Air/liquid Culture and Exposure Technique: Preliminary Results of a Prevalidation Study

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Abstract

The increasing demand for assessing inhalation toxicity hazards calls for new testing strategies comprising both in vitro and in vivo assays. For this purpose, we are currently evaluating the air/liquid interface (ALI) exposure strategy where cells are exposed to toxic gases at the air/liquid interface. The human carcinoma alveolar epithelial cell line A549, grown on microporous membranes, was exposed to selected test atmospheres in a system enabling at the same time steady state nutrification, humidification and direct gas exposure. Under coordination of Fraunhofer ITEM were assessed the intra- and inter-laboratory reproducibility and predictive capacity of the method by characterizing the toxicity of four gases, i.e. NO_2, SO_2, formaldehyde, and ozone. The aims of this study are: optimisation and refinement of experimental protocols; generation of standard operating procedures; assessment of reproducibility within and between laboratories; establishment of test acceptance criteria; determination of the in vitro vs. in vivo dose-response relationships. After transfer of the method, optimization of protocols and experimental procedures the four partners started testing of the gases. Each gas together with an online analytical monitoring system was passed from one lab to the next. The test design comprised one hour gas exposure followed by direct determination of cytotoxicity (electrical current exclusion method, CASY®) and genotoxicity (COMET assay). So far, the project has proved satisfying transferability of the test method.

Organization and management

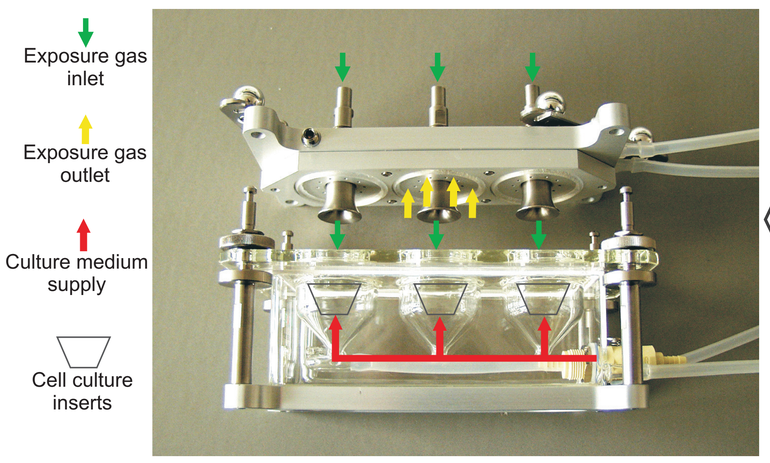
Funding: Bundesministerium für Bildung und Forschung

Coordination: Fraunhofer ITEM Institut Toxikologie und Experimentelle Medizin

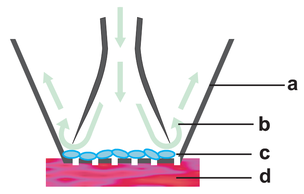
BAuA Bundesanstalt für Arbeitsschutz und Arbeitsmedizin; BfR Risiken erkennen – Gesundheit schützen; HELMHOLTZ ZENTRUM FÜR UMWELTFORSCHUNG UFZ

ALI-exposure system

Exposure module for static medium supply (VITROCELL)



Exposure system for one insert (Falcon, BD) in detail



a Cell culture insert

b Exposure atmosphere (Gases), continuous flow

c Cells grown on microporous membranes

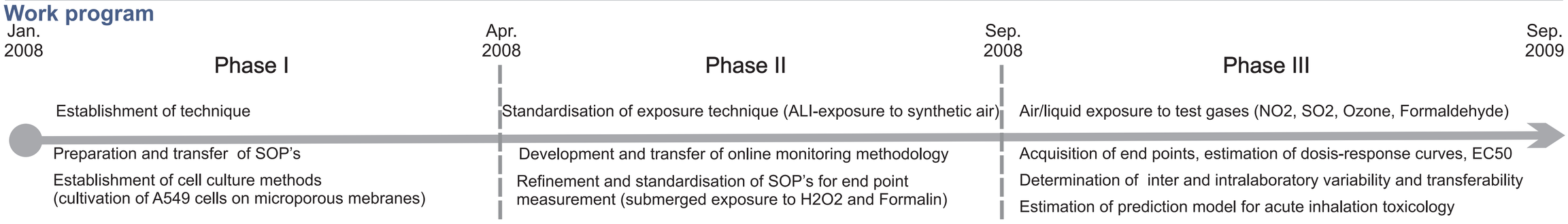
d Culture medium below the membrane

Test gases

	Nitrogen dioxide	Sulfur dioxide	Ozone	Formaldehyde
Molecular formula	**NO_2**	**SO_2**	**O_3**	**CH_2O**
Molar mass	46 g/mol	64 g/mol	48 g/mol	30 g/mol
Toxicity	very toxic, corrosive	toxic, corrosive	very toxic, genotox. potential	toxic, probably cancerogenic
MAK* Value	5 ppm	0,5 ppm	0,1 ppm	0,3 ppm
Recalculation	1 ppm = 1,86 mg/m^3 (SATP+)	1 ppm = 5,58 mg/m^3 (SATP)	1 ppm = 1,94 mg/m^3 (SATP)	1 ppm = 1,21 mg/m^3 (SATP)
Source	atmosphere, burning of biomass,exhaust gas	atmosphere, burning of S-containing fuels, preservatives	atmosphere, laser printer, copy machine, water purification	atomosphere, motor vehicle, preservative in cosmetic, furniture, cigarette smoke, desinfection
Safety and Handling	causes burns, keep away from combustible material	hazardous reactions with oxidizing agent	causes burns, keep away from combustible material, instable	poymerisation to Paraformaldehyde, flammable

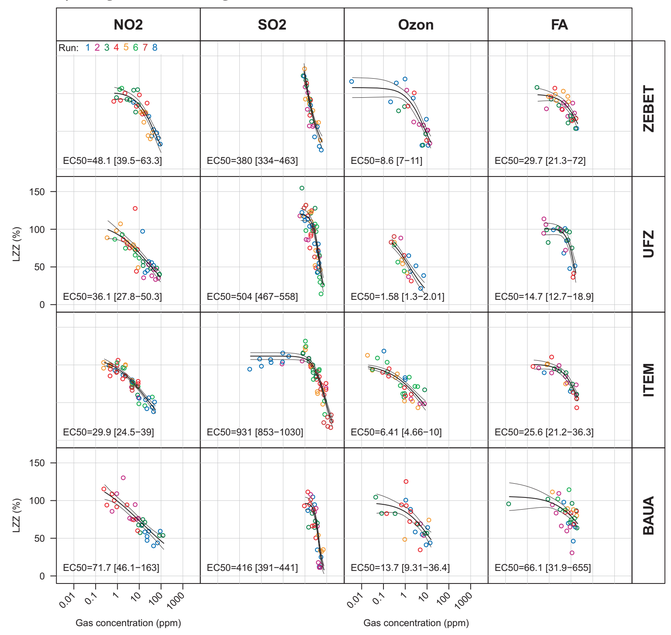
* TLV -Threshold Limit Value
\+ SATP - Standard Ambient Temperatureand Preasure (25 C, 1000 Hpa)

Work program

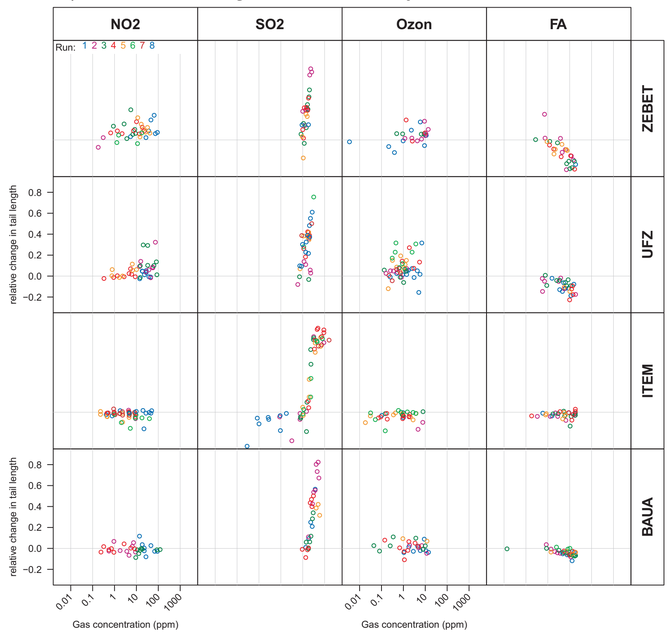


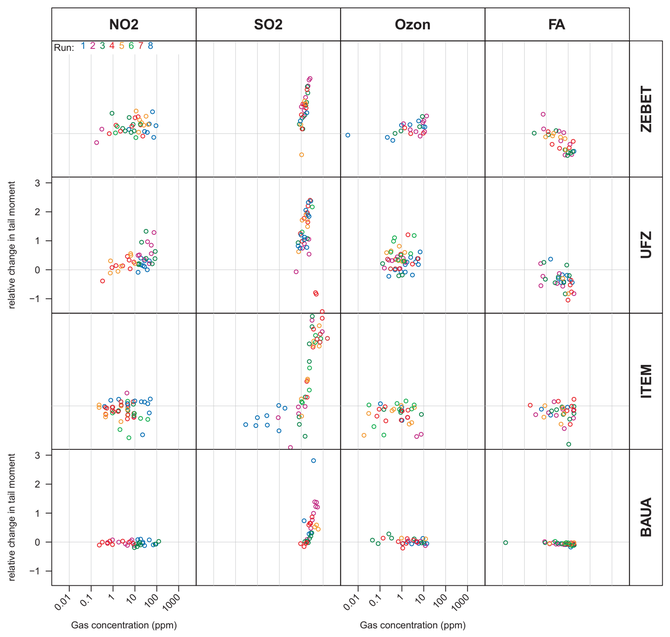
Experimental results

1). **Cytotoxicity**: electrical current exclusion methods



2). **Genotoxicity**: Comet Assay





in vitro vs. *in vivo*

Gas	In vitro, EC50	In vivo, LC50, (Rat)
NO_2	29,9 – 71,7 ppm	88 ppm/4h
SO_2	380 - 931 ppm	2520 ppm/4h
Ozone	1,58 – 13,7 ppm	4,8 ppm/4h
Formaldehyde	14,7 – 66,1 ppm	150 ppm/4h

Outlook

- Evaluation of intra and inter-laboratories reproducibility and transferability
- Enlargement of databases in further extended prevalidation study: additional test substances and toxicologica end points (e.g. Inflammation)
- Development of a first prediction model

Acknowledgments

This project was financially supported by Federal Ministry of Education and Research, Germany [BMBF-0313963]

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