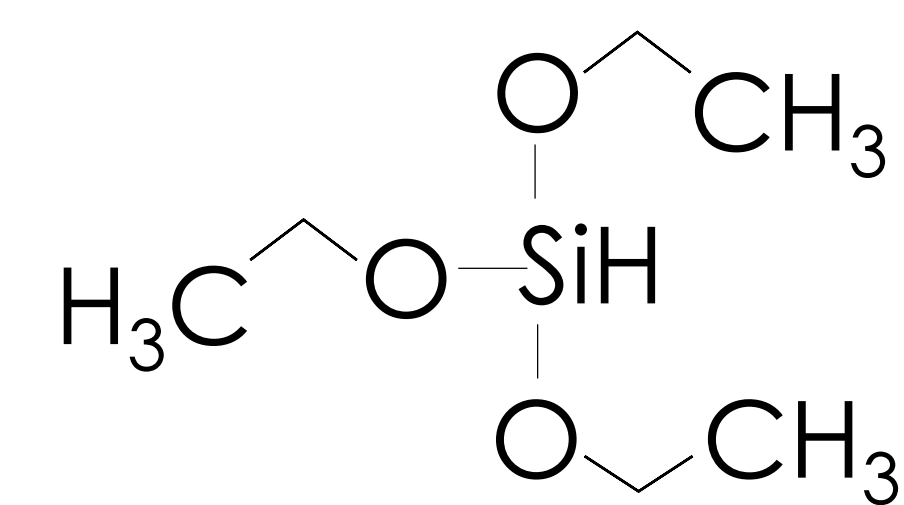


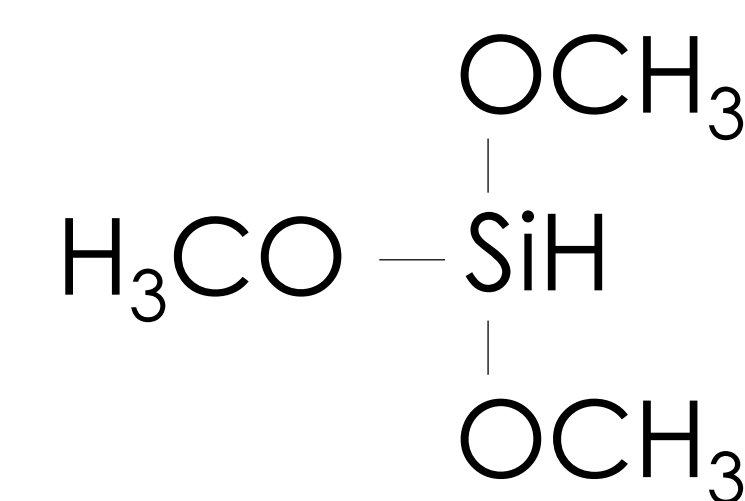
Abstract: Approaches to efficiently and effectively assess the toxicity of chemicals on the human respiratory tract using *in vitro* systems would provide useful information to inform product development and risk management decisions. Presented here is an approach to help better understand the appropriate *in vitro* system to use and the biological markers to monitor based on the test chemical under evaluation. In this study, BEAS-2B cells (a human bronchial epithelial cell line) were exposed to various concentrations (0.72ppm, 25ppm, and 85ppm) of triethoxysilane vapor at the air-liquid interface using a capillary dosage unit coupled to a VITROCELL 6/4 exposure module. Triethoxysilane is an industrial chemical classified as a GHS category 2 inhalation toxicant based on rat acute inhalation toxicity testing. A significant concentration-dependent decrease in cell viability (resazurin-based assay) and increase in cytotoxicity (lactate dehydrogenase assay) was observed after exposure to triethoxysilane (test chemical) and nitrogen dioxide (positive control) as compared to clean air (negative control). A significant increase in expression of inflammatory markers, determined by Meso Scale Discovery technology, was observed at 25ppm. Additional work is underway to test other silanes that vary only in their carbon length to determine if this *in vitro* system can detect the decrease in toxicity that correlates with increasing carbon-chain length and to determine the advantages of using a 2D cell line (BEAS-2B cell) versus a 3D human reconstructed tissue model. Overall, these results will evaluate the utility of an *in vitro* system to predict the likelihood of a chemical to cause portal-of-entry effects on the human respiratory tract and could be a useful approach to rank chemical toxicity.

Experimental Design

Test chemicals



Triethoxysilane
Inhalation toxicity:
GHS 2



Trimethoxysilane
Inhalation toxicity:
GHS 1

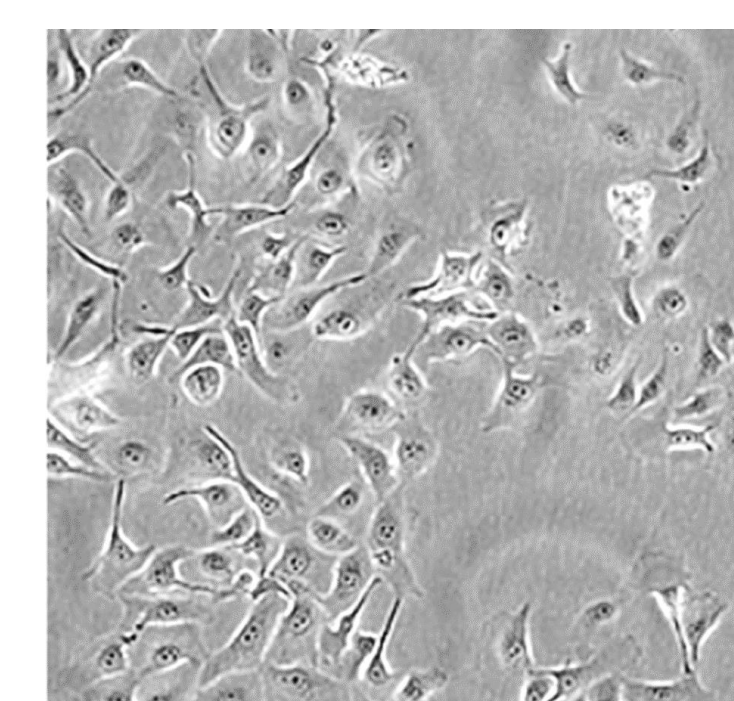


Vapor Generation

- Vapor generated using capillary dosage system where the fluid is thermally heated, evaporated, and injected dynamically into a gas stream
- Stability monitored by gas chromatography-flame ionization detector (GC-FID) and total hydrocarbon (THC) analyzer
- Different concentrations obtained by using values measured by THC analyzer

Exposure

- 1 hour air-liquid interface exposure of human bronchial epithelial cell line
- Test concentrations: 0.72, 25, and 85 ppm based on LC50 data
- 3 biologically independent runs with 4 replicates each
- Positive control: Nitrogen dioxide
- Negative controls: Clean air (CA) and incubator control (IC)



BEAS-2B
Human bronchial epithelial
cell line

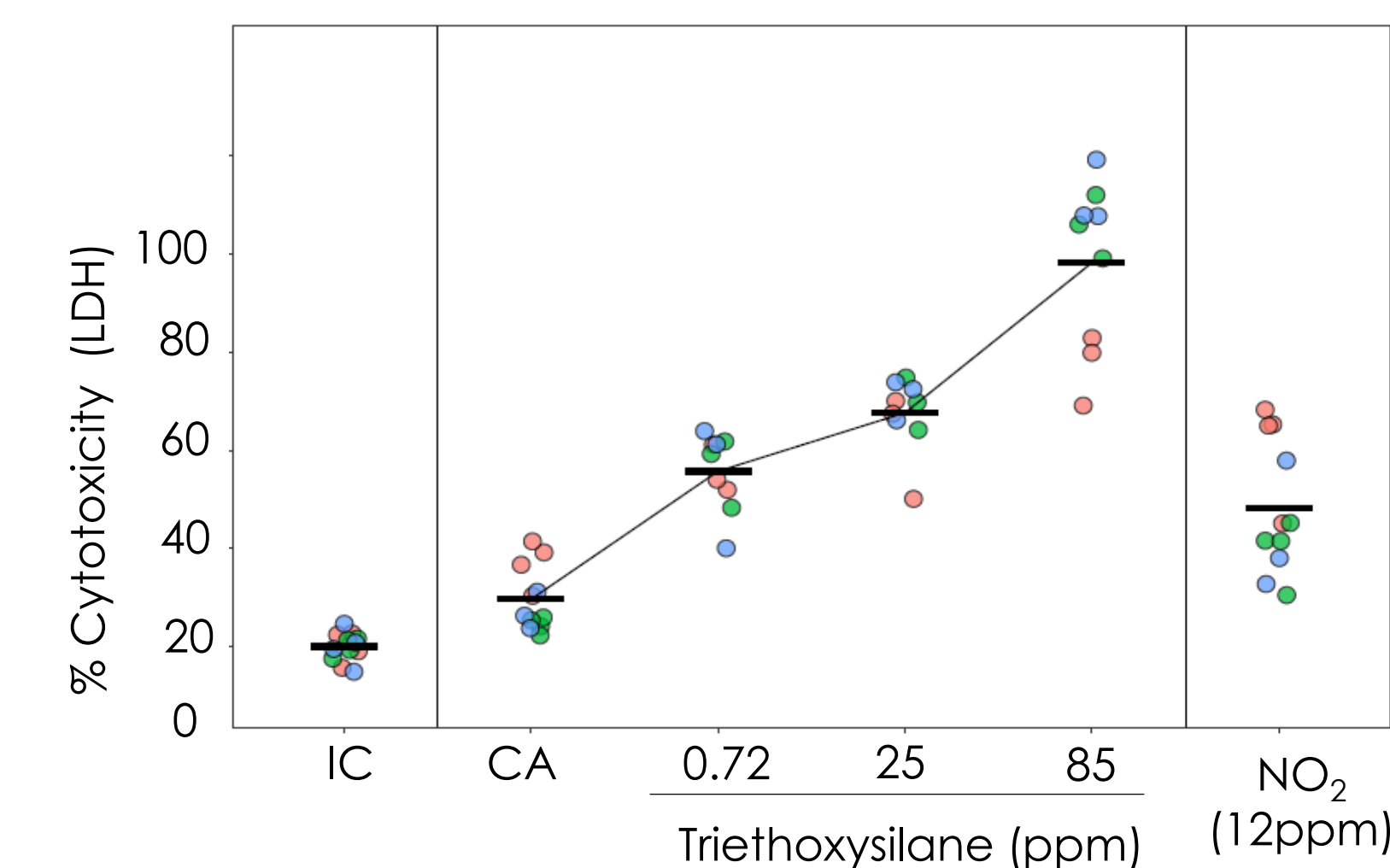


VITROCELL® 6/4 module

Results

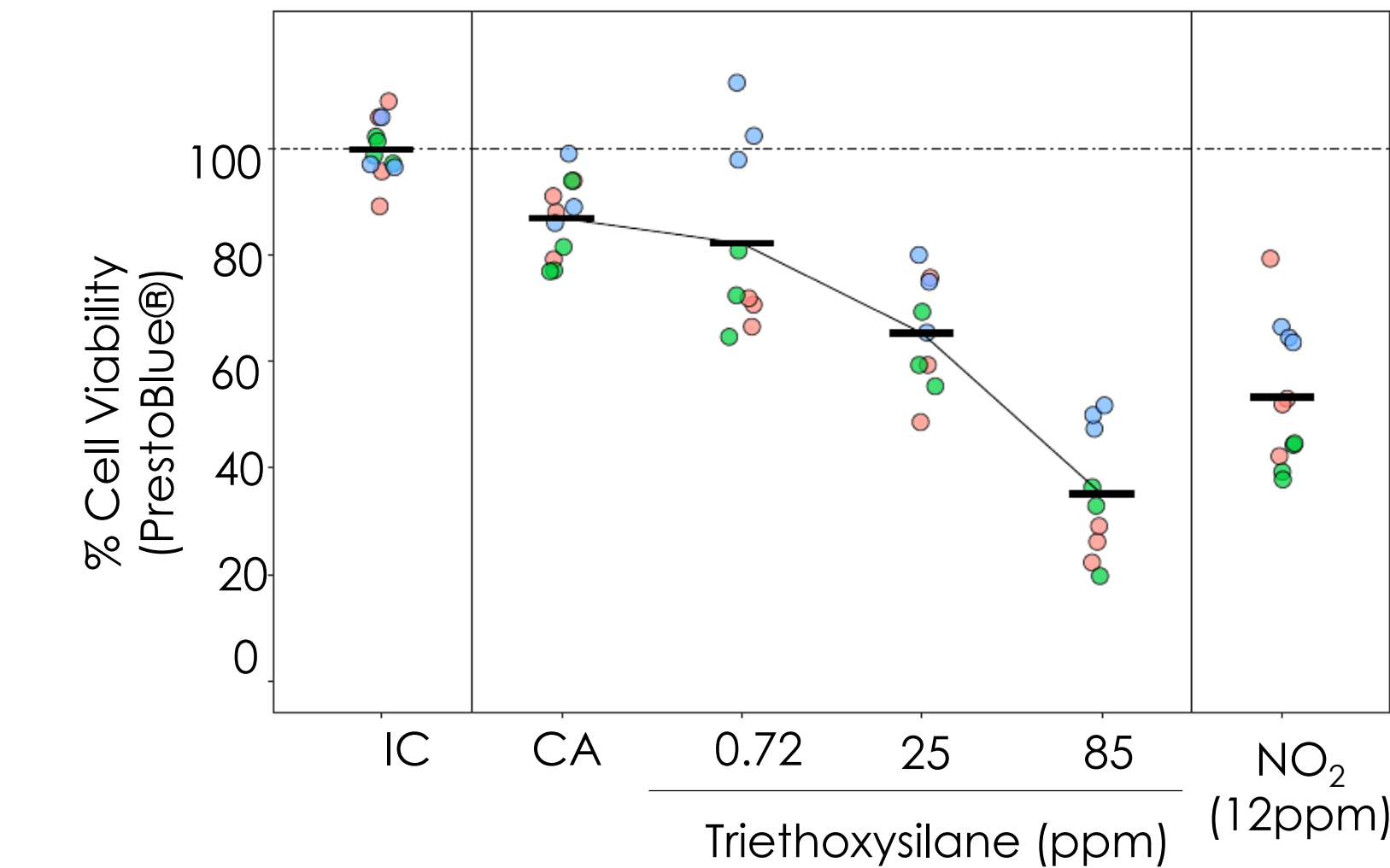
Cytotoxicity

(Lactate dehydrogenase (LDH),
release 30 minutes post-exposure)



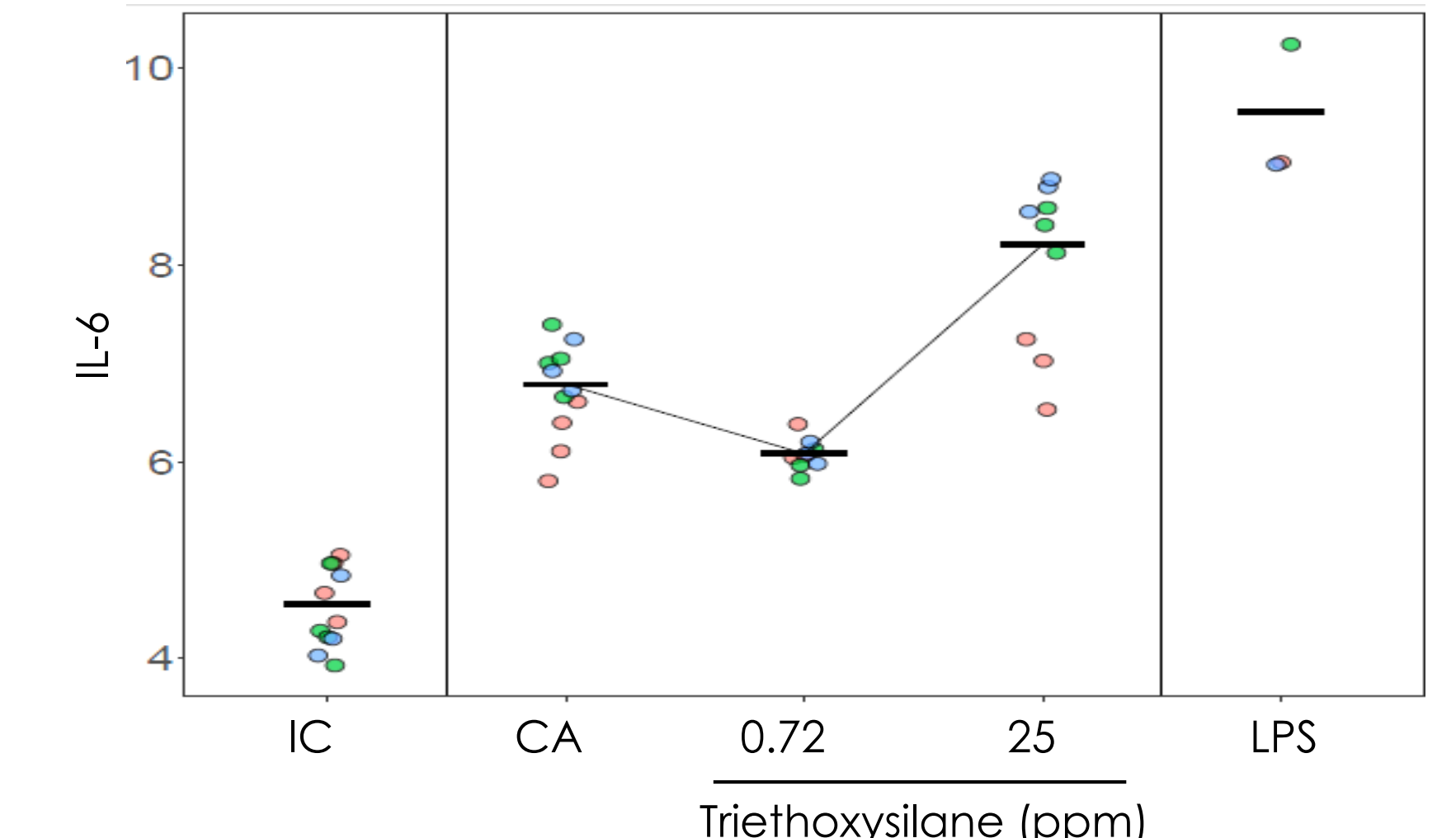
Cell viability

(Resazurin-based assay,
20-24 hours post-exposure)

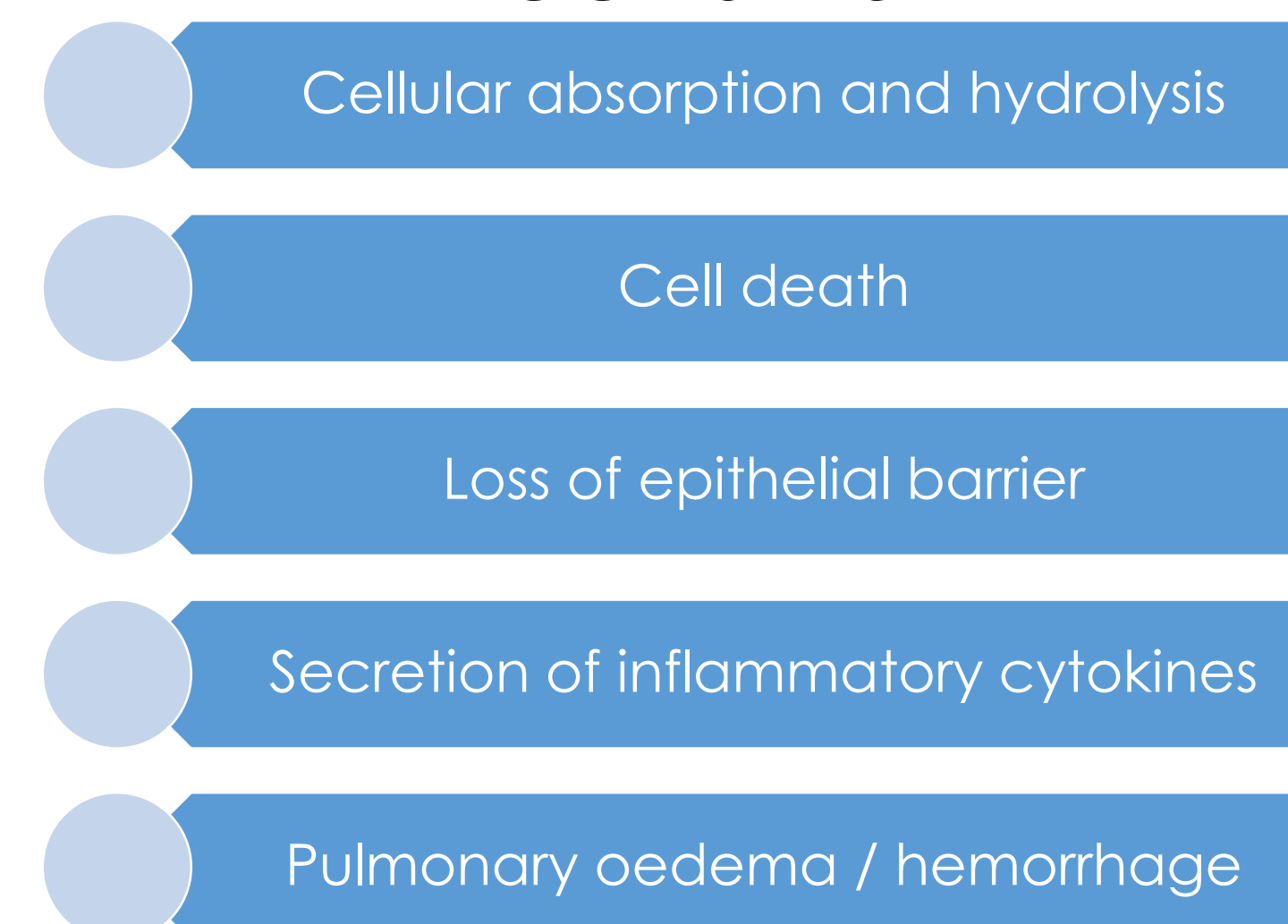


Inflammation

(Cytokine release,
20-24 hours post-exposure)



Mechanism



- References:
- <https://echa.europa.eu/de/registration-dossier/-/registered-dossier/6310/7/3/3>
 - <https://echa.europa.eu/de/registration-dossier/-/registered-dossier/16105/7/3/3>
 - <https://pubchem.ncbi.nlm.nih.gov/compound/6327710#section=NIOSH-Toxicity-Data&fullscreen=true>
 - <https://pubchem.ncbi.nlm.nih.gov/compound/6327710#section=Toxicity>
 - <https://echa.europa.eu/de/registration-dossier/-/registered-dossier/5339/7/3/3>

Cellular concentration of test chemical

Exposure concentration triethoxysilane (ppm)	Concentration of triethoxysilane in cells (ICP-AES*) (µg)				Avg µg
	N=1	N=2	N=3		
0.72	/	/	/		
25	2.6	3.3	3.8		3.2
85	15.4	17.4	20.8		17.9

Exposure concentration triethoxysilane (ppm)	Concentration of triethoxysilane in medium (µg)				Avg µg
	N=1	N=2	N=3		
0.72	/	/	/		/
25	2.8	2.5	2.3		2.5
85	5.9	6.3	5.8		6.0

*ICP-AES: Inductively coupled plasma-atomic emission spectroscopy

Observations

- A concentration-dependent cytotoxicity and cell viability response was observed
- A statistically significant release of IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and TNF-α was observed for exposure of BEAS-2B cells to 25 ppm triethoxysilane compared to clean air (CA)

Next Steps

- Test additional materials:
 - Silanes: Trimethoxysilane
 - Positive control(s): Triton-X and nitrogen dioxide
 - Negative control(s): Clean air, incubator control, and citrate buffered saline
- Include other cell systems:
 - Cell lines
 - Three dimensional reconstructed human tissue models
- Include dosimetry modeling