Novel biomarkers to characterize exposure to aldehydes from e-vapor products based on stable-isotope constituents

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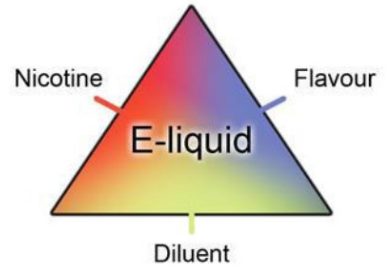
Strategy of stable-isotope labeling

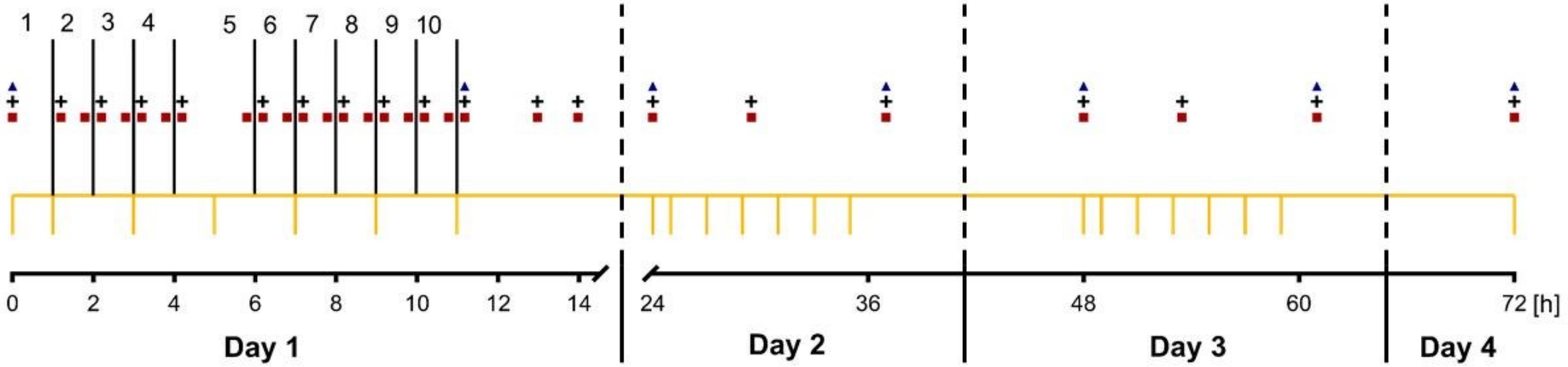
- E-Cigarette use-related uptake is difficult to distinguish from other sources



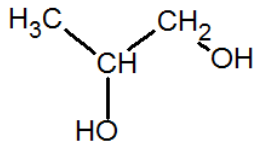
- Stable-isotope tracers are used as the “gold” standard method in MS for understanding kinetics, uptake and distribution of various compounds in living organisms
- 10% replacement of the e-liquid with stable isotope labeled constituents.
  - Differentiate between E-cig related levels of PG, VG and Nic and those from other sources
  - Aerosol analysis of labeled carbonyls specific for degradation of PG and VG
  - Measurement of labeled biomarkers of exposure (BoEx) to assess the metabolic fate of compounds formed from the main constituents in the body

Clinical study

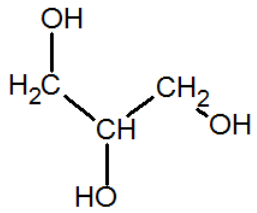
- 20 healthy experienced e-cigarette vapers + 5 healthy smokers (control group)
  - Male: age 21 - 60 years; BMI 18 – 30 kg/m^2
  - Vapers: e-liquid consumption ≥ 1.5 mL and no dual use
  - Smokers: > 10 cigarettes per day
  - 3 subgroups:
    5 smokers of conventional cigarettes
    10 vapers at low wattage (10 W)
    10 vapers at high wattage (18 W)



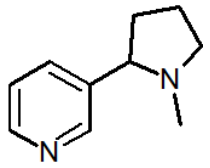
Preparation of stable-isotope labelled test items



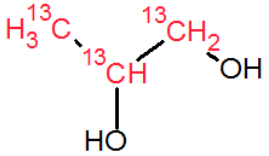
Propylene glycol (PG)



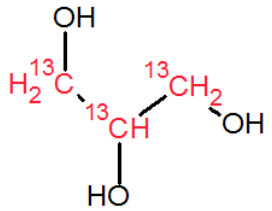
Glycerol (VG)



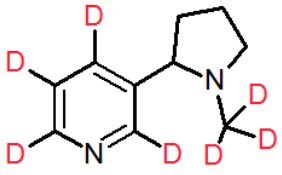
Nicotine (Nic)



$^{13}C_3$-PG



$^{13}C_3$-VG



D_7-Nic

- Customized e-liquid for the study from Happy Liquid (Munich, Germany):
  PG and VG 50:50 (w/w); 1.2 % Nic; American Blend flavor
- Replacement: 10% with isotope labeled constituents
- Test e-cigarette: Eleaf iStick TC 40 W (W adjustable) with Aspire Nautilus mini tank 2mL 1.8 Ω
- Non-filter combustible cigarettes (10 mg tar; 0.8 mg Nic; 10 mg CO (ISO))
- Cigarette spike: 13.4 mg $^{13}C_3$-PG, 13.6 mg $^{13}C_3$-G, 0.32 mg D_7-nicotine
- Spiking solution evenly distributed along the central axis of the tobacco rod using a needle-armed syringe
- Labeled compounds purchased from Aptochem (Montreal, Canada)
- Purity taken into account for spiking

Characterization of labelled main constituents

ACCEPTED MANUSCRIPT

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

Anne Landmesser, MSc, Max Scherer, PhD, Nikola Pluym, PhD, Mohammadi Sarkar, Prof, Jeffery Edmiston, PhD, Reinhard Niessner, Prof, Gerhard Scherer, Prof ✉

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Abstract

Introduction

An important basis for risk estimation for e-cigarette users is a well-founded dosimetry. The objective of this study was to assess the applicability of stable-isotope e-liquid ingredients for exposure studies in vapers.

Methods

E-cigs with 10% of labeled propylene glycol (PG), glycerol (G) and nicotine was used by 20 experienced vapers under controlled (Part A) and free (Part B) conditions. In Part A, 10 subjects vaped at 10 W and another 10 subjects at 18 W power setting of the e-cig. In Part B, the same subjects used the same product *ad libitum* in their usual environment. Five smokers, smoking 10 non-filter cigarettes, spiked with labeled PG, G and nicotine, served as positive control during Part A. PG, G, nicotine and its metabolites were measured in plasma, urine and saliva.

Results

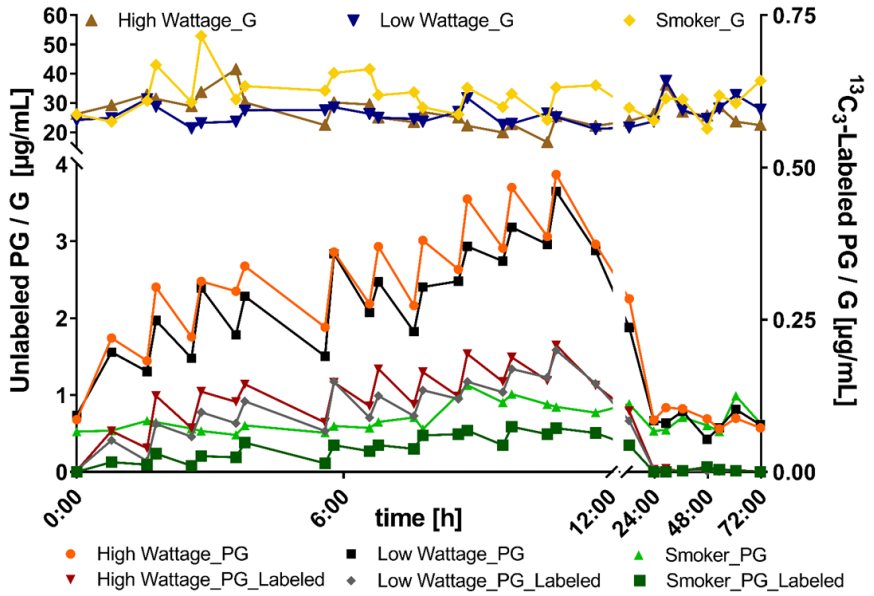
Peak nicotine levels (sum of measured labeled and unlabeled) in plasma were lower in vapers (15.8 to 19.6 ng/mL) than in smokers (36 ng/mL). The labeled plasma nicotine levels were ten times lower than the unlabeled, reflecting the ratio in the e-liquid. PG levels in plasma and urine also reflected the vaping activities in Part A, while G in these body fluids showed no association with vaping.

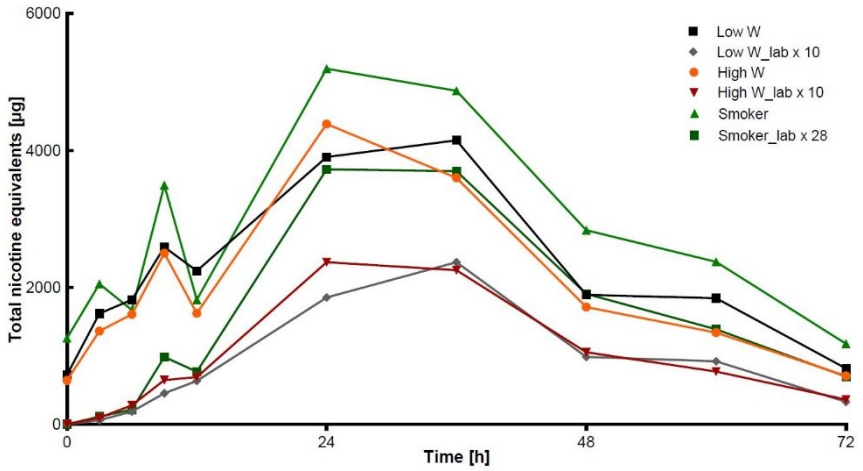
Conclusions

This proof of concept study shows that the application of labeled e-liquid ingredients allows the accurate quantification of the dose of nicotine and PG when other nicotine and tobacco products were used simultaneously. Unchanged G was not assessable by this approach.

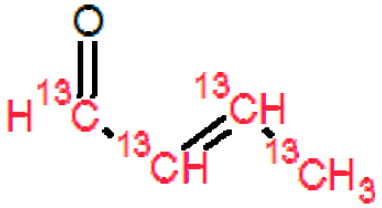
Implications

This approach allows the investigations of the absorption of potential PG-, G- and nicotine-derived vapor constituents (e.g. aldehydes and epoxides) by vaping. Appropriate studies are in progress in our laboratory.

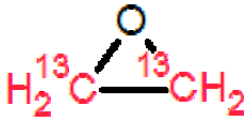




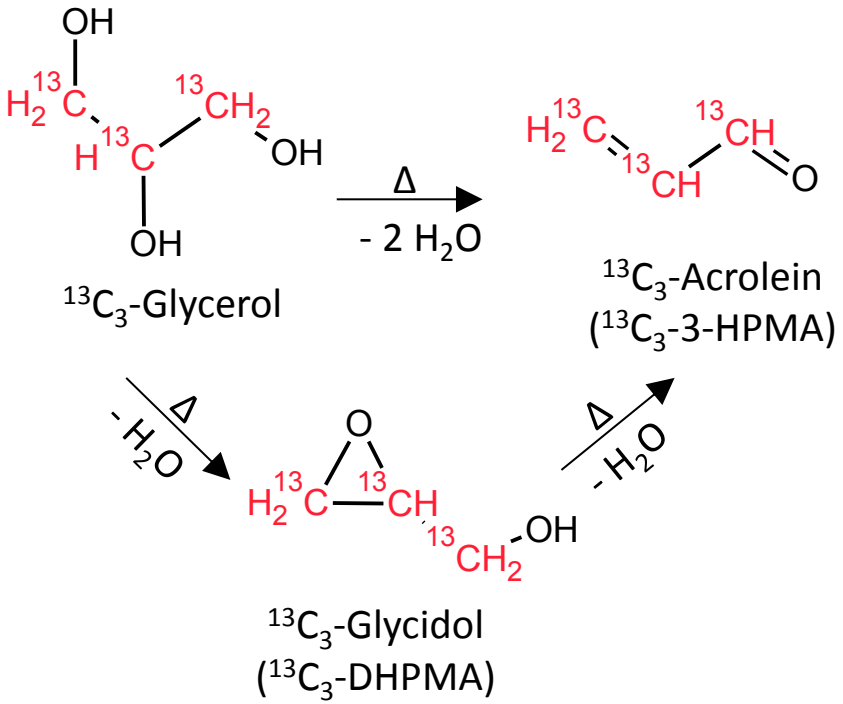
Thermal degradation (pyrolysis) of PG / G



$^{13}C_4$-Crotonaldehyde
($^{13}C_4$-HMPMA)



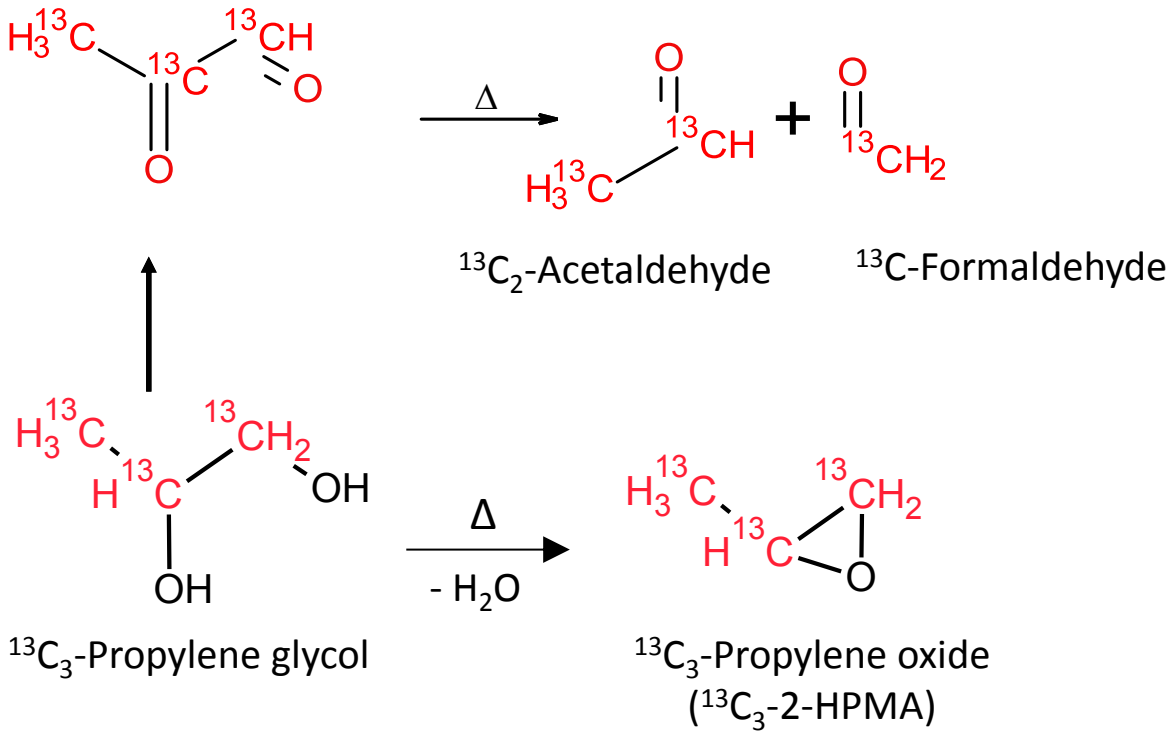
$^{13}C_2$-Ethylene oxide
($^{13}C_2$-HEMA)



$^{13}C_3$-Glycerol

$^{13}C_3$-Acrolein
($^{13}C_3$-3-HPMA)

$^{13}C_3$-Glycidol
($^{13}C_3$-DHPMA)



$^{13}C_2$-Acetaldehyde

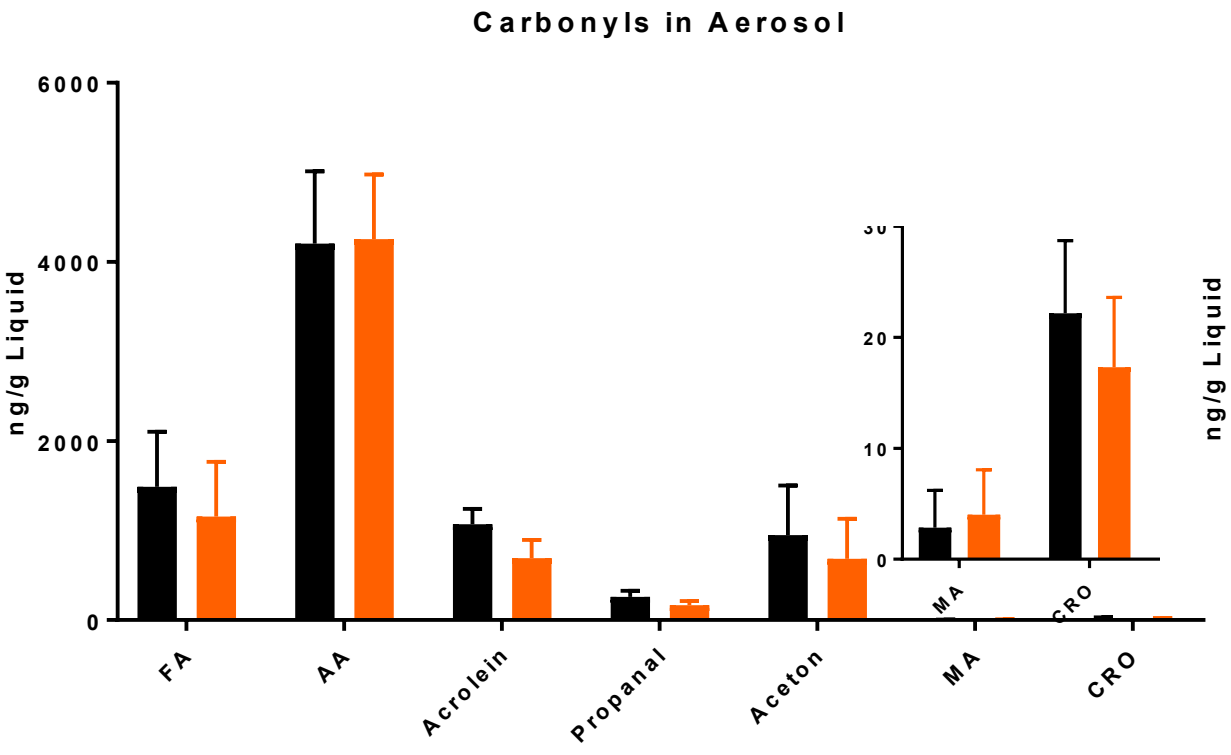
^{13}C-Formaldehyde

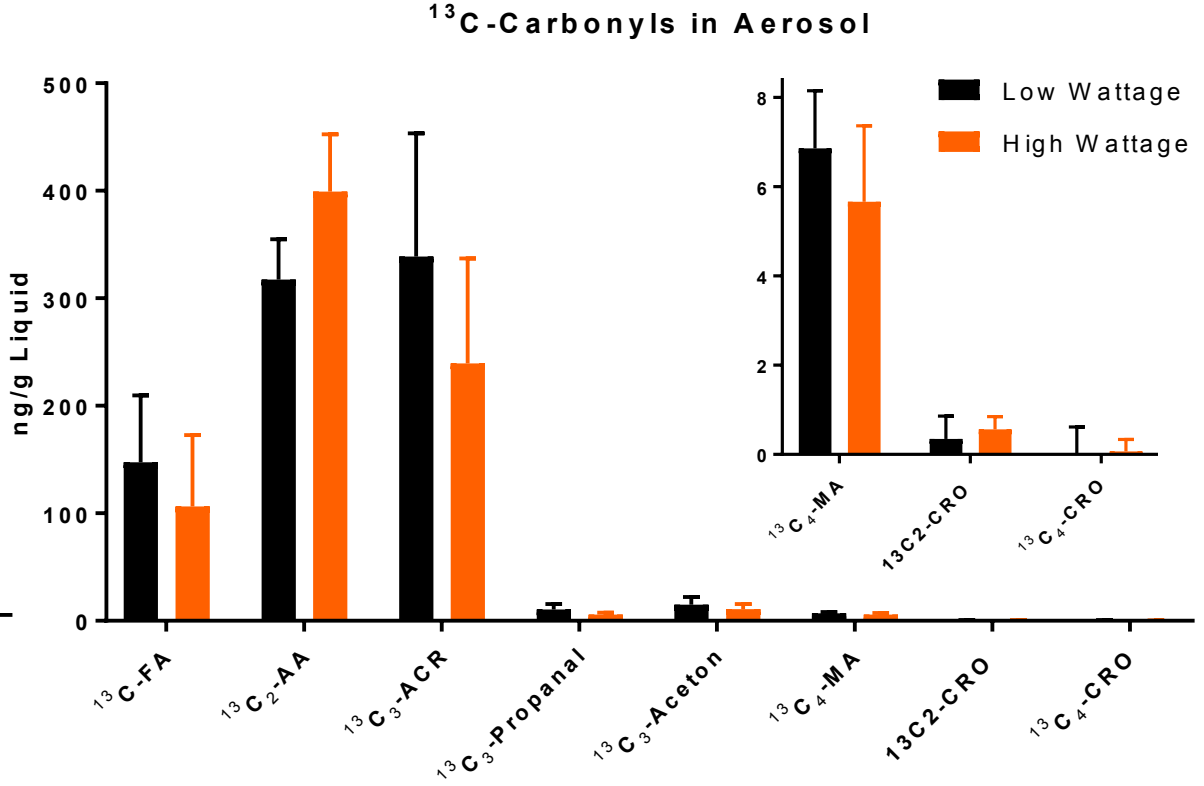
$^{13}C_3$-Propylene glycol

$^{13}C_3$-Propylene oxide
($^{13}C_3$-2-HPMA)

Sleiman, M., *et al.* Emissions from Electronic Cigarettes: Key Parameters Affecting the Release of Harmful Chemicals. *Environ. Sci. Technol.* **2016**

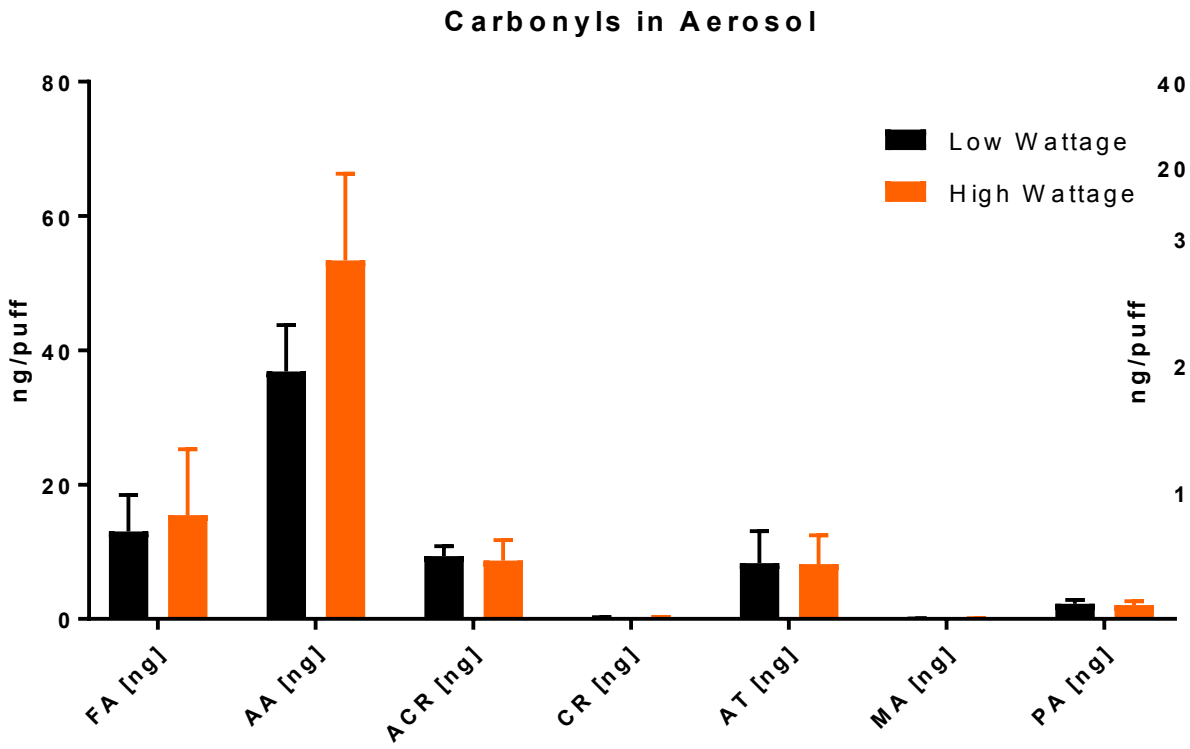
Carbonyls - Aerosol analysis

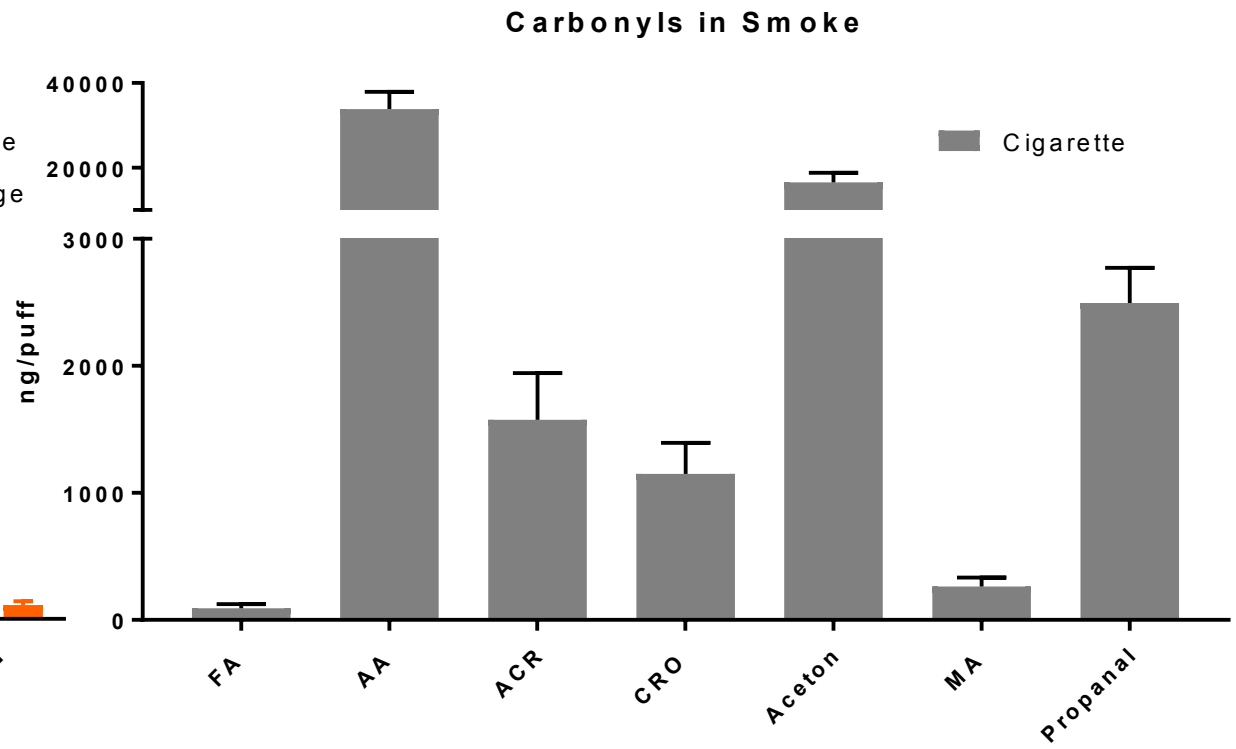




Aerosol analysis revealed formation of (labeled) FA / AA / Acrolein and to a lower extent of other carbonyls from PG / VG using the test e-cigarette at 10 W / 18 W

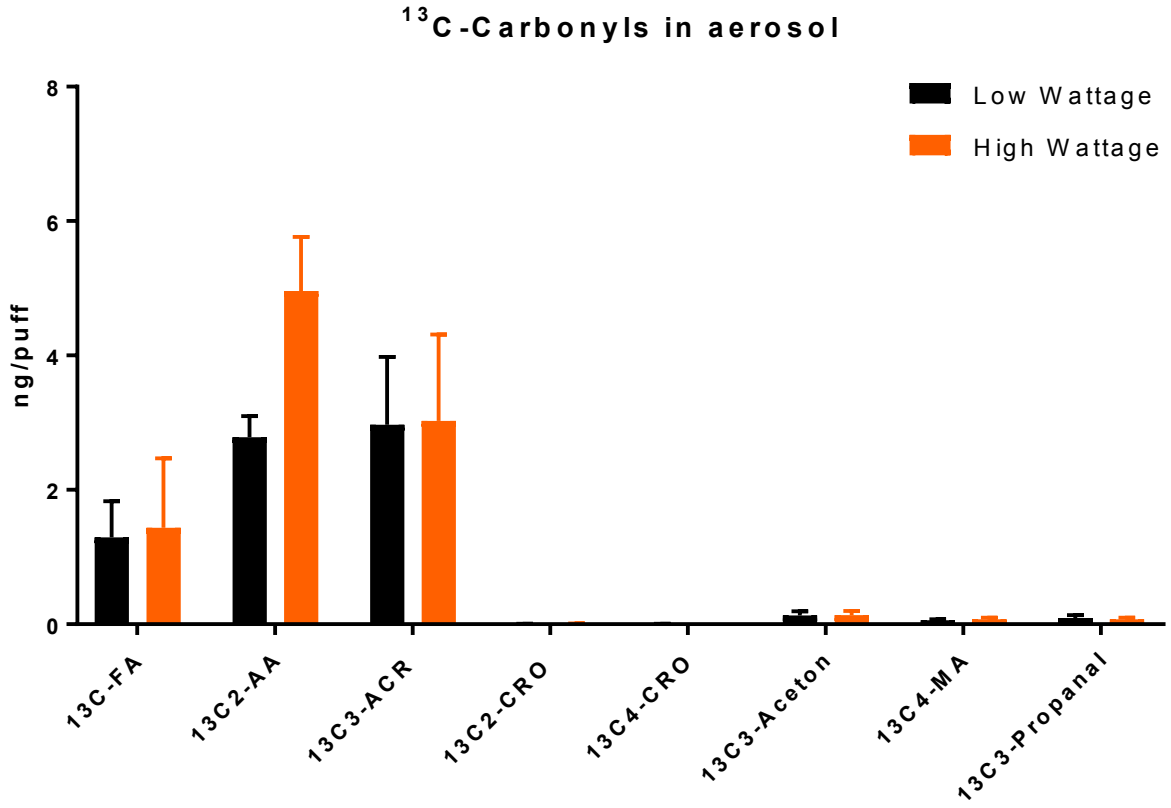
Carbonyls – smoke vs. aerosol



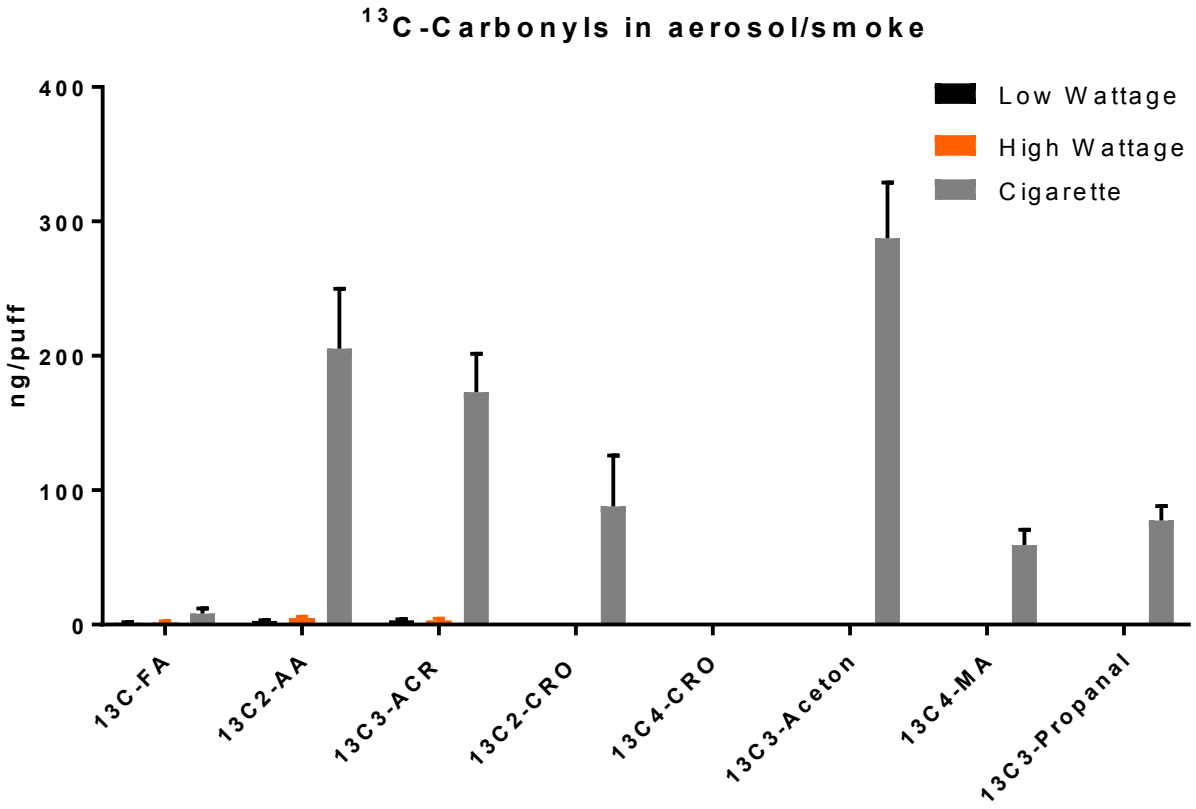


Comparison aerosol and smoke on a per puff basis:
Much higher carbonyl levels in smoke (between x 10 for FA and x 1000 for AA)

Carbonyls – smoke vs. aerosol



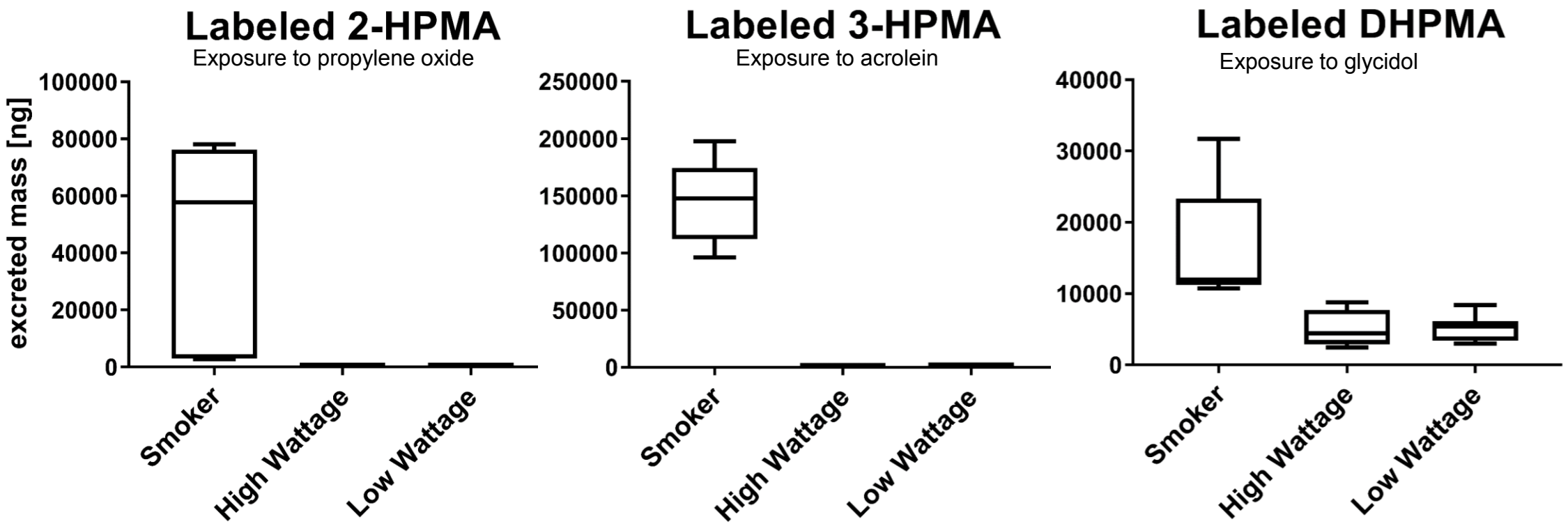
Carbonyls – smoke vs. aerosol



- Minor formation of FA / AA during e-liquid vaporization
- Much higher levels under combustion of CCs (per puff comparison: labeled FA/AA in smoke 20-1000 times higher compared to e-vapor)

Biomarkers of exposure – Mercapturic acids

Mercapturic acids (MAs) as specific BoEx to propylene oxide, acrolein, glycidol:



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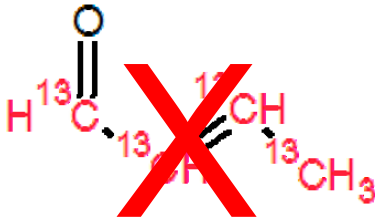

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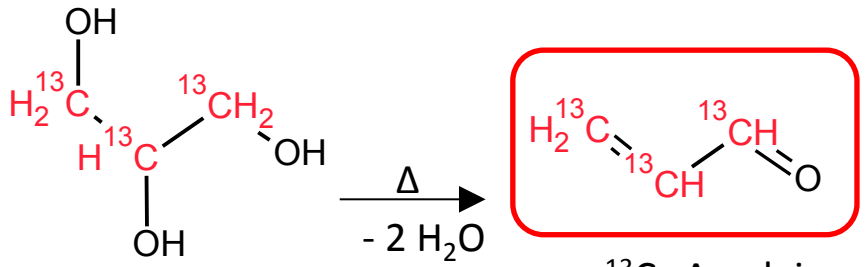
($^{13}C_4$-HMPMA)

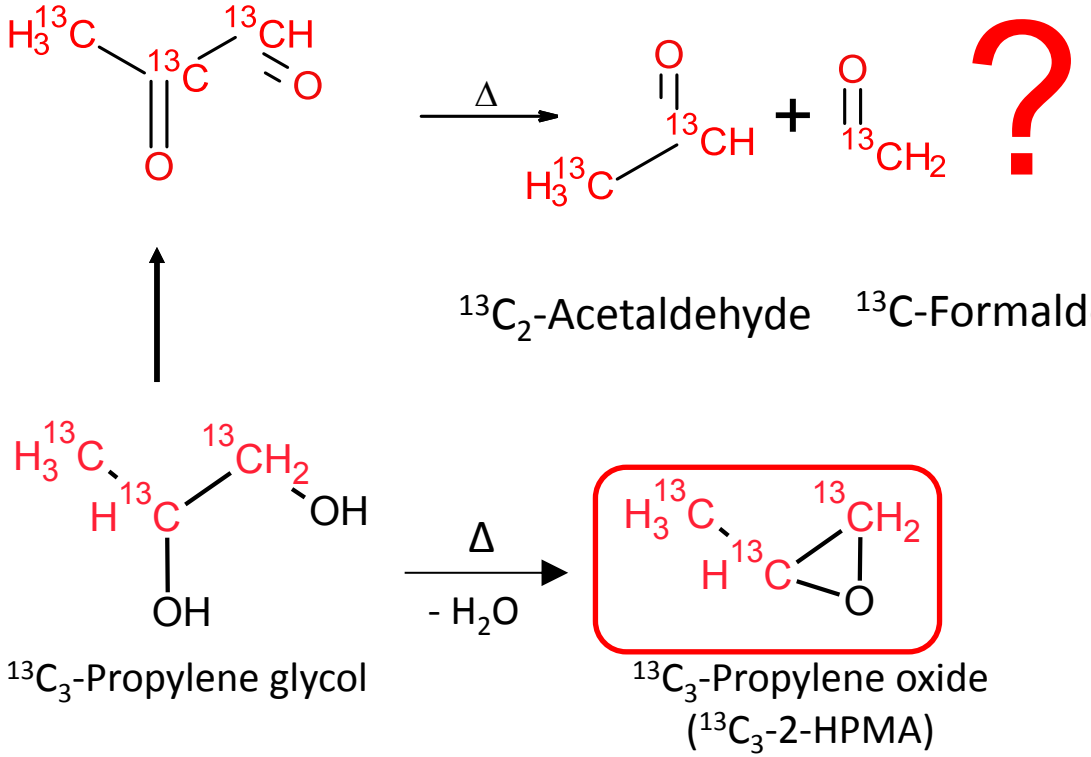
$^{13}C_2$-Ethylene oxide
($^{13}C_2$-HEMA)



$^{13}C_3$-Glycerol

$^{13}C_3$-Acrolein
($^{13}C_3$-3-HPMA)

$^{13}C_3$-Glycidol
($^{13}C_3$-DHPMA)



$^{13}C_2$-Acetaldehyde + ^{13}C-Formaldehyde

$^{13}C_3$-Propylene glycol

$^{13}C_3$-Propylene oxide
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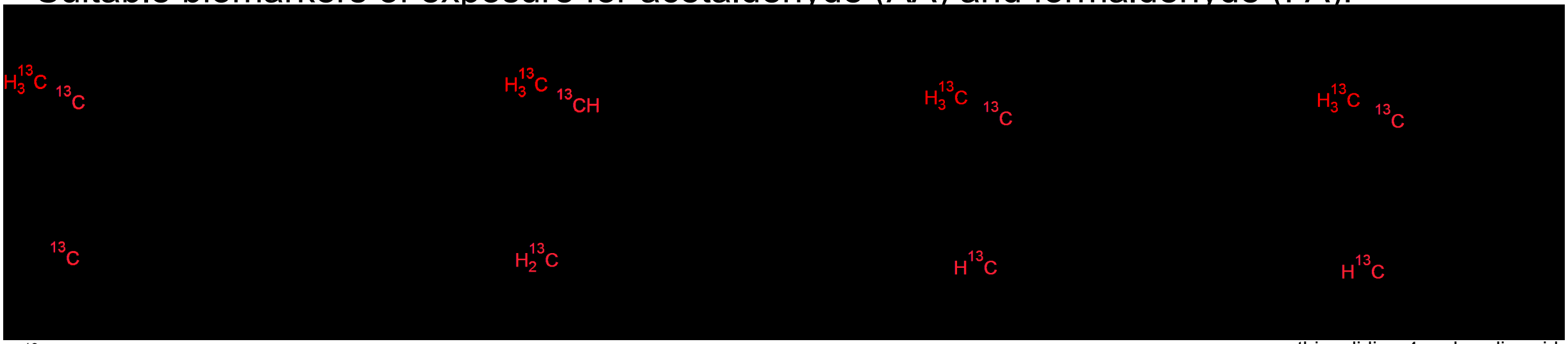
Sleiman, M., *et al.* Emissions from Electronic Cigarettes: Key Parameters Affecting the Release of Harmful Chemicals. *Environ. Sci. Technol.* **2016**

Biomarkers of exposure to AA / FA: MTCA and TCA

Suitable biomarkers of exposure for acetaldehyde (AA) and formaldehyde (FA):



^{13}C-FA Cys

Reischl, R., et al. (2012) Anal Bioanal Chem, 404, 1779-1787; Kera, Y., et al. (1985) Agents Actions, 17, 48-52.

thiazolidine-4-carboxylic acid (TCA)

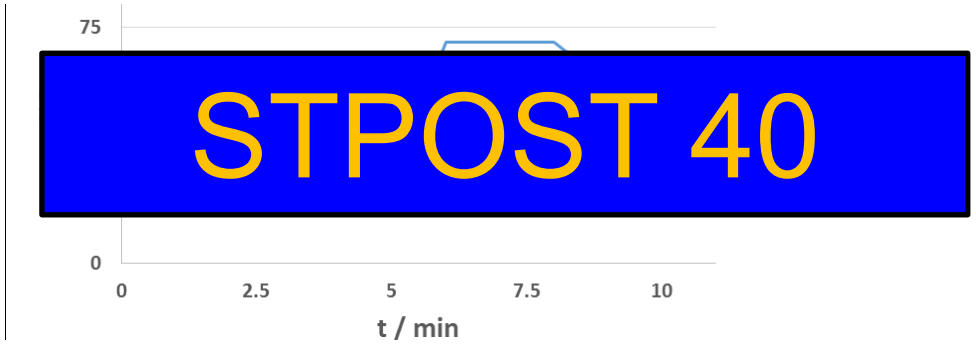
Analytical method

LC-MS/MS: Sciex 6500$^+$Qtrap (MS); Shimadzu Nexera X2 (UHPLC)

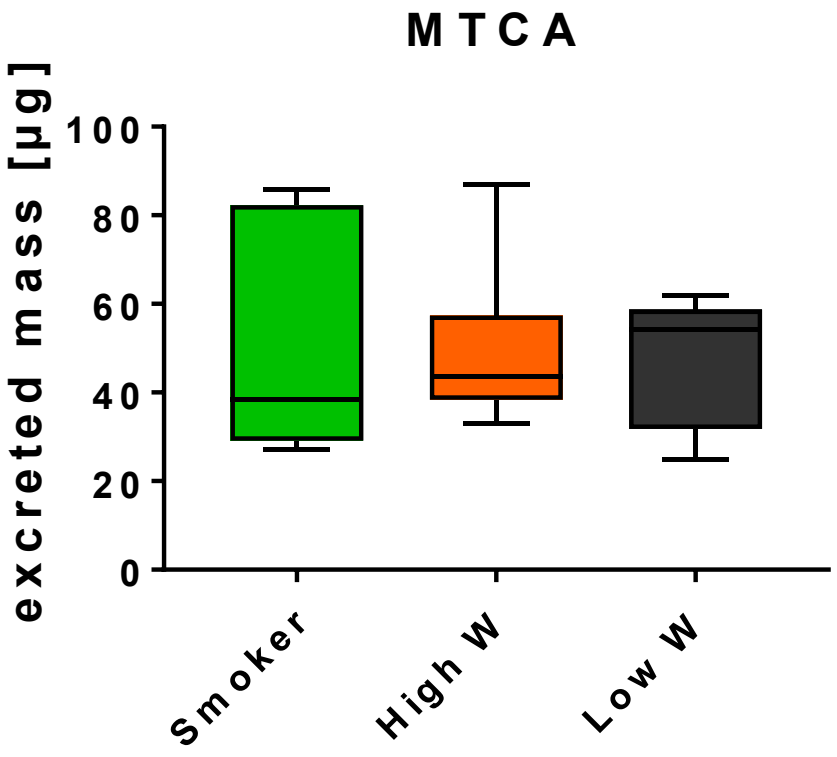
Chromatographic separation: Phenomenex Kinetex EVO C18

(150 x 2.1 mm, 5 µm); Injection volume: 10 µL; Flow rate: 0.7 mL/min

0.1% ammonium acetate (A) – ACN with 0.1% formic acid (B)

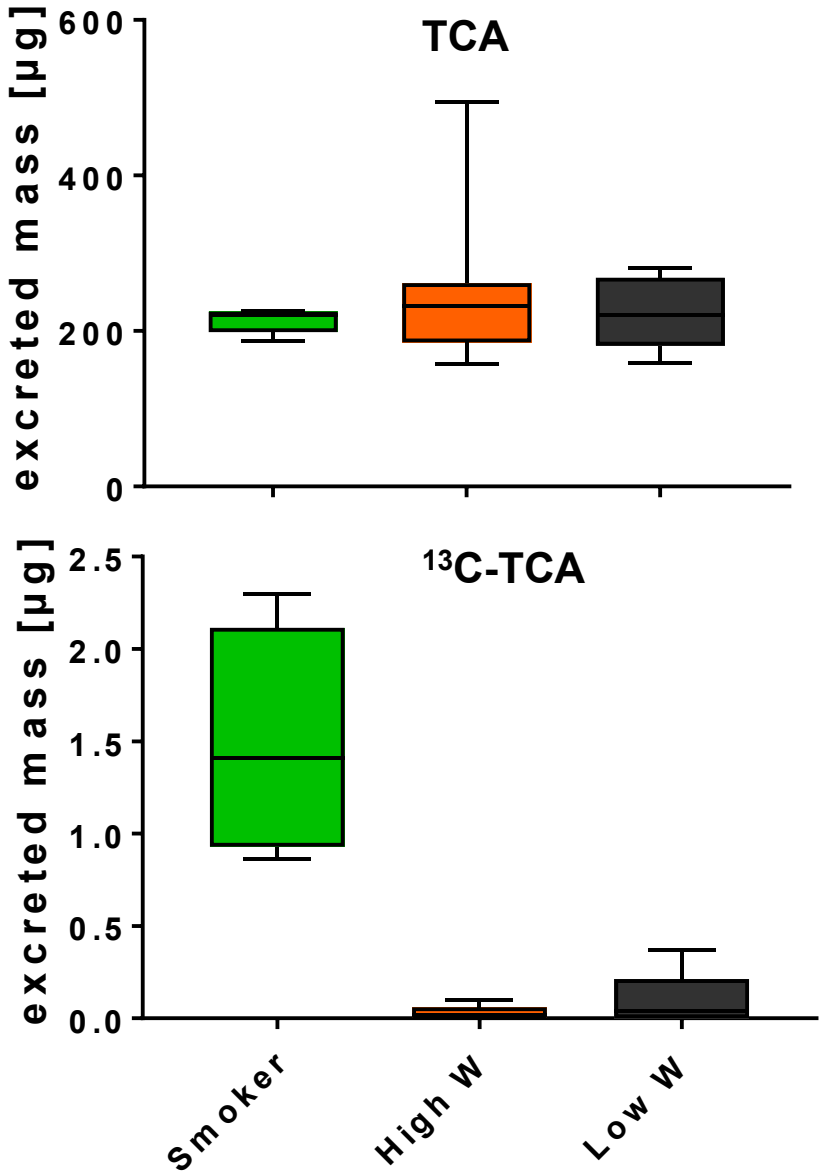


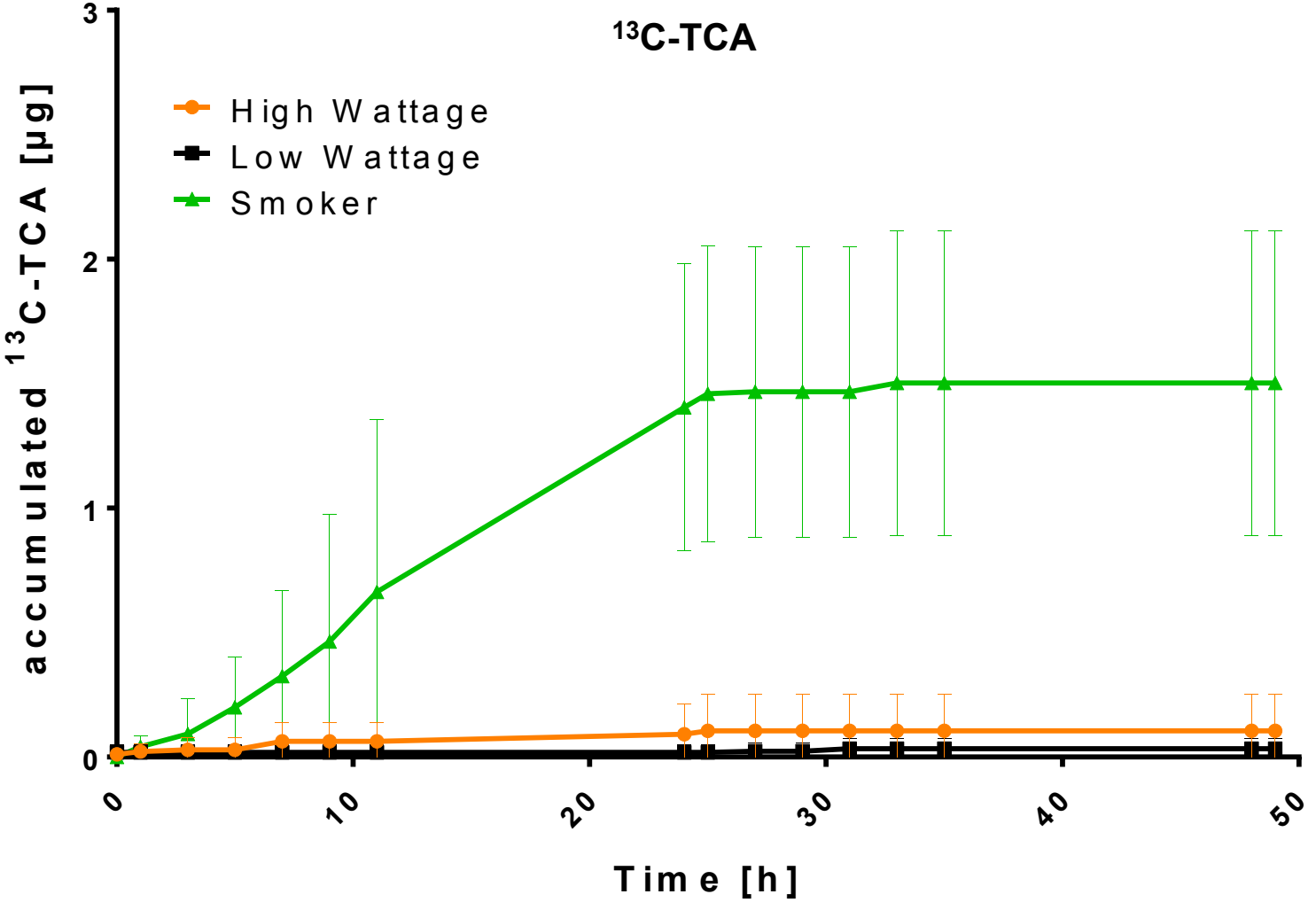
Analysis of MTCA as BoEx for AA



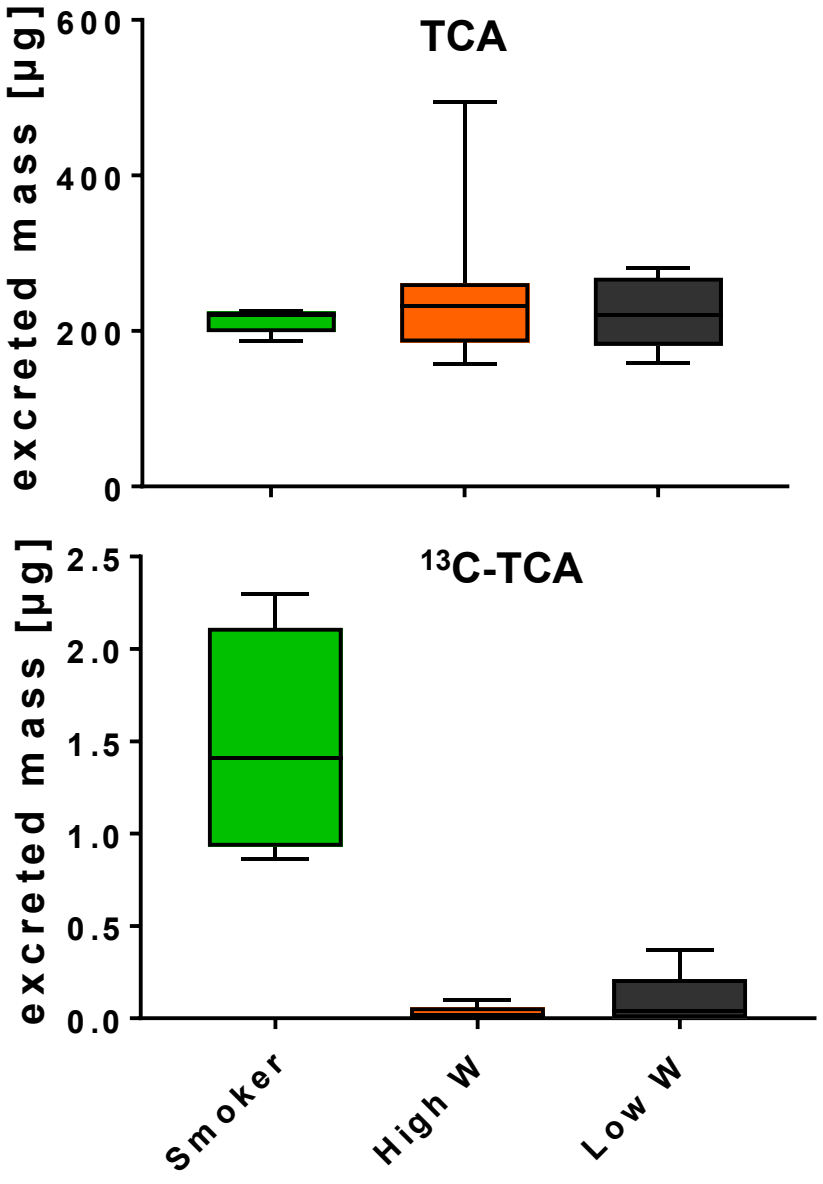
- Labeled MTCA < LOQ
- No vaping/smoking related effects on levels of MTCA observed
- Stability in urine problematic for MTCA

Analysis of TCA as BoEx for FA





Analysis of TCA as BoEx for FA



- No correlation with vaping/smoking for labeled + unlabeled MTCA and unlabeled TCA
- No significant increase of labeled TCA detected in vapers
- Labeled TCA with significant increase in smokers' urine compared to baseline

Conclusion

- Stable-isotope labeling of e-cig and CC was applied in a clinical study under controlled conditions
- Aerosol analysis revealed formation of (labeled) FA / AA / Acrolein and to a lower extent of other carbonyls from PG / VG using the test e-cigarette at 10 W / 18 W
- Carbonyl concentrations much higher in CCs (CCs >> 18 W ≈ 10 W)
- Bioanalysis: MAs of acrolein (3-HPMA) and propylene oxide (2-HPMA) were found in their labelled form in urine of smokers; MA of glycidol (DHPMA) already observed under vaping conditions (in both groups)
- LC-MS/MS method was developed for urinary analysis of MTCA (BoEx to AA) and TCA (BoEx to FA)

Conclusion

- Labeled TCA with significant increase in smokers' urine compared to baseline
- E-vapor burden not sufficient to detect an increase in the BoEx MTCA/TCA
- Even in smokers no impact on unlabeled BoEx levels observable → other sources for FA/AA dominate the levels for the BoEx (also: MTCA problematic in terms of stability)
- Labeled FA concentration in smokers resulted in a significant increase in urinary TCA as also shown for labeled acrolein, propylene oxide and glycidol

➢ Labeled TCA, 2-HPMA, 3-HPMA, DHPMA identified as suitable BoEx for risk assessment of e-vapor products considering potential PG/VG degradation

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Aptochem

Francois Deschamps

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