

# The INSPIRE Initiative: Assessing the potential toxicity of surfactants using human respiratory cell-based *in vitro* systems

Andreas O. Stucki<sup>1</sup>, Monita Sharma<sup>1</sup>, Nuria Roldan<sup>1</sup>, Sandra Verstraelen<sup>2</sup>, An Jacobs<sup>2</sup>, Karen Hollanders<sup>2</sup>, Jo Van Laer<sup>2</sup>, Sylvie Remy<sup>2</sup>, Evelien Frijns<sup>2</sup>, Amy J. Clippinger<sup>1</sup>  
<sup>1</sup>PETA Science Consortium International e.V., Stuttgart, DE; <sup>2</sup>Flemish Institute for Technological Research (VITO), Environmental Intelligence Unit, Mol, BE



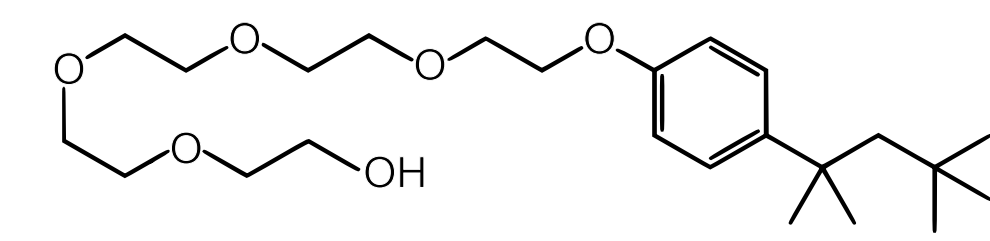
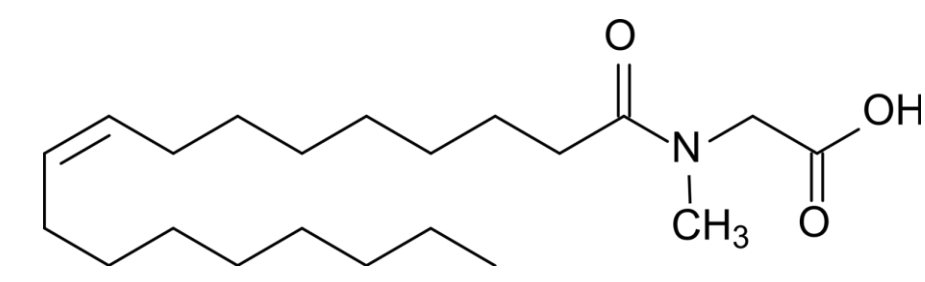
## Background & purpose

Surfactants are essential ingredients found in products such as household cleaners, personal care items, and medicines, and they function to, for example, help water and oil mix, create foam, and/or remove dirt. As widely used and commercially important chemicals, understanding the potential toxicity of surfactants is a key focus for regulatory agencies. Surfactants may interact with cell membranes, potentially compromising membrane integrity and leading to general cytotoxicity. To evaluate potential respiratory toxicity of these chemicals, a human bronchial epithelial cell line (BEAS-2B) and a bronchial reconstructed human respiratory epithelium (RHRE; MucilAir™, Epithelix, Switzerland) were exposed to two surfactants: Triton X-100 (non-ionic surfactant; CAS Number: 9036-19-5) and oleoyl sarcosine (anionic surfactant; CAS Number: 110-25-8). This work is a part of the INSPIRE (*In vitro* System to Predict REspiratory toxicity) Initiative that aims to build scientific confidence in human cell-based methods to predict respiratory toxicity in humans.

## Study design

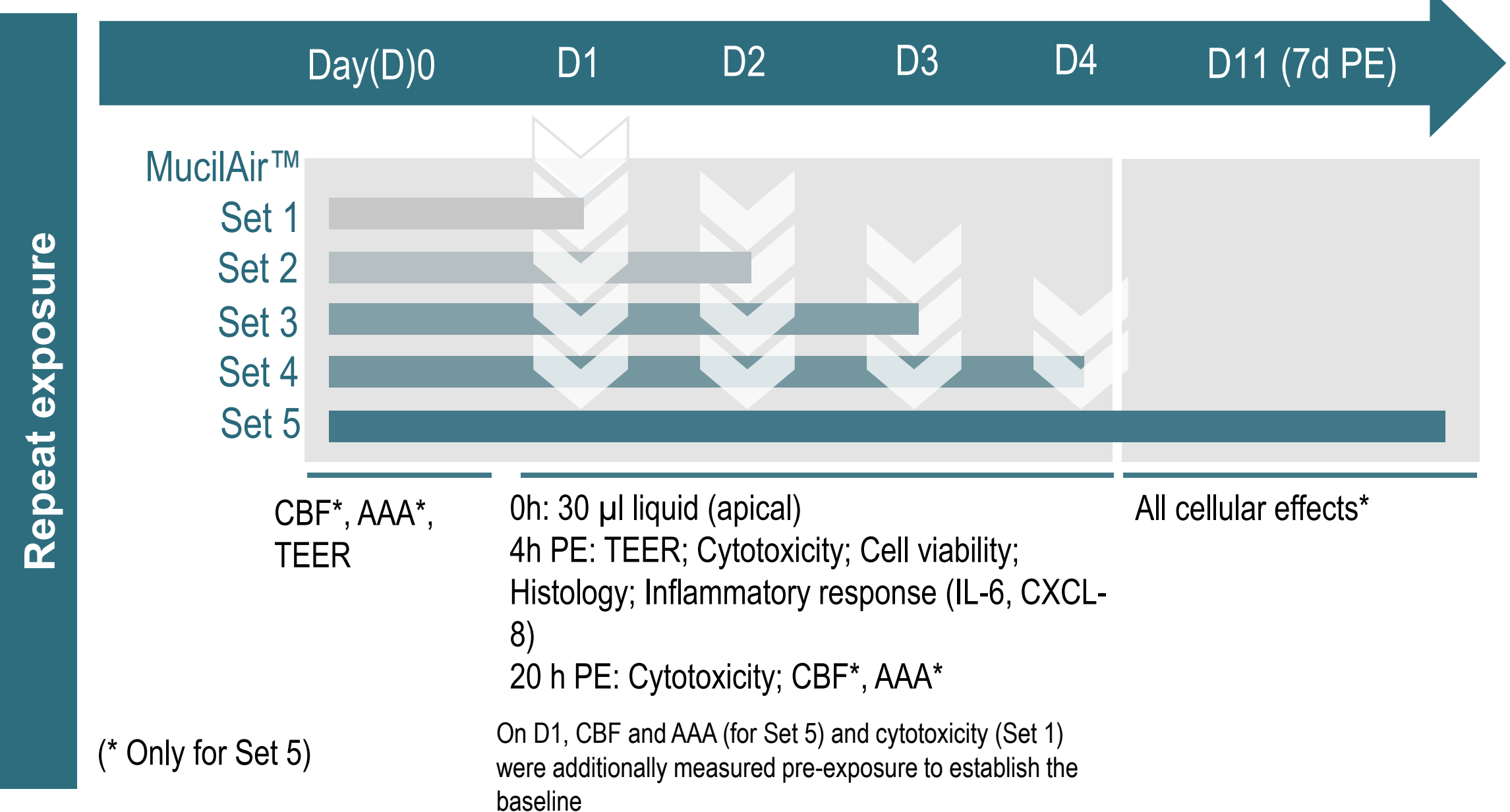
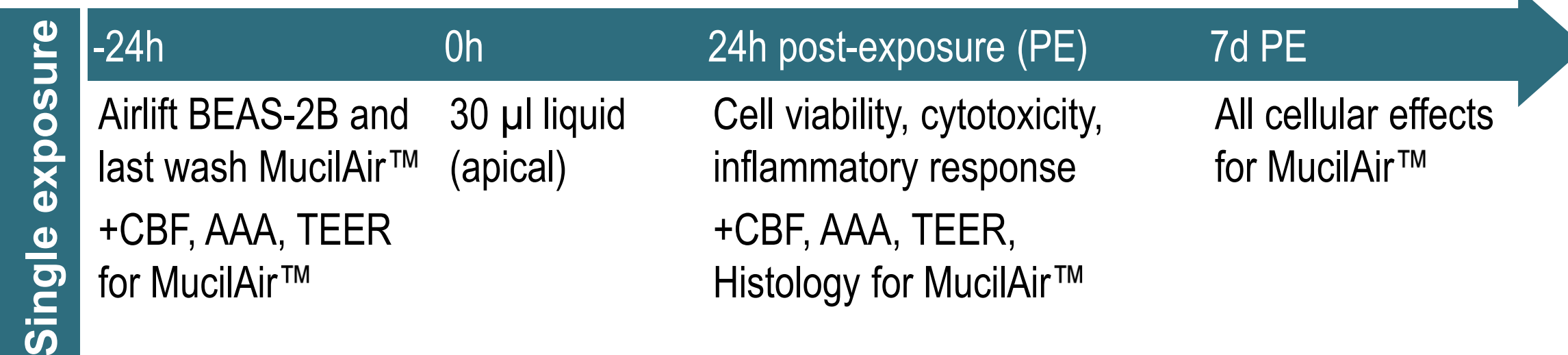
### Oleoyl sarcosine

### Triton X-100

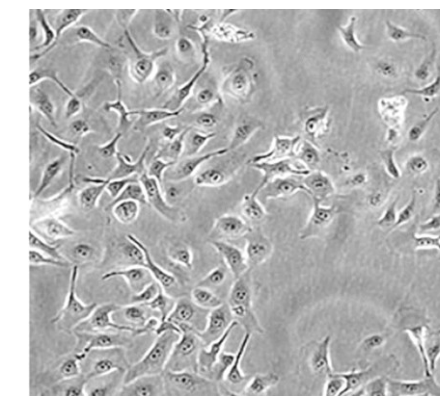


Oleoyl sarcosine	Triton X-100
Anionic surfactant; Negatively charged	Non-ionic surfactant; Not charged
Insoluble in water	Soluble in water
Critical micelle concentration 0.026 g/L (74 µM)	Critical micelle concentration 0.17 g/L (260 µM)
Acute tox (inhalation): Cat. 4	Acute tox (inhalation): No cat.
Skin irritation: Cat. 2 / Eye Irritation: Cat. 2	Skin irritation: Cat. 2 / Eye Irritation: Cat. 1

Cellular effects	BEAS-2B	MucilAir™
Cell viability (PrestoBlue®)	✓	✓
Cytotoxicity (LDH release)	✓	✓
Inflammatory markers (IL-6, CXCL-8)	✓	✓
Cilia beat frequency (CBF) and average active area (AAA)		✓
Barrier integrity (Transepithelial electrical resistance; TEER)		✓
Histology (H&E staining)		✓



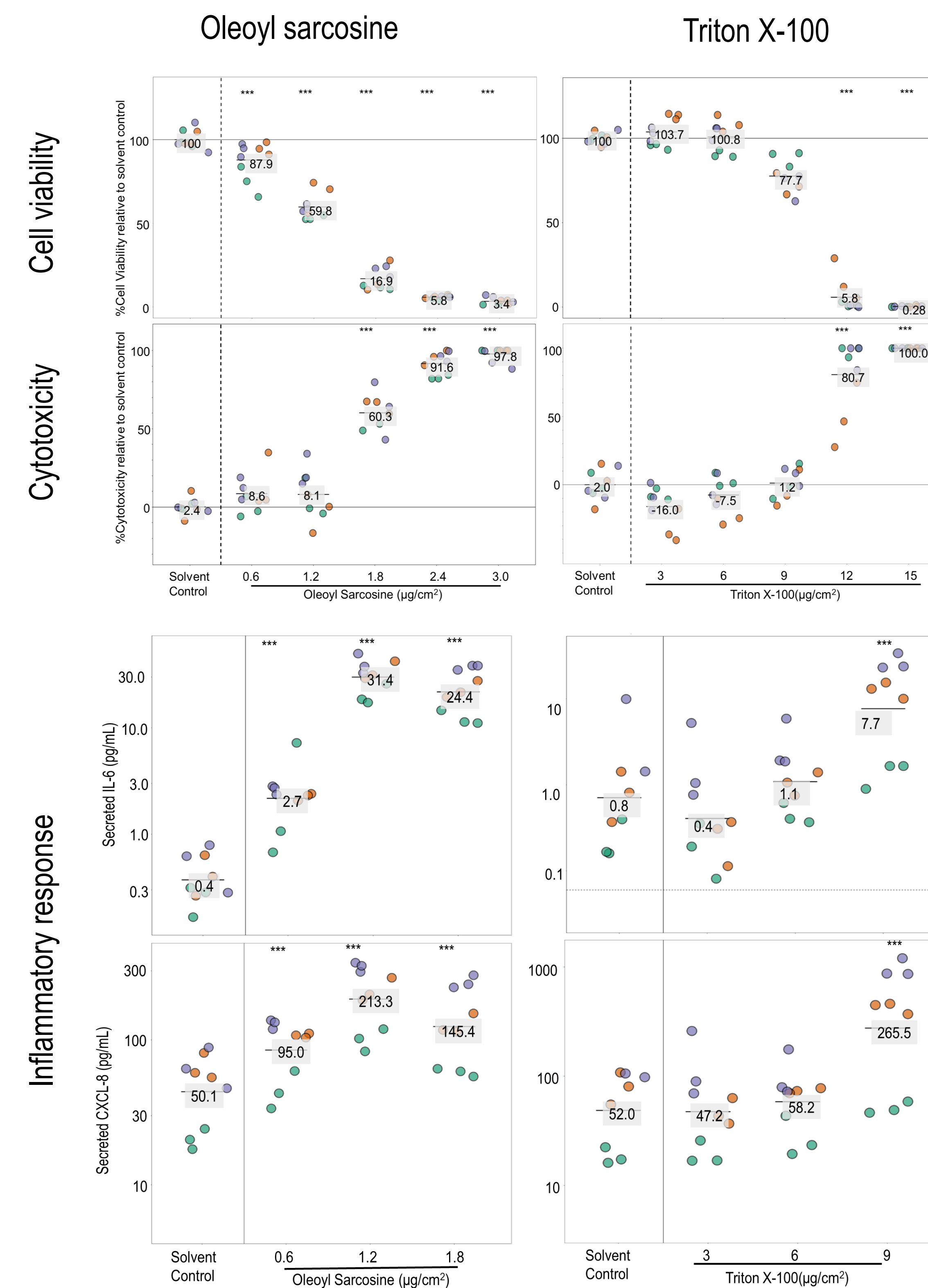
## Results BEAS-2B – single exposure



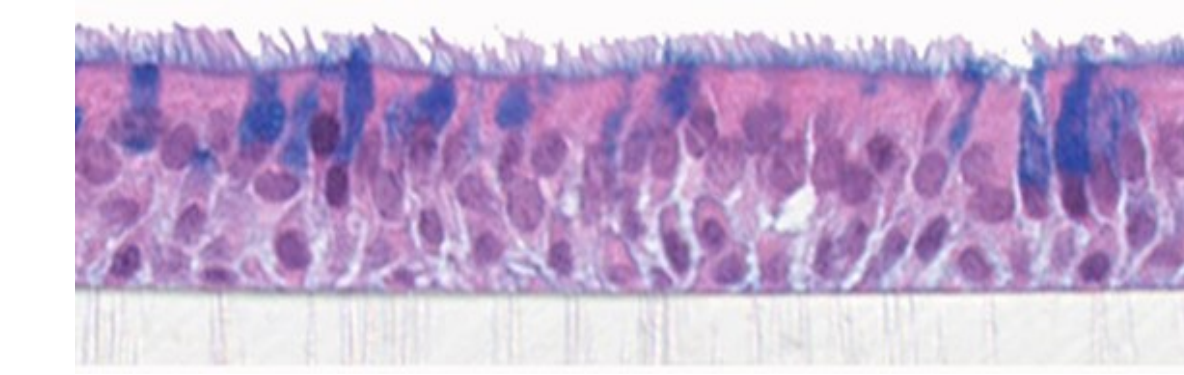
Human bronchial epithelial cell line

### Observations

- For both surfactants, significant concentration-dependent cellular effects were observed for every readout.
- Oleoyl sarcosine exposures showed cellular effects at lower concentrations than Triton X-100.



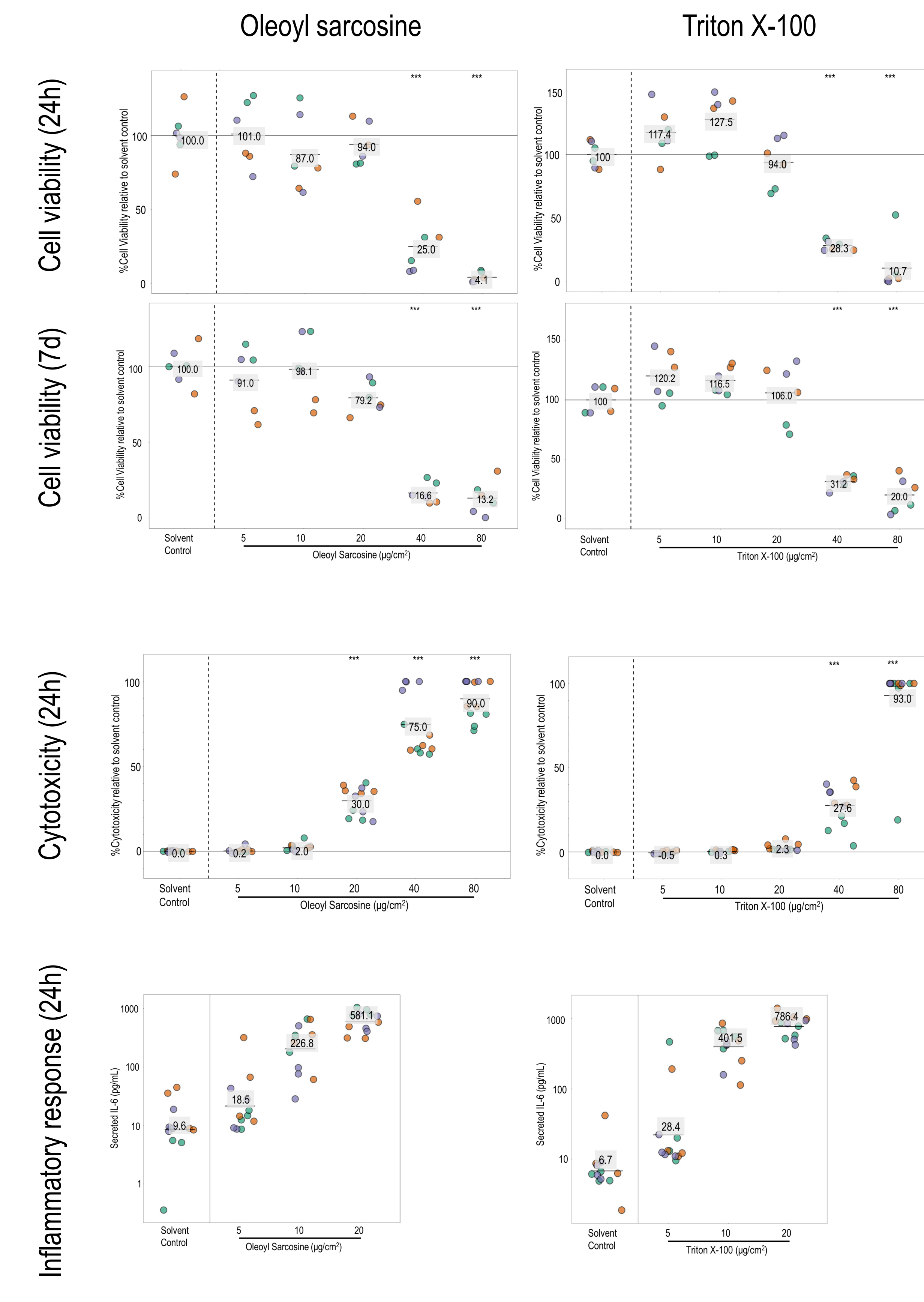
## Results MucilAir – single exposure



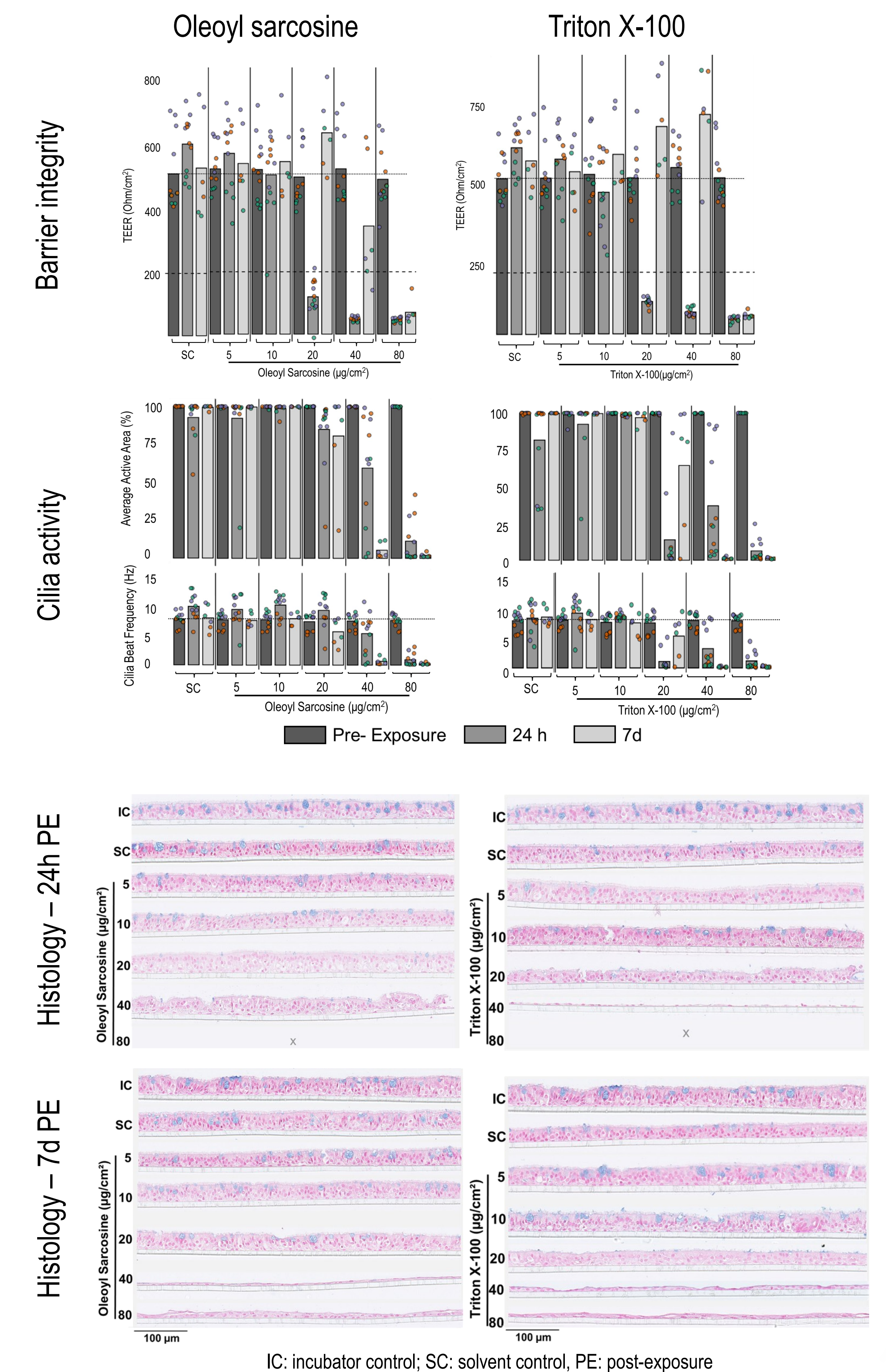
MucilAir cell line

### Observations

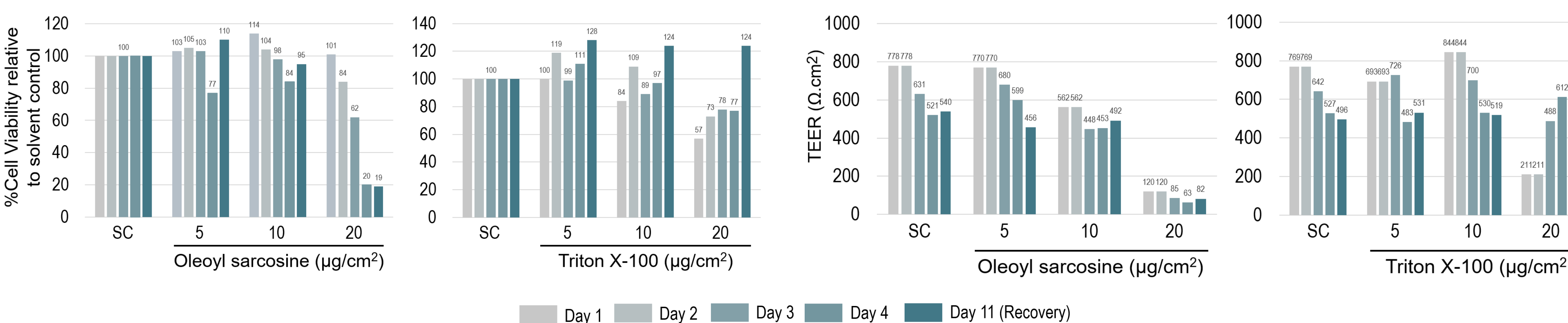
- For both surfactants, significant concentration-dependent effects were observed for every cellular effect assessed.
- The two highest concentrations showed severe and long-lasting effects, as seen at 7 days post-exposure.
- Unlike in BEAS-2B cells, both surfactants show cellular effects at comparable concentrations in MucilAir™.



### Observations



## Preliminary results MucilAir – repeat exposure



## Conclusions & next steps

Concentration-dependent cellular effects were observed for both surfactants in all experimental settings. BEAS-2B seem to be more sensitive than MucilAir™, which may be attributed to the lack of mucus and the overall lower tissue thickness. Preliminary repeat exposure results may indicate an additive effect in oleoyl sarcosine (only at the highest concentration) that is not observed in Triton X-100 exposure. Testing of these surfactants in an alveolar test system and 13 additional surfactants in MucilAir™ is planned. These results will further inform an ongoing multi-laboratory study testing up to 30 chemicals in MucilAir™.

Scan QR code for more information or contact AndreasS@thePSCI.eu

