Human cell-based in vitro systems to assess respiratory toxicity of chemicals

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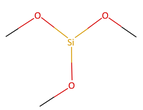
Abstract

Inhalation is a major route by which human exposure to substances can occur. Resources have therefore been dedicated to optimize human-relevant *in vitro* approaches that can accurately and efficiently predict the toxicity of inhaled chemicals for robust risk assessment and management. In this study, called the INSPiRE Initiative (*IN vitro* System to Predict REspiratory toxicity), two- and three-dimensional (2D and 3D) systems were used to predict the ability of chemicals to cause portal-of-entry effects on the human respiratory tract. Human bronchial epithelial cell line (BEAS-2B) and reconstructed tissue model (MucilAir™, Epithelix) were exposed to silanes (triethoxysilane (TES, Globally Harmonized System classification (GHS) 2) and trimethoxysilane (TMS, GHS 1)) at the air-liquid interface in a VITROCELL® 6/4 system. Nitrogen dioxide was included as a positive control, and sodium chloride (0.9%), incubator control, or nitrogen gas (N_2) as negative controls. Endpoints assessed include cell viability (Prestoblue™ assay), cytotoxicity (lactate dehydrogenase assay), and secretion of inflammatory markers (electrochemiluminescence immunoassay, Meso Scale Discovery) and, for the 3D tissues, morphology (hematoxylin and eosin staining), barrier integrity (transepithelial electrical resistance), and cilia beating frequency (SAVA system) were also examined. The results show a concentration-dependent decrease in cell viability and an increase in cytotoxicity after 30 minutes and 19-24h exposure of BEAS-2B cells to TES (1, 50, and 150 ppm) and TMS (1, 25, and 85ppm) as compared to N_2. An increase in secretion of inflammatory markers (including interleukin (IL)-2, IL-6, IL-8, and tumor necrosis factor-alpha) was observed for both silanes in BEAS-2B cells at middle concentrations with no change at the lowest concentration (1ppm). In 3D tissues, a concentration-dependent decrease in cell viability and increase in cytotoxicity was observed at 19-24h post exposure. The active cilia beating areas and barrier integrity were also reduced in a concentration-dependent manner at 19-24h post exposure. Studies are underway to assess additional test chemicals in both systems. The results of this project can be used to better understand the usefulness of different test systems and to predict the likelihood of a chemical to cause portal-of-entry effects on the human respiratory tract and inform regulatory decision-making.

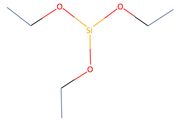
Project details

Phase1	**Pilot study assessing TES in BEAS-2B cells**	**Purpose of Phase 1:** • To generate preliminary data to optimize study design
Phase 2	**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 to the apical side 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 (4 single donors (not pooled)) • Assessing additional endpoints (TEER, CBF, and histology) • Adding 7 day recovery period

Test chemicals: Silanes



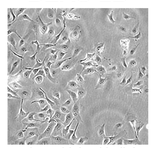
Trimethoxysilane
(TMS, GHS 1, CAS# 2487-90-3)



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

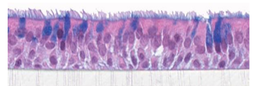
Test systems and endpoints

BEAS-2B



Human bronchial epithelial cell line

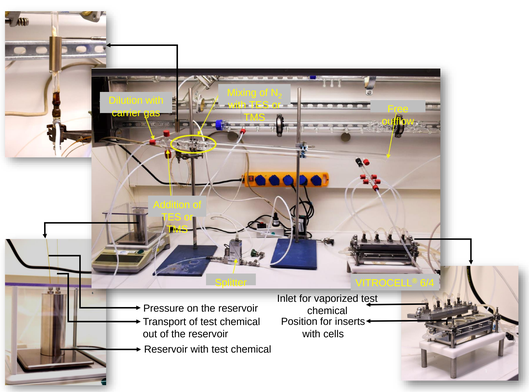
MucilAir™



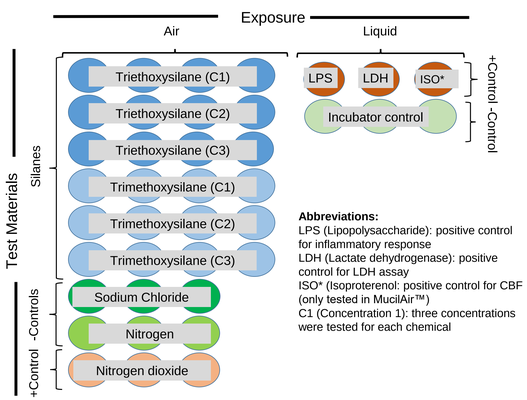
3D Human bronchial epithelial tissue model

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

Exposure set-up



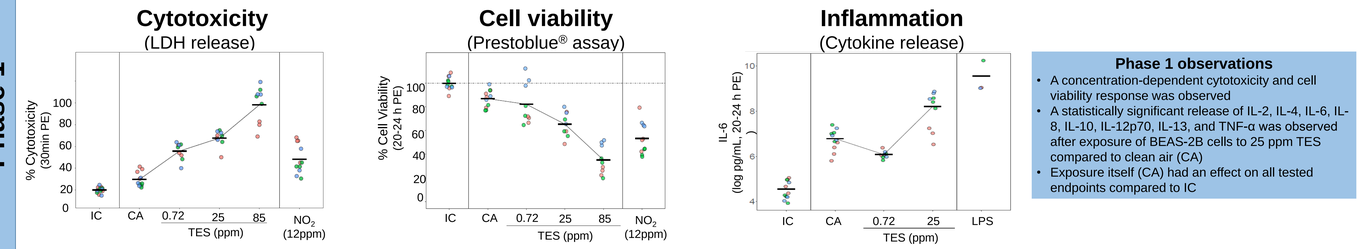
Experimental set-up



Abbreviations:
LPS (Lipopolysaccharide): positive control for inflammatory response
LDH (Lactate dehydrogenase): positive control for LDH assay
ISO* (Isoproterenol: positive control for CBF (only tested in MucilAir™)
C1 (Concentration 1): three concentrations were tested for each chemical

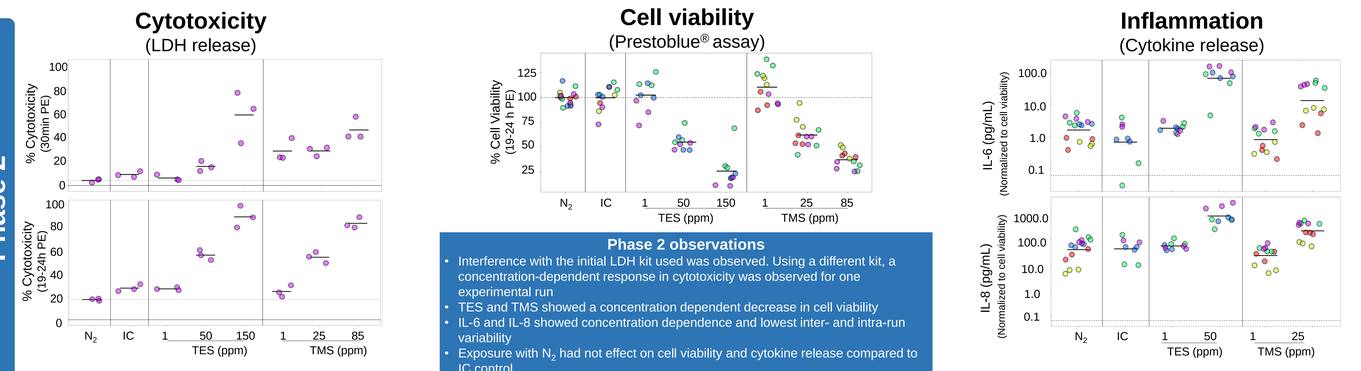
Phase 1

Results



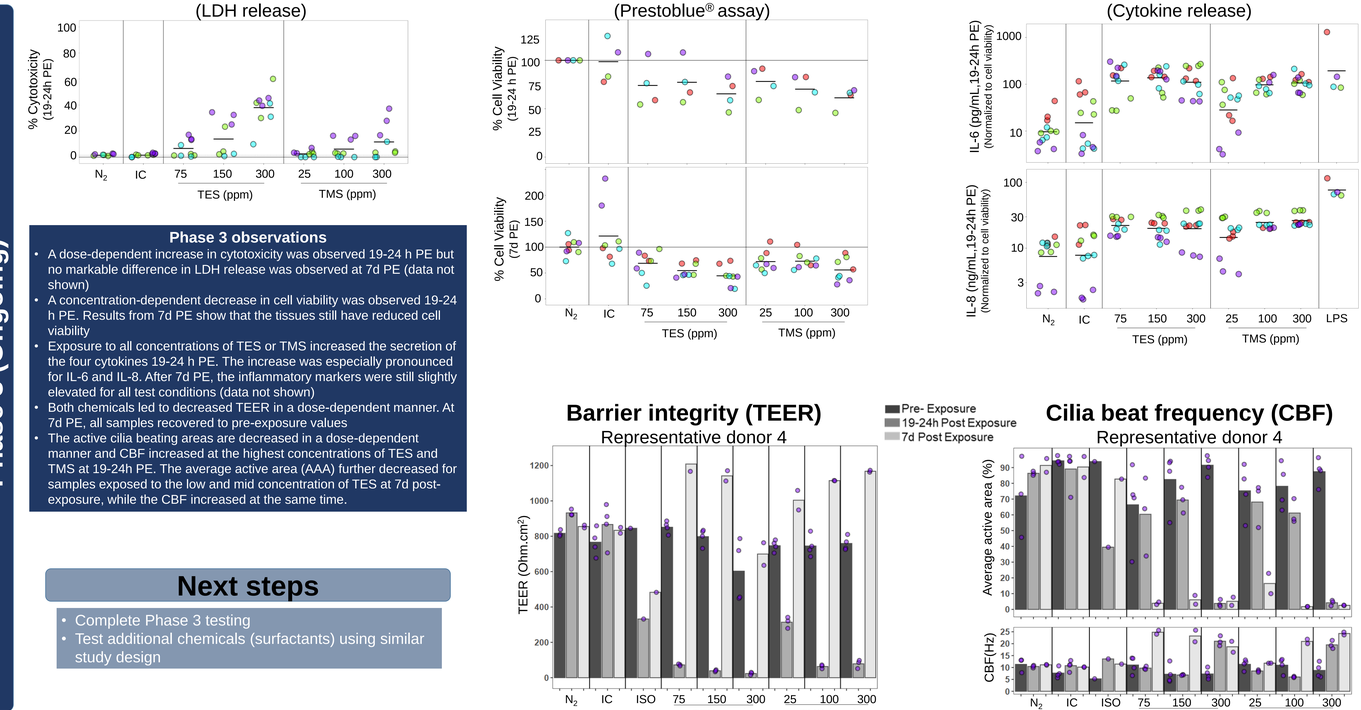
Phase 1 observations
- A concentration-dependent cytotoxicity and cell viability response was observed
- A statistically significant release of IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and TNF-α was observed after exposure of BEAS-2B cells to 25 ppm TES compared to clean air (CA)
- Exposure itself (CA) had an effect on all tested endpoints compared to IC

Phase 2



Phase 2 observations
- Interference with the initial LDH kit used was observed. Using a different kit, a concentration-dependent response in cytotoxicity was observed for one experimental run
- TES and TMS showed a concentration dependent decrease in cell viability
- IL-6 and IL-8 showed concentration dependence and lowest inter- and intra-run variability
- Exposure with N_2 had not effect on cell viability and cytotoxicity release compared to IC control

Phase 3 (Ongoing)



Phase 3 observations
- A dose-dependent increase in cytotoxicity was observed 19-24 h PE but no markable difference in LDH release was observed at 7d PE (data not shown)
- A concentration-dependent decrease in cell viability was observed 19-24 h PE. Results from 7d PE show that the tissues still have reduced cell viability
- Exposure to all concentrations of TES or TMS increased the secretion of the four cytokines 19-24 h PE. The increase was especially pronounced for IL-6 and IL-8. After 7d PE, the inflammatory markers were still slightly elevated for all test conditions (data not shown)
- Both chemicals led to decreased TEER in a dose-dependent manner. At 7d PE, all samples recovered to pre-exposure values
- The active cilia beating areas are decreased in a dose-dependent manner and CBF increased at the highest concentrations of TES and TMS at 19-24h PE. The average active area (AAA) further decreased for samples exposed to the low and mid concentration of TES at 7d post-exposure, while the CBF increased at the same time.

Next steps

- Complete Phase 3 testing
- Test additional chemicals (surfactants) using similar study design