An alternative model for testing of acute respiratory local toxic and physiological effects based on in vitro and isolated perfused lung (IPL) technologies

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Background and objective

Due to the demand for further developments of predictive, robust alternative models in inhalation toxicology, a combined in vitro / ex vivo model was set up to cover both local acute toxic effects and respiratory physiological effects from inhalable powders.

Experimental design

The in vitro and ex vivo approaches are based on an air-lifted interface (ALI) cell culture model and an isolated perfused rat lung (IPL) model including exposure to highly concentrated aerosols generated from small amounts of micronized powders.

The in vitro inhalation model included a human lung alveolar epithelial cell model (A549) in an optimized exposure device (P.R.I.T.® ExpoCube®), determination of cytotoxicity and dosimetry considerations. During inhalation testing with the isolated perfused lung, physiological respiratory parameters such as tidal volume, dynamic lung compliance and relative increase in lung weight were determined, thus offering insight into acute physiological respiratory effects.

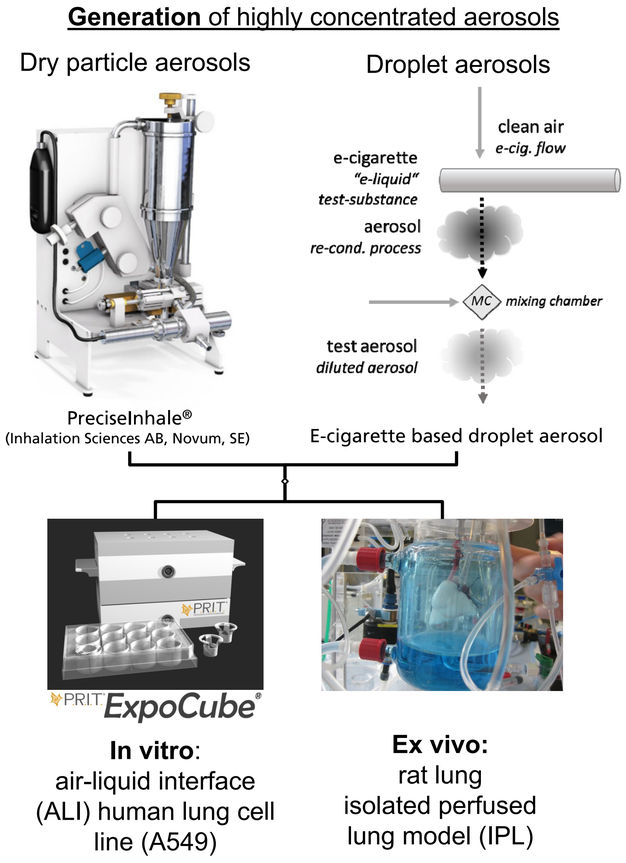


Figure 1 : **Experimental design.** Two technical processes were used for generation of highly concentrated dry particle or droplet aerosols. Aerosols from these processes where both tested using in vitro and ex vivo approaches based on ALI cultures from human lung cells (A549) or isolated perfused rat lungs (IPL).

Results

- Five commercial fungicides, sodium dodecyl sulphate (SDS) and *n*-dodecane were used as test items and positive/negative controls for acute local lung toxicity, respectively.
- Small amounts of testing materials of less than 500 mg only were needed in each model to establish dose-response curves (in vitro) or testing results (ex vivo).
- ED_{50}-values from in vitro testing were correlated to LD_{50} values from acute rat inhalation in vivo testing and showed promising predictivity.
- Similarly, exposure to harmful test items as well as to the positive control SDS resulted in formation of oedema and for SDS also in acute decrease of lung function parameters in the IPL model as a measure for acute respiratory toxicity.
- Hence, the inhalation effects from toxic fungicides could primarily be assigned to cell toxicity.

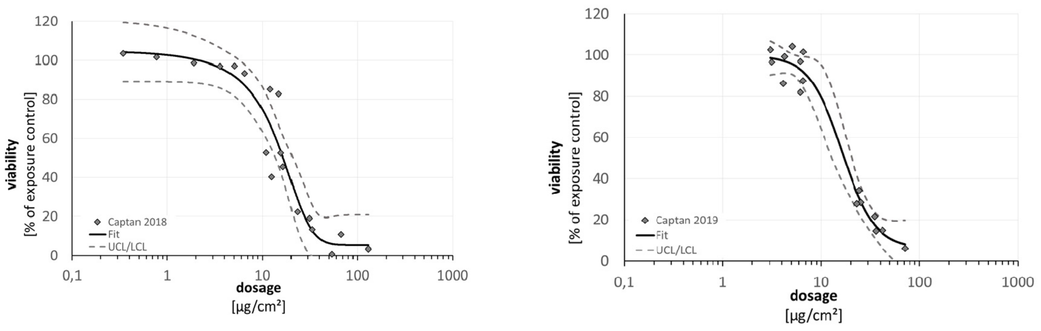
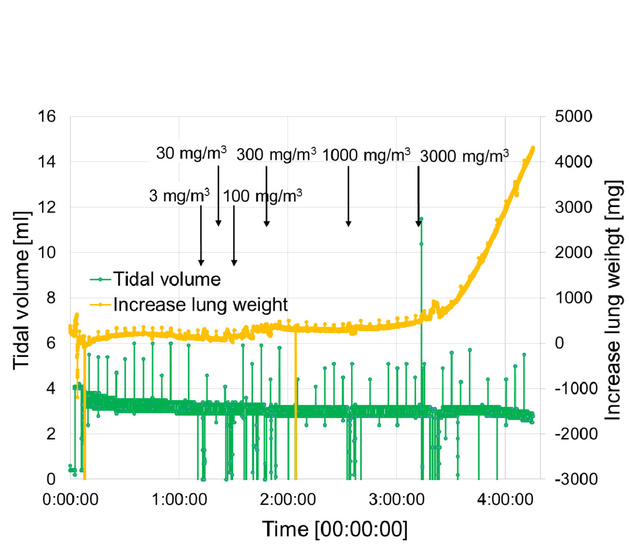


Figure 2 : **Example from dose-response in vitro testing using dry particle aerosols.** Captan was tested using the in vitro ALI assay and cellular viability measurement (WST-1, 24h) as an indicator for toxicity. Left: first experimental set resulting in an ED_{50} of 16.48 µg/cm²; right: repetition experimental series (18 months later) resulting in an ED_{50} of 17.21 µg/cm²). 140 mg resp. 54 mg material consumption for these experiments.



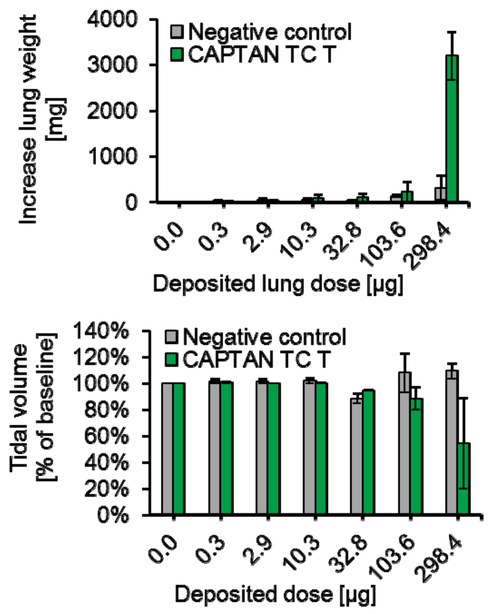


Figure 3 : **Ex vivo testing design** and typical results for increase in lung weight as marker for cellular toxicity and tidal volume additionally indicating impairment of breathing mechanics/lung surfactant function

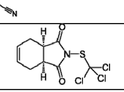
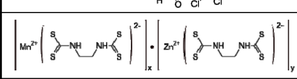
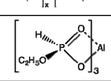
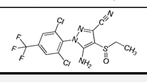
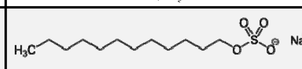
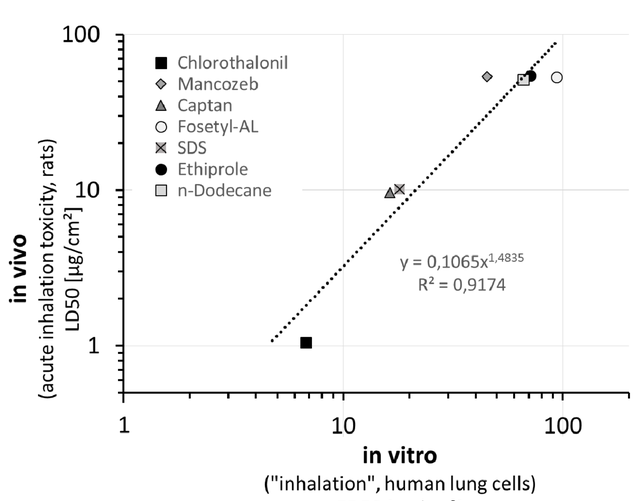
	Compound	Chemical structure	Solubility [mg/l]	Symbol (overall toxicity)	LC_{50} (in vivo) [mg/l]	ED_{50} (in vitro) [µg/cm²]	Toxic dose, estimated (ex vivo) [µg/lung]
Test Materials (crop agents)	Chlorothalonil		1		0.1	6.76	~ 88 µg
	Captan		3.77		0.92	16.28	~ 298 µg
	Mancozeb		6		5.14	45.02	>> 215 µg
	Fosetyl-AL		770000		5.11	106.56	> 423 µg
	Ethiprole		9.2		5.21	71.02	(presumably > 184 µg)
Controls	Sodium dodecyl sulphate (SDS)		150000		0.975	18.03	~ 16 µg
	n-dodecane		0.18		4.91	66.11	(presumably ~ 3410 µg)

Table 1 : Test compounds and results from complementary in vitro / ex vivo toxicity testing



	Compound	In vivo (inhalation)	Ex vivo [4] (IPL)	
		harmful (+/-)	toxicity	breathing effects
Test Materials	Chlorothalonil	+	+	+[1]
	Captan	+	+	+[1]
	Mancozeb	-	-	-
	Fosetyl	-[2]	-	+
	Ethiprole	-	-	No reliable data
Controls	SDS	+	+	++
	n-dodecane	-[3]	-	++

1) Effects on breathing mechanics due to development of cellular toxicity-related pulmonary oedema
2) Breathing effects known from literature
3) Breathing effects expected due to chemical structure
4) Results based on 1 – 4 IPL experiments per substance

Figure 4 : **In vitro / in vivo and ex vivo / in vivo correlations.** Rat LD_{50} values were calculated from aerosol concentrations [mg/l] to inner lung surface load [µg/cm²] using the MPPD model (left). Summary of ex vivo results (IPL) correlating qualitative effects on toxicity and breathing in exposed IPLs to in vivo reference data (acute inhalation tests according to literature).

Conclusions

In summary, the complementary in vitro / ex vivo model appeared very promising to investigate both acute local lung toxicity and respiratory physiological effects from inhalation of bulk powder material using only small amounts of material with short study times and meaningful predictivity:

- **In vitro model**: promising "quantitative" prediction of respiratory/inhalation-related toxicity; calculation of mean in vivo inner lung surface load indicates relevance of in vitro dosage range.
- **Ex vivo model**: "qualitative" toxicity of the substances, in addition detection of impairment of breathing mechanics.
- **Possible perspective**: in vitro -> ex vivo -> in vivo tiered approach for acute inhalation toxicity testing for aiming at replacement, reduction and refinement of animal experimentation ("3R principles").

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