Epithelix

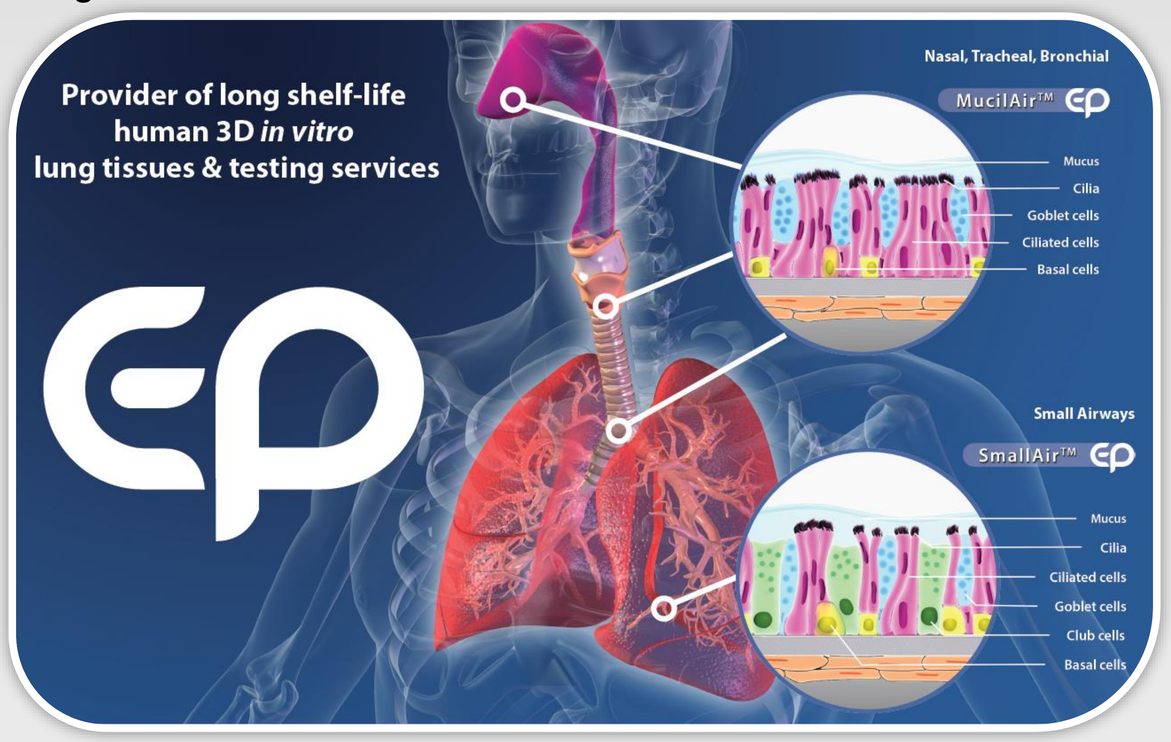
in vitro Solutions for Respiratory Diseases and Chemical Testing

Respiratory irritants cause reversible up-regulation of pro-inflammatory cytokines on human nasal mucosa reconstituted *in vitro*

Faten Hussein (1), Bernadett Boda (1), Ludovic Wiszniewski (1), Song Huang (1) & Samuel Constant (1)
(1) Epithelix Sàrl, 18 chemin des aulx, 1228 Plan-les-Ouates, Geneva, Switzerland

Respiratory irritants are considered as substances of higher risk, at the same level as carcinogens, mutagens and toxic chemicals for reproduction. However, until now there is no validated *in vitro* cell model for identifying the respiratory chemical irritants. The aim of this study is to develop an *in vitro* cellular assay for identification of respiratory chemical irritants based on human 3D nasal airway epithelium (MucilAir™). Epithelia were reconstituted with primary human nasal cell polled from 14 donors. MucilAir™ is not only morphologically and functionally differentiated; but it can also remain at a homeostatic state for more than one year, allowing repeated dose and long term toxicity testing.

11 chemical compounds belonging to 3 classes (irritants ”H335”, Fatal if inhaled ”H330”, and non-toxic chemicals through inhalation) were tested. The cytotoxic effects of these chemicals were assessed by several endpoints: TEER measurement, cilia beating monitoring, LDH release, morphological observation, etc... Pro-inflammatory cytokines, IL-8 and IL-6 were used as biomarkers for discriminating these molecules. Interestingly, at sub-toxic doses, only the respiratory irritants up-regulated reversibly the secretion of IL-8 and IL-6 upon acute challenge.



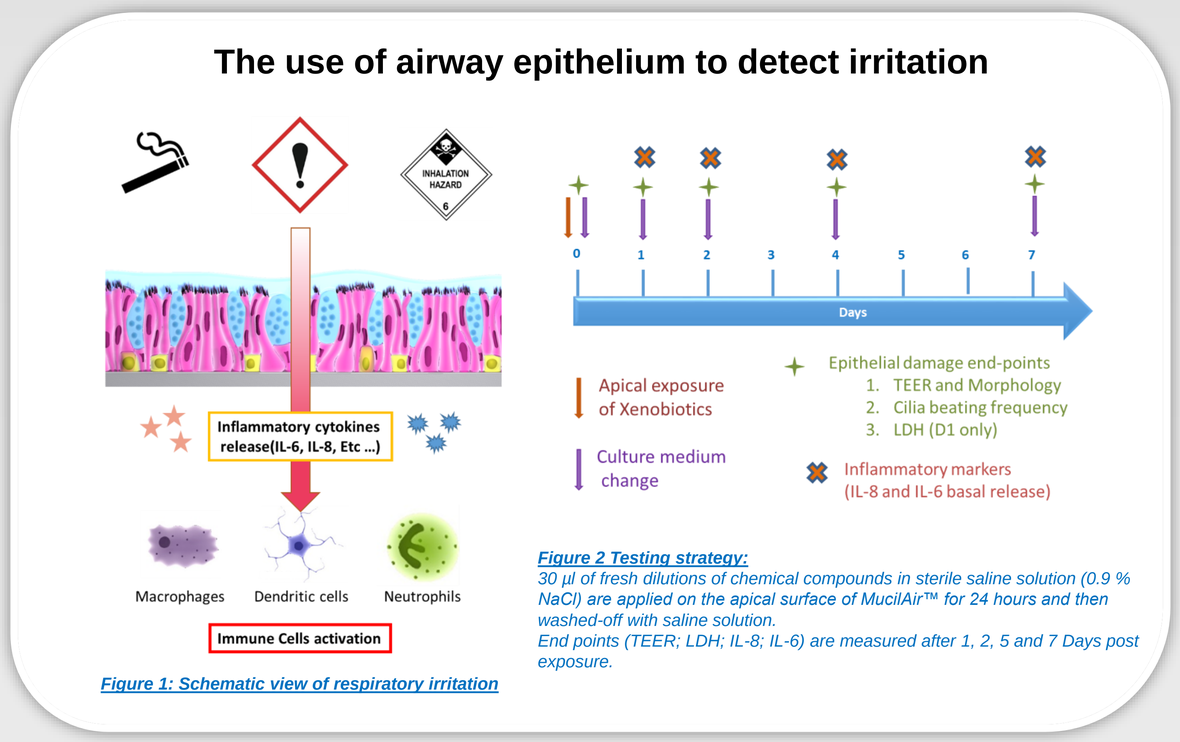


Figure 1: Schematic view of respiratory irritation

Figure 2 Testing strategy:
30 µl of fresh dilutions of chemical compounds in sterile saline solution (0.9 % NaCl) are applied on the apical surface of MucilAir™ for 24 hours and then washed-off with saline solution.
End points (TEER; LDH; IL-8; IL-6) are measured after 1, 2, 5 and 7 Days post exposure.

Sub-toxic dose selection

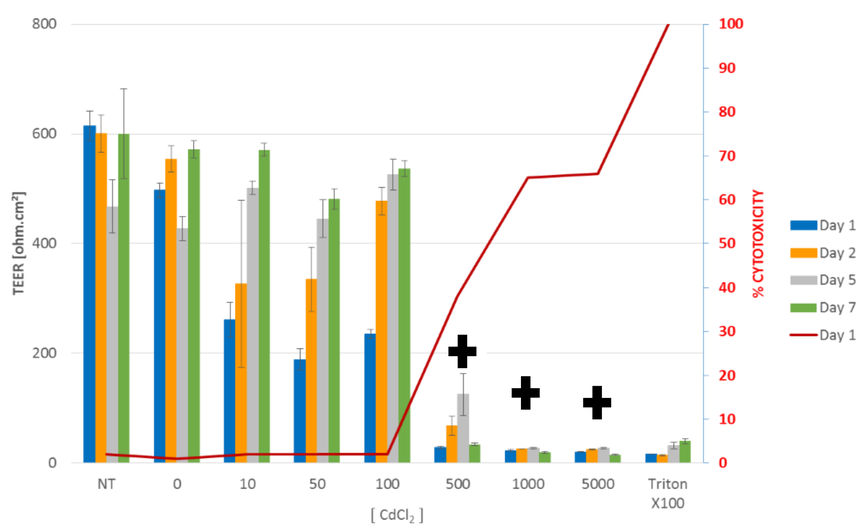


Figure 3: *Representative selection of toxic doses of $CdCl_2$ on MucilAir™ based on TEER and LDH release (N=3; data represent mean values ± sem).*

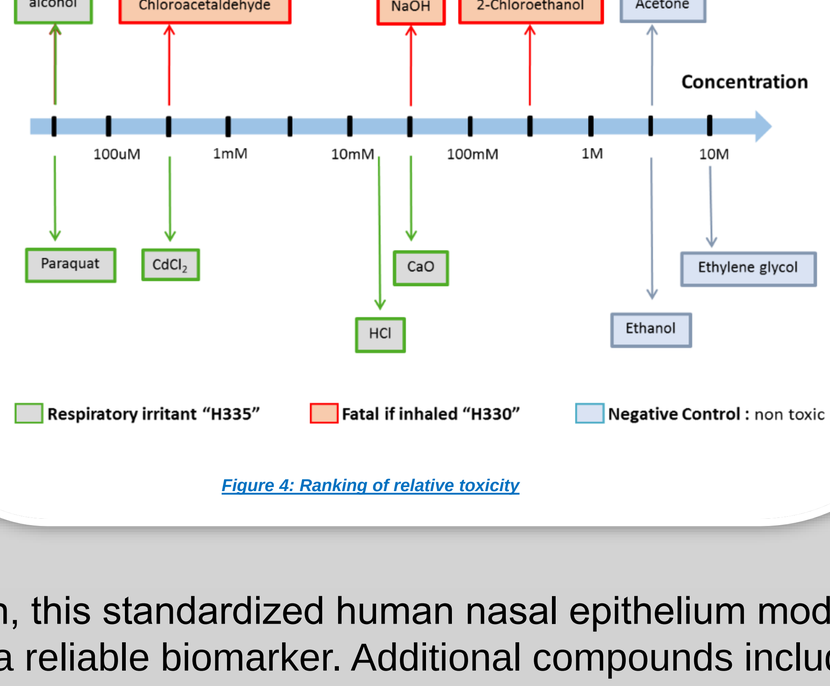


Figure 4: Ranking of relative toxicity

Detection of irritation markers

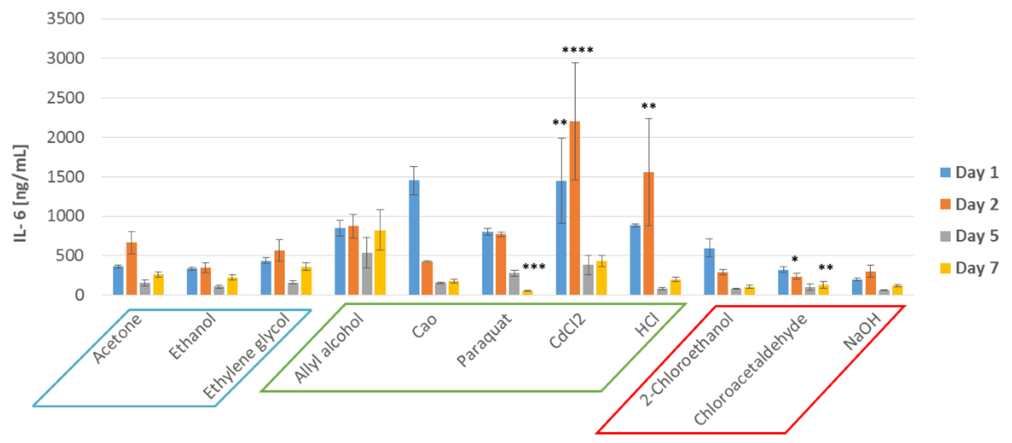


Figure 5: *Comparative IL-6 release of non-toxic vs respiratory irritants and toxic non-irritants compounds at the highest tolerated dose after 1, 2, 5 and 7 Days post exposure (N=3; data represent mean values ± sem).*

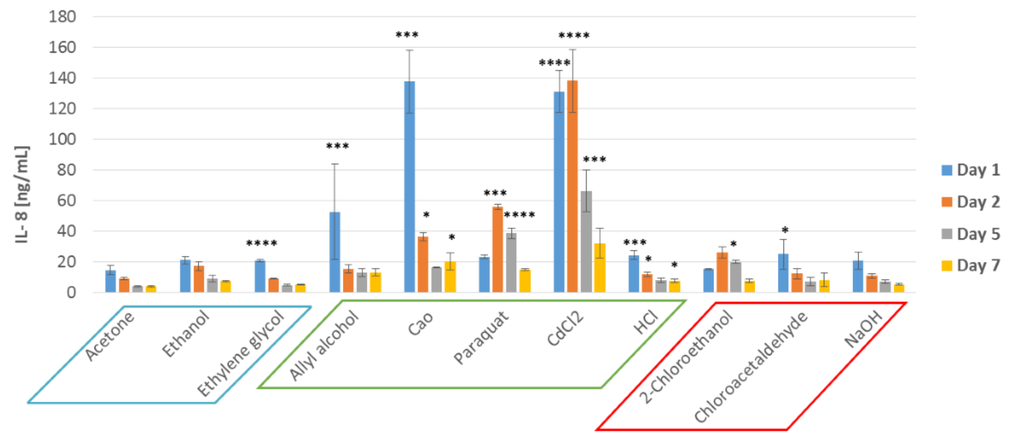


Figure 6: *Comparative IL-8 release of non-toxic vs respiratory irritants and toxic non-irritants compounds at the highest tolerated dose after 1, 2, 5 and 7 Days post exposure (N=3; data represent mean values ± sem).*

Conclusion

As conclusion, this standardized human nasal epithelium model MucilAir™ is a promising platform for identifying the respiratory irritants and IL-8 seems to be a reliable biomarker. Additional compounds including drug compounds will be tested in the future to further validate this novel approach.