IN VITRO APPROACH FOR ASSESSING RESPIRATORY TOXICITY IN HUMAN LUNG CELLS

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Abstract

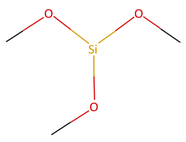
Approaches to efficiently and effectively assess the toxicity of chemicals on the human respiratory tract using in vitro systems would provide useful information to inform product development and risk management decisions. Presented here is an approach to help better understand the appropriate in vitro 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 (LDH) assay) was observed after exposure to the 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.

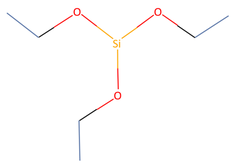
Additional work is underway to test other substances, including silanes that vary only in their carbon length to determine if this in vitro system can detect the decrease in toxicity that correlates with increasing carbon-chain length, and to determine the advantages of using a 2D cell line (BEAS-2B cell) versus a 3D human reconstructed tissue model (MucilAir). Overall, these results demonstrate the utility of in vitro systems 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.

Test chemicals

Silanes

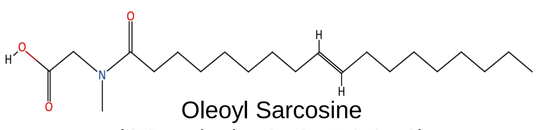


Trimethoxysilane
(TMS, GHS 1, CAS# 2487-90-3)

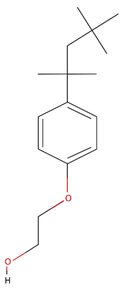


Triethoxysilane
(TES, GHS 2, CAS# 998-30-1)

Surfactants



Oleoyl Sarcosine
(OS, Anionic, CAS#110-25-8)

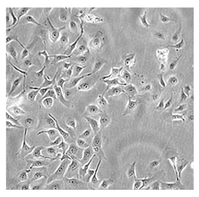


Triton X-100
(TX100, Non-ionic, CAS#9002-93-1)

Test system(s) and endpoints

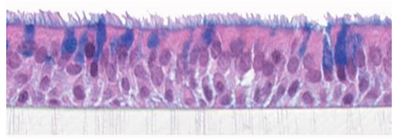
BEAS-2B: Human bronchial epithelial cell line

Endpoints:
- Cell viability (PrestoBlue®)
- Cytotoxicity (LDH)
- Inflammatory markers [interleukin (IL)-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL- 12p70, IL-13, interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α)]

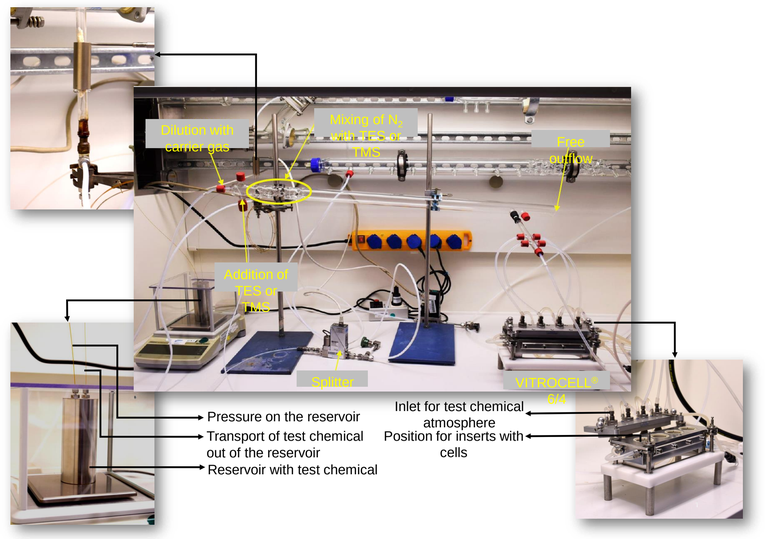


MucilAir™: 3D Human bronchial epithelial tissue model

Endpoints:
- Cell viability (PrestoBlue®)
- Cytotoxicity (LDH)
- Cilia beat frequency (CBF)
- Morphology (H&E staining)
- Barrier integrity (TEER)
- Inflammatory markers (IL-2, IL-6, IL-8, TNF-α)



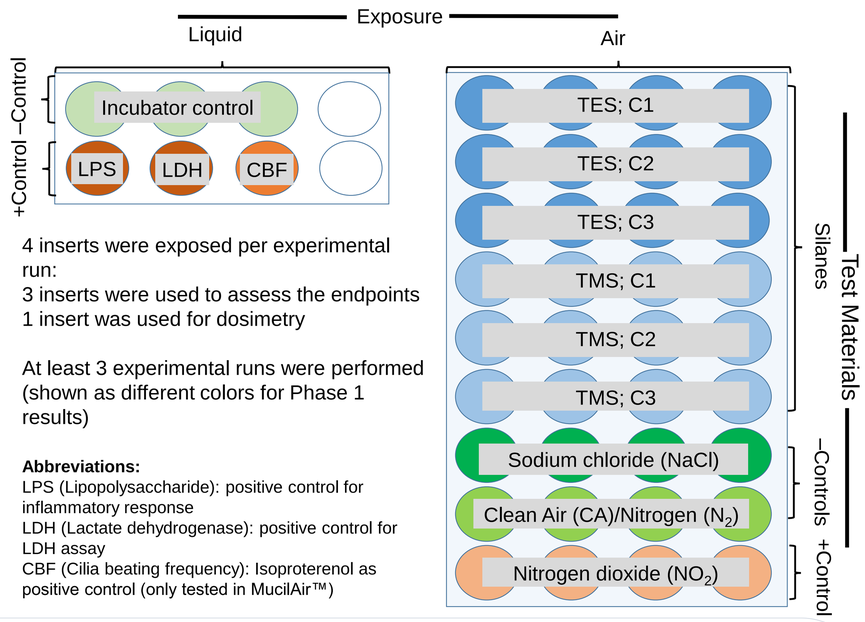
Exposure set-up for silanes



Project details

	Differences between project phases	
Phase1 (Completed)	**Assess the toxicity of TES in BEAS-2B cells**	
Phase 2 (Ongoing)	**Assess the toxicity of silanes and surfactants in BEAS-2B cells**	**Key differences between Phase 1 and Phase 2:** • Reducing exposure time from 1hr to 30min • Additional test substances (TMS and surfactants) • Adding "true" negative control (sodium chloride) • Using nitrogen as a carrier control • Testing only four inflammatory markers (IL-2, IL-6, IL-8, TNF-α) • Not adding media after exposure • Removed bovine pituitary extract from cell media
Phase 3 (Ongoing)	**Assess the toxicity of silanes and surfactants in MucilAir™**	**Key differences between Phase 2 and Phase 3:** • Using a 3D model • Assessing additional endpoints (TEER, CBF, and histology) • Adding 7 day recovery period

Experimental set-up for silanes



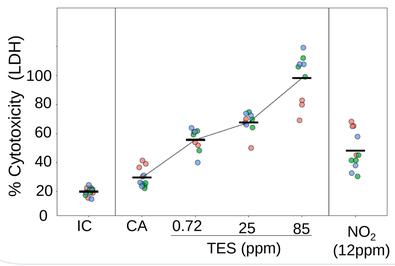
4 inserts were exposed per experimental run:
3 inserts were used to assess the endpoints
1 insert was used for dosimetry

At least 3 experimental runs were performed (shown as different colors for Phase 1 results)

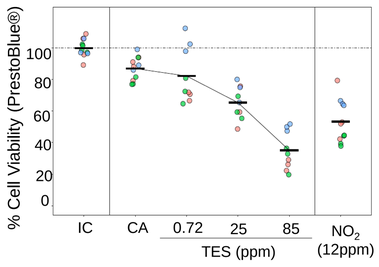
Abbreviations:
LPS (Lipopolysaccharide): positive control for inflammatory response
LDH (Lactate dehydrogenase): positive control for LDH assay
CBF (Cilia beating frequency): Isoproterenol as positive control (only tested in MucilAir™)

Phase 1 (Complete)

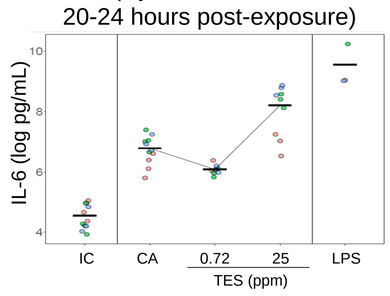
Cytotoxicity
(LDH release,
30 minutes post-exposure)



Cell viability
(Prestoblue® assay,
20-24 hours post-exposure)



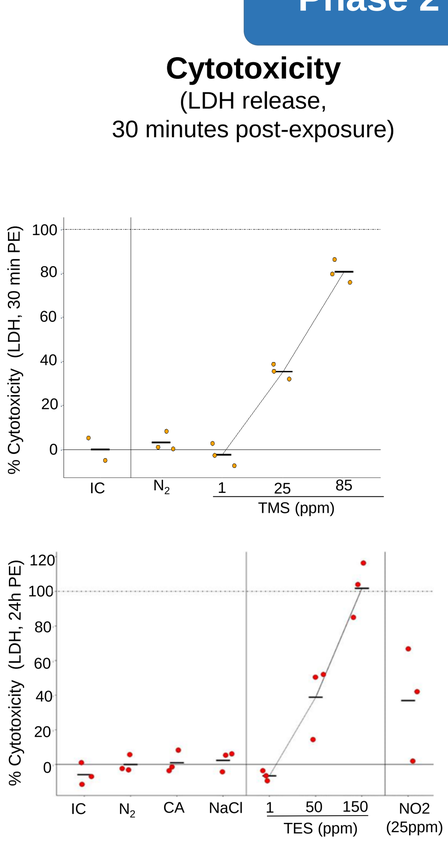
Inflammation
(Cytokine release,
20-24 hours post-exposure)



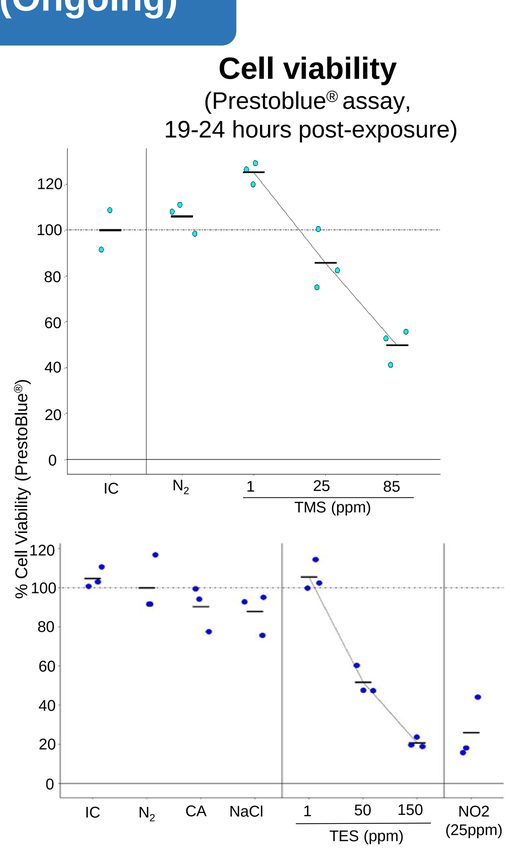
Observations
- The three different colored dots in the graphs represent three separate experiments (with three replicates within each experiment).
- A concentration-dependent cytotoxicity and cell viability response was observed
- A statistically significant release of IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and TNF-α was observed after exposure of BEAS-2B cells to 25 ppm TES compared to CA

Phase 2 (Ongoing)

Cytotoxicity
(LDH release,
30 minutes post-exposure)



Cell viability
(Prestoblue® assay,
19-24 hours post-exposure)

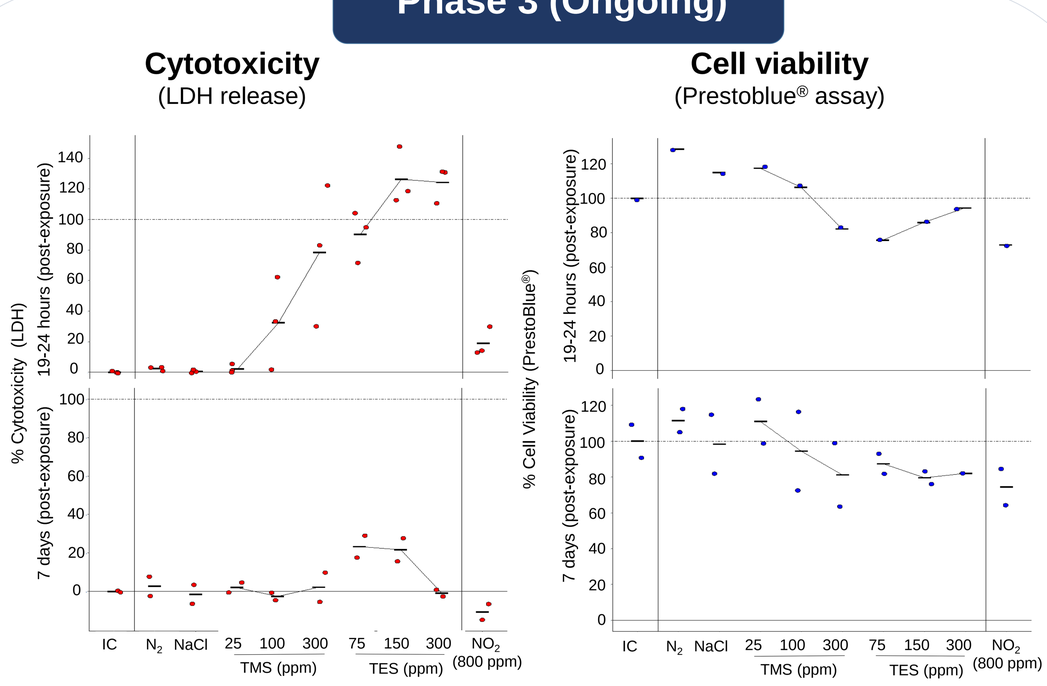


Observations and next steps
- Results presented are from one experimental run
- A concentration-dependent cytotoxicity and cell viability response was observed but this experiment will be repeated 2 more times to establish statistical significance
- Next steps include repeating these experiments and testing surfactants

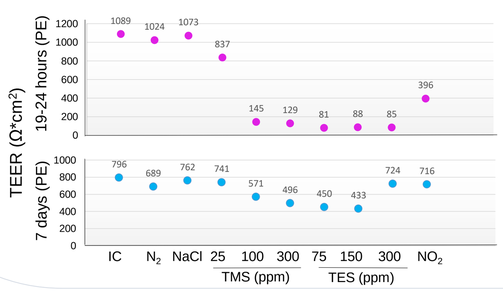
Phase 3 (Ongoing)

Cytotoxicity
(LDH release)

Cell viability
(Prestoblue® assay)



Barrier integrity
(TEER)



Observations and next steps
- Results presented are from one experimental run
- Work is ongoing to troubleshoot variability, and may be a result of issues with consistent exposure.
- Next steps include repeating these experiments and testing surfactants