

Assessing the exposure to 'smoke' from electronic cigarettes by biomarkers

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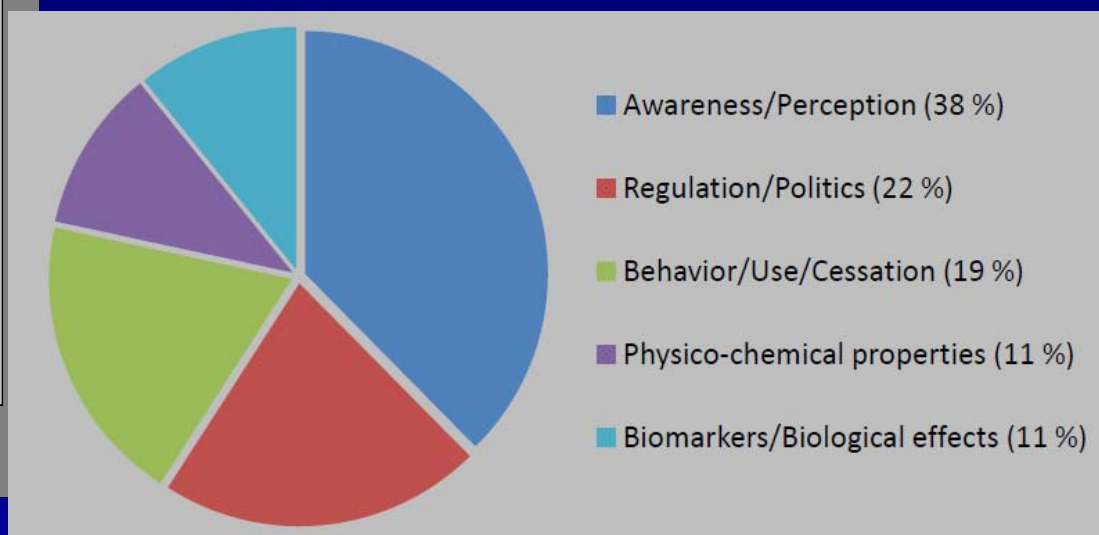
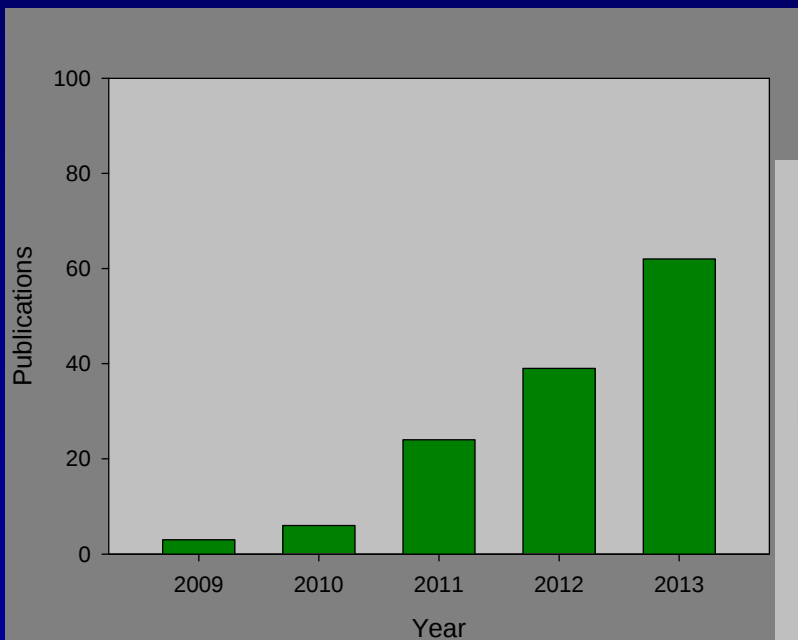
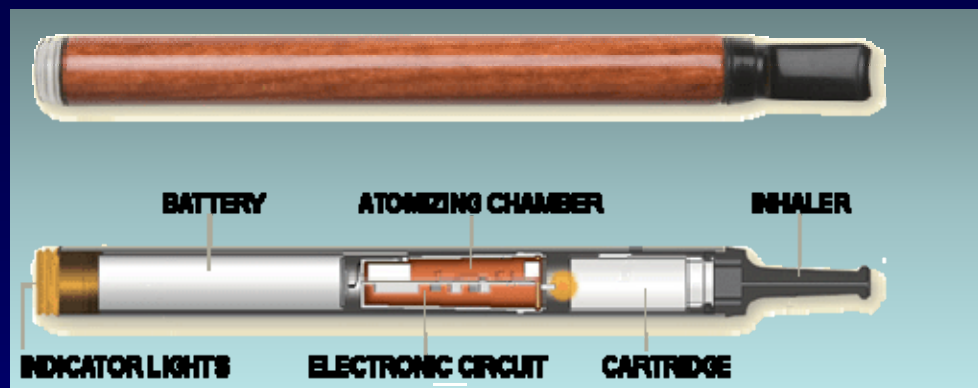
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ST07

Overview

- **How are e-cigarettes reflected in the scientific literature?**
- **Composition and properties of the aerosol of e-cigarettes**
- **How could the exposure dose to the aerosol of e-cigarettes be assessed?**
- **Which biomarkers could be used for e-cigs users?**
- **First results from a pilot study with e-cigs**
- **Outlook**

E-cigarettes: Reflection in the scientific literature



E-cigarettes: Composition and properties of the aerosol

- The aerosol is formed at 60 - 250 °C from a liquid
- Composition of the e-liquid:
 - Excipients: Propylene glycol (PG), glycerol (G), some water
 - Nicotine: 0 – 25 mg/ml
 - Flavors: Tobacco aroma, pyrazines, menthol, various extracts from plants, many more ...
 - Contaminants: Other nicotine alkaloids, nicotine/PG/G degradation products, TSNA, PAHs, diethylene glycol, propylene oxide, others
- Composition of the aerosol:
 - In principle all liquid constituents, plus ...
 - Thermal decomposition products from liquid constituents: formaldehyde, acetaldehyde, acrolein, propylene oxide, diethylene glycol (all very low, when e-cig is working 'normally')
- Properties of the aerosol:
 - Particle size: Submicron range (similar to cigarette smoke, but broader size distribution)
 - Particle conc.: Lower than in cigarette smoke (more unprotonated nicotine in the vapor phase → 'harsher smoke')

E-cigarettes: Dosimetry/Exposure assessment

■ External dose/exposure:

- **Yields** (what is the base: per puff, per liter ?)
- **Consumption** (what is the base: number of puffs per hour, day, ...?)
- **Dose estimate:** puffs/d x puff volume (ml) x yield (mass/ml) = **amount/d**

■ Internal dose/exposure:

- **Routes of uptake:** Inhalation, buccal absorption, ingestion
- **Biomarker of exposure (BoE):**
 - Nicotine and metabolites
 - Excipients: Propylene glycol (PG), glycerol (G)
 - Flavor compounds
 - (Toxic contaminants)
- **Biological matrix for BoE:**
 - Blood (serum/plasma)
 - Urine (spot, 24-h-urine)
 - Saliva
 - Exhaled breath
- **Dose estimate:** BoE level x conversion factor = **amount/d**

E-cigarettes: Feasibility (Pilot) study

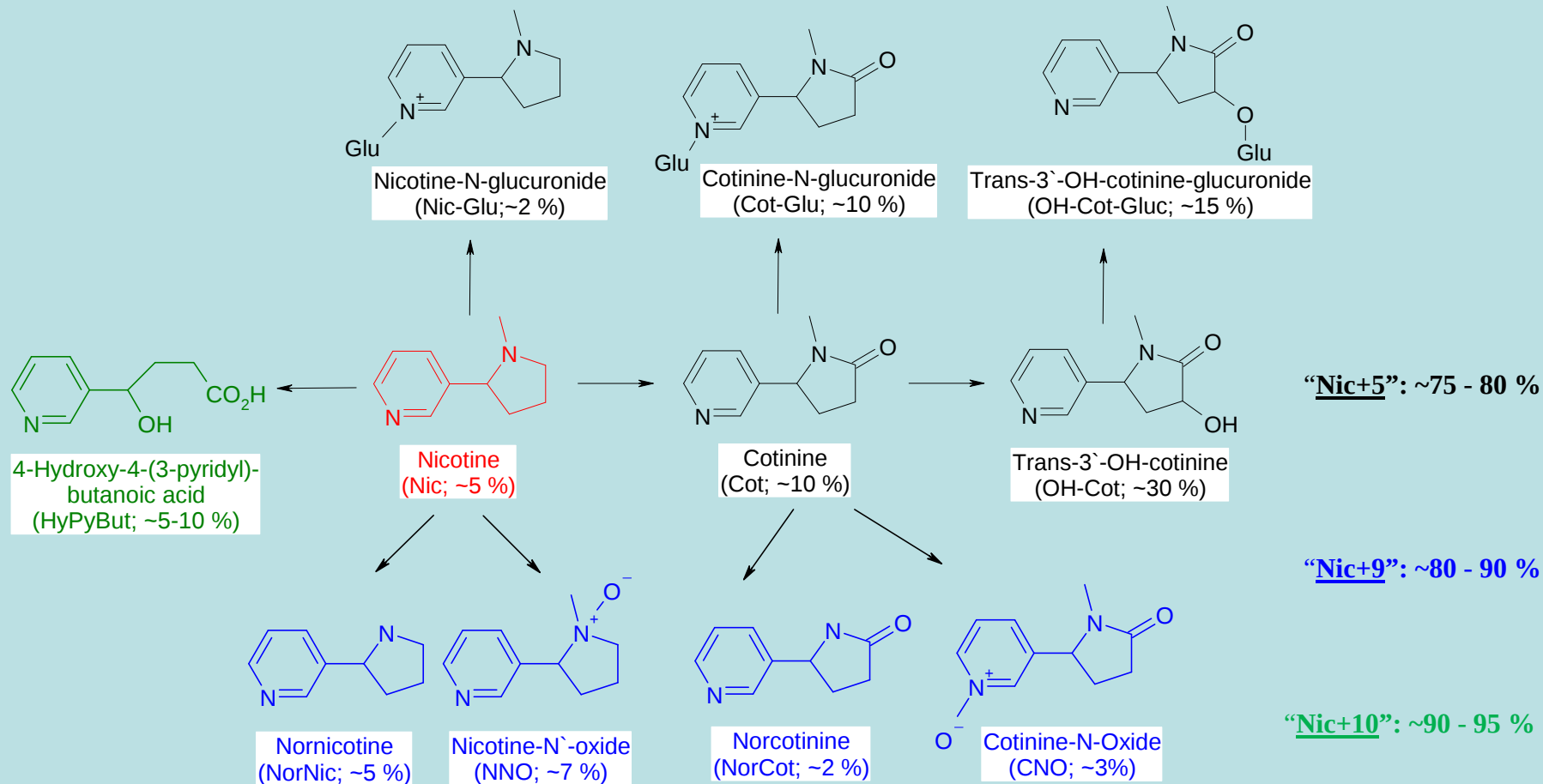
■ Objectives:

- Quantify the uptake of nicotine from e-cigarettes by suitable biomarkers of exposure
- Quantify the uptake of PG and G from e-cigarettes by suitable biomarkers of exposure

■ Research approach:

- Apply available (salivary cotinine and hydroxycotinine) and improved (“Nic+10” in urine) methods for the determination of nicotine uptake
- Develop, validate and apply a new method for PG and G uptake
- Perform a small study for collecting suitable samples
 - 5 subjects smoke e-cigarettes (on one day without nicotine, on another day with nicotine), single puffs from the e-cigarettes were recorded in a protocol
 - 6 Urine fractions were collected every 4 – 6 h (over 24 h)
 - 6 Saliva samples were collected every 4 – 6 h (over 24 h)

Urinary nicotine equivalents: “Nic+10”



Analytical method: PG and G in body fluids

Sample preparation

1. Add internal standards ($^{13}\text{C}_3$ -G and $^{13}\text{C}_2$ -PG) to 100 μl urine (plasma or saliva) and evaporate
2. Derivatize with acetic acid anhydride
3. Evaporate and re-dissolve in acetonitrile

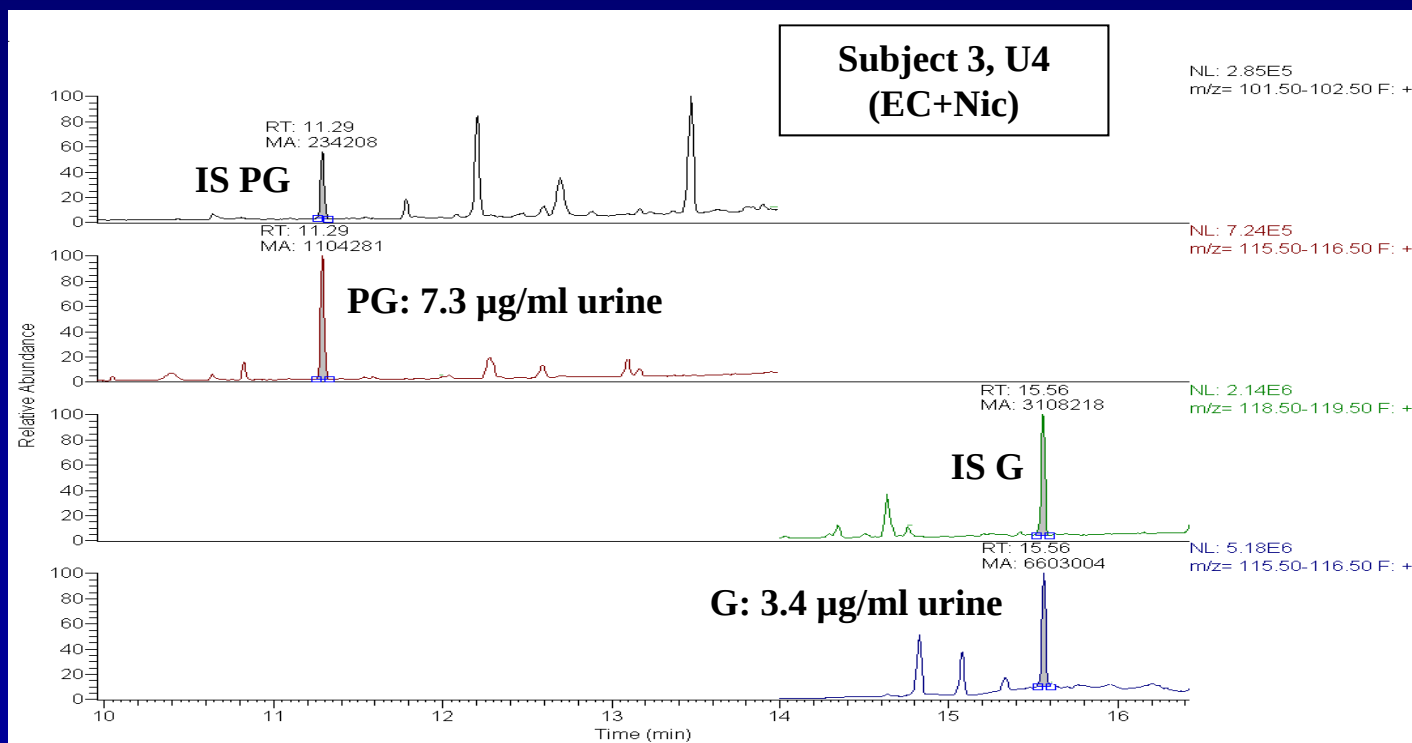


GC-MS analysis

4. Inject 1 μl in GC-MS
5. Separate on DB Wax 30 m x 0.25 mm column
6. MS conditions: EI, SIM mode

Method characteristics

	PG	G
LOD ($\mu\text{g/ml}$)	0.02	0.07
LOQ ($\mu\text{g/ml}$)	0.05	0.2
Linear range ($\mu\text{g/ml}$)	0.05 - 10	0.2 - 50
Conjugates	< 5 %	~ 30 %



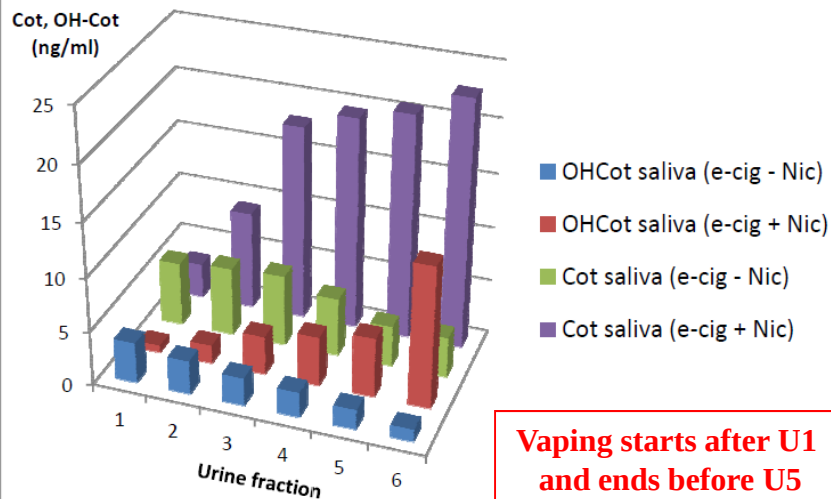
E-Cigarettes: Results of pilot study (1)

Analysis of the e-liquid
("USA- Mix")

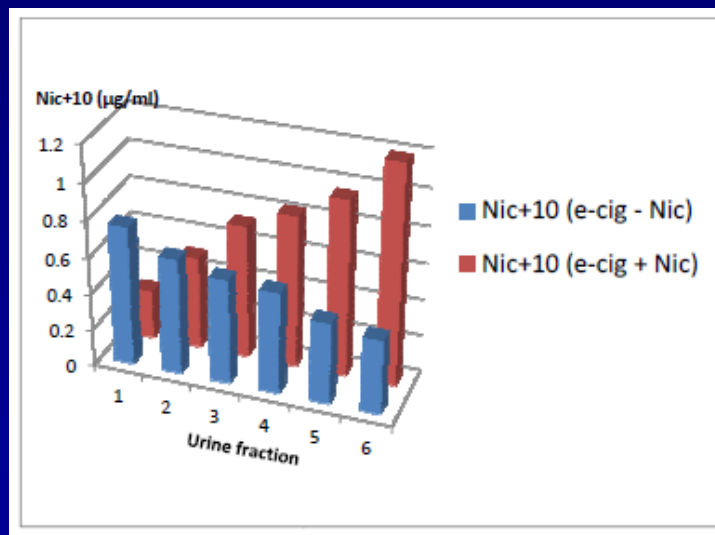
		-Nic	+ Nic
PG:G	Label	60:40	60:40
	Found	39:61	37:63
Nicotine (mg/ml)	Label	0	18
	Found	0	2.6

'Vaping behavior' (average (range) of 5 subjects)

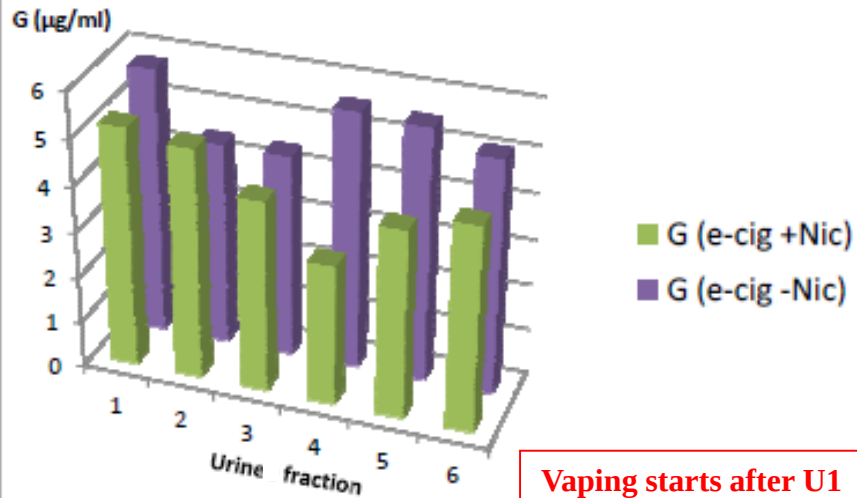
Number of vaping sessions (/d /type)	7.6 (5 – 10)
Duration of session (min):	15 (10 – 20)
Number of puffs per session:	12 (5 – 15)
Total puff number, EC+Nic:	78 (34 – 106)
“ EC – Nic:	91 (66 – 133)



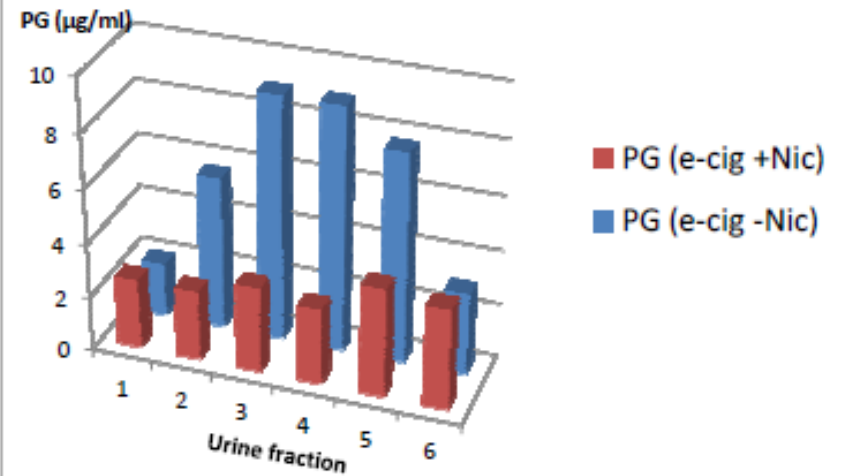
Vaping starts after U1
and ends before U5



E-Cigarettes: Results of pilot study (2)



Vaping starts after U1
and ends before U5



E-Cigarettes: Results of pilot study (3)

Comparison between vaping and smoking			
	E-Cigarettes (EC)	Conventional cigarettes (CC, 25 smokers)	Ratio (CC/EC)
Cotinine in saliva (ng/ml)	22.9	240.8	10.5
OH-Cot in saliva (ng/ml)	13.1	53.2	4.1
Nic+10 (mg/d)	1.05	20.72	19.7
Δ PG (mg/d) EC <u>with</u> nicotine	3.36	1.18	0.35
Δ PG (mg/d) EC <u>without</u> nicotine	7.07		0.17

Note: The various CC/EC ratios for the nicotine metabolites are probably due to the fact that no steady-state levels during vaping were achieved.

Outlook

- The application of biomarkers of exposure to e-cigarette users ('vapers') is possible and also useful in order to get reliable dose information
- Apart from the exposure to nicotine, also the exposure to the excipients (PG, G) and flavors can be determined by suitable biomarkers. Background exposure to these compounds is an issue and has to be considered in the study design
- It is of superior importance to assess the dose absorbed through the lungs (deeply inhaled fraction) by suitable biomarkers
- Future human studies with e-cigarettes will have to include also suitable biomarkers of effect

Thank you for your attention !