Evaluation of biological effects of airborne material using an improved cell-based *in vitro* approach

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### Introduction

*In vitro* approaches for evaluation of biological effects of airborne materials are under development for several years. Meanwhile, biological test systems including cell-lines, primary cultures or *ex vivo* tissues such as PCLS and air-lifted interface (ALI) cultures are used for these purposes. However, until now, there have been still limitations with regard to the applicability and relevance during exposure of ALI cultures towards airborne material. These limitations for airborne testing lead to extended and unfavorable practical needs compared to usual work with liquid test compounds. Therefore, they represent a clear obstacle for harmonization, standardization, further dissemination and faster development of *in vitro* methods for testing inhalable compounds in their airborne state.

### Results

#### Efficient individual exposure of ALI cultures in a stagnation flow alignment

- Efficient deposition of small particles from aerosols
- Acute toxicity testing of volatile organic / gaseous compounds
- Dose-responsive effects from dry particle aerosols

#### Observation of cells during exposure by fluorescence

- Prevention of irrelevant secondary route of exposure

**Figure 1:** particle deposition rates from different aerosols in the size range below 2000 nm

**Figure 2:** Acute toxic response from human lung cells (A549) to a set of volatile organic compounds and in vitro / *vivo* correlation according to data from the ECHA data base.

**Figure 3:** Toxic (cell viability, black) and irritative (*IL-8* secretion, red) response from human lung cells (A549) to a dry particle aerosol (sodium dodecyl sulfate)

**Figure 4:** Experimental setup for generation, particle monitoring and exposure of ALI cultures to a variety of different test aerosols including aerosols emerging from consumer product use, such as heating applications of cosmetic products (e.g. hair care products)

**Figure 5:** Kinetic effects of small aerosol on exposed human lung cells as followed by live fluorescence staining of the mitochondria.

### Objectives

By simultaneous realization of (1) an efficient exposure alignment (stagnation flow), (2) an efficient particle deposition from aerosols without harming the exposed tissue (thermophoresis technique), (3) complete work in standard consumable multiwell-plates (4) the possibility to observe the biological effects during exposure non-invasively (*live* fluorescence staining) and (5) technically safeguarding the relevant route of airborne exposure exclusively, these limitations are specifically addressed and improved for exposure of ALI cultures to airborne material in the P.R.I.T.® ExpoCube®.

### Conclusions

- Handling and testing quality is clearly improved by use of commercial consumables.
- Testing of volatile chemicals resulted in a promising in vitro / *in vivo* correlation.
- Observation of exposed cells during exposure documents fast cellular response to aerosol compounds.
- Effective particle deposition also for small particles < 1 μm.
- Cell-based in vitro testing using ALI cultures can be applied to a range of different testing materials and testing scenarios including gases, aerosols an complex mixtures.
- The compactness of the design and a large range of applicability improve the *in vitro* potential to test inhalable materials in a relevant way.