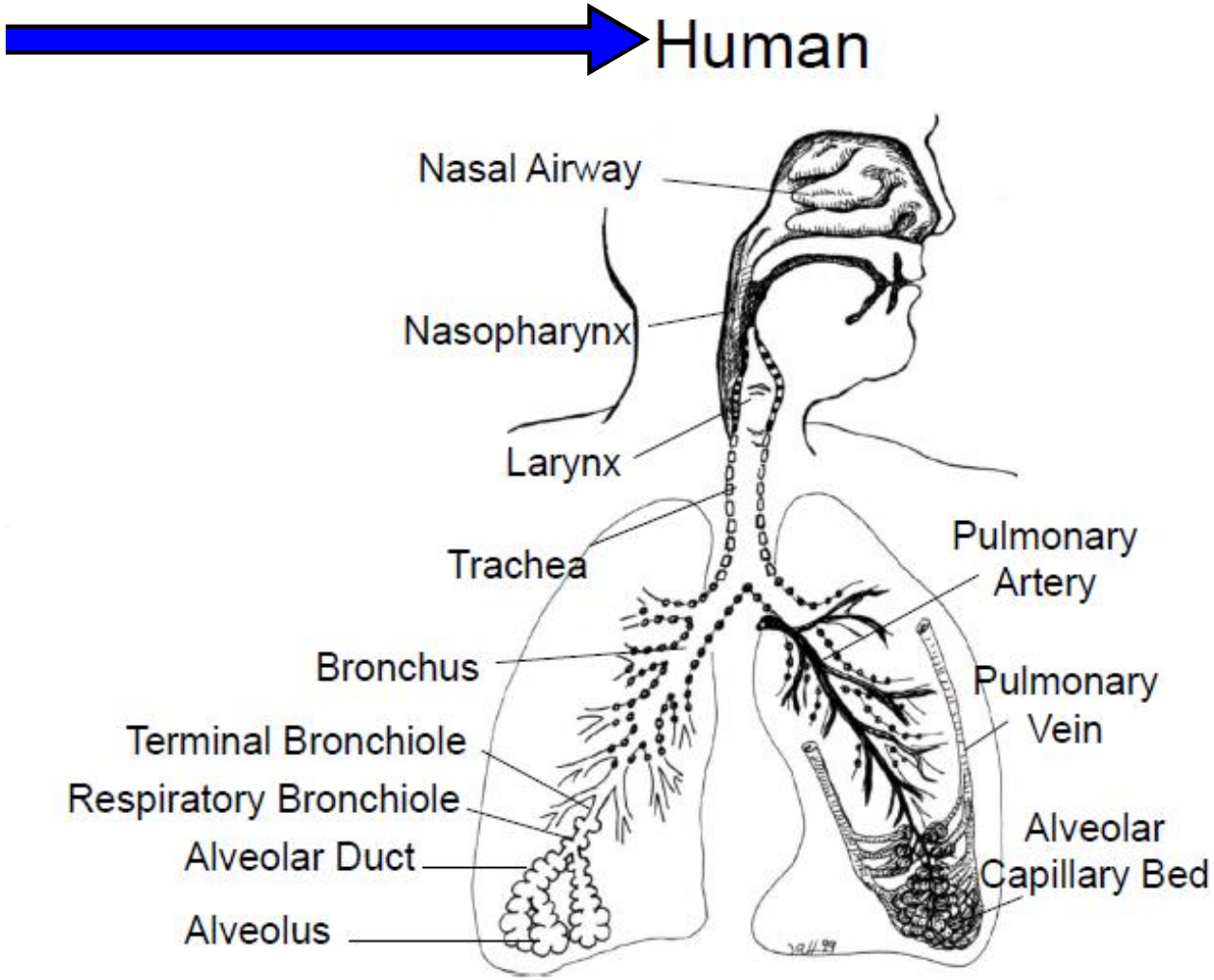
Integrating New Approach Methodologies (NAMs) to Assess the Risk to Human Health from Inhaled Materials

Shannon Krieger
Jon Hotchkiss and Dan Wilson
Toxicology and Environmental Research and Consulting
The Dow Chemical Company

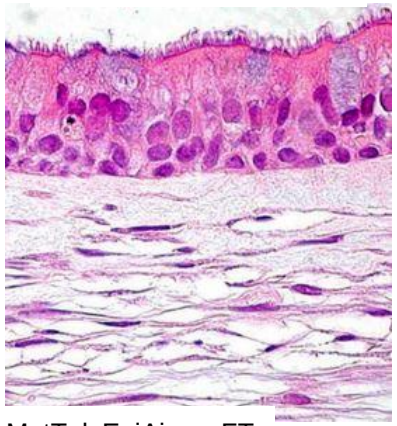
Seek Together™

What is the goal?

In Silico
In Vitro



Human Airway
3D ALI Culture



MatTek EpiAirwayFT

Inhalation Testing

- Inhalation is a major route of human exposure
- Unique interface between environment and systemic circulation
  - Upper (URT) and lower (LRT) respiratory tract important
- Exposure-response data for hazard identification
  - Integration of material properties, deposition, absorption, transport, metabolism and elimination
- Identify critical responses to inhaled materials
  - Portal of entry effects
    - Cells and tissues of URT and LRT
  - Systemic effects
    - Internal organs and tissues

Dose is the Key: What is the Dose?

Inhaled dose

Concentration x minute ventilation x duration

- Rat: (mg/L) x (*0.78 L/min ·kg*) x min = mg/kg
- Mouse: (mg/L) x (*1.533 L/min ·kg*) x min = mg/kg
- Human: (mg/L) x (*0.089 L/min ·kg*) x min = mg/kg

Assumes 100% deposition and absorption

Deposited dose

Fractional Deposition x Inhaled Dose

- Better – often quite good for particles (use MPPD model)

Absorbed dose

Mass transport (flux) x Deposited Dose

- Even better – requires knowledge of regional deposition, mass transport
  - Response modified by local metabolism and or sensitivity of cell populations

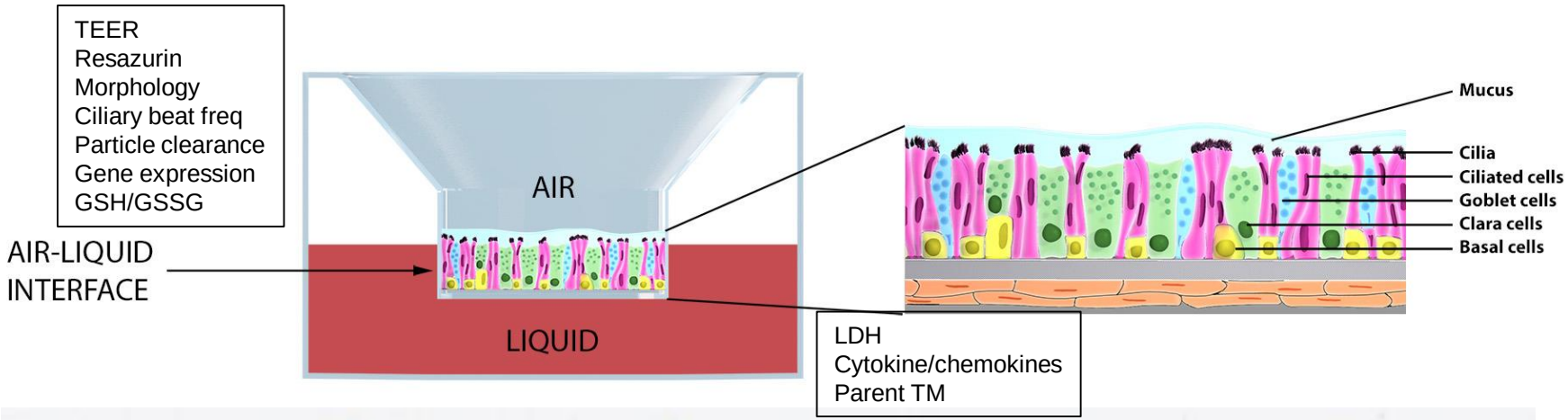
Cellular dose

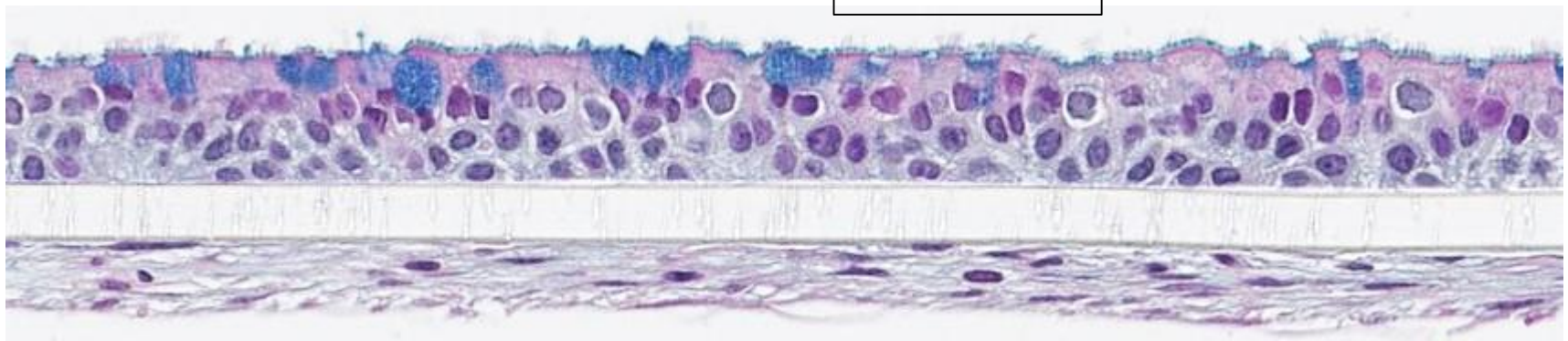
Dose/unit area or dose/cell

Human 3D Airway Model

Differentiated airway epithelium is fed from the basolateral surface while the apical epithelial surface possessing beating cilia and active mucous secretion is exposed to the test atmosphere

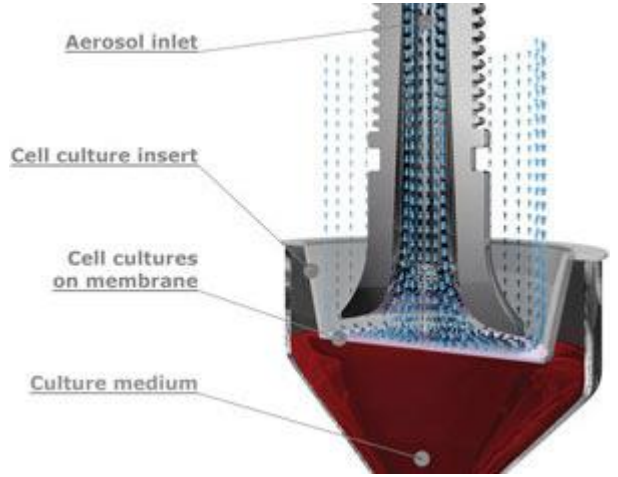
Schematic representation of SmallAir™-HF





www.epithelix.com

Exposure Methods



Cultures are exposed to test materials by:

- Direct application of test material to apical surface of the culture (50 µL)
- Cloud exposure – single or multiple episodic liquid aerosol exposures
- Continuous exposure to test atmosphere (gases, vapors or solid aerosols)

Experimental Design – Range-finding Study

DAY 0 - DOSE
Pipet or Cloud

→

DAY 1 - WASH
TEER, Cytotoxicity, Viability, IL-6, IL-8

→

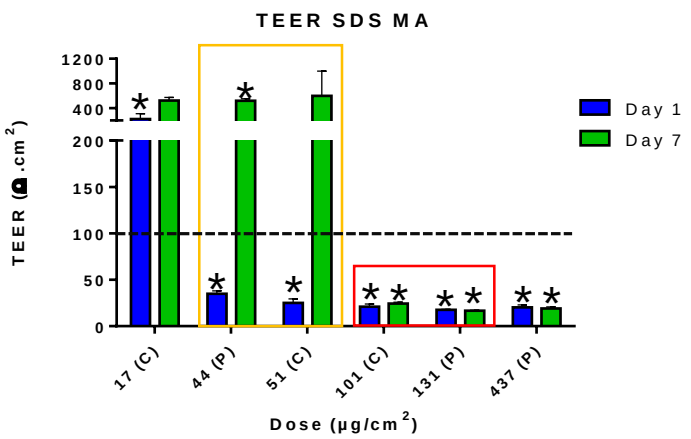
DAY 7 – STUDY TERMINATION
TEER, Cytotoxicity, Viability, IL-6, IL-8 Histopathology & IHC

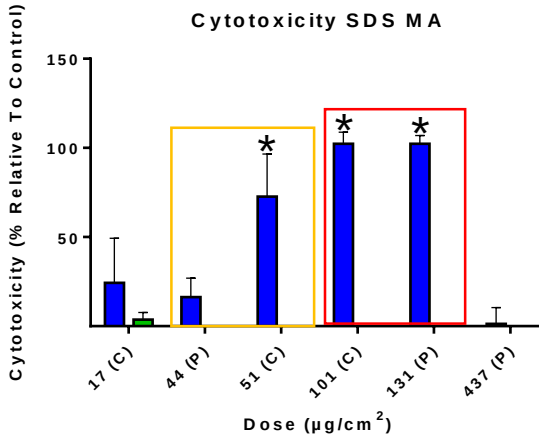
24h Exposure

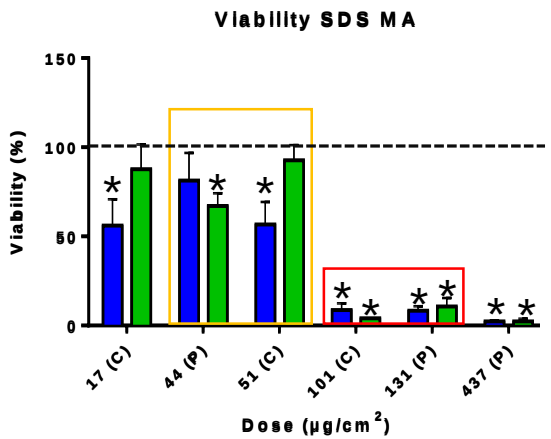
1wk Recovery

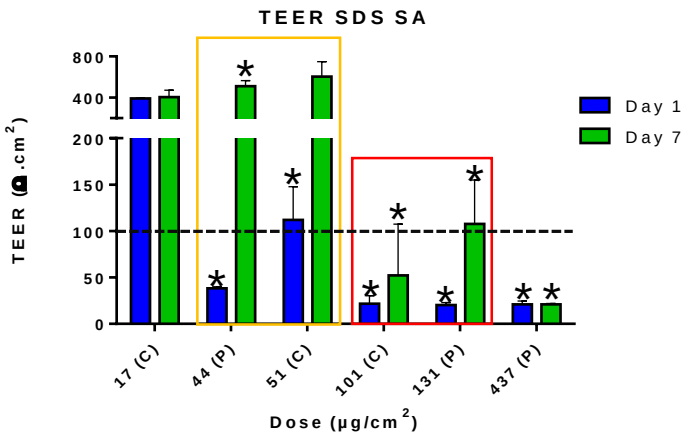
Exposure Route	Concentration (mM)			Dose (ug/cm²)		
	Low	Mid	High	Low	Mid	High
Cloud	50	150	300	17	51	101
Pipet	1	3	10	44	131	437

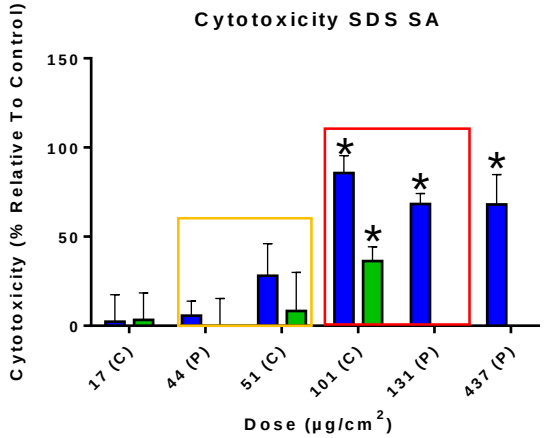
SDS Range-finding

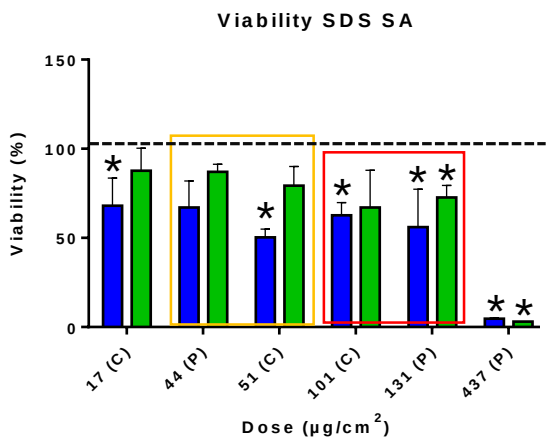




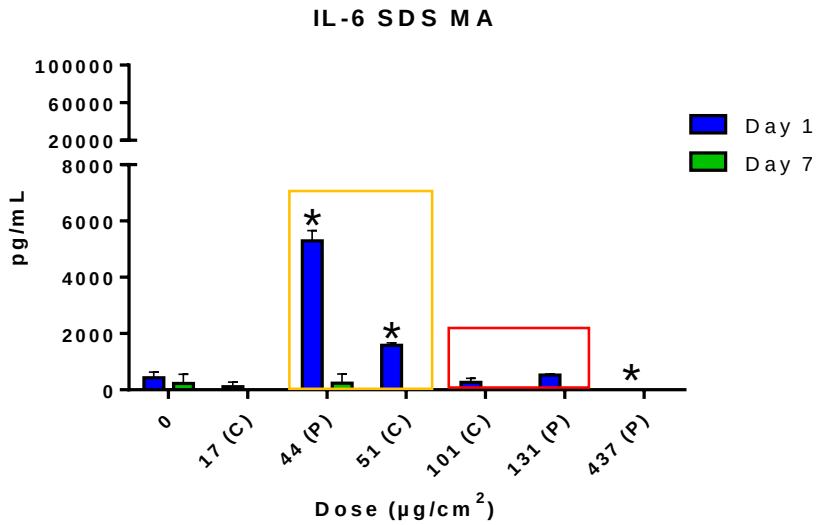


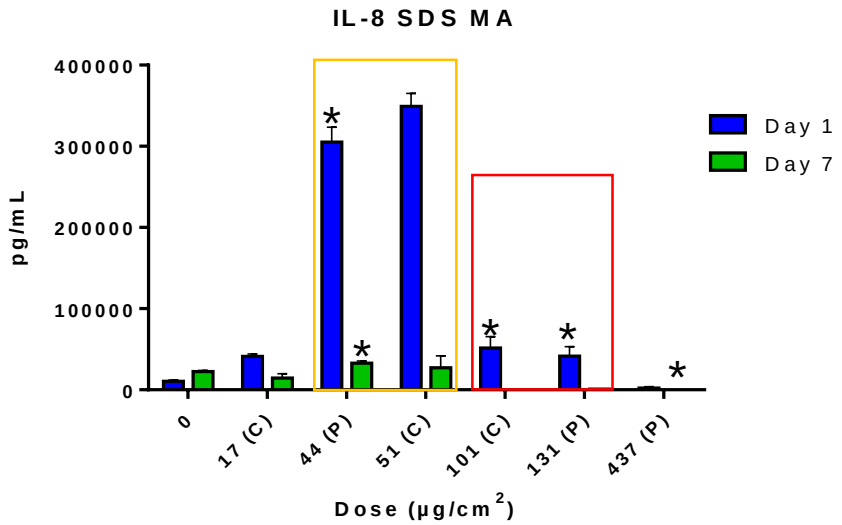


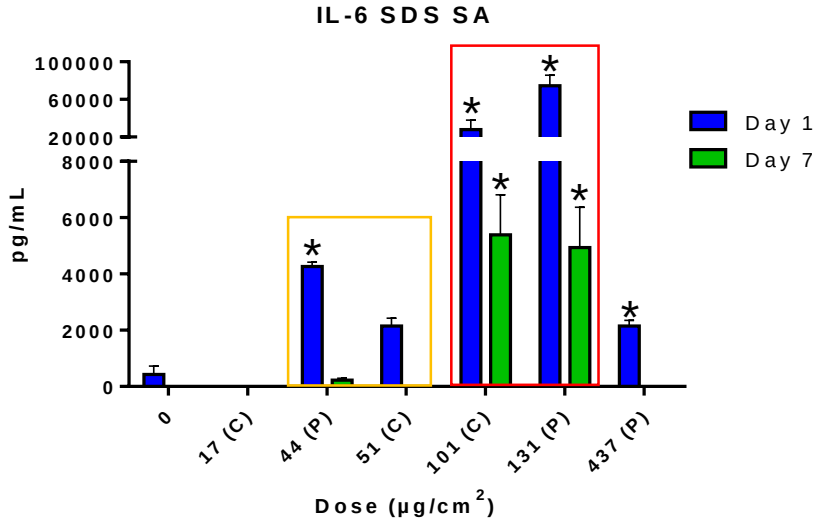


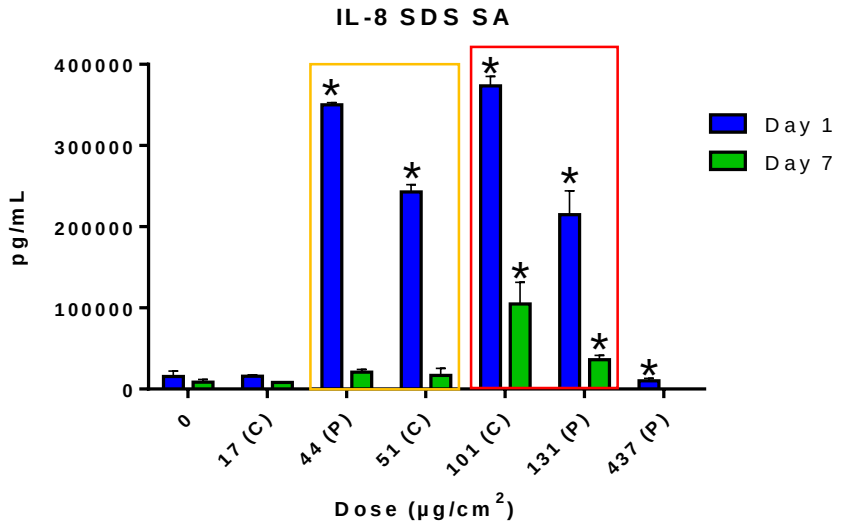


SDS Range-finding

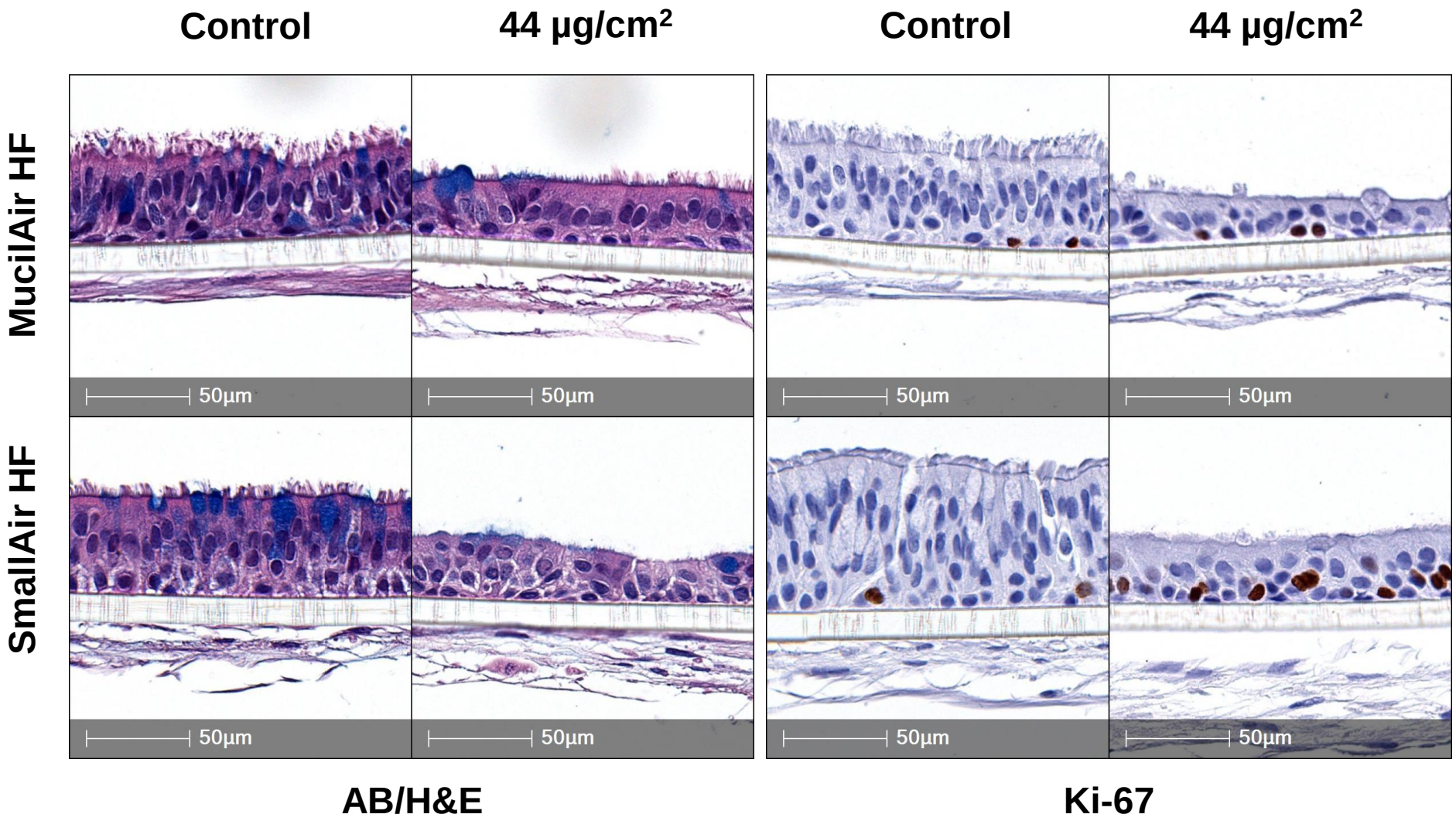








SDS Range-finding



Experimental Design – Definitive Study

DAY 0 - DOSE
Pipet or Cloud

→

DAY 1 - WASH
TEER, Cytotoxicity, Viability, IL-6, IL-8

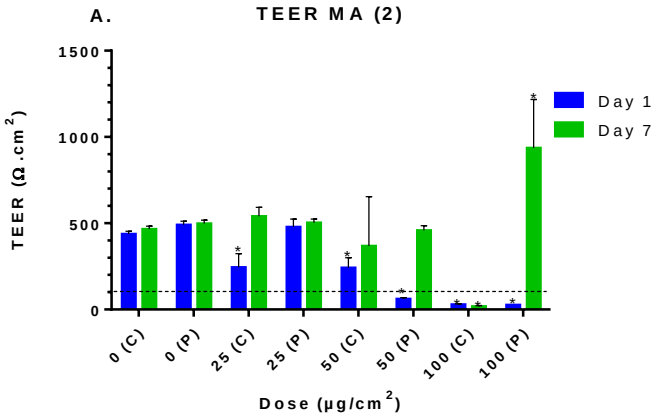
→

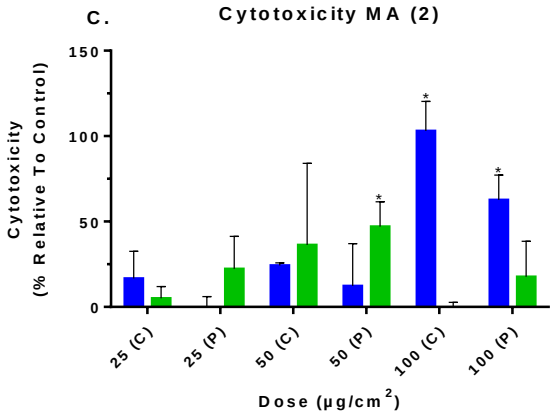
DAY 7 – STUDY TERMINATION
TEER, Cytotoxicity, Viability, IL-6, IL-8 Histopathology & IHC

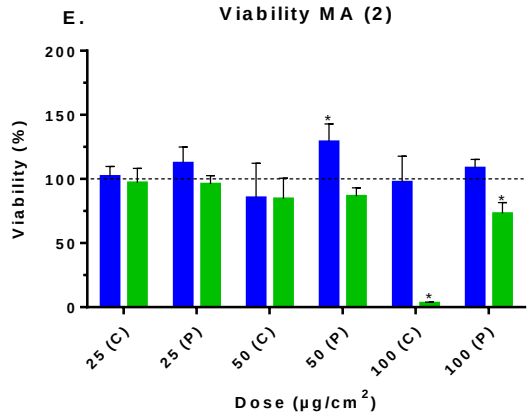
24h Exposure | **1wk Recovery**

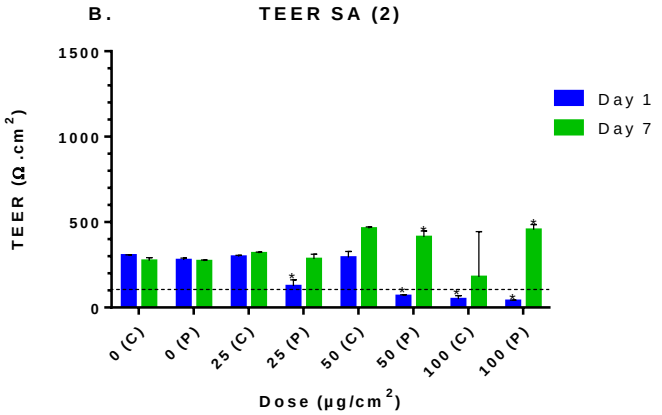
Exposure Route	Concentration (mM)			Dose (ug/cm²)		
	Range-Finding Study					
Cloud	50	150	300	17	51	101
Pipet	1	3	10	44	131	437
	Definitive Study					
Cloud	74	148	296	25	50	100
Pipet	0.6	1.1	2.3	25	50	100

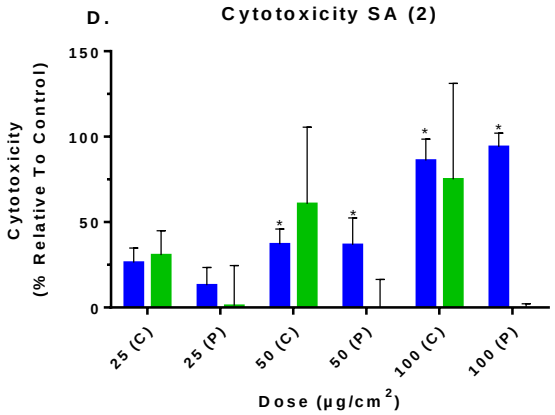
SDS Definitive

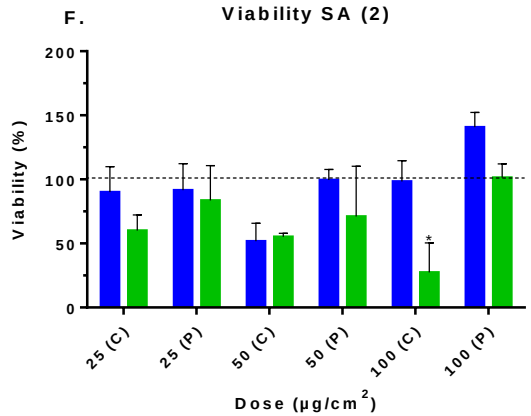




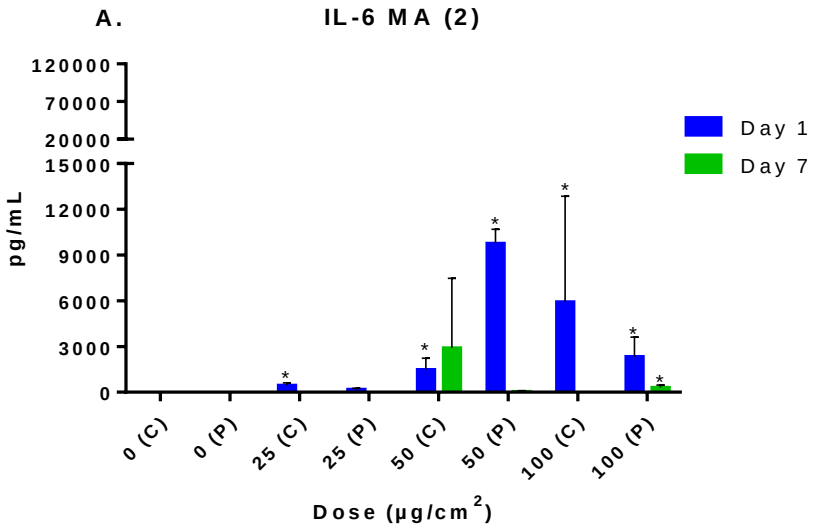


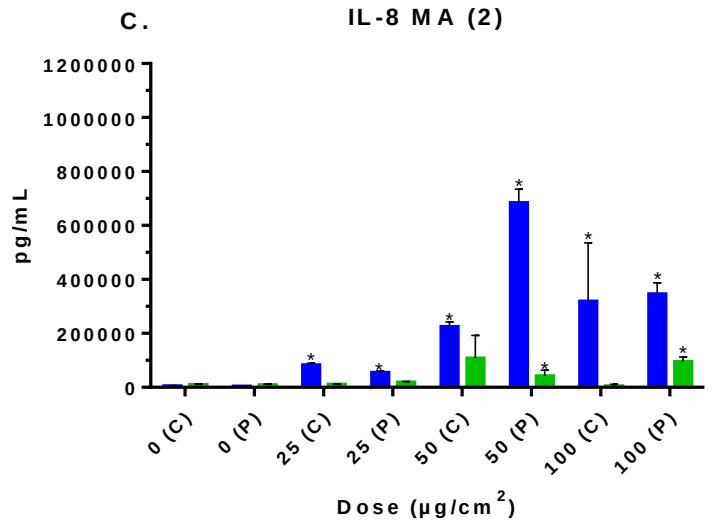


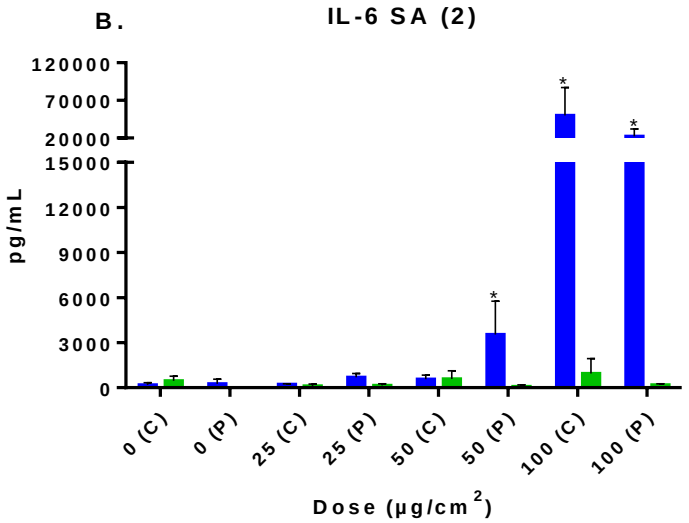


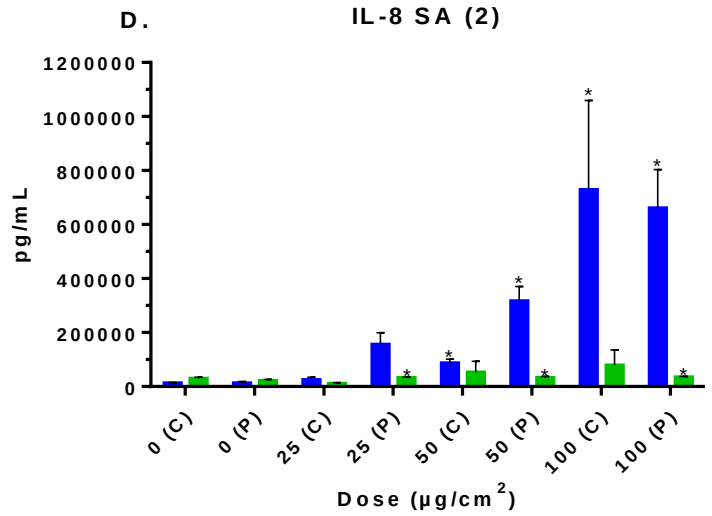


SDS Definitive

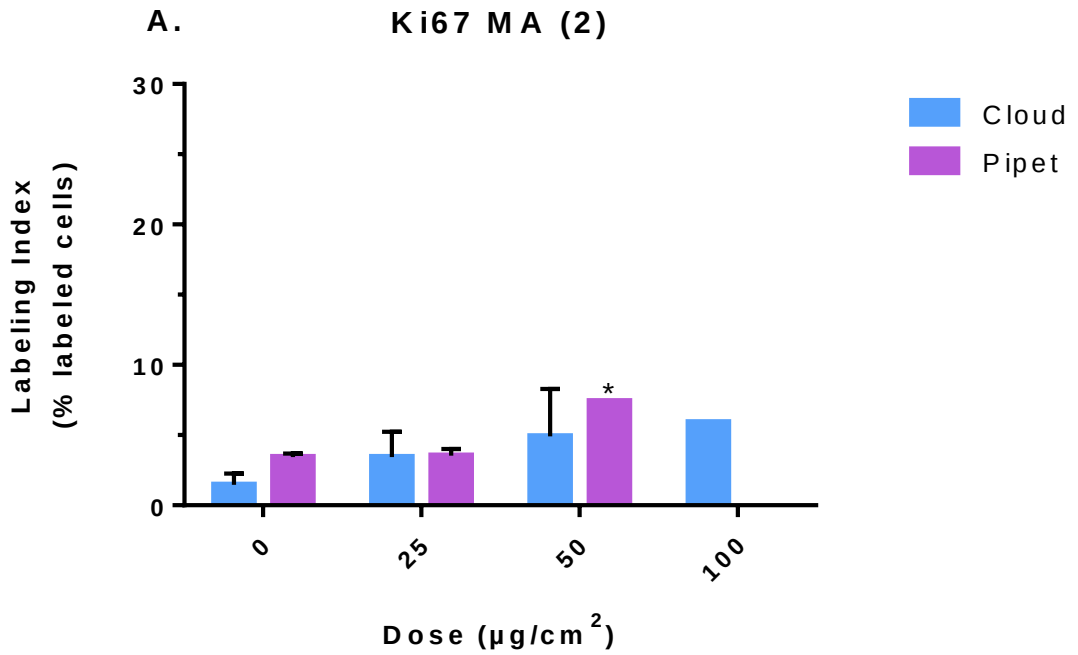


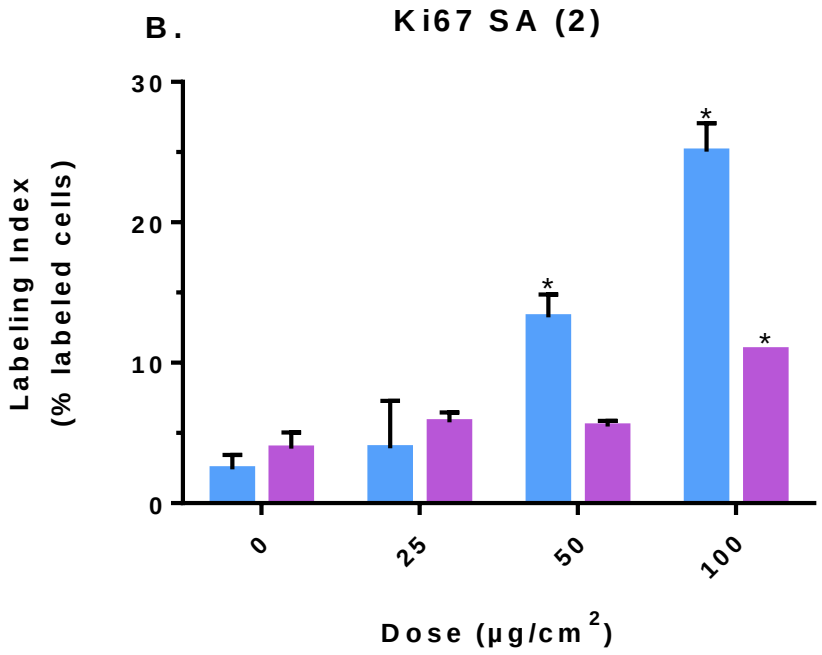






SDS Definitive





Conclusions

- Similar exposure-response profiles observed in MA and SA
  - when dose/unit cell surface area are equivalent between direct application and aerosol exposure
- SA were more resistant to direct acting toxicants than MA
- These results underscore the need for multiple endpoints to characterize the acute exposure-response profiles of airway epithelial cultures
- These data also confirm the need to use deposited/absorbed dose and not exposure concentration when assessing the acute toxicity of inhalable materials using in vitro systems

DOW®

Cheminformatics

In silico identification of molecular initiating events

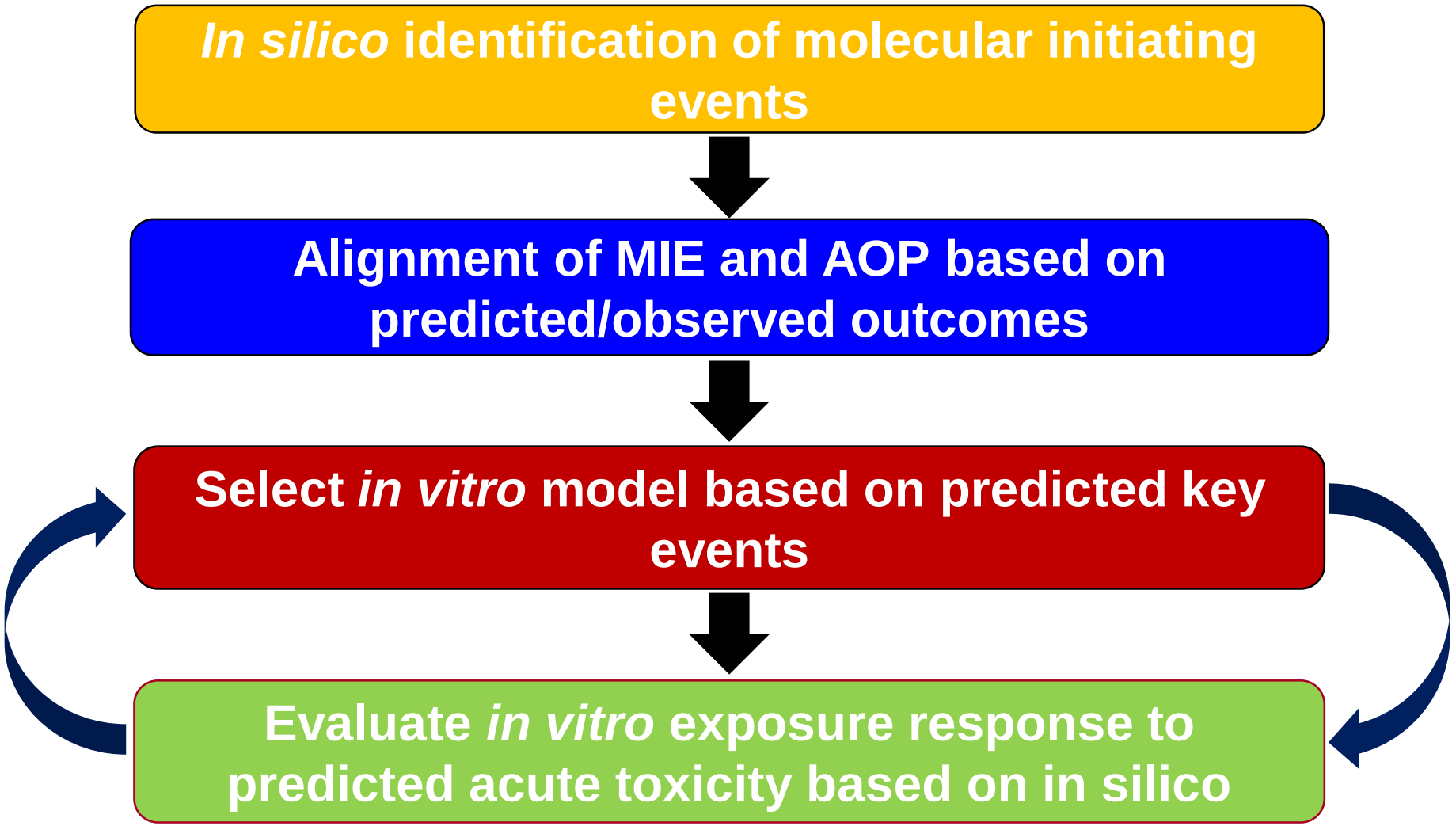
In-house Profilers

- Reactives (facile)
- Surfactants
- Chelants
- Hydrophobics
- Anticoagulants
- Denaturants
- Receptor-mediated MOAs:
  - Cholinergics
  - Serotonergics
  - GABAminergics
  - Glycinergics
  - Mitochondria inhibitors

Small molecule docking-based models

- Docking
  - Aromatase
  - GABA
  - Aromatic hydrocarbon receptor (AhR)
  - Nicotinic AChR
  - Muscarinic AChR
  - Acetylcholinesterase
  - TSPO
  - μ-Opioid receptor
  - Uricase
  - Xanthine Oxidase

Proposed Inhalation IATA



Opportunities and Challenges.....

- Computational mechanistic profiling to determine MIE
- Ascertain whether MIE is preferentially specific to inhalation *vs* oral routes of exposure
- If it is, use specialized 3D respiratory *in vitro* models
- If not, use route-agnostic *in vitro* models appropriate to MIE
  - or use read-across to oral data with assessment of bioavailability
- Simplification/standardization of alternative model systems is key to regulatory acceptance
- **Knowing tissue dose – *in vitro* and *in vivo* will ultimately determine if exposure-response is equivalent across platforms**

DOW®

Thank you

Seek

Together™