



Exhaled breath in health and disease

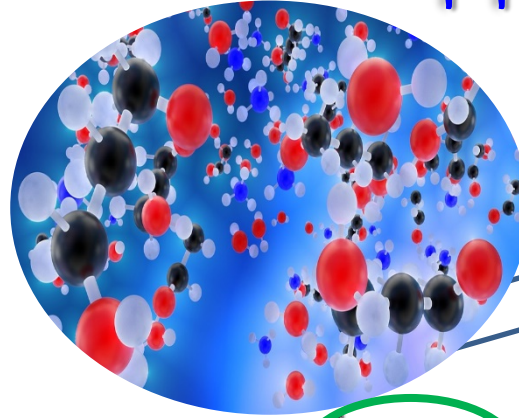
Agnieszka (Agi) Smolinska
Department of Pharmacology and Toxicology
Maastricht University, The Netherlands

08.11.2018

Exhaled breath applications



Maastricht University

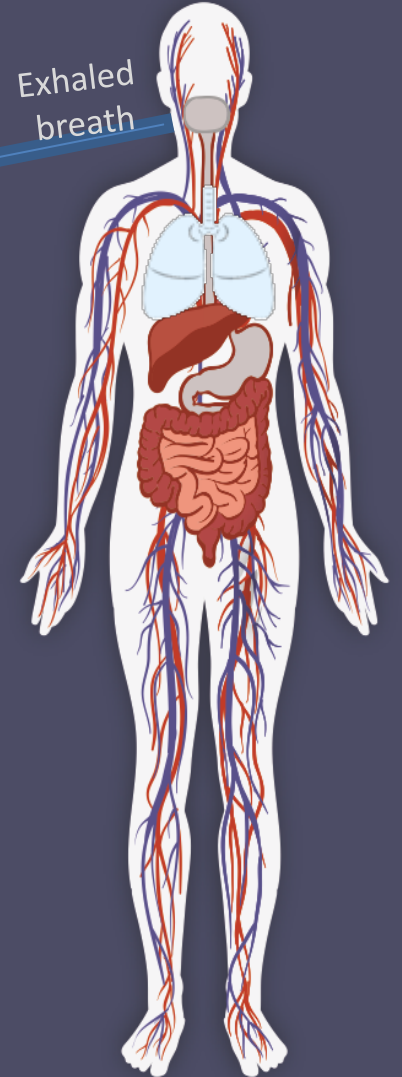


Exhaled
breath

Lungs

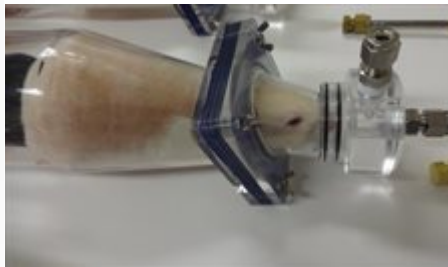
Liver

GI track

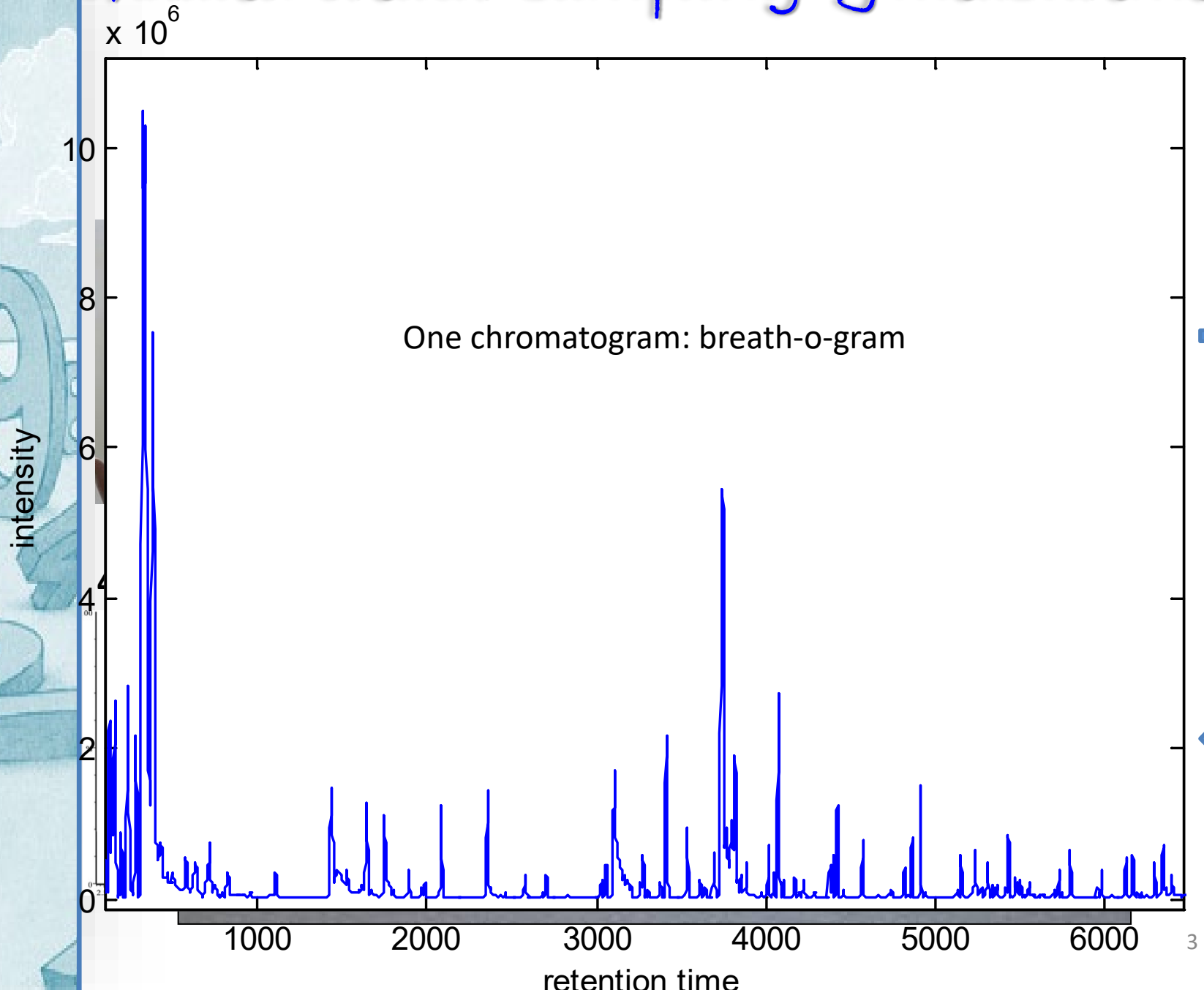


Sheep

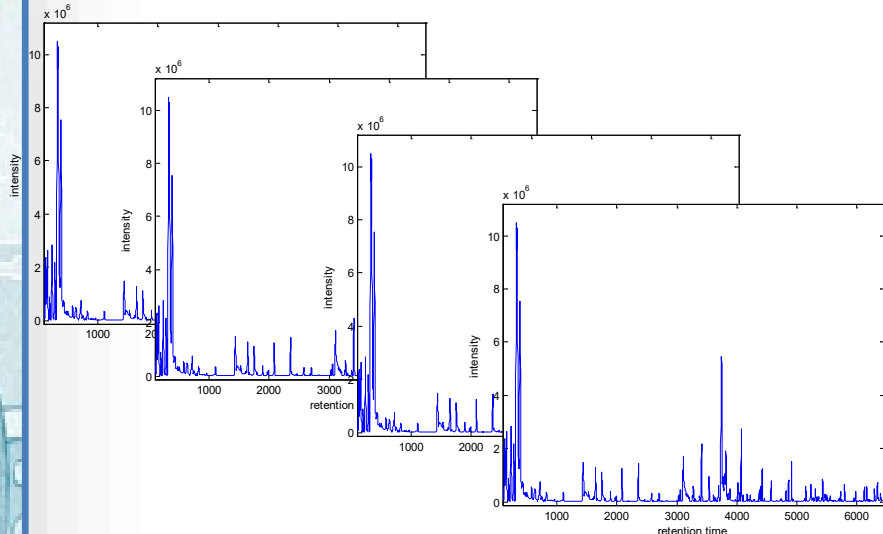
Mice



Exhaled breath sampling & measurement




Exhaled breath data



Intensity

retention time

Compounds/meta data

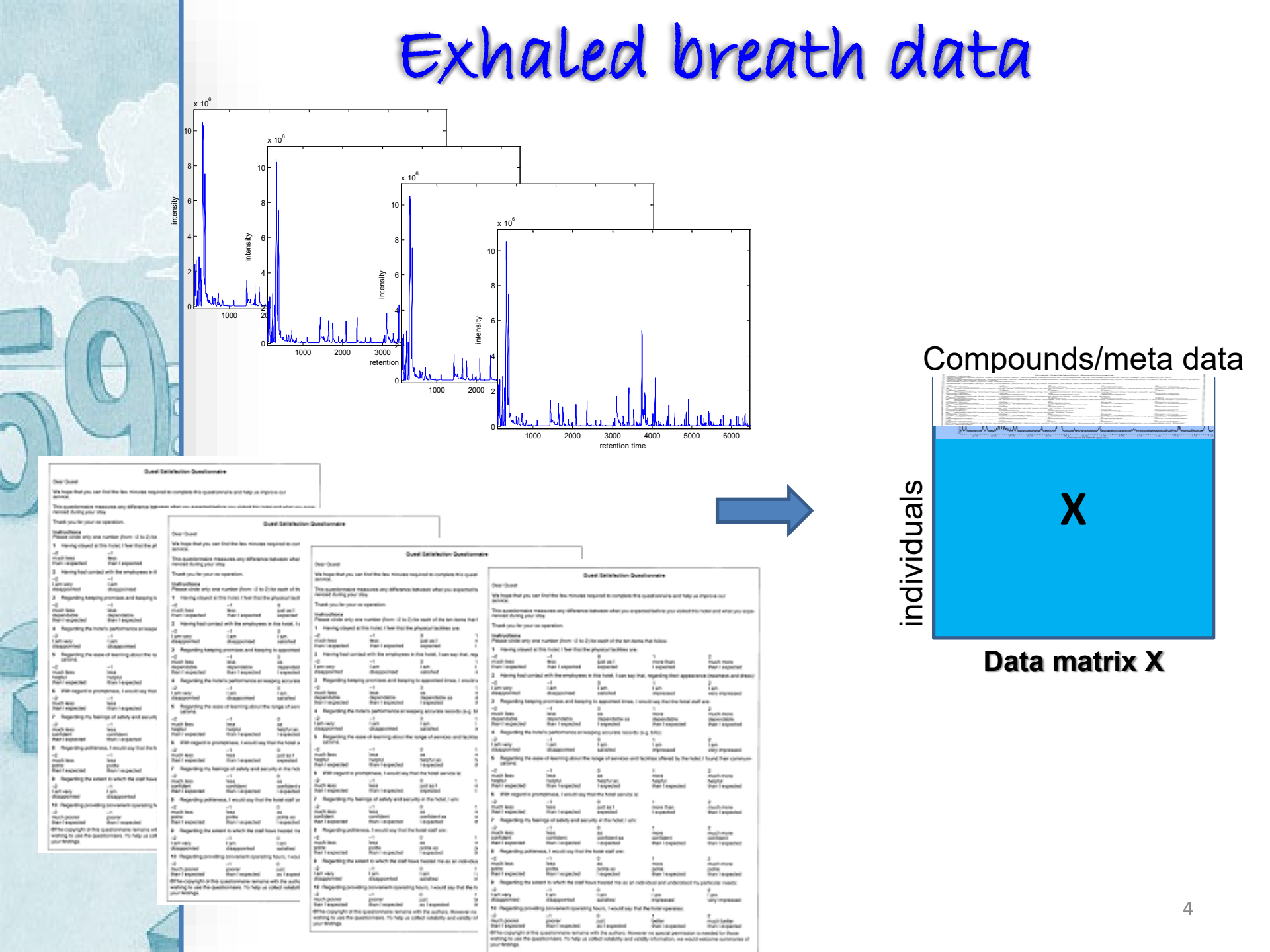


individuals

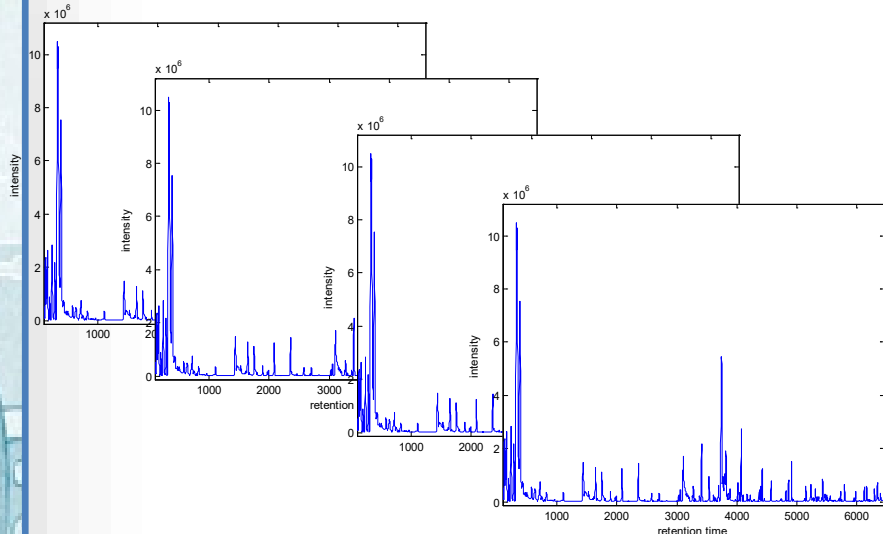
X

Data matrix X

4




Exhaled breath data



Intensity

retention time

Compounds/meta data

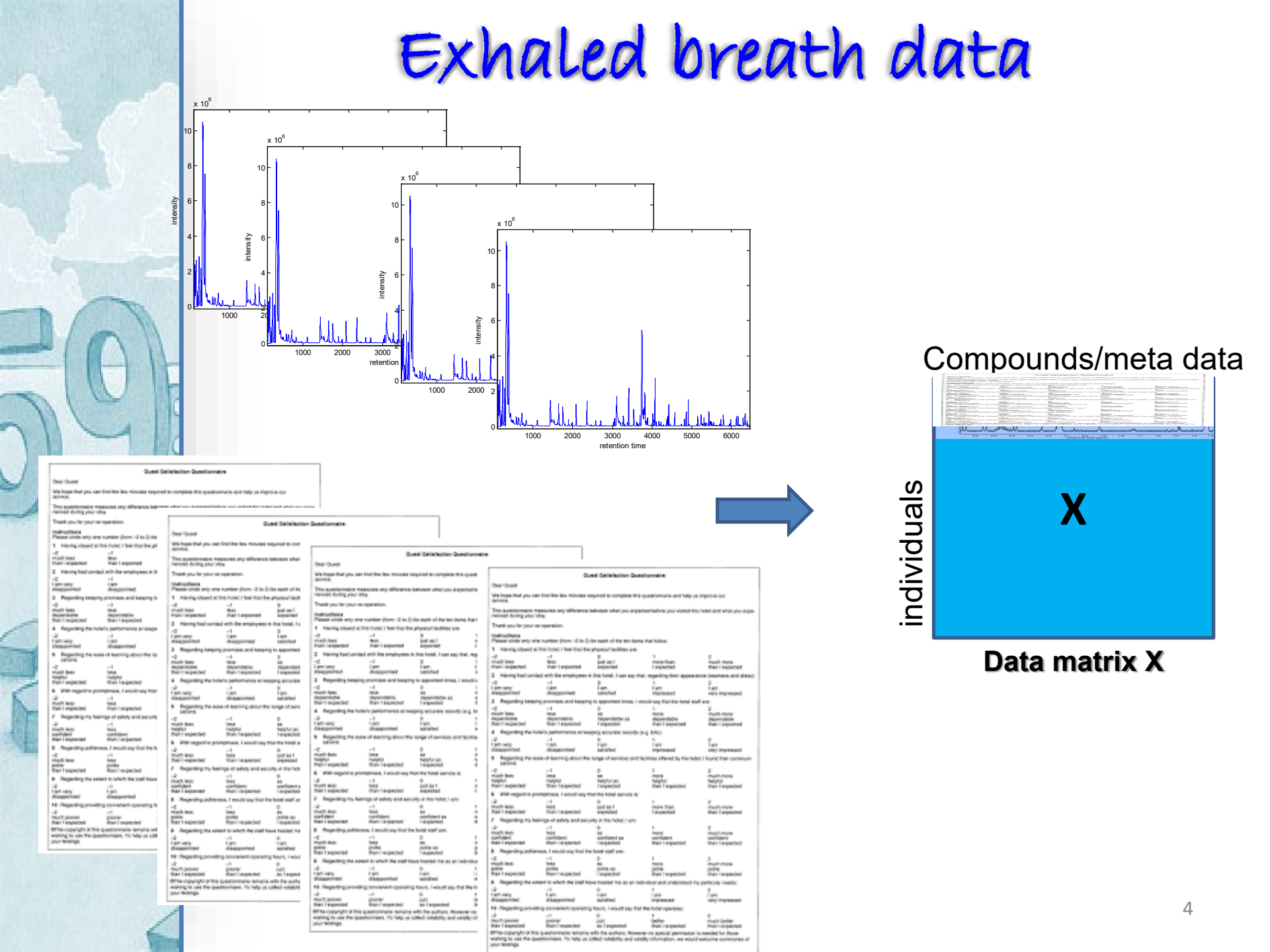


individuals

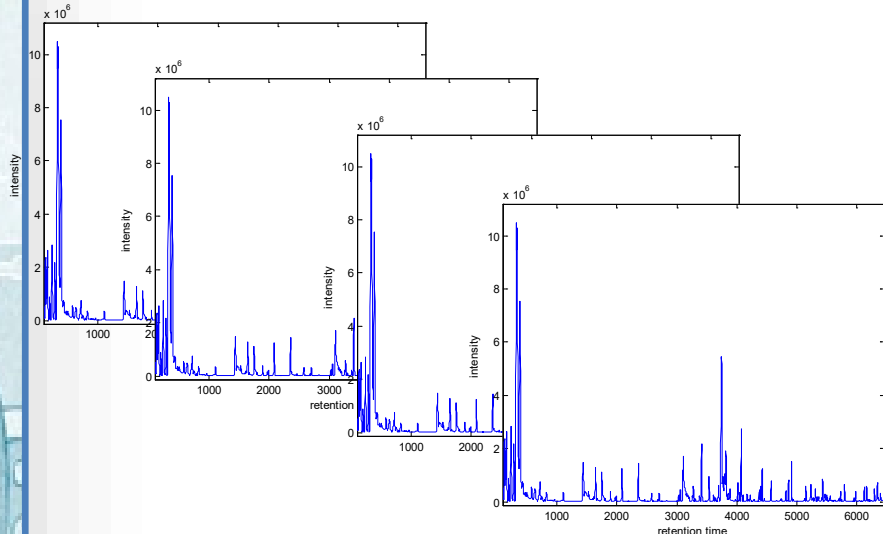
X

Data matrix X

4




Exhaled breath data



Intensity

retention time

Compounds/meta data



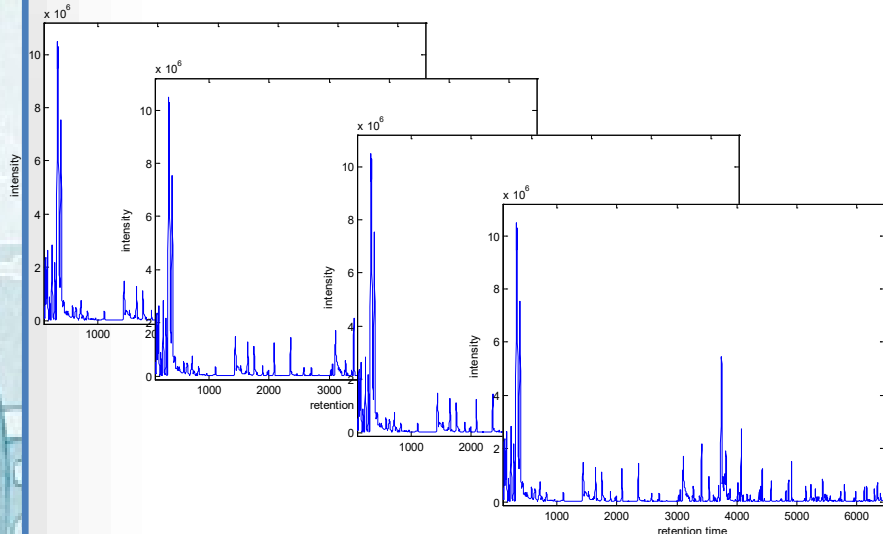
individuals

X

Data matrix X

4


Exhaled breath data



Intensity

retention time

Compounds/meta data



individuals

X

Data matrix X

4



Statistics

Machine Learning

Multivariate analysis

Bioinformatics

Data mining

BIOSTATISTICS

Big data

Exhaled breath: statistical procedure

Data collection

Data transformation

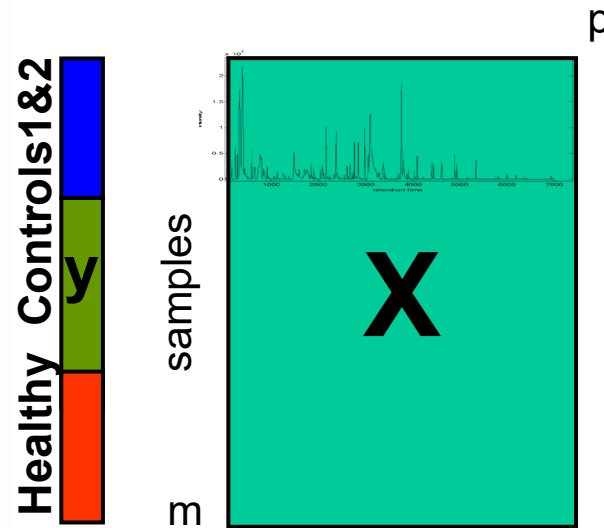
Data visualization



Data Integration

Data analysis

Exhaled breath: statistical procedure



splitting

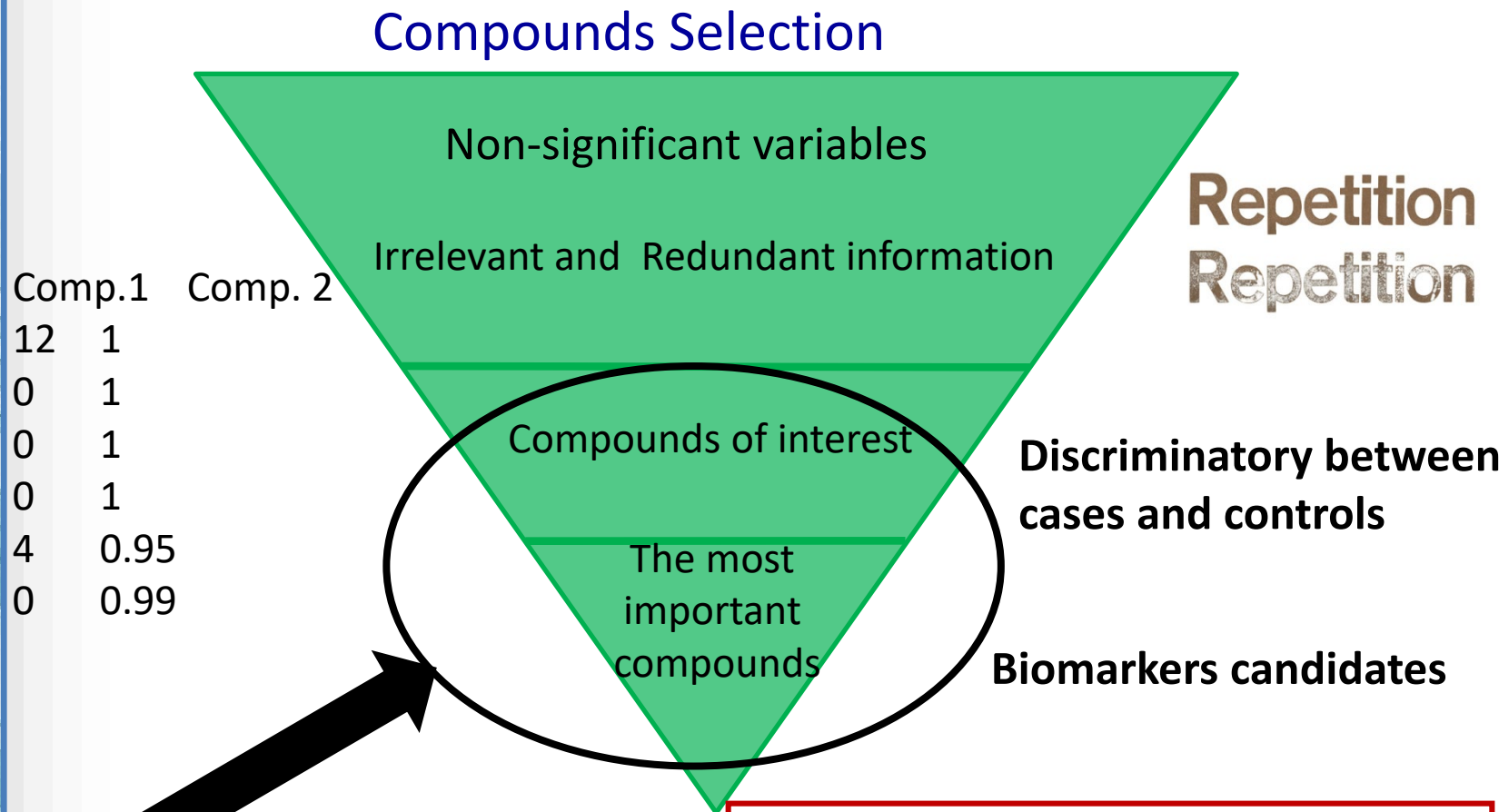
If possible validation
in independent (new)
individuals



Optimize a statistical method
(selection of important
compounds)

validate a statistical method (are
my compounds real?)

Exhaled breath: statistical procedure



We want to get this part
through compounds selection

Different machine learning algorithms:

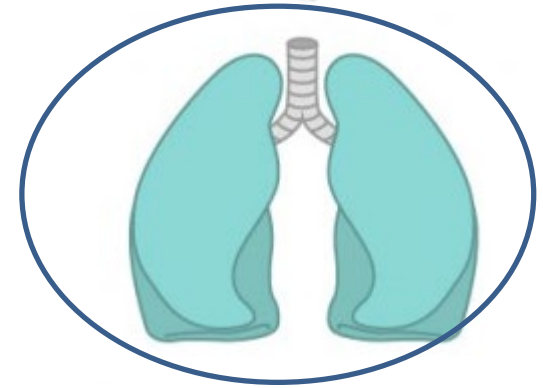
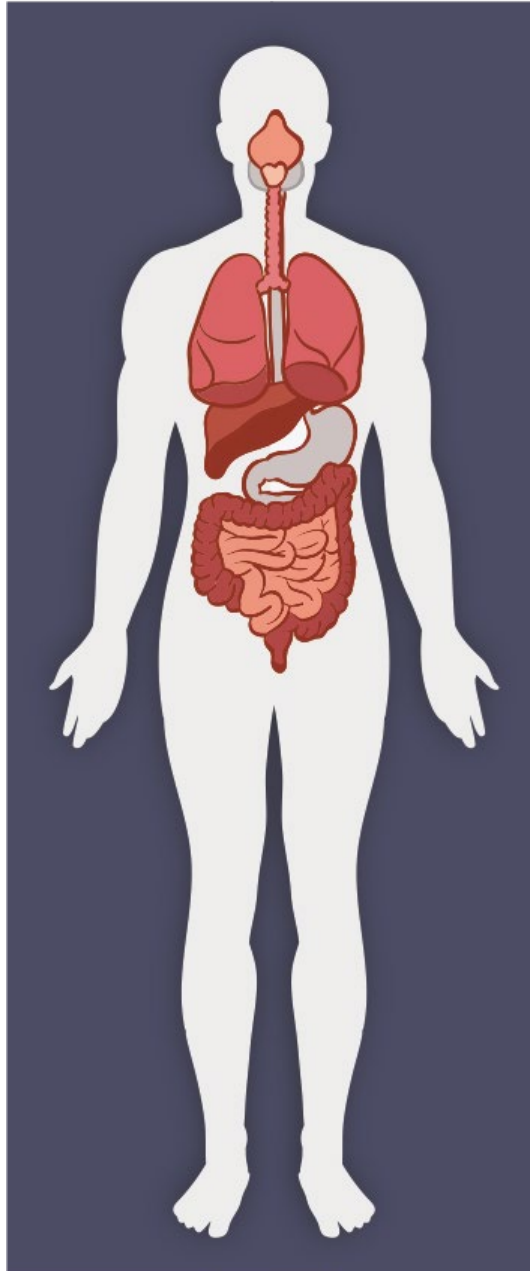
Discriminant Analysis

PLS-DA

Random Forest, Gradient boosting

ANOVA-PCA

Exhaled breath: examples

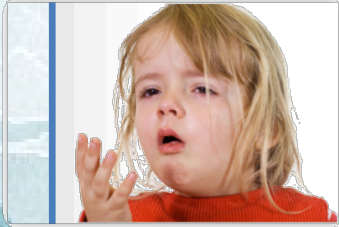


Lungs malfunctions



Gut/Bowel malfunctions
GI track

Exhaled breath for asthma in children



- ☐ asthma is an inflammatory process
- ☐ the most common illness in childhood
- ☐ diagnosis made at age 6
- ☐ wheezing or true asthma in preschool children needed
- ☐ prediction of asthma in preschool children

Wheeze or asthma



?



Exhaled breath for asthma in children

- ❑ 252 children participated in the study
- ❑ exhaled breath collection: preschool children 2-6 years old
- ❑ each child sampled 3-7 times → 1074 samples
- ❑ 3 groups: healthy, wheezeing, asthma (defined at age 6)

Exhaled breath for asthma in children

Splitting: training and validation



Optimizing: selecting significant compounds



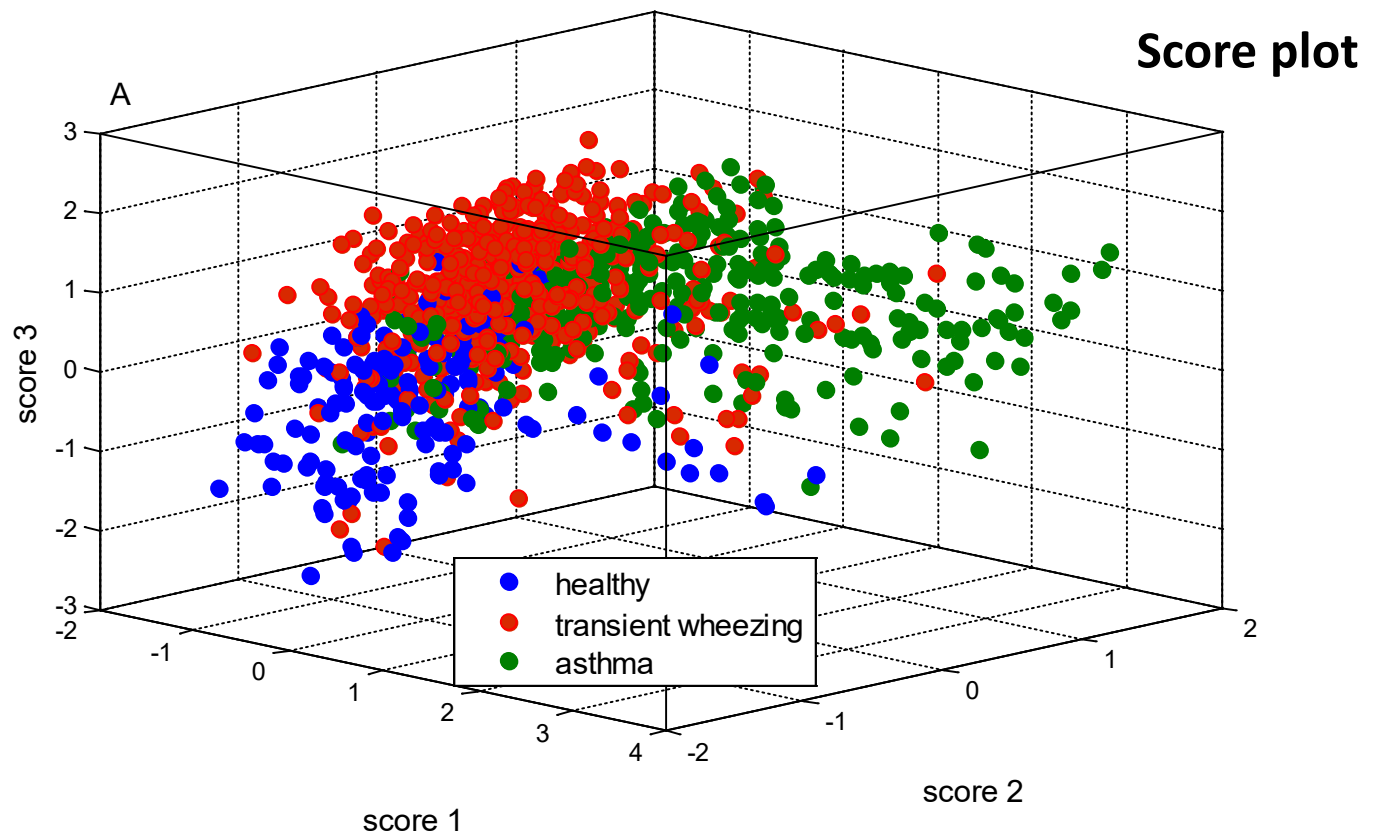
Validation: are the compounds real



Visualization: are there groups?

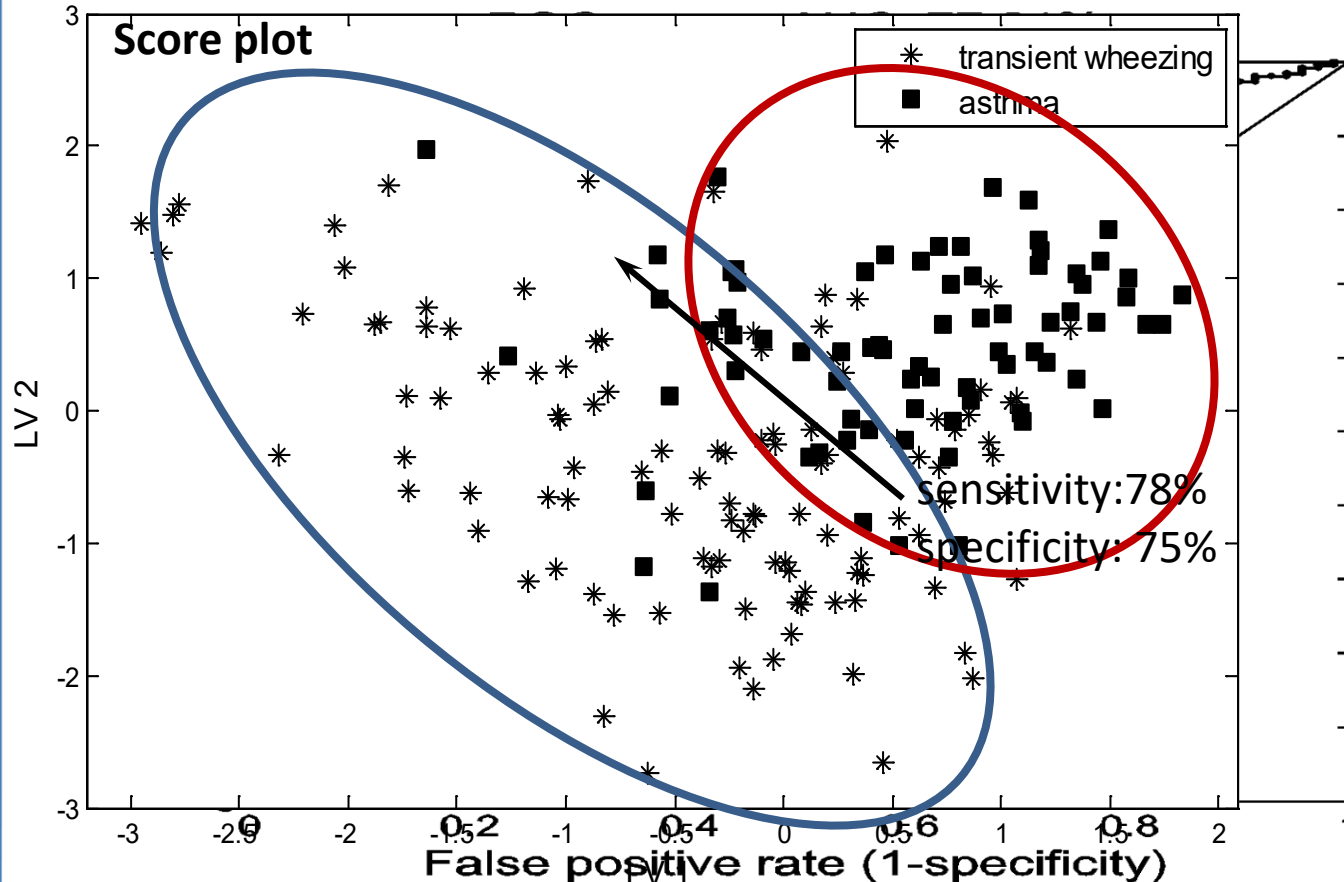
Asthma in children: Results

- ❑ Each point is a breath-o-gram (breath sample)
- ❑ **17 volatile metabolites** selected by Random Forest classifier
- ❑ **Important: 1074 samples from 252 children included**



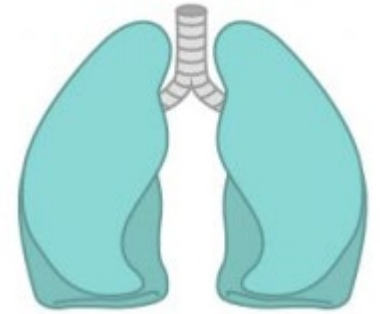
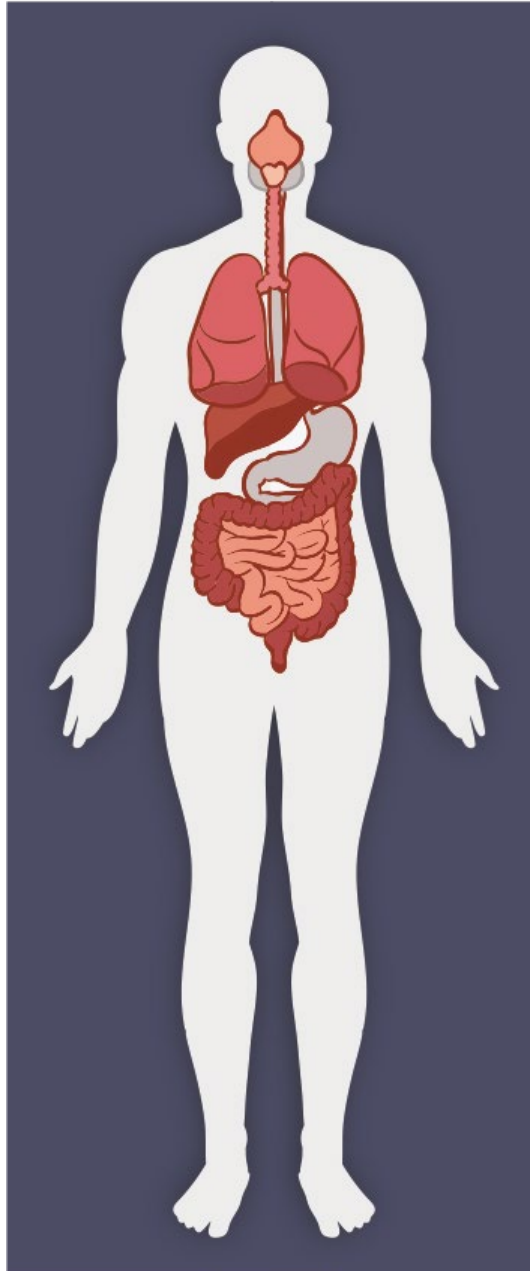
Asthma in preschool children: results

- ❑ Each point is a breath-o-gram: (wheezing and asthma at age 2)
- ❑ Differences based on **17 volatile metabolites**
- ❑ Important: prediction of asthma in preschool children (**age 2**) → 76% correct!!

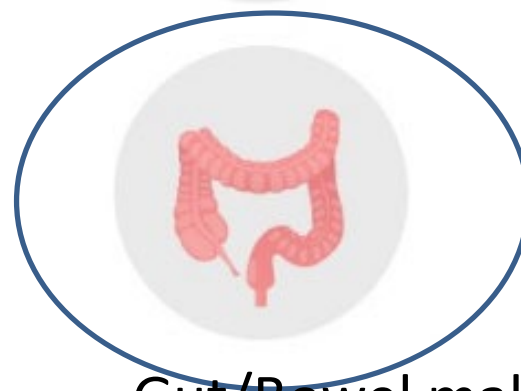


Validation samples

Exhaled breath: examples

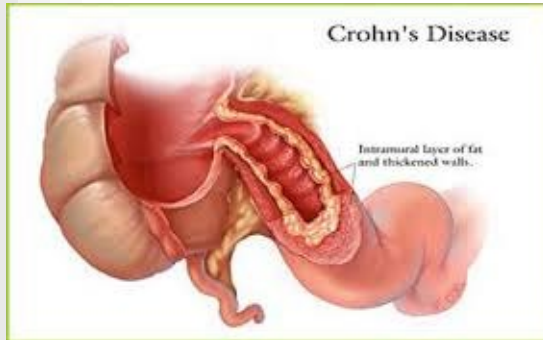


Lungs malfunctions



Gut/Bowel malfunctions
GI track

Exhaled breath: Crohn's disease



- ☐ Crohn's disease (CD) → Inflammatory Bowel Disease
- ☐ Chronic & relapsing (active and remission)
- ☐ Abdominal pain, vomiting, diarrhea, bleeding, severe internal cramps, muscle spasms in the region of the pelvis
- ☐ Ileocolonoscopy → gold standard

Exhaled breath: Crohn's disease

Can exhaled breath analysis discriminate active from inactive stage of CD

- ☐ Faeces for Calprotectin, blood for CRP, Harvey Bradshaw Index (Clinical activity indices) & exhaled air
- ☐ Evaluation of disease activity: Harvey Bradshaw Index (HBI) CRP and FC:
 - ☐ active disease: $FC > 250 \mu\text{g/g}$
 - ☐ remission disease: $HBI \leq 4$ & $CRP < 5 \text{ mg/l}$ & $FC < 100 \mu\text{g/g}$
- ☐ Data:
 - ☐ 140 real CD active
 - ☐ 135 real CD remission

Exhaled breath: Crohn's disease

Splitting: training and validation



Optimizing: selecting significant compounds



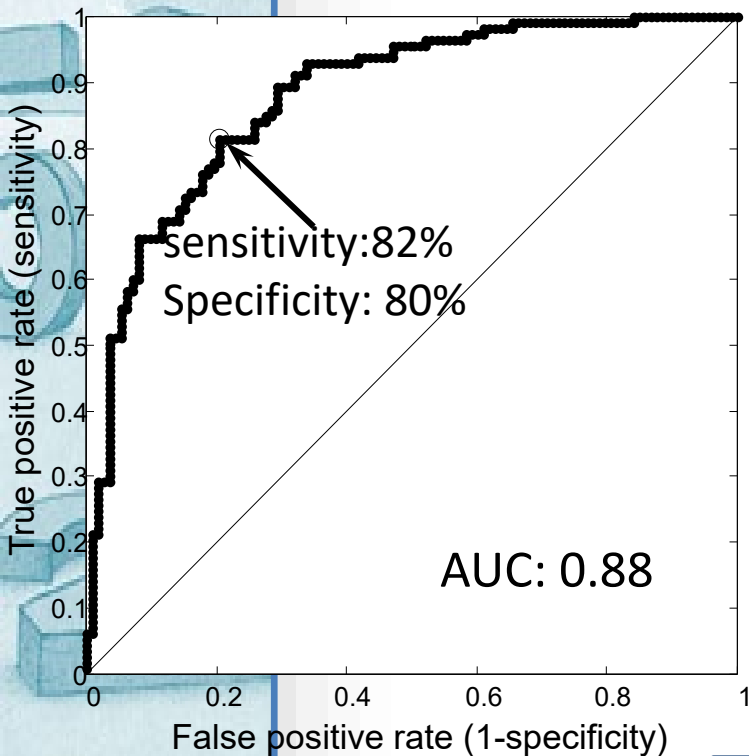
Validation: are the compounds real



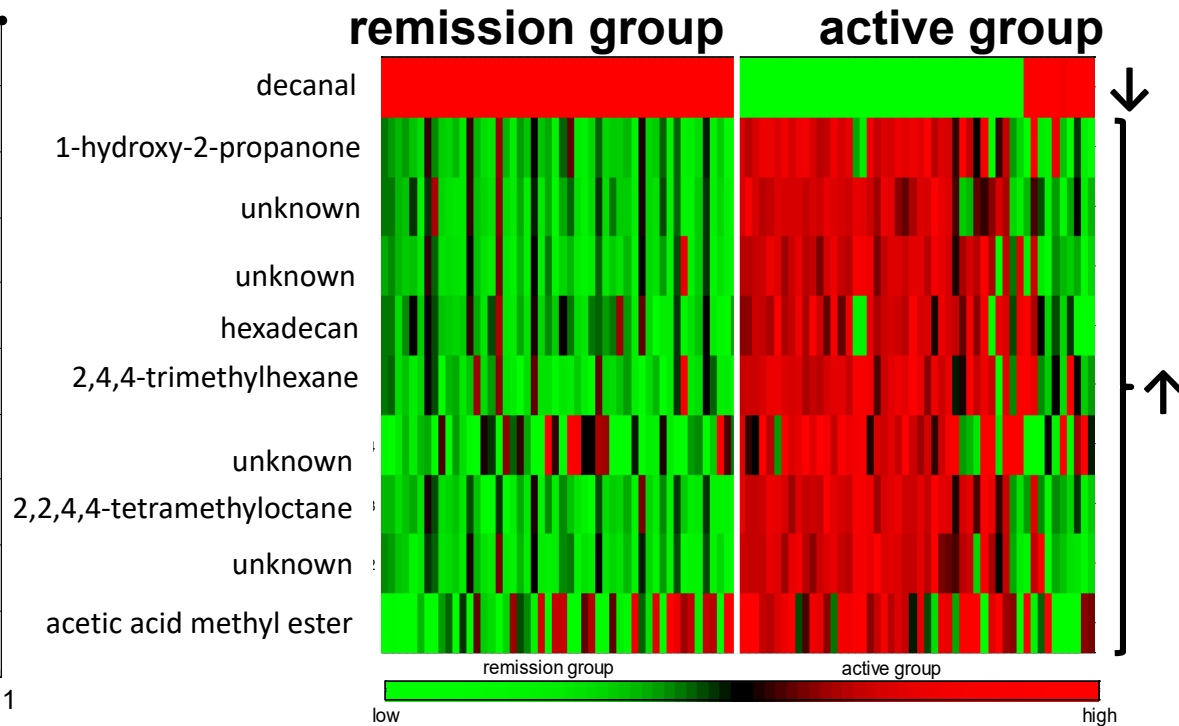
Visualization: how the compounds change in groups?

Exhaled breath: Crohn's disease

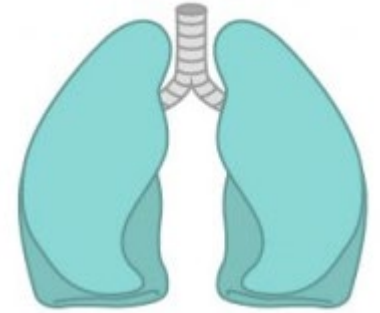
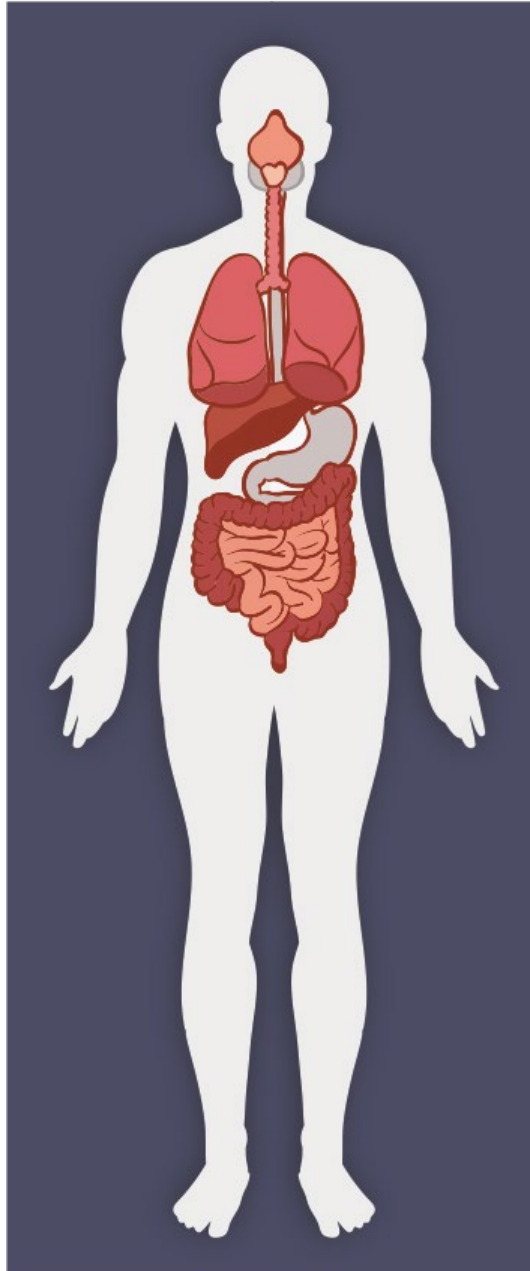
- 10 volatile metabolites selected via Random Forests analysis



validation samples



Exhaled breath: examples



Lungs malfunctions

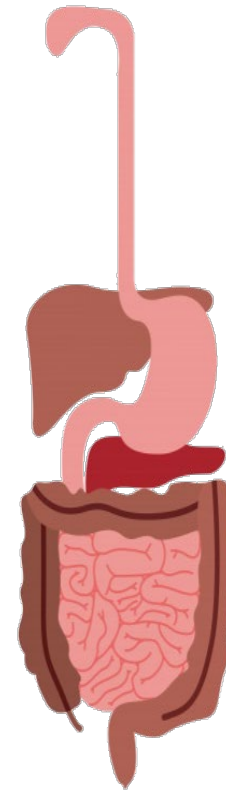
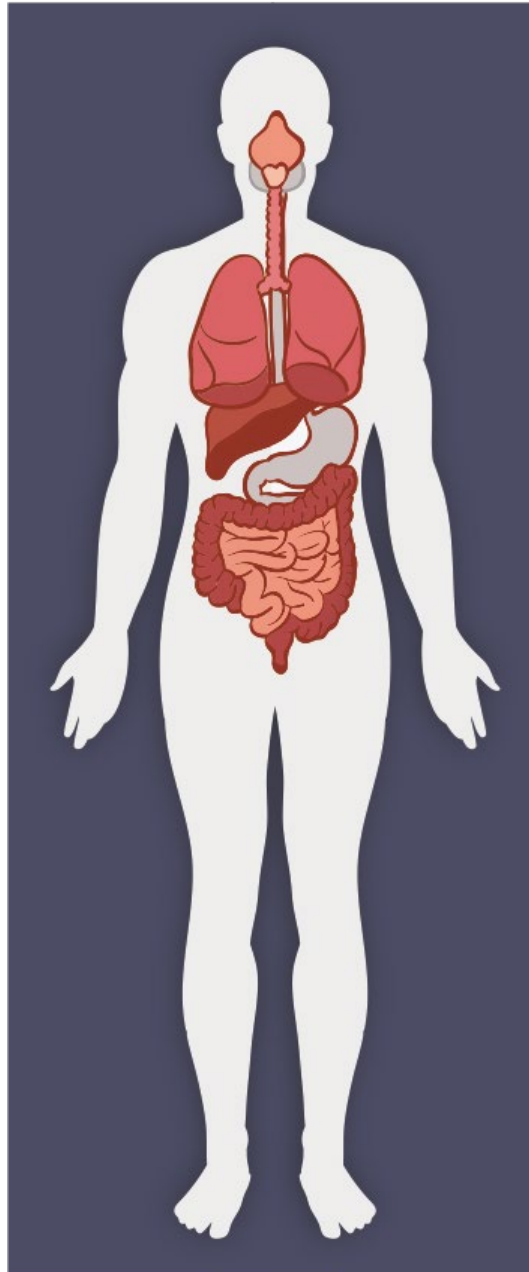


Gut/Bowel malfunctions



GI track

Exhaled breath: examples



DIET

GI track



Exhaled breath: infant formula



- ❑ 29 males (cross-over, double blinded)
- ❑ 6 time points (0, 30, 60, 120, 180 & 240)
- ❑ 2 formulas milk: active and control

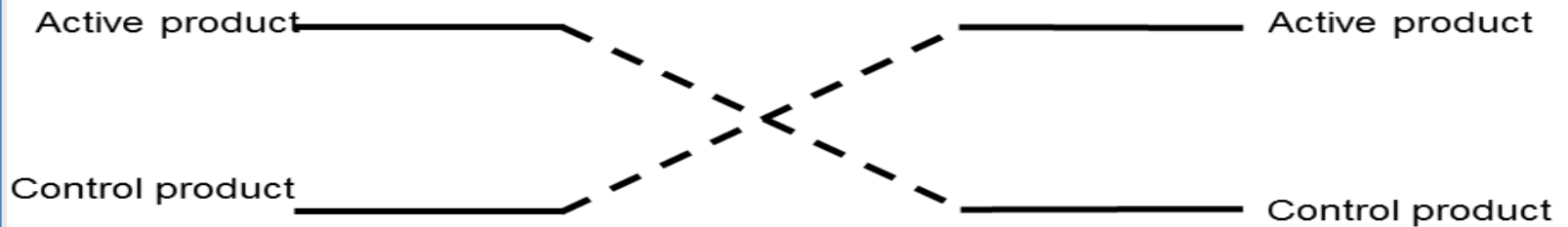


Large phospholipid coating

Commercial milk: Nutricia

Is there difference?

Exhaled breath: infant formula



Active vs. Control at each time point

Exhaled breath: infant formula

Splitting: training and validation



Optimizing: selecting significant compounds



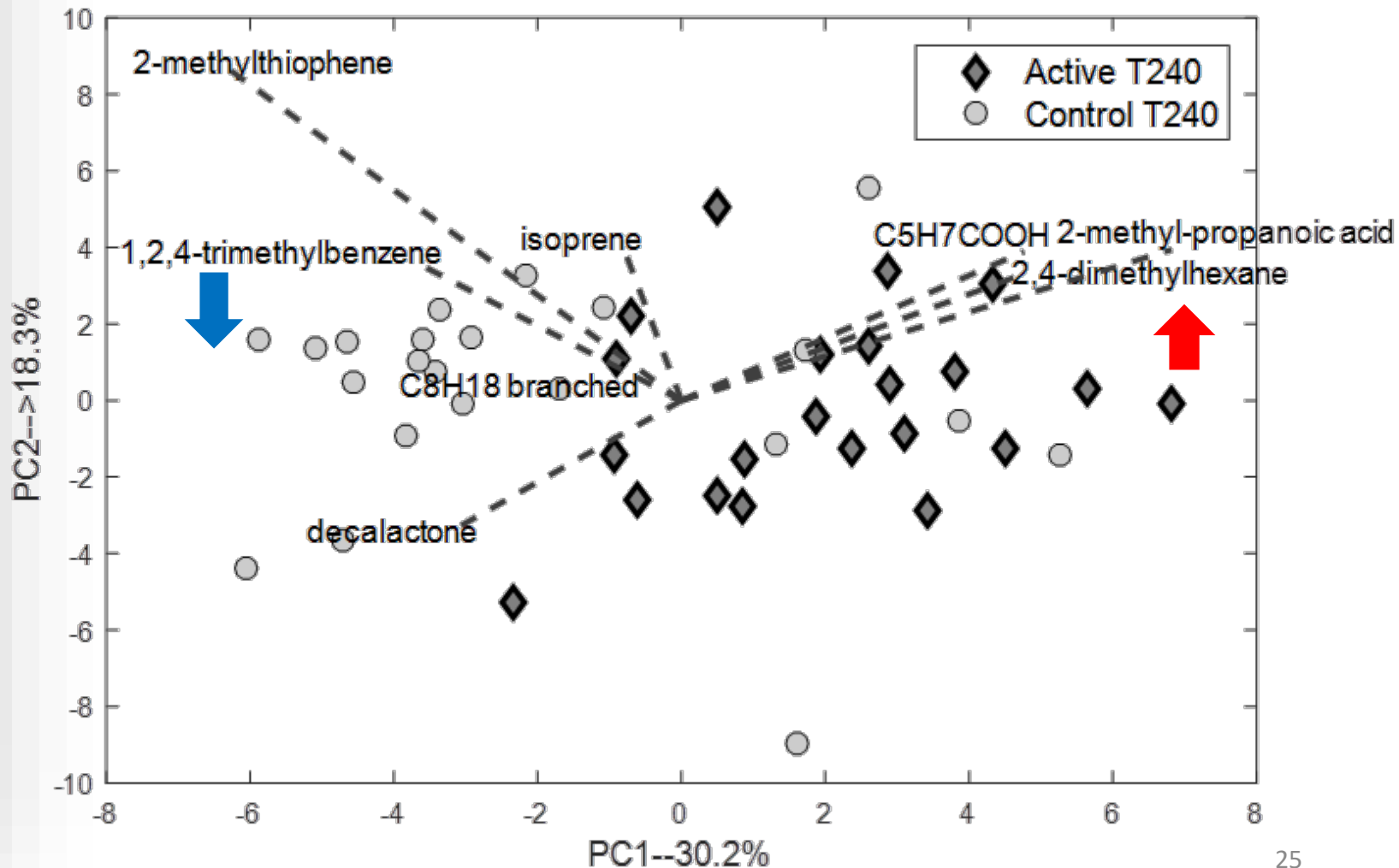
Validation: are the differences real



Visualization: are there groups?

Exhaled breath: infant formula

- significant differences only at T240 → 8 compounds selected (p-value<0.0001)



Exhaled breath: examples

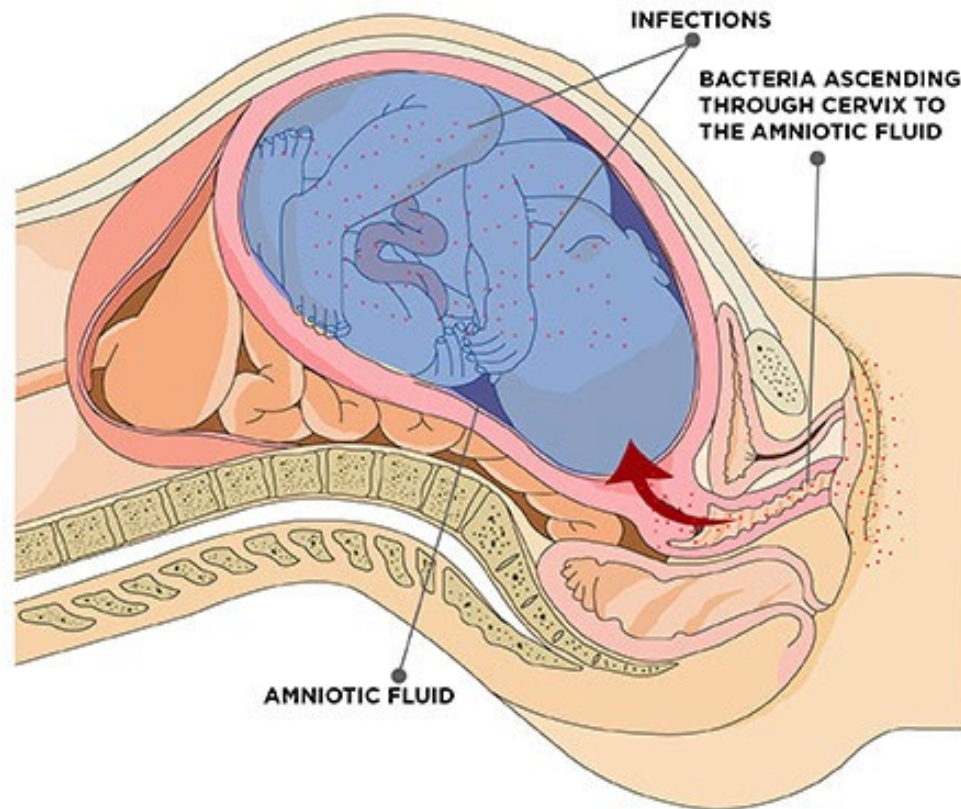


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Animal model

Exhaled breath: chorioamnionitis

Chorioamnionitis



Exhaled breath: chorioamnionitis

Injection of *Ureaplasma parvum*



Tedlar bag



Inhaling



☐ Breath at baseline and daily for 6-7 days

☐ 4 animals in January 2017 and 4 animals in July 2017



Discovery set



External validation set

Exhaled breath: chorioamnionitis

Splitting: training and validation



Optimizing: selecting significant compounds



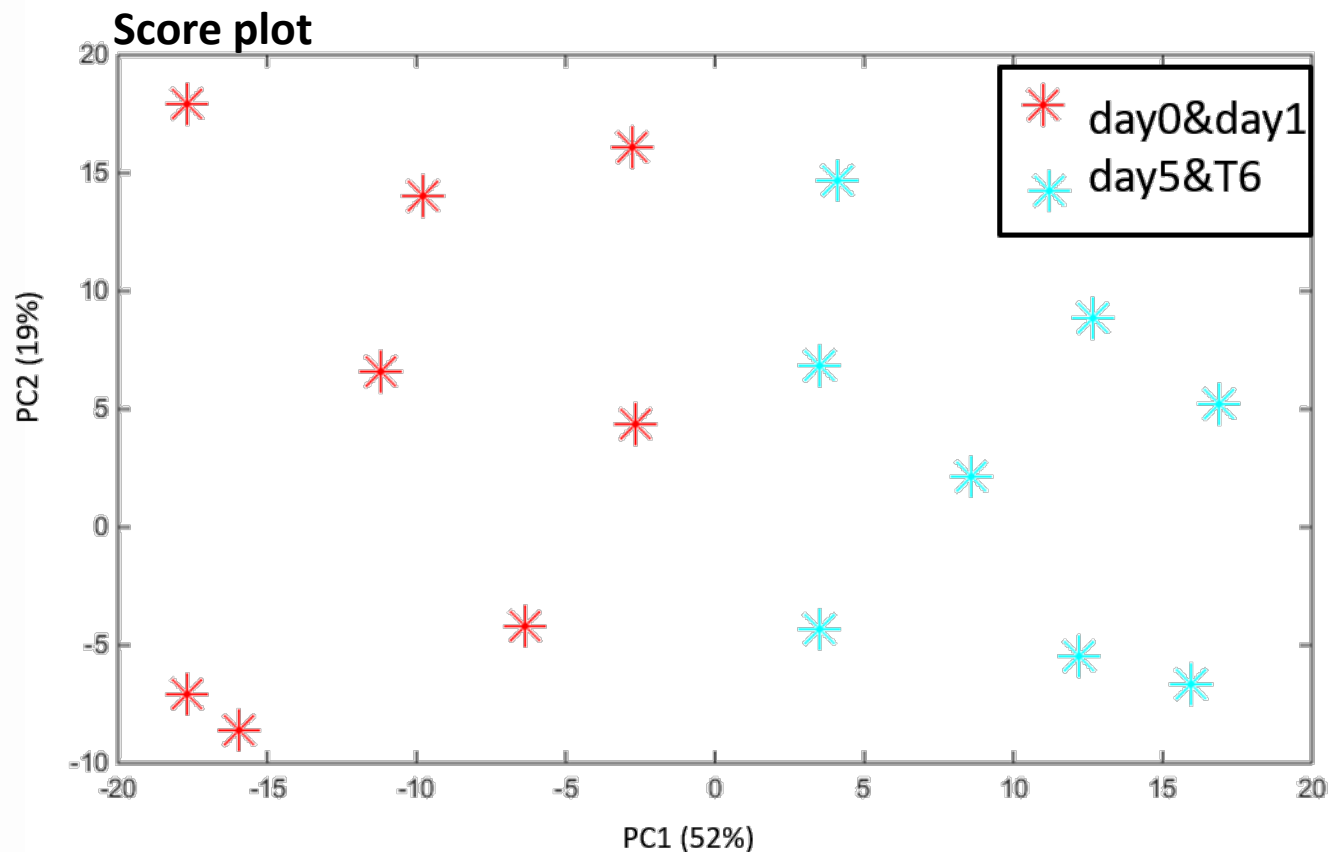
Validation: are the compounds real



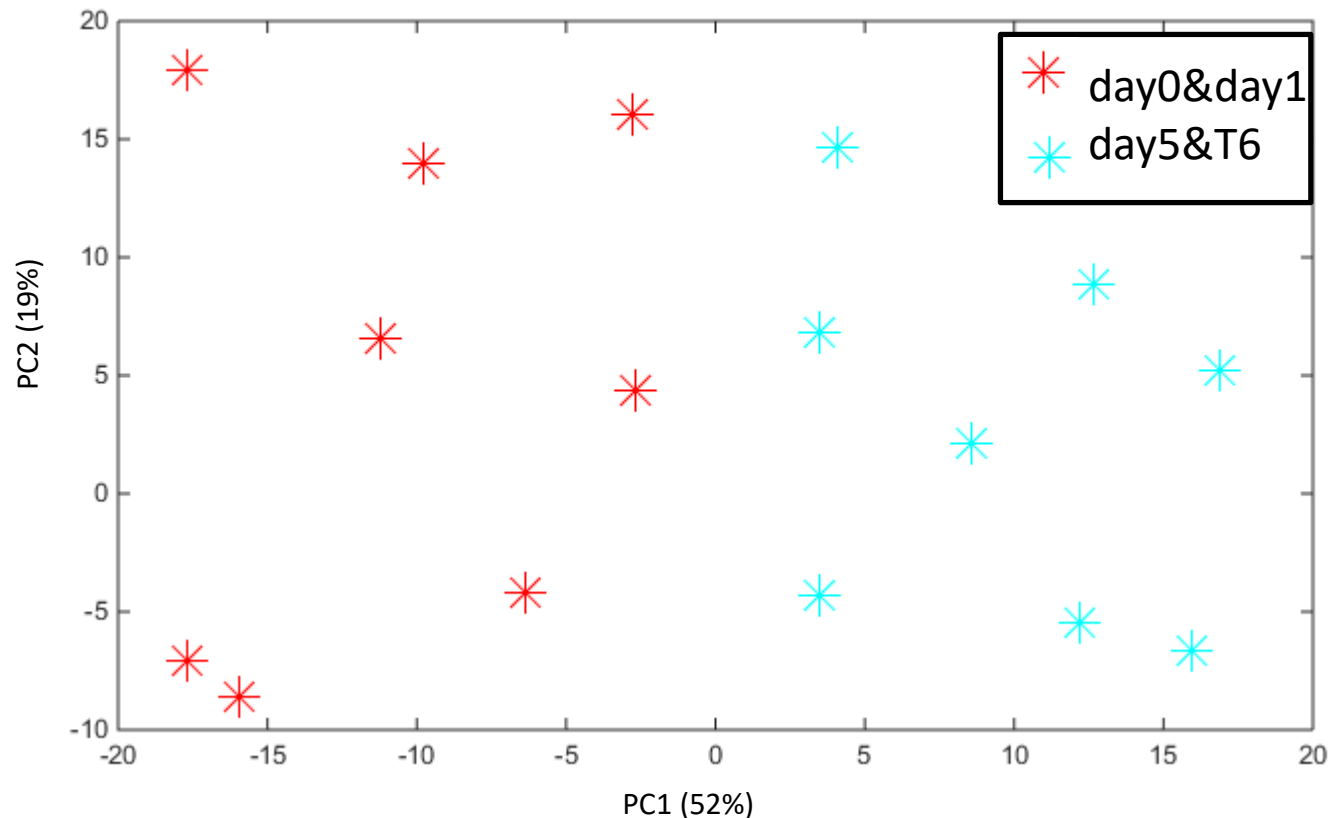
Visualization: are there groups?

Exhaled breath: chorioamnionitis

- 19 volatiles selected as the most discriminatory between **no infection** and **infection** → **DISCOVERY SET**



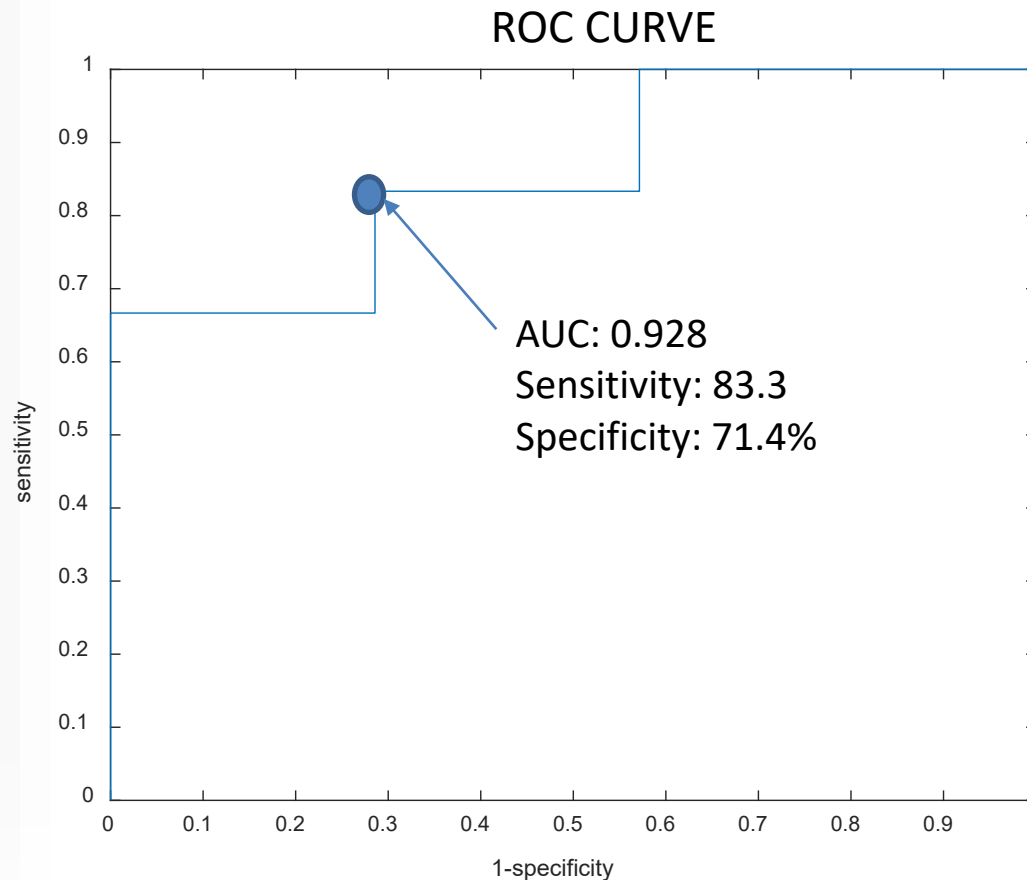
Exhaled breath for chorioamnionitis validation



- ❑ differences based on 19 volatiles selected as the most discriminatory between **no infection** and **infection**

Exhaled breath: chorioamnionitis validation

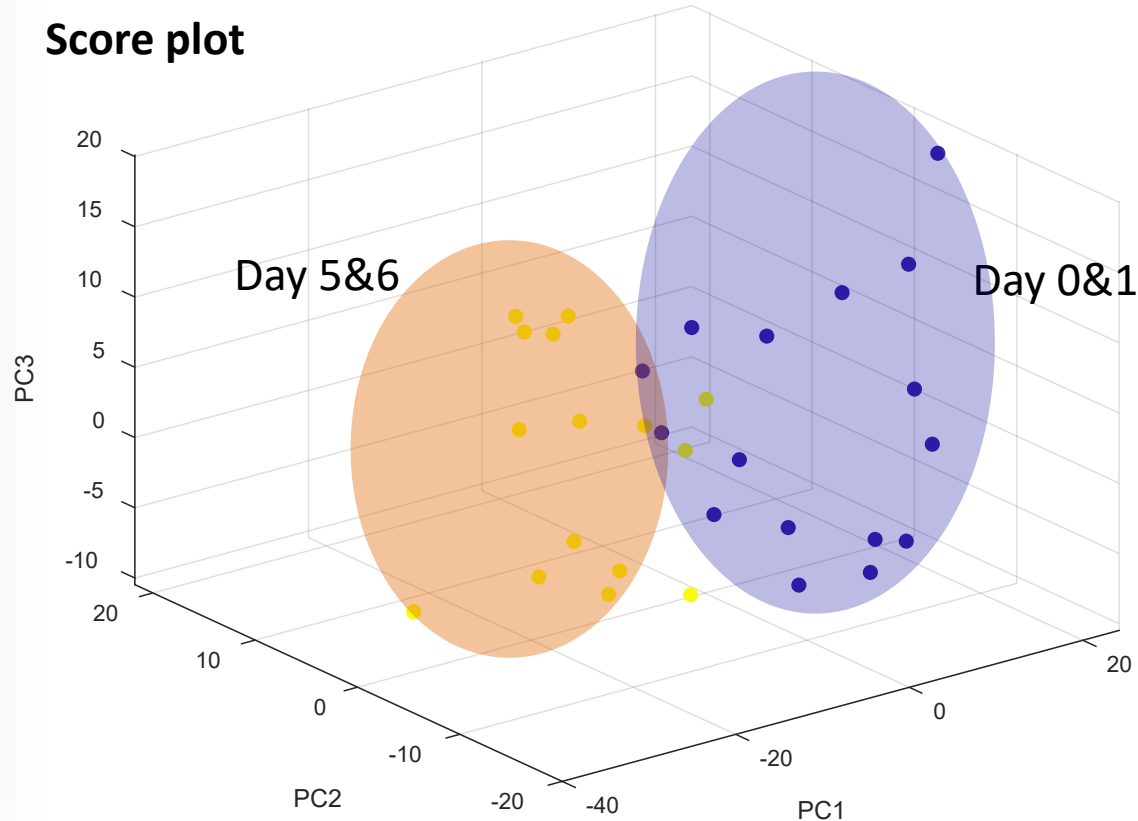
- Validated on the second set of sheep (measured in July)



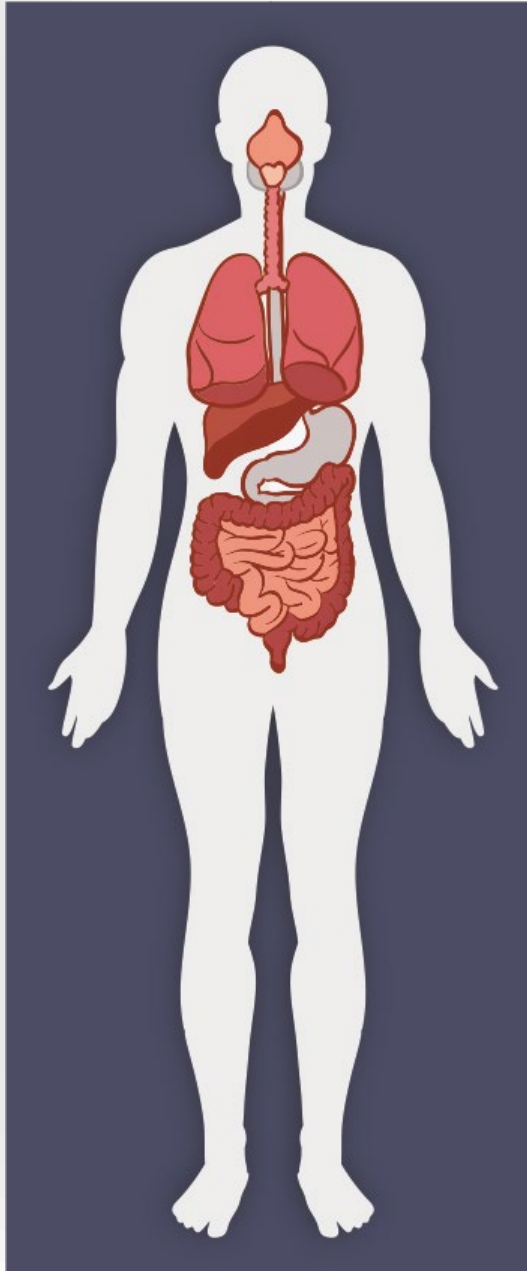
Independent validation!

Exhaled breath: chorioamnionitis visualization

- ❑ All sheep → Discover & Validation
- ❑ 19 volatiles used between **no infection** and **infection**



Summary



- ❑ Exhaled breath goes beyond lungs
- ❑ Exhaled breath related to various malfunctions
- ❑ Application in several large real life cohorts plus animal model
- ❑ Breath Data are complex thus special care with data analysis



Acknowledgment

Frederik-Jan van Schooten

Agnes Boots

Jan Dallinga

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John Penders

Edward Dompeling

Tim Wolfs

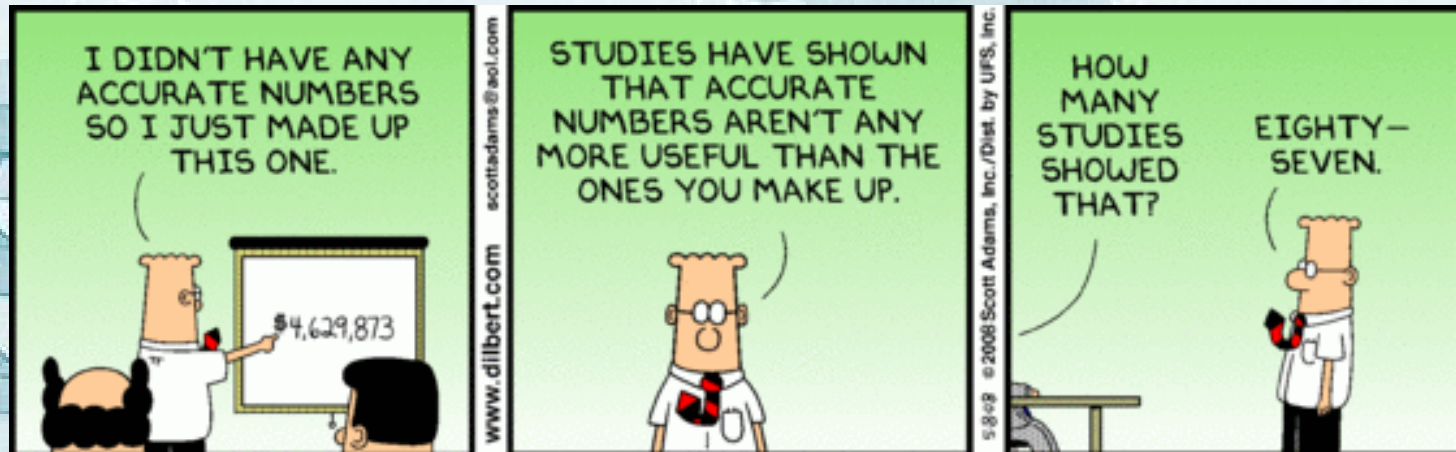
Ad Masclee

Marieke Pierik

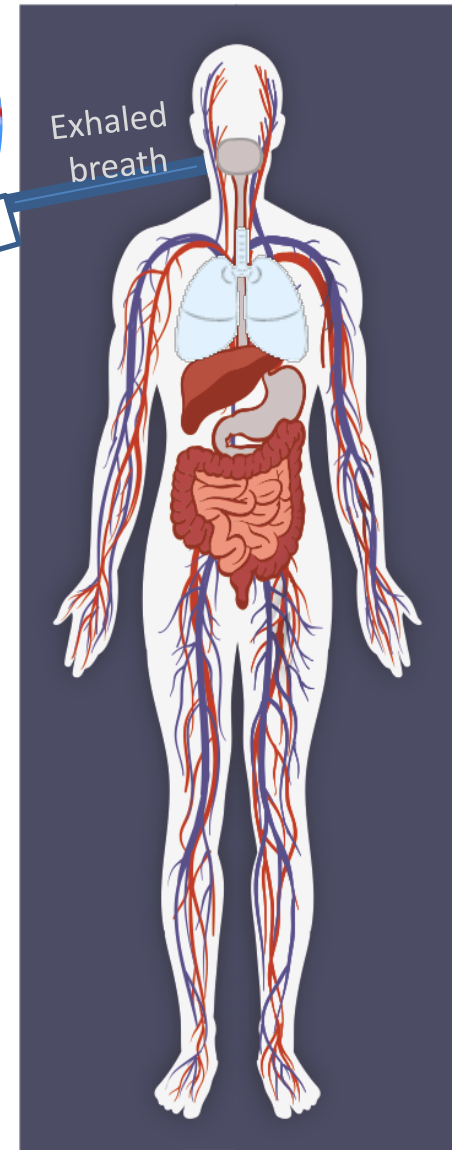
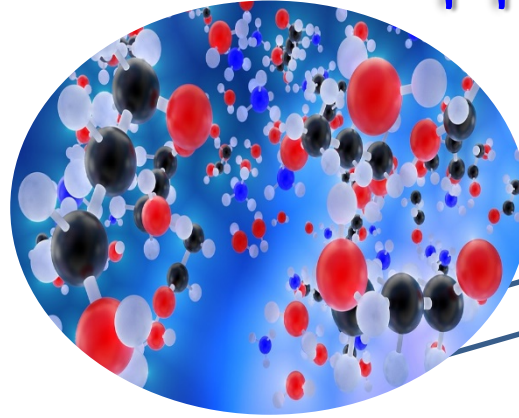
Ger Koek

Questions?

Thank you for your attention



Exhaled breath applications



Respiratory and airway diseases

Liver diseases

Kidney diseases

GI track diseases

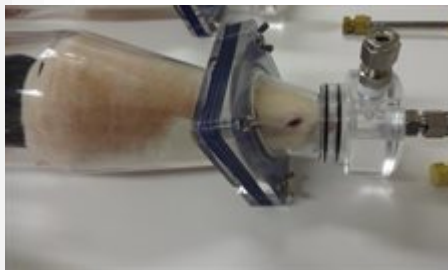
Exhaled breath applications



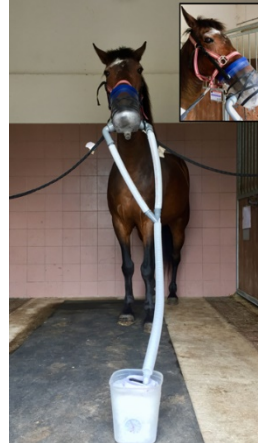
Dolphins



Goats



Mice and rats



Horses



Calves and bovine