



# Investigating Impacts of Cannabis Tobacco and Vapes on Lung Cells Using GC×GC-MS

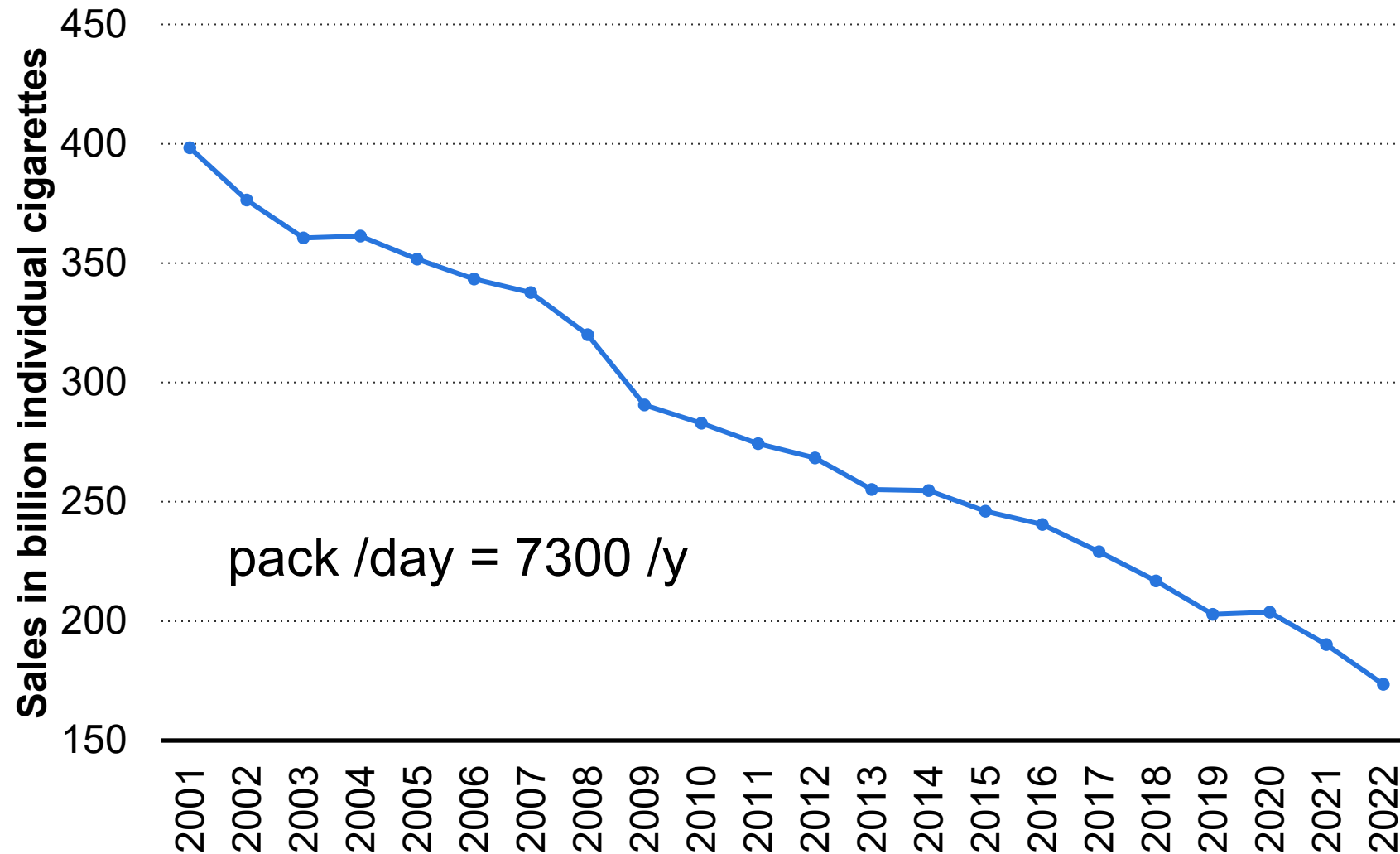
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# Introduction

- Smoking: major cause of coronary heart disease, stroke, lung cancer, and chronic obstructive pulmonary diseases.
  - E cigarettes / vaping are gaining popularity and are relatively un-studied
  - Cannabis use is increasing, many see it as “safe” and many questions about this health exposure
- 
- My lab:
    - Mandate to study exposures
    - We have a customizable smoking machine
    - We have a glassblower



# Cigarette sales in USA



Decreasing but...

Still a huge number



Note(s): United States; 2001 to 2022

Further information regarding this statistic can be found on [page 8](#).

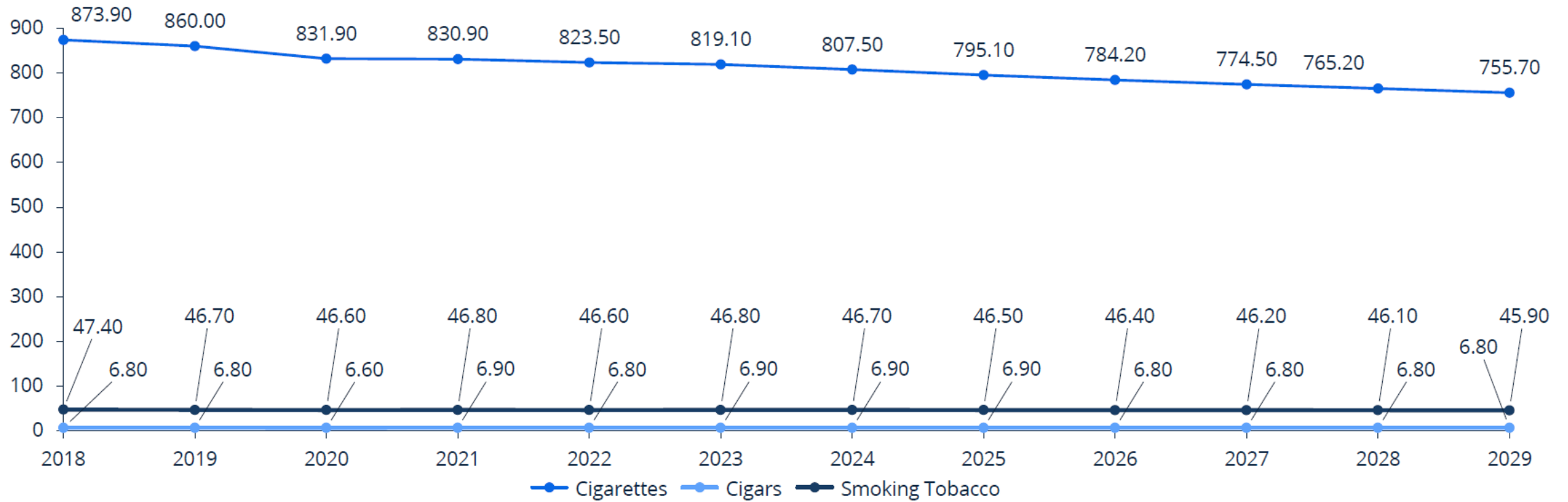
Source(s): Federal Trade Commission; ID 1092860



# Tobacco Products volume sales per capita have been declining steadily

Market Size: Worldwide (4/6)

Average volume per capita in pieces



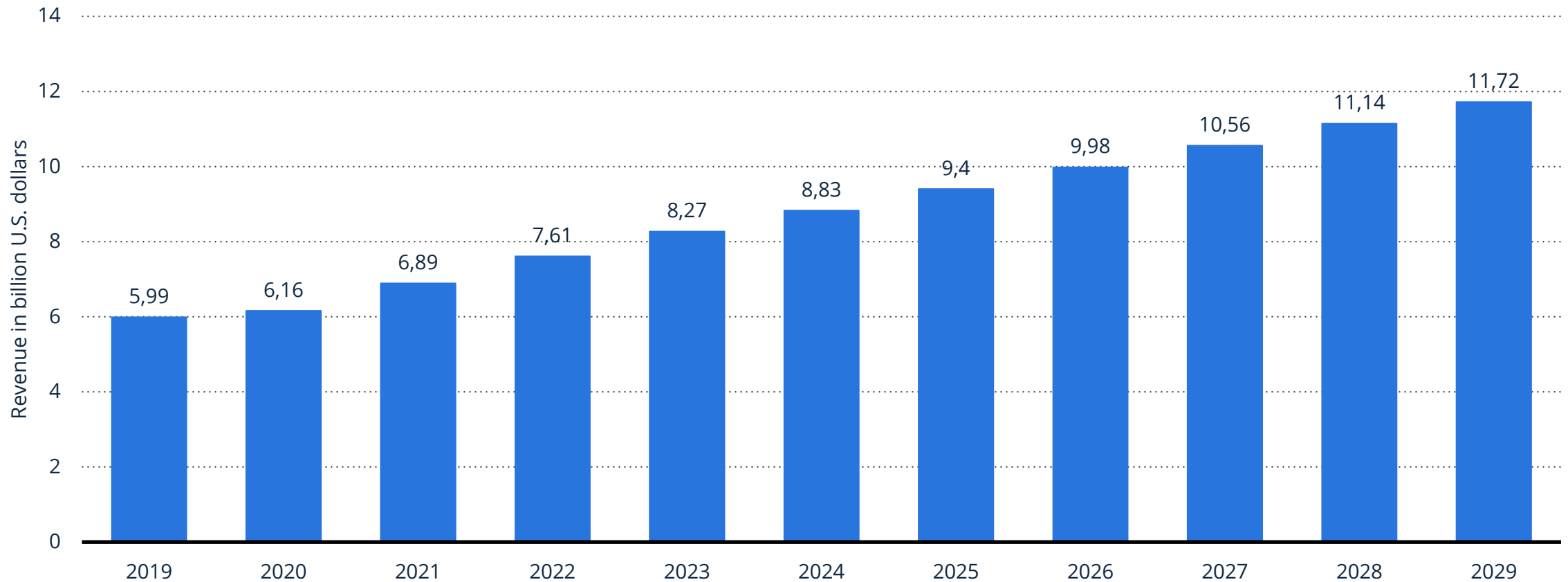
13 Notes: Included are Cigarettes, Cigars, and Smoking Tobacco

Sources: Statista Market Insights 2024



# Revenue of the e-cigarettes industry in the U.S. 2019-2029 (in billion U.S. dollars)

Revenue of the e-cigarettes industry in the U.S. 2019-2029

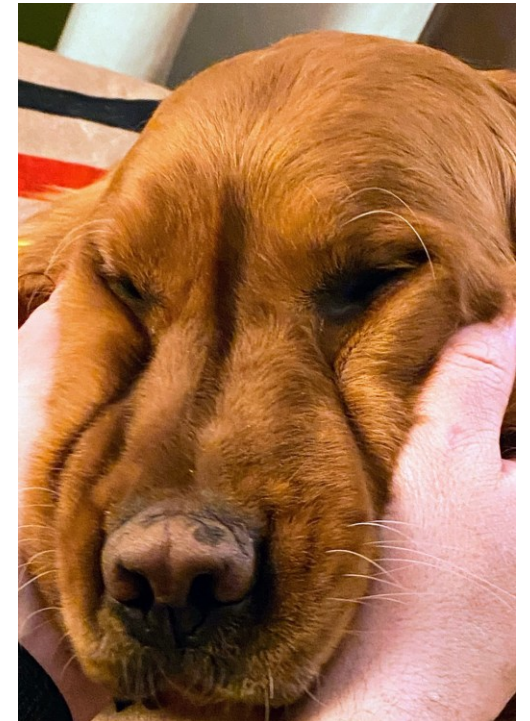
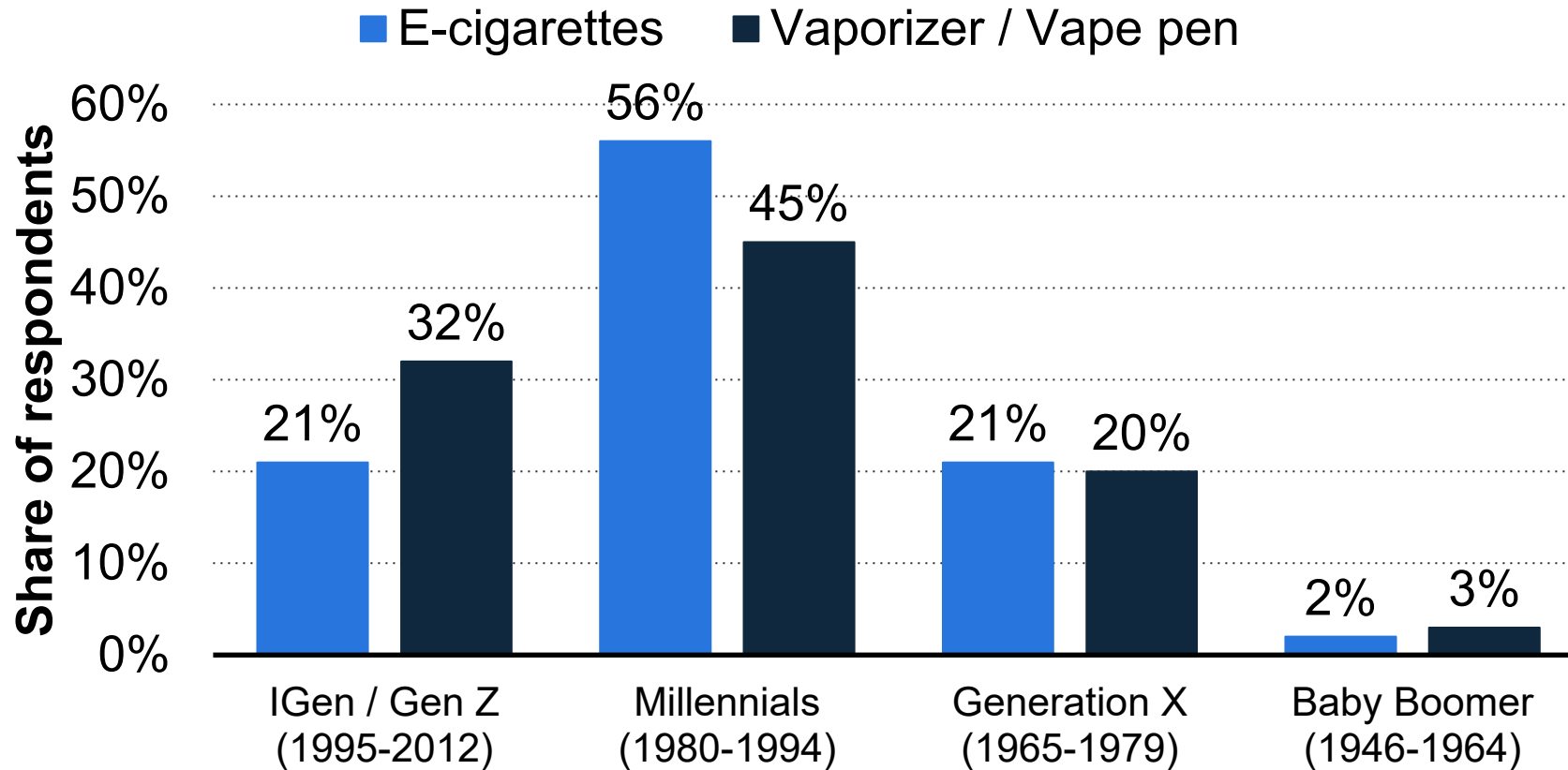


# Ages of Vape / e-cigarette users



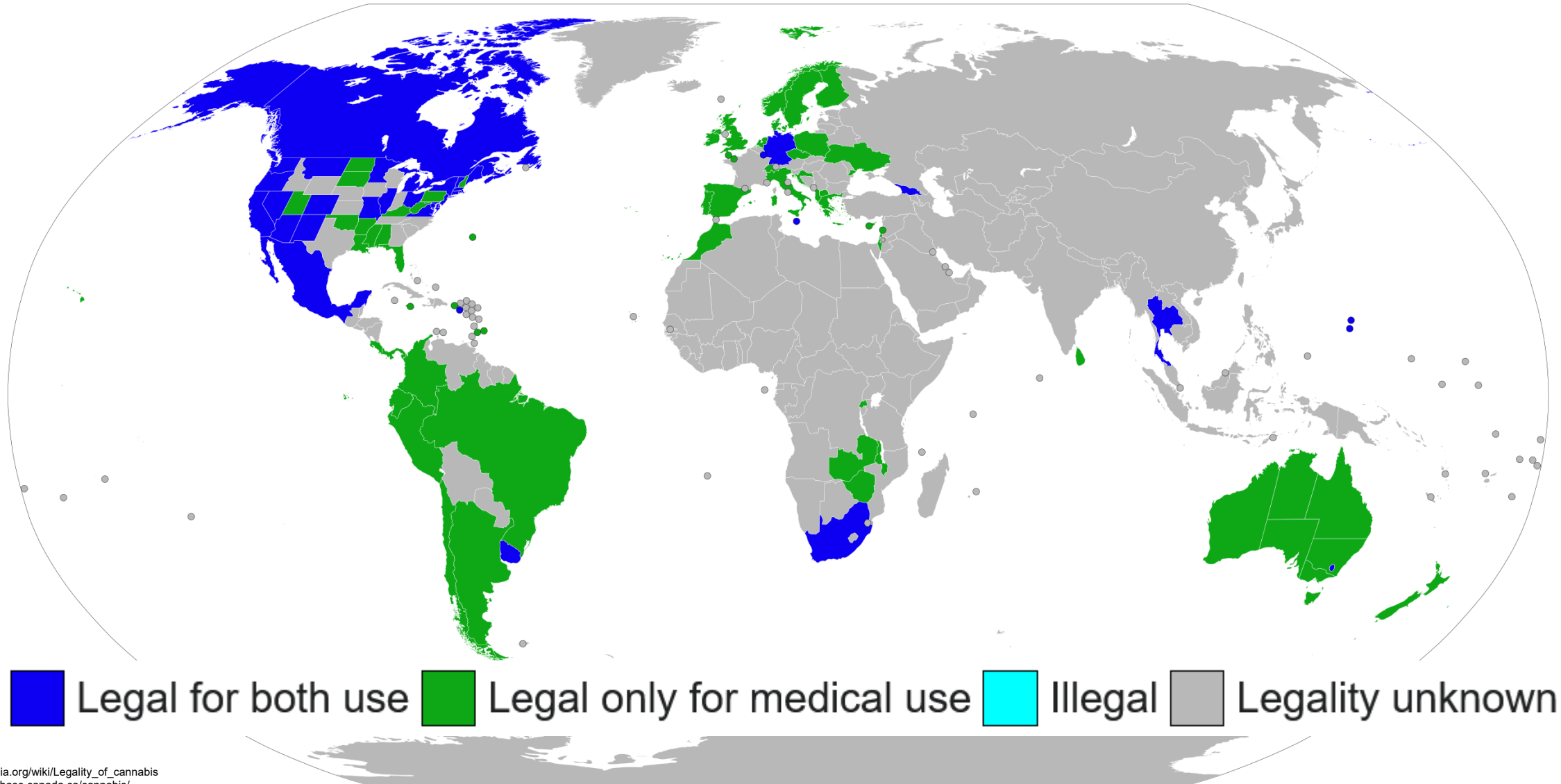
~14% of high school

~5% of middle school

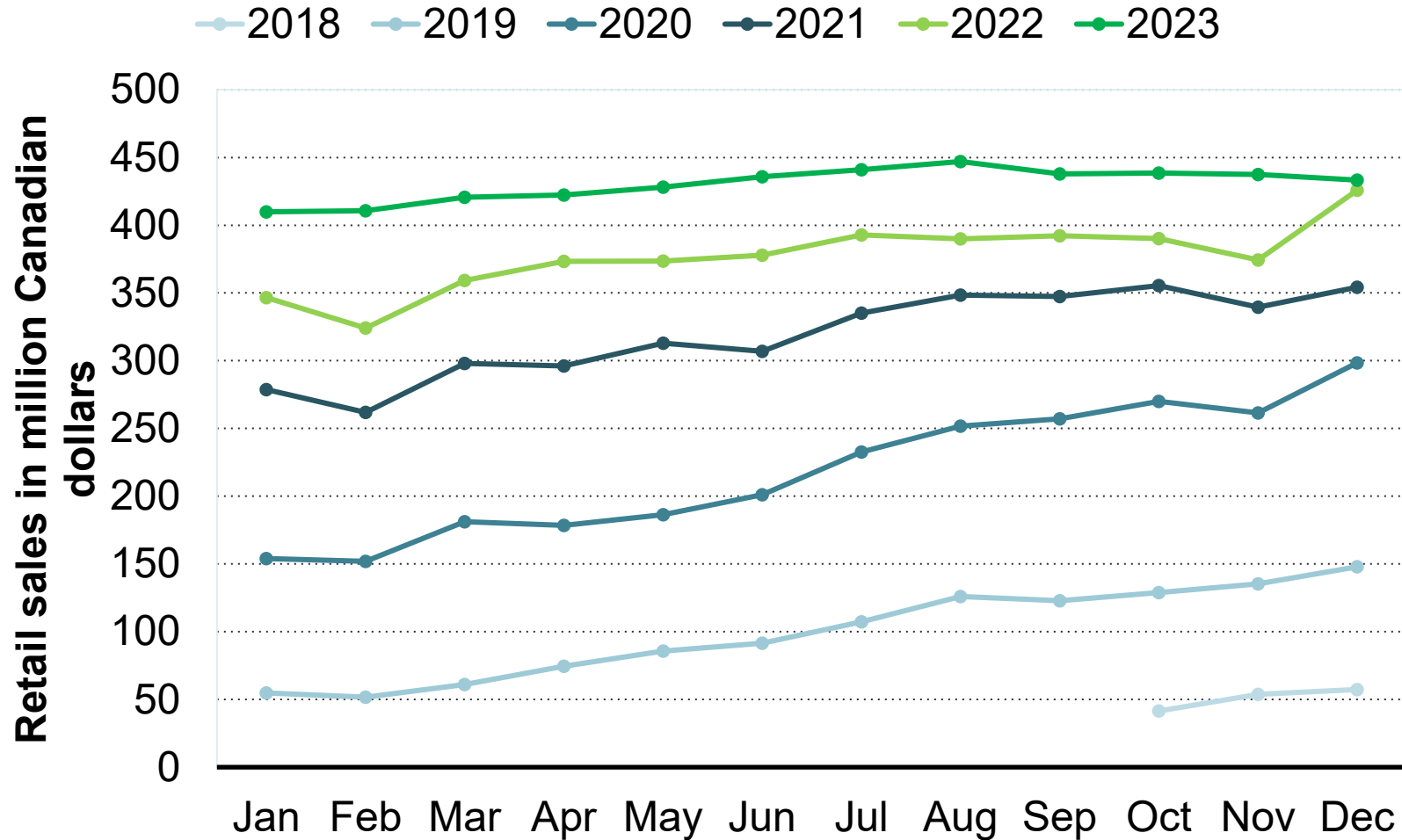


**Note(s):** United States; April 4, 2023 to March 24, 2024; 18-64 years; 60,327 respondents; Respondents who can legally buy tobacco  
Further information regarding this statistic can be found on [page 8](#).  
**Source(s):** Statista Consumer Insights; ID 443152

# Status of cannabis possession for recreational / medicinal use



# Legal cannabis sales - Canada



~65% dried flower

~25% inhalable extracts



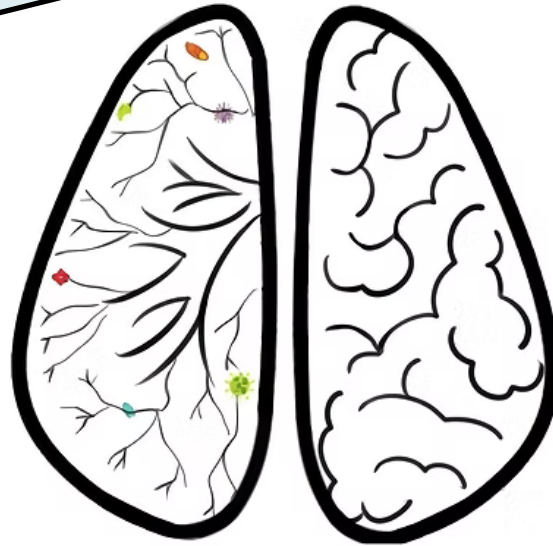
Note(s): Canada; October 2018 to December 2023

Further information regarding this statistic can be found on [page 8](#).

Source(s): StatCan; ID 1045766

# Enter Ritu and Paul

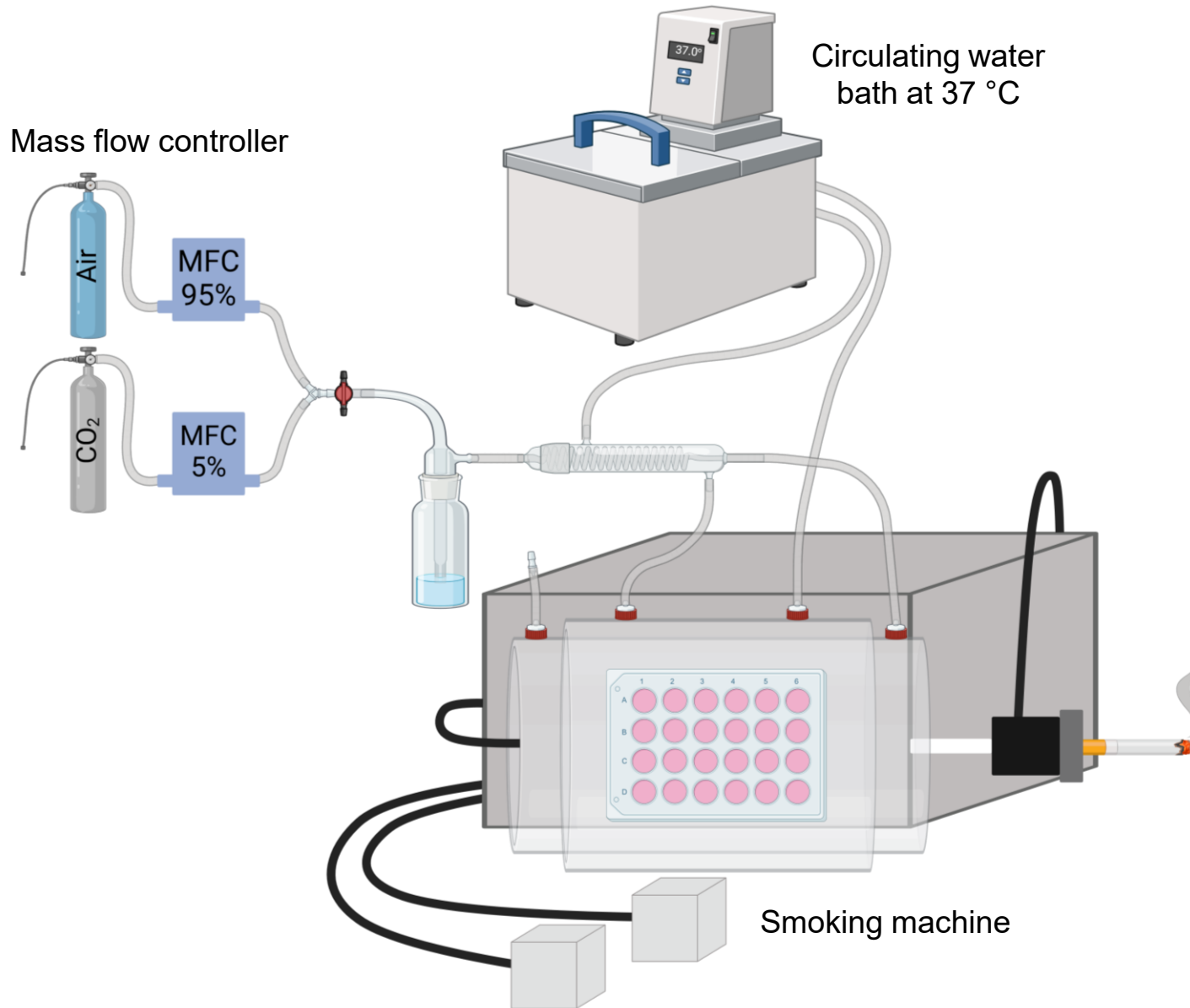
**Can we use your smoking machine to expose our lung cells ?**



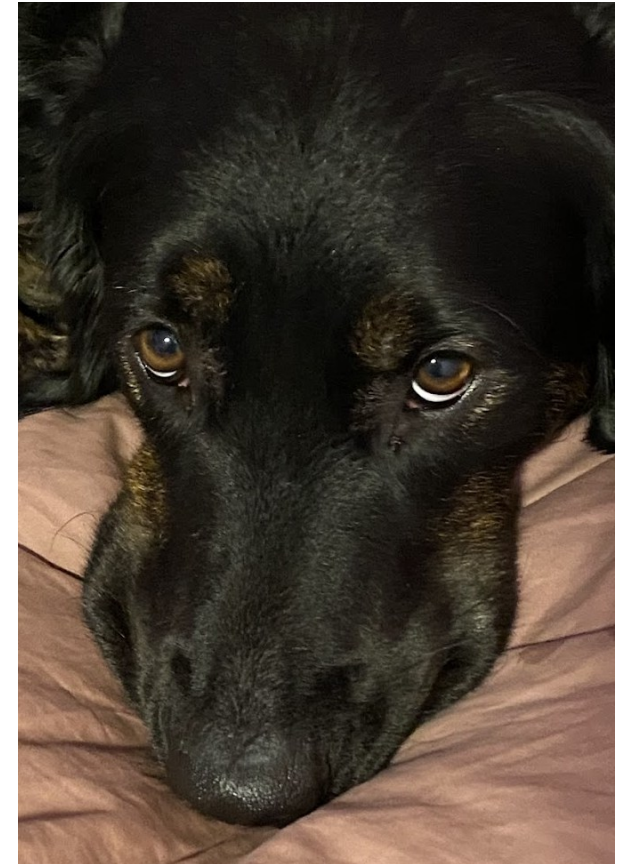
Lung | Brain | Microbiota  
THE FORSYTHE LAB

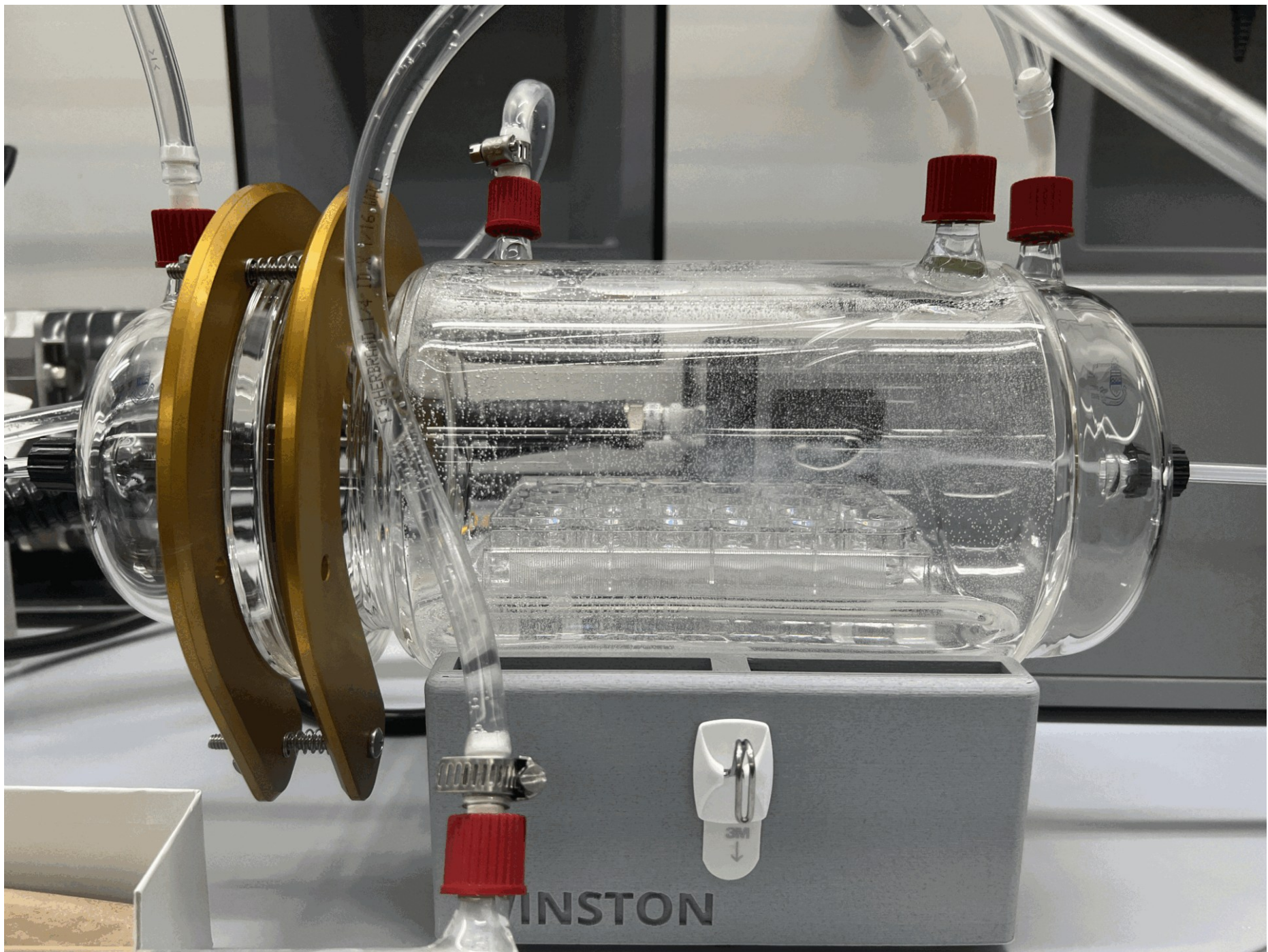


# Smoking machine customization

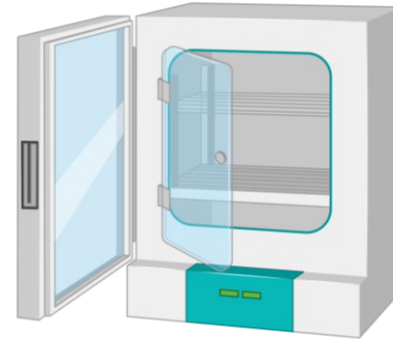
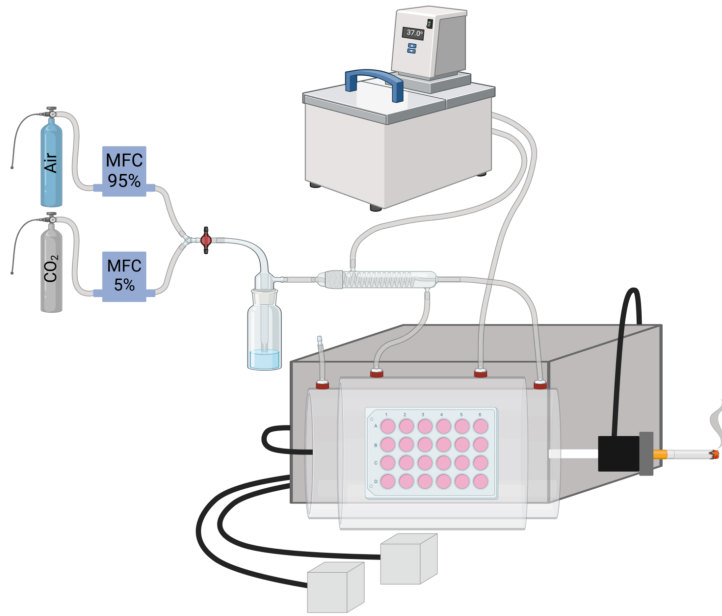


- Custom-built glass chamber
  - Water jacket (control temp)
  - Extra air flow (keep cells happy)

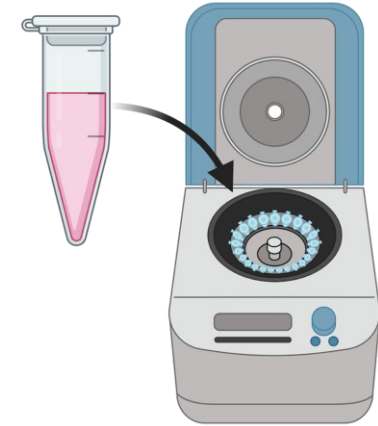




# Methodology



Incubation



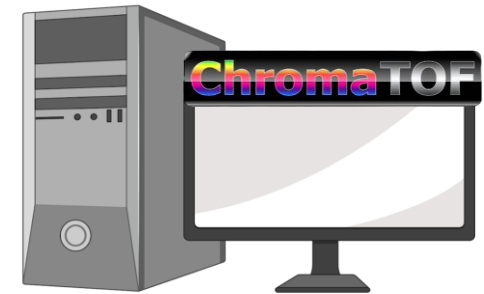
Extraction



Derivatization

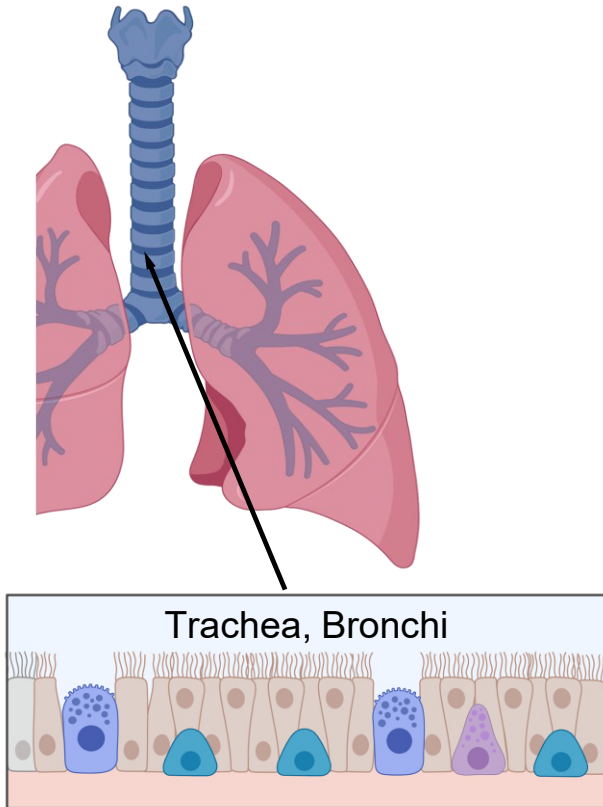


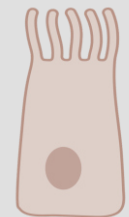

GCxGC-TOFMS



Data processing

# Experiment 1: Incubation Time



Lung Cell Type	Smoke Exposures	Incubation Time
 Human Bronchial Epithelial Cells (HBEC)	Control	2 Hours of incubation
	Cigarette	
 Epithelial-derived Pulmonary Neuroendocrine Cells (ePNEC)	Vape	6 Hours of incubation

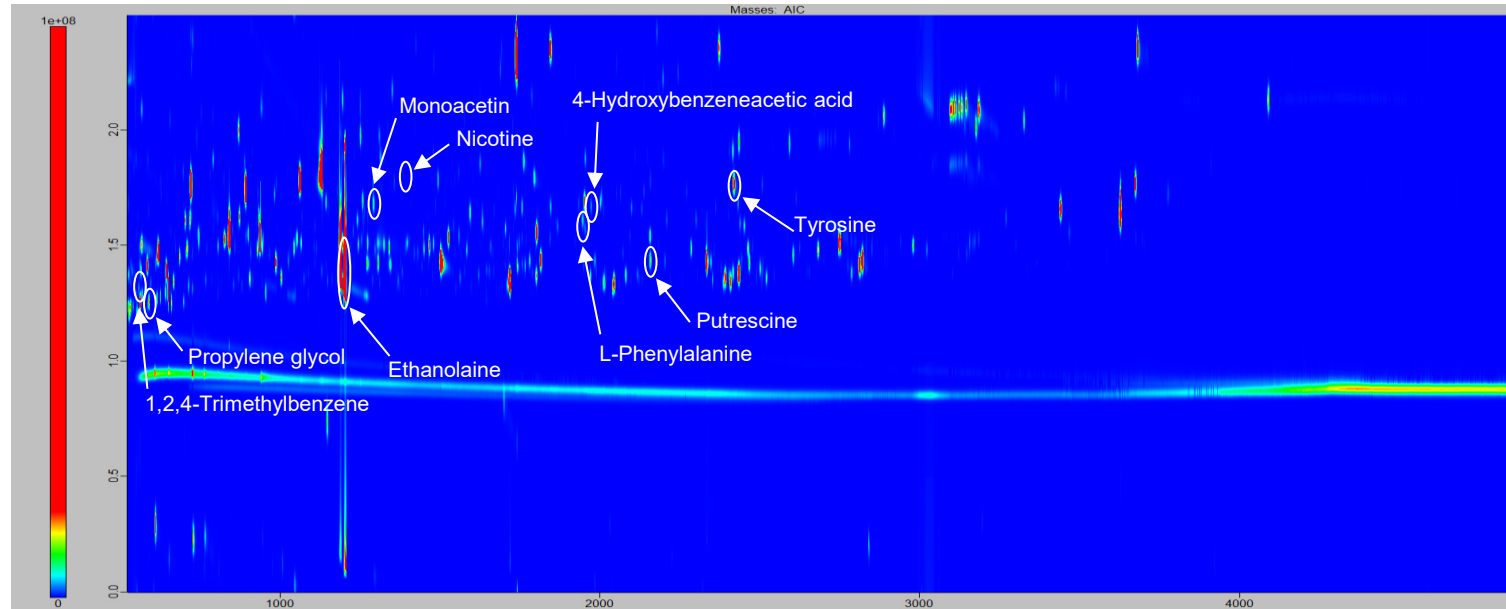
2h and 6h

After 2h see changes in mRNA; after 6h see changes in proteins

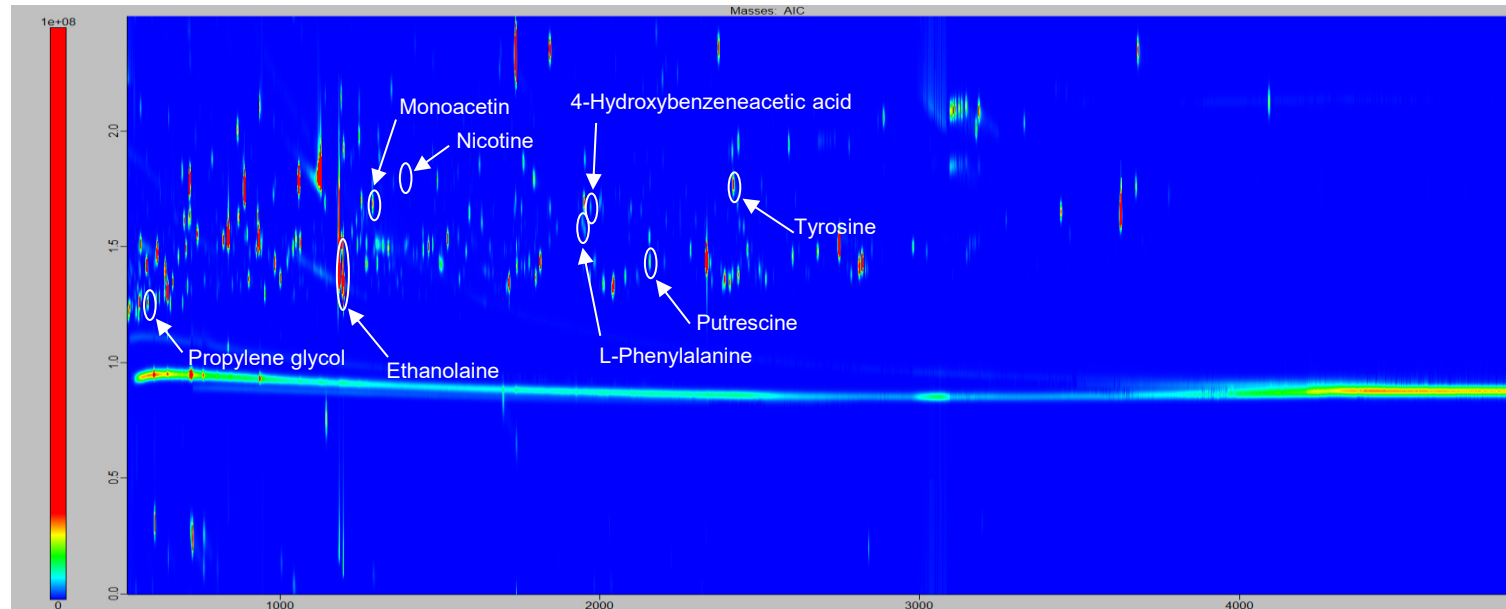
# ePNEC Cigarette Chromatograms



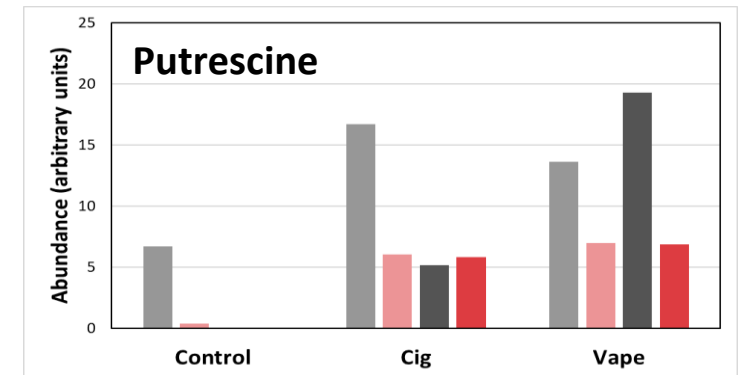
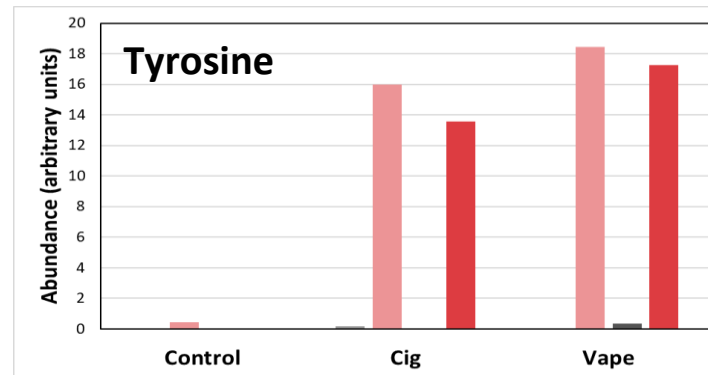
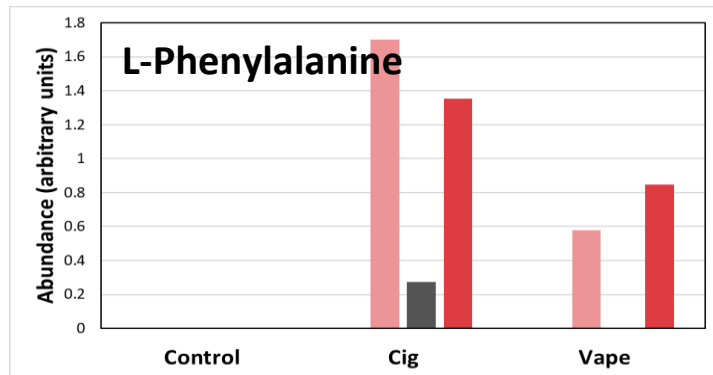
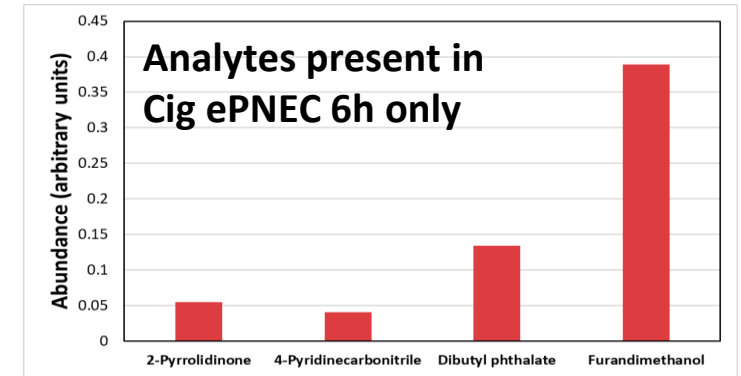
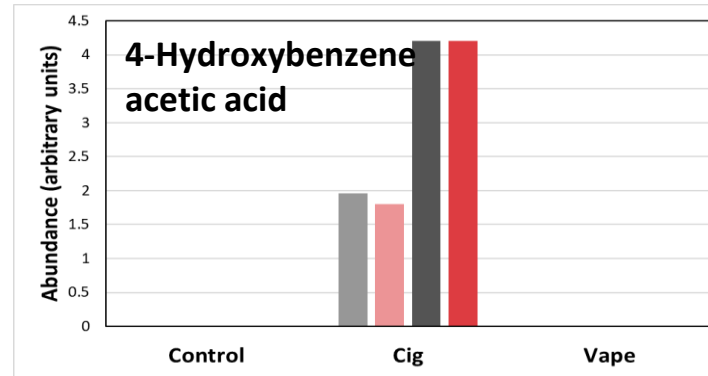
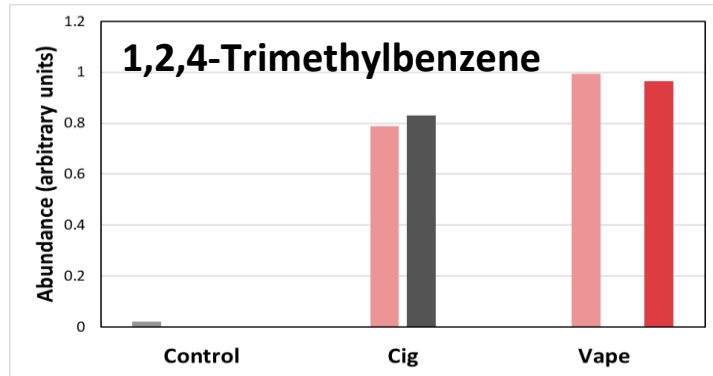
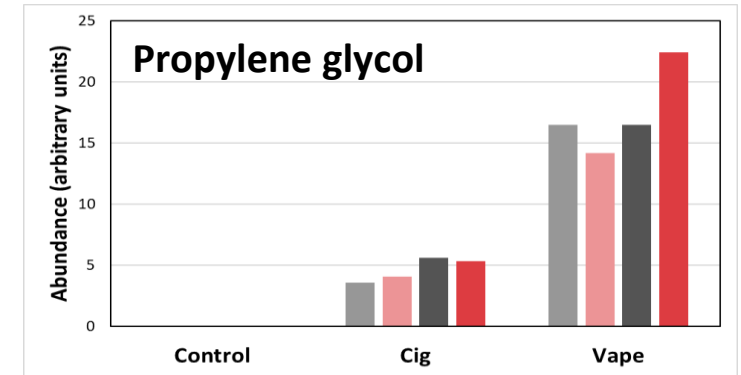
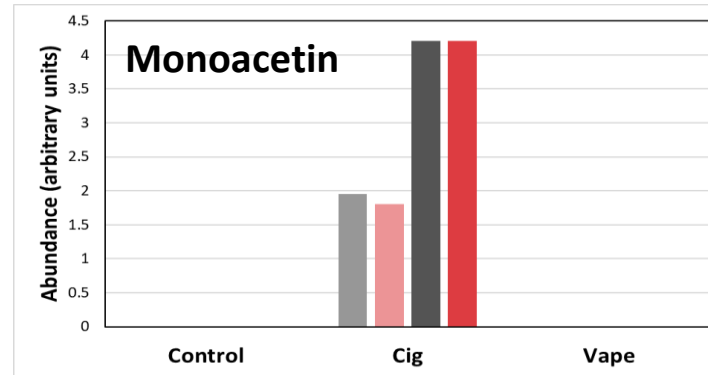
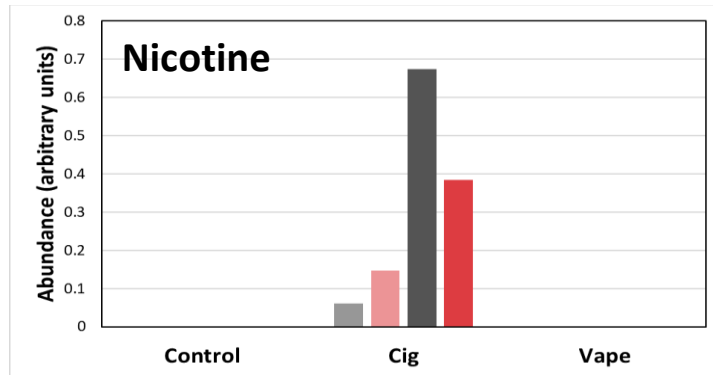
2 h



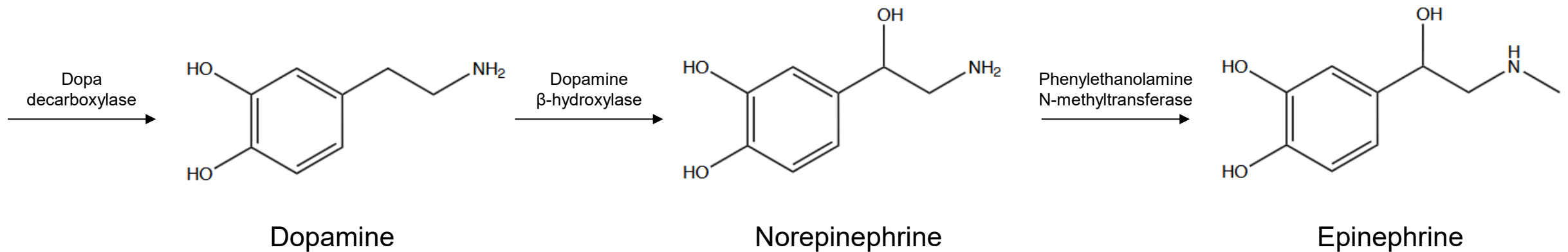
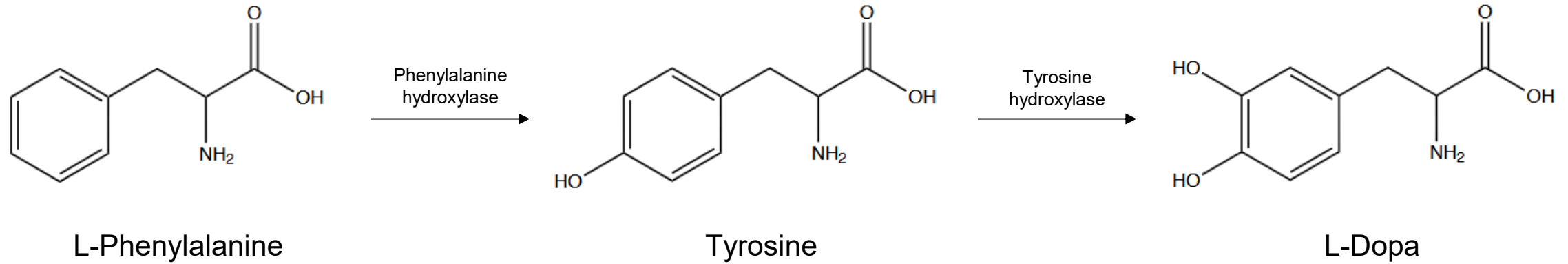
6 h



# Compound Abundances

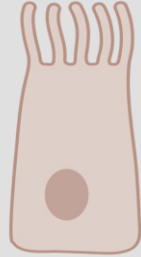


# Phenylalanine and Tyrosine are exciting!



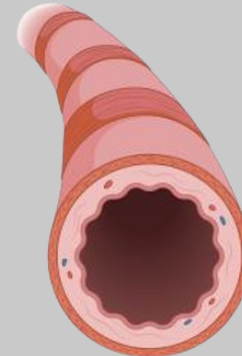
# Experiment 2: Health Conditions

Lung Cell Type



Human Bronchial Epithelial Cells (HBEC)

Health Conditions



Healthy

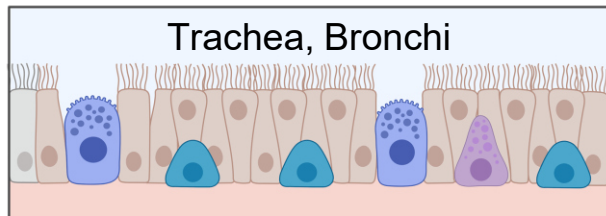
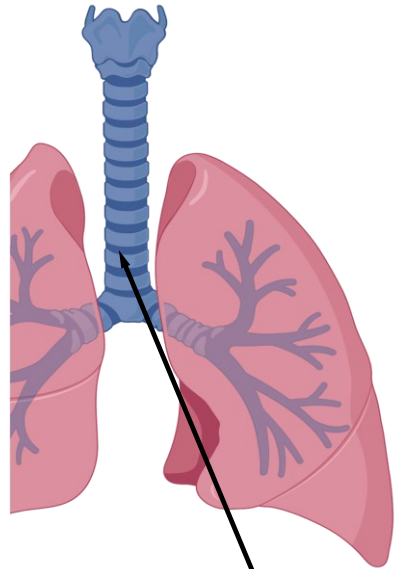
Smoke Exposures

Control

Cigarette

Vape

Cannabis



Epithelial Pulmonary Neuroendocrine Cells (ePNEC)

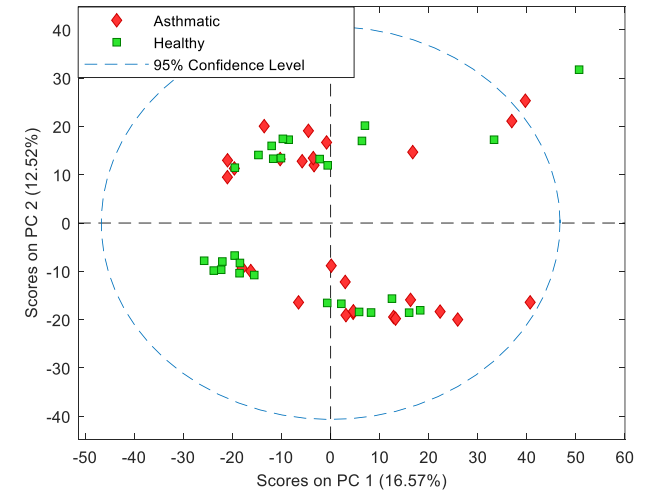
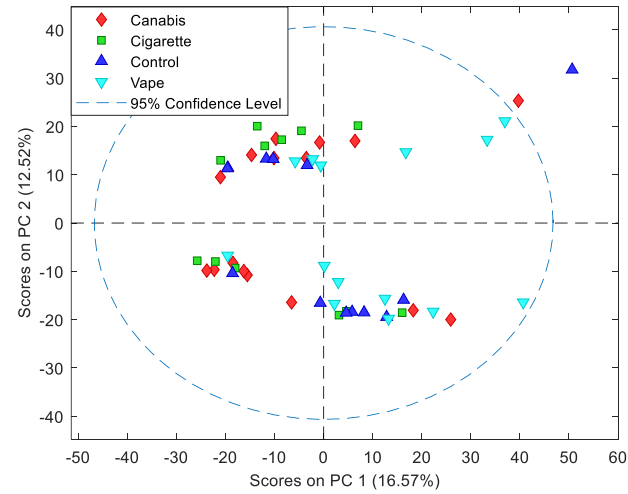
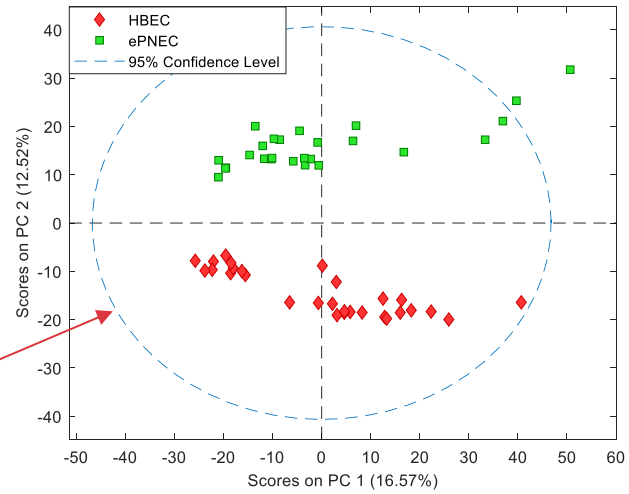


Asthmatic

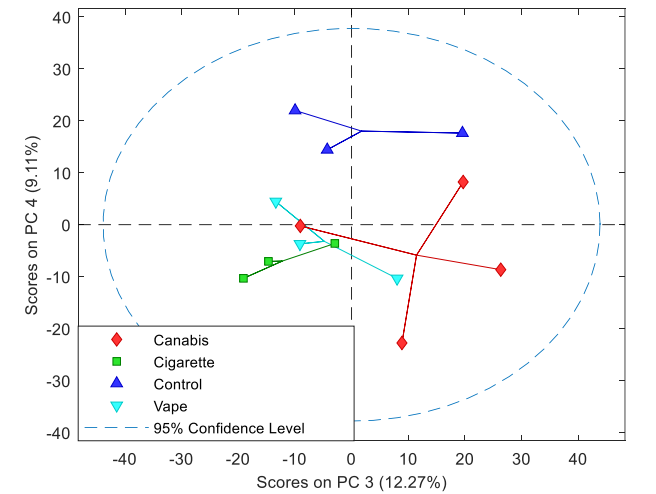
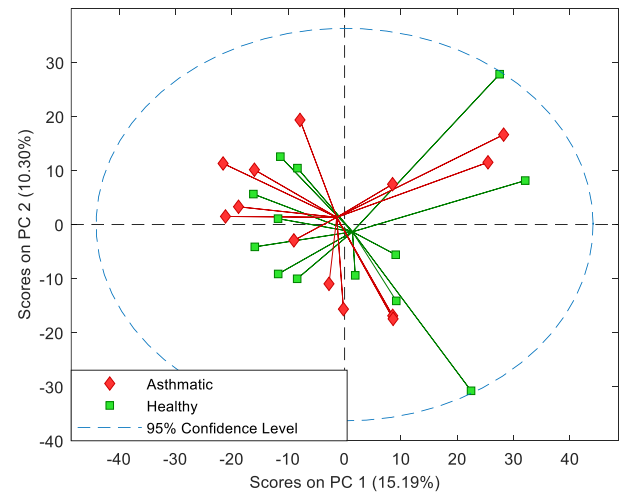
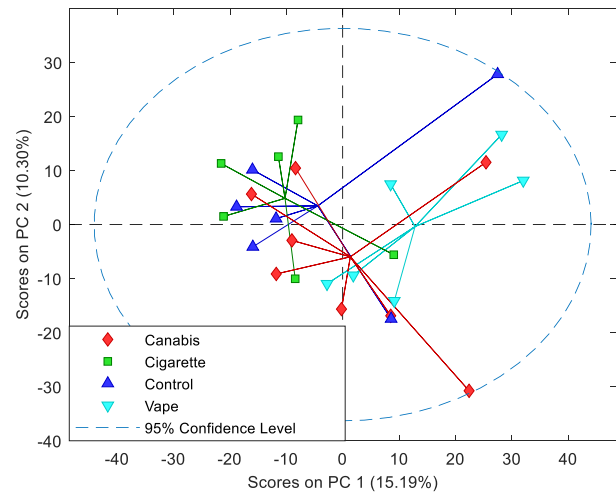
# Results

55 media samples  
× 2043 variables

Difference in cell  
types is dominant



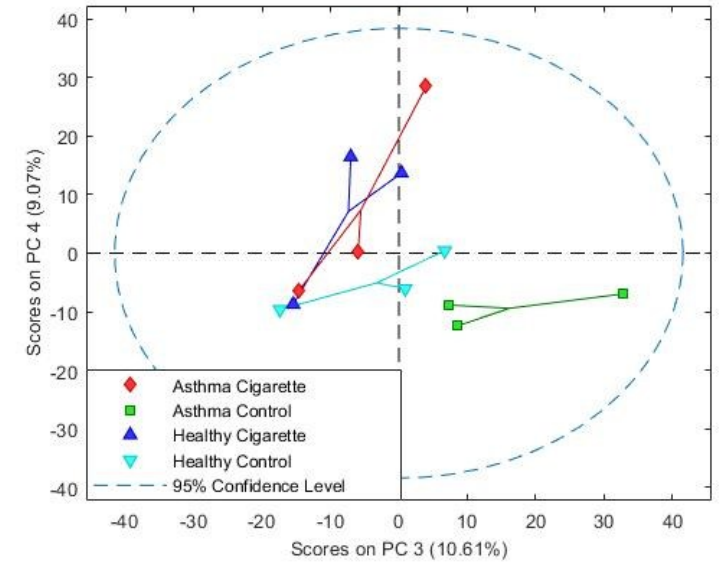
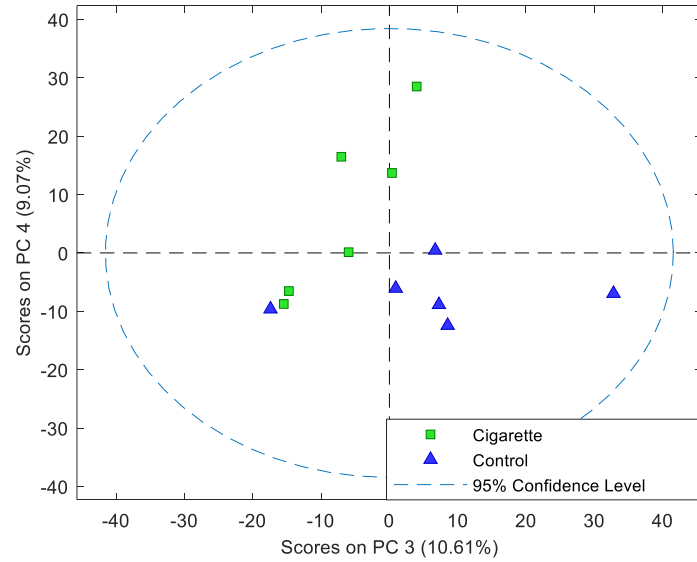
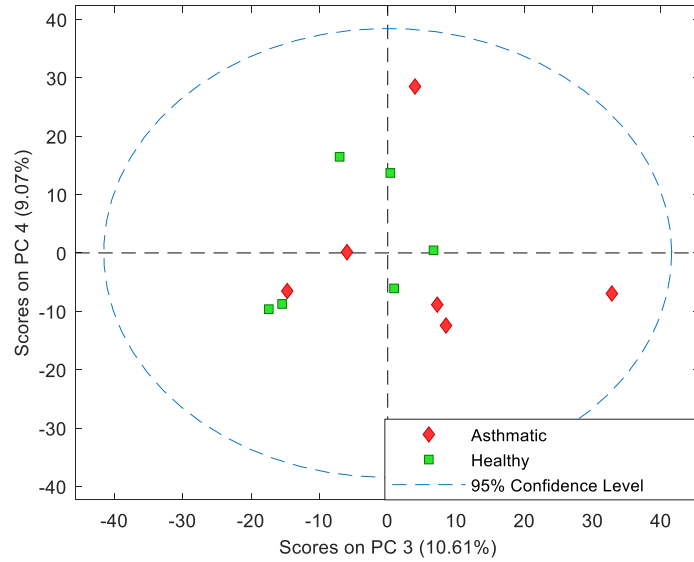
26 ePNEC only  
× 1807 variables



13 asthmatic × 1807 variables

# Control vs Cigarette

12 samples × 1807 variables

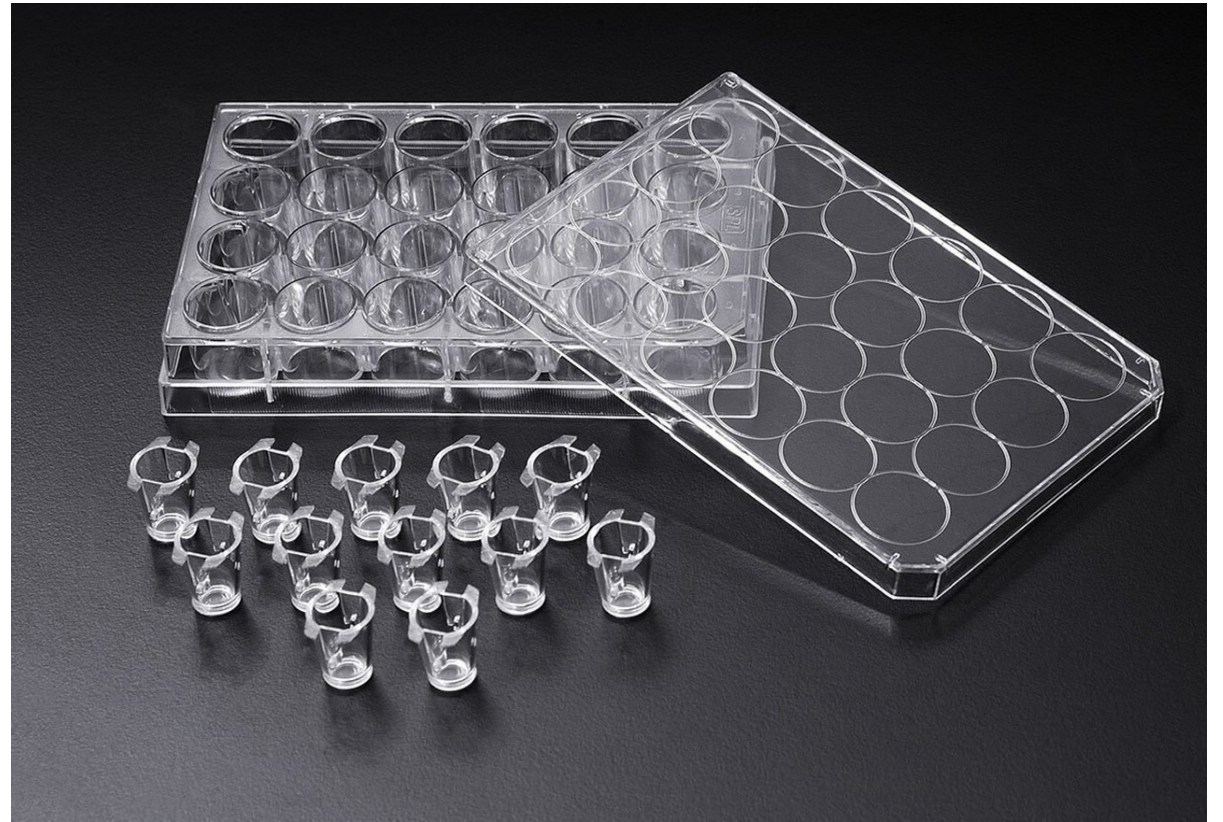


# Cool, but...

- Gradients in smoke exposure???
- Smoke entering media or cells?
- Plates as source of exposure afterwards?

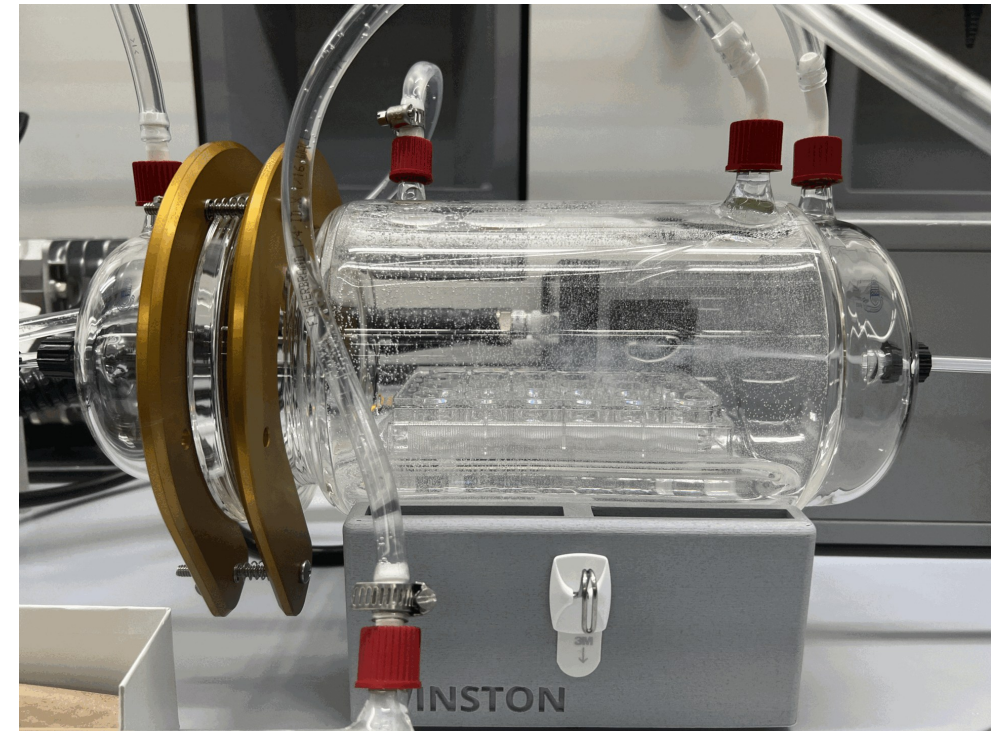
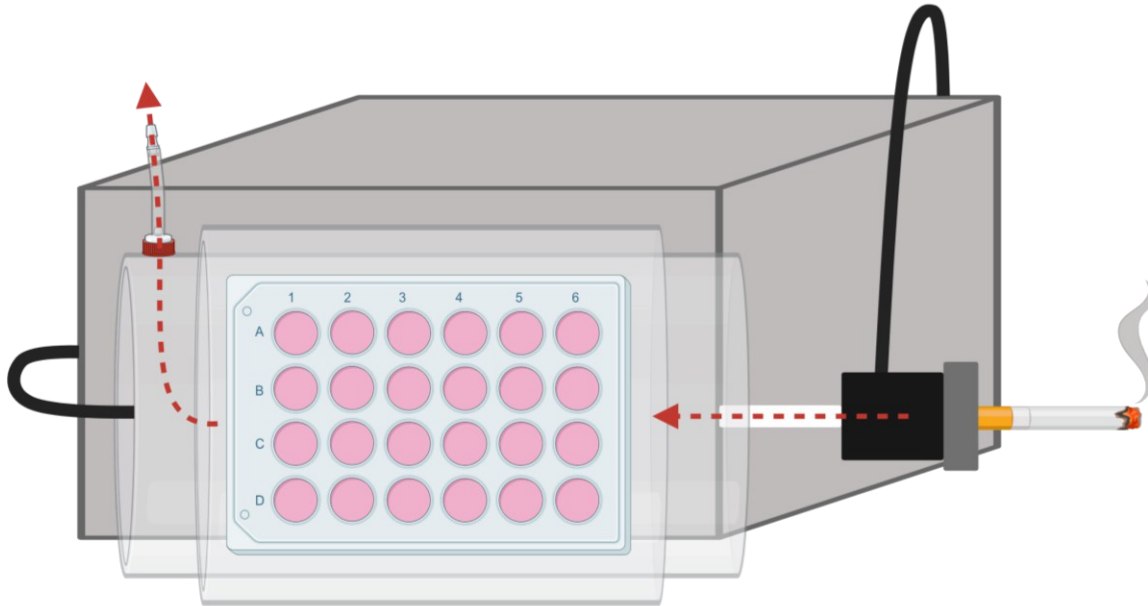
To address last 2 points

Engineered 3D printed PC enclosure



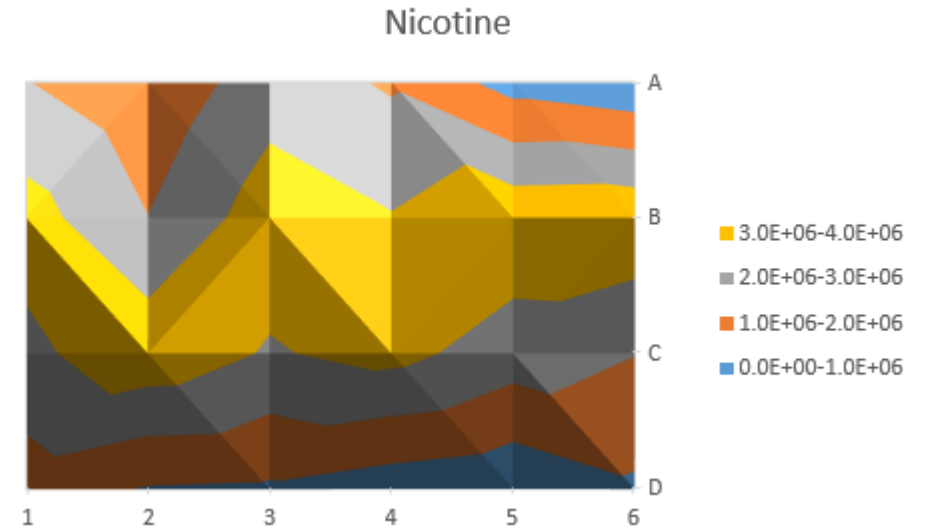
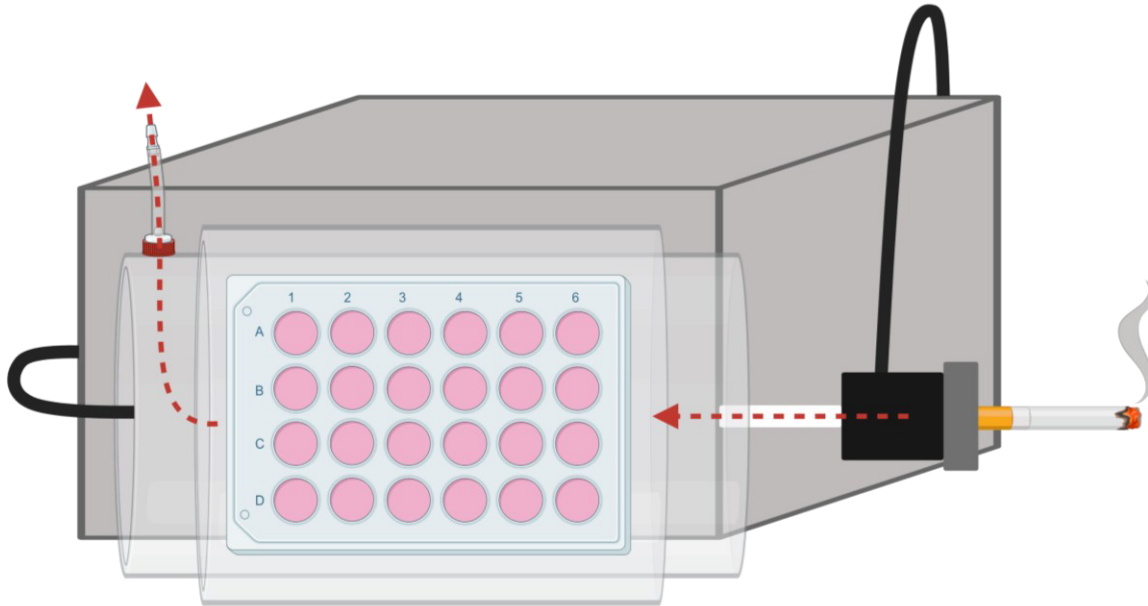
# Smoke Gradient

- Is smoke evenly distributed across the 24-well plates?

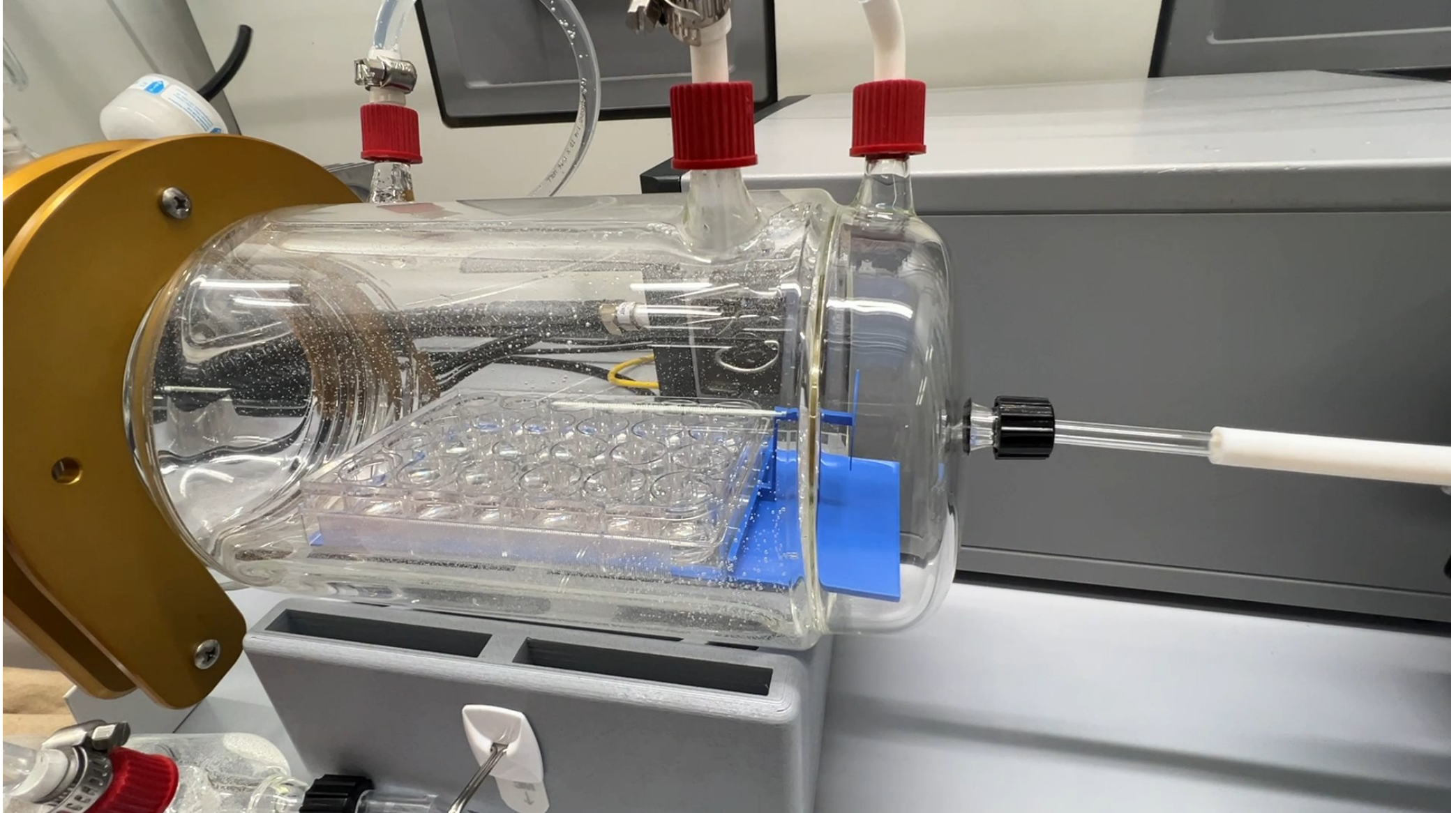


# Smoke Gradient

- Is smoke evenly distributed across the 24-well plates?



# Smoke Gradient



# Conclusions

- GC×GC-MS, allows us to observe impacts of smoking/vaping on metabolism of HBEC and ePNEC cells.
- With a few refinements, it's worth doing a bigger study
- Hopefully the density of dog images satisfies the organizers/audience



# Acknowledgements

