PETA SCIENCE CONSORTIUM INTERNATIONAL e.V.

Assessing Respiratory Toxicity in Human Cell-based *In Vitro* Systems

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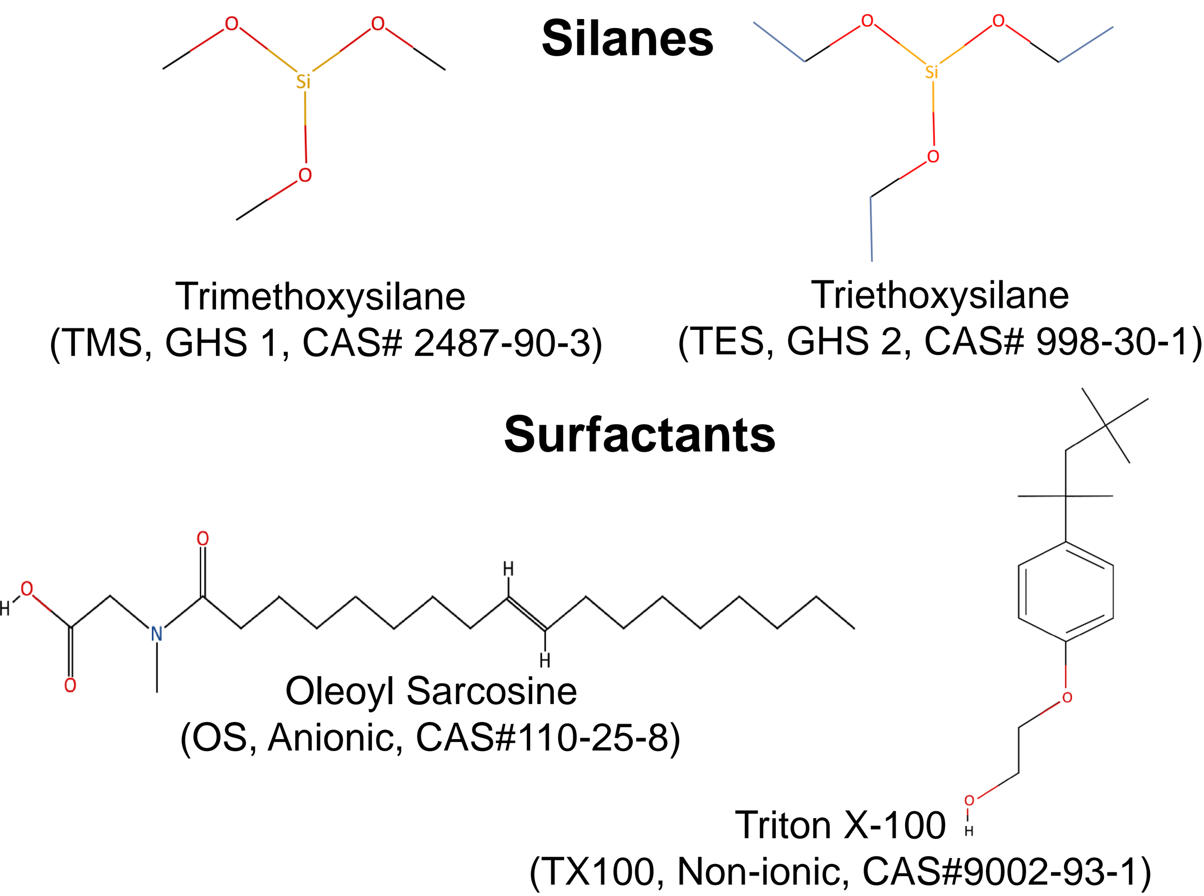
Abstract: Risk assessment and management relies on approaches that can accurately and efficiently predict the toxicity of chemicals in humans. Inhalation is a major route by which exposure to substances can occur, and is an area where resources have been dedicated to optimize human-relevant *in vitro* approaches. In this study, called the INSPiRE Initiative (*IN vitro* System to Predict REspiratory toxicity), a two-dimensional (2D) human bronchial epithelial cell line (BEAS-2B) and a three-dimensional (3D) human reconstructed tissue model (MucilAir™, Epithelix) were used to predict the ability of chemicals to cause portal-of-entry effects on the human respiratory tract. The human cell-based systems were exposed to different concentrations of silanes (triethoxysilane (TES) and trimethoxysilane (TMS)) using a capillary dosage method and surfactants (Triton X-100 and/or oleoyl sarcosine) using atomization, at the air-liquid interface in a VITROCELL® 6/4 exposure module. Nitrogen dioxide (NO_2) was included as a positive control and sodium chloride and clean air (CA) or nitrogen gas (N) as negative controls. Endpoints assessed include cell viability (Prestoblue™ assay), cytotoxicity (lactate dehydrogenase assay; LDH), and expression of inflammatory markers (electrochemiluminescence immunoassay, Meso Scale Discovery) and, for the 3D tissues, morphology (hematoxylin and eosin (H&E) staining), barrier integrity (transepithelial electrical resistance, TEER), and cilia beat frequency (SAVA system) were also examined. Preliminary studies demonstrated a concentration-dependent decrease in cell viability and an increase in cytotoxicity after 1 hour exposure of BEAS-2B cells to TES (0.72ppm, 25ppm, and 85ppm) as compared to CA. A significant increase in expression of inflammatory markers (including interleukin (IL)-6, IL-8, IL-2, and tumor necrosis factor-alpha (TNF-α) was observed at 25ppm of TES. Studies are underway to assess additional test chemicals and endpoints in both systems. The results of this project can be used to better understand the usefulness of different test systems and, therefore, help guide selection. The results can also be used to predict the likelihood of a chemical to cause portal-of-entry effects on the human respiratory tract and inform regulatory decision-making.

Test chemicals

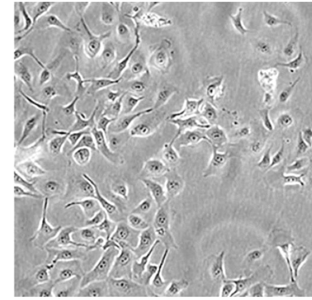


Test system(s) and endpoints

BEAS-2B: Human bronchial epithelial cell line

Endpoints:

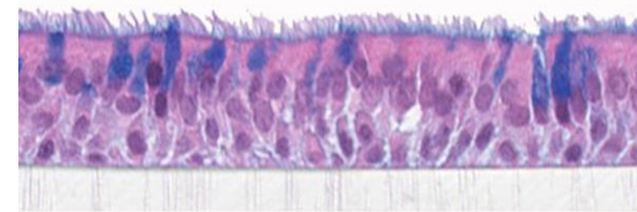
- Cell viability (PrestoBlue®)
- Cytotoxicity (LDH)
- Inflammatory markers (IL-2, IL-6, IL-8, TNF-α)



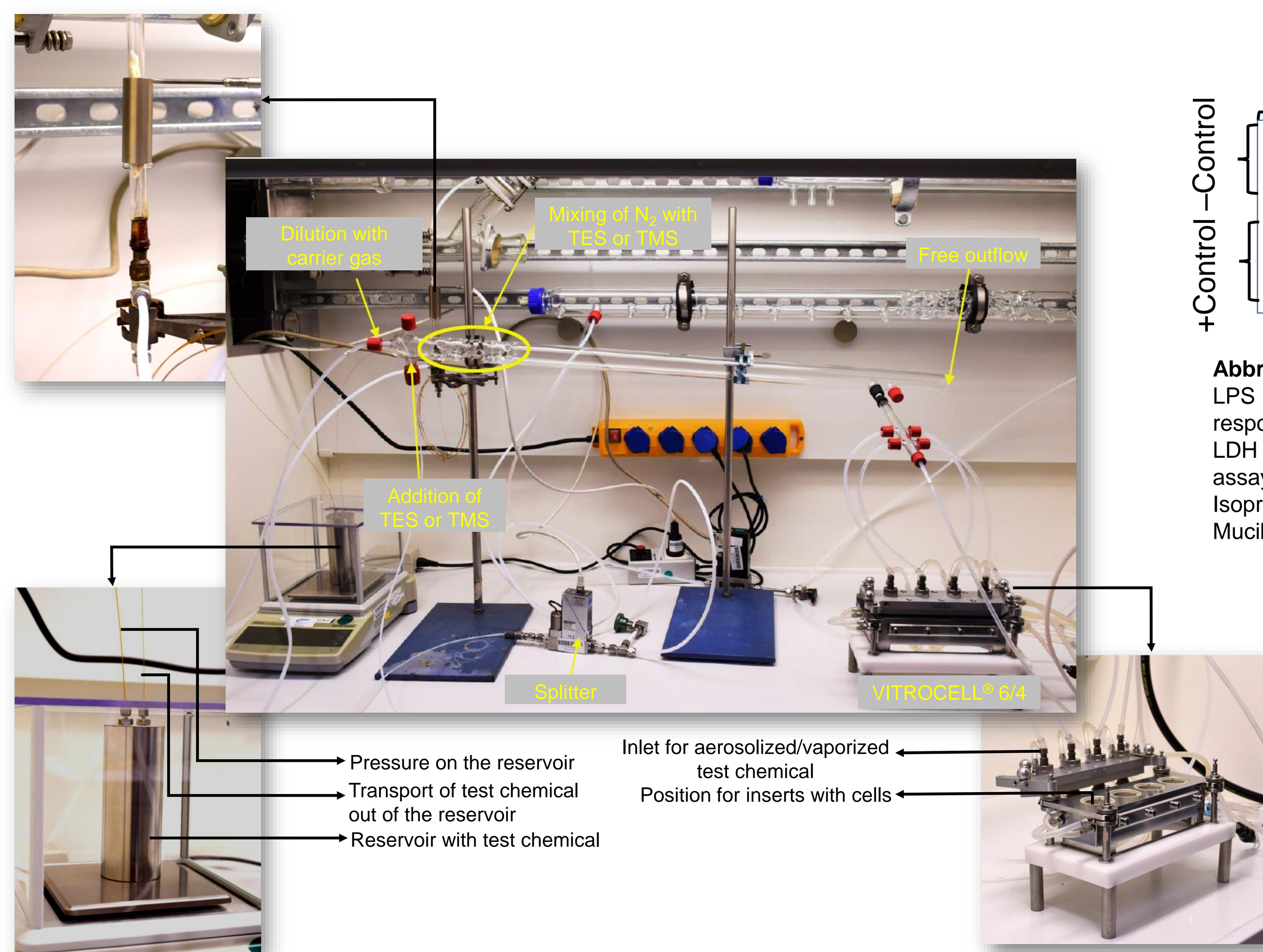
MucilAir™: 3D Human bronchial epithelial tissue model

Endpoints:

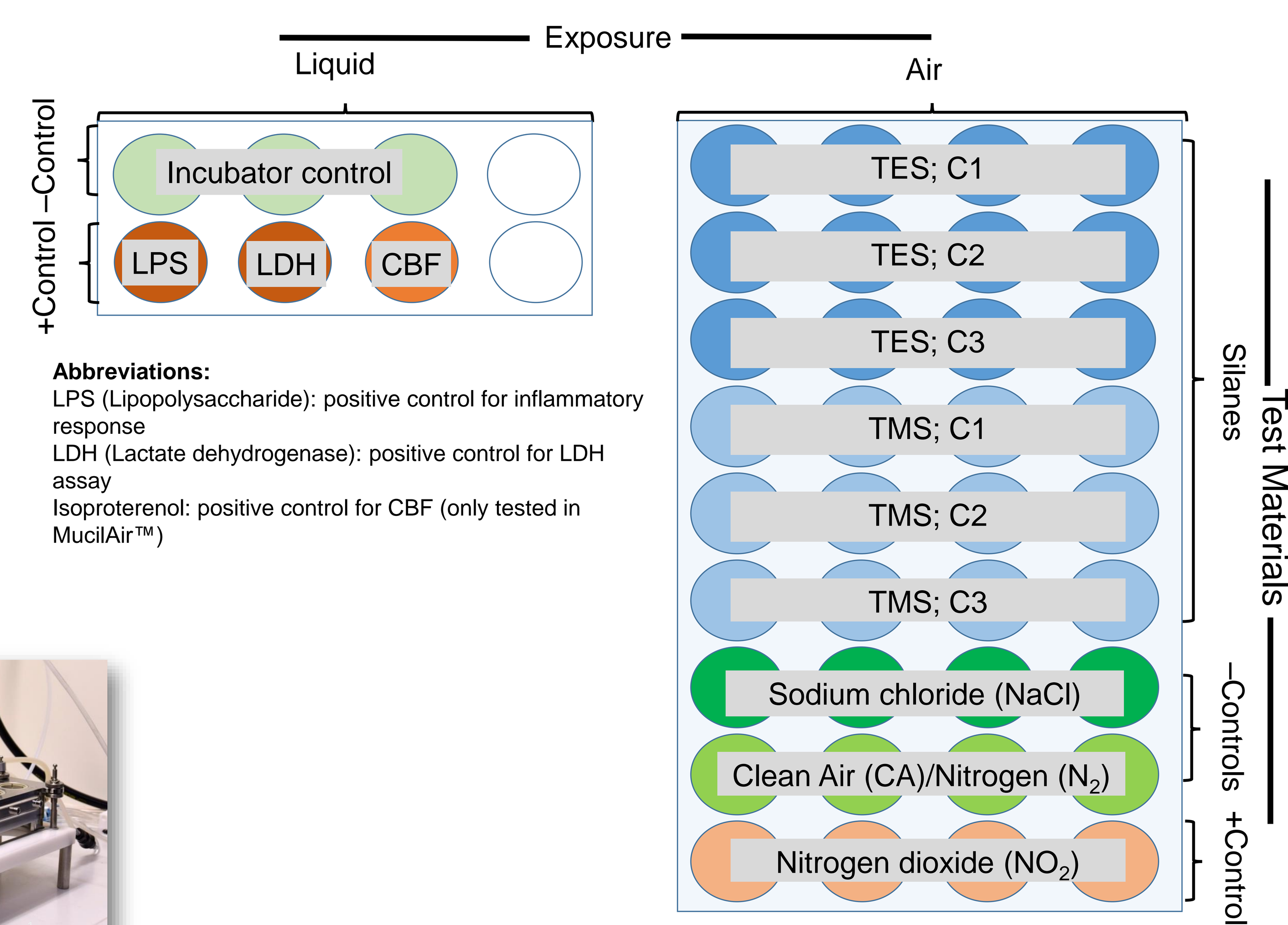
- Cell viability (PrestoBlue®)
- Cytotoxicity (LDH)
- Cilia beat frequency (CBF)
- Morphology (H&E staining)
- Barrier integrity (TEER)
- Inflammatory markers (IL-2, IL-6, IL-8, TNF-α)



Exposure set-up for silanes



Experimental set-up for silanes



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

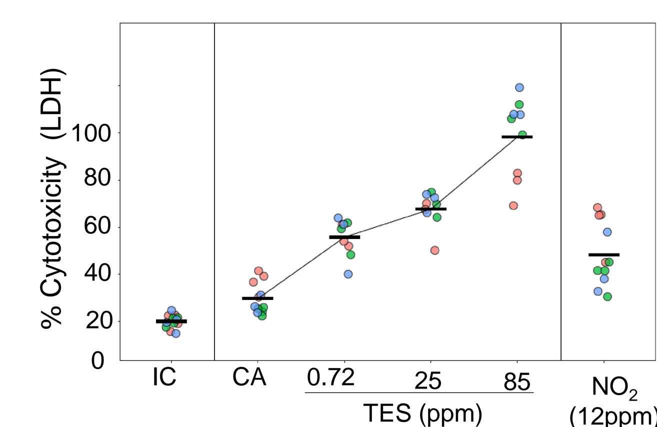
Project details

		Differences between project phases
Phase1 (Completed)	Assess the respiratory toxicity of TES in BEAS-2B cells	
Phase 2 (Ongoing)	Assess the respiratory toxicity of silanes and surfactants in BEAS-2B cells	**Key differences between Phase 1 and Phase 2:** • Reduce 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 cytokines (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 respiratory 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

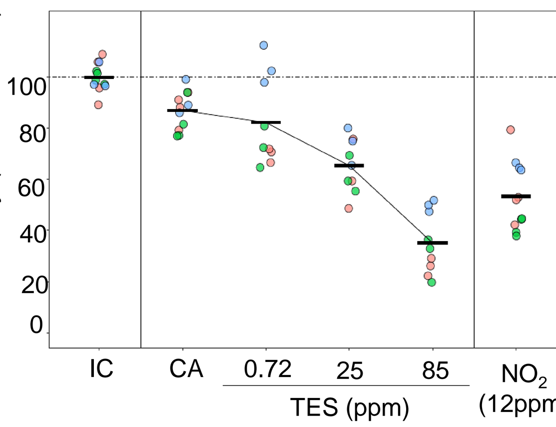
Results

Phase 1 (Complete)

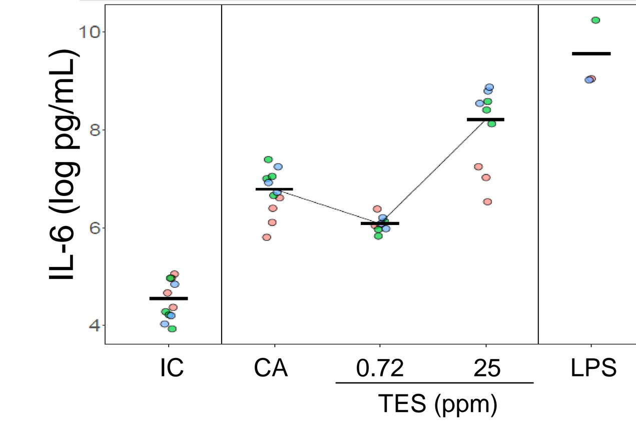
Cytotoxicity (LDH release, 30 minutes post-exposure)



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



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

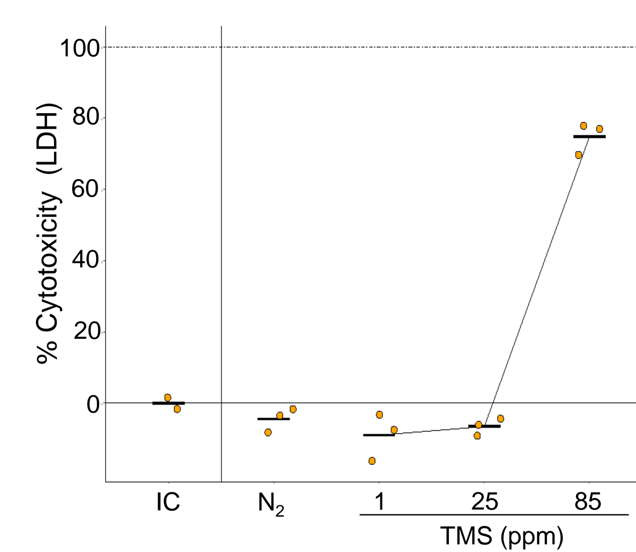


Observations

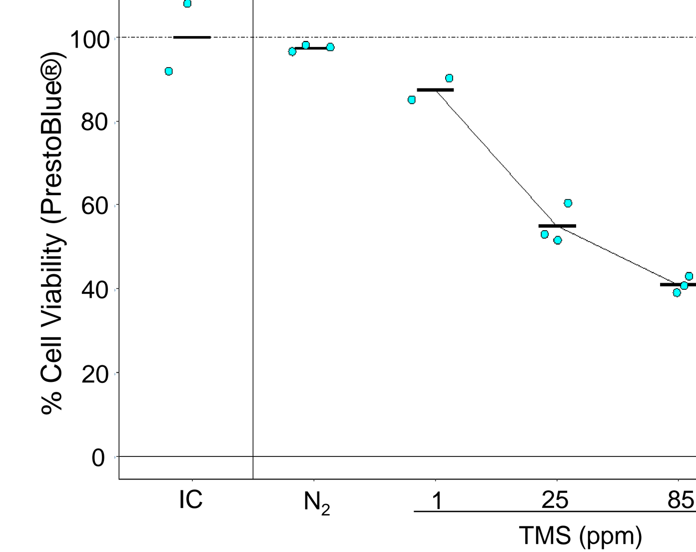
- A concentration-dependent cytotoxicity and cell viability response was observed
- A statistically significant release of IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and TNF-α was observed after exposure of BEAS-2B cells to 25 ppm TES compared to CA

Phase 2 (Ongoing)

Cytotoxicity (LDH release, 30 minutes post-exposure)



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

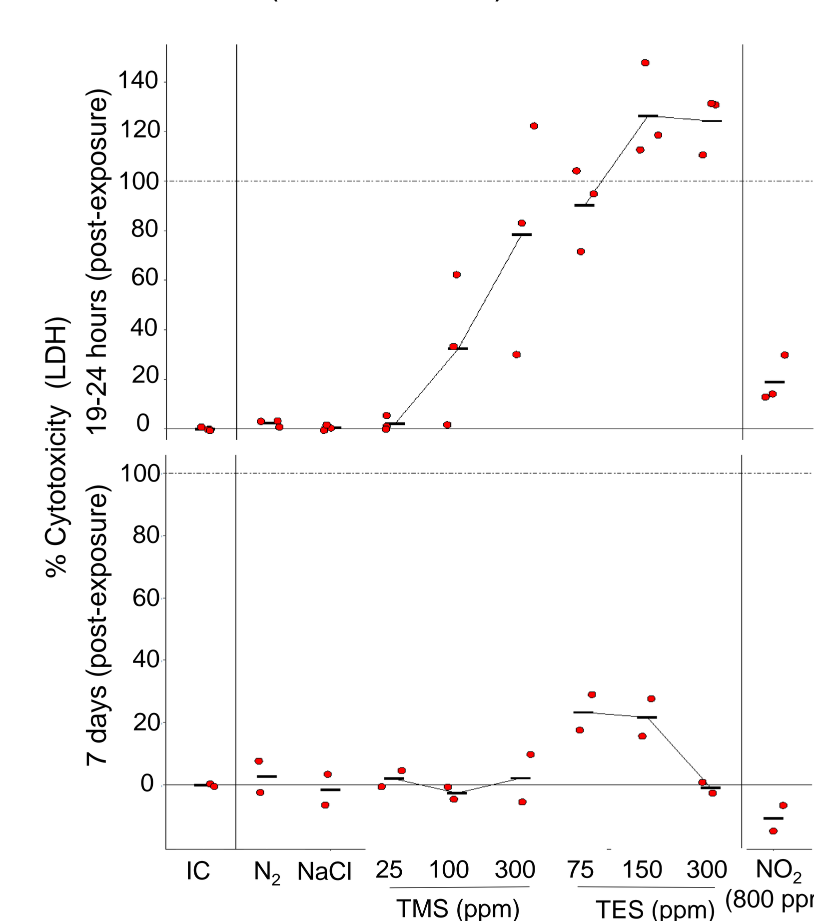


Observations and next steps

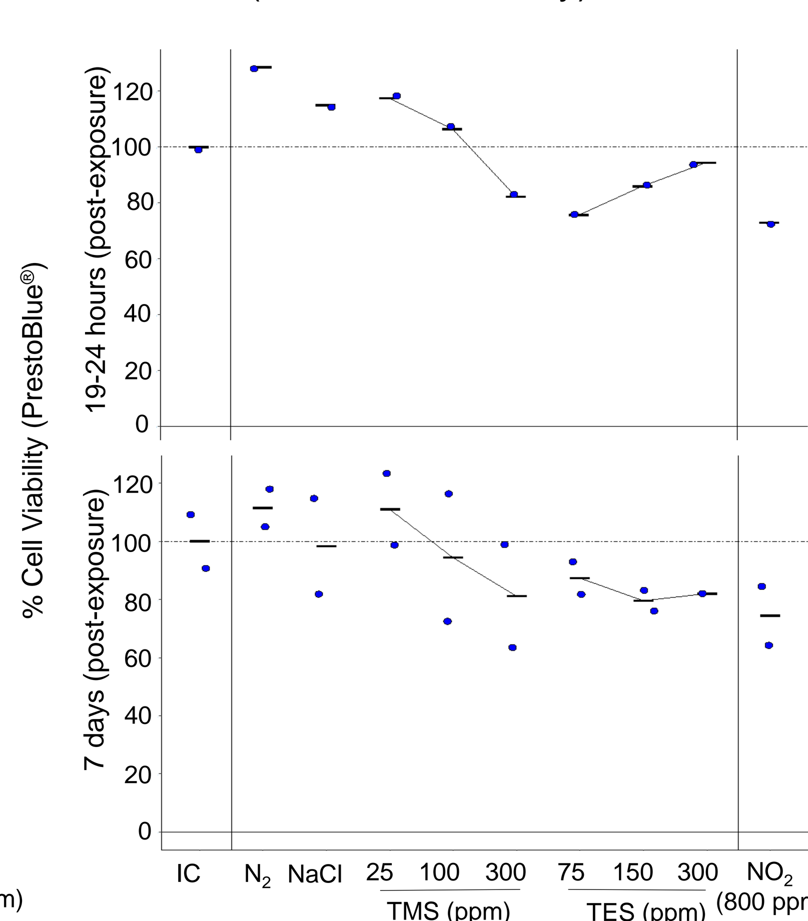
- Results presented are from one experimental run
- A concentration-dependent cytotoxicity and cell viability response was observed but this experiment will be repeated 2 more times to establish statistical significance
- Next steps involve testing TES and surfactants

Phase 3 (Ongoing)

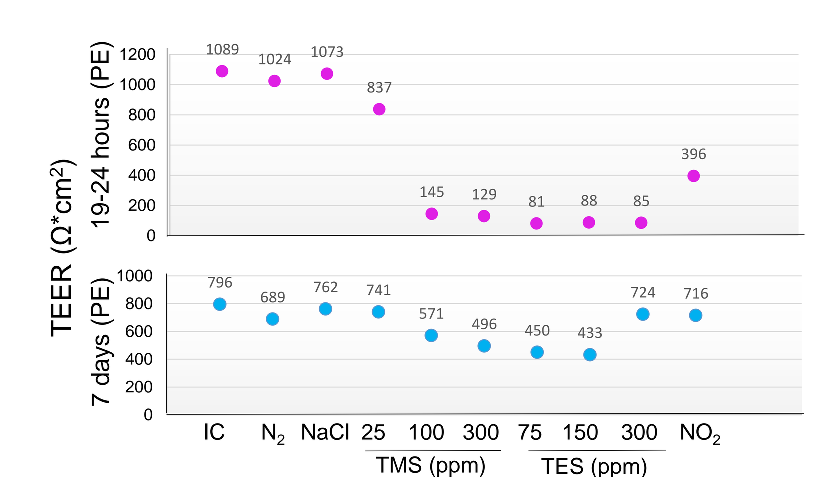
Cytotoxicity (LDH release)



Cell viability (Prestoblue® assay)



Barrier integrity (TEER)



Observations and next steps

- Results presented are from one experimental run
- We observed variability, potentially as a result of issues with consistent exposure. Work is ongoing to troubleshoot variability
- Next steps include repeating this experiment and testing additional chemicals

Discussion

The goals of this study are to show how *in vitro* assays can be used to provide information about potential human health effects and to guide future study design by better understanding the value of testing these chemicals in a 2D versus a 3D model system and of assessing various endpoints.