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NAMS AOPS-COPD II: IN VITRO ASSESSMENT OF MUCUS HYPERSECRETION WITH QUANTITATIVE AOP MODELING (COMBINATION OF 3D IN VITRO MODEL AND MATHEMATICAL MODELING FOR RISK ESTIMATION)

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Outline

- NAMs for disease risk assessment
- AOP-based in vitro testing
- Quantitative AOP modeling
- In vitro dose vs in vivo dose?
- Gaps between NAMs and RW

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NAMs for disease assessment

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NAMs for disease risk assessment
For future replacement of animal testing

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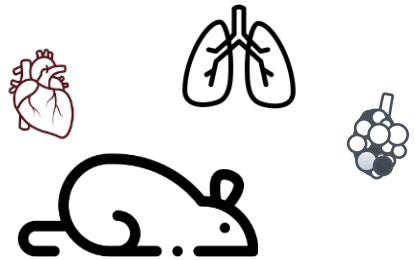
Disease risk assessment with animal testing



Animal disease models

- Species difference
  - Low predictivity of human outcomes
- Needs for animal welfare (3Rs)
  - **Replace**
  - Reduce
  - Refine

with NAMs (?)

in vitro human cell cultures
- High content screening
- 3D models
- Organ(s) on chip
- … etc.

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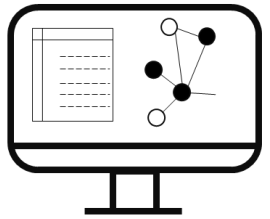

in silico (computational) tools
- (Q)SAR
- Read Across
- Mathematical models
- Machine learning
- …etc.

- Use of human cell cultures
- Combination with computational tools

- Still challenging due to
  - Complexity of living system
    → Human relevant in vitro system?
  - Complexity of disease onset process
    → Adverse outcome pathway (AOP)?

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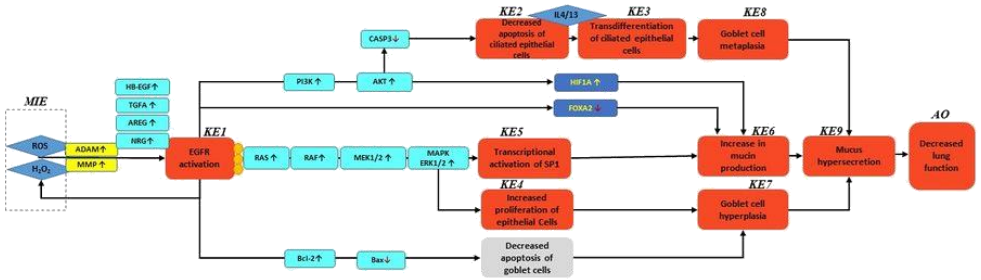
NAMs for disease risk assessment
AOP for lung function decrease (AOP148)

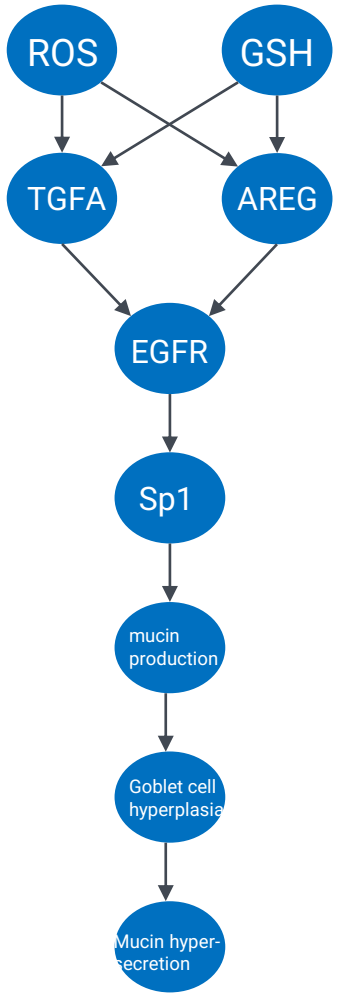
Original structure of AOP 148



- Originally submitted to AOP-wiki by PMI and BAT.
- ROS (MIE) eventually leads to “Decreased lung function” (AO).

- We modified the AOP structure, as some of the KEs were difficult to build as in vitro assay.

AOP 148 for this study



Oxidative stress (MIEs)

Signal transduction
(KEs observable from **acute phase**)

Changes in phenotype
(KEs observable in **chronic phase**,
not inducible by single exposure)

Adverse Outcome
(tentative setting)

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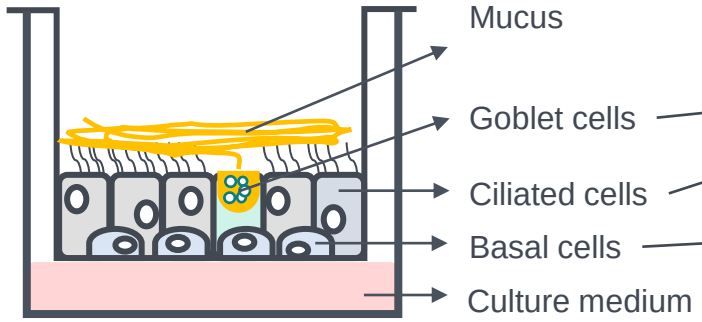
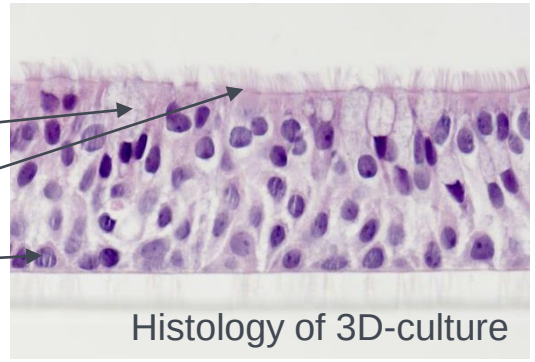
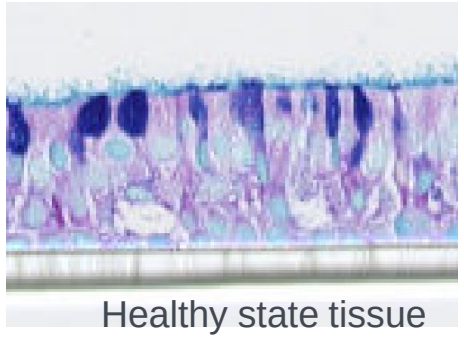
NAMs for disease risk assessment
- in vitro 3D-cultured bronchial epithelial cells



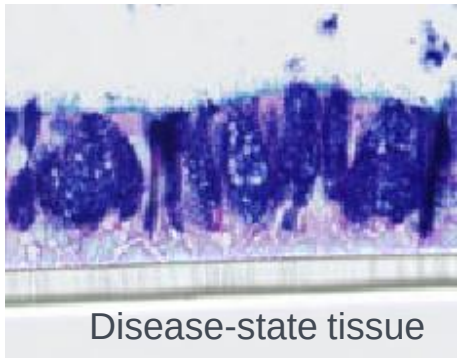


Histology of 3D-culture

Mucin staining



Healthy state tissue



Disease-state tissue

After 2-week IL-13 treatment

3D-cultured human bronchial epithelial cells (HBEC);

- ✓ are relevant with actual human bronchial tissue structure and cell types.
- ✓ recapitulate muco-ciliary clearance.
- ✓ are inducible of disease-state by interleukin- 4 /13 treatment.
- ✓ are capable of repeated exposure study.

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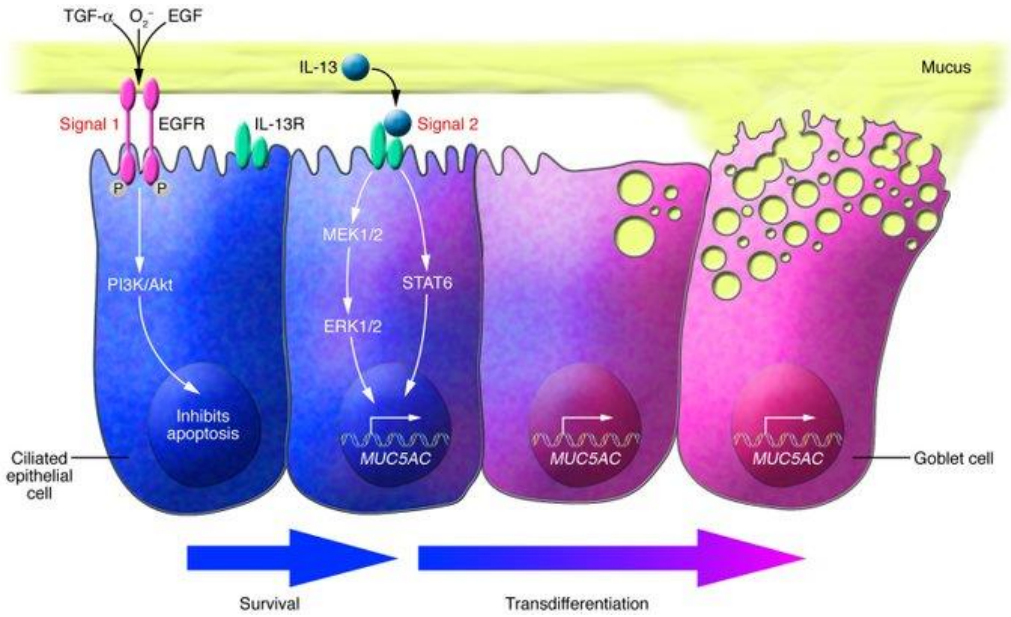
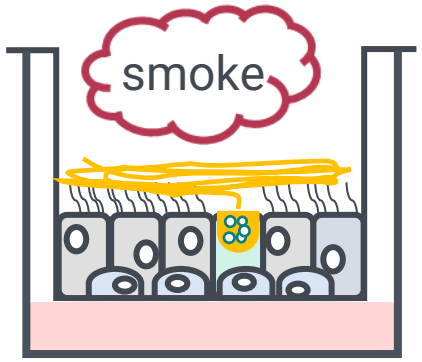
NAMs for disease risk assessment
Immune cell co-culture system to induce disease-state

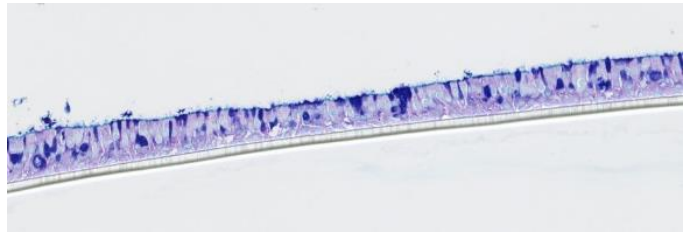


https://doi.org/10.1172/JCI27690.

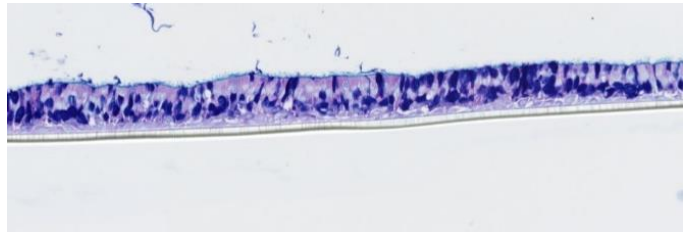
IL-4 and IL-13 are responsible for the induction of goblet cells.
Epithelial cells are not the source of such cytokines.

Immune cells, as a source of the cytokines, are introduced to the culture system.





Mono-culture failed to induce disease-state



Co-culture system was able to induce disease-state

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In Vitro Testing

Study design and results

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Experimental design

Cells: 3D-bronchial epithelial cells with immune cells

Donors: 6 donors

Replicate: 3 insert/dose/donor

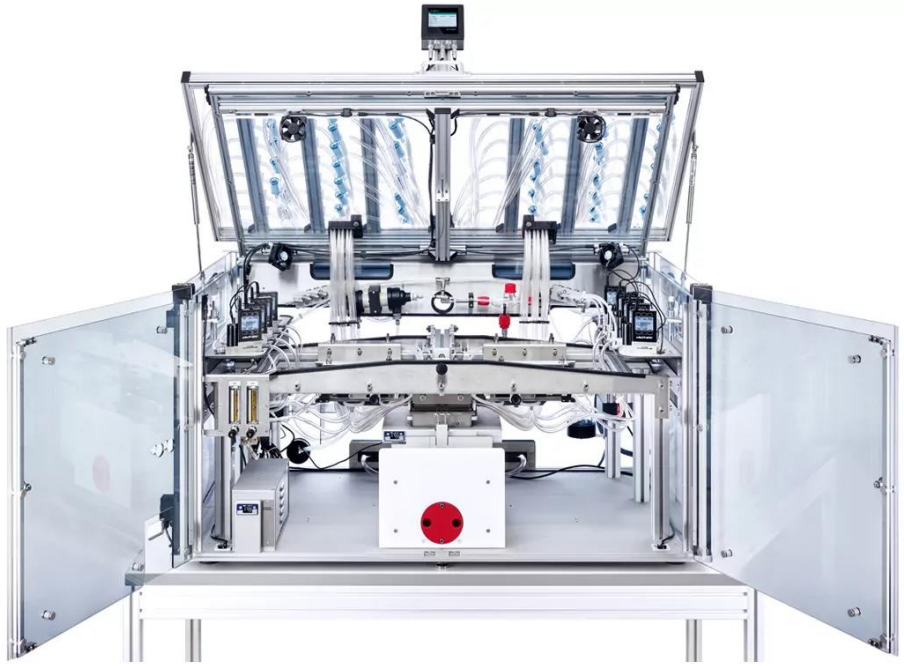
Test product: 1R6F reference cigarette

Exposure apparatus: Vitrocell® 24/48

Dose: 0, 1.25, 1.8, 3.3 μg/mL Nic.equivalent conc.

Exposure repetition: 6 (Mon, Wed, Fri, 2 wks)

Sampling and harvest: 1 hour post exposure



In vitro inhalation exposure module for 24 well inserts (vitrocell.com)

Endpoints: ROS, GSH(MIEs), AREG and TGFa (EGFR ligands), EGFR activation (KE1), SP1 activation(KE2), Mucus production (KE3), Goblet cell meta/hyperplasia (KE4), Mucus hypersecretion (AO) __ total 9 endpoints

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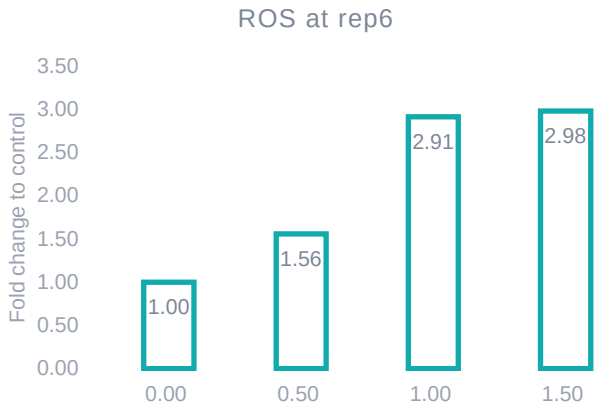
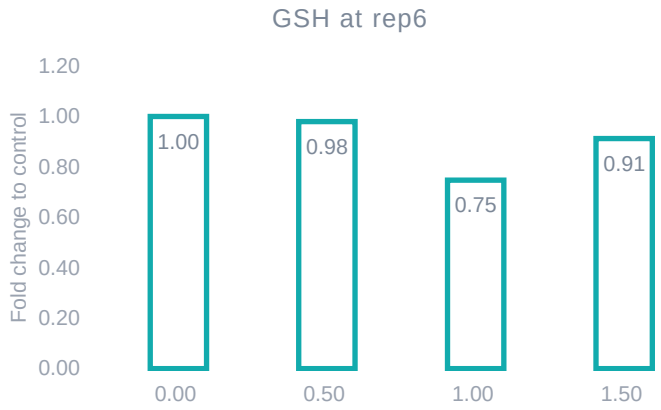
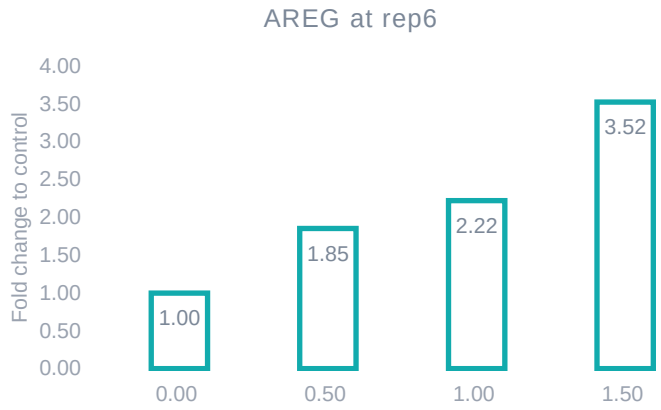
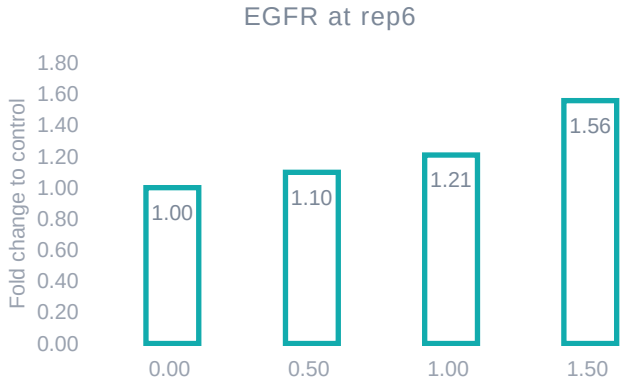
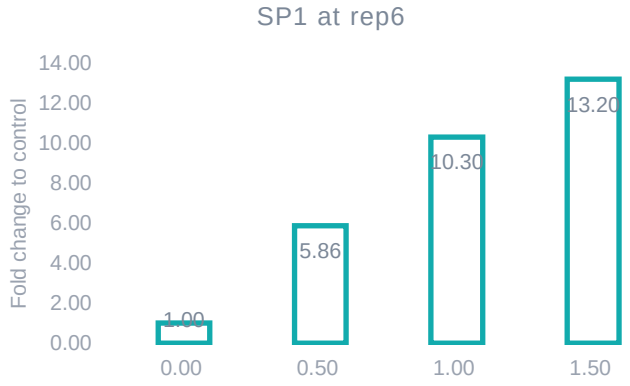
In vitro test result – acute endpoints all donor mean at exposure repetition 6











Acute-phase KEs are activated by cigarette smoke exposure

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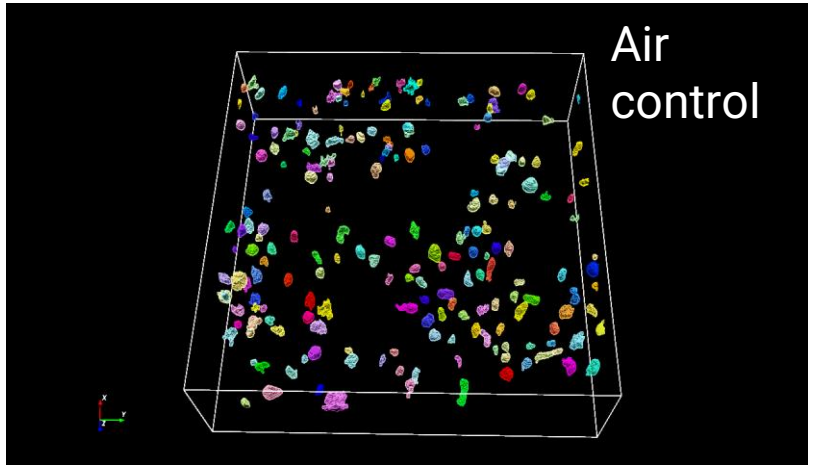
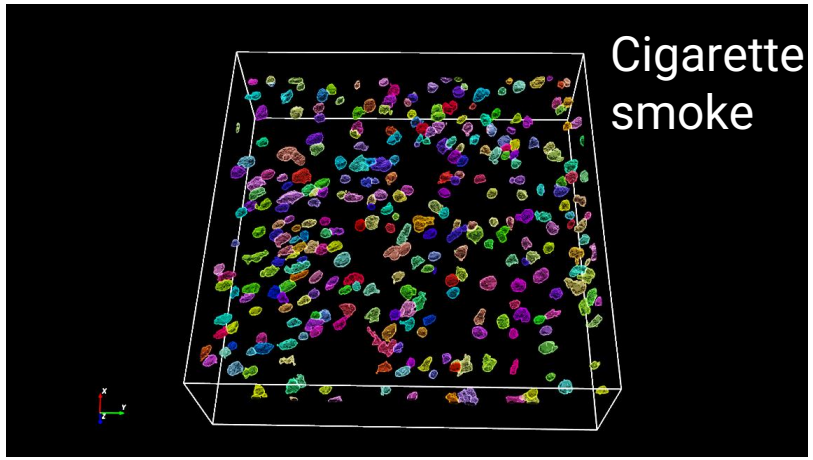
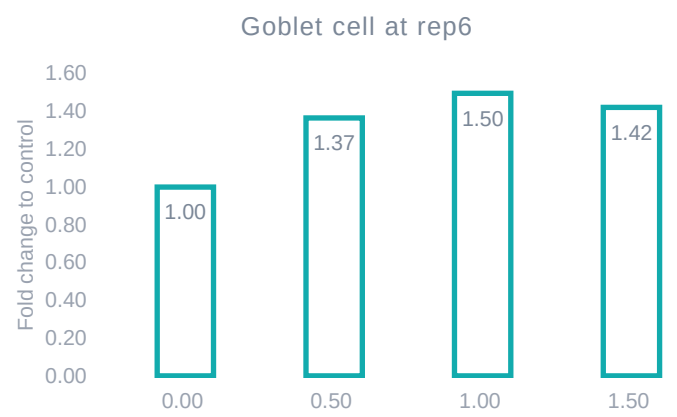
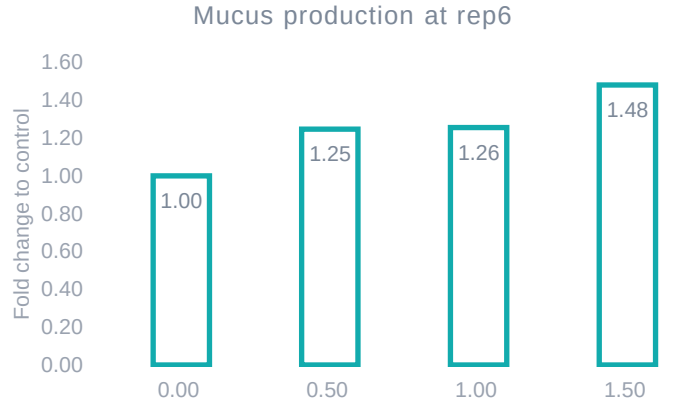
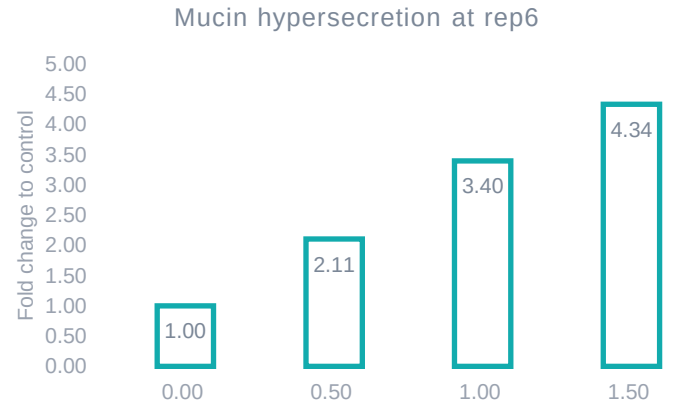
In vitro test –chronic endpoints
all donor mean at exposure repetition 6











Mucin hypersecretion was significantly induced by repeated exposure of cigarette smoke.

Increase of Goblet cells
(confocal IHC, 300 μm x 300 μm, example image)

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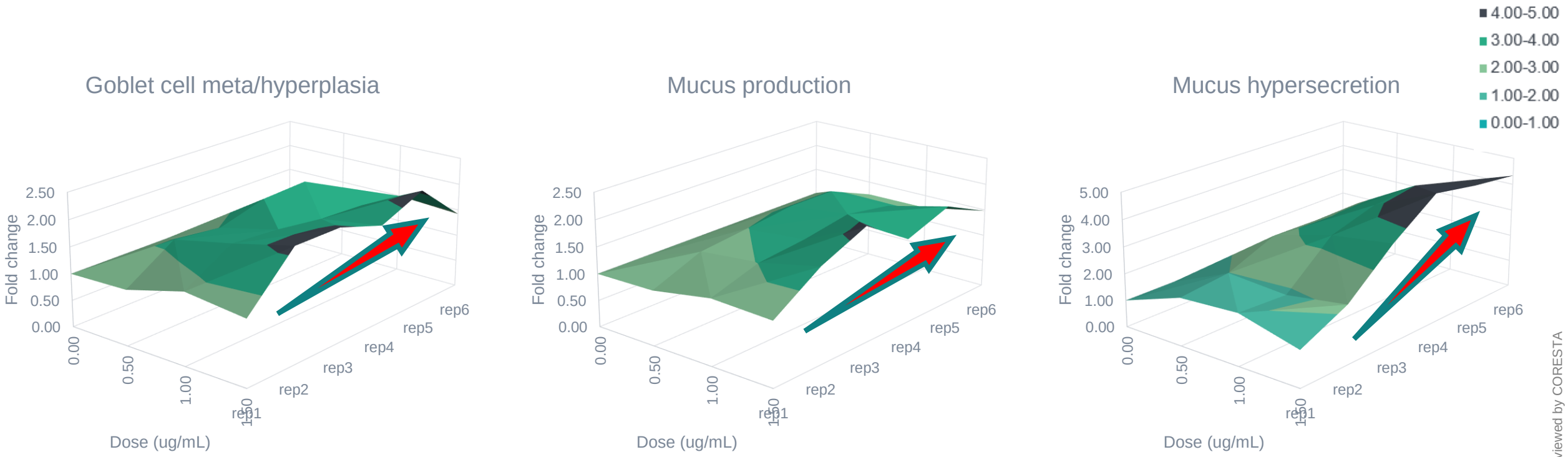
Mucus hypersecretion was accelerated by exposure repetition



Effects of chronic exposure to cigarette smoke could be well reproduced.

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Testing with multiple donors could reflect individual variability?

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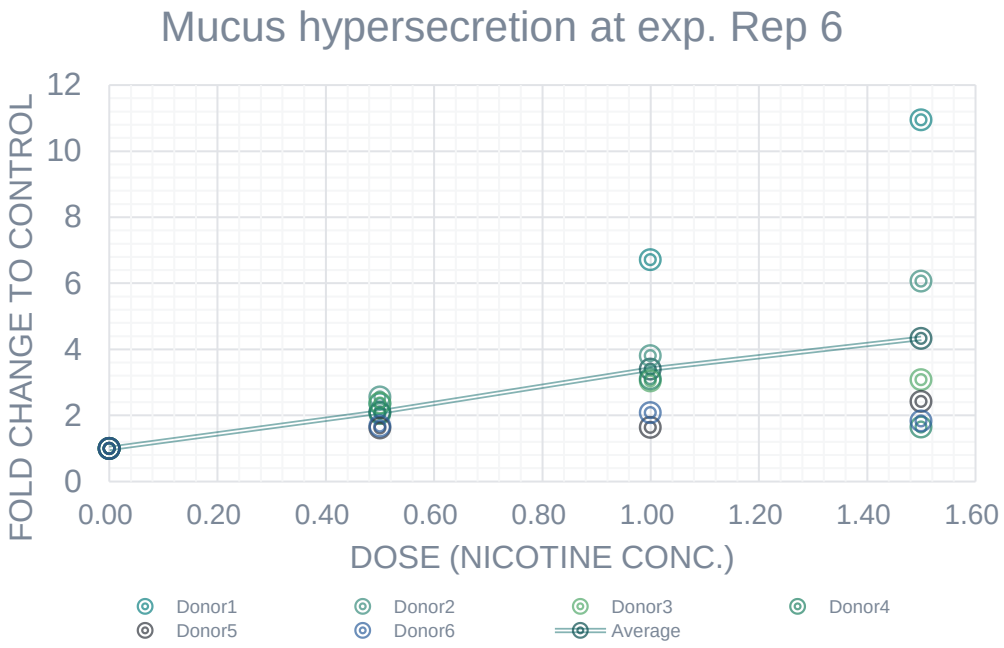

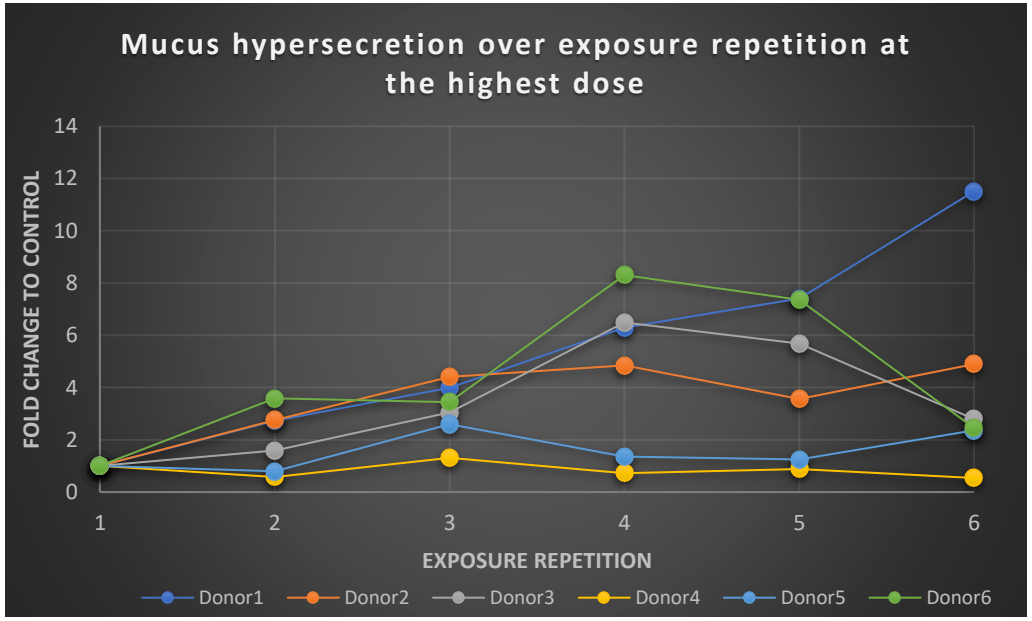

Induction of mucin hypersecretion highly varied across donors

may reflect real world situation? (e.g., not all smokers contract smoking related diseases)

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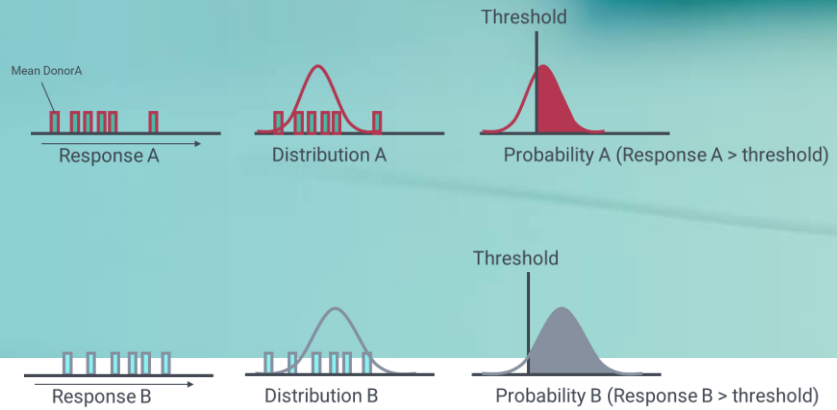
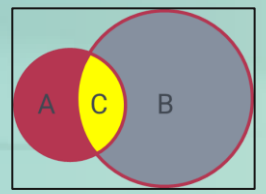
QUANTITATIVE AOP

Probabilistic modeling for disease risk assessment





$$P(B|A) = \frac{\text{C}}{\text{A}}$$

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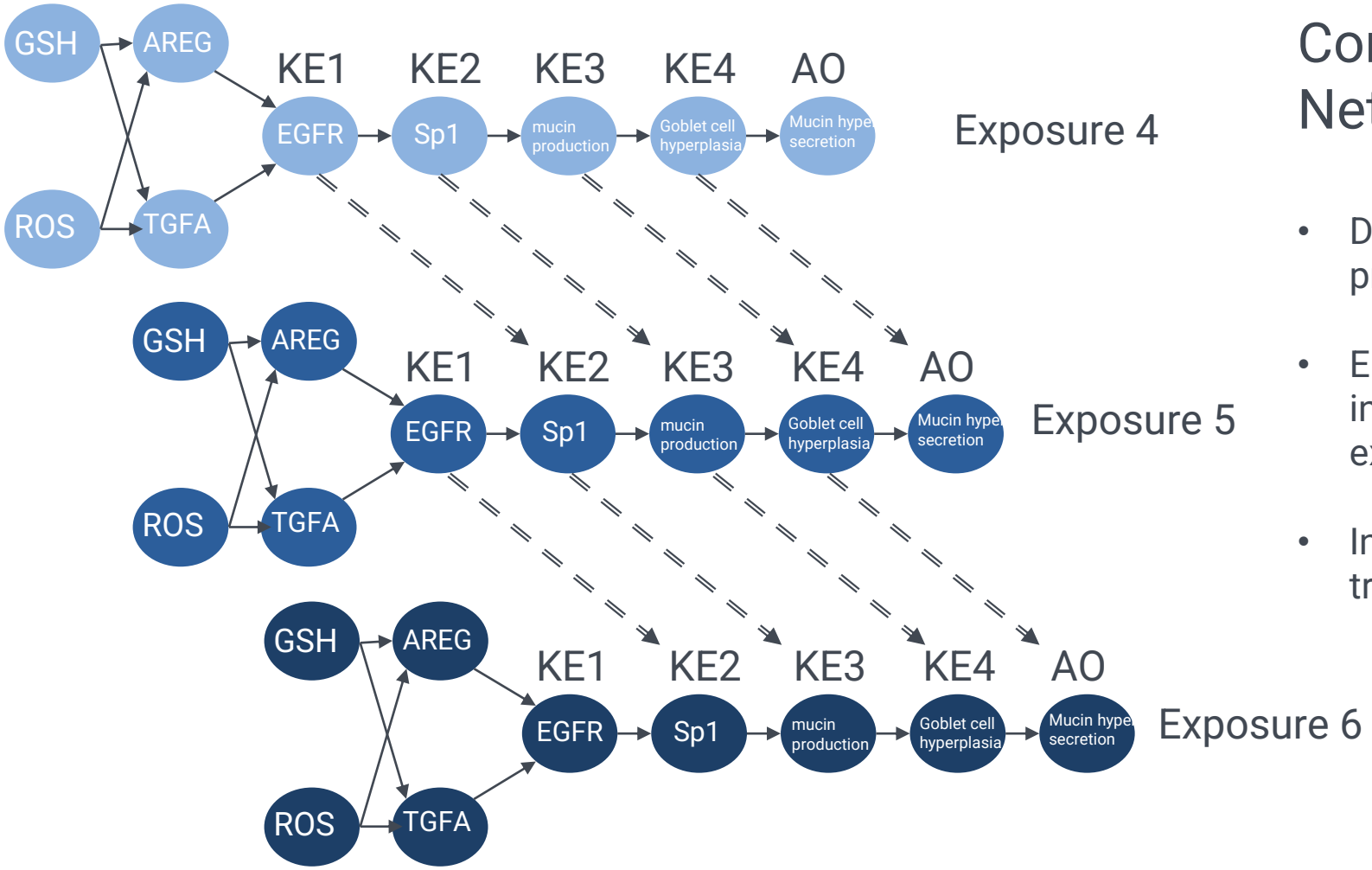
Transform Mean and SD of in vitro test results into Conditional transition probability



Concept of Dynamic Bayesian Network (DBN) model

- DBN model is able to capture the influence of previous exposure on the next.
- E.g. the result at exposure repetition 1 influences the probability calculation at exposure repetition 2.
- Incidence of AO is therefore calculated as transition (and conditional) probability.

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AO probability is likely to be higher in the later stage exposure repetition

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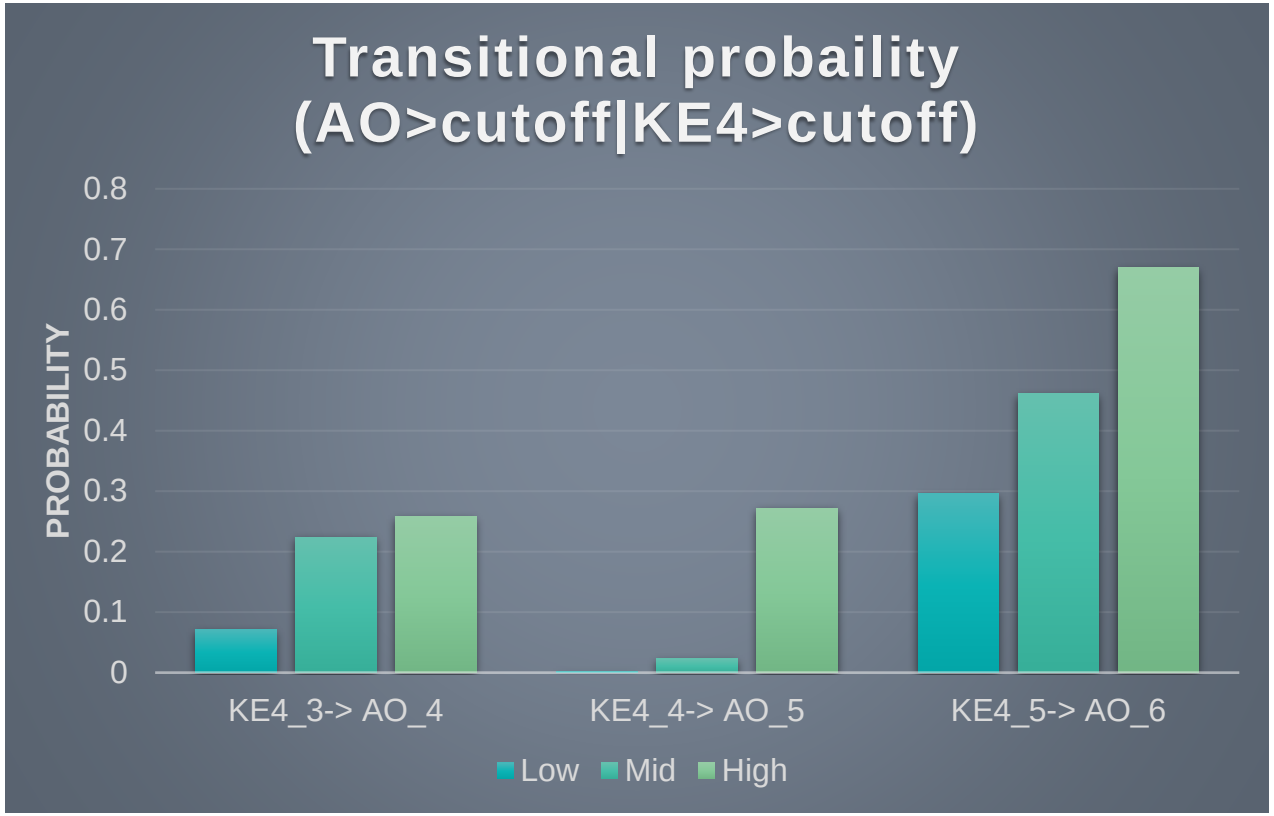

Dose dependent and mostly time-dependent increase in the probability of AO occurrence.

Relative risk assessment can be performed if RRP data is available with the same experimental setup.

Probability depends on:
AOP structure / In vitro data / cutoff setting/ Selected donors*
(probability in the left graph was calculated by average of random pickup of 4 out of 6 donors)

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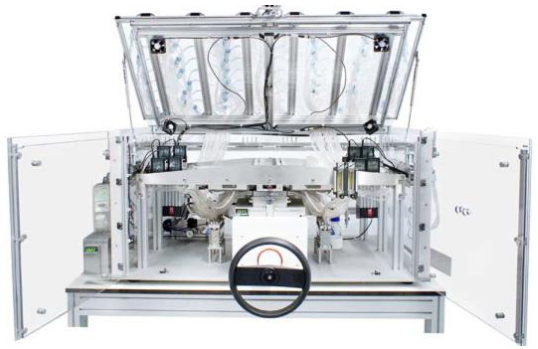
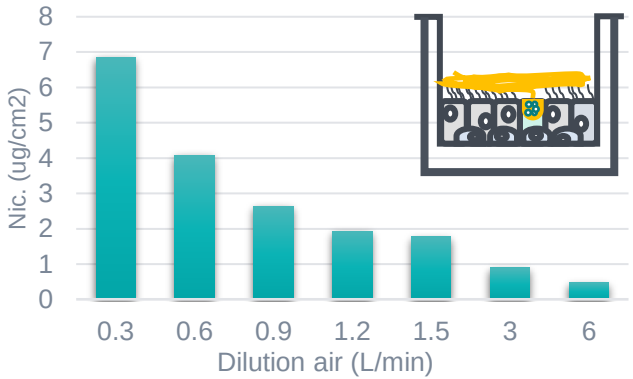
In Vitro Dose vs In Vivo Dose ?





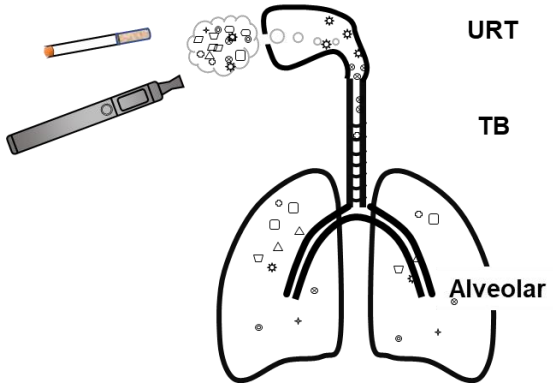
Dosimetry in in vitro test conditions

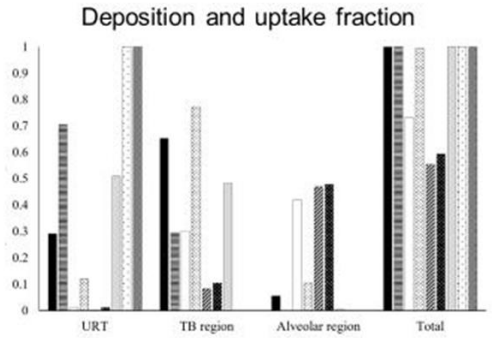
approx.

0.5~1.5 µg Nic. / cm²

per day

Deposition model





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Predicted deposition in TB region

1.5 µg Nic. / cm² / day

in case of 1 pk (20 cigs) /day of 10 mg Tar cigarette

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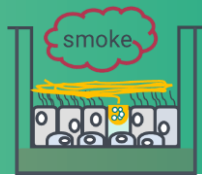
Gaps between Real world and NAMs.



	Real world	NAMs (current study)
Site of disease onset	Trachea, Bronchi, Alveolar	Bronchi
Number of Cell type	> 5	2
Duration of disease onset	> Decades	> 2 weeks
Subjects	Population-wide	Limited donors (6 in this study)

Should we completely fill the gaps in NAMs? OR
Gain the predictive accuracy with limited dataset?

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Summary

- AOP-based *in vitro* assay system for disease risk assessment was developed.
  - Repeated exposure of whole cigarette smoke induced mucus hypersecretion.

- Bayesian network modeling of in vitro test results were performed.
  - Causal chain of AOP and time series of repeated exposure were integrated.
  - in vitro test results (mean and SD) were converted into probability of AO occurrence.

<u>Link to real world situation should be investigated further.</u>

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Appendix. Donor information

57Y Male Caucasian	→ low response
73Y Female Black	→ early onset
65Y Female Caucasian	→ low response
62Y Female Black	→ responsive donor
50Y Male Hispanic	→ responsive donor
56Y Male Caucasian	→ early onset

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