CS8: The popcorn lung – overview and experimental challenges
Tanja Hansen1, Jan Boel2, Harry Vrieling2, Johannes Schimmin2, Stefanie Klima2, Sylvia Escher1, Katherina Sewald1, Jan Knebel1, Detlef Ritter2

1 Department of Preclinical Pharmacology and In Vitro Toxicology, Fraunhofer ITEM, Hannover, Germany
2 Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands
3 Division of Toxicology, Leiden University, The Netherlands
4 In vitro Toxicology and Biomedicine, Faculty of Science, University of Konstanz

Background
The alpha-diketone diacetyl (2,3-butanedione) was defined as lead compound of this case study. The compound is known to induce the so-called “popcorn lung” which was frequently observed among microwave popcorn manufacturing employees who inhaled the butler flavor vapor of diacetyl.

Bronchiolitis obliterans (BO) is a disease that results in obstruction of the smallest airways of the lungs (bronchioles) due to inflammation. The airway epithelium is initial target of injury. Alpha-diketones are known to have a high electron affinity and are able to transfer electrons which lead to ROS production and oxidative stress. Beta-diketones induce effects in the respiratory tract after inhalation exposure but also neurotoxic effects. Gamma-diketones are known to form pyrroles with amines (via nucleophilic addition). This step is assumed to be the obligatory for the expression of gamma-diketone neurotoxicity.

Submerse test systems
Peripheral neurons from iPSC - Neurite growth assay

Case Study Compounds
α- diketones
β- diketones
γ- diketones

• Use NAMs to reduce the uncertainty of a read-across approach e.g. by providing data on a shared ADP/mode of action.
• How far are selected NAMs able to differentiate the α, β, and γ diketone specific toxicity?

Air-liquid exposure strategy
Primary bronchial epithelial cells (PBECs) and precision cut lung slices (PCLuS) are exposed at the air-liquid interface using the P.R.I.T.® ExpoCube® exposure device. For exposures to gases or vapours, it seems reasonable to evaluate the applied dose during such experiments based on the general calculation of the dosage as

\[ D = c \times t \times Q \]

with the dosage D, the concentration c, the exposure time t and the exposure volume flow Q, which quantifies the flow that is conducted over the surface of the exposed cells or tissues.

Mechanism of diacetyl-induced airway injury

Normal
Injury, inflammatory response
Repair response, fibroblast proliferation
Extracellular matrix deposition
BO/Fibrosis

Innate and adaptive immune responses
Cytotoxic stress, DNA damage
Repair response, fibroblast proliferation
Extracellular matrix deposition

Experiments with PBECs and PCLuS target the first steps at the level of epithelial injury and inflammatory response.

Readouts:
• Cytotoxicity (e.g. LDH, WST)
• Epithelial integrity (TEER)
• Cytokine release
• Gene expression (TempOSeq)

CAVE: High volatility of the case study compounds leads to dosing uncertainties → qualitative data only