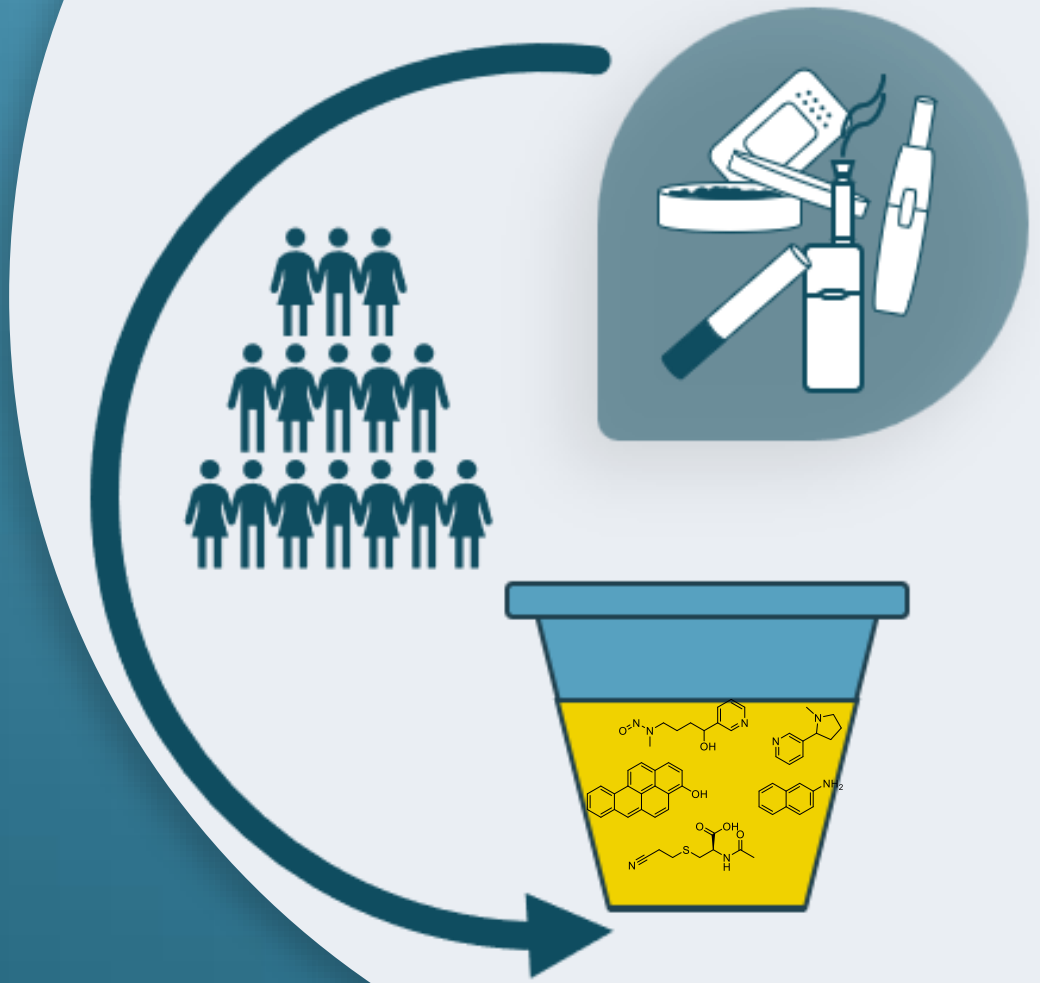
Beyond cotinine: Implementing a comprehensive biomarker panel for nicotine product use monitoring in Europe



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Human Biomonitoring in Europe

- Harmonisation at the European level
- Agree on a harmonised method portfolio
- Laboratory network
- Identification / Closure of data gaps
- Establish reference values
- Link exposure to health outcomes
- Monitor time trends
- Observe effectiveness of regulatory measures

PARC T4.1.2.: Selected biomarkers of exposure for PARC Aligned Studies

Partnership for the Assessment of Risks from Chemicals

PARC T4.1.2.: Selected biomarkers of exposure for PARC Aligned Studies

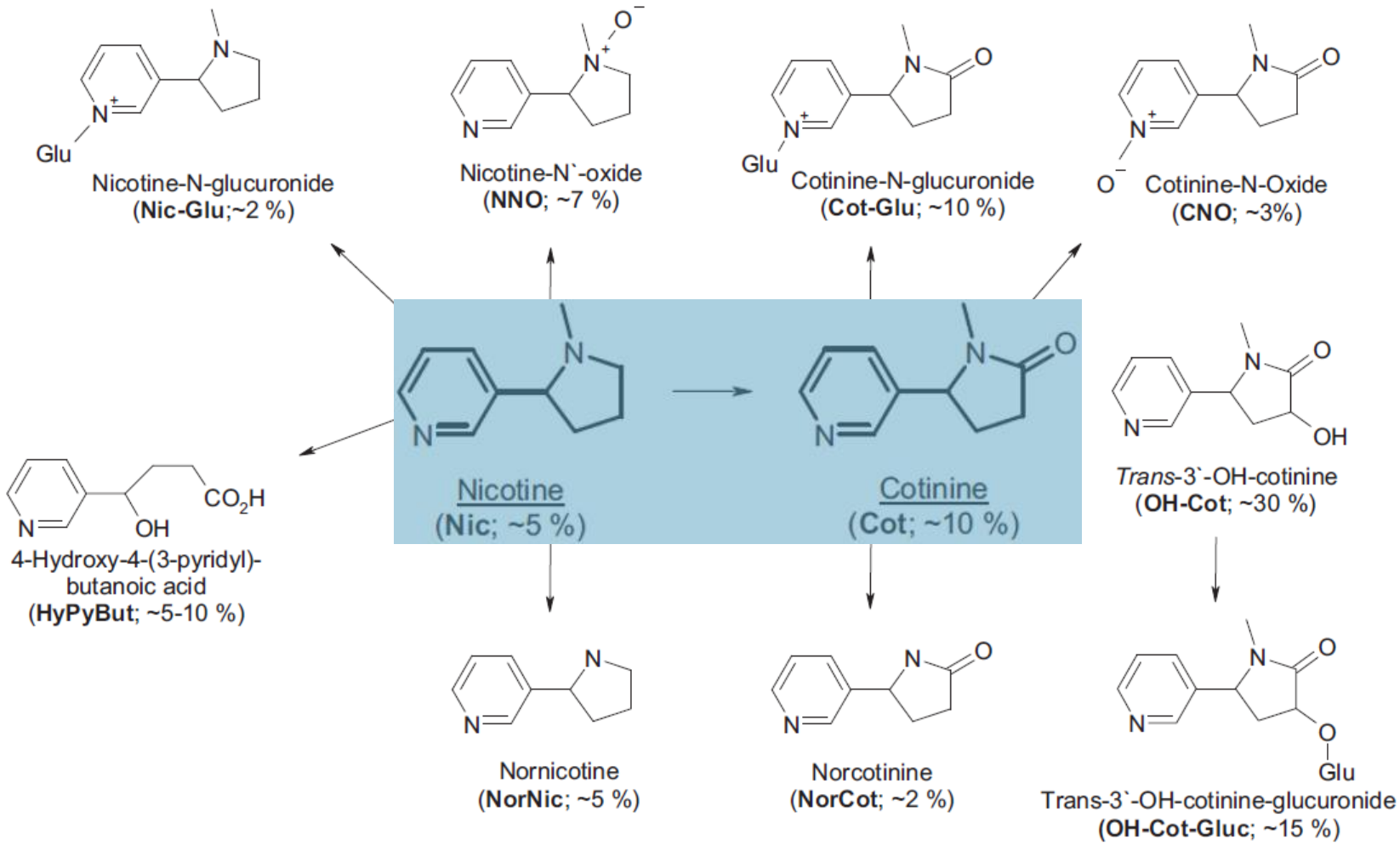
PARC Partnership for the Assessment of Risks from Chemicals

Co-funded by the European Union

This partnership has received funding from the European Union's Horizon Europe research and innovation program under Grant Agreement No 101057014.

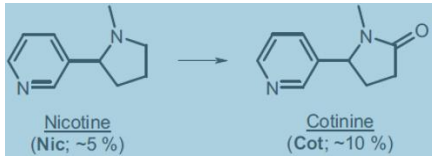
- Bisphenols
- Phthalates
- PFAS
- Pesticides
- Metals
- Cotinine

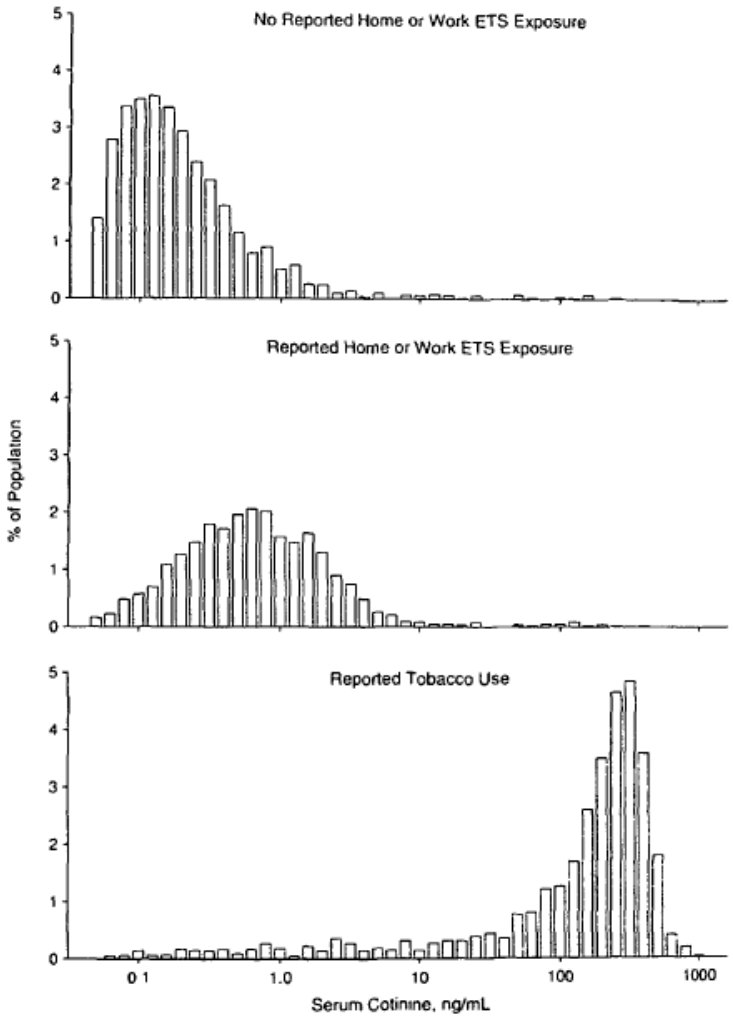
Nicotine metabolism

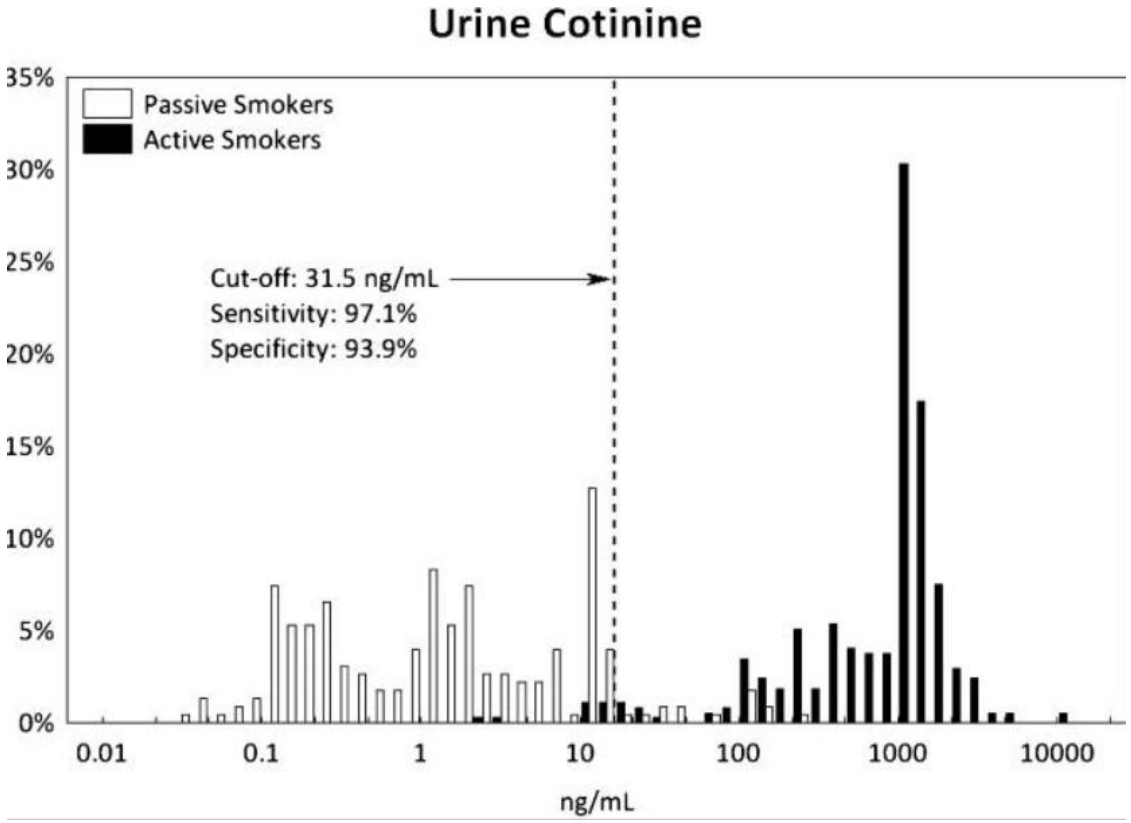


M. Piller et al. / J. Chromatogr. B 951– 952 (2014) 7– 15

Cotinine: Biomarker of smoking & passive smoke exposure







Benowitz, Epidemiol Rev Vol 18, No 2, 1996; Goniewicz ML et al. Comparison of urine cotinine and the tobacco-specific nitrosamine metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and their ratio to discriminate active from passive smoking. Nicotine Tob Res. 2011

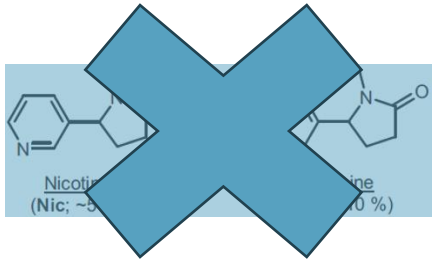
The changing landscape of nicotine products

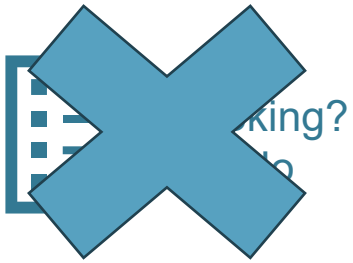
Combustible cigarette (CC)

Heated tobacco products (HTP)

Electronic cigarette (EC)

Snus





Nicotine pouches (NP)

Identification of product-use specific biomarkers

- Exclusive, sole users of 5 different nicotine product categories and non-users as control
- 3 days confinement (ad lib use, diet-controlled)
- Biospecimens: 24h-urine, plasma, saliva, erythrocytes, and exhaled breath

General study objective

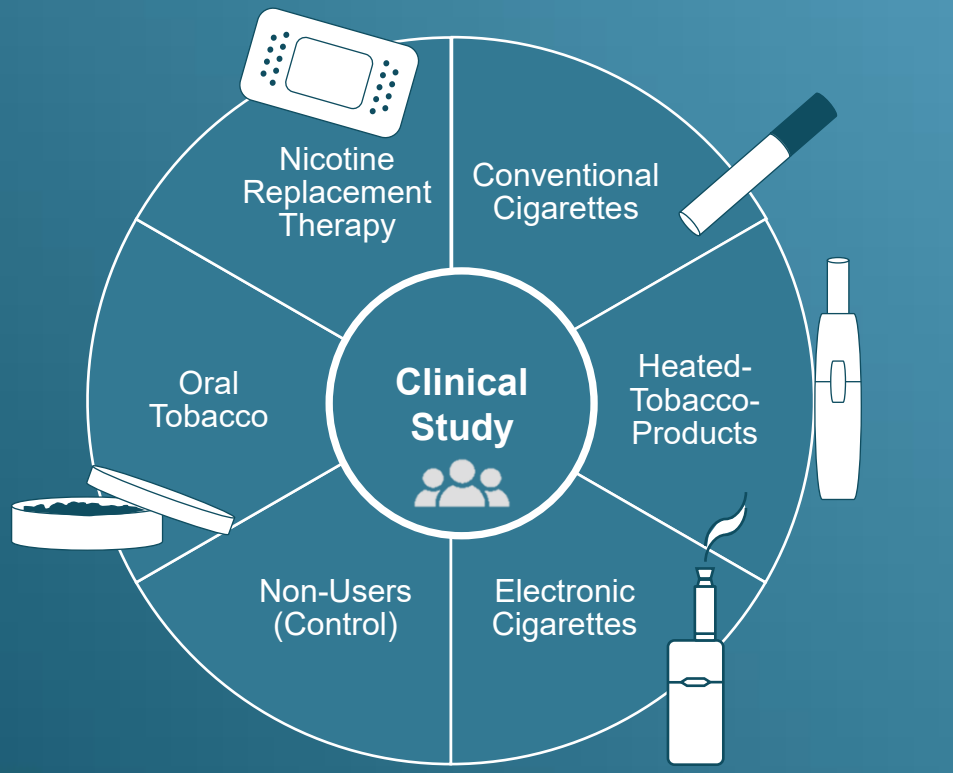
Identify biomarkers or biomarker patterns capable to discriminate between different nicotine product user groups and non-users. Focus on differentiation users of new products ⟷ smokers and non-users.

Top-Down Approach

Non-targeted identification of compounds/metabolites elevated in one use group by high-resolution LC-MS (**Exposomics**)

↓

Verification by targeted, quantitative analyses



10 subjects per group

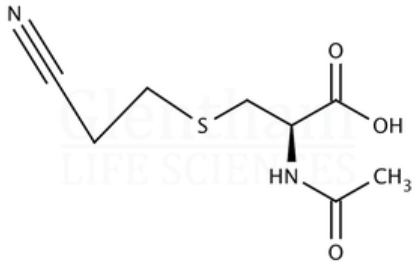
Day 1 → Day 2 → Day 3

Sibul et al (2021): Identification of biomarkers specific to five different nicotine product user groups: Study protocol of a controlled clinical trial. *Contemporary Clinical Trials Communications*, 22

Identification of product-use specific biomarkers: CC

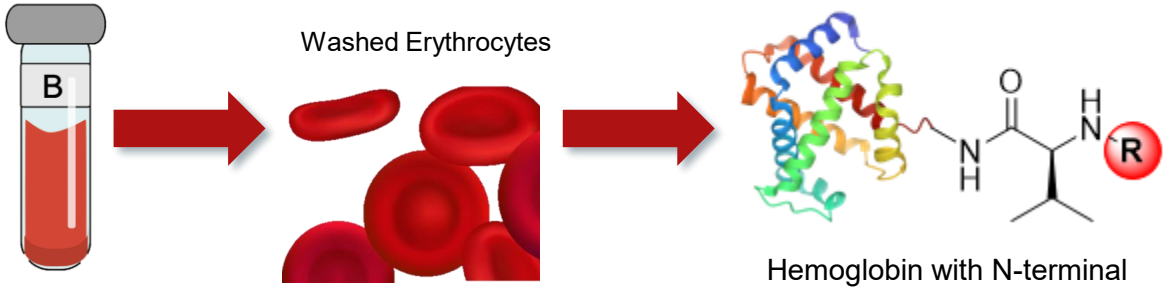
Acrylonitrile exposure

- Biomarker in urine



2-Cyanoethyl mercapturic acid (2CyEMA)

- Cyanoethyl valine hemoglobin adduct



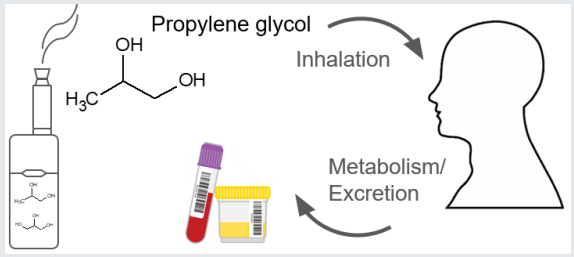
Whole blood

Hemoglobin with N-terminal adduct (R)

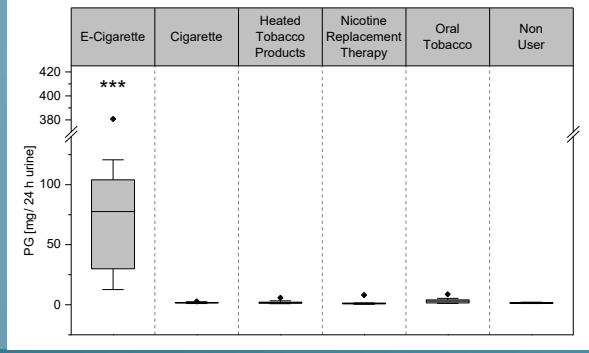
- Exposure to the combustion product acrylonitrile is highly specific to smoking
- 2CyEMA in urine is the ideal candidate to discriminate between combustion and non-combustion products
- The US Centers for Disease Control (CDC) proposed a cut-off of 7.32 ng/mL to classify people as smokers of CC
- The hemoglobin adduct formed from acrylonitrile (CeVal) has a detection period of 60-80 days
- CeVal is capable to detect past smoking over a longer time-frame compared to 2CyEMA
- 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

Identification of product-use specific biomarkers: EC/HTP/Pouch use

- **Biomarkers for the verification of vaping ECs**



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



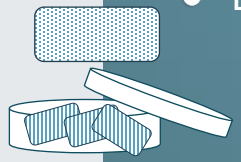
- **Biomarkers for the verification of HTP use**
  - Mercapturic acids (Acrylamide, Acrolein, Crotonaldehyde)
  - TSNAs (total NNAL, total NAB)

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

- **Biomarkers for the verification of SLT use**
  - Minor tobacco alkaloids (AB, AT)
  - TSNAs (total NNAL, total NAB)

- 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

- **Biomarkers for the verification of NP use**
  - Nicotine metabolites (cotinine)



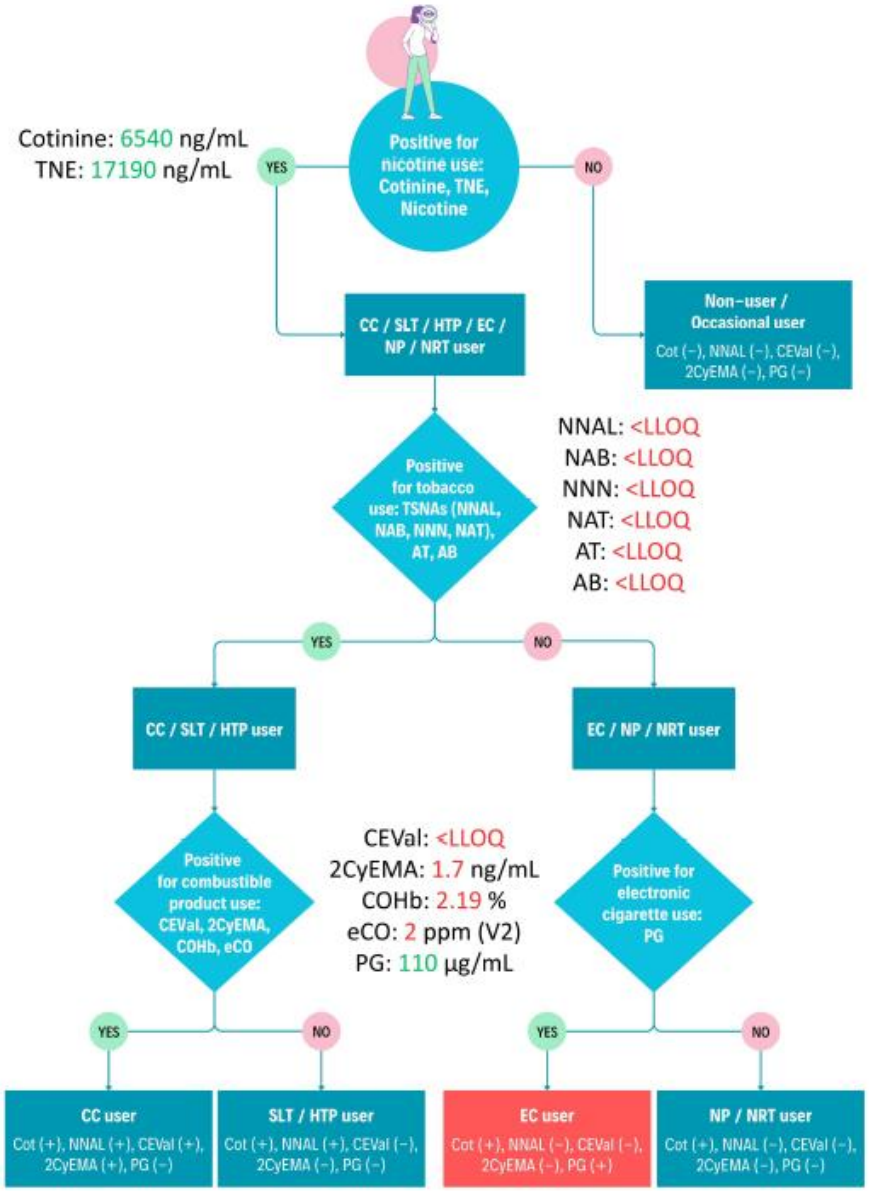
- Exposure to only nicotine, lack of tobacco-specific as well as combustion markers

Biochemical verification of product-use status

Identification of non-compliant subjects in a study with 60 smokers, 60 former smokers and 60 non-smokers:

Biomarkers of exposure

Nicotine use	**Cotinine** + Robust threshold level available + Moderate detection period (3 - 4 days)	**TNE** + Broader coverage - No threshold level available	
Tobacco use	**NNAL** + Robust threshold level available + Long detection period (30 - 90 days)	**NAB, NAT, NNN, AT, AB** + Supportive information for NNAL - Short detection periods	
CC use	**CEVal** + Threshold level + Long detection (60 - 80 d)	**2CyEMA** + Robust threshold - Short detection (30 h)	**eCO, COHb** + Rapid, inexpensive - Short detection (2 - 8 h)
EC use	**Propylene Glycol (PG)** + Specific and sensitive detection of EC use - Short detection period (4 - 8 h); no robust threshold currently available		



Sajid et al. J. Environ. Expo. Assess. 2026, 5, 7

US PATH Study

PATH Population Assessment of Tobacco and Health

- US Congress gave FDA authority to regulate tobacco products in 2009 (Tobacco Control Act)
- Population Assessment of Tobacco and Health (PATH) Study is a national longitudinal study of tobacco and nicotine use and how it affects the health of people in the United States since 2013
- Research Objectives:
  - Use transitions between products / use prevalence of different nicotine/tobacco products
  - Product initiation
  - Dual and poly-use of different products
  - How people quit the product(s)
  - Users' and non-users' product risk perceptions
  - Track potential behavioral and health impacts, including biomarkers of exposure and harm on an individual level and for the general population (public health)

https://pathstudy.nih.gov/

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US PATH Study

PATH Population Assessment of Tobacco and Health

Questionnaires

use, demographics, socioeconomics, poly use, nicotine dependence,

risk perceptions, secondhand smoke exposure, health…

Biomarkers

Biomarkers of exposure:
Nicotine + metabolites, tobacco-specific nitrosamines, OH-PAHs, metals, VOC metabolites (mercapturic acids)…

Biomarkers of potential harm: sICAM, IL6, CRP, PGF2a…

https://www.icpsr.umich.edu/sites/nahdap/home

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Conclusion

- Moving beyond cotinine for accurate differentiation of nicotine/tobacco product use
- Broader biomarker panel established for accurate exposure & risk assessment of smoking and nicotine product use

Tobacco-specific nitrosamine: NNAL
Propylene glycol

VOC metabolites: Mercapturic acids
2CyEMA, AAMA, 3HPMA, 3HMPMA

VOCs have several sources beyond smoking/tobacco:

Mercapturic acids provide valuable information regarding the exposure to VOCs of health concern for general public

Conclusion

- Moving beyond cotinine for accurate differentiation of nicotine/tobacco product use
- Broader biomarker panel established for accurate exposure & risk assessment of smoking and nicotine product use
- Ask the right questions

PATH Population Assessment of Tobacco and Health

Smoking?
Yes/No

Questionnaires in health surveys should take into account the evolving product landscape of nicotine/tobacco products

Conclusion

- Moving beyond cotinine for accurate differentiation of nicotine/tobacco product use
- Broader biomarker panel established for accurate exposure & risk assessment of smoking and nicotine product use
- Ask the right questions

PATH Population Assessment of Tobacco and Health

Suitable **biomarkers and questionnaires** can be introduced into HBM survey with manageable effort

Complementing cotinine with **specific biomarkers** like TSNAs, mercapturic acids and PG will significantly improve the understanding of **prevalence** and **health risks** of **nicotine/tobacco product use** for **European citizens**

Acknowledgment

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