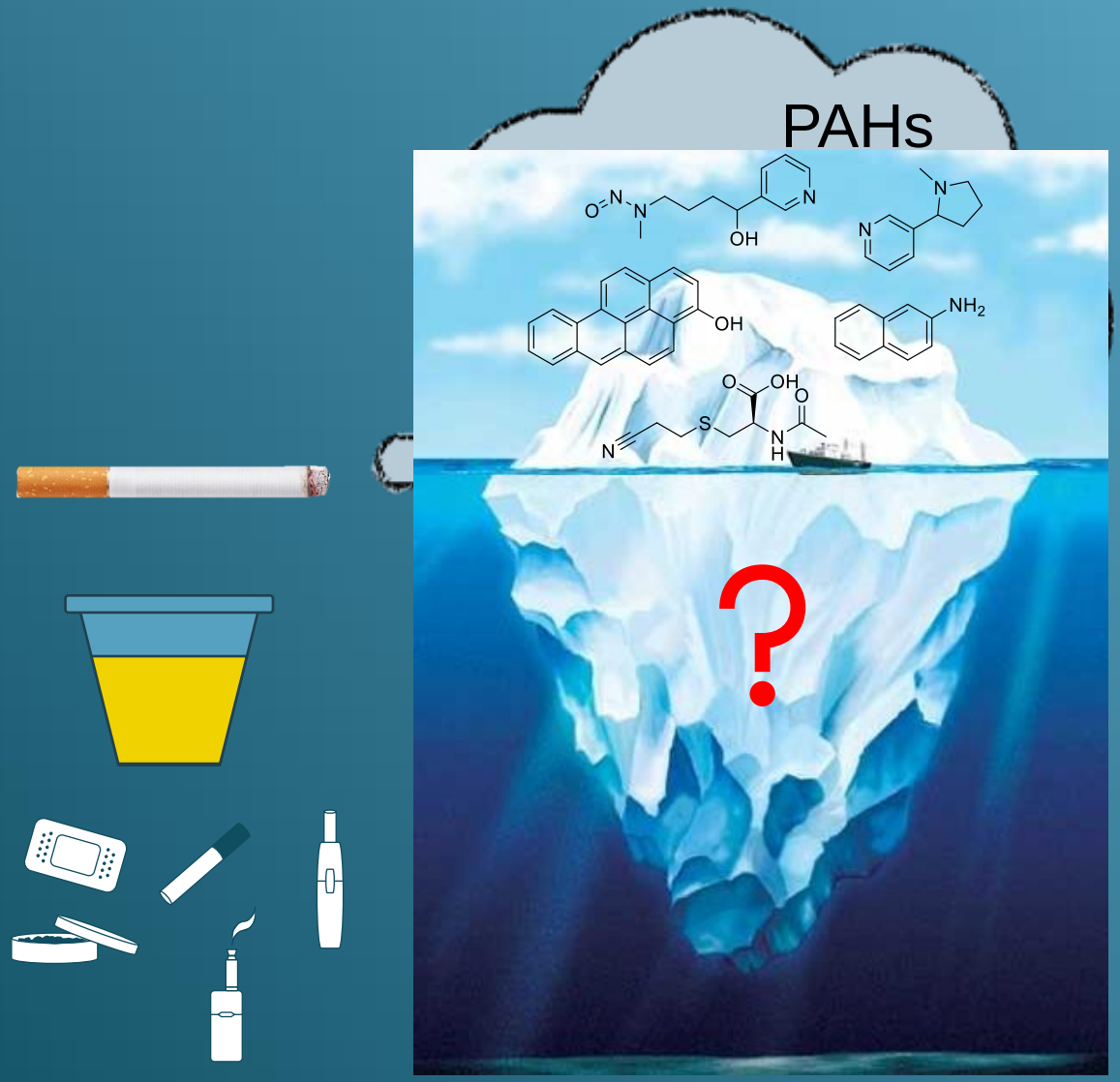
Revealing the urinary exposome of smokers, vapers, HTP users and pouch consumers by high-resolution LC-MS – an important step in the identification of use-specific biomarkers of exposure

A. Kachhadia, N. Pluym, T. Burkhardt, G. Scherer, M. Scherer

8 – 12 October 2023 | CORESTA SSPT 2023 | Cancun, Mexico

The Exposome

- Exposome: the totality of human environmental exposures[1]
- Numerous harmful&potentially harmful constituents (HPHCs) in tobacco smoke
- Current studies in tobacco harm reduction measure biomarkers of exposure (BoE) related to these HPHCs
- Targeted analysis of urinary metabolites of PAHs, VOCs, TSNAs, nicotine, …
- Problem: Those BoEs related to combustion and (some more, some less) specific to smoking combustible cigarettes



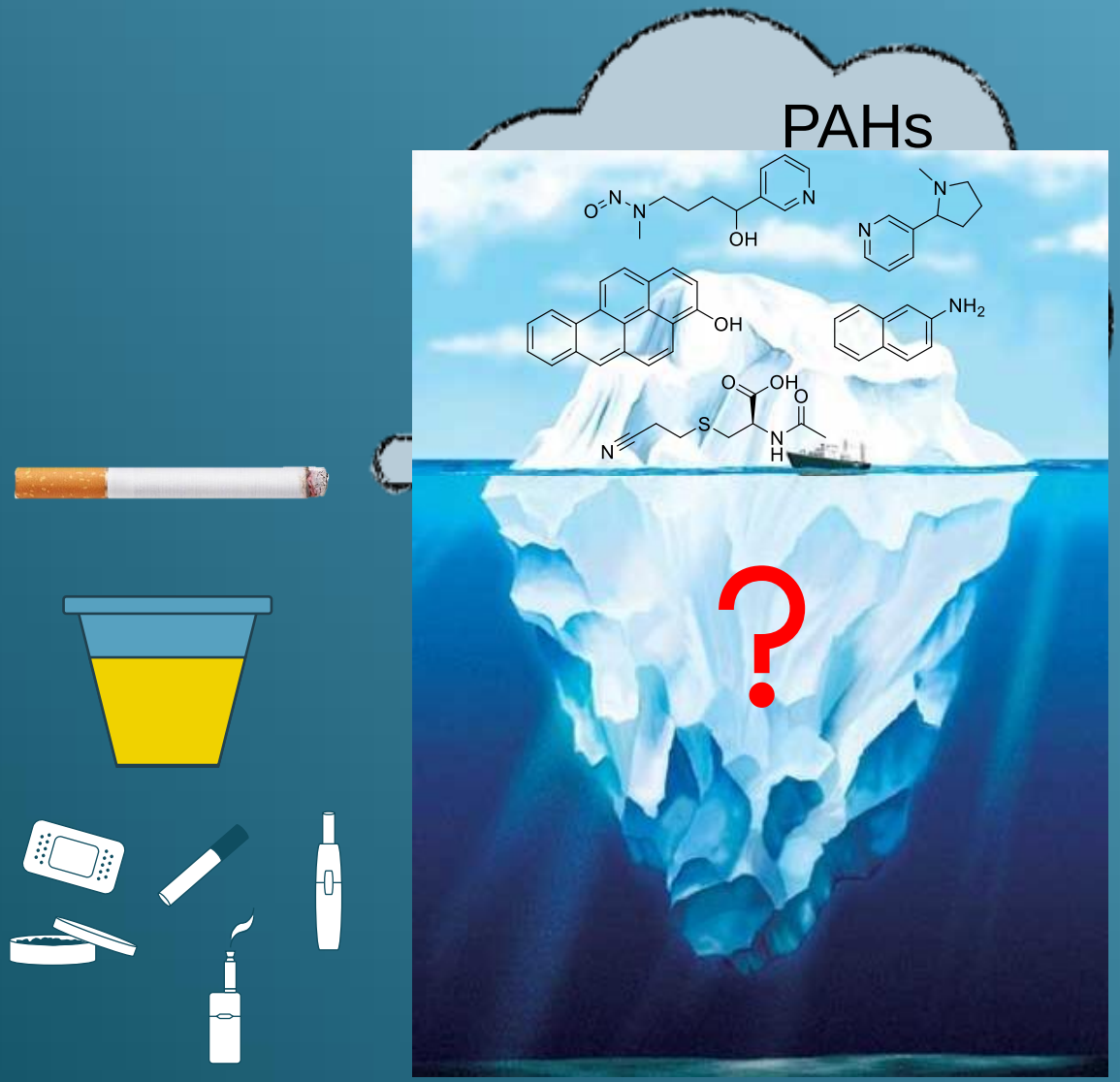
[1] Wild CP. Complementing the genome with an “exposome”: the outstanding challenge of environmental exposure measurement in molecular epidemiology. Cancer Epidemiol Biomarkers Prev 2005;14:1847–50.

The Exposome

Study objective

Decipher the urinary exposome to identify BoE which are capable of differentiating the consumers of different nicotine products

EXPOSURE	**SPECIFICITY**
RISK EVALUATION	**COMPLIANCE**



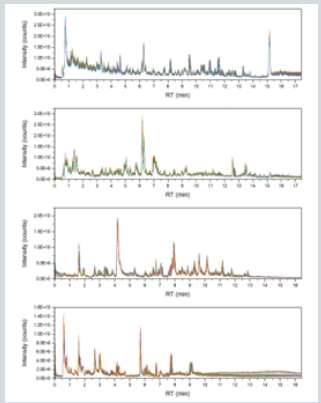
[1] Wild CP. Complementing the genome with an “exposome”: the outstanding challenge of environmental exposure measurement in molecular epidemiology. Cancer Epidemiol Biomarkers Prev 2005;14:1847–50.

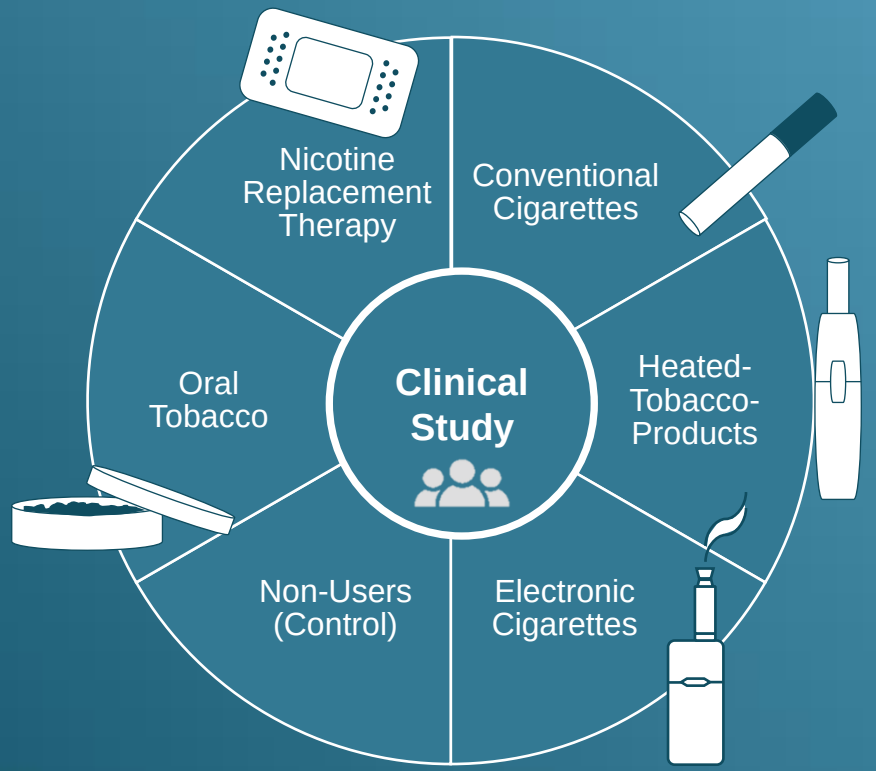
Clinical Study

- 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, blood, saliva, exhaled breath and exhaled breath condensate

Non-targeted analysis in 24h urine by LC-MS (Thermo Qexactive HF-X)





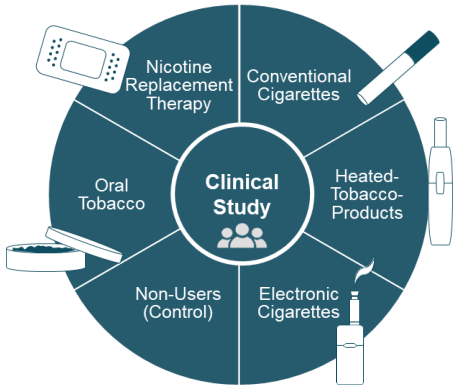


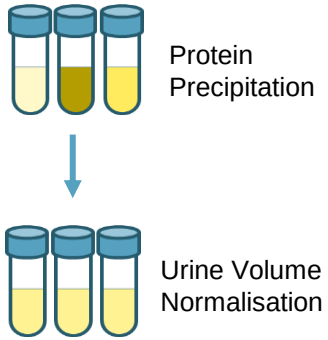
10 subjects per group

Day 1 → Day 2 → Day 3

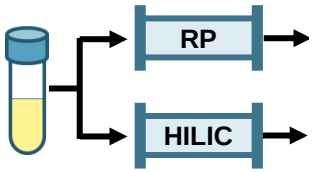
Sibul et al (2021): Identification of biomarkers specific to five different nicotine product user groups: Study protocol of a controlled clinical trial. *Contemporary Clinical Trials Communications*, 22

Non-targeted analysis – Workflow

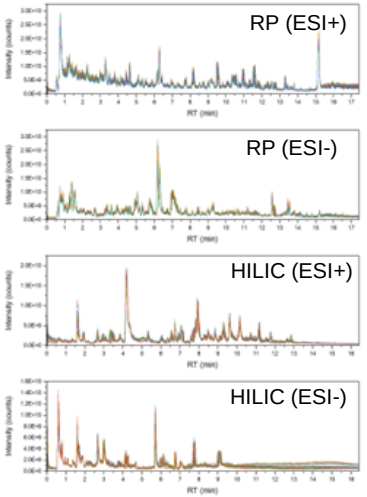




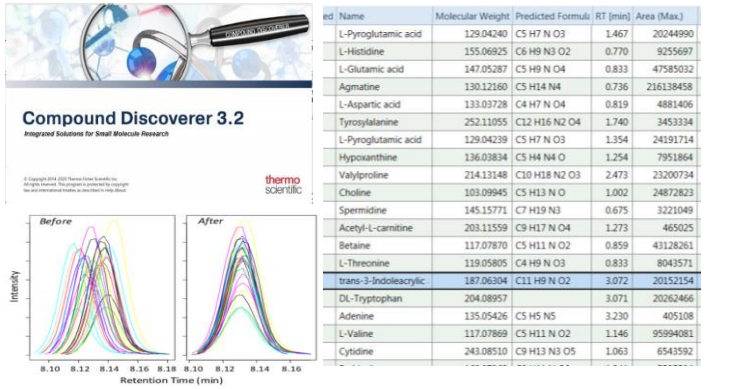
Sample processing



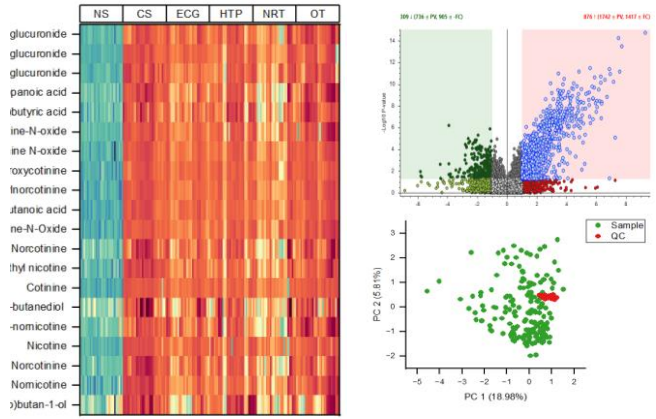




Data acquisition



Data pre-processing:
RT alignment / Intensity correction by QC



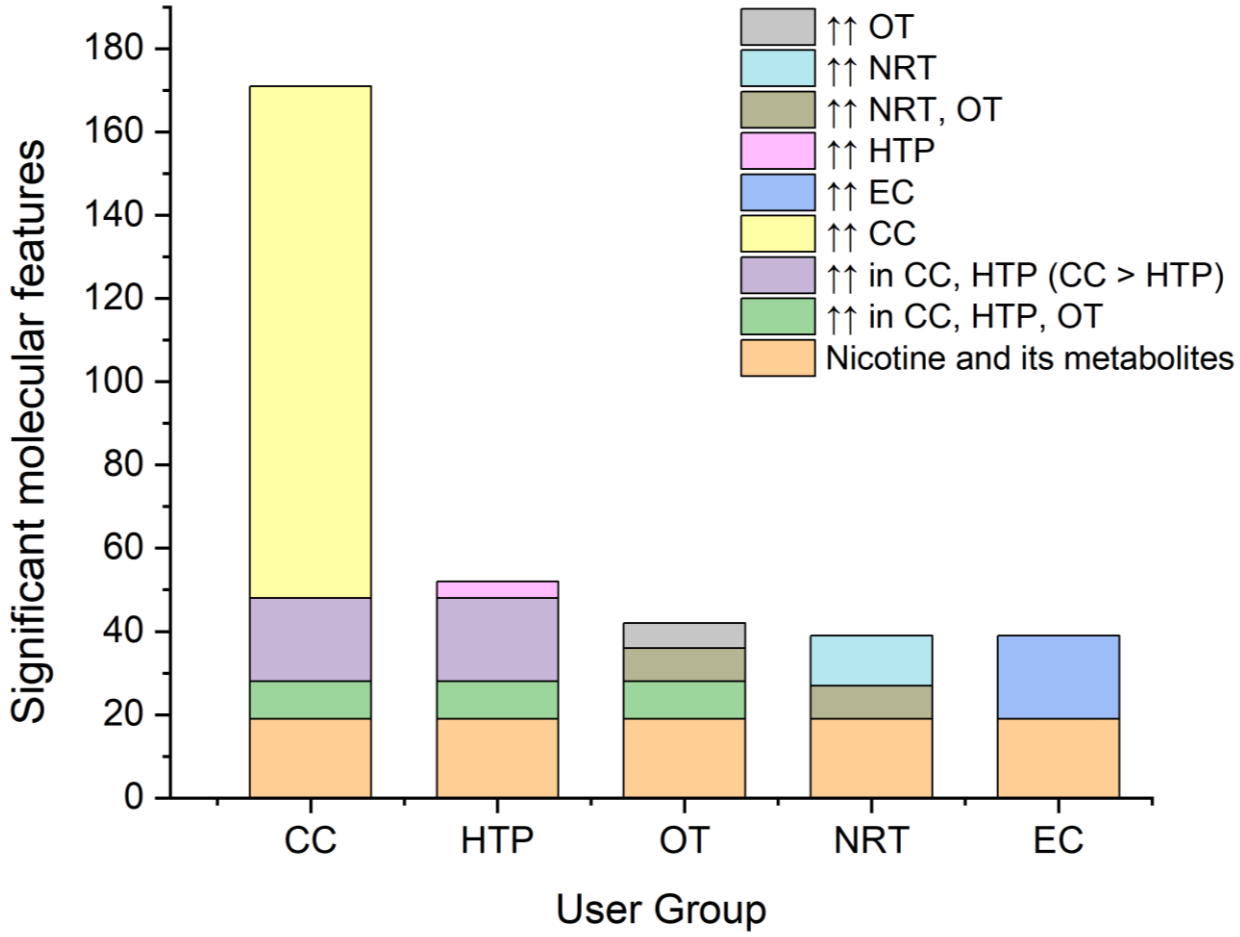
Statistical analysis: multi-group comparison:
$p < 0.05$; fold change (FC) > 2

Metabolite identification:

- Spectral information (exact mass / fragmentation / retention time)
- 3 Levels of confirmation (Level 1 > Level 2 > Level 3):
  - Level 1: Reference standard
  - Level 2: Spectral library
  - Level 3: In-Silico Fragmentation

Urinary Exposome

- A total of 208 molecular features specific to one nicotine product user group vs. NU (↑)
- Nicotine+metabolites identifed for all nicotine use groups (proof-of-concept 👍)
- Majority of elevated features in CC vs NU
- Specific features found for CC (123), NRT (12), EC (7), OT (6), HTP (4)
- Some features elevated in more than one group (e.g., NRT+OT; CC+HTP)

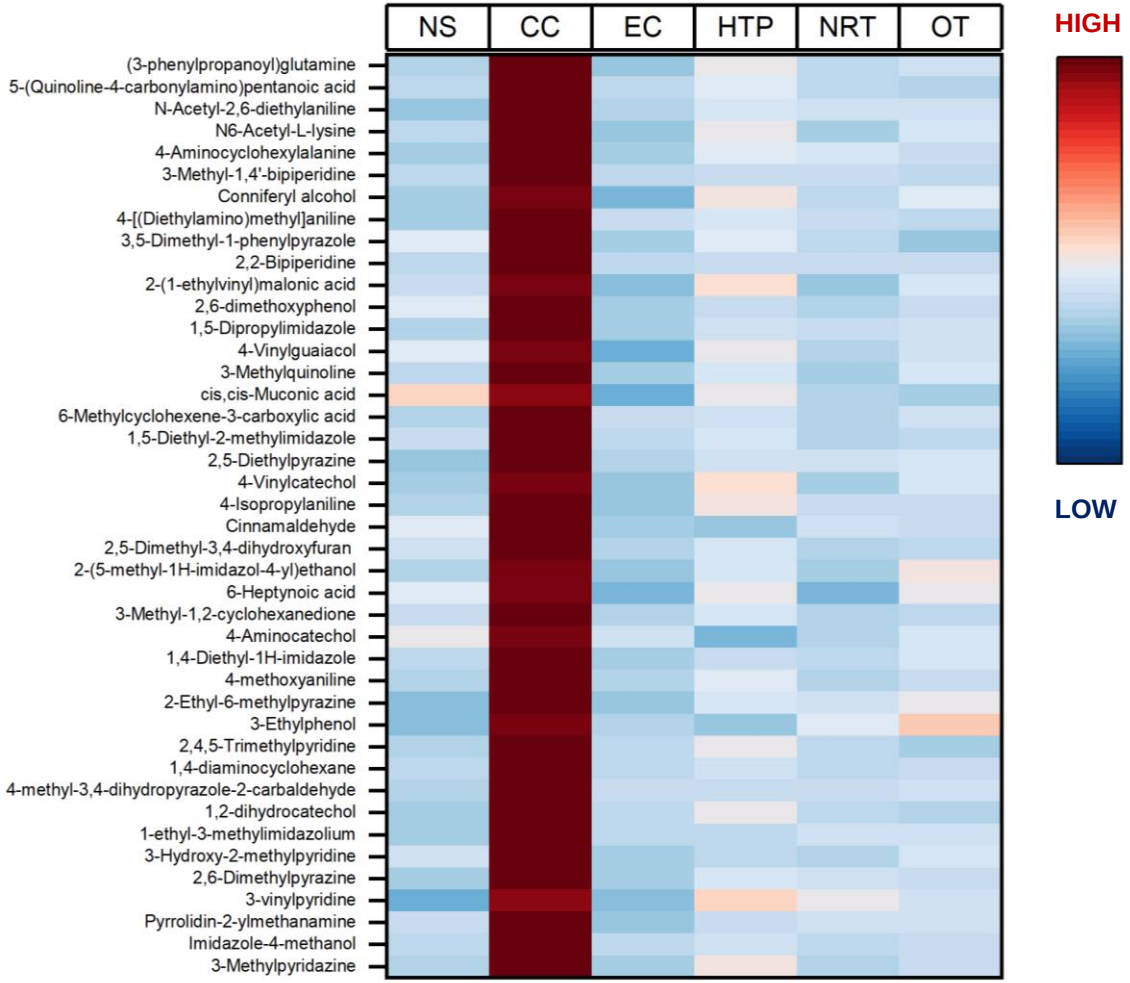


BoE specific to

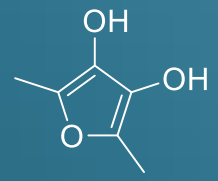
CC



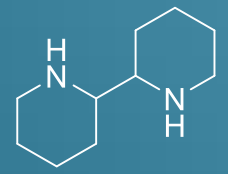
Biomarkers specific to CC users







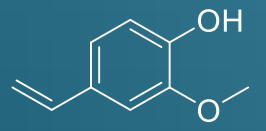
2,6-Dimethyl-3,4-dihydroxyfuran:
2,6-Dimethylfuran metabolite (?)



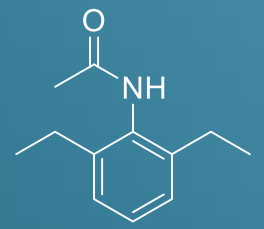
2,2'-Bipiperidine



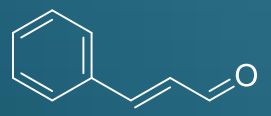
4-Vinylpyridine



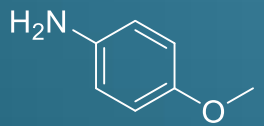
4-Vinylguaiacol



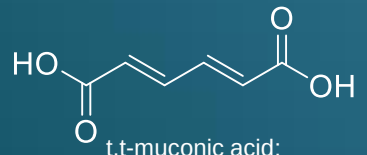
N-Acetyl-2,6-diethylaniline:
2,6-diethylaniline metabolite (?)



Cinnamaldehyde



4-Methoxyaniline

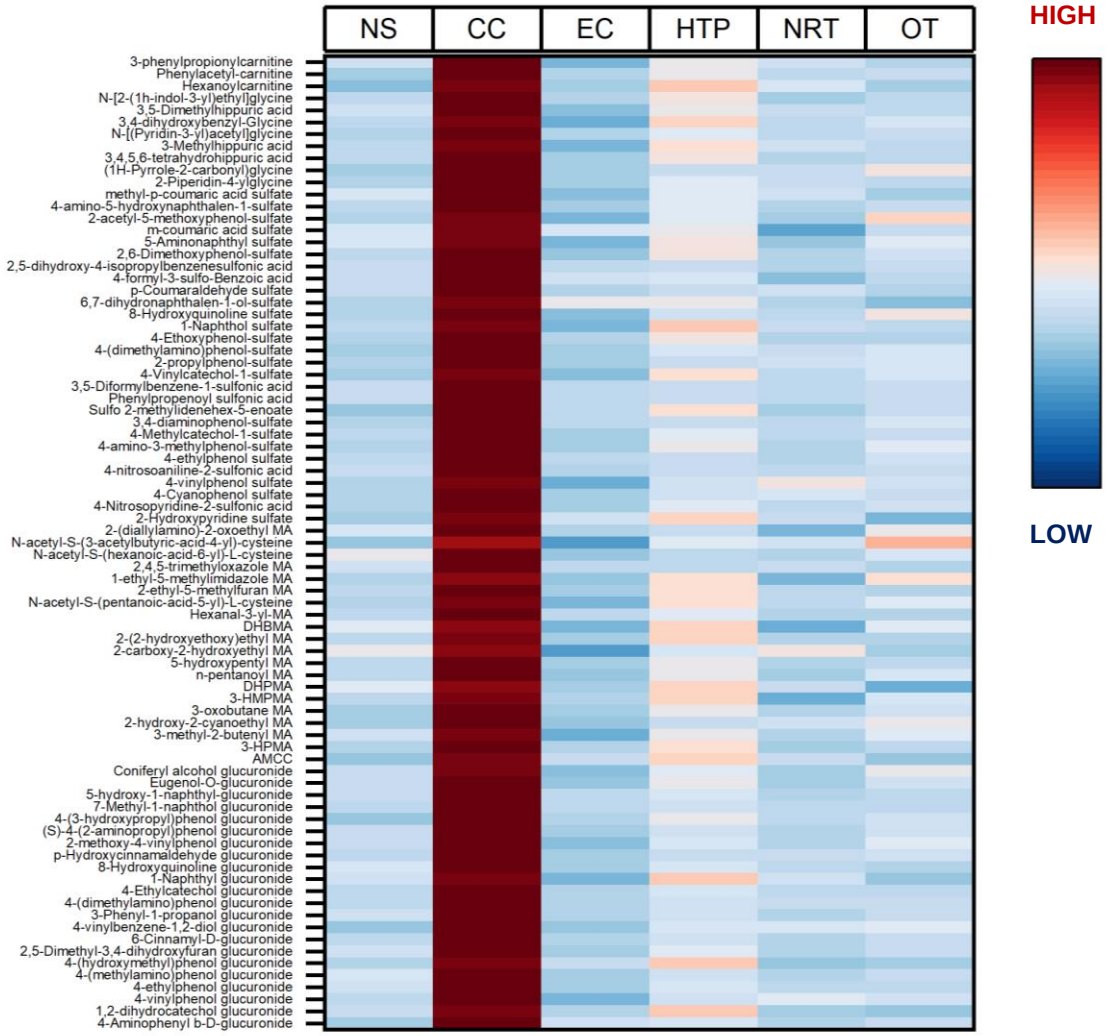


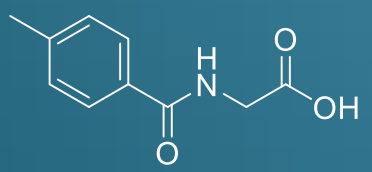
t,t-muconic acid:
Benzene metabolite



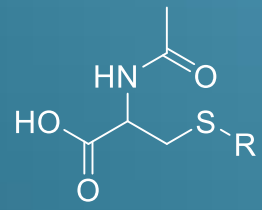
Heptynoic acid

Biomarkers specific to CC users – Phase II conjugates



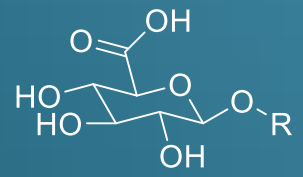


11 Hippuric acids:
E.g., 4-Methylhippuric acid:
p-Cresol metabolite



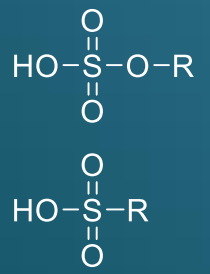
29 Mercapturic acids:

Acrolein, dimethylformamide, crotonaldehyde, OH-alkenals, OH-alkanes, carbonyls



30 Glucuronides:

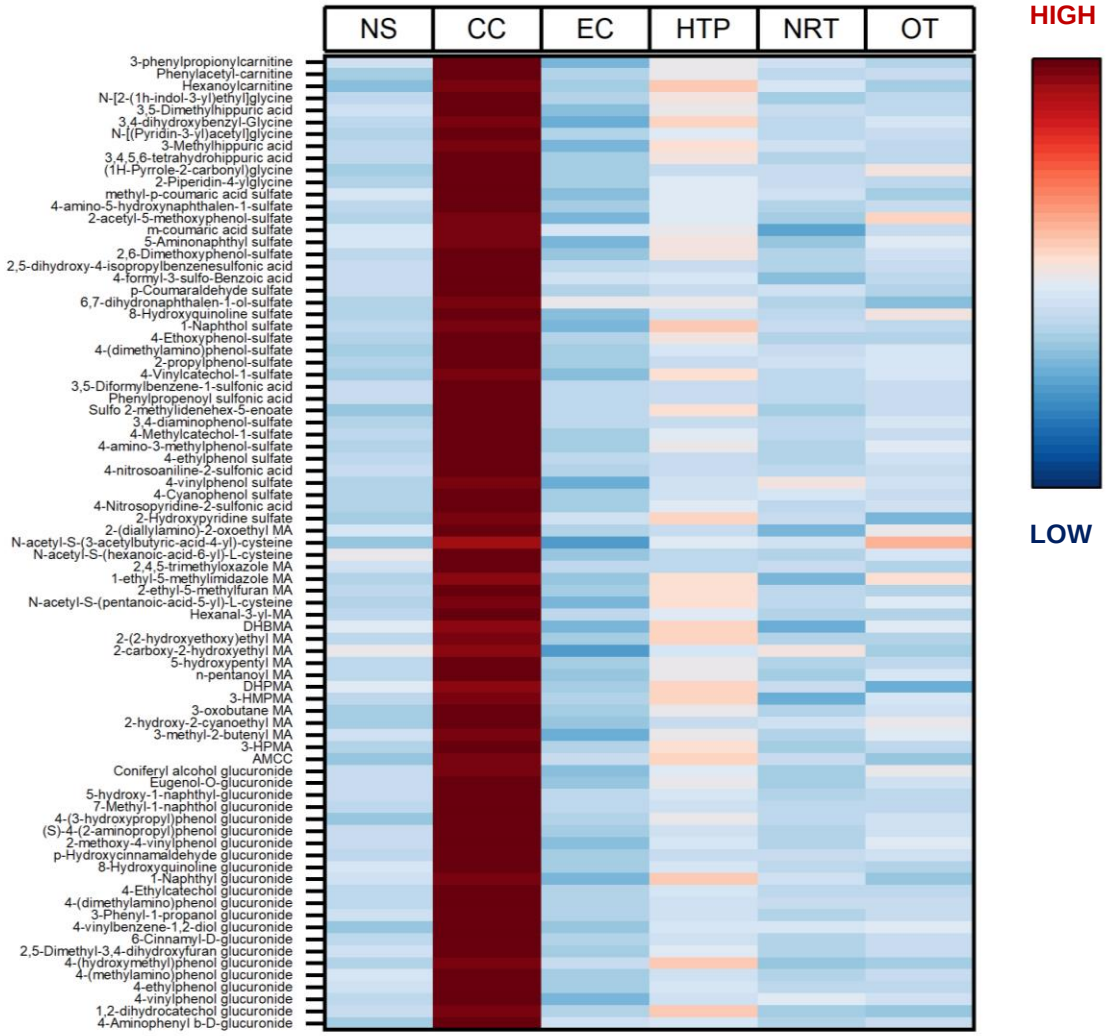
PAHs
Alcohols, Phenols
Dimethylfuran
Aromatic amines (4-ABP)



30 Sulfates:

Aromatic amines
PAHs
Pyridines
Phenols

Biomarkers specific to CC users – Phase II conjugates



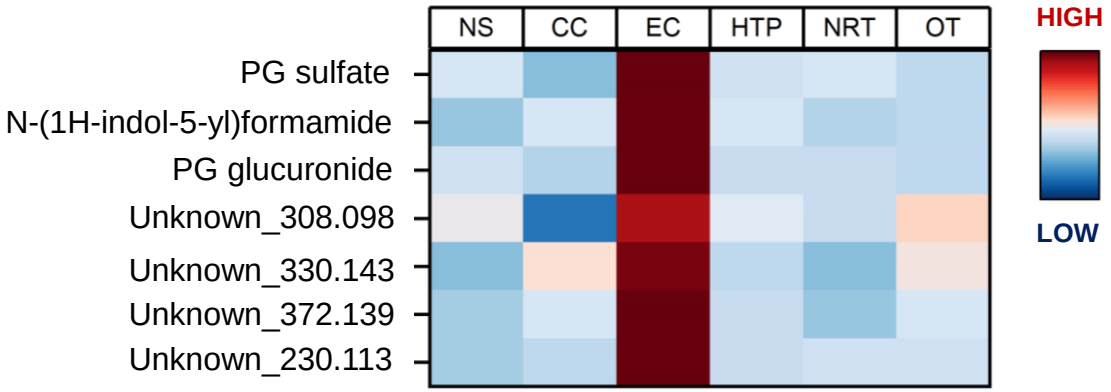
- Metabolites, especially Phase II conjugates, related to several chemical classes of toxicologically relevant compounds:
- VOCs: Aldehydes, Ketones, Epoxides, Alkenes, Alkanes
- Alcohols, phenols
- PAHs
- Aromatic amines
- Heterocyclic compounds
- Alkaloids

BoE specific to

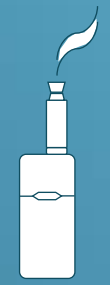
EC



Biomarkers specific to EC users



Proposed Compound	Molecular Formula	MW	FC: EC/NS	Correlation with EC use (g liquid/d)
Propylene glycol (PG) sulfate	C3H8O5S	156.01	3.7	0.77
PG glucuronide	C9H16O8	252.09	7.5	0.82
N-(1H-indol-5-yl)formamide	C9H8N2O	160.06	4.5	0.66
Unknown_230.113	C7H14N6O3	230.11	5.0	0.77
Unknown_308.098	C12H18N2O6	308.10	3.4	0.57
Unknown_330.143	C14H22N2O7	330.14	13.7	0.35
Unknown_372.139	C13H20N6O7	372.14	25.5	0.66



- PG conjugates elevated in EC users only
- Dose-response with EC use confirms specificity
- Results confirmed by targeted LC-MS/MS analysis of PG[1]

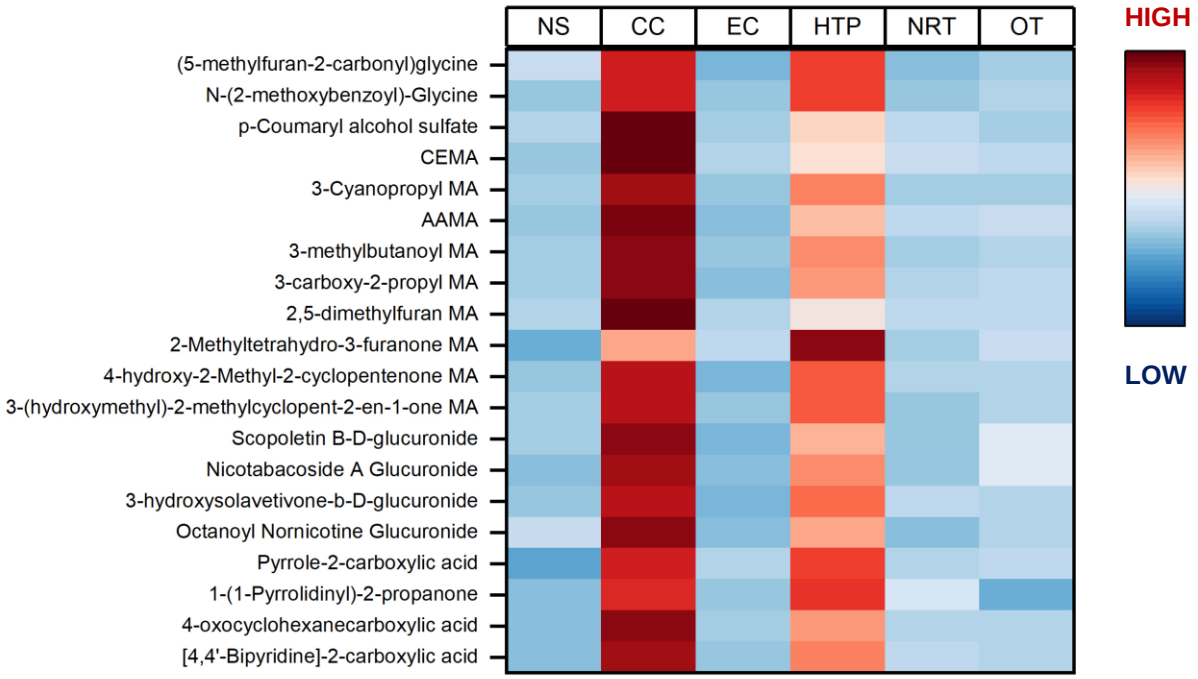
Burkhardt et al (2021): 1,2-Propylene Glycol: A Biomarker of Exposure Specific to e-Cigarette Consumption. *Separations*, 8, 180.

BoE specific to

HTP



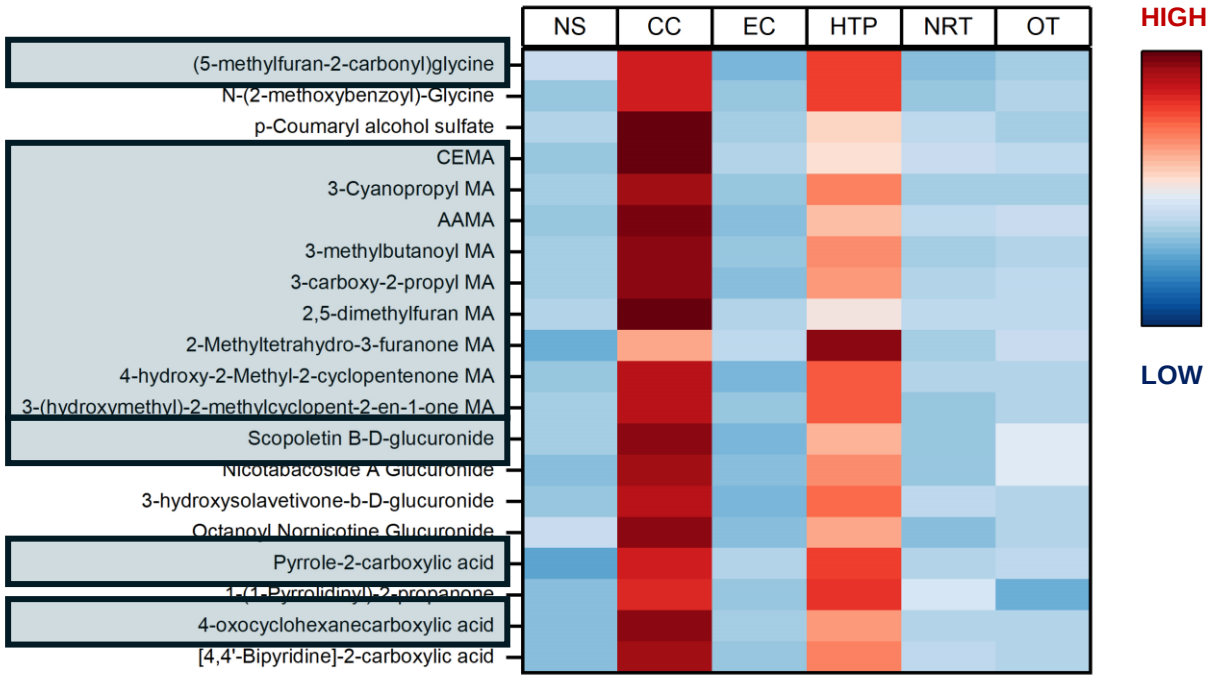
Biomarkers specific to HTP users





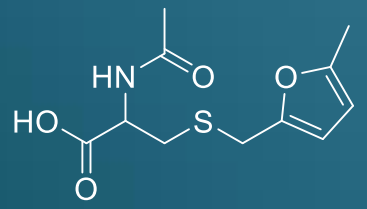
- 20 metabolites significantly elevated in CC and HTP users compared to NU and all other groups
- Unique profile for HTP users due to significant difference CC / HTP users

Biomarkers specific to HTP users

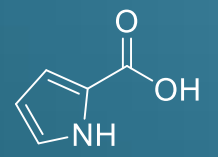




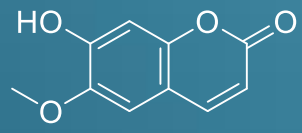
- 9 Mercapturic acids show this unique profile
  - CEMA (acrylonitrile)
  - AAMA (acrylamide)
- Several tobacco constituents



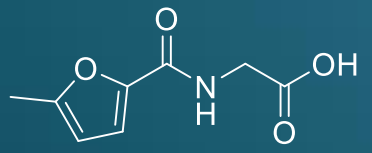
2,5-Dimethylfuran MA:
Metabolite of 2,5-dimethylfuran



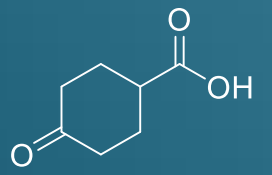
Pyrrole-2-carboxylic acid:
tobacco smoke constituent



Scopoletin:
tobacco constituent



(5-Methylfurancarbonyl)glycine:
Metabolite of 2,5-dimethylfuran



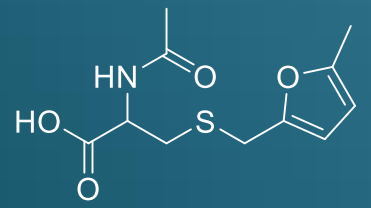
4-Oxocyclohexanecarboxylic acid

Biomarkers specific to HTP users

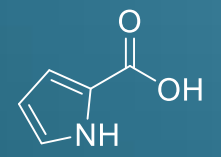
Proposed Compound	Molecular Formula	MW	FC: CC/NS	FC: HTP/NS	Correlation with CC use (CPD)	Correlation with HTP use (sticks/d)
Pyrrole-2-carboxylic acid	C5H5NO2	111.0	3.7	3.2	0.29	0.27
1-(1-Pyrrolidinyl)-2-propanone	C7H13NO	127.1	14.3	13.3	0.57	0.45
4-oxocyclohexanecarboxylic acid	C7H10O3	142.1	11.3	3.7	0.23	0.28
(5-methylfuran-2-carbonyl)glycine	C8H9NO4	183.1	2.6	2.3	0.62	0.65
[4,4'-Bipyridine]-2-carboxylic acid	C11H8N2O2	200.1	266.3	31.8	0.67	0.69
N-(2-methoxybenzoyl)-Glycine	C10H11NO4	209.1	3.8	3.3	0.39	0.60
CEMA	C8H12N2O3S	216.1	31.2	2.9	0.79	0.65
3-Cyanopropyl MA	C10H15NO3S	229.1	22.6	5.8	0.64	0.45
p-Coumaryl alcohol sulfate	C9H10O5S	230.0	71.4	3.4	0.76	0.39
AAMA	C8H14N2O4S	234.1	5.6	2.1	0.68	0.36
3-methylbutanoyl MA	C10H17NO4S	247.1	40.8	7.2	0.60	0.57
3-carboxy-2-propyl MA	C9H15NO5S	249.1	33.2	6.0	0.58	0.62
2,5-dimethylfuran MA	C11H15NO4S	257.1	32.9	2.0	0.53	0.35
2-Methyltetrahydro-3-furanone MA	C10H15NO5S	261.1	2.8	6.6	0.64	0.65
4-hydroxy-2-Methyl-2-cyclopentenone MA	C11H15NO5S	273.1	9.4	5.2	0.60	0.76
3-(hydroxymethyl)-2-methylcyclopent-2-en-1-one MA	C12H17NO5S	287.1	17.3	8.5	0.64	0.59
Scopoletin B-D-glucuronide	C16H16O10	368.1	7.9	2.5	0.80	0.22
Nicotabacoside A Glucuronide	C20H30O8	398.2	35.0	8.3	0.84	0.72
3-hydroxysolavetivone-b-D-glucuronide	C21H30O8	410.2	16.7	7.1	0.68	0.73
Octanoyl Nornicotine Glucuronide	C23H34N2O7	450.2	22.1	3.5	0.74	0.65



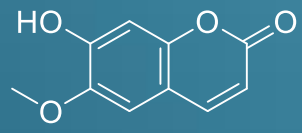
- CC >> HTP > NU/EC/NRT/OT
- Dose-response to CC/HTP use indicates specificity
- Results confirmed in targeted analysis (STPOST18)



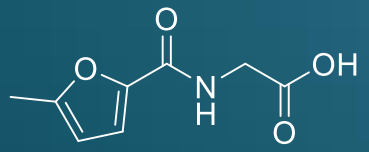
2,5-Dimethylfuran MA:
Metabolite of 2,5-dimethylfuran



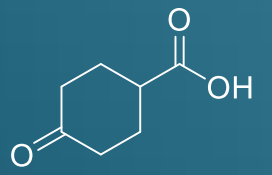
Pyrrole-2-carboxylic acid:
tobacco smoke constituent



Scopoletin:
tobacco constituent

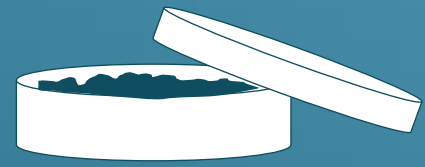


(5-Methylfurancarbonyl)glycine:
Metabolite of 2,5-dimethylfuran

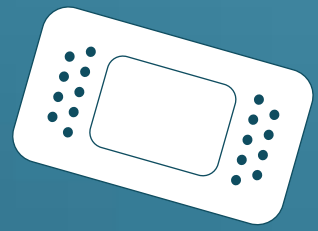


4-Oxocyclohexanecarboxylic acid

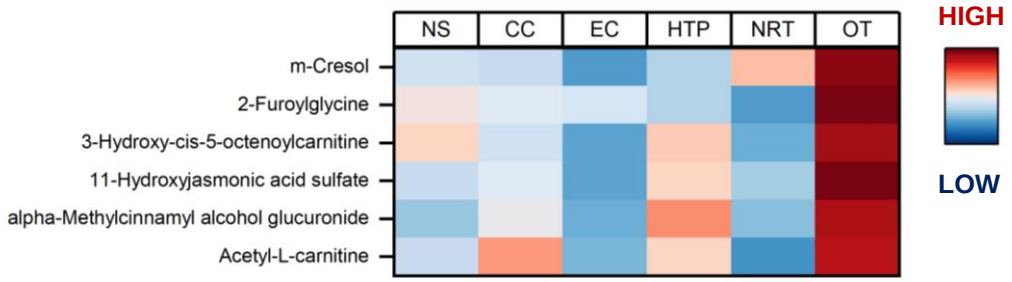
BoE specific to

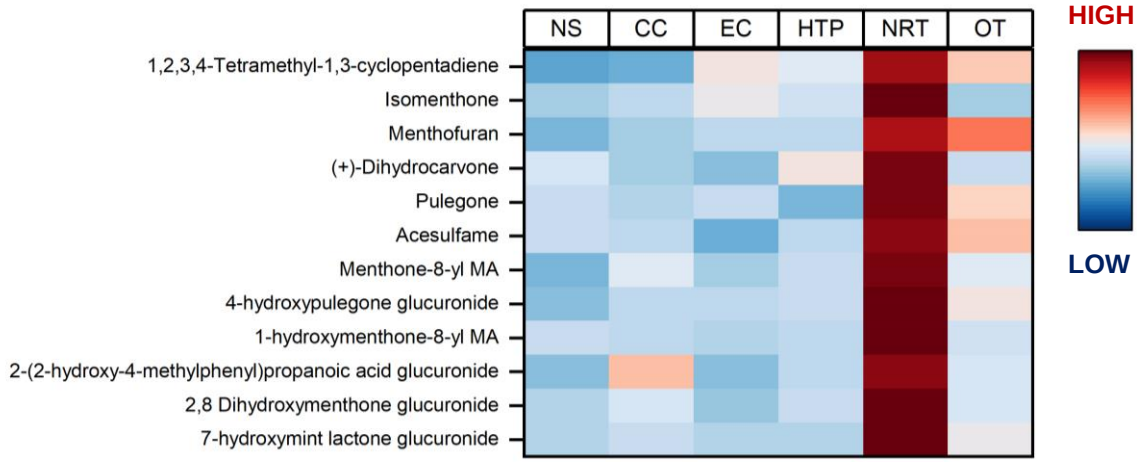


OT & NRT



Biomarkers specific to OT & NRT users





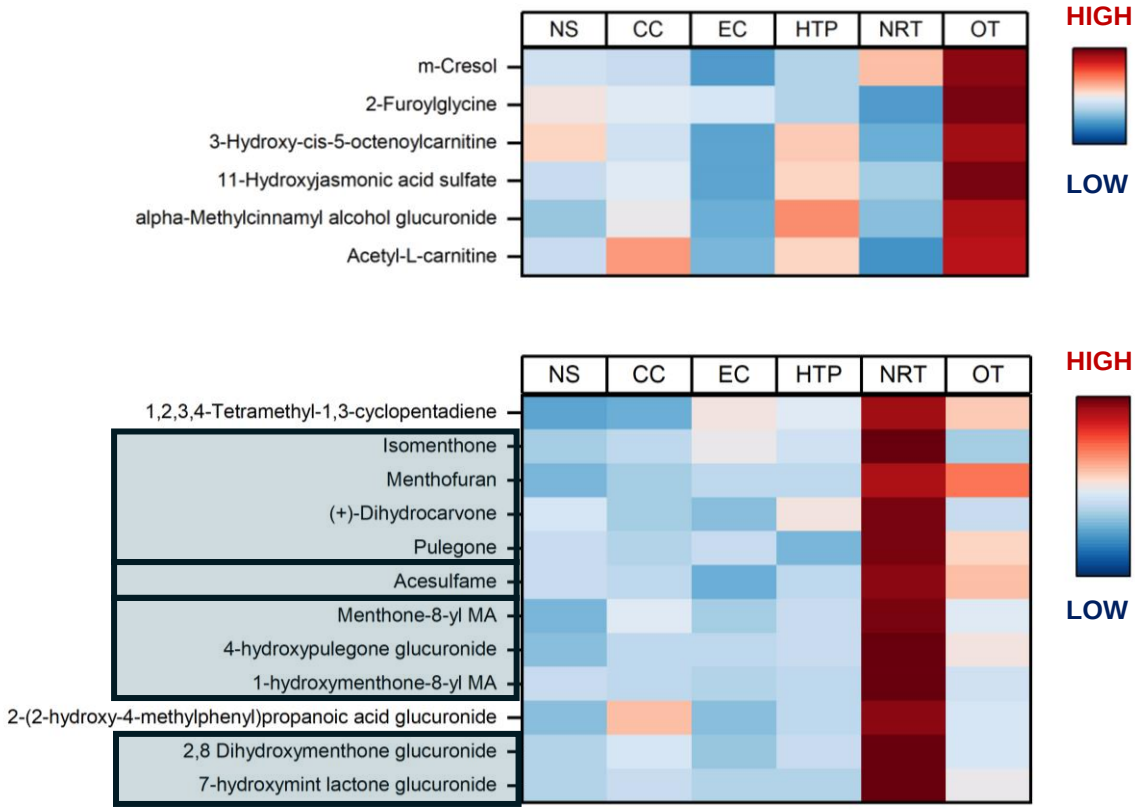


- OT group consisted primarily of snus users
- 6 compounds elevated in OT group only



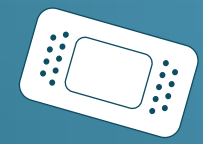
- NRT group consisted of users of nicotine gum and nicotine inhalers
- 12 compounds elevated in NRT group only
- Mostly monoterpenoid flavors and their metabolites

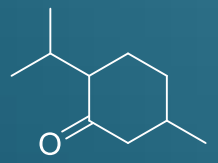
Biomarkers specific to OT & NRT users



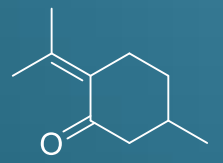


- OT group consisted primarily of snus users
- 6 compounds elevated in OT group only

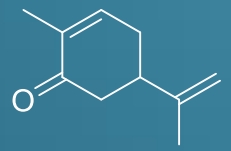




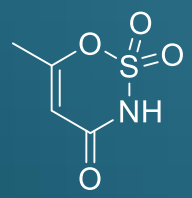
Menthone



Pulegone

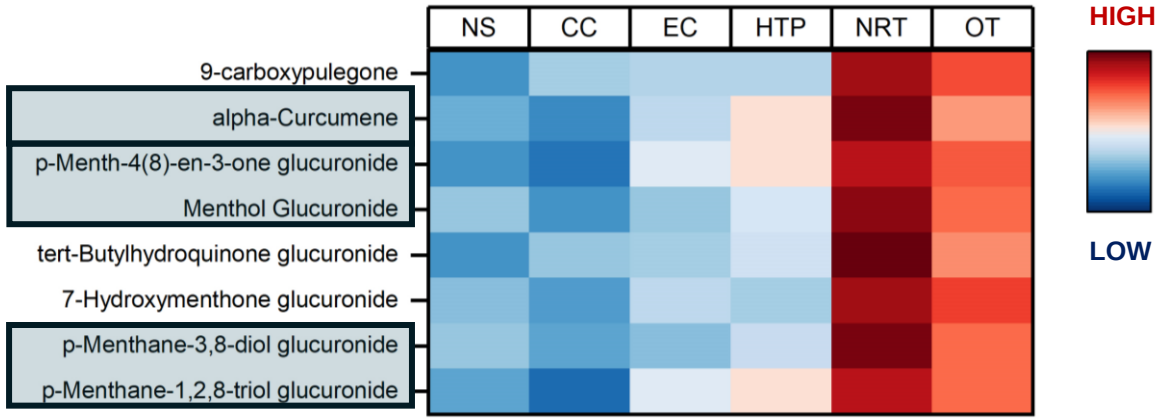


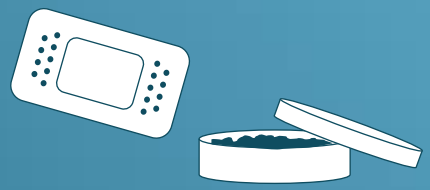
Carvone



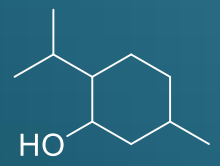
Acesulfame

Biomarkers specific to OT & NRT users

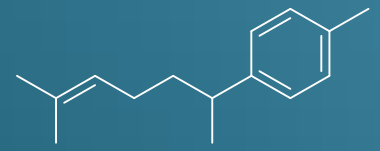




- 8 compounds elevated in both OT & NRT users
- Mostly menthol metabolites + other flavors



Menthol



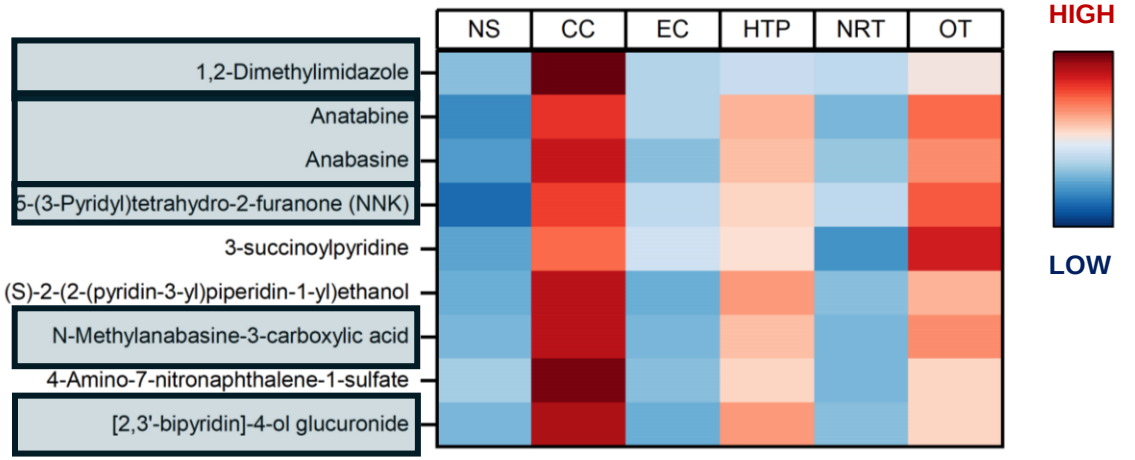
Curcurmene

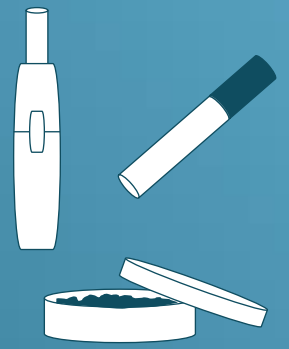
BoE specific to

tobacco-derived products



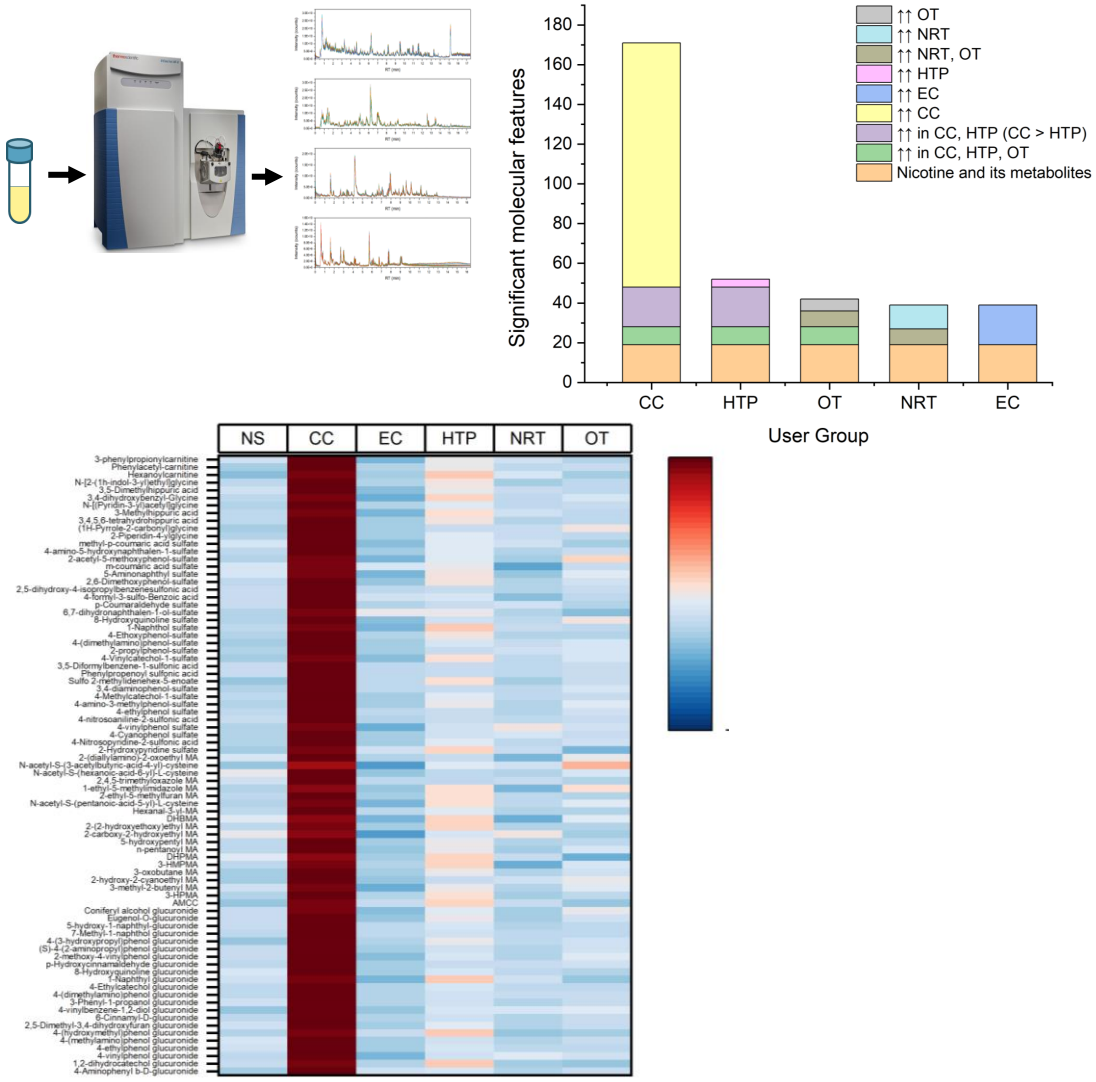
Biomarkers specific to tobacco-derived products





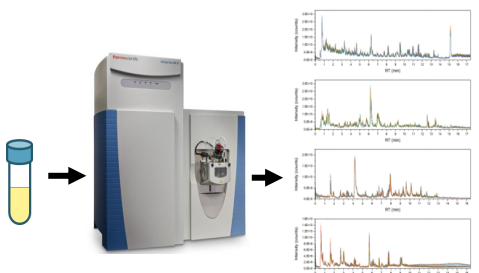
- 9 BoE identified as specific to tobacco derived product use of CC, HTP, OT
- Minor alkaloids anatabine/anabasine
- TSNAs
- Imidazole
- 2,3‘-Bipyridine

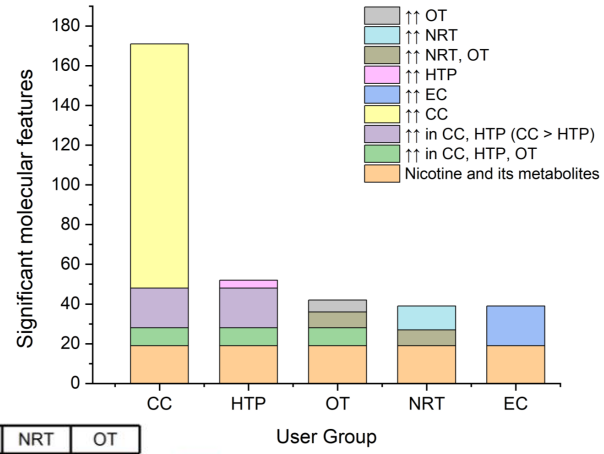
Conclusion

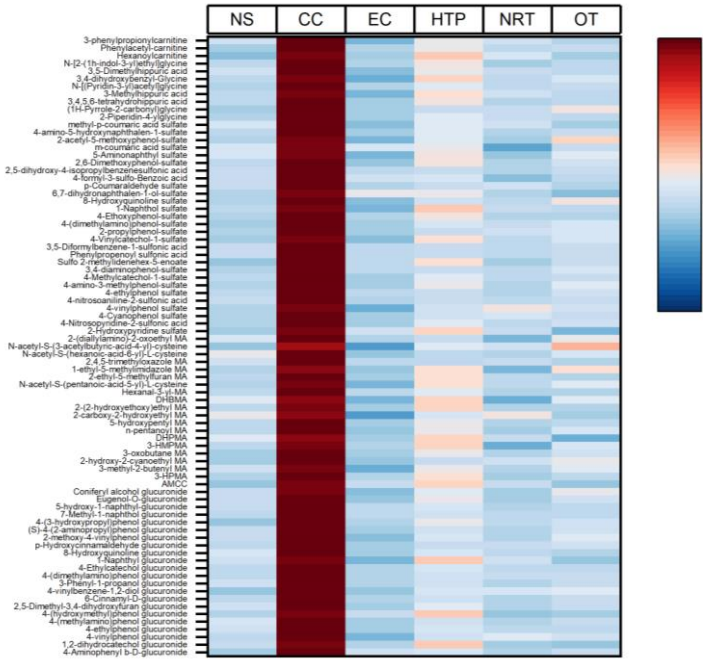


- Highly sensitive nontargeted method for urine analysis capable of detecting metabolites in the low ng/mL range
- Decipher the urinary exposome related to the use of 5 different nicotine&tobacco products
- **Smoker** exposome shows by far the highest burden to harmful compounds
- Minor alkaloids & TSNAs indicate use of tobacco-derived products

Conclusion

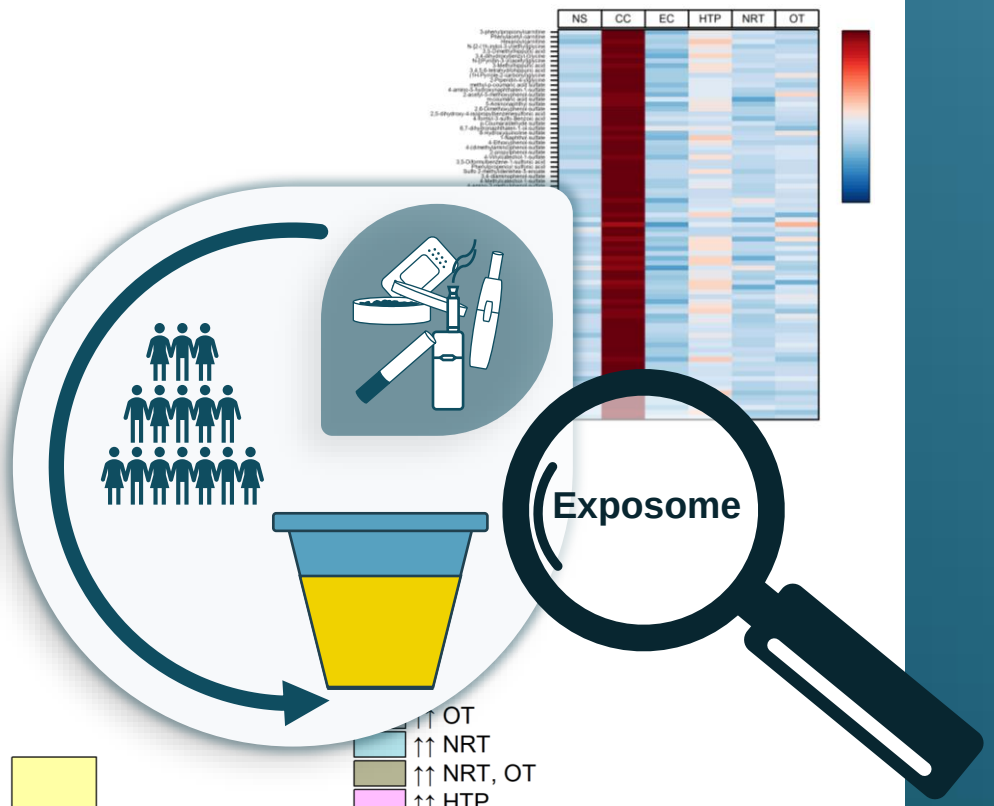


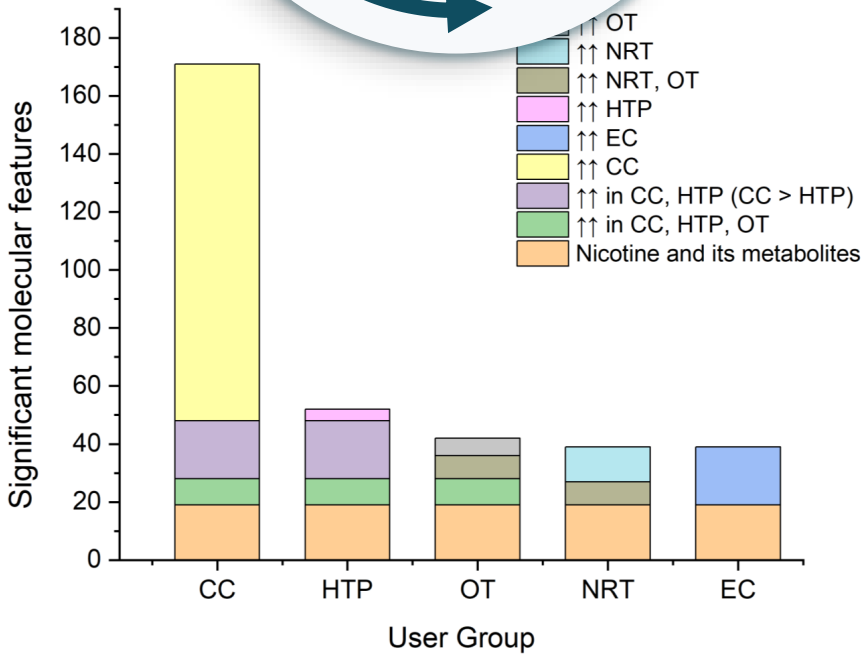




- **EC**: PG in urine as vaping-specific BoE
- **NRT / OT**: Unique flavor profile
- Panel of mercapturic acids potential to stratify **HTP** from other products including CC
- Powerful & unique approach to identify use-specific BoE for new nicotine products

Outlook

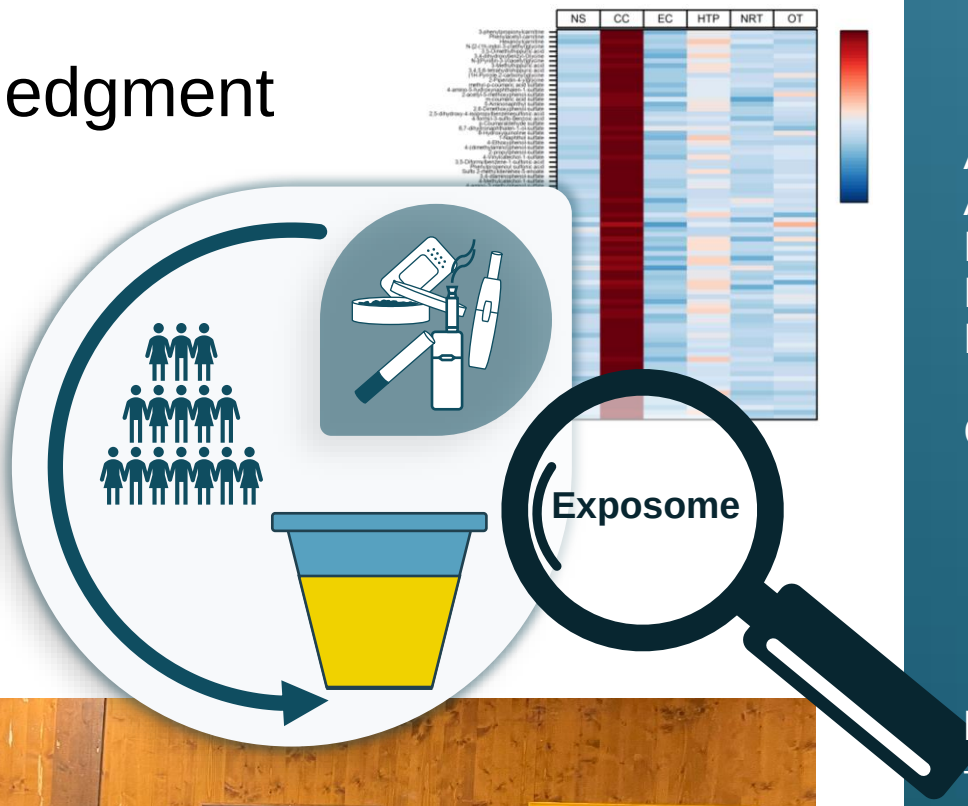




Specificity of the identified biomarkers needs confirmation by targeted analysis with larger sample sizes & in different populations

Implementation of specific BoE as a key tool for unambiguous verification of subjects' compliance
&
robust categorization of the products in the risk-continuum in future THR studies

Acknowledgment





ABF Team
Alpesh Kachhadia (PhD candidate)
Dr. Therese Burkhardt
Dr. Max Scherer
Prof. Dr. Gerhard Scherer

CTC North Hamburg: Clinical study conduct

FOUNDATION FOR A SMOKE-FREE WORLD

Funding:

This study was funded with a grant from the Foundation for a Smoke-Free World (FSFW), a US nonprofit 501(c)(3) private foundation. The contents, selection, and presentation of facts, as well as any opinions expressed herein are the sole responsibility of the authors and under no circumstances shall be regarded as reflecting the positions of the Foundation for a Smokefree-World, Inc. FSFW accepts charitable gifts from PMI Global Services Inc. (PMI); under FSFW's Bylaws and Pledge Agreement with PMI, FSFW is independent from PMI and the tobacco industry.