Two- and three-dimensional human cell-based *in vitro* systems to assess respiratory toxicity of silane vapors

Andreas O. Stucki[1], Monita Sharma[1], Sandra Verstraelen[2], An Jacobs[2], David Poelmans[2], Sylvie Remy[2], Frederick Maes[2], Evelien Frijns[2], Karen Hollanders[2], Lieve Geerts[2], Stefaan Voorspoels[2], Jo Van Laer[2], Amy J. Clippinger[1]

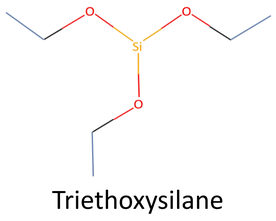
[1]PETA Science Consortium International e.V., Germany; [2]Flemish Institute for Technological Research (VITO), Belgium

Correspondence: AndreasS@thePSCI.eu

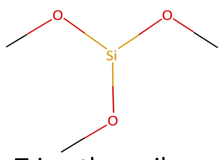
Introduction

Triethoxysilane (TES) and trimethoxysilane (TMS) are known inhalation toxicants that are widely used as reducing and coupling agents with applications in corrosion protection, adhesion promotion, and surface modifications. However, due to their rapid hydrolyzation (seconds in water, minutes in air) and the low vapor pressure (exist solely as vapors at 25°C), conventional, submerged *in vitro* methods cannot be used to assess the respiratory toxicity. In this study, the INSPiRE (*IN vitro S*ystems to Predict REspiratory toxicity) Initiative, two- and three-dimensional (2D and 3D) systems were used to predict the ability of vaporized silanes to cause portal-of-entry effects on the human respiratory tract. The Initiative aims at getting a better understanding of how the use of *in vitro* systems may inform regulatory decision-making.

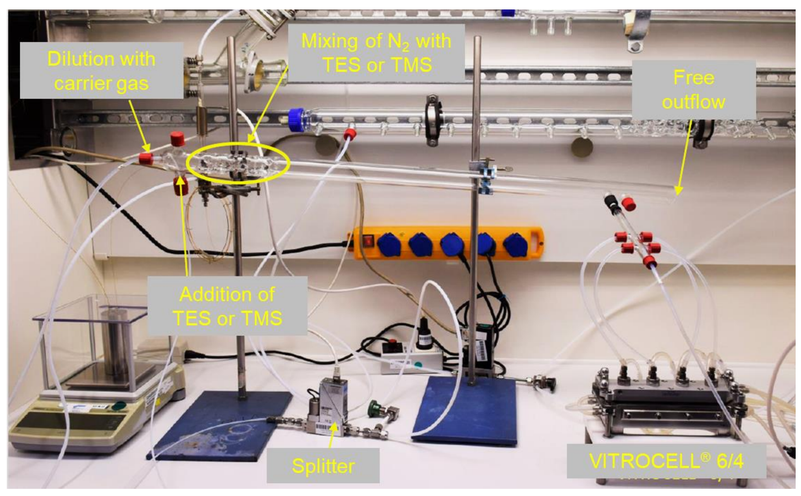
Test chemicals and exposure set-up



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



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

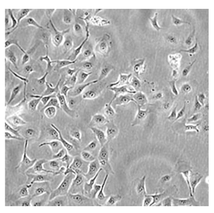


Study design

Exposure at air-liquid interface
30 minutes
↓
Incubation in fresh basal medium
19-24 hours
↓
Assess endpoints
↓
Recovery (MucilAir™ only)
6 days
↓
Assess endpoints

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)		✓

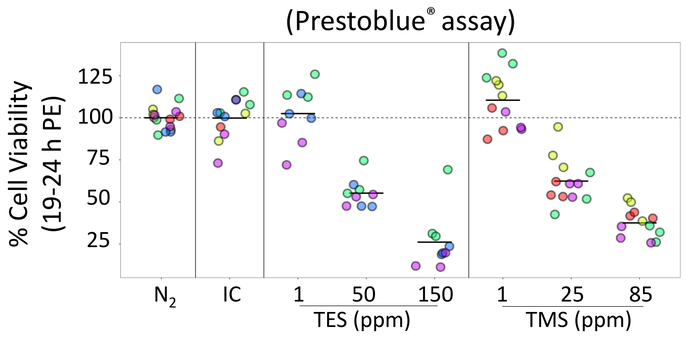
BEAS-2B cells exposed to silane vapors – 2D model



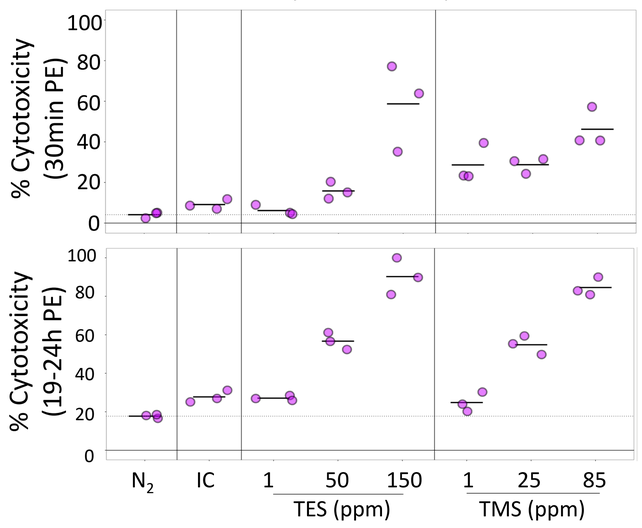
BEAS-2B
Human bronchial epithelial cell line

PE = post-exposure
Different colors = separate experimental runs

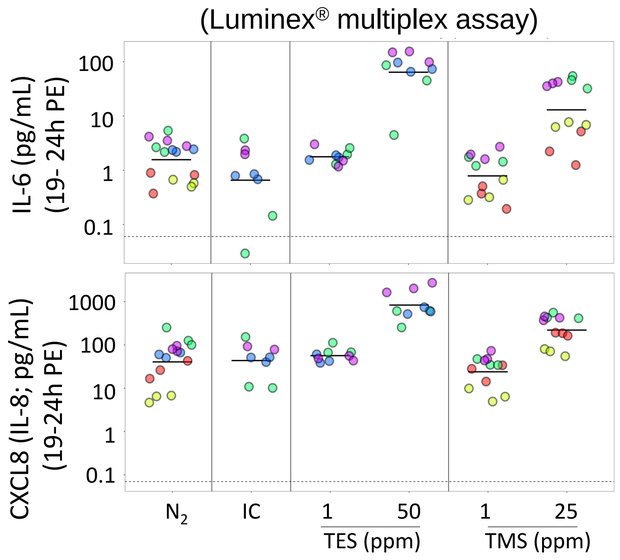
Cell viability
(Prestoblue® assay)



Cytotoxicity
(LDH release)



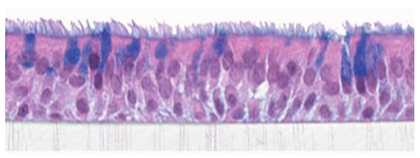
Inflammatory markers
(Luminex® multiplex assay)



Observations – 2D model

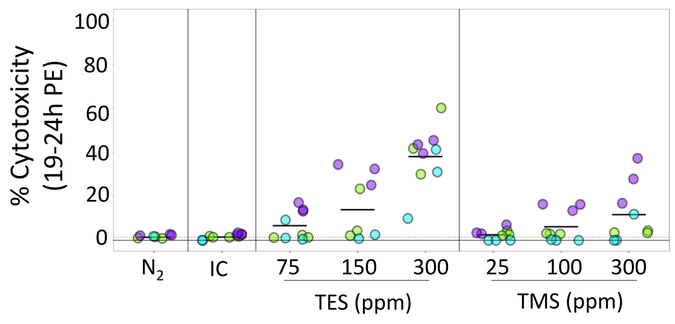
- For both chemicals, no change in cell viability, cytotoxicity, or inflammatory markers was observed at the lowest concentration but concentration-dependent effects were observed at the middle and high concentrations.
- Interference with the initial LDH kit used was observed. Using a different kit, a concentration-dependent cytotoxicity was observed for one experimental run
- Of the four inflammatory markers assessed, IL-6 and CXCL8 (IL-8) were most robust and sensitive.

MucilAir™ tissues exposed to silane vapors – 3D model

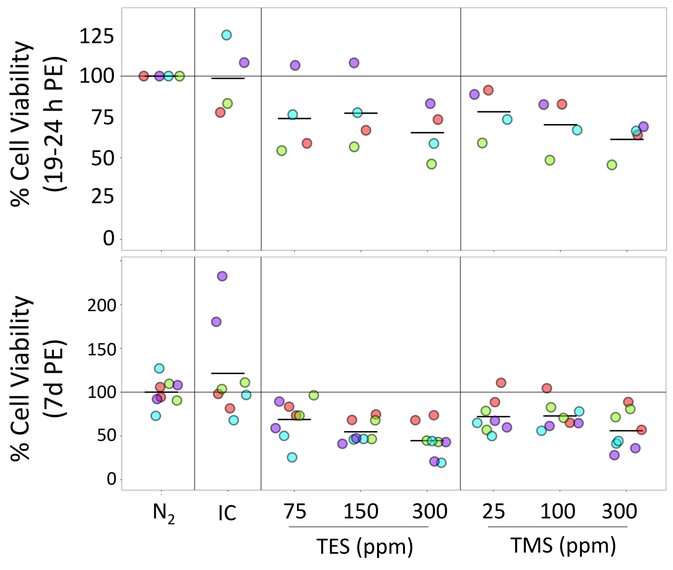


MucilAir™
Reconstructed human bronchial epithelial tissue
(Different colors = 4 donors)

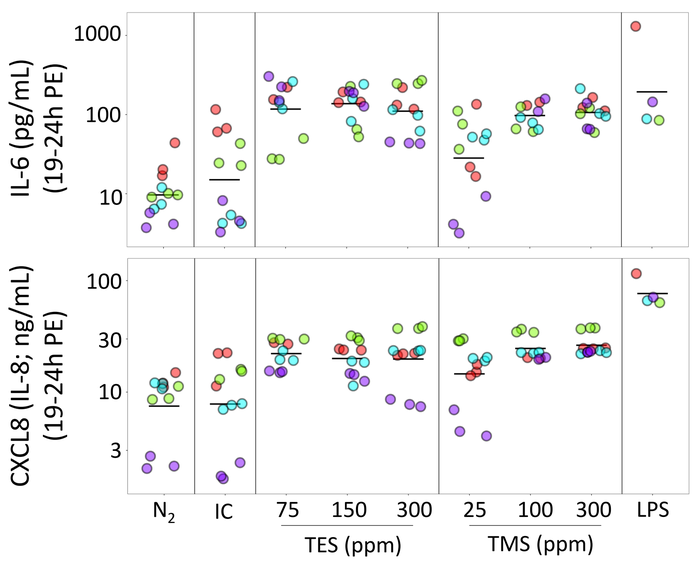
Cytotoxicity (LDH release)



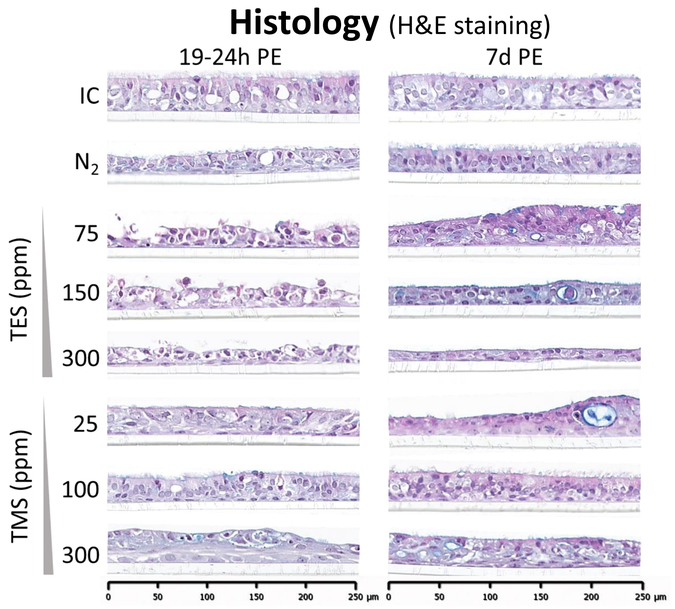
Cell viability (Prestoblue® assay)



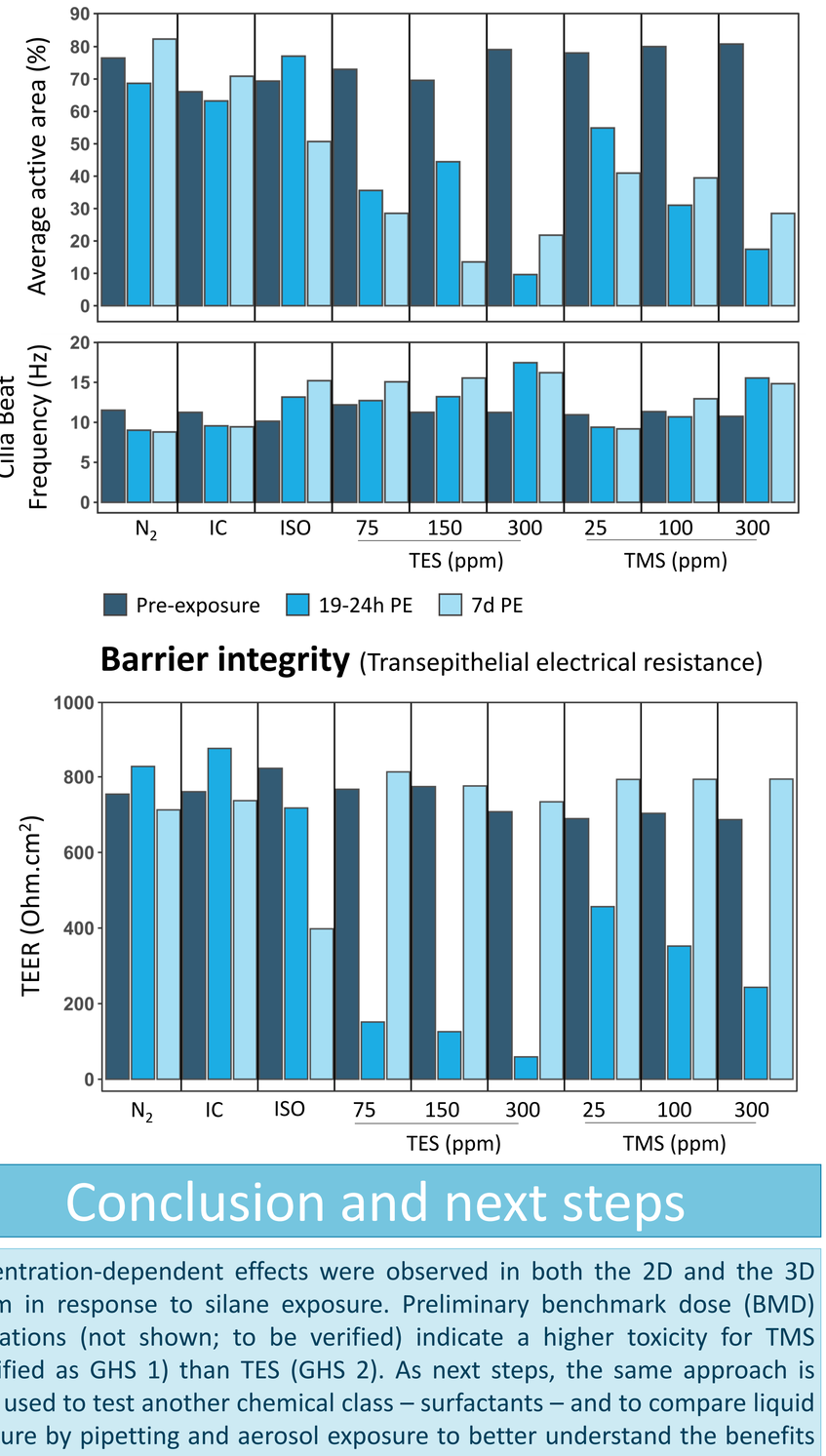
Inflammatory markers (Luminex® multiplex assay)



Histology (H&E staining)



Cilia active area and beat frequency



Barrier integrity (Transepithelial electrical resistance)

Observations – 3D model

- A concentration-dependent increase in cytotoxicity and decrease in cell viability was observed 19-24 h PE. Results from 7d PE show that the tissues still have reduced cell viability but no markable difference in LDH release was observed.
- Exposure to all concentrations of TES or TMS increased the secretion of IL-6 and CXCL8 (IL-8) 19-24 h PE. After 7d, the inflammatory markers were still slightly elevated for all test conditions.
- 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 cilia activity remains low after the recovery period of 7 days.
- Histology revealed mild to severe destruction of cilia and cellular structures at 19-24h PE to TES and TMS. Tissue remodeling occurs during the recovery period of 7 days with some restoration of original tissue structures.

Conclusion and next steps

Concentration-dependent effects were observed in both the 2D and the 3D system in response to silane exposure. Preliminary benchmark dose (BMD) calculations (not shown; to be verified) indicate a higher toxicity for TMS (classified as GHS 1) than TES (GHS 2). As next steps, the same approach is being used to test another chemical class – surfactants – and to compare liquid exposure by pipetting and aerosol exposure to better understand the benefits of each method. Eventually, efforts will be taken to come up with human-equivalent concentrations