

Biomarkers of exposure specific to e-vapor products based on stable-isotope labelled ingredients – study design

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Outline

- Background and Objectives

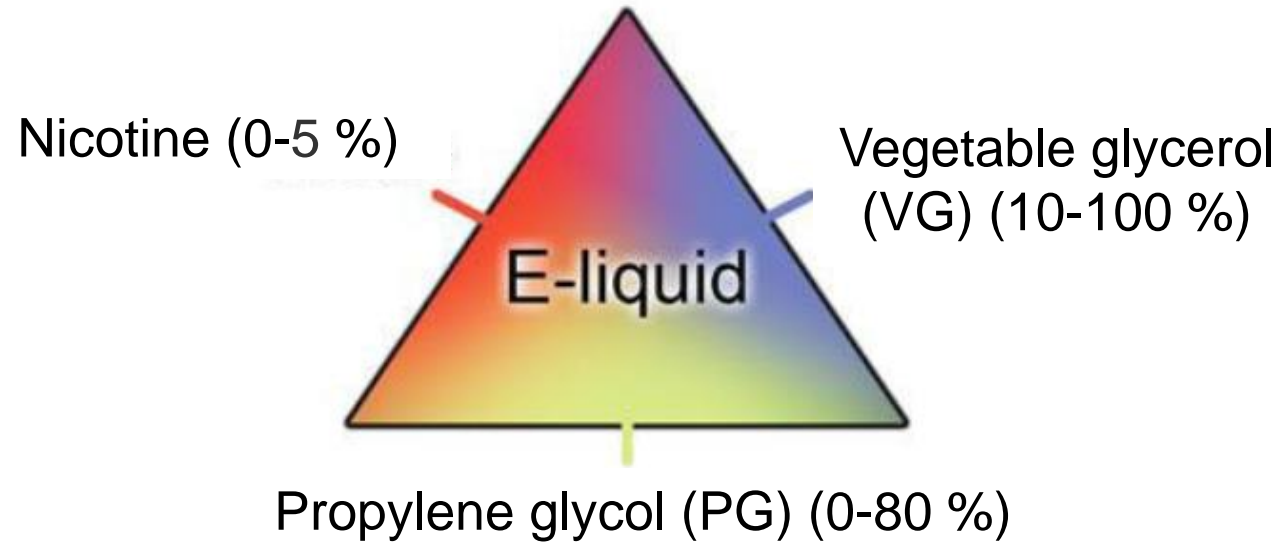
- Clinical Study
 - ✓ Study design
 - ✓ Sample collection procedure Part A
 - ✓ Sample collection procedure Part B

- Summary

Background and Objectives

Composition of the e-liquids

➤ Main ingredients:

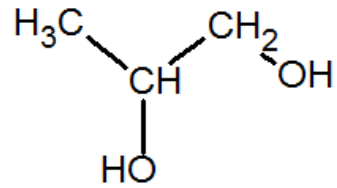


- Water (1-20 %)
- Flavours (3-5 %)

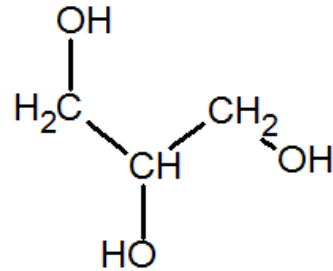
Strategy of stable-isotope labelling

- Problem: e-liquid ingredients are ubiquitous (exogenous/endogenous)
- E-Cigarette use-related uptake 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
 - Partial replacement (10%) of the e-liquid with stable isotope labelled e-liquid. Use MS to distinguish between E-cig related levels of PG, G and N and those from other sources
 - By measuring known, labelled biomarkers, this approach allows the quantitative assessment of the absorption, metabolism and further fate of PG, G, and N as well as compounds formed from these precursors in the human body.

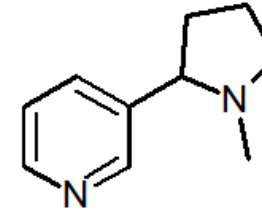
Isotopic labelled compounds



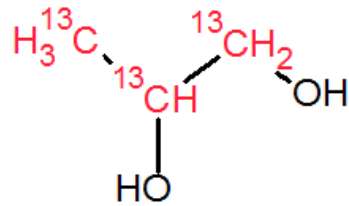
Propylene glycol (PG)



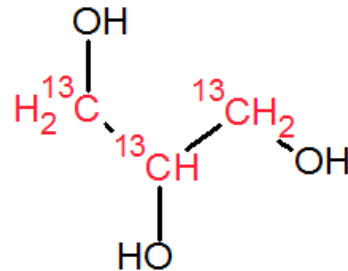
Glycerol (VG)



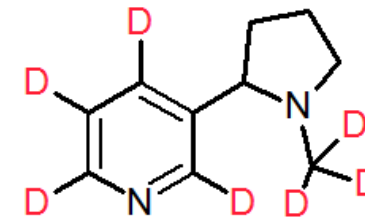
Nicotine (N)



$^{13}\text{C}_3$ -Propylene glycol



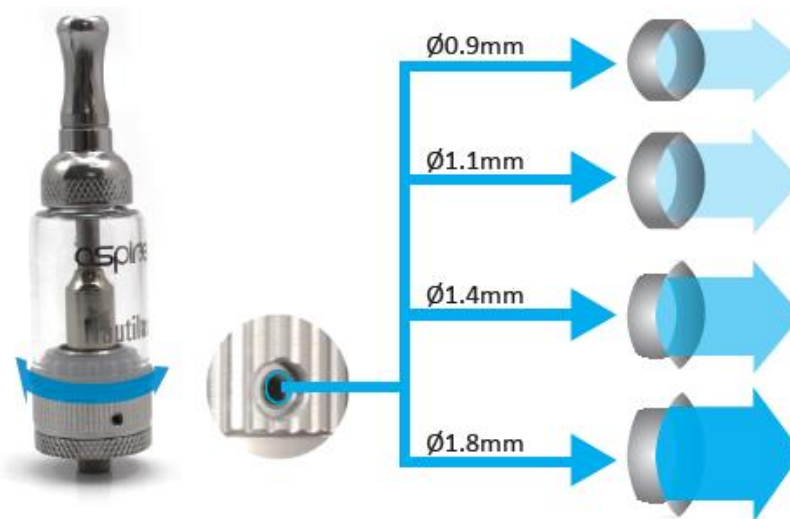
$^{13}\text{C}_3$ -Glycerol



Nicotine- d_7

- Customized e-liquid for the study (manufactured by Happy Liquid):
PG and VG 50:50 (w/w); 1.2 % N; American Blend flavor
- Replacement: 10% with isotope labelled ingredients PG, VG and N

E-Leaf iStick 40 W



Objectives

- Clinical study with 25 healthy male subjects
- Measuring of smoke related analytes in plasma, saliva, urine and sputum
 - Nicotine + 10 metabolites¹
 - Propylene glycol and glycerol
 - Metabolites of potentially occurring toxicants²
 - Tobacco-specific nitrosamines
- Integration of the labelled analytes in existing methods
- Method development:
 - Aerosol analysis
 - Determination of PG and VG in liquids as well as in human samples
 - High-sensitive method for nicotine and metabolites in plasma
 - Analysis of labelled MTCA and TCA (metabolites of acetaldehyde and formaldehyde, respectively) in human urine samples
 - Analysis of labelled mercapturic acids derived from acrolein, crotonaldehyde, ethylene oxide, propylene oxide, glycidol

¹ Piller et al. *Journal of Chromatography B*, March 1st, (951-52) 7-15

² Pluym et al. *Anal Bioanal Chem*, 3, 3.

Study design

Clinical study

- 20 healthy experienced e-cigarette vapers + 5 healthy smokers
 - Male: age 21- 60 years
 - e-Liquid consumption \geq 1.5 mL
 - Divided into 3 groups (5 smokers of conventional cigarettes, 2 e-cigarette vaper subgroups: 10 vapers low wattage; 10 vapers high wattage)

- Part A (stationary)
 - 4 days confined in the clinic
 - Diet controlled

- Part B (ambulatory)
 - 3 days at the subjects whereabouts
 - Free use of the test device with labelled liquid
 - Every evening visit in the clinic

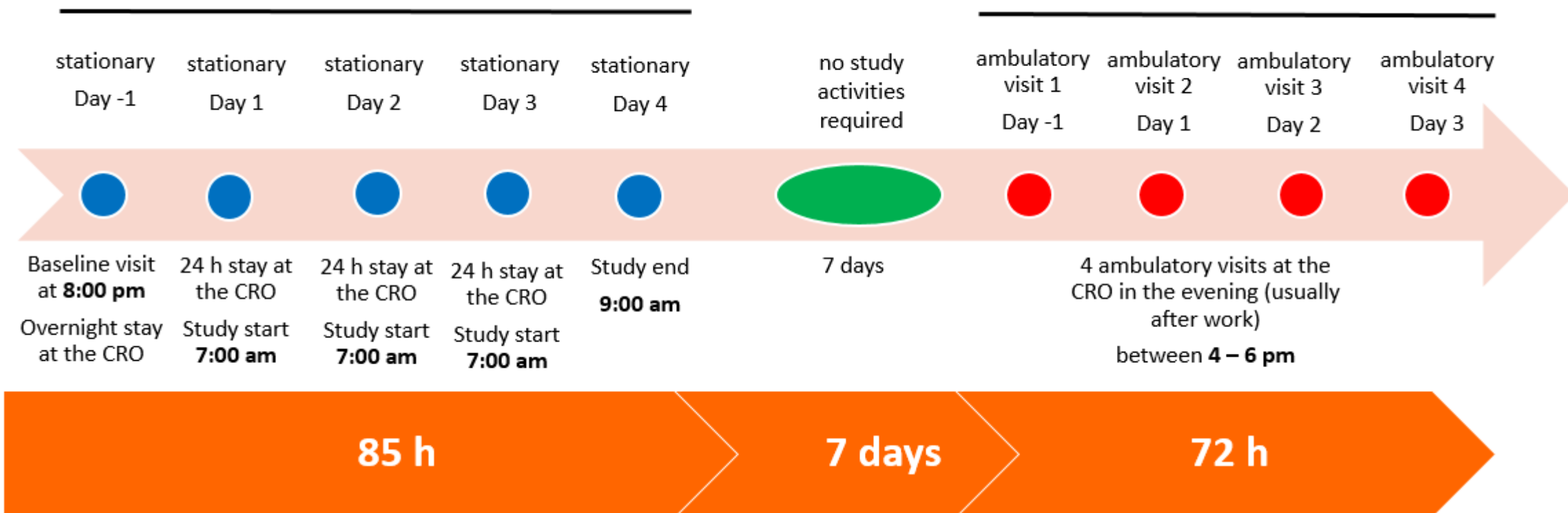
Clinical study – E-Vapors

- 10 Subjects low wattage (10 W), 10 subjects high wattage (18 W)
- E-liquid consumption ≥ 1.5 mL
- American blend flavour; 12 mg/ml Nic, PG/VG (1/1)
- 10% isotope labelled ingredients $^{13}\text{C}_3$ -PG, $^{13}\text{C}_3$ -VG and d_7 -N
- No dual users!



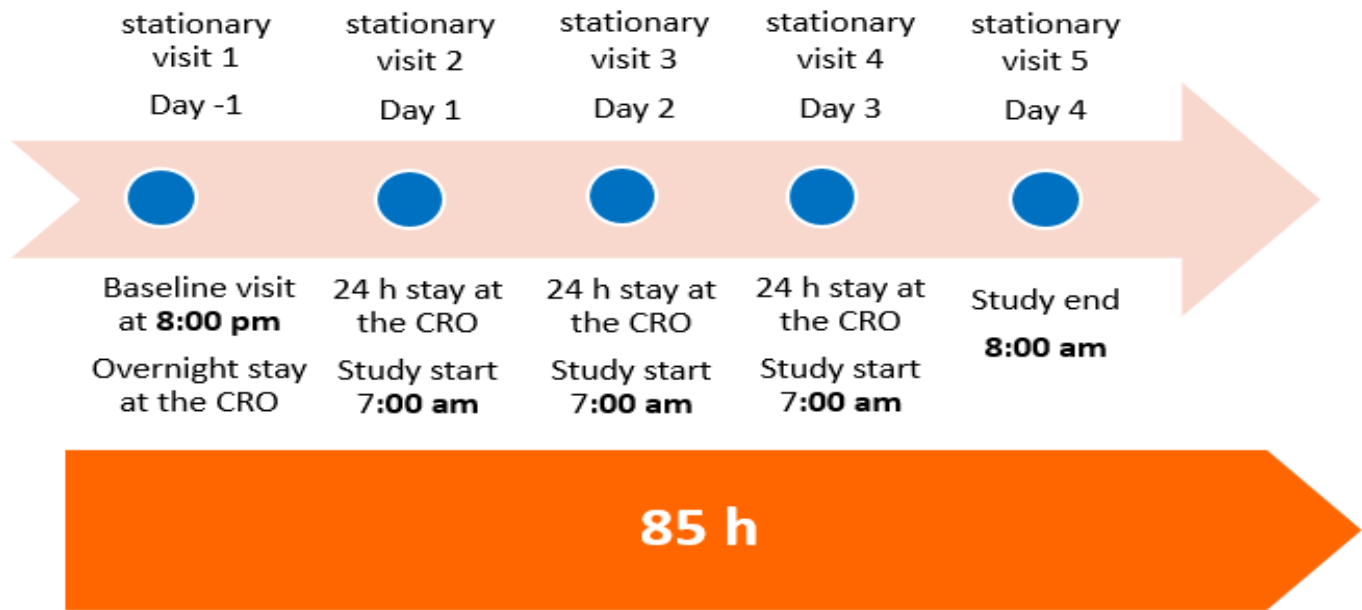
Part A

Part B



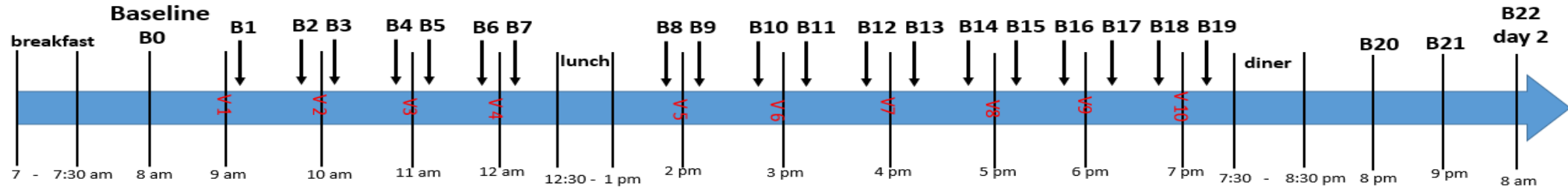
Clinical study – Smokers (Control group)

- 5 subjects
- > 10 cigarettes per day for at least the past 6 months
- Non-filter cigarette spiked with stable isotopes:
 - 10 mg tar
 - 0.8 mg Nic
 - 10 mg CO
 - 104 mg $^{13}\text{C}_3\text{-PG}$
 - 126 mg $^{13}\text{C}_3\text{-G}$
 - 2.4 mg $\text{d}_7\text{-N}$



Clinical study design: Blood (Saliva, Sputum) sample collection - Part A -

Part A Day 1:

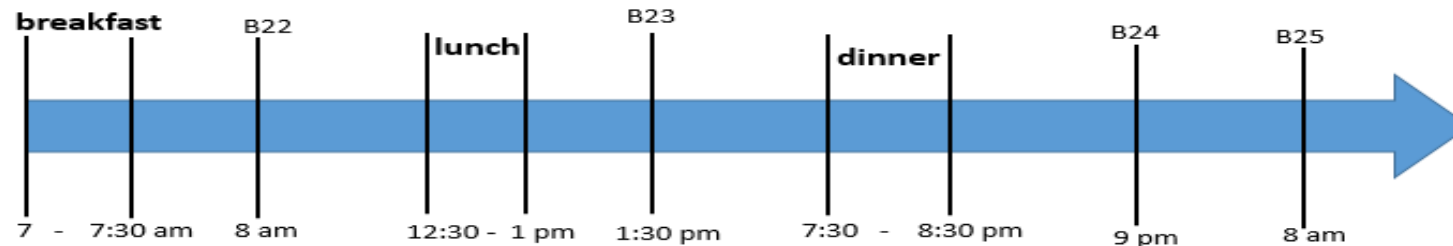


V = Vaping session

B = Blood draw

- Blood samples: before and after each vaping session
- Saliva samples: after each vaping session
- Non-induce sputum samples: in the morning, after the last vaping session and in the evening

Part A Day 2 - 4:

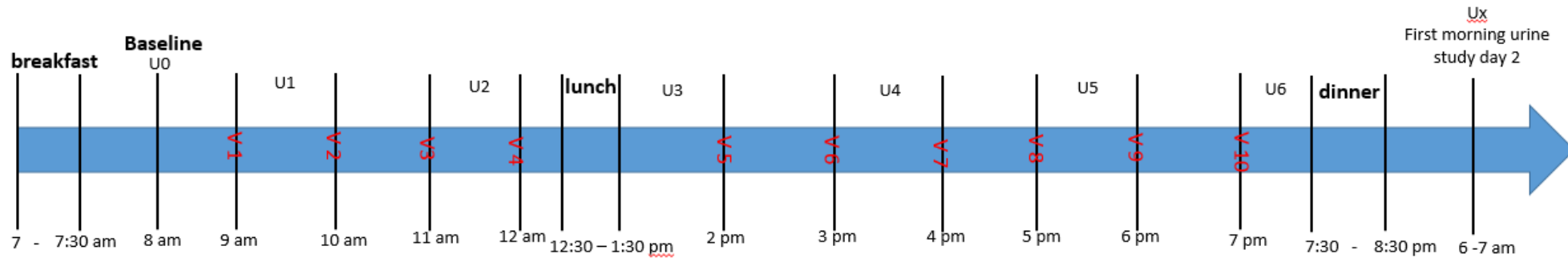


B = Blood draw

- Blood samples: in the morning, noon and evening
- Saliva samples: in the morning, noon and evening
- Non-induce sputum samples: in the morning and in the evening

Clinical study design: Urine sample collection - Part A -

Part A Day 1 - 4:



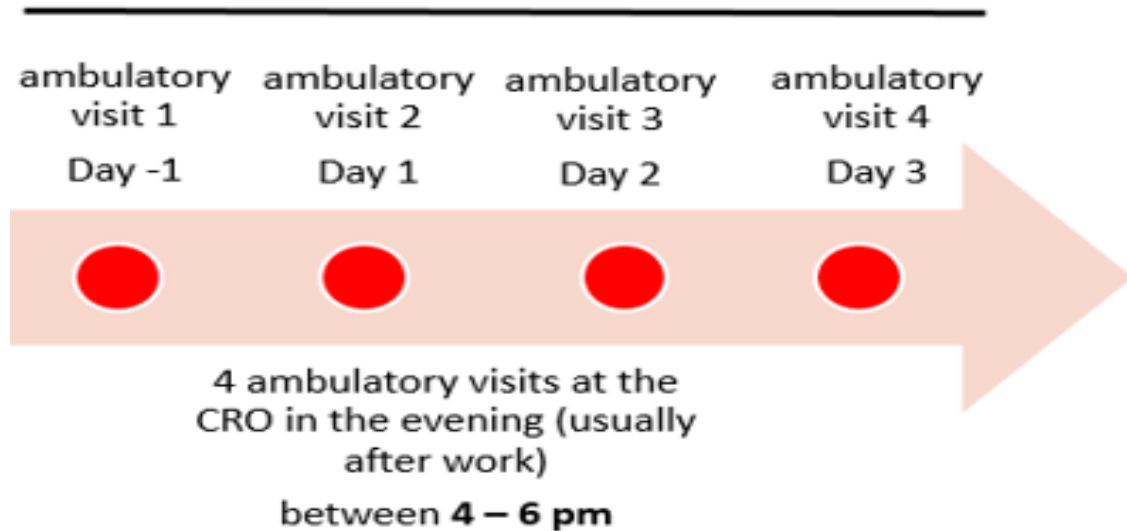
V = Vaping session

Ux = Urine fractions

- Urine samples: one urine fraction every 2 hours

Clinical study design: Sample collection - Part B -

Part B



- Blood samples: One blood draw per subject during the ambulatory visit
- Urine samples: The morning urine and a spot urine during the ambulatory visit was collected
- Saliva samples: Saliva samples were collected in the morning and during the ambulatory visit
- Sputum samples: Sputum samples were collected at the beginning (day -1) and end of part B (day 3)

Summary

- A 2-part clinical study (ethical approval received from the ethical commission Hamburg) was successfully conducted in order to assess biomarkers of exposure specific to e-vapor products based on stable-isotope labelled ingredients:
 - ✓ Part A: 4-days confinement under strictly controlled conditions
 - ✓ Part B: 3 days in an ambulatory setting
- Partial replacement (10%) of the e-liquid with stable isotope labelled e-liquid
 - ✓ $^{13}\text{C}_3\text{-G}$
 - ✓ $^{13}\text{C}_3\text{-PG}$
 - ✓ $\text{d}_7\text{-N}$
- E-Vapors were split into 2 groups with different vaping conditions:
 - ✓ Low wattage (10 W)
 - ✓ High wattage (18 W)
- Inclusion of a positive control group (CC smokers) into the clinical study required for investigation of potential pyrolysis products from PG/G as well as demonstration of the applicability of the analytical approach (proof of concept)

Study results will be presented in

ST 24

**For more information on method details for Nic
(+metabolites) PG/G, and mercapturic acid analysis visit
our poster**

STPOST 43

Acknowledgement

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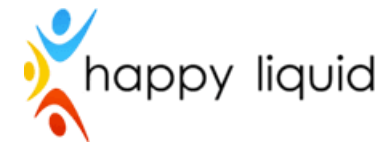
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Happy Liquid

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