

# Acute inhalation toxicity of volatile organic compounds – application of an improved cell-based *in vitro* procedure

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## Situation

- Cell-based *in vitro* methods to assess the biological effects of inhalable compounds are based on “air-lifted interface” (ALI) cell culture protocols where cultures from cell lines, primary cells or *ex vivo* sources such as PCLS (precision cut lung slices) are exposed efficiently to airborne material.
- The **basic ALI procedure has been successfully prevalidated** as a short-term “acute” exposure protocol for chemical gases.
- Although good predictability for highly toxic gaseous compounds or non-toxic inert gases could be demonstrated, it was **not clear until now, if highly hydrophobic or low-toxic volatile organic compounds (VOCs) can also be assessed by cell-based methods *in vitro*** under these conditions with regard to a relevant estimate of their acute inhalation toxicity characteristics *in vivo*.

## Objectives

### Establishment of a simple and robust experimental concept.

- to set up a small compound **test substance matrix** from the data base of the European chemicals agency (ECHA) including **VOCs differing in hydrophobicity and acute *in vivo* inhalation toxicity**,
- to carry out cell-based testing including vaporized compounds, **an improved air-liquid interface *in vitro* procedure** (PRIT ExpoCube) with human lung cells (A549) and read-out of acute cytotoxicity after **short-term “acute” exposures**,
- to **compare *in vitro* toxicity data to acute *in vivo* inhalation toxicity data** from the ECHA data base.

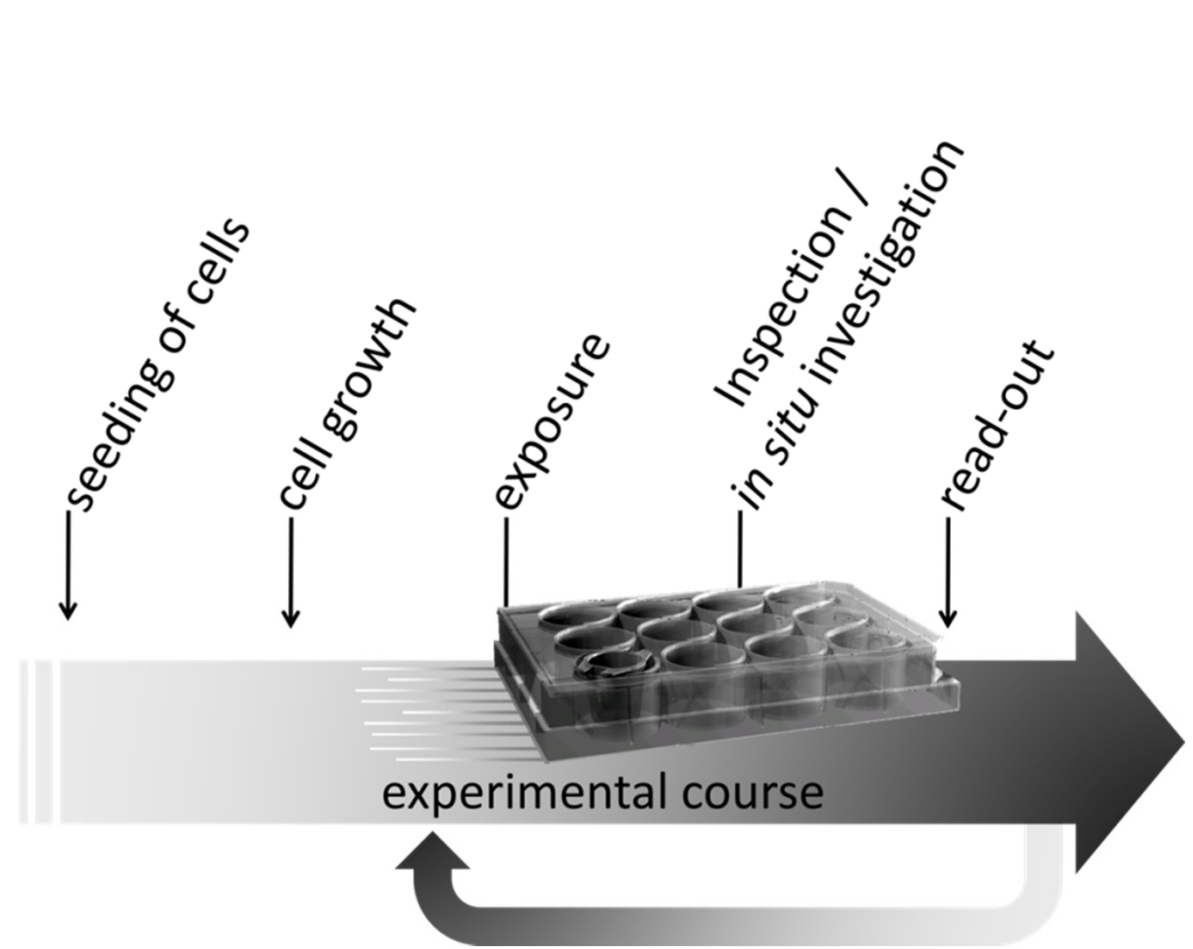
## Approach

### Application of the concept with a first set of volatile organic compounds.

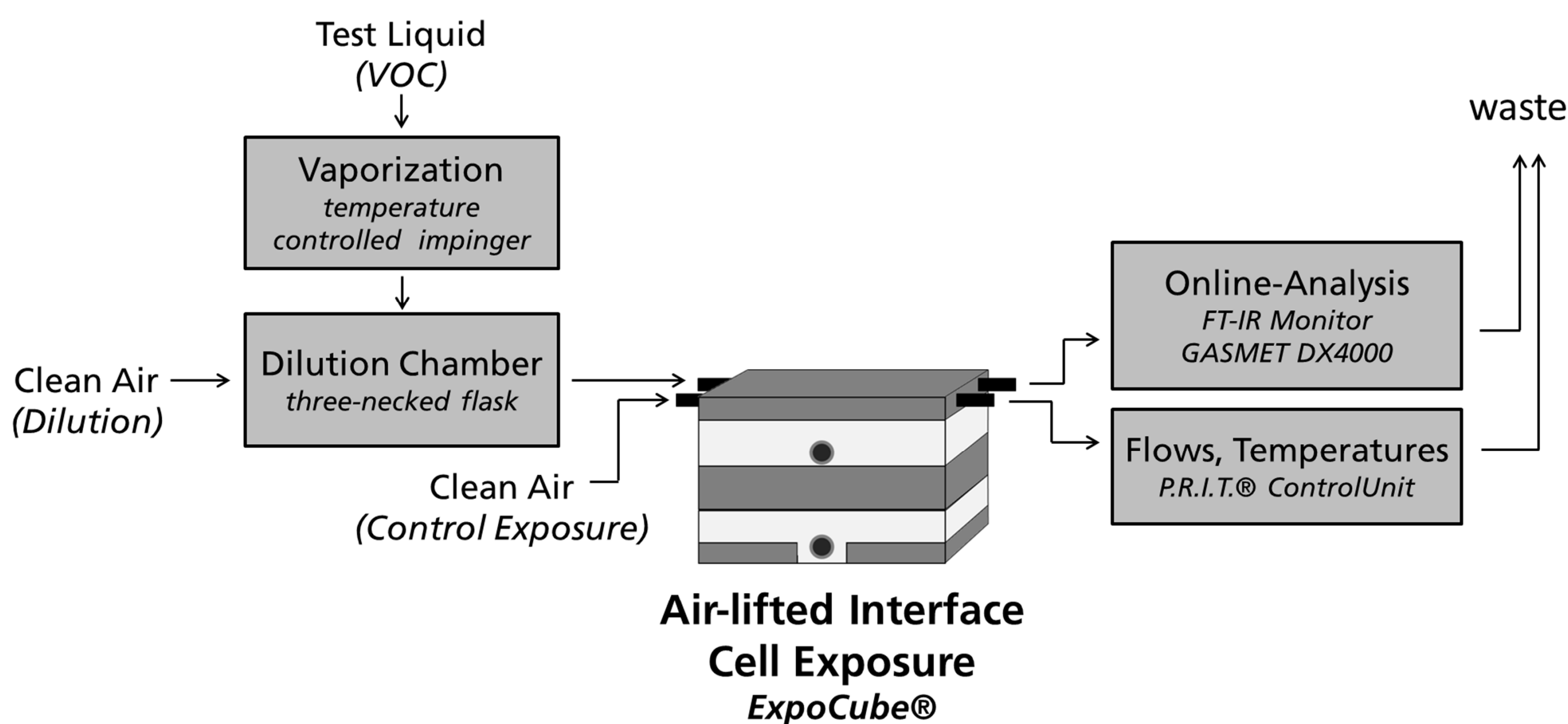
- Generation of VOC vapors by in-line, temperature-controlled vaporization from small substance volumes (~ 5 ml liquids).
- Simple and efficient aerosol exposure using the P.R.I.T.-ALI ExpoCube®, including application of **standard multiwell plates** throughout the whole testing procedure („all-in-one-plate“- workflow). (Ritter and Knebel, Adv. in Tox. (2014), <http://dx.doi.org/10.1155/2014/185201>)
- Online quantification of exposure concentrations by FT-IR analysis.

## Approach (cont'd)

- “All-in-one-plate”-workflow.



- Experimental setup.



## Results

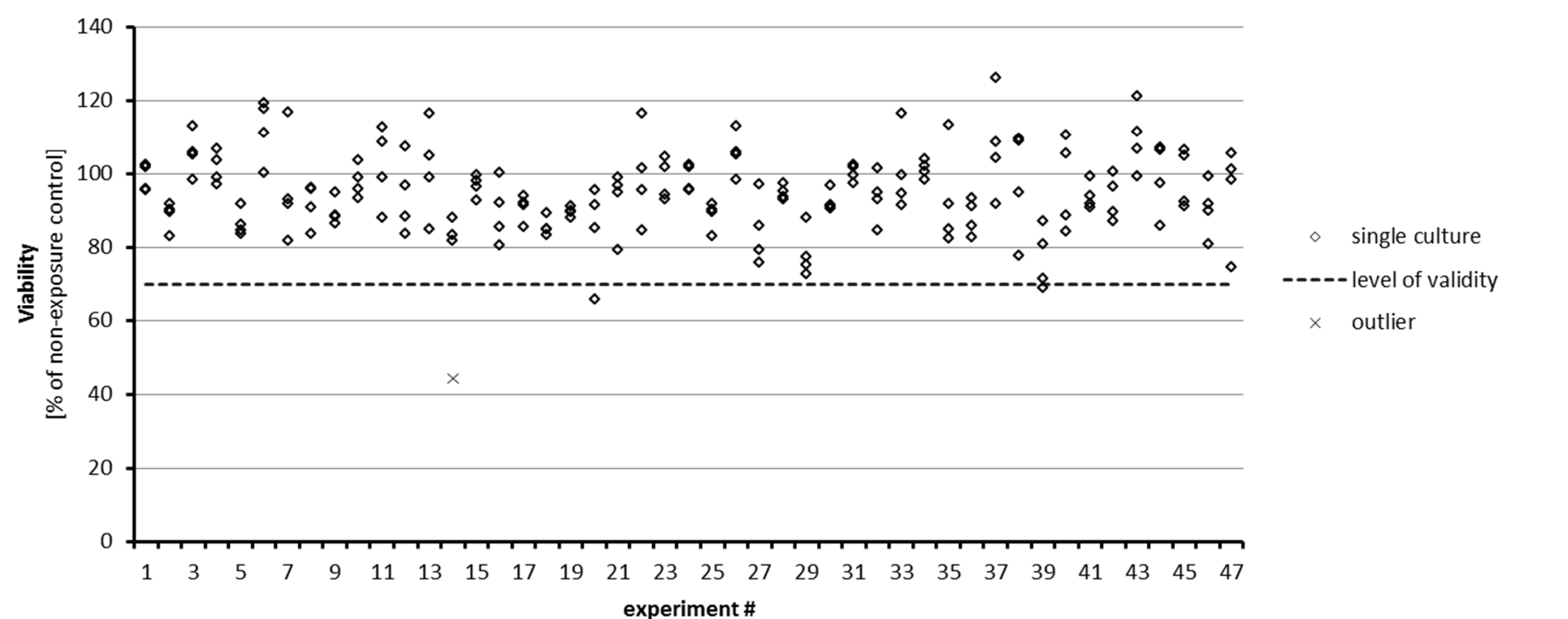
- 4 compound test matrix from ECHA data base

| Substance                | CAS      | water solvability [g/l] / 20 °C | boiling point [°C]           | vapour pressure [hPa / °C] | Acute inhalation toxicity LC <sub>50</sub> (rat / mouse, 4h) [mg/l] |
|--------------------------|----------|---------------------------------|------------------------------|----------------------------|---------------------------------------------------------------------|
| tert. butylhydroperoxide | 75-91-2  | 100-150 <sup>1)</sup>           | > 75 (decomp.) <sup>2)</sup> | 50,78 hPa / 25 °C          | 1.2 - 1.8 (n=3)                                                     |
| 2-methylpropan-1-ol      | 78-83-1  | 70-95                           | 108                          | 13.6 - 1.8 hPa / 25 °C     | 15 - 26 (n=4)                                                       |
| toluene                  | 108-88-3 | 0.52 - 0.59                     | 106 - 110                    | 29 -30 hPa / 20 °C         | 12.5 - 28.8 (n=5)                                                   |
| styrene                  | 100-42-5 | 0.20 - 0.32                     | 143- 146                     | 6 - 8 hPa / 20 - 25 °C     | 11.8 (n=7)                                                          |

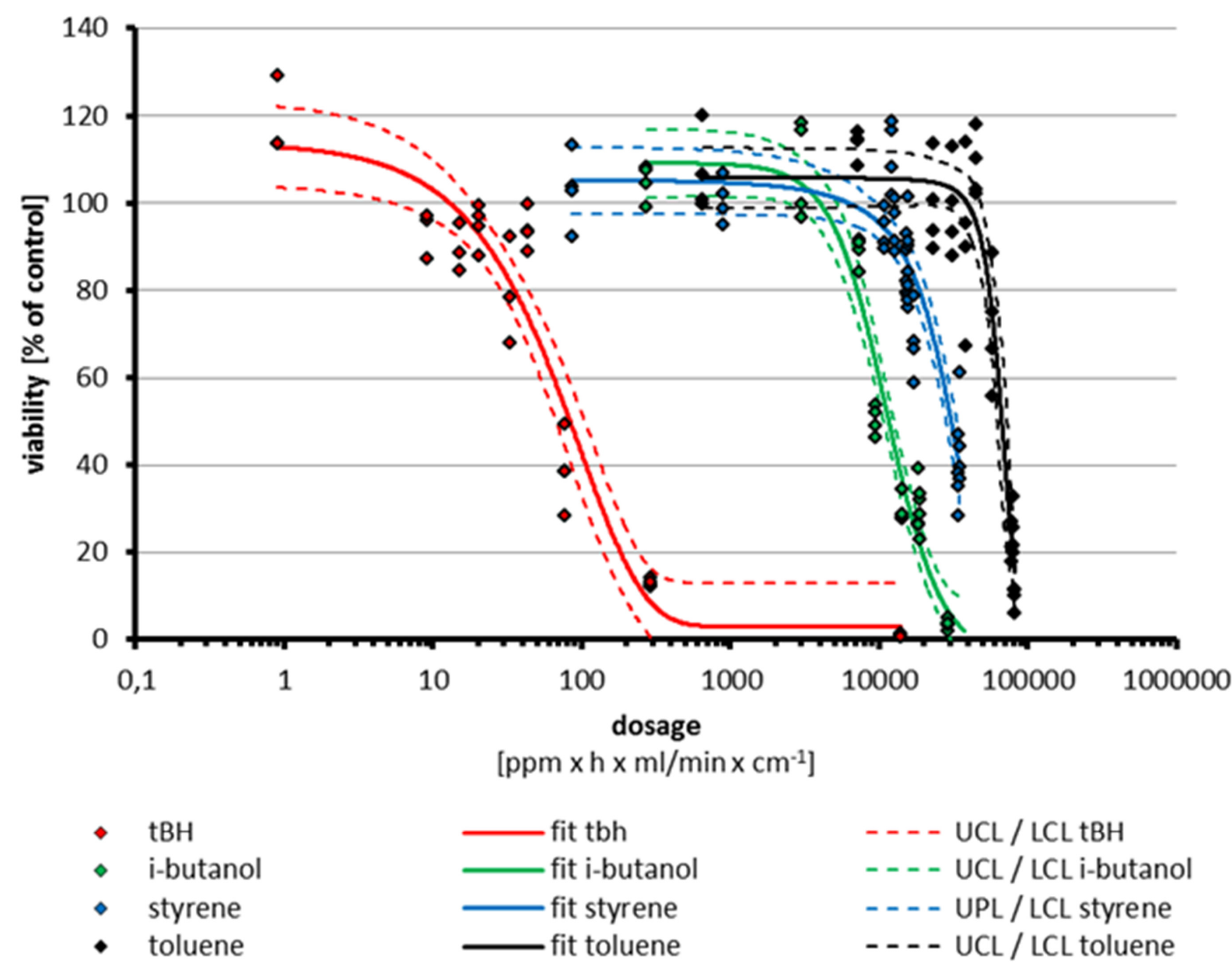
Ref.: <sup>1)</sup> RA report, CBS, Bilthoven, Netherlands, 2006; <sup>2)</sup> gestis.itrust.de, others: [www.echa.eu](http://www.echa.eu)

## Results (cont'd)

- Exposure controls for determination of experimental validity



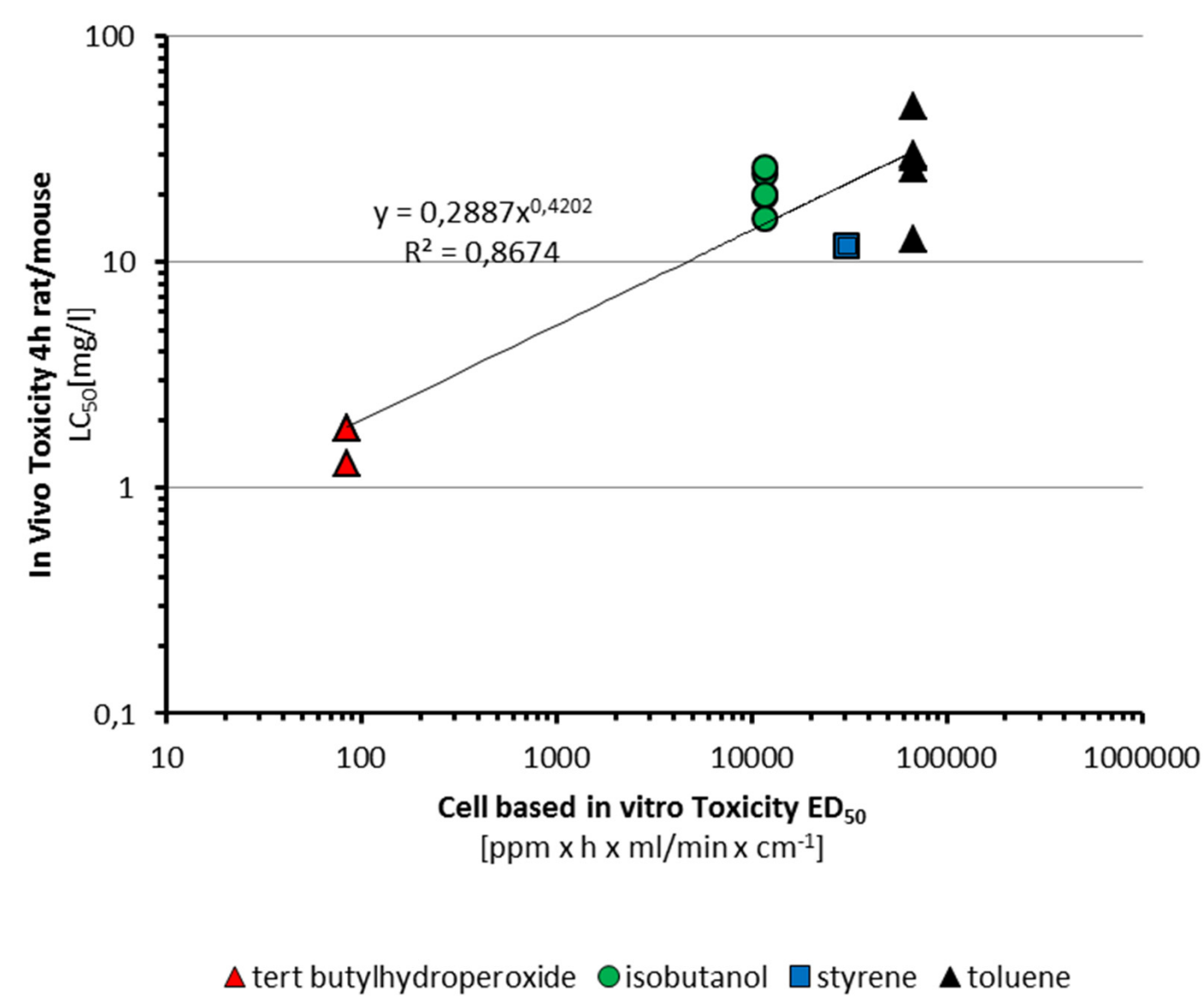
- Establishment of dose-response curves



- Substance characteristics

|                                                               | hydrophilic | hydrophobic |
|---------------------------------------------------------------|-------------|-------------|
| "toxic" (GHS/CLP acute inhalation toxicity class 2)           | ✗           |             |
| "less toxic" (GHS/CLP acute inhalation toxicity class 4 or 5) | ✗           | ✗ ✗         |

- ED<sub>50</sub> values (*in vitro*) and LC<sub>50</sub> values (*in vivo*) for *in vitro* / *in vivo* correlation



## Conclusion

- In vitro* ED<sub>50</sub> values could be determined for all gases** including low-toxicity, highly hydrophobic compounds (styrene, toluene) with high reproducibility using a short –term “acute” exposure protocol.
- High and low toxicity VOCs could be clearly discriminated;** toxicity was not only correlated to hydrophicity / water solvability.
- First *in vitro* dataset showed **good correlation to *in vivo* data**.
- A **promising approach for acute toxicity screening of inhalable gases and VOCs *in vitro*** by cell-based methods.