

# In vitro non-target screening of gaseous/volatile irritants after evaporation from products

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People are **exposed by inhalation to multiple substances** evaporating from sources such as furniture, clothes, or others in indoor environment or at workplaces. Although much has been done regarding improvement of overall air-quality, it is still hard to gain insight into **potentially adverse effects**, when people complain about health constraints upon inhalation, but there is no clear source of potentially harmful airborne material.

As a first step in the direction of designing an *in vitro* inhalation non-target screening approach, **three different biological test systems were included in a collective *in vitro* inhalation test strategy** and characterized with respect to detectability of effects when applied to evaporations from fabric in the range of safety values as defined by regulation and OEKO-TEX standard using formaldehyde and allyl isothiocyanate as representatives for a harmful substances.

## Methods

- Sampling **gaseous evaporations from test materials** (10 cm x 10 cm) in a circular, closed volume flow under defined conditions (time, temperature, humidity).
- Online (non-target) process control and analysis of evaporation from products by FT-IR.
- Testing of biological effects using **3 *in vitro* inhalation models** with related endpoints based on air-liquid interface (ALI) cultures:
  - In vitro* inhalation toxicity model** (human lung A549 epithelial cells, viability, mitochondrial membrane potential, stress, release of IL-8).
  - In vitro* inhalation sensory irritation model** (transfected TRPA1-receptor cells)
  - In vitro* inhalation inflammation model<sup>1)</sup>** (transfected TLR4 cells).
- Depending on the model, *in vitro* inhalation exposure included exposure times between 5 and 60 minutes and a post-exposure incubation period (24h).

Table 1: definition of 4 test cases with relevance for real product contamination based on the OEKO-Text standard limit value of 10 mg/kg fabric for many volatile organic compounds (VOCs)<sup>2)</sup>

Case	potential product contamination		resulting experimental test concentration
	FA [mg/m <sup>2</sup> ]	AITC [mg/m <sup>2</sup> ]	FA or AITC [ppm]
1	8.5	45.1	1
2	42.6	225.4	5
3	85.2	450.8	10
4	230.2	1217.2	27

## Results

- Online analysis by FT-IR** represented expected experimental concentrations with **recovery of about 100 %** of the controlled contaminations
- The *in vitro* inhalation toxicity model indicated **concentration dependent biological effects** corresponding to the test cases 2 to 4. Viability decreased during test cases 3 and 4, cellular stress and release of IL-8
- The *in vitro* inhalation sensory irritation model indicated **receptor activation of the TRPA1 receptor by AITC** already at an experimental concentration of 1 ppm (test case 1) and higher and by FA at 5 ppm and higher.
- The *in vitro* inhalation inflammation model indicated **activation of the TLR4 receptor** as a result of AITC exposure at both tested cases 2 and 3.

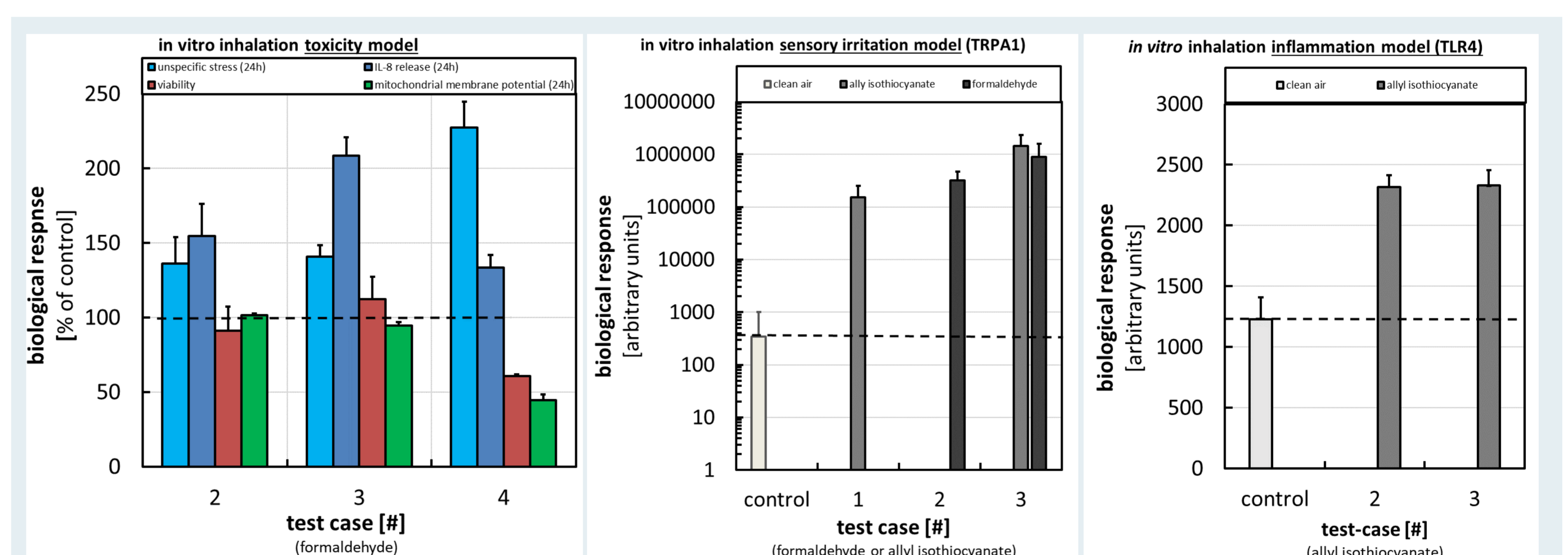


Figure 2: results from 3 *in vitro* inhalation models with test cases 1 to 4 (model substances formaldehyde or allyl isothiocyanate)

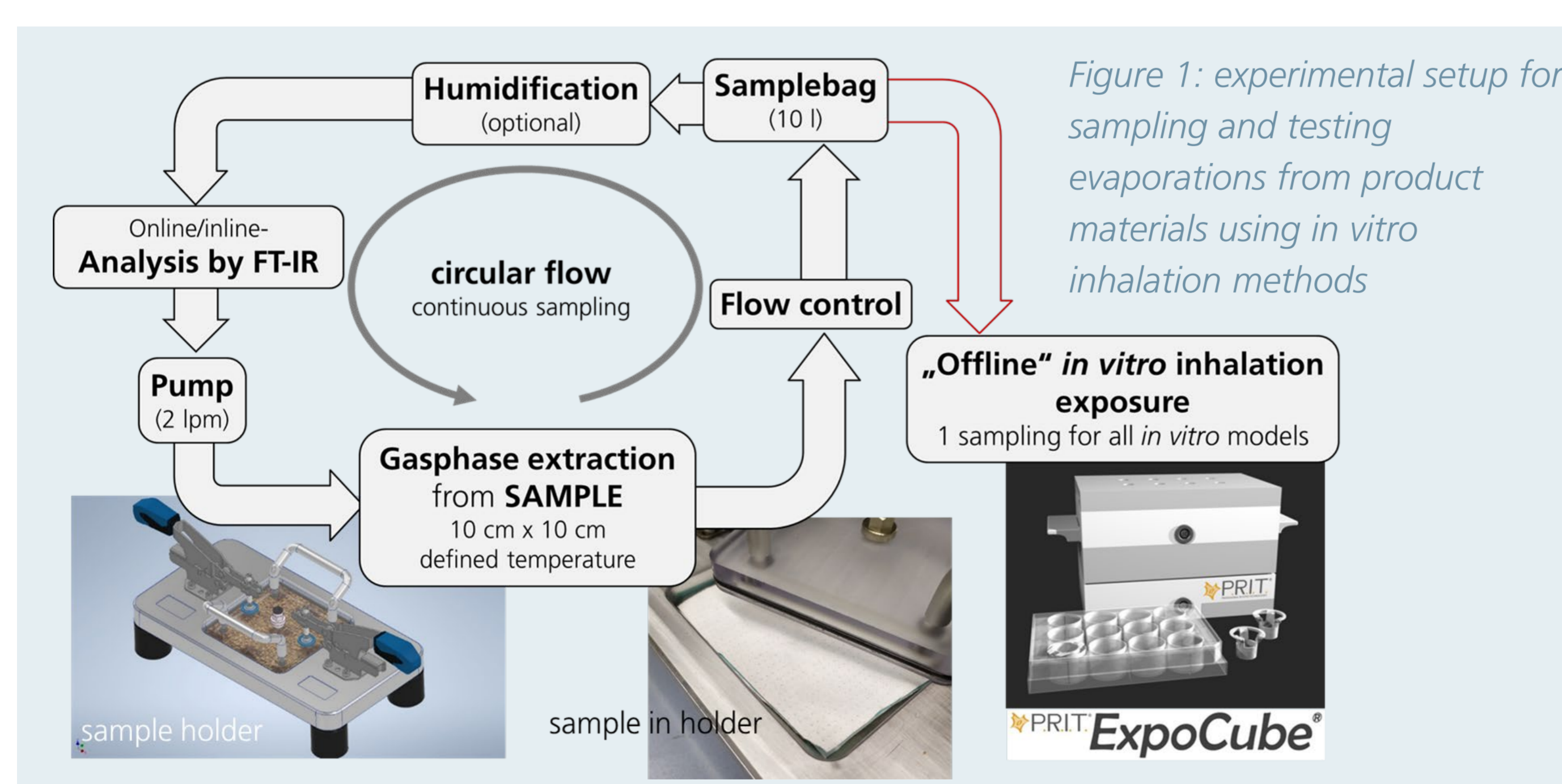


Figure 1: experimental setup for sampling and testing evaporations from product materials using *in vitro* inhalation methods

## Case study

- 4 cases based on **OEKO-TEX Standard 100<sup>2)</sup>**
- Calculated for a potentially contaminated t-shirt fabric (160 g/m<sup>2</sup>) in the range of the **VOC contamination limit value of 10 mg/kg fabric**.
- Simulation by controlled contamination of inert glass-fiber material.
- Formaldehyde (FA) and allyl isothiocyanate (AITC)** were used as known toxicants for controlled contamination experiments.

## Conclusion and Perspective

- Detection of potentially adverse biological effects in a relevant testing scenario** for evaporation of gaseous compounds from fabric products of filters.
- Sensitivity** of the *in vitro* inhalation setup was **enough to detect effects in the range of limit values** based on the OEKO-TEX Standard<sup>2)</sup>.
- Promising **experimental perspective to evaluate yet unknown reasons for irritating evaporations from products**.
- Further specification of the method could be done with respect to sampling technique, range of biological endpoints or applicability to different kinds of test materials when specific product types (furniture, carpets, clothing, etc.) are of interest.

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