The INSPIRE Initiative: Using human cell-based *in vitro* systems to assess respiratory toxicity of surfactants delivered as liquid or aerosols

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Background and purpose

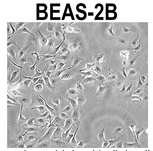
Inhalation is a major route through which substances can cause toxicity, and *in vitro* testing approaches are being used to study the effects of inhaled chemicals. The INSPIRE Initiative (*IN vitro* System to PredIct REspiratory toxicity) aims at building scientific confidence in these methods to predict respiratory toxicity in humans. By testing four chemicals belonging to two different classes (silanes and surfactants), the goal of this initiative is to help identify relevant cellular effects, generation and exposure methods, and model systems that may be most appropriate for use, depending on the purpose of testing. The work presented here builds upon recently published data for the testing of silane vapors (Sharma *et al.* 2023) and focuses on surfactant testing. Surfactants are of interest to regulatory agencies because of their use in various products (e.g., soaps, adhesives, and herbicides) as emulsifiers, wetting agents, detergents, foaming agents, or dispersants. Surfactants can interact with the amphiphilic cell membranes compromising membrane integrity and causing general cytotoxicity. When inhaled, surfactants can also perturb the resident surface lining fluid in the lower respiratory tracts that may lead to a collapse of the alveoli (atelectasis). In this study, a human bronchial epithelial cell line (BEAS-2B) and a reconstructed human tissue model (MucilAir™) were used to assess the toxicity of two surfactants —Triton X-100 (non-ionic surfactant; CAS Number: 9002-93-1) and oleoyl sarcosine (anionic surfactant; CAS Number: 110-25-8)—exposed to cells as aerosols or liquids (pipetting).

Methods

BEAS-2B cells were seeded on 24-well PET cell culture inserts and airlifted before exposures. For liquid exposures, five different concentrations of Triton X-100 or oleoyl sarcosine were pipetted (30 µL each) on the apical side of both cell systems. For aerosol exposures, Triton X-100 was diluted in ultrapure water and oleoyl sarcosine was diluted in 10% ethanol and aerosol atmosphere was generated using a collision atomizer. The cell systems were placed in a VITROCELL® 6/4 system and exposed to the surfactants for 30 minutes. Aerosol particle size distribution was assessed using Aerodynamic Particle Sizer (APS). The deposited concentration of both surfactants was determined using liquid chromatography - mass spectrometry (LC-MS). Cellular effects were assessed ~24 hours after exposure. These included cytotoxicity (lactate dehydrogenase release; data not shown), cell viability (using resazurin-based PrestoBlue® assay), secretion of interleukins-6 (IL-6) and -8 (CXCL-8; MSD V-PLEX Assay). Benchmark concentration (BMC) modelling was performed using U.S. EPA's Benchmark Dose Software (BMDS) online. In addition, to understand the effects of these chemicals through other routes of exposure, both surfactants were assessed for skin and eye irritation using *in vitro* Organisation for Economic Co-operation and Development (OECD) test guidelines (TG) 431 and 439, and 492B, respectively.

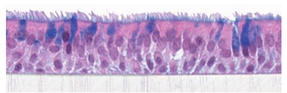
Test systems and cellular effects

BEAS-2B



Human bronchial epithelial cell line

MucilAir™

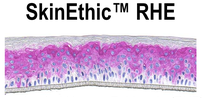


Human bronchial epithelial tissue model

SkinEthic™

Reconstructed human cornea-like epithelium (RhCE)

SkinEthic™ RHE

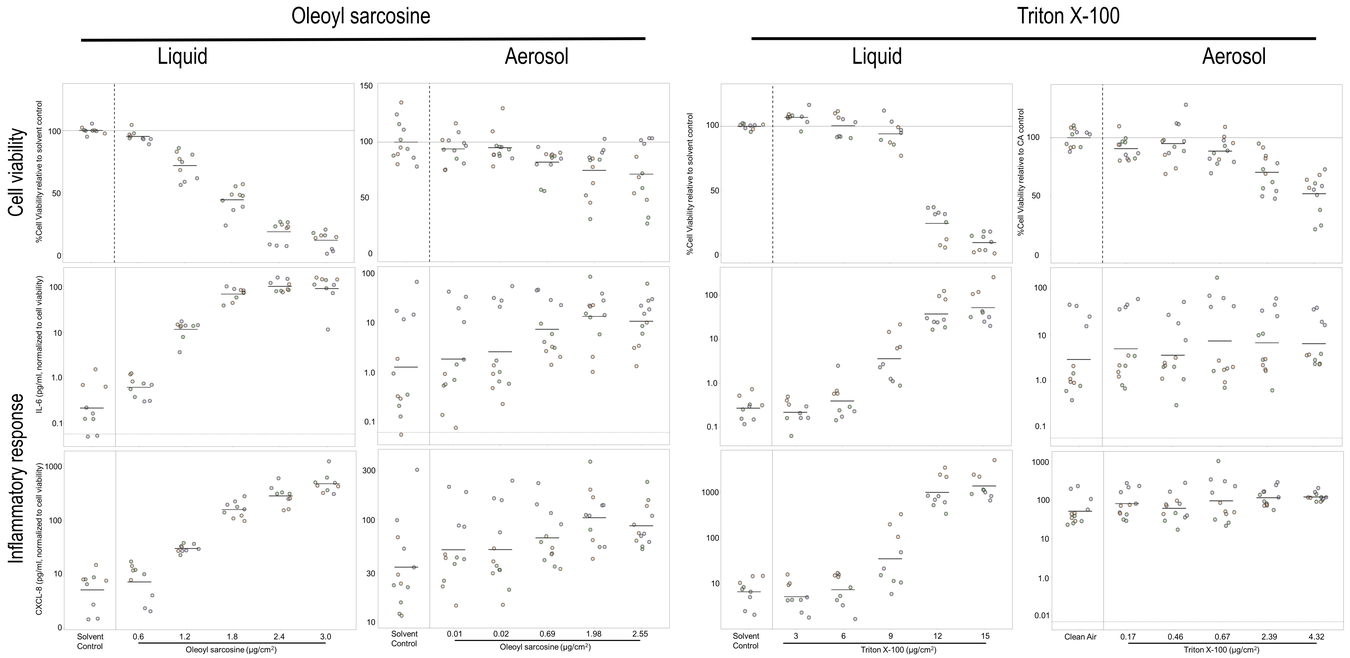


Reconstructed Human Epidermis (RHE)

Cellular effects	BEAS-2B	MucilAir	SkinEthic RHE	SkinEthic HCE
Cell viability (PrestoBlue®)	✓	✓		
Cytotoxicity (LDH, data not shown)	✓	✓		
Inflammatory markers (IL-6 and IL-8)	✓	✓		
Cilia beating frequency (CBF) and average active area (AAA)		✓		
Barrier integrity (TEER)		✓		
Morphology (H&E staining)		✓		
Skin irritation/corrosion (OECD 431 and 439)			✓	
Eye irritation/corrosion (OECD 492B)				✓

Results

Assessment of respiratory toxicity of surfactants in BEAS-2B cells

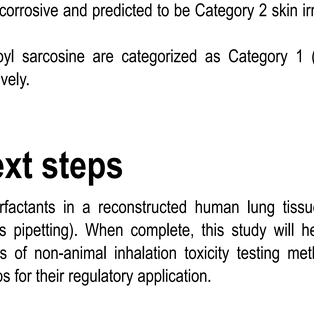


Benchmark concentration lower bound (BMCL)

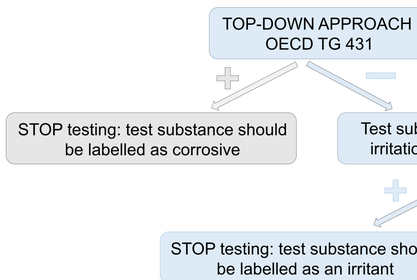
	Triton X-100		Oleoyl sarcosine	
	Liquid (µg/cm²)	Aerosol (µg/cm²)	Liquid (µg/cm²)	Aerosol (µg/cm²)
PrestoBlue	6.922	0.462	0.336	0.541
LDH	7.568	0.749	0.872	2.657*
IL-6	3.470	NA	0.478	NA
CXCL-8	5.021	NA	0.639	5.499*

*value higher than highest dose tested; NA, not available

Examples of BMC modelling



Assessment of skin irritation/corrosion potential of surfactants: OECD TG 431 and 439



Chemical	% viability (3 min)	% viability (60 min)	Prediction
Triton X-100	64.08 ± 1.80	56.29 ± 2.80	Non-corrosive
Oleoyl sarcosine	103.10 ± 7.34	95.96 ± 7.16	Non-corrosive

Chemical	% viability ± SD	Prediction
Triton X-100	1.49 ± 0.35	Category 2
Oleoyl sarcosine	2.19 ± 0.14	Category 2

Assessment of eye irritation/corrosion potential of surfactants: OECD TG 492B

Chemical	% viability (5 min)	% viability (16 min)	% viability (120 min)	Prediction
Triton X-100	28.0 ± 3.32	1.7 ± 0.07	0.5 ± 0.06	Category 1
Oleoyl Sarcosine	20.4 ± 7.16	56.1 ± 4.11	2.7 ± 0.065	Category 2

Observations

- For oleoyl sarcosine, a concentration-dependent decrease in cell viability was observed for liquid exposures. A concentration-dependent increase in IL-6 and CXCL-8 was observed for all tested concentrations. IL-6 levels were the highest and seemed to plateau at the two highest concentrations.
- For Triton X-100, a significant decrease in cell viability was observed only at the two highest concentrations for liquid exposures. IL-6 and CXCL-8 levels showed a concentration-dependent increase at all concentrations for liquid exposures.
- Aerosolization of highly viscous surfactants was challenging leading to a higher variability observed in aerosolized samples versus liquid application. Nevertheless, the results were similar to liquid applications.
- Benchmark concentration (BMC) modeling revealed higher BMCL for liquid exposures of Triton X-100 compared to aerosol exposures. However, the opposite was observed for oleoyl sarcosine. In general, the difference of BMCLs from liquid and aerosol exposures differed at maximum by one order of magnitude.
- According to TG 431, both chemicals were non-corrosive and predicted to be Category 2 skin irritants based on TG 439.
- According to TG 492B, Triton X-100 and oleoyl sarcosine are categorized as Category 1 (serious eye damage) and Category 2 (eye irritation), respectively.

Next steps

Experiments are underway to assess the two surfactants in a reconstructed human lung tissue model using different modes of exposure (aerosolization versus pipetting). When complete, this study will help to increase scientific confidence in and identify the usefulness of non-animal inhalation toxicity testing methods to inform regulatory decision-making as well as remaining gaps for their regulatory application.