Analysis of aldehydes, propylene oxide and glycidol in tobacco smoke and e-cigarette vapor and their respective urinary biomarkers

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Introduction

We recently performed a clinical study with e-cigarette users (vapers) and smokers. The stable-isotope labeled e-cigarette constituents propylene glycol, glycerol, and nicotine in the test e-cigarette were used in order to investigate the e-cigarette specific uptake of these compounds as well as their degradation products, which may occur during vaping. As a positive control, a smoking group was included, which consumed spiked cigarettes with the same stable-isotope labeled constituents in a similar way to the vaping group.

We observed elevated levels of the mercapturic acids (MA) of the epoxides ethylene oxide (HEMA), propylene oxide (2-HPMA), and glycidol (DHPMA) in urine of smokers. In contrast, only the MA corresponding to glycidol was found in the vapers at lower concentrations compared to smokers. The metabolites for ethylene oxide and propylene oxide were not detectable in vapers. In conclusion, a specific uptake of glycidol was observed after vaping the test product.

In order to be able to correlate these biomarker data with smoke and aerosol concentrations, we developed a GC-MS method for the quantification of epoxides after liquid trapping.

This poster presentation summarizes the results of the epoxide analyses in smoke and vapor, linking the smoke/aerosol data to the obtained metabolite levels in urine.

Clinical Study

- 25 healthy male Caucasian volunteers, aged 21 to 60 years; BMI: 18 – 30 kg/m^2
- 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
- Defined smoking session: 1 non-filter cigarette at a puff interval of 30 s and puff duration of 4 s

Test items:

- E-cigarette: Eleaf iStick TC 40 W with Aspire Nautilus mini 2mL 1.8 Ω tank; American Blend flavor e-liquid PG/G 50/50, 12 mg/mL nicotine (Happy Liquid, Germany) including 10 % (w/w) of $^{13}C_3$-PG, $^{13}C_3$-G, D_7-nicotine
- Combustible cigarette: non-filter cigarette spiked with 13.4 mg $^{13}C_3$-PG, 13.6 mg $^{13}C_3$-G, and 0.32 mg D_7-nicotine

Characterization of test items:

- Machine derived smoke/aerosol yields from 10 replicates:
  20 puffs (EVP) or 7 puffs (CC), 30 sec puff interval, 4 s puff duration, 55 mL square wave puff

➢ Machine-smoking/vaping resembled the regimen set in the clinical study

➢ EVP aerosol was analyzed at 10 W (low wattage condition) and 18 W (high wattage condition)

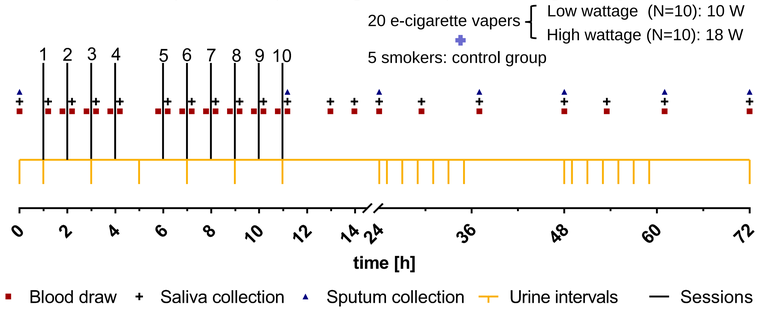

Figure 1: Time scheme for the clinical study. Lines 1 – 10 indicate time points for the vaping/smoking session. Sample collection is marked with various symbols. The clinical study is described in detail in [1].

References:

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Formation of aldehydes and epoxides in cigarette smoke and e-cigarette (EVP) aerosol

VOCs such as the aldehydes formaldehyde (FA), acetaldehyde (AA), acrolein (ACR), or the epoxides propylene oxide (PO) and glycidol are of particular interest in the risk assessment of EVPs due to their possible formation from G and PG in the aerosol according to Figure 2. The aforementioned VOCs are constituents of the gas phase of cigarette smoke but also originate from various other environmental sources resulting in high background levels, especially for FA and AA [2]. Reported FA and AA concentrations in e-cigarette aerosol highly vary depending on the used EVP but also on the methodology and issues with background levels are frequently discussed [3]. Hence, introducing the stable-isotope labeled constituents $^{13}C_3$-PG and $^{13}C_3$-G into the e-liquid as potential precursors helps to unequivocally assess the formation of VOCs specific to the use of the EVP.

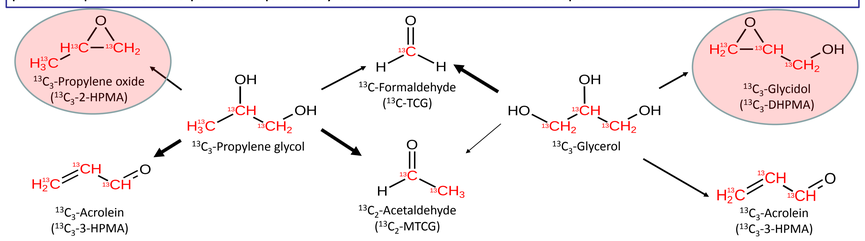

Figure 2: Postulated formation of the aldehydes and epoxides investigated in the current study from PG and G according to [4, 5]. The main routes are illustrated with bold arrows. The corresponding biomarkers are given in brackets.

- **Analytical challenges** to overcome for the analysis of propylene oxide and glycidol from smoke/aerosol:
  - ➢ Several trapping experiments performed under varying conditions (filter trapping, different organic solvents with varying trapping temperatures)
  - ➢ Several chromatographic conditions (GC-MS) assessed to combine both analytes in one chromatographic run.

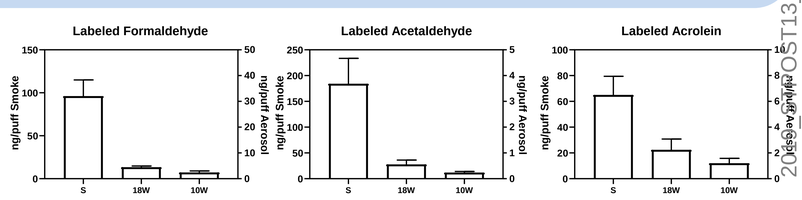

Figure 3: Labeled FA, AA, and ACR determined from smoke (test combustible cigarette) and aerosol (test EVP). Aerosol levels were determined at 10 W and 18 W mimicking the conditions of the clinical study. Note the different scales of the left y-axis for smoke and the right y-axis for aerosol.

- Higher aldehyde levels at 18W compared to 10 W on per puff basis due to more aerosol produced
- Formation of aldehydes under smoking conditions (combustion) more pronounced compared to aerosol of EVP by a factor of 30 (ACR, FA) and 400 (AA) (Figure 3)

➢ Higher temperature during combustion leads to increased degradation of PG/G to FA, AA, ACR

Table 1: Mean concentrations of labeled FA, AA, ACR in aerosol

Analyte	Mean ± SD (ng/g liquid) 10 W	Mean ± SD (ng/g liquid) 18 W
^{13}C-FA	150 ± 34	162 ± 23
$^{13}C_2$-AA	15 ± 4	20 ± 6
$^{13}C_3$-ACR	80 ± 30	81 ± 32

➢ Formation of 15 – 162 ng/g liquid of labeled aldehydes in aerosol of test EVP (AA < ACR < FA)

➢ Estimation of EVP specific uptake per subject based on average liquid consumption (1.4 g, 10% replacement) in the study: 2200 ng FA, 250 ng AA, 1140 ng ACR

Uptake and excretion of VOCs deriving from PG and G after vaping – Identification of suitable (labeled) biomarkers of exposure

The toxicants of interest are part of the daily diet and/or even formed endogenously, which results in high background levels. Our approach using $^{13}C_3$-labeled PG and G allowed us to distinguish between background and smoke/vaping related uptake measuring suitable biomarkers of exposure (BoEx) in urine. A new method based on LC-MS/MS was developed and validated for BoEx to FA and AA (Figure 4). Labeled mercapturic acids (MA) were successfully included into a previously established LC-MS/MS method [6].

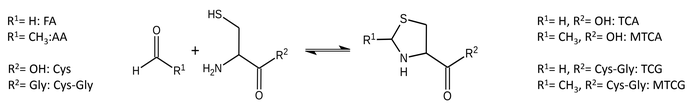

Figure 4: Formation of (methyl)-thiazolidine carboxylic acid (M)TCA and of (methyl)-thiazolidine carbonylglycine (M)TCG in the human body by cyclization of FA and AA, respectively, with Cys(-Gly) [7, 8].

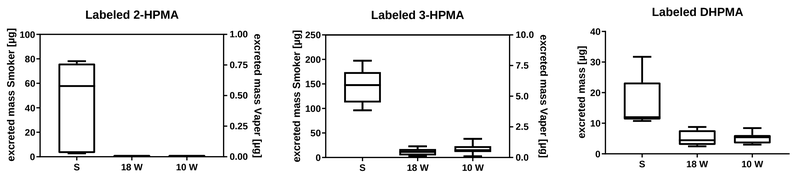

Figure 6: Box plots for labeled 2-HPMA, 3-HPMA and DHPMA in urine of smokers and vapers (at 18 W and 10 W). Excreted mass in urine until 48h after start of the clinical study. Note the different scales of the left y-axis for smokers and the right y-axis for vapers regarding the plots for 2-HPMA and 3-HPMA.

- Labeled 2-HPMA only found in smokers; labeled 3-HPMA at much higher levels in smokers (Figure 6)
- Uptake of labeled ACR from EVP estimated from machine-derived vaping to 114 ng already led to detectable levels of labeled 3-HPMA in vapers' urine
- Labeled DHPMA was observed in smokers and vapers (Figure 6).

Summary & Conclusion

- FA, AA, ACR found in their labeled form in aerosol of the test EVP and to a much higher extent in smoke
- Labeled metabolites of FA (TCA/TCG), ACR (3-HPMA), glycidol (DHPMA) and PO (2-HPMA) were detected in urine of smokers.
- Analytical challenges in the determination of epoxides (propylene oxide, glycidol) from aerosol/smoke.
- Labeled TCA/TCG and 3-HPMA in much lower levels but still detectable in urine of vapers but no 2-HPMA observed in vapers. DHPMA was found in vapers as well as smokers

➢ ***TCA/TCG for FA, 2-HPMA for PO, 3-HPMA for ACR and DHPMA for glycidol identified as suitable BoEx for the risk assessment of e-cigarettes (EVPs)***

➢ ***Stable-isotope labeling of the main e-liquid constituents proved its suitability to understand the formation and uptake of several VOCs under cigarette and EVP use***

➢ ***Linking machine-derived smoke and aerosol concentrations of the labeled compounds with BoEx levels in smokers and vapers may reveal additional information in the risk assessment of EVPs***

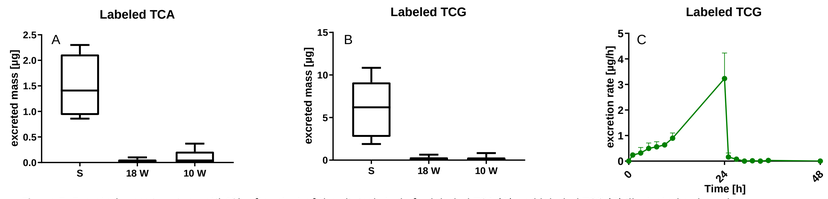

Figure 5: Excreted mass in urine until 48h after start of the clinical study for labeled TCA (A) and labeled TCG (B) illustrated as box plots. Excretion rates of smokers in urine for TCG (C).

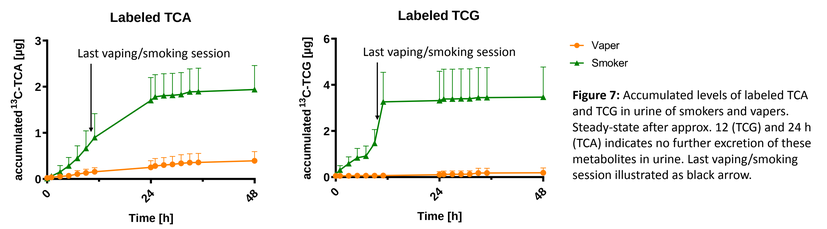

Figure 7: Accumulated levels of labeled TCA and TCG in urine of smokers and vapers. Steady-state after approx. 12 (TCG) and 24 h (TCA) indicates no further excretion of these metabolites in urine. Last vaping/smoking session illustrated as black arrow.

- (M)TCA and (M)TCG in similar concentrations detected in smokers and in vapers (not shown)

➢ Representing the endogenous background levels of FA/AA

- Labeled MTCG and MTCA was not found in any group
- MTCA not stable in our investigations (investigations for MTCG on-going)

➢ MTCA/MTCG not suitable for exposure assessment of AA from smoking/vaping

- A correlation between smoking and BoEx levels for labeled TCA/TCG was observed
- Excretion peak after last smoking session (Figure 5C); accumulation in urine complete after 12/24h (Figure 7)
- Labeled TCA and TCG were found in smokers and at much lower levels in vapers after consumption of the test items (excreted mass after 48h; Smoker: 1.5 µg ^{13}C-TCA; 6.0 µg ^{13}C-TCG; Vaper: 0.1 µg ^{13}C-TCA; 0.2 µg ^{13}C-TCG)
- Uptake of labeled FA from EVP estimated to 220 ng (machine-vaping) sufficient to detect labeled TCA and TCG (around LOQ)
- Higher levels of labeled FA in smokers readily detectable in smokers' urine as ^{13}C-TCA and ^{13}C-TCG

Outlook

➢ Further development and validation of suitable analytical method(s) for determination of the epoxides propylene oxide and glycidol in aerosol and smoke

➢ Further investigations to identify a suitable biomarker for AA exposure from smoking and vaping