Biomarkers of exposure specific to e-vapor products based on stable-isotope labeled ingredients

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Introduction

E-vapor products (EVPs) consumption has steadily increased worldwide over the past decade. Despite the increasing popularity of EVPs, little information exists on the fate of the main ingredients glycerol (G), propylene glycol (PG) and nicotine (Nic) during EVP use. Currently there are no biomarkers available to differentiate exposure from EVPs relative to other confounders (e.g. other tobacco products, food, etc.). To overcome this problem, we took advantage of the stable-isotope labelling approach as the "gold" standard in mass spectrometry-based analysis of kinetics, uptake and distribution of various compounds in living organisms. In the current study, the e-liquid was partially replaced (10%) with stable-isotope labeled $^{13}C_3$-PG, $^{13}C_3$-G and Nic-d_7.
By measuring known biomarkers, this approach allows the quantitative assessment of the absorption, metabolism and further fate of PG, G and Nic as well as compounds such as acrolein (ACR), propylene oxide (PO) or glycidol that may be formed from the precursors in the e-liquid (or endogenously from the absorbed labeled precursors).

Clinical Study

- 25 healthy male Caucasian volunteers, aged 21 to 60 years; BMI: 18 – 30 kg/m²
- 20 experienced vapers of e-cigarettes: ≥ 1.5 ml/d of nicotine containing e-liquid and no dual use
- Vapers divided into low wattage group (vaping at 10 W) and high wattage group (vaping at 18 W)
- 10 vaping/smoking sessions on Day 1 (Figure 1)
- Defined vaping session: 10 puffs at a puff interval of 30 s and puff duration of 4 s
- 5 current smokers (positive control): ≥ 10 cigarettes/d
- Smoking session: 1 non-filter cigarette spiked with labeled PG, G, and Nic

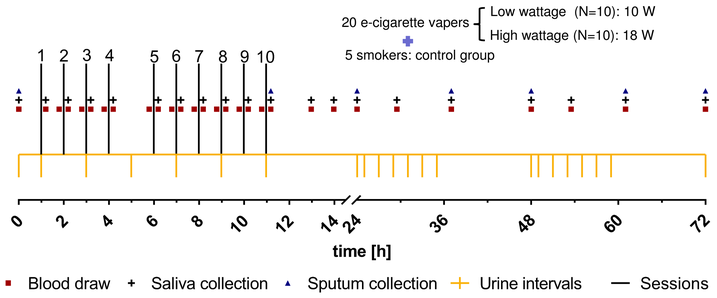

Figure 1: Time scheme for the clinical study. Lines 1 – 10 indicate time points for the vaping/smoking session. Sample collection is marked with various symbols.

Analysis of propylene glycol and glycerol in plasma/urine

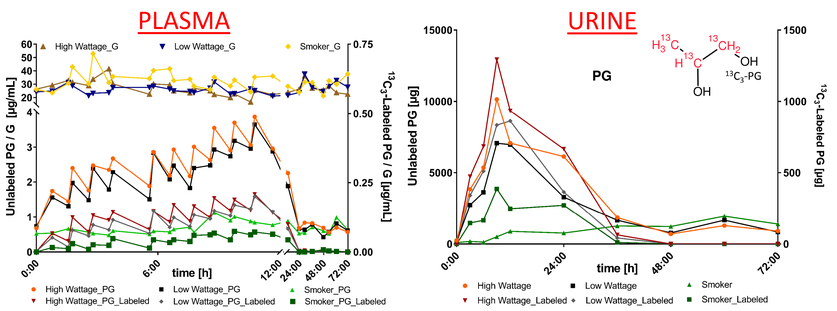

Figure 2: Plasma levels (left) and urine levels (right) of PG/G (labeled/unlabeled; G for urine not shown) in all groups. LC-MS/MS analysis according to [1].

- PG levels in plasma of vapers show a vaping session-dependent pattern for both labeled and unlabeled PG
- No difference in PG concentrations between low and high W groups
- Background for unlabeled PG of ≈ 0.5 – 1.0 µg/mL in plasma ↔ no background for labeled $^{13}C_3$-PG
- Urinary excretion of PG completed after 36 hours
- No alterations in unlabeled G levels (Figure 2, upper curves). Labeled G not detectable in plasma/urine

References:
[1] Landmesser, A., et. al. Biomarkers of exposure specific to e-vapor products based on stable-isotope labelled ingredients – methods. CORESTA SSPT Kitzbühel (Austria) **2017**
[2] Scherer, G., et al. Relationship between machine-derived smoke yields and biomarkers in cigarette smokers in Germany. *Regulatory Toxicology and Pharmacology* **2007**
[3] Piller, M., *et al.* Simple, fast and sensitive LC–MS/MS analysis for the simultaneous quantification of nicotine and 10 of its major metabolites, *Journal of Chromatography* B. **2014**
[4] Sleiman, M., *et al.* Emissions from Electronic Cigarettes: Key Parameters Affecting the Release of Harmful Chemicals. *Environ. Sci. Technol.* **2016**
[5] Pluym, N., *et al.* Analysis of 18 urinary mercapturic acids by two high-throughput multiplex-LC-MS/MS methods. *Anal Bioanal Chem*. **2015**

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Determination of Nic + metabolites in plasma, saliva, and urine

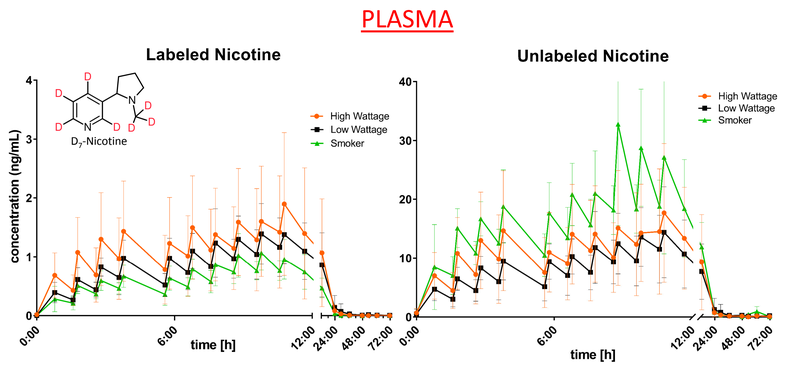


Figure 3: Plasma concentrations of labeled D_7-Nic (left) and unlabeled Nic (right) for all three groups. LC-MS/MS analysis according to [2] with modifications.
10 % of the e-liquid were replaced with stable-isotope labeled $^{13}C_3$-PG, $^{13}C_3$-G, and D_2-nicotine (cf. Chapter "preparation of test items"). Error bars represent 95 % confidence interval. Therefore, differences between groups not significant (p >> 0.05).

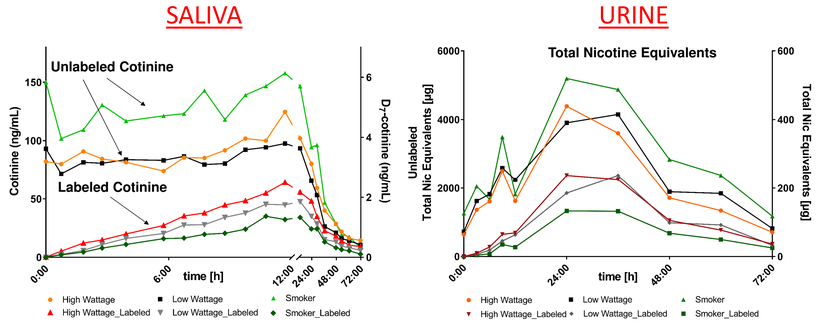


Figure 4: Saliva concentrations of cotinine (labeled/unlabeled) (left) and total nicotine equivalents (TNE) in urine (labeled/unlabeled) (right) in all groups.
TNE: nicotine, cotinine, 3-hydroxycotinine, nicotine-glucuronide, cotinine-glucuronide, 3-hydroxycotinine-glucuronide, nornicotine, norcotinine, nicotine-*N*-oxide, cotinine-*N*-oxide, 4-hydroxy-(3-pyridyl) butanoic acid. Saliva analysis according to [2]; Urine determination according to [3].

Table 1: Area under curve (AUC) and maximum concentrations (C_{max}) in plasma for Nic and PG, both labeled and unlabeled for smokers, low wattage and high wattage vapers

	Smokers (N = 5)				Low wattage vapers (N = 10)				High wattage vapers (N = 10)			
	Nic	D_7-Nic	PG	$^{13}C_3$-PG	Nic	D_7-Nic	PG	$^{13}C_3$-PG	Nic	D_7-Nic	PG	$^{13}C_3$-PG
AUC[1]	12.02	0.44	0.142	0.034	7.05	0.73	1.07	0.077	8.45	0.89	1.31	0.093
C_{max}[2]	35.1	0.96	1.59	0.090	14.4	1.38	4.27	0.21	17.7	1.90	4.83	0.24

[1]: AUC for Nic [ng/mL * h]; AUC for PG [µg/mL * h]
[2]: C_{max} for Nic [ng/mL]; C_{max} for PG [µg/mL]

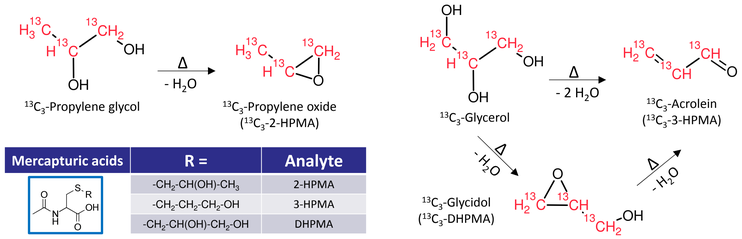
- Mean Nic concentrations (labeled and unlabeled) slightly higher in high W group compared to low W group and peak concentrations after each session with higher variations in high W group (Figure 3)
- Smokers had similar levels in labeled Nic and higher levels of unlabeled Nic compared to vapers
- Plasma C_{max} and AUC in vapers for labeled Nic was ≈10-fold lower compared to unlabeled Nic (Table 1)
  - ➢ reflects 10 % replacement in e-liquid
- No background at study start for labeled nic-metabolites, neither for cotinine in saliva nor for TNE in urine (Figure 4)
  - ➢ Smoking/vaping was allowed until evening before study start -> background for unlabeled metabolites

Preparation of test items

- ❑ Test e-cigarette: Eleaf iStick TC 40 W (adjustable wattage)
- ❑ Atomizer Aspire Nautilus mini 2mL 1.8 Ω tank
- ❑ Custom-made e-liquid (Happy Liquid, Munich, Ger)
- ❑ American Blend flavor
- ❑ PG/G 50/50 (v/v), 12 mg Nic/mL
- ❑ Non-filter combustible cigarette
- ❑ 10 mg tar, 0.8 mg Nic, 10 mg CO (ISO yield)

- 10 % of the e-liquid replaced with a mixture of $^{13}C_3$-PG/$^{13}C_3$-G 50/50 (v/v) + D_2-nicotine (12 mg/mL)
- Labeled compounds purchased from Aptochem (Montreal, Canada); certified purity „as is":
  - $^{13}C_3$-PG (96.6 %); $^{13}C_3$-G (99.2 %), D_7-Nic (96.8 %)
  - Purity taken into account for spiking
- Cigarette spike: 13.4 mg $^{13}C_3$-PG, 13.6 mg $^{13}C_3$-G, and 0.32 mg D_7-nicotine
- Spiking solution evenly distributed along the central axis of the tobacco rod using a needle-armed syringe

Quantification of thermal degradation products of PG and G: LC-MS/MS analysis of mercapturic acids derived from propylene oxide, acrolein and glycidol in urine



Mercapturic acids	R =	Analyte
	$-CH_2-CH(OH)-CH_3$	2-HPMA
	$-CH_2-CH_2-CH_2-OH$	3-HPMA
	$-CH_2-CH(OH)-CH_2-OH$	DHPMA

Figure 5: Selected thermal degradation products of PG and G according to [4]. The corresponding MAs are given in brackets.

Propylene oxide, acrolein and glycidol are of particular interest due to their possible thermal degradation from G and PG according to Figure 5. Acrolein is a constituent of the gas phase of cigarette smoke and part of the daily diet as well as endogenous formation, which results in high background levels. Exposure to propylene oxide originates mostly from cigarette smoke while glycidol is taken up from various sources. Our approach using $^{13}C_3$-labeled G and PG allowed us to distinguish between background and smoke related uptake measuring mercapturic acids (MAs) in urine as metabolic end-products. Labeled MAs were successfully included into a previously established LC-MS/MS method [5].

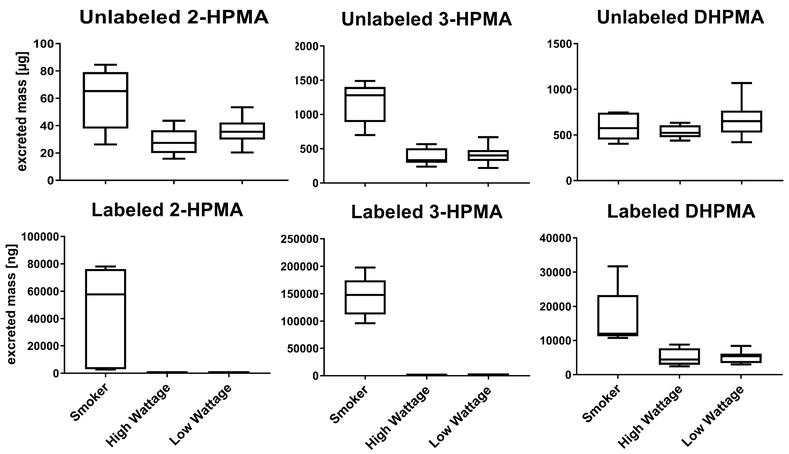


Figure 6: Box plots for 2-HPMA, 3-HPMA, and acrolein in urine of smokers, vapers at high wattage and vapers at low wattage. Excreted mass until 48 hours after start of the vaping/smoking sessions.

- Higher concentrations of unlabeled 2-HPMA / 3-HPMA in smokers compared to vapers. There were no differences in unlabeled DHPMA
- Labeled 2-HPMA / 3-HPMA were only found in smokers
- Labeled DHPMA was observed in all three groups

Summary

- Vaping-dependent increases were observed for Nic and PG in all matrices
- No significant differences were noted between low and high wattage for Nic or PG
- Labeled MAs of acrolein (3-HPMA) and propylene oxide (2-HPMA) were quantifiable in urine of smokers but not in vapers
- ➢ Smoker subgroup adequately served as positive control for monitoring potential degradation products
- Labeled MA of glycidol (DHPMA) was detected in urine of vapers and smokers

Conclusion

- ➢ **Measurement of stable-isotope labeled metabolites in various body fluids revealed:**
  - **1) e-vaping specific internal dose of the main ingredients and**
  - **2) presence of their thermal degradation products and their further metabolism in the human body**
- ➢ **A stable-isotope labeling approach can be useful for toxicological evaluations of e-cigarettes particularly for constituents that are confounded by other sources of exposure**
- ➢ **This approach allows the quantitative assessment of the absorption, metabolism and excretion of PG, G and Nic as well as compounds formed from these precursors (either in the vapor or endogenously) such as acrolein, propylene oxide or glycidol**