Acute inhalation toxicity *in-vitro* and *ex-vivo* test battery prior to regulatory OECD 403 studies

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Objective

New crop protection agents are usually tested for acute inhalation toxicity in-vivo according to OECD Test Guideline 403. In order to reduce the amount of animals and test compound needed, this project aimed at the implementation of ex-vivo and in-vitro screening tests that can be used to estimate the acute inhalation toxicity prior to regulatory OECD 403 studies. Therefore, two reference compounds with known toxicity, chlorothalonil (CAS 1897-45-6) and mancozeb (CAS 8018-01-7), were tested applying two complementary methods: the rat isolated perfused lung (IPL) model and in-vitro using human lung epithelial cells under air-liquid-interphase (ALI) conditions with the P.R.I.T.®-ALI Technology.

Methods

Cell culture model: The A549 human lung cell line was purchased from a commercial supplier (ATCC; LGC Promochem). Cells were routinely taken from a stock pool and grown in 75cm² flasks by use of Dulbecco´s MEM medium (Seromed, Berlin) supplemented with 10% FCS and antibiotics. During a cell passage an aliquot of the cells was then seeded on microporous membranes (Inserts, BD Falcon; 0.4µm pore size; growth area ~1cm²).

Cell Exposure: Air–lifted interface cultures from A549 cells were exposed to the test substances using the P.R.I.T.® ExpoCube®.

WST-1 assay: Cells were analyzed with regard to viability (WST-1) after a 24h post-exposure re-incubation period under cell-specific conditions inside an incubator.

Rat isolated perfused lung (IPL) model: Lungs were prepared from Male Wistar Han (Crl:WI(Han)) rats (Charles River, Sulzfeld, Germany) and positioned in a commercial IPL system (Hugo Sachs Elektronik –Harvard Apparatus GmbH). To test the potential of the test items to induce acute pulmonary toxicity, IPLs were exposed to increasing concentrations of the test items (3 to 3000mg/m³). In order to determine pulmonary viability, the respiratory parameters tidal volume (V_T), resistance (RL), dynamic lung compliance (Cdyn) and the relative increase in lung weight were determined in IPL experiments.

Aerosolization of the test compounds: Generation of aerosols for the in-vitro and ex-vivo model was carried out using the PreciseInhale® (Inhalation Sciences AB, Novum, SE) device.

Results

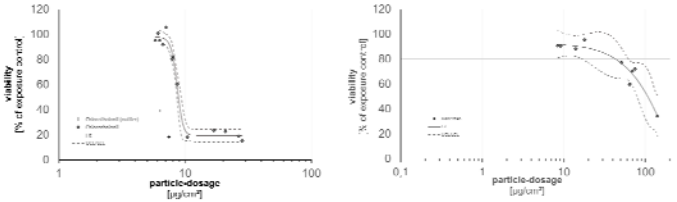

Figure 1: Dose response relationship after exposure of A549 cells to dry particle powder from chlorothalonil (left) and mancozeb (right).

Figure 2: EC_{50} values after exposure of human lung cells (A549) towards dry powder aerosols from the test compounds and analysis of cellular viability in compare to a concurrent clean air exposure control 24 hours after exposure; (EC = Effective Concentration, UCL = Upper Confidence Limit; LCL = Lower Confidence Limit)

[µg/cm²]	Chlorothalonil	Mancozeb
EC_{50} value	8.85	107.12
UCL (95%)	9.30	143.50
LCL (95%)	8.61	79.23

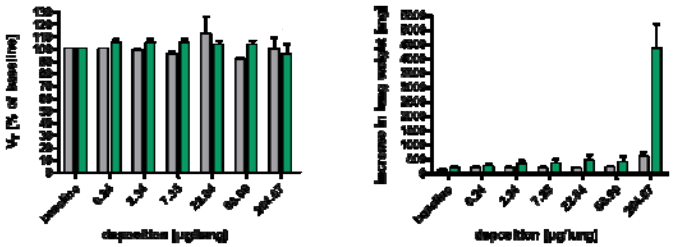

Figure 3: IPLs were exposed to chlorothalonil (green) or clean air control (grey) in increasing concentrations. Tidal volume (V_T) (left) and lung weight (right) were measured at baseline and at the end of every experiment. For every exposure step, V_T and lung weight were measured right before the start of the following exposure. Means + SEM, n=3. deposited dose given as means.

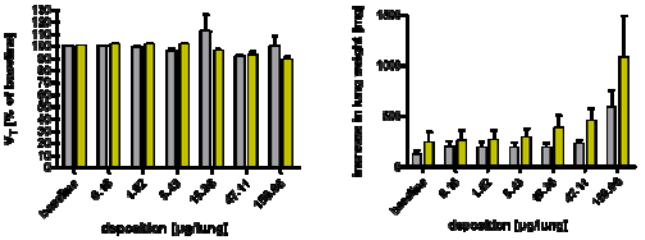

Figure 4: IPLs were exposed to mancozeb (light green) or clean air control (grey) in increasing concentrations. Tidal volume (V_T) (left) and lung weight (right) were measured at baseline and at the end of every experiment. For every exposure step, V_T and lung weight were measured right before the start of the following exposure. Means + SEM, n=4. deposited dose given as means..

- Exposure to test substances in-vitro resulted in dose dependent reduction of cell viability and establishment of dose response curves (Figure 1)
- In-vitro EC_{50} values documented toxic potentials for chlorothalonil and a significantly lower toxic potential (≈10 fold) of mancozeb on human lung cells (Figure 2).
- Lung function, as shown exemplary for V_T, was largely unaffected by exposure to the test items (Figure 3,4).
- IPL exposure to the test items resulted in dose-dependent increase in lung weight and thus pulmonary oedema formation (Figure 3,4).
- In both assays (in-vitro/ex-vivo) mancozeb showed a significantly lower toxic potential than chlorothalonil.

Conclusion

Both assays will be used in the future to predict the acute toxic potential of further substances with known and unknown toxic potential to broaden the data base and verify the reliability of this test battery.

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