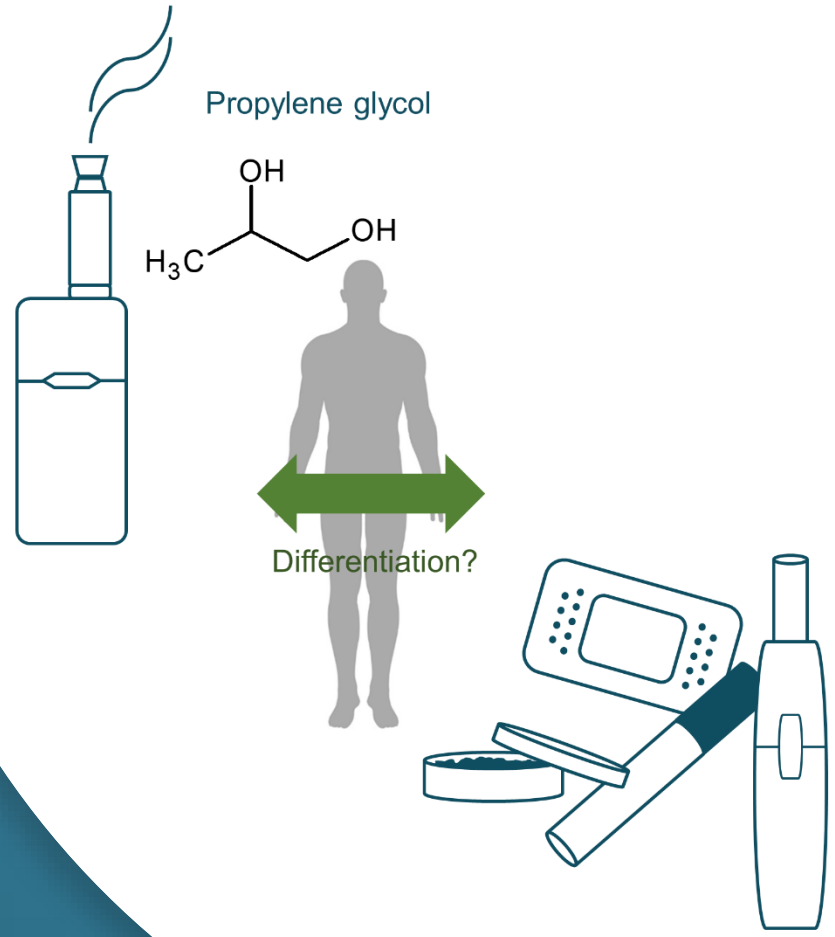
Propylene glycol as a specific biomarker for e-cigarette use Showcase of a novel Orbitrap-MS-based exposomics approach



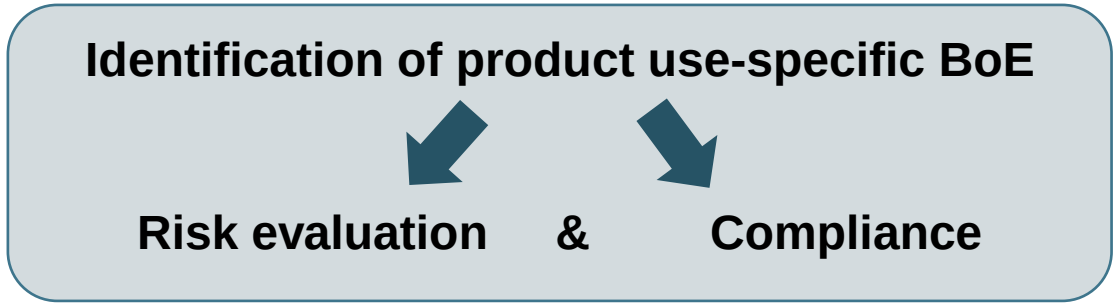
N. Pluym, T. Burkhardt, A. Kachhadia, G. Scherer, M. Scherer

Analytisch-Biologisches Forschungslabor GmbH, Semmelweisstr. 5, 82152 Planegg, Germany

ISES 2022, Lisbon, 26-Sep-2022

Background

- Variety of new tobacco and nicotine products were introduced over the past decade
- Especially E-cigarettes (ECs) gained a lot attention as potentially reduced risk products
- ECs discussed as cessation tools in tobacco harm reduction
- Long-term (epidemiological) data needed to evaluate health risks from EC use and harm reduction potential for smokers after switching
- Lack of use-specific biomarkers of exposure (BoE) (not only for EC but all other new products)



Study Design & Objectives

- 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

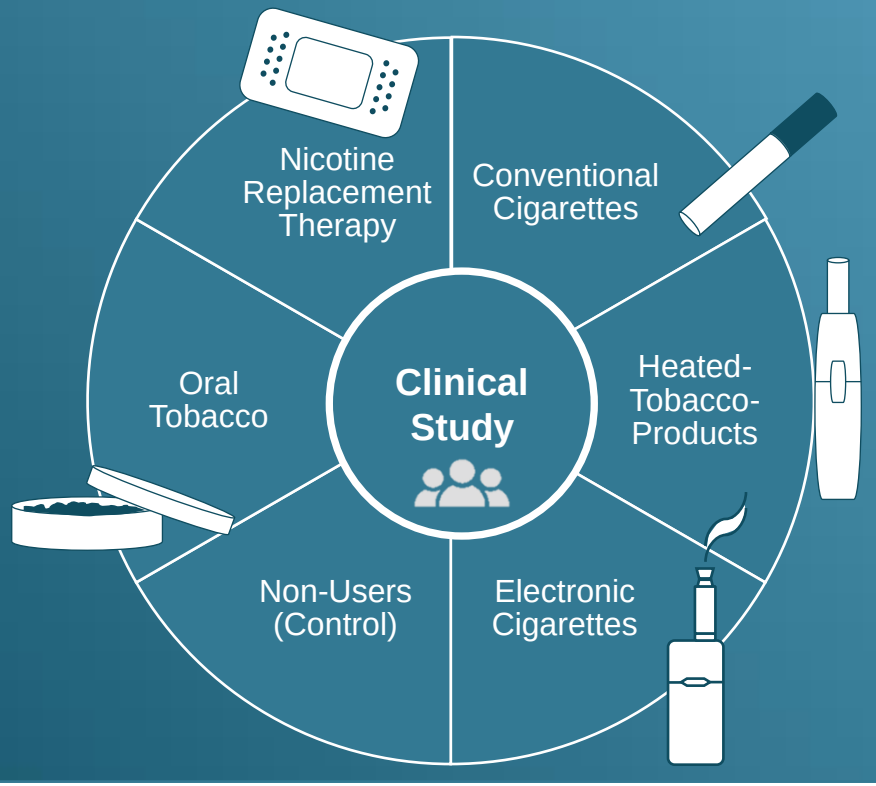
Identify biomarkers or biomarker patterns capable to discriminate between different nicotine product user groups and non-users. Focus on differentiation users of new products ⟷ smokers and non-users.

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

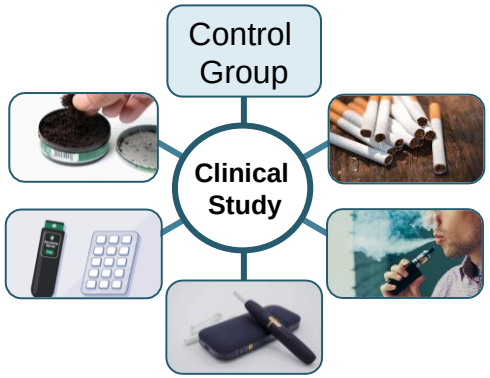


10 subjects per group

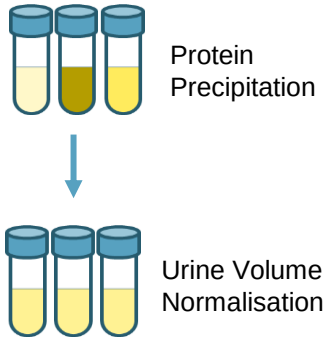
Day 1 → Day 2 → Day 3

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

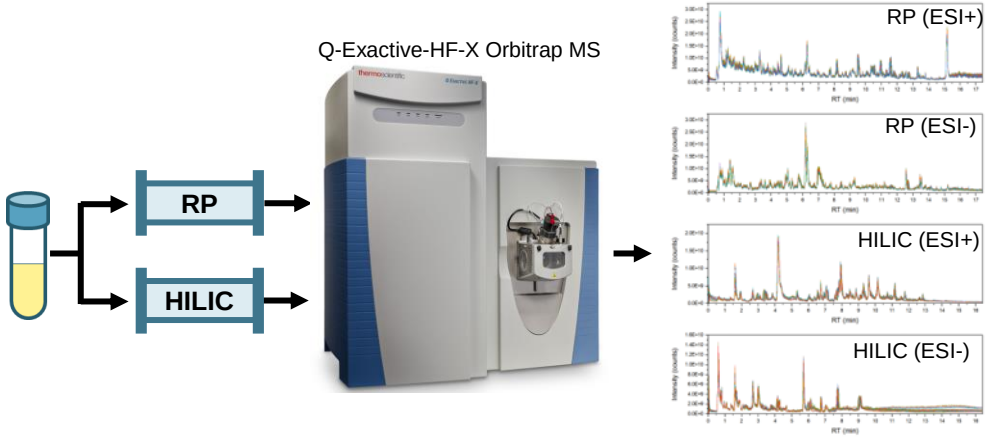
Exposomics – Workflow



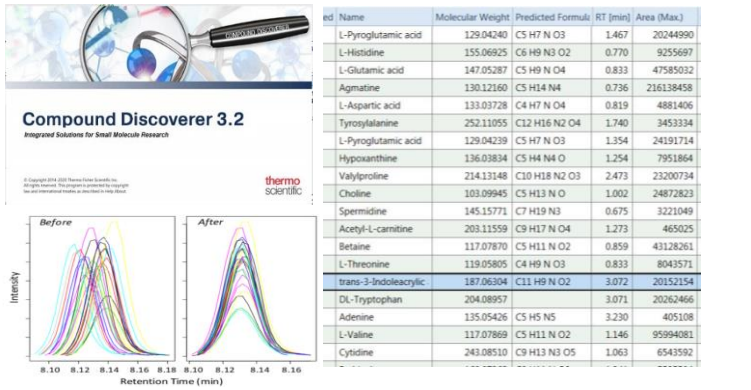
Sample collection



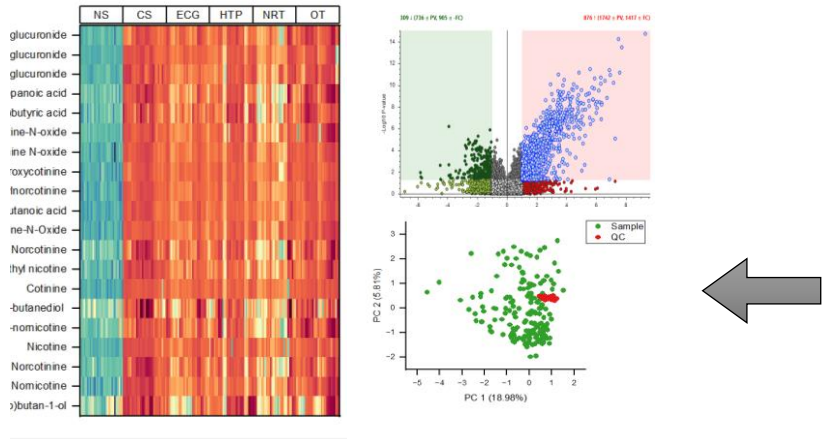
Sample preparation



Data acquisition



Data pre-processing: RT alignment / Intensity correction by QC, NL and frag ion search

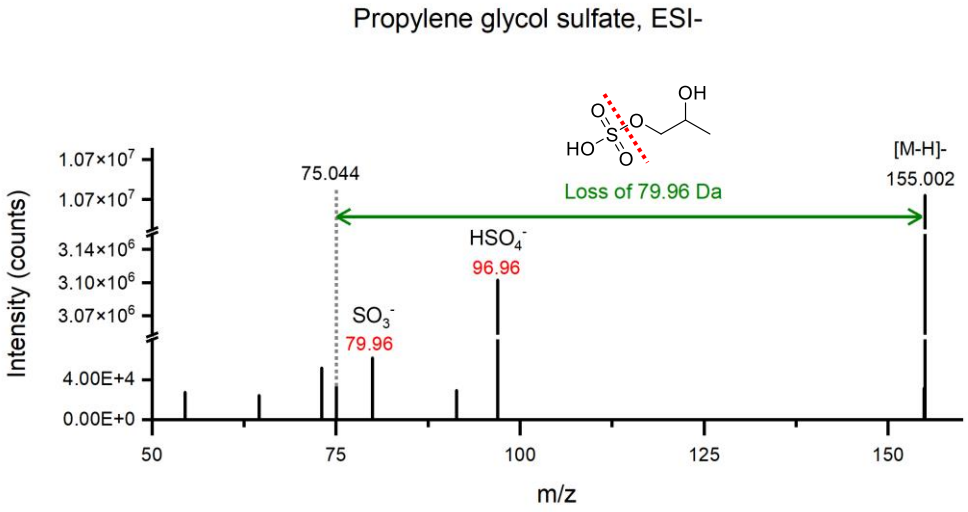


Statistical analysis: PCA, Volcano plot
threshold for features with significant differences compared to NS and S: $p < 0.05$; fold change > 2
Heat map data visualization (Originlab)

Metabolite identification

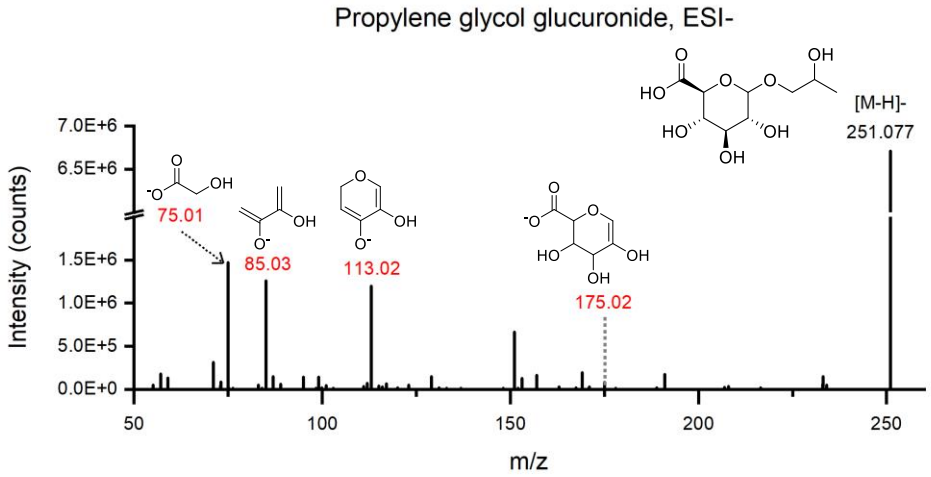
Exposomics – Metabolite Identification

1. Spectral Information
   - Neutral losses
   - Characteristic fragment ions
   - Isotopic pattern
2. MS Spectral library
   - NIST 2020 MS
   - mzCloud
   - Human Metabolome Database
   - In-house spectral library
3. In-silico Fragmentation
   - Metfrag, SIRIUS, CSI:FingerID



Sulfate conjugates:

- **Neutral loss of 80 Da**
- **Characteristic fragment Ion of 80 / 97 Da**

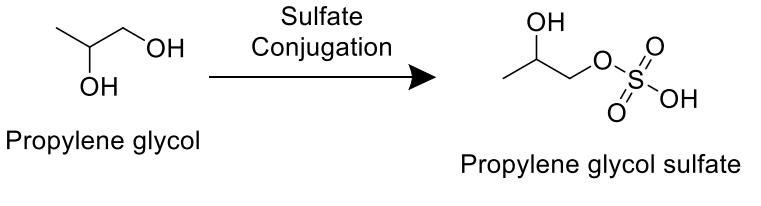


Glucuronide conjugates:

- **Characteristic fragment Ion of 75 / 85 / 113 / 175 Da**

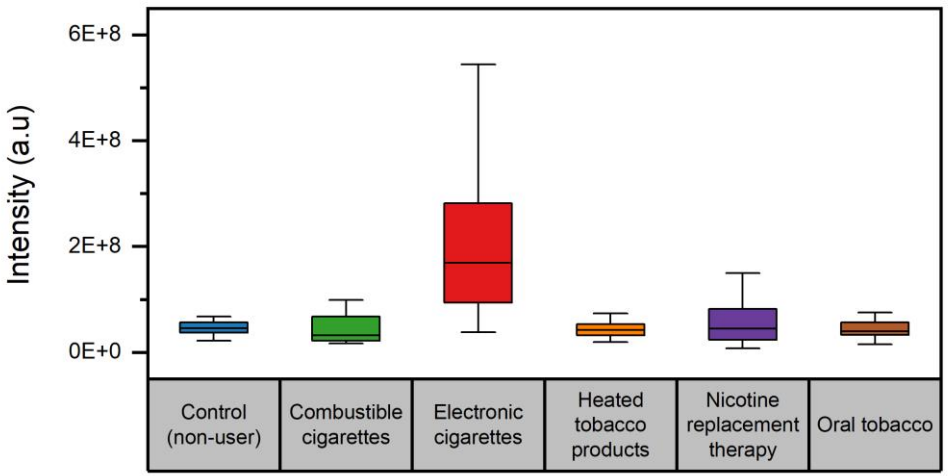
Exposomics – Group comparison

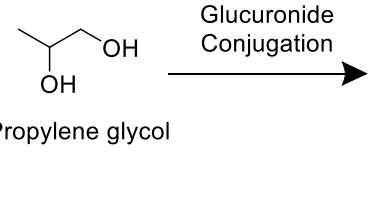
- Sulfate and glucuronide conjugates of proylene glycol (PG) significantly higher in urine of EC users



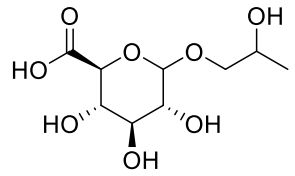
Sulfate Conjugation

Propylene glycol sulfate

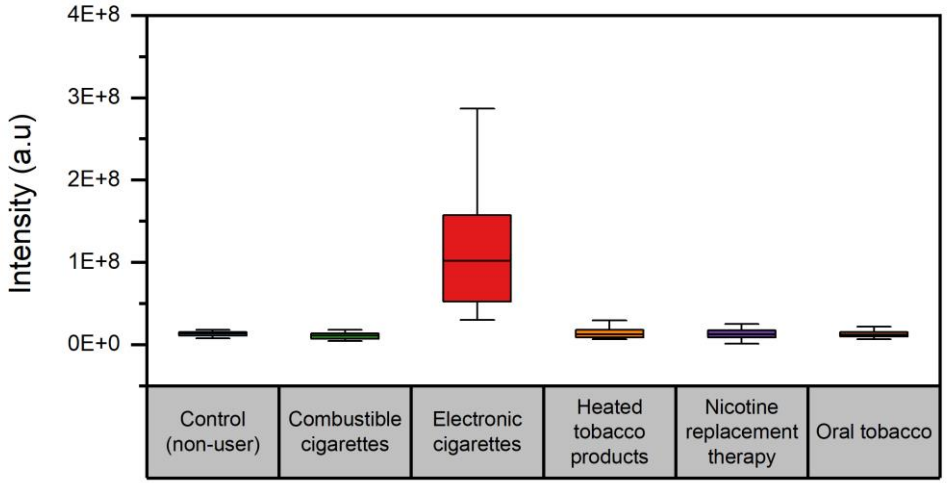




Glucuronide Conjugation



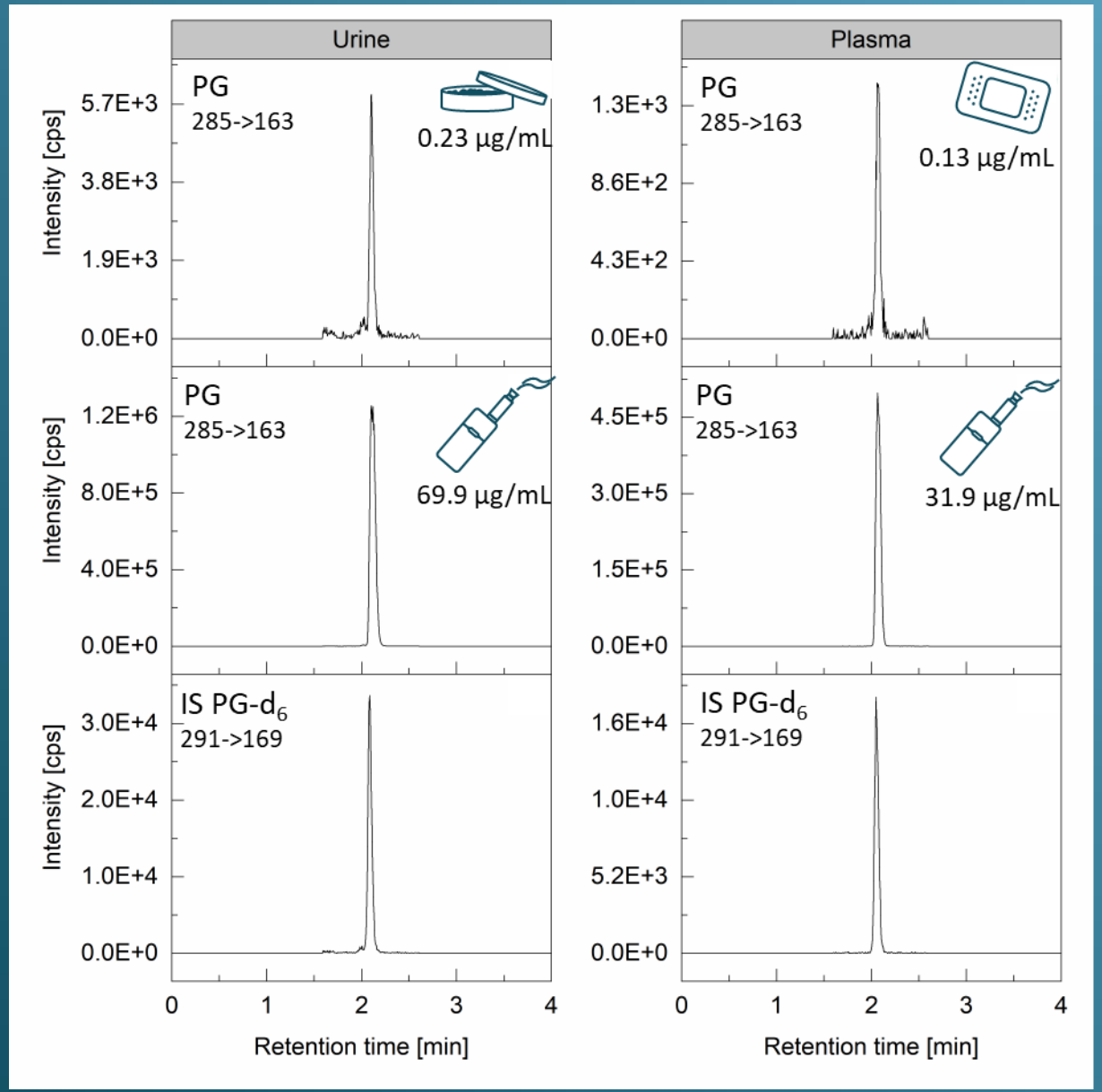
Propylene glycol glucuronide



- Development of a targeted LC-MS/MS method for quantification of PG in urine and plasma

Quantitative LC-MS/MS method

- 25 µL sample (plasma or urine) + 10µL IS (PG-d_6) + 500 µL 4M NaOH + 100µL benzoyl chloride + 2mL pentane for 15 min (Schotten-Baumann reaction)
- Quench with 10% aq. Gly
- Evaporation & reconstitution in ACN
- LC-MS/MS analysis:
  Agilent 1100 HPLC / Sciex 4000 MS/MS
- Successful method validation according to FDA Guidelines on Bioanalytical MV
- Accuracy: 97-101% / Precision: <10% CV
- LLOQ of 0.1 µg/mL



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

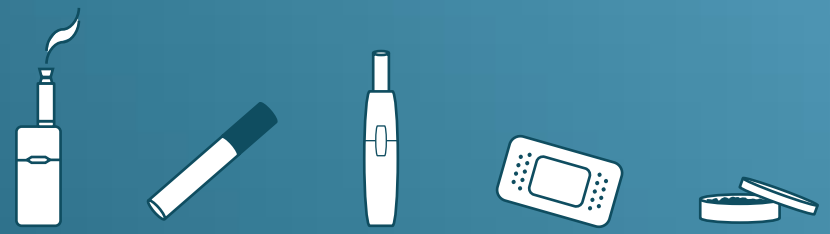
Results of PG analysis in **urine**

- **Significant differences** in PG excretion of **e-cigarette** users compared to other nicotine product user groups and non-users ($p < 0.001$, Mann-Whitney U Test, 24 h urine, Day 3)
- Data for Day -1 (uncontrolled conditions) confirm these findings
- Recent publication (Eissenberg lab VCU) supports our findings that EC users show elevated PG levels in urine

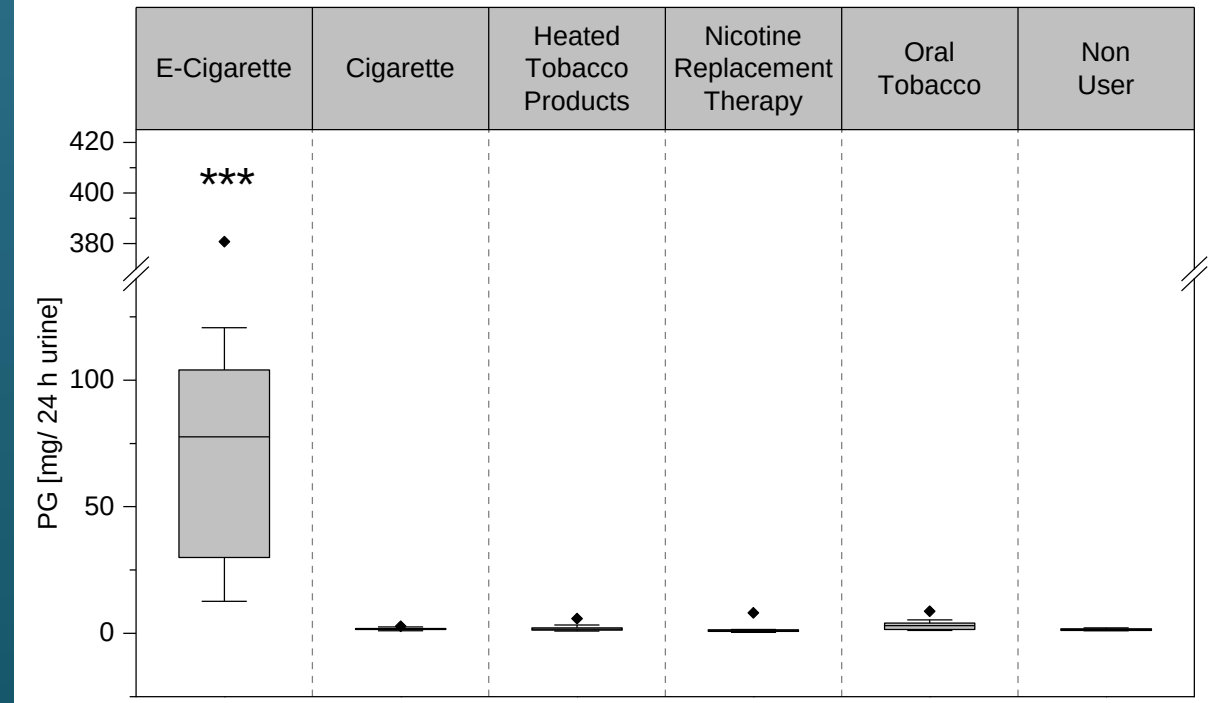
Hiler et al (2020): Are urine propylene glycol or vegetable glycerin markers of e-cigarette use or abstinence? *Tobacco Regulatory Science*, 6 (4)

Differentiation between **e-cigarette users** and other **nicotine user groups** based on PG content in urine

Current findings suggest **elevated exposure** to **PG by vaping** also under **real-life conditions**



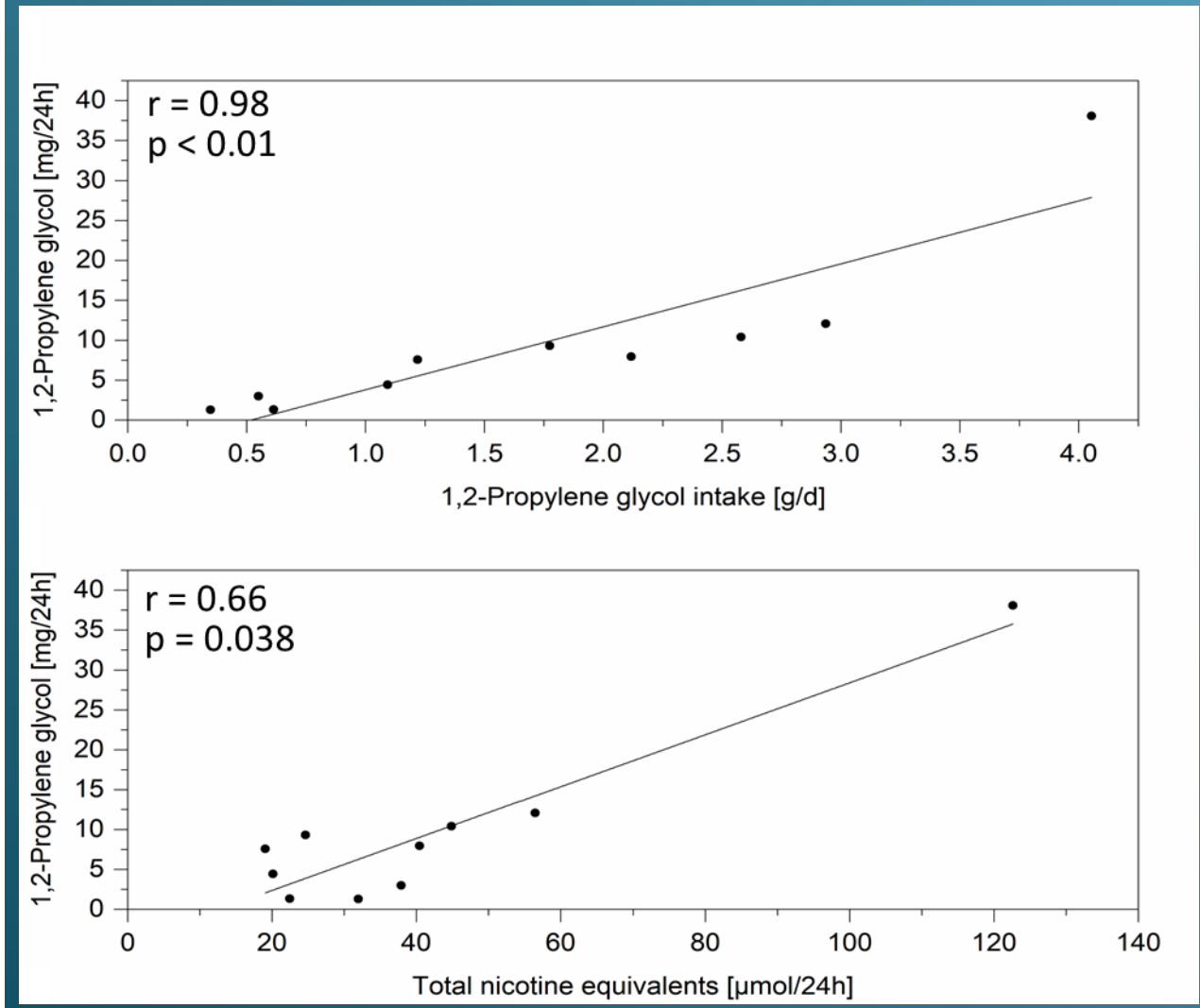
Control



Results of PG analysis in **urine**

- Intake of PG from ECs depends on individual use behavior, liquid composition (PG content), device characteristics (wattage, temperature)
- Urinary PG excretion was correlated with the calculated PG intake based on e-liquid consumption
- **Very strong correlation** between **intake** and **PG** in 24h urine
- **Strong correlation** between **total nicotine equivalents** and **PG** in 24h urine

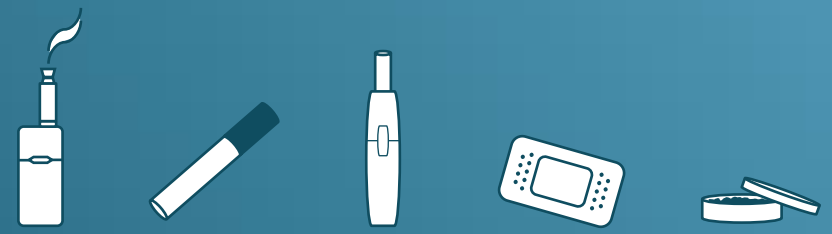
Clear dose-response relationship between urinary PG and EC consumption

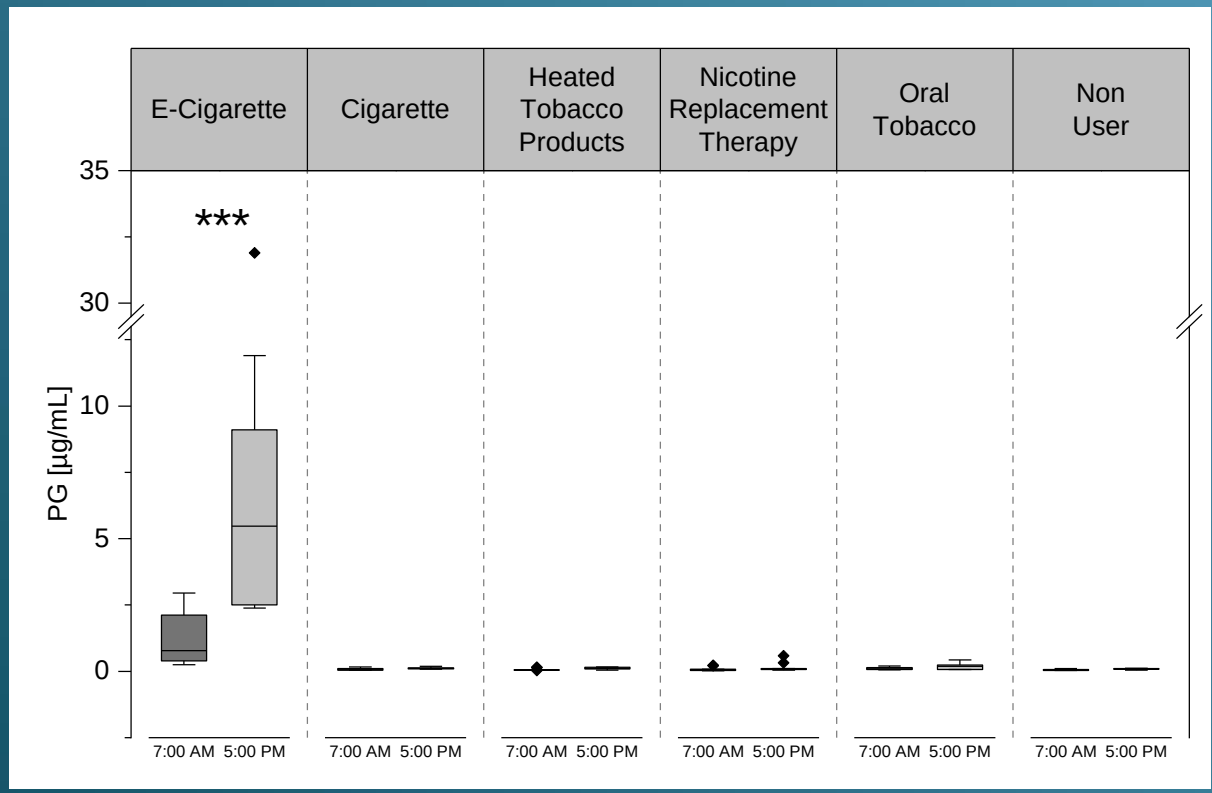


Results of PG analysis in **plasma**

- **Significant differences** in PG content of **e-cigarette** users compared to other nicotine product user groups and non-users ($p < 0.001$, Mann-Whitney U test, plasma day 3)
- Increase in PG plasma concentrations during the day in correlation to e-cigarette use (no use before 8 AM)

Differentiation between **e-cigarette users** and other **nicotine product user groups** based on the PG content in plasma

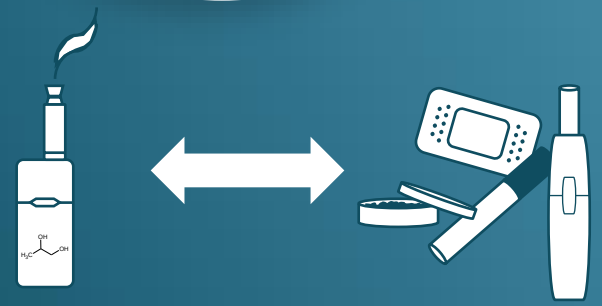




Conclusion

- Identification of PG conjugates as significantly elevated in urine of EC users compared to smokers, and sole users of other nicotine/tobacco products as well as non-users by non-targeted **exposomics** platform
- Confirmation by quantitative (targeted) LC-MS/MS in urine and plasma
- (Very) strong correlation between urinary PG excretion & EC consumption
- **Propylene glycol: a specific biomarker of EC use**
- Monitor EC use compliance in exposure assessments under real-life conditions (field and epidemiological studies)

PG identified by untargeted Orbitrap-MS and confirmed by targeted LC-MS/MS analysis as specific BoE for vaping



Differentiation based on PG level in urine and plasma

Acknowledgment

Funding:

FOUNDATION FOR A SMOKE-FREE WORLD

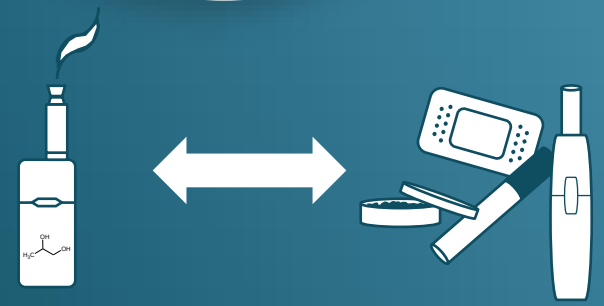
Clinical Study Conduct:
Ralf Freese, Stefan Schoder

CTC North

ABF Team:
Alpeshkumar Kachhadia, Marta Latawiec,
Dr. Therese Burkhardt, Fabian Pilz,
Prof. Dr. Gerhard Scherer, Dr. Max Scherer

abf

PG identified by untargeted Orbitrap-MS and confirmed by targeted LC-MS/MS analysis as specific BoE for vaping



Differentiation based on PG level in urine and plasma

abf