CS8: The popcorn lung – overview and experimental challenges

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Background

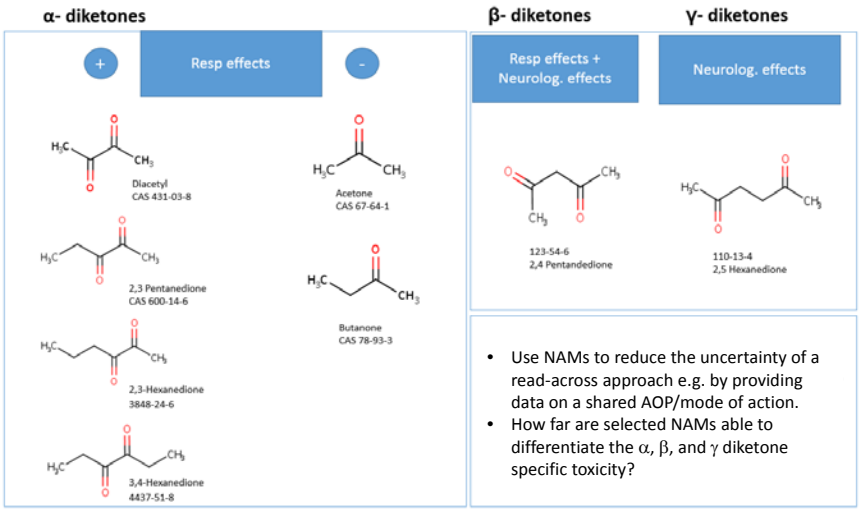
The alpha-diketone diacetyl (2,3- butanedione) was defined as lead compound of this case study. The compound is known to induce the so called "**popcorn lung**" which was frequently observed among microwave popcorn manufacturing employees who inhaled the butter flavor vapor of diacetyl.

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 steps is assumed to be the obligatory for the expression of gamma-diketone **neurotoxicity**.

Case Study Compunds

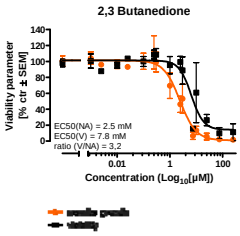


- Use NAMs to reduce the uncertainty of a read-across approach e.g. by providing data on a shared AOP/mode of action.
- How far are selected NAMs able to differentiate the α, β, and γ diketone specific toxicity?

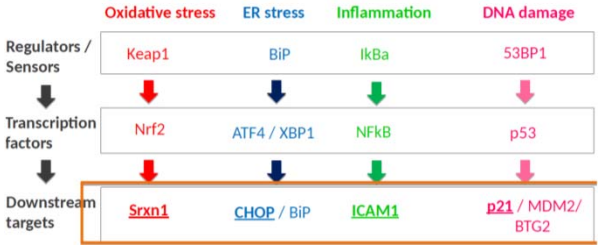
Submerse test systems

Peripheral neurons from iPCS - Neurite growth assay

2,3-butanedione	Ratio (V/NA) = 3.2	specific
2,3-pentandione	Ratio (V/NA) = 6	specific
2,3-hexandione	Ratio (V/NA) = 3.9	specific
3,4-hexandione	Ratio (V/NA) = 2.4	unspecific
2,4-pentandedione	Ratio (V/NA) = 7.3	specific
2,5-hexanedione	Ratio (V/NA) = 5.3	specific
Acetone	Ratio (V/NA) = 1	unspecific
Butanone	Ratio (V/NA) = 1	unspecific



Stress pathway responses in HepG2 cells



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

Air-liquid exposure strategy

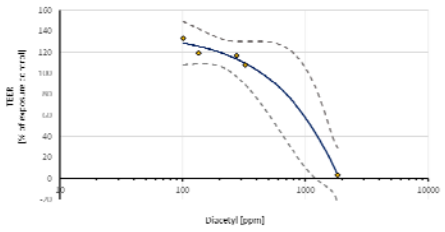
Primary bronchial epithelial cells (PBECs) and precision cut lung slices (PCLuS) are exposed at the air-liquid interface using the P.R.I.T.® ExpoCube® exposure device.

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

$$D = c \times t \times Q$$

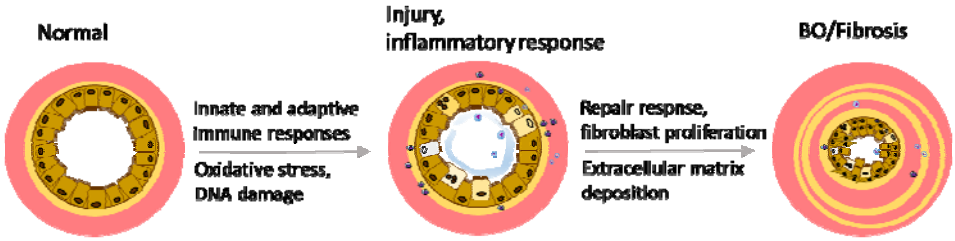
with the dosage D, the concentration c, the exposure time t and the exposure volume flow Q, which quantifies the flow that is conducted over the surface of the exposed cells or tissues.

P.R.I.T.® ExpoCube® exposure device



Reliable dose-response relationship

Mechanism of diacetyl-induced airway injury



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)

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www.eu-toxrisk.eu/