# The INSPIRE Initiative: Using *in vitro* systems to predict respiratory toxicity of surfactants by pipetting and aerosol exposure

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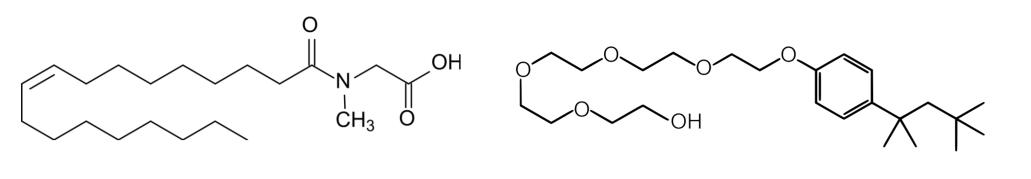
Triton X-100



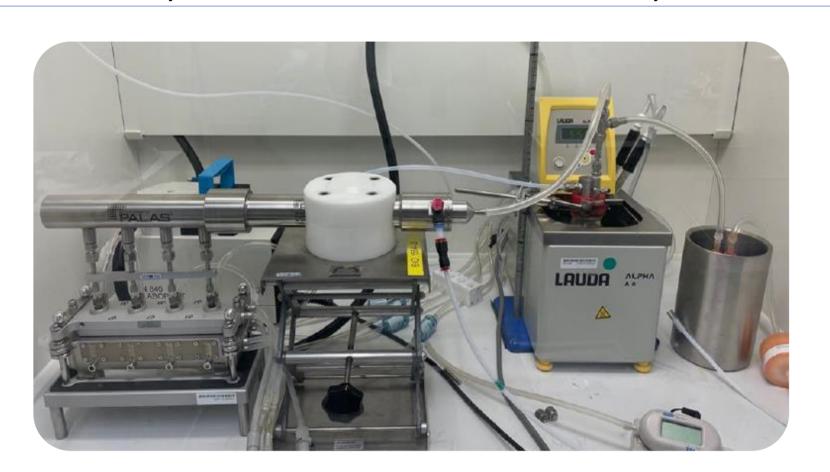
#### Study design

#### Oleoyl sarcosine

#### Triton X-100



Anionic surfactant; Negatively charged	Nonionic 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 Skin irritation: Cat. 2 / Eye Irritation: Cat. 2	Acute tox (inhalation): No cat. Skin irritation: Cat. 2 / Eye Irritation: Cat. 1



## BEAS-2B Human bronchial epithelial cell line Grown on 24-well inserts (0.4 µm pores) ALI 24 hours prior exposure



#### MucilAir<sup>TM</sup>

Reconstructed human bronchial epithelium Grown on 24-well inserts (1 µm pores)

Cellular effects	BEAS-2B	MucilAir
Cell viability (PrestoBlue®)	$\checkmark$	$\checkmark$
Cytotoxicity (LDH release)	<b>√</b> *	<b>√</b> *
Inflammatory markers (IL-6, CXCL-8*)	$\checkmark$	<b>√</b> *
Cilia beat frequency (CBF) and average active area (AAA)		<b>√</b> *
Barrier integrity (Transepithelial electrical resistance; TEER)		✓
Histology (H&E staining)		✓
		*not shown on poster

-24h	0h	24h post-exposure (PE)	7d PE	
Airlift BEAS-2B and last wash MucilAir	30 µl liquid or	Cell viability, LDH, inflammatory markers	All cellular effects for MucilAir	
+CBF, AAA, TEER for MucilAir	30 min aerosol exposure	+CBF, AAA, TEER, Histology for MucilAir		



#### In vitro skin irritation

Oleoyl sarcosine

1G	% viability (3 min)	$103.10 \pm 7.34$	$64.08 \pm 1.80$
OECD	% viability (60 min)	$95.96 \pm 7.16$	$56.29 \pm 2.80$
OE	Prediction	Non-corrosive	Non-corrosive
39		Oleoyl sarcosine	Triton X-100
TG 439	% viability	2.19 ± 0.14	1.49 ± 0.35
DECD	Prediction	Category 2	Category 2



431

#### In vitro eye irritation

æ		Oleoyl sarcosine	Triton X-100
492B	% viability (5 min)	20.4 ± 7.16	$28.0 \pm 3.32$
TG	% viability (16 min)	56.1 ± 4.11	$1.7 \pm 0.07$
OECD	% viability (120 min)	$2.7 \pm 0.065$	$0.5 \pm 0.06$
ō	Prediction	Category 2	Category 1

### Results BEAS-2B — liquid and aerosol-Oleoyl sarcosine Liquid Aerosol **Cell viability** Inflammatory response 0.02 0.69 1.98 2.55 Control Oleoyl sarcosine (µg/cm²) Oleoyl sarcosine (µg/cm²) Triton X-100 Liquid Aerosol Cell viability 000 nse respo Inflammatory 0.1 Triton X-100 (µg/cm<sup>2</sup>) Triton X-100 (µg/cm<sup>2</sup>)

## Results MucilAir – liquid Oleoyl sarcosine Triton X-100 24h PE Triton X-100 (µg/cm²) 120 H **7**d viability Cell control Triton X-100 (µg/cm²) Oleoyl sarcosine (µg/cm²) Barrier integrity ■ 24h before exposure ■ 24h post-exposure ■ 7d post-exposure SC **\***Ш 5 Histology Goding and Broader of Matheway the on the State of the \*Experimental run #2; IC: incubator control; SC: solvent control



#### Observations

- Concentration-dependent decreases in cell viability and inflammatory markers cells was observed in BEAS-2B cells for liquid exposures to both oleoyl sarcosine and Triton X-100.
- Aerosolization of highly viscous surfactants and measuring the deposited dose are challenging and lead to a higher variability observed in aerosolized samples for BEAS-2B cells. Nevertheless, a concentration-dependent effect was observed for cell viability with both surfactants and inflammatory cytokines with oleoyl sarcosine.
- Concentration-dependent effects were observed for liquid exposures of MucilAir at slightly higher concentrations than for BEAS-2B cells at 24 hours post-exposure. Concentration-dependent effects were also observable 7 days post-exposure, indicating a substantial loss of cells at higher concentrations that was confirmed by histology. Interestingly, the epithelial barrier was compromised at concentrations above 20 µg/cm² 24 hours post-exposure but was able to recover at 7 days except for the highest concentration.
- The surfactants were also tested *in vitro* for their skin and eye corrosion/irritation potential. OECD TGs 431 and 439 predicted the surfactants to be GHS category 2 skin irritants and OECD TG 492B predicted oleoyl sarcosine and Triton X-100 as GHS category 2 and 1 eye irritants, respectively.

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