user groups

HNB = HTP, OT = SLT, NG = Nicotine gum,
NU = Non-user



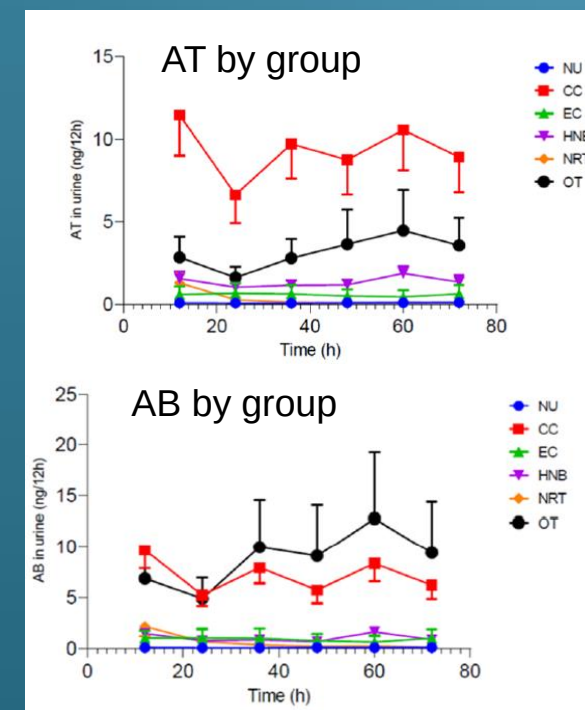
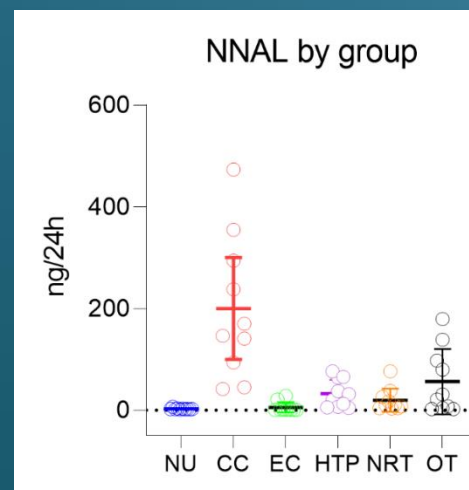
Ref. G. Scherer, N. Pluym, M. Scherer, Intake and Uptake of Chemicals Upon Use of Various Tobacco/Nicotine Products: Can Users be Differentiated by Single or Combinations of Biomarkers?, Contributions to Tobacco & Nicotine Research 30(4) (2021) 167-198.

Decision tree



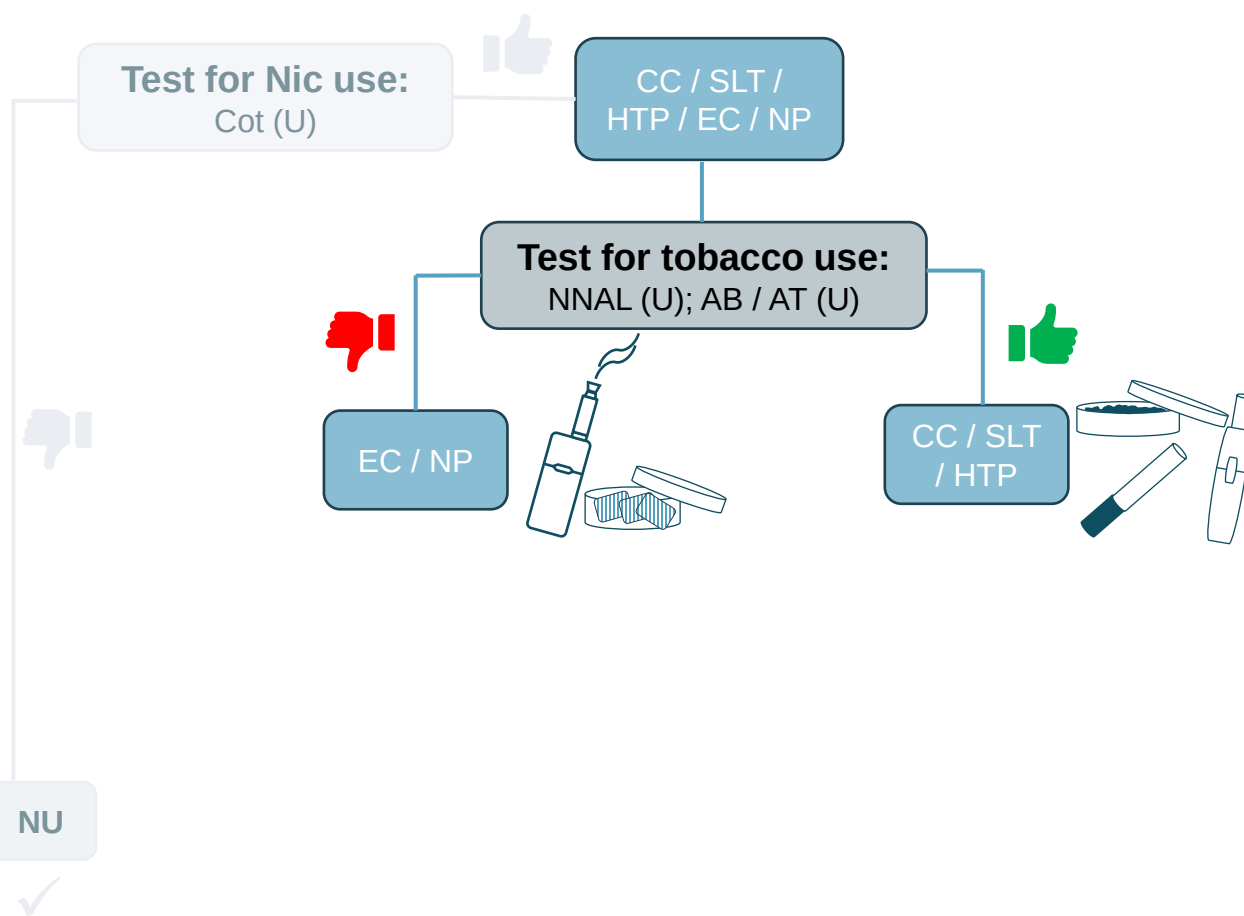
NNAL, AB/AT uptake by different nicotine/tobacco user groups

HNB = HTP, OT = SLT, NG = Nicotine gum



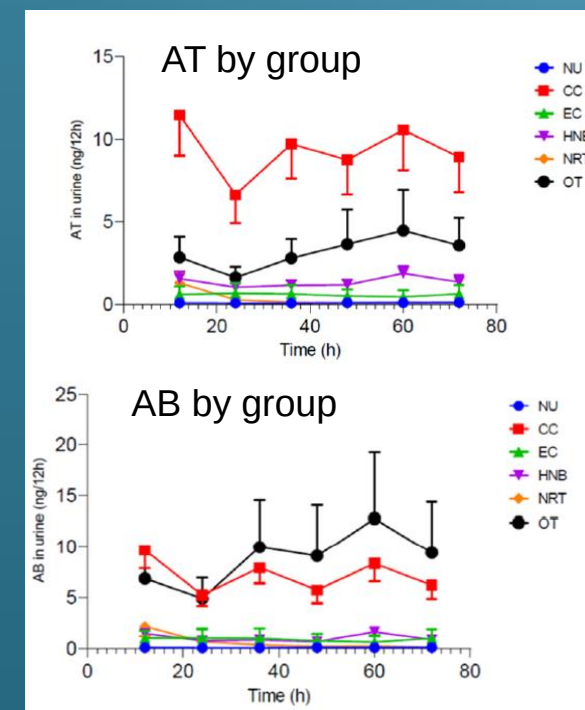
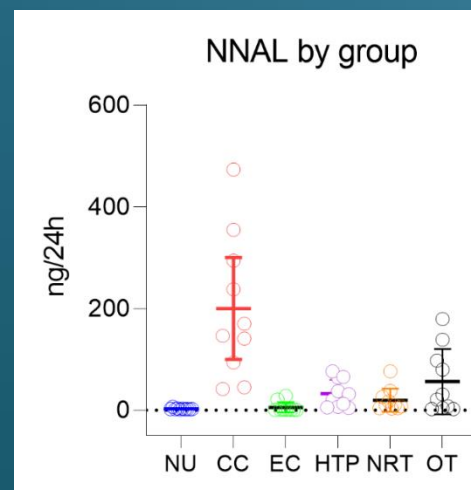
Ref. G. Scherer, M. Scherer, J. Mutze, T. Hauke, N. Pluym, Assessment of the Exposure to Tobacco-Specific Nitrosamines and Minor Tobacco Alkaloids in Users of Various Tobacco/Nicotine Products, *Chem Res Toxicol* 35(4) (2022) 684-693.

Decision tree



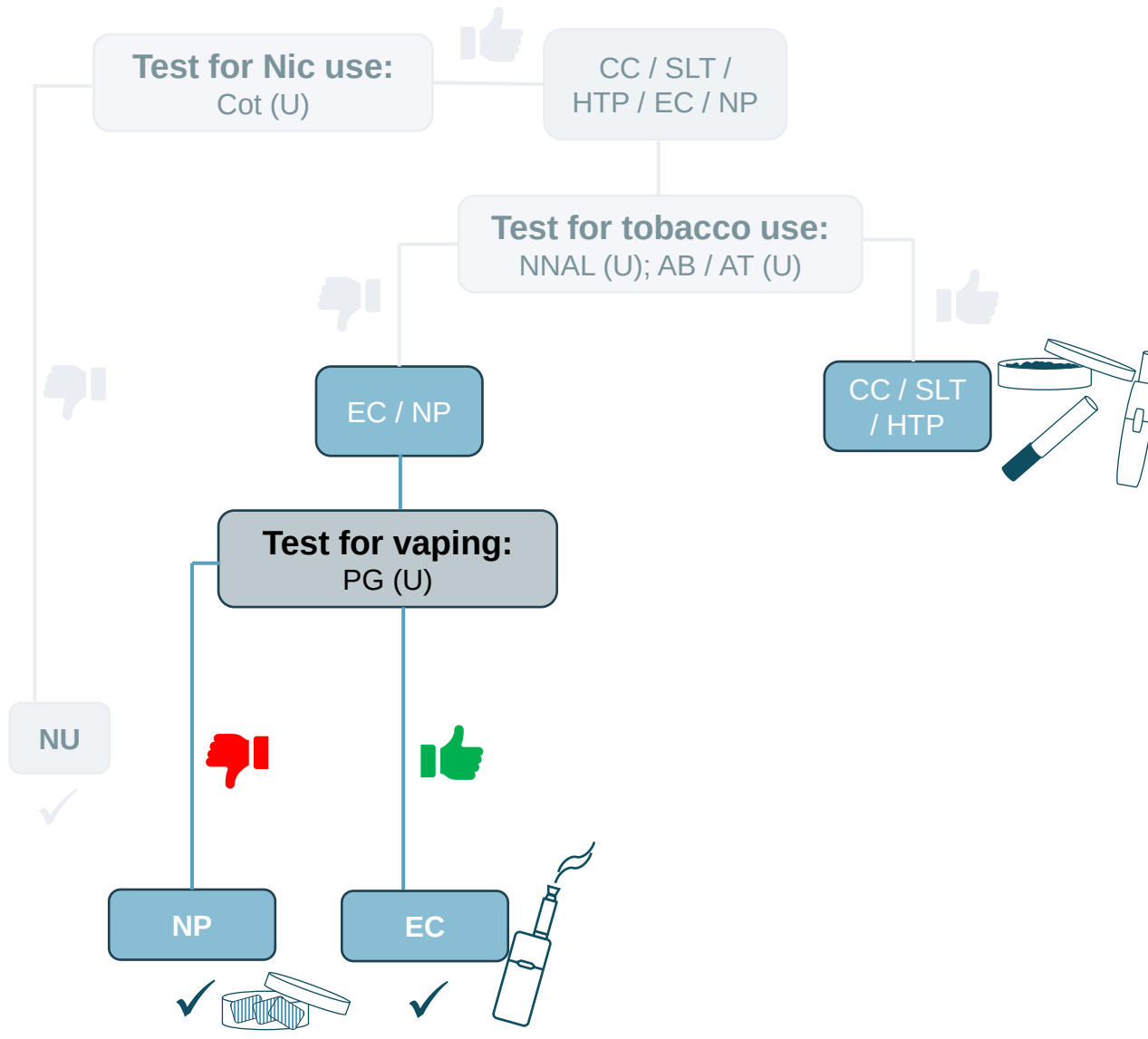
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HNB = HTP, OT = SLT, NG = Nicotine gum

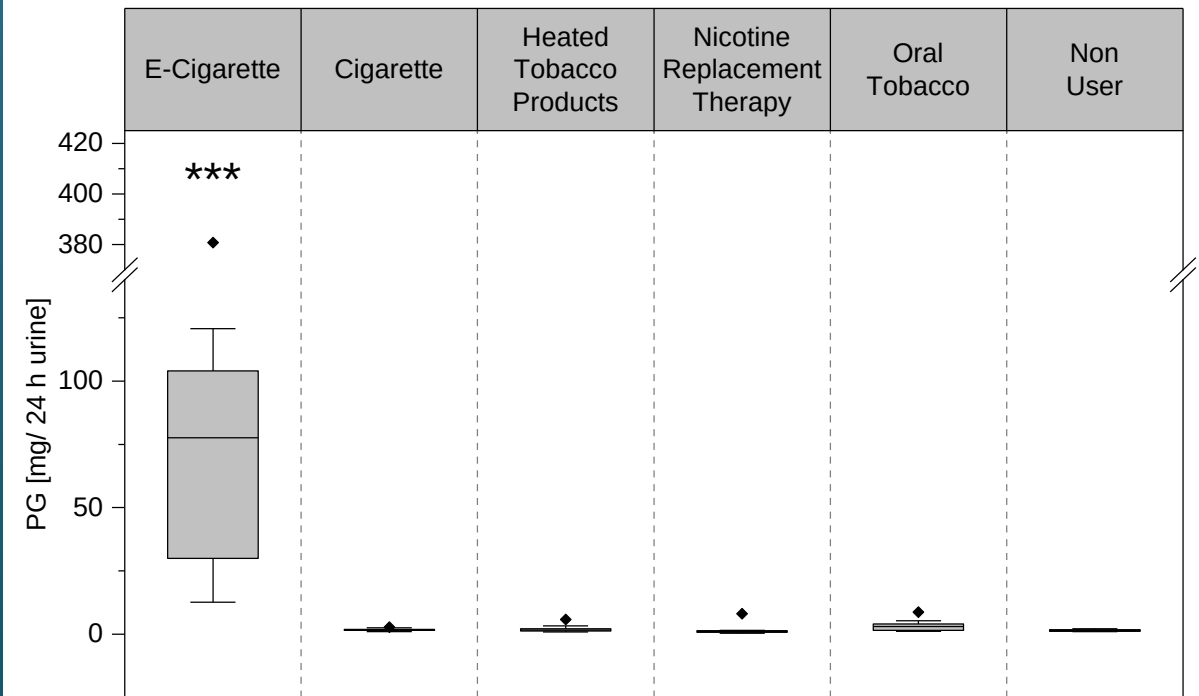


Ref. G. Scherer, M. Scherer, J. Mutze, T. Hauke, N. Pluym, Assessment of the Exposure to Tobacco-Specific Nitrosamines and Minor Tobacco Alkaloids in Users of Various Tobacco/Nicotine Products, *Chem Res Toxicol* 35(4) (2022) 684-693.

Decision tree

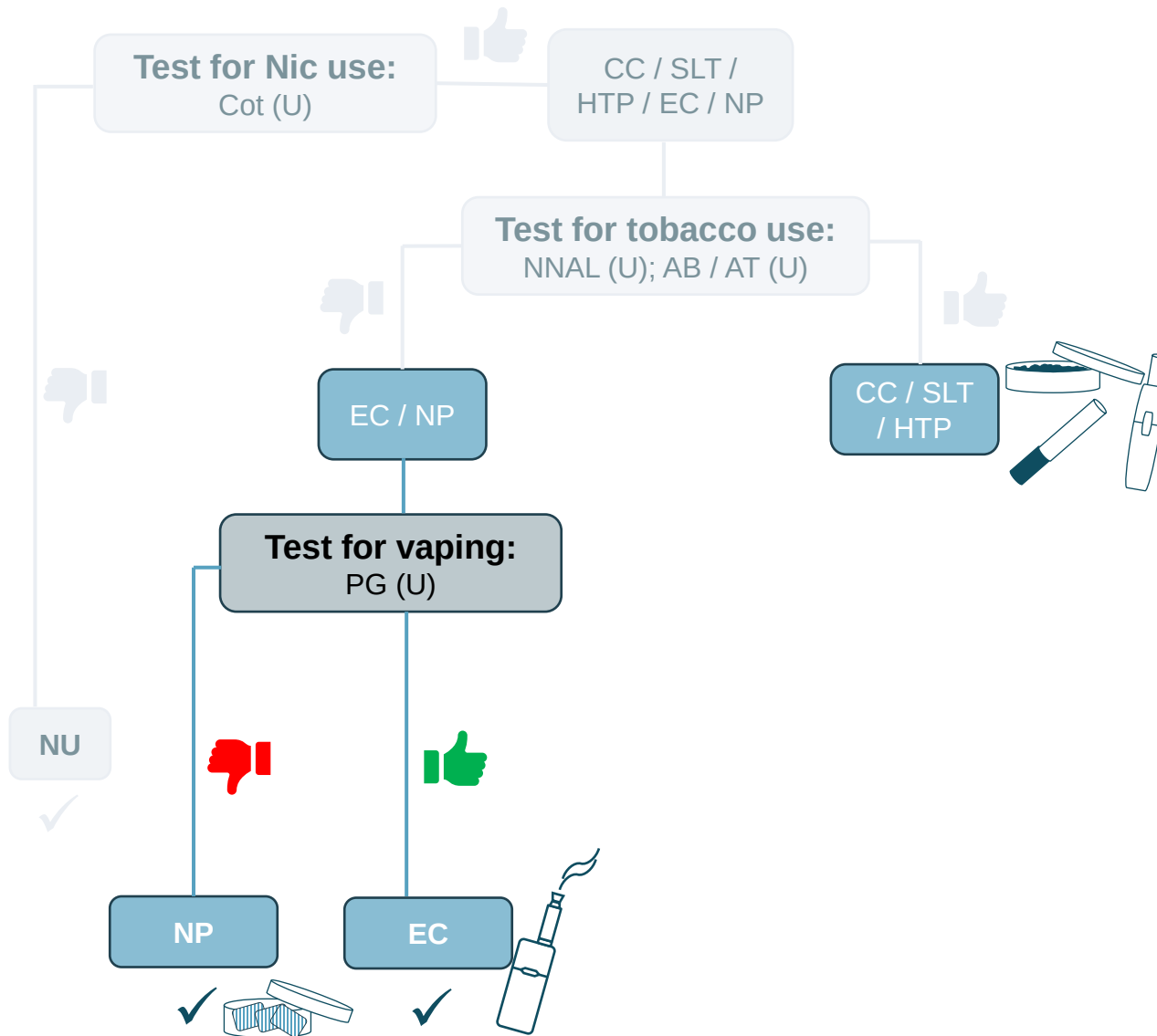


PG as specific biomarker to discriminate EC vaping from other groups

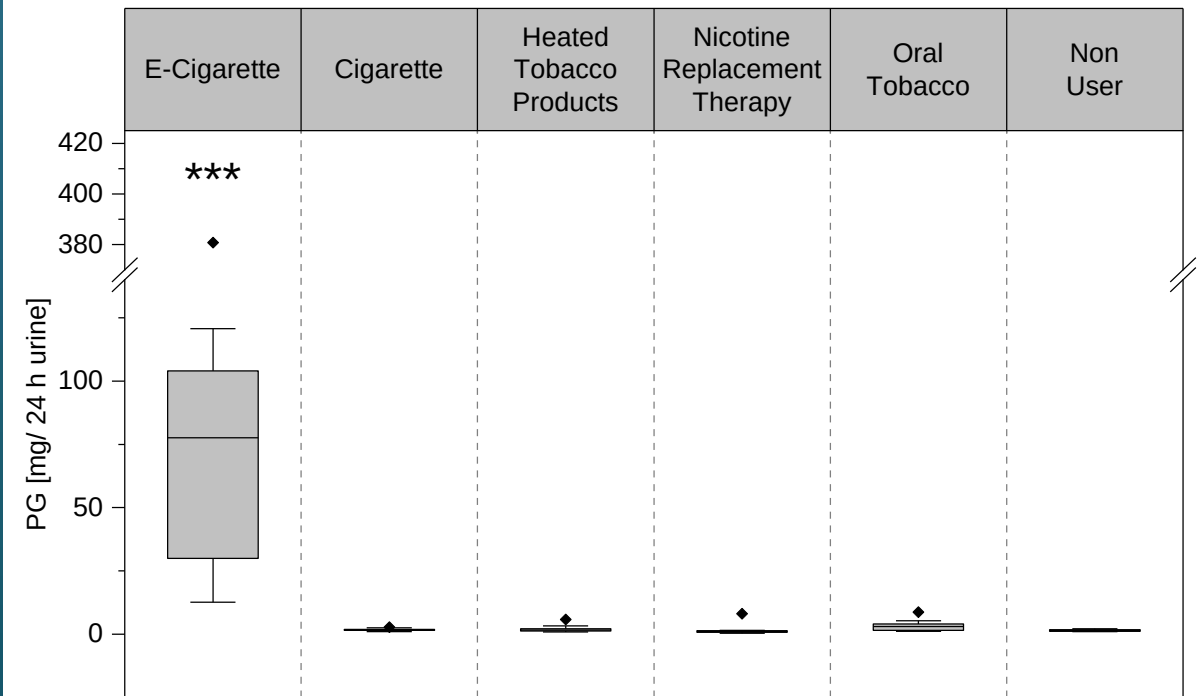


Ref. T. Burkhardt, N. Pluym, G. Scherer, M. Scherer, 1,2-Propylene Glycol: A Biomarker of Exposure Specific to e-Cigarette Consumption, Separations 8(10) (2021) 180.

Decision tree

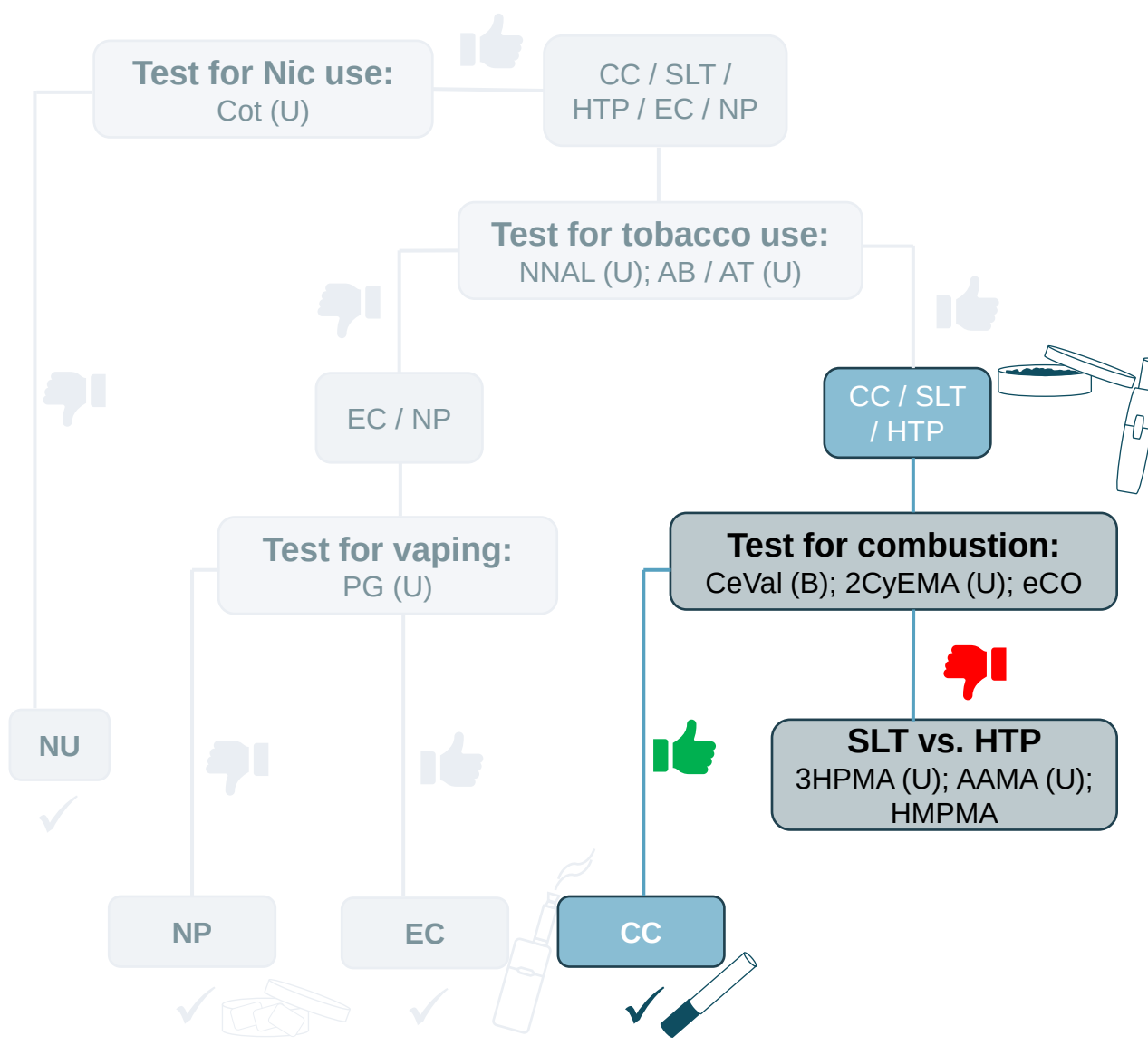


PG as specific biomarker to discriminate EC vaping from other groups



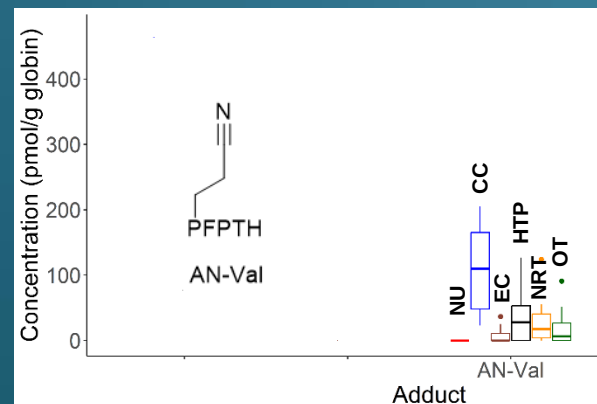
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Decision tree



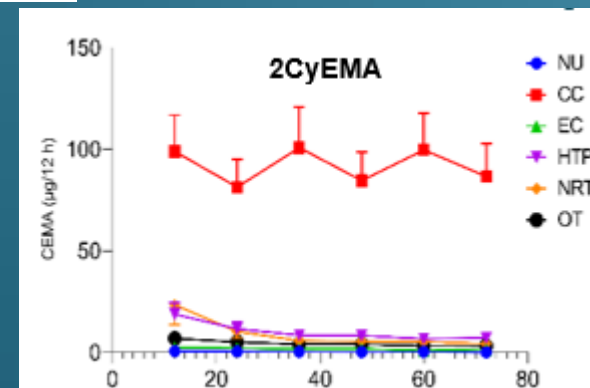
Combustion markers to differentiate smoking

AN = CeVal, OT = SLT, NRT = Nicotine gum

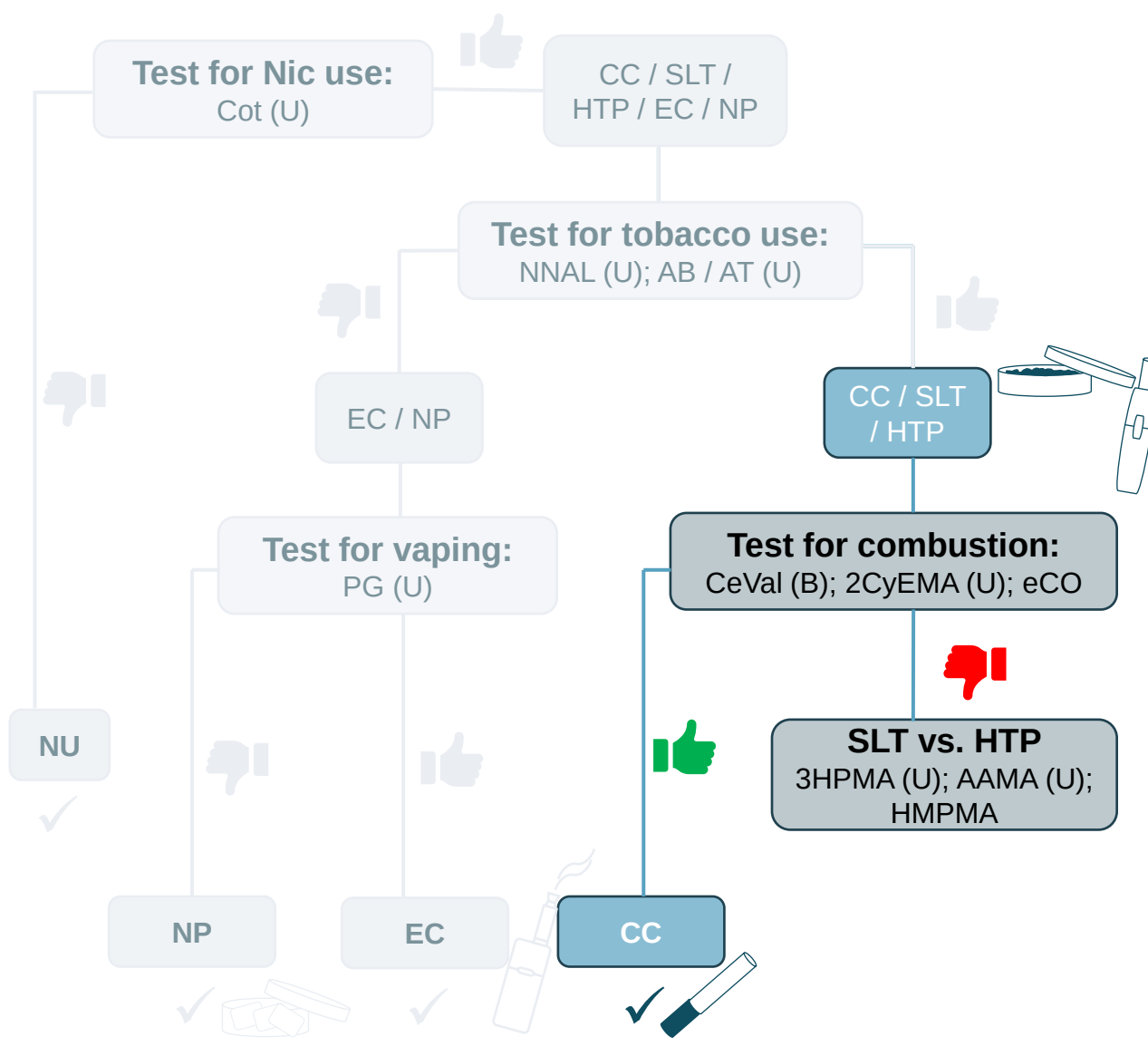


Ref. F. Pilz et al. *Journal Chromatography B* (2024) under revision

Ref. G. Scherer, N. Pluym, M. Scherer, *Comparison of urinary mercapturic acid excretions in users of various tobacco/nicotine products, Drug Test Anal* 15(10) (2023) 1107-1126.

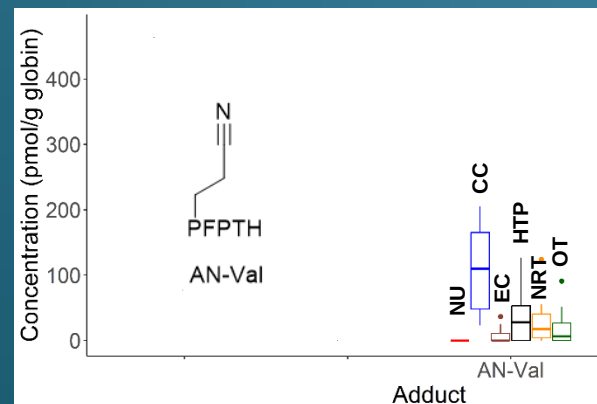


Decision tree



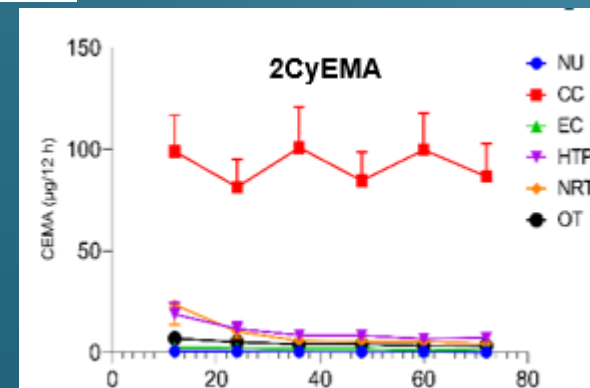
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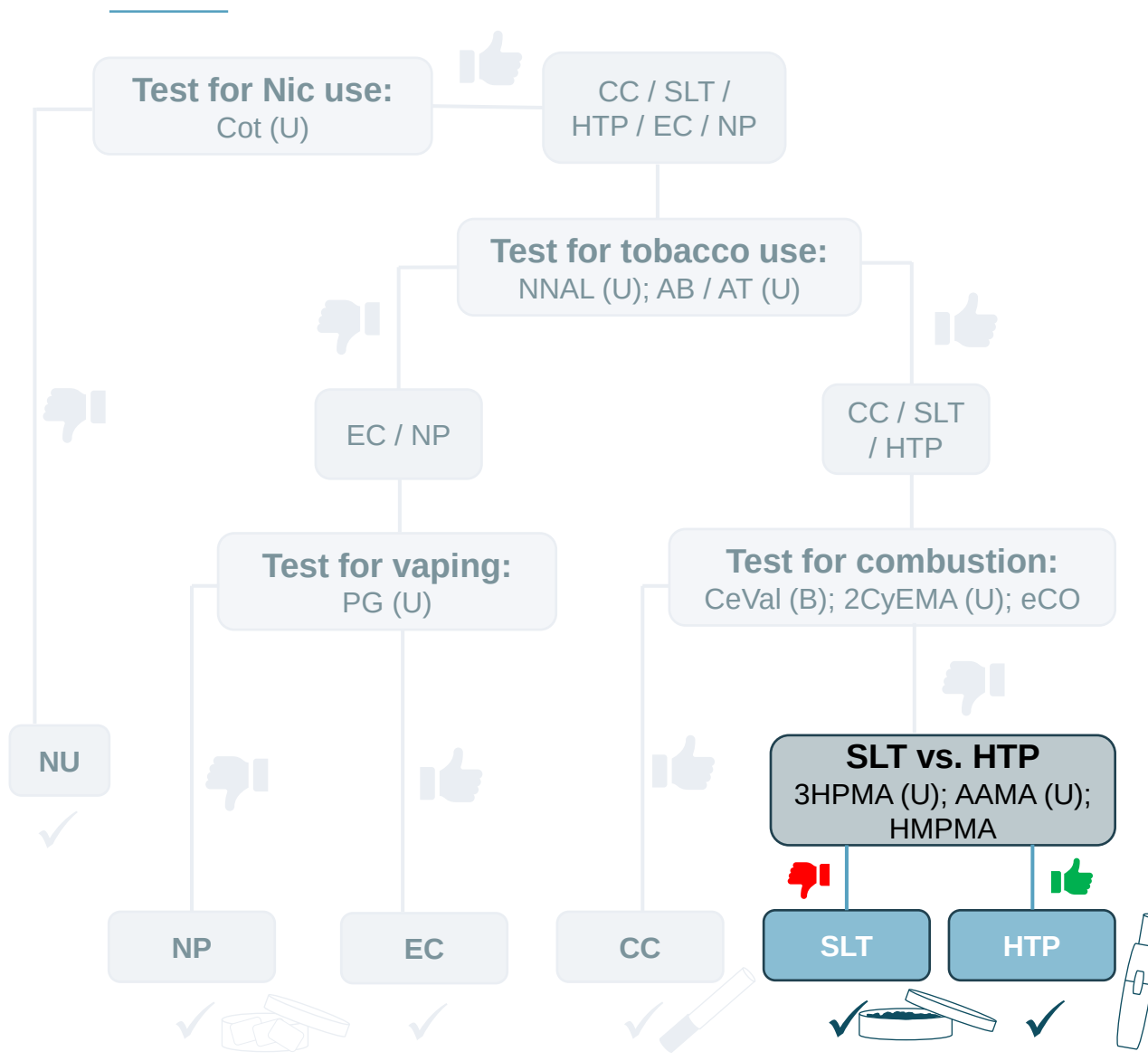


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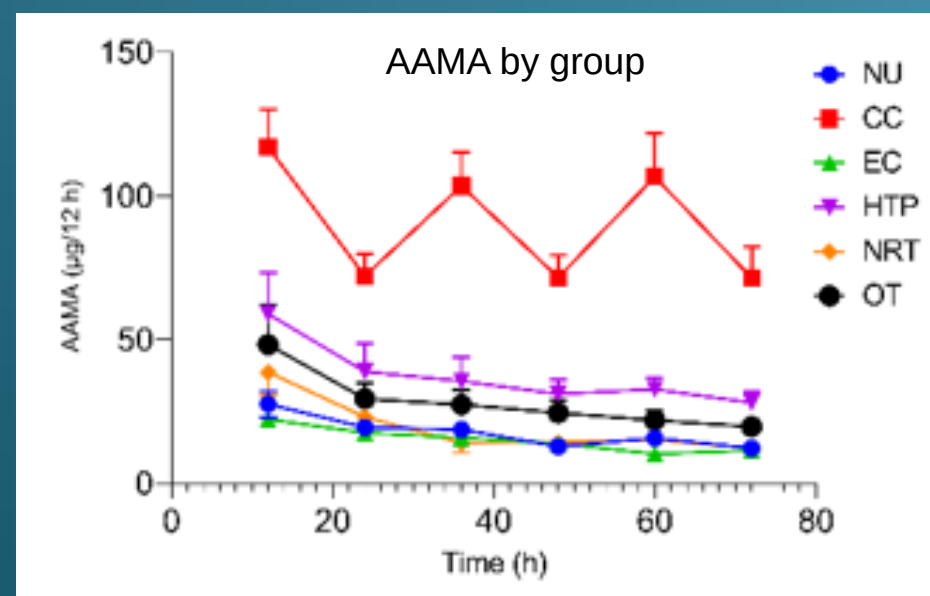


Decision tree



AAMA as potential biomarker to differentiate SLT from HTP group

OT = SLT, NRT = Nicotine gum



Ref. G. Scherer, N. Pluym, M. Scherer, Comparison of urinary mercapturic acid excretions in users of various tobacco/nicotine products, *Drug Test Anal* 15(10) (2023) 1107-1126.

Conclusion

- Identification of biomarkers for compliance to the exclusive use of one product category is challenging
- The presented decision tree is intended to help identify suitable compliance marker
- Modifications are certainly required depending on the study design and groups to be distinguished
- A number of biomarkers and their combinations could be useful for long-term field studies
- Cut-off values are only available for a few biomarkers (Cot, 2CyEMA, eCO)
- Additional data are needed to establish missing cut-off values
- Biomarker ratios may increase the certainty instead of the absolute concentrations of a biomarker panel to distinguish between product user groups in the future

How to differentiate between nicotine / tobacco user groups?



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Candidates for Biomarkers of Compliance (4)

Additional Potential Markers

- Volatile Organic Compounds (VOCs)
 - Mercapturic acids of **Acrolein** (3HPMA), **Crotonaldehyde** (3HMPMA), **Isoprene** (IPMA3), **Benzene** (PhMA)
- Aromatic Amines
 - **2-Aminonaphthalene** (2-Nap), **4-Aminobiphenyl** (4-ABP)
- Hydroxylated Polycyclic Aromatic Hydrocarbons (OH-PAHs)
 - **1-hydroxy-naphthalene** (1-OH-Nap)
- Tobacco-Specific Nitrosamines (TSNAs)
 - **Total NNAL, total NAB**
- Minor Tobacco Alkaloids
 - **Anabasine, anatabine, nicotine**

VOCs, Aromatic Amines, OH-PAHs

- Half-life and confounding factors such as dietary intake as well as occupational and ambient air exposure needs to be considered
- Product-specificity to a certain nicotine user group has to be given
- 3HPMA showed elevated levels in both CC smokers and HTP users, albeit at a much lower degree in the latter

Minor tobacco alkaloids and TSNAs

- Specific to discriminate tobacco user (CC, HTP, SLT) from non-tobacco user groups (EC, NP)
- Suitability of Nicotelline is limited by very short half-life (2-3 h)
- NNAL seems superior with an average half-life of 10 – 40 days