



E-cigarettes: Research at the BfR

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Outline



Analytics of e-cigarettes



Toxicology of flavors in e-liquids



JUUL

- Carbonyles
- Benzoic acid
- Vapor development
- Nicotine content in vapor
- Human studies in cooperation with LMU Munich

Analysis of E-Liquids

- Cooperation with CVUA Sigmaringen and CVUA Karlsruhe

Cooperations with CVUA Sigmaringen and Karlsruhe



Characterization of 50 E-Liquids:

- **Vitamin E and Vitamin-E-acetate**
 - at CVUA Sigmaringen (LC-DAD and LC-FLD)
 - at BfR (LC-MS/MS)
- **Vitamin C (CVUA Sigmaringen)**
 - Vitamin E in total : 14 quantified
 - 0.004 – 0.490 ng/mg
 - Vitamin E acetate in total : 1 quantified (Karlsruhe)
 - 0.316 ng/mg
- Correctness of labelling and packaging (CVUA Sigmaringen, RP Tübingen)

JUUL

Vapor: development & nicotine content

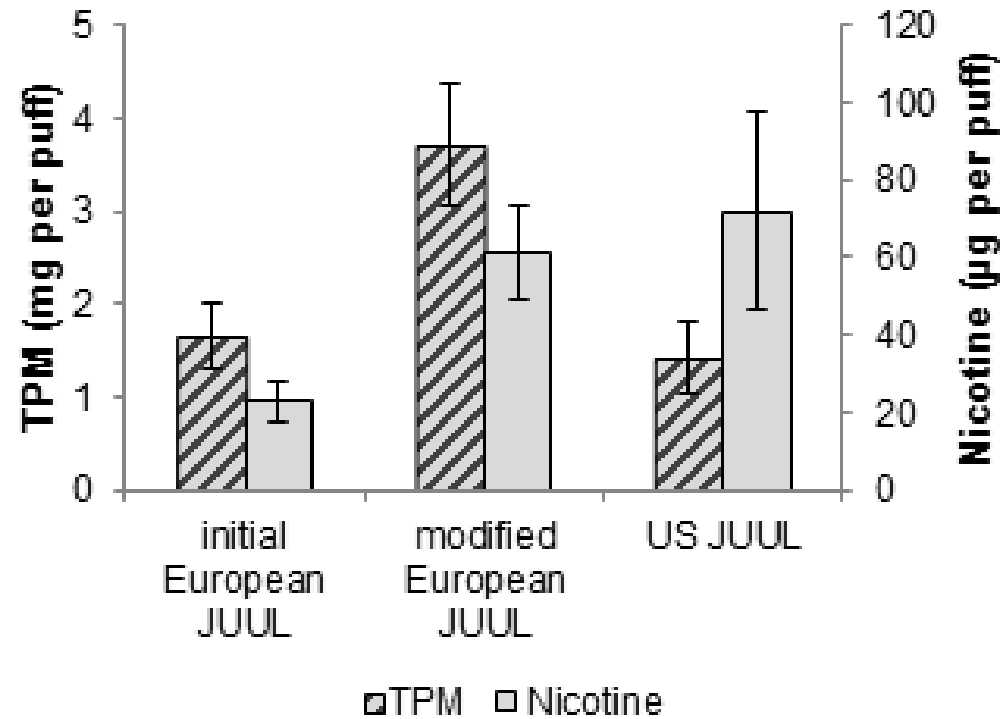
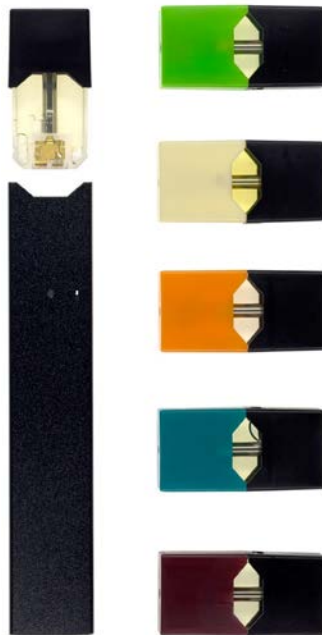


Figure 2. Vapor generation (displayed as total particulate matter, TPM) and nicotine release per puff of the initial European version, the modified European version, and the US version.

Publication: Mallock et al. (2020): Trendy e-cigarettes enter Europe: chemical characterization of JUUL pods and its aerosols.

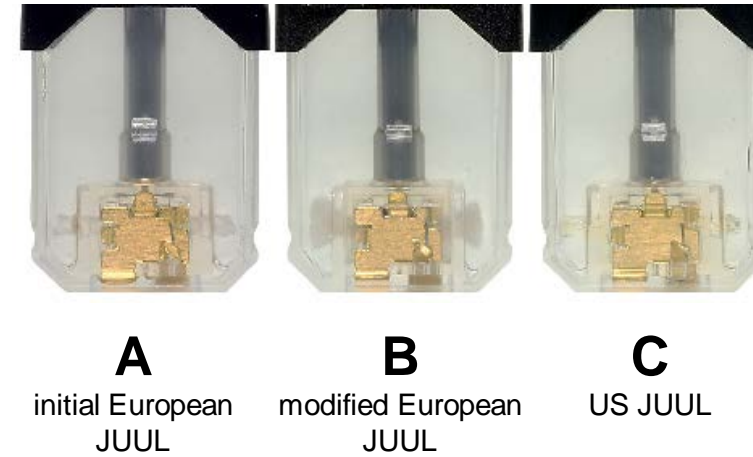


Figure 3. Initial EU and US pods (a and c) contained the same wick material. Modified EU pods (b) had a different wick material that performed better in supplying e-liquid to the coils, resulting in an increased and more stable vapor generation.

Conclusions:

- Shortly after initial product launch, the product was modified
 - Vapor generation increased
 - Nicotine release per puff increased

JUUL

Study on nicotine delivery by JUUL e-cigarette (Nicotide study)



- Human trial in cooperation with LMU Munich
- E-cigarette users and cigarettes smokers
- Blood sampling during consumption at certain time points
- Measurement with LC-MS/MS

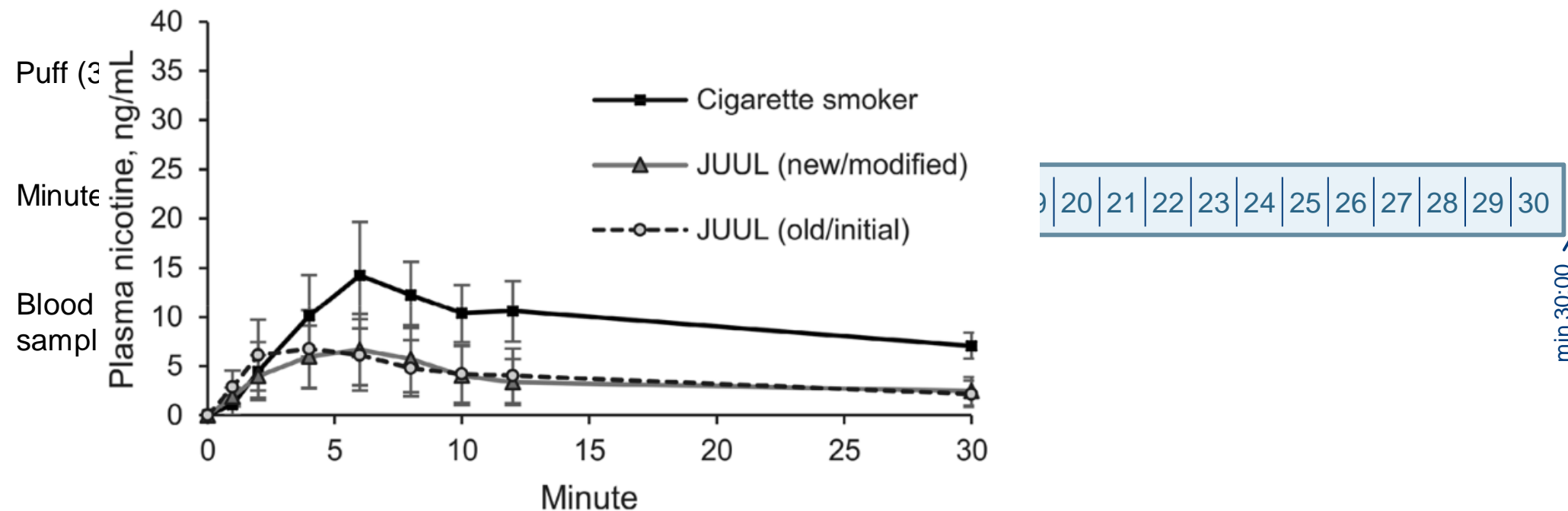


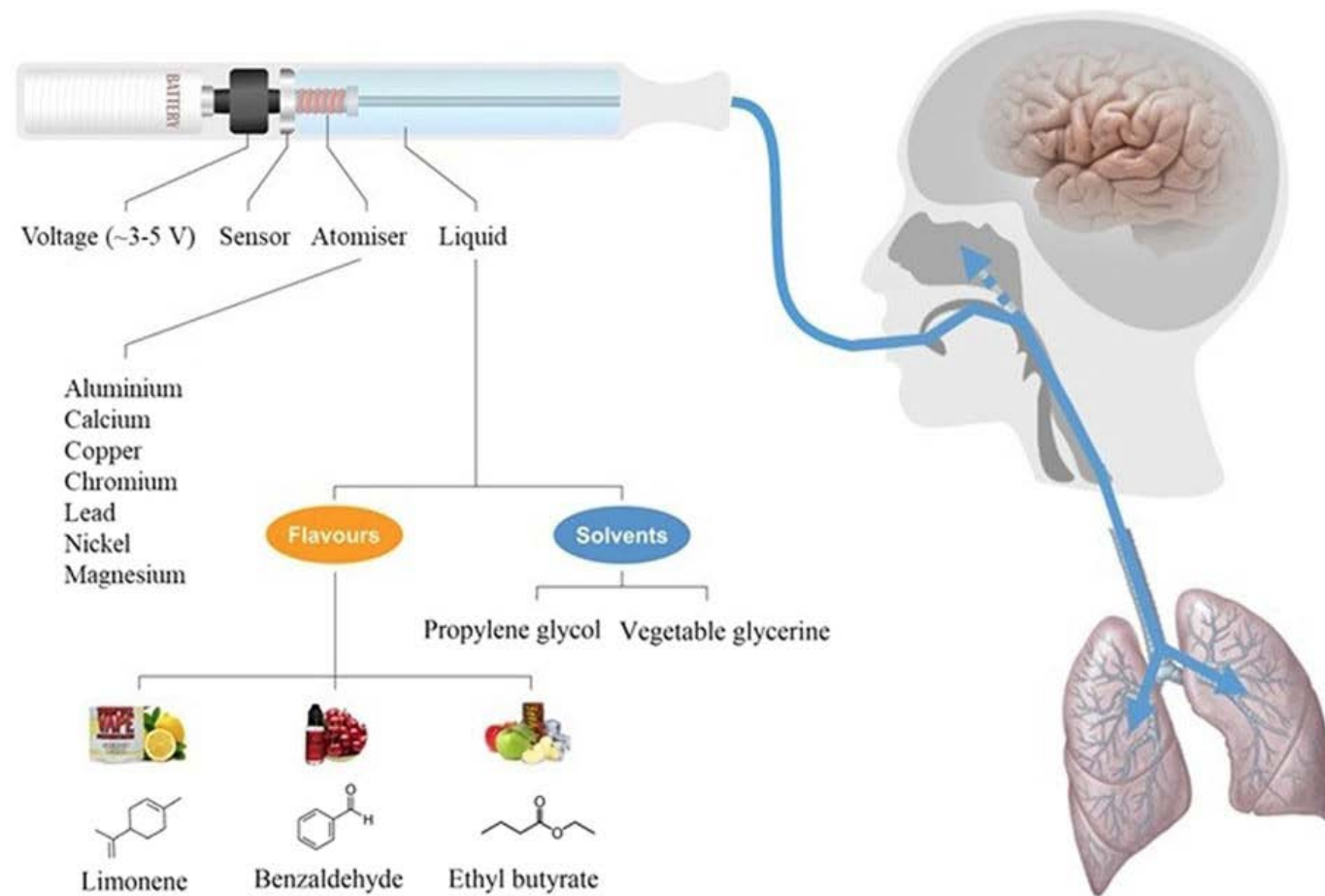
Figure 4: Arithmetic means and 95% confidence interval of the plasma curves for three groups

Publication: Mallock et al. (2021): Nicotine delivery and relief of craving after consumption of European JUUL e-cigarettes prior and after pod modification

Toxicology of flavors in e-liquids



- Flavors below 0.1 % do not have to be claimed on the packaging
- Flavors are generally recognized as safe (GRAS) for oral consumption → Respiratory effects?



Dinu et al. Science of Food (2020) 4:15; <https://doi.org/10.1038/s41538-020-00075-y>

In vitro studies: Cytotoxicity of e-liquids

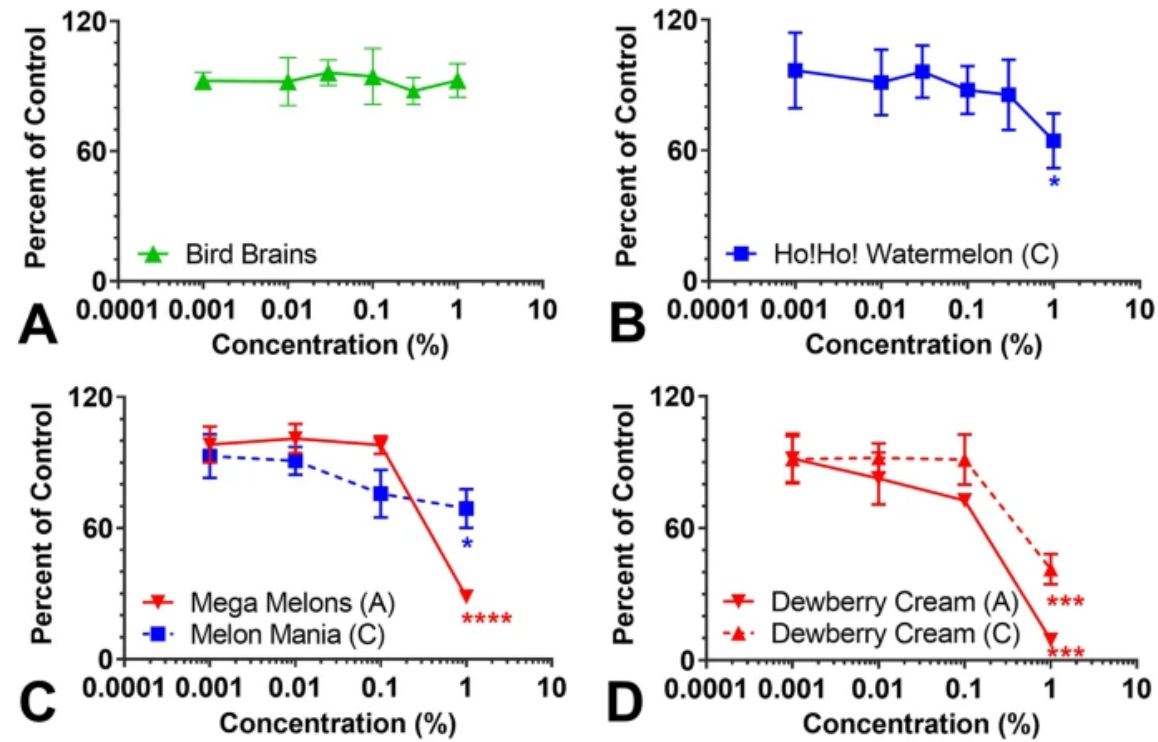


Figure 6: Cytotoxic refill fluids identified using mouse neural stem cells. (A–D) Representative MTT concentration-response curves for products that were: (A) not cytotoxic, (B) cytotoxic reaching IC_{70} , and (C,D), highly cytotoxic reaching IC_{50} .

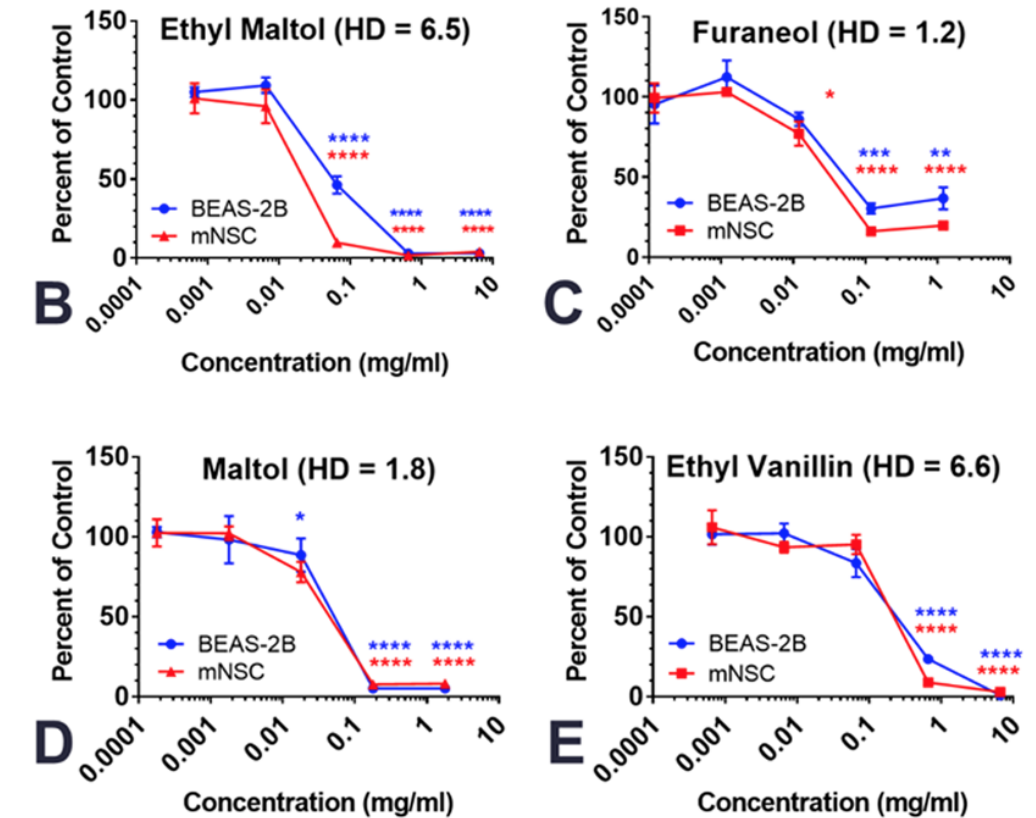


Figure 7: Concentration-response curves of authentic standard chemicals present in the highest concentrations in the two most toxic refill fluids and their clones.

Hua et al. (2019): <https://doi.org/10.1038/s41598-019-38978-w>

Conclusion: More research needed for safety of flavor compounds in e-cigarettes

Study layout



Analyt	Anzahl Proben	Gehalt (µg/mL)
Limonen	40	1.6 - 953
Linalool	38	4.6 - 588
Benzylalkohol	27	1.6 - 7570
Geraniol	16	2.6 - 780
Geranial	10	1.6 - 232
Citronellol	8	7.4 - 59
Cinnamal	7	3.2 - 87
Eugenol	7	2.9 - 79
+ Vanillin	Literature	Literature

Preparation of self-made e-liquids:

- DIN ISO 17375:2020-09
- 69/29 % PG/VG, 1 % water, 1 % ethanol containing one flavour

Which of the following e-cigarette liquid variants do you use at least on a monthly basis?
(MULTIPLE ANSWERS POSSIBLE)
(% - EU27 + UK)

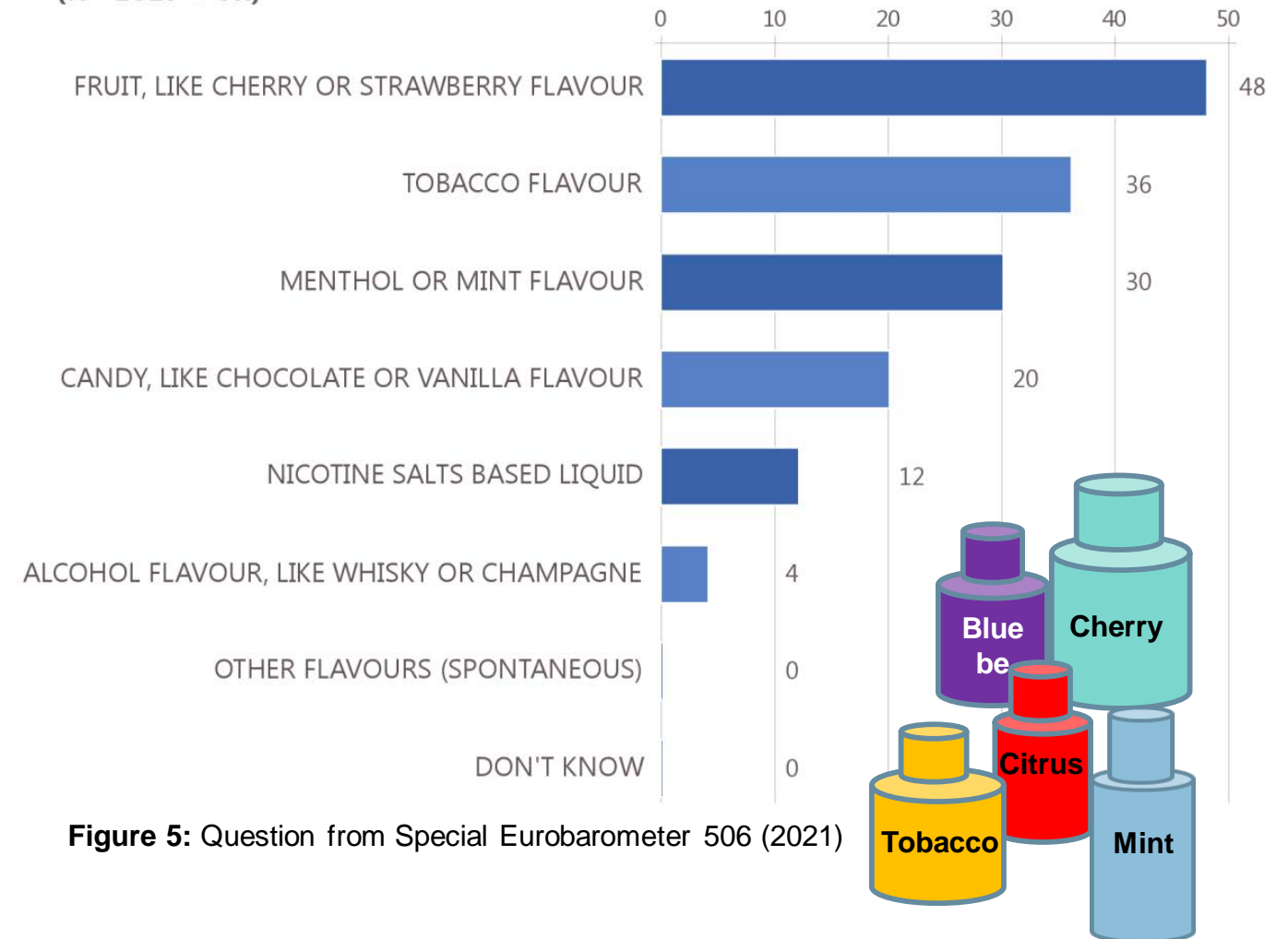


Figure 5: Question from Special Eurobarometer 506 (2021)

Study layout



E-cig	1	2	3	1-3
Mean liquid consumption in g (± SD)	1.46 (± 0.13)	1.40 (± 0.05)	1.43 (± 0.04)	1.43 (± 0.10)

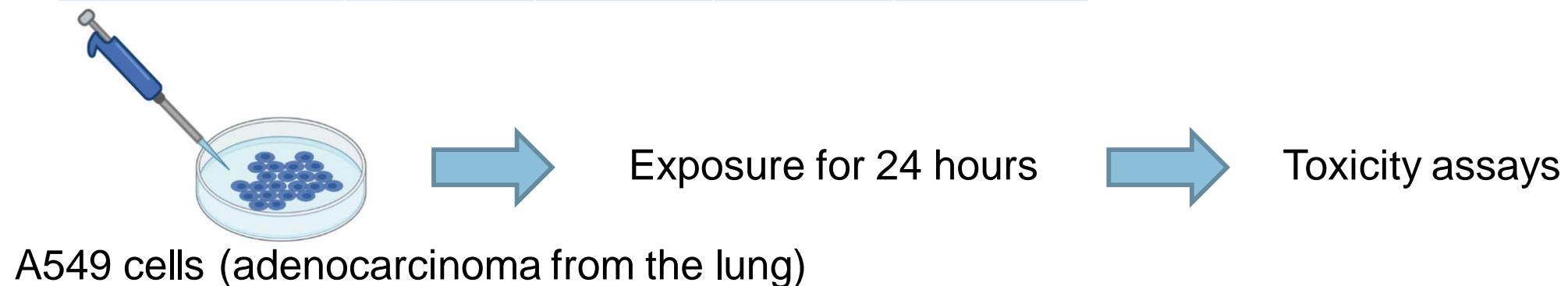
Vaping machine: Borgwaldt LM4E

Puffing regime: CORESTA No. 81:

- Puff volume: 55 mL
- Puff duration: 3 s
- Puff frequency: 30 s

Vaping procedure:

- Generated emissions collected in impinger, filled with cell culture medium (conditioned medium)
- 100 puffs in total



Preliminary results



Pure flavour compounds in cell culture medium

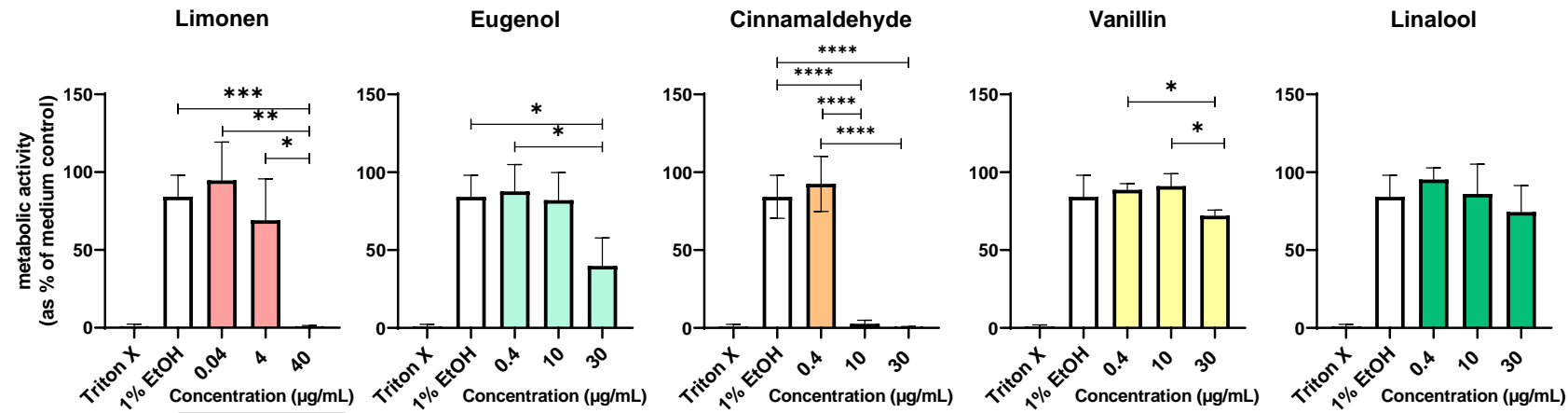


Figure 8: Metabolic activity measured following a 24 hour exposure to Triton X (positive control), vehicle control 1% ethanol or unvaped flavours in cell culture medium compared to medium control. *** p<0.001, **p<0.01, *p<0.05. N=3, n=3

Flavour compounds in e-liquids – Aerosol collected in cell culture medium

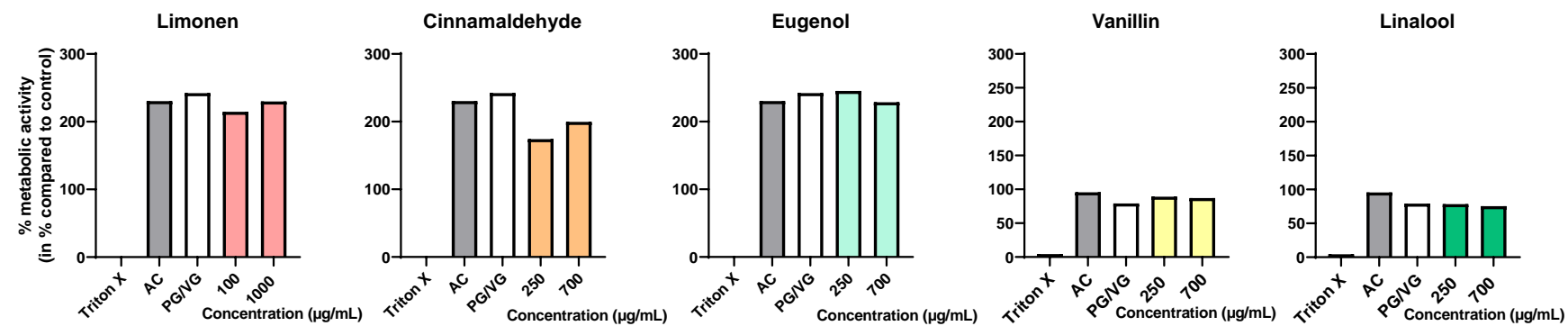


Figure 9: Metabolic activity measured after a 24 hour exposure to air control (AC), vehicle control PG/VG or conditioned medium with self-made e-liquids containing one flavour. N=1, n=3

Thank you for your attention

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