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Lung surfactant inhibition – an alternative acute inhalation toxicity test

Jorid Birkelund Sørli and Emilie Da Silva

jbs@nfa.dk
eds@nfa.dk

Endpoint replacement

- **Acute inhalation toxicity**

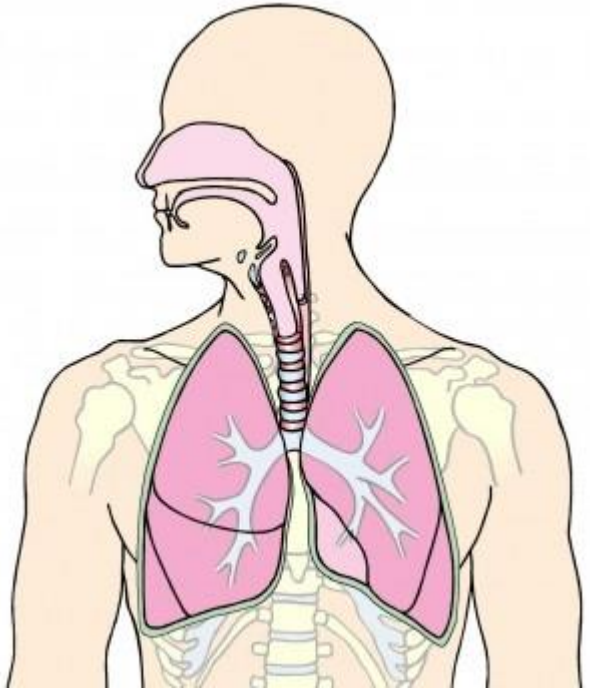
- Accepted tests OECD TG 403/436/433
- Endpoints: death (403 and 436) or evident toxicity (433)

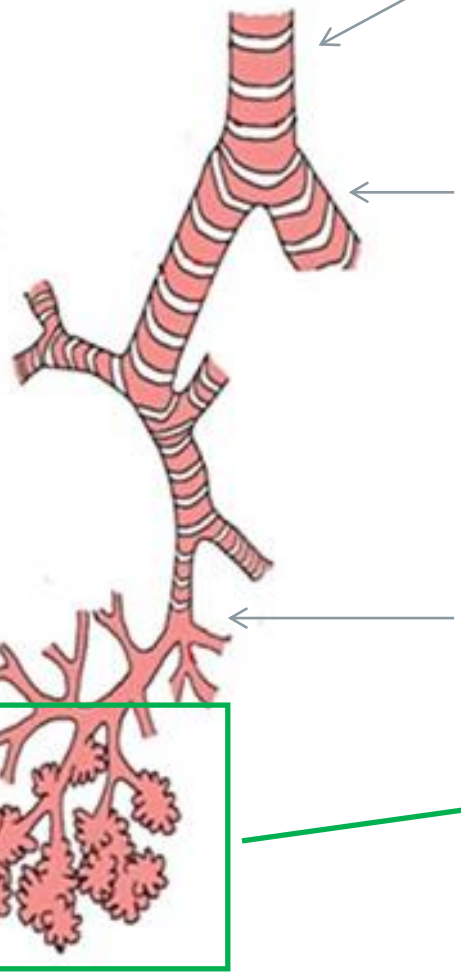
- No accepted alternatives

Impregnation product	In vitro test	In vivo test	Correlation	Toxic for humans
"Wood impregnation"	Yes	Yes	Yes	Yes
"Stain repellent super"	Yes	Yes	Yes	Yes
"Liquid stain protection"	Yes	Yes	Yes	Yes
"Faceal oleo MG"	Yes	Yes	Yes	Yes
"HG textile"	Yes	Yes	Yes	Yes
"HG leather "	Yes	Yes	Yes	Yes

Sørli et al, 2015

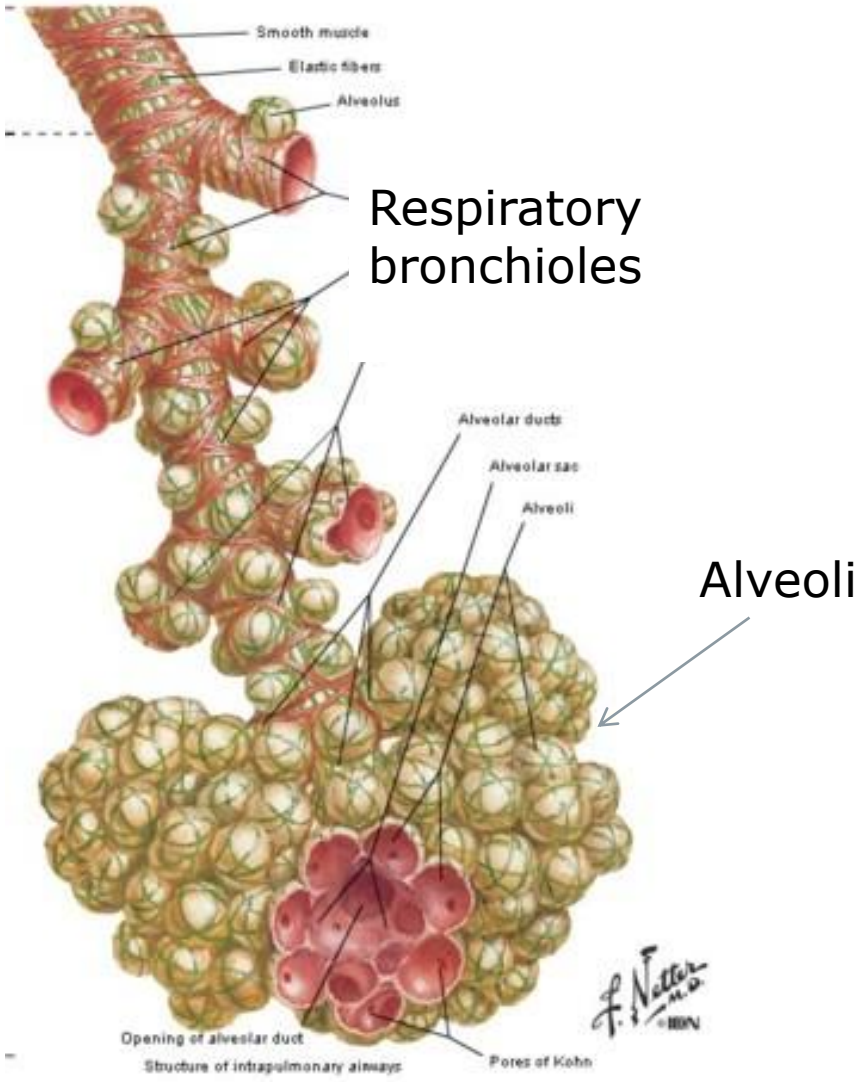
The airways





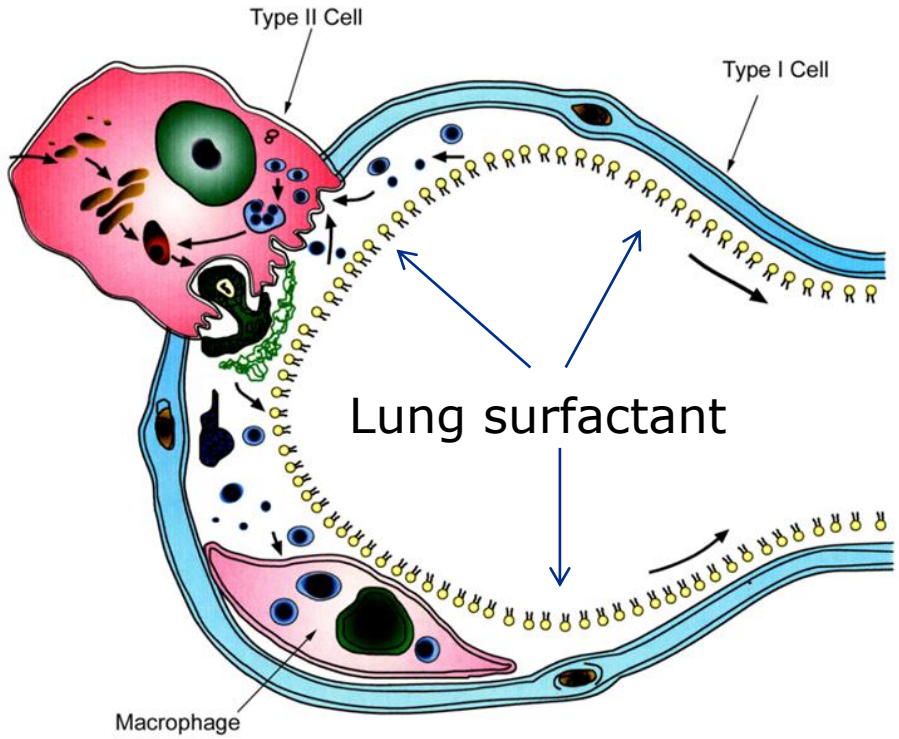
Generation 17-23
respiration

Adapted from Ungaroet al, 2012



http://www.sharinginhealth.ca/respiratory/respiratory.html

Alternative integrated testing strategy gap



- Most **cell-based** lung toxicity assays do not incorporate lung surfactant

BUT

- Lung surfactant has a vital function in the lungs

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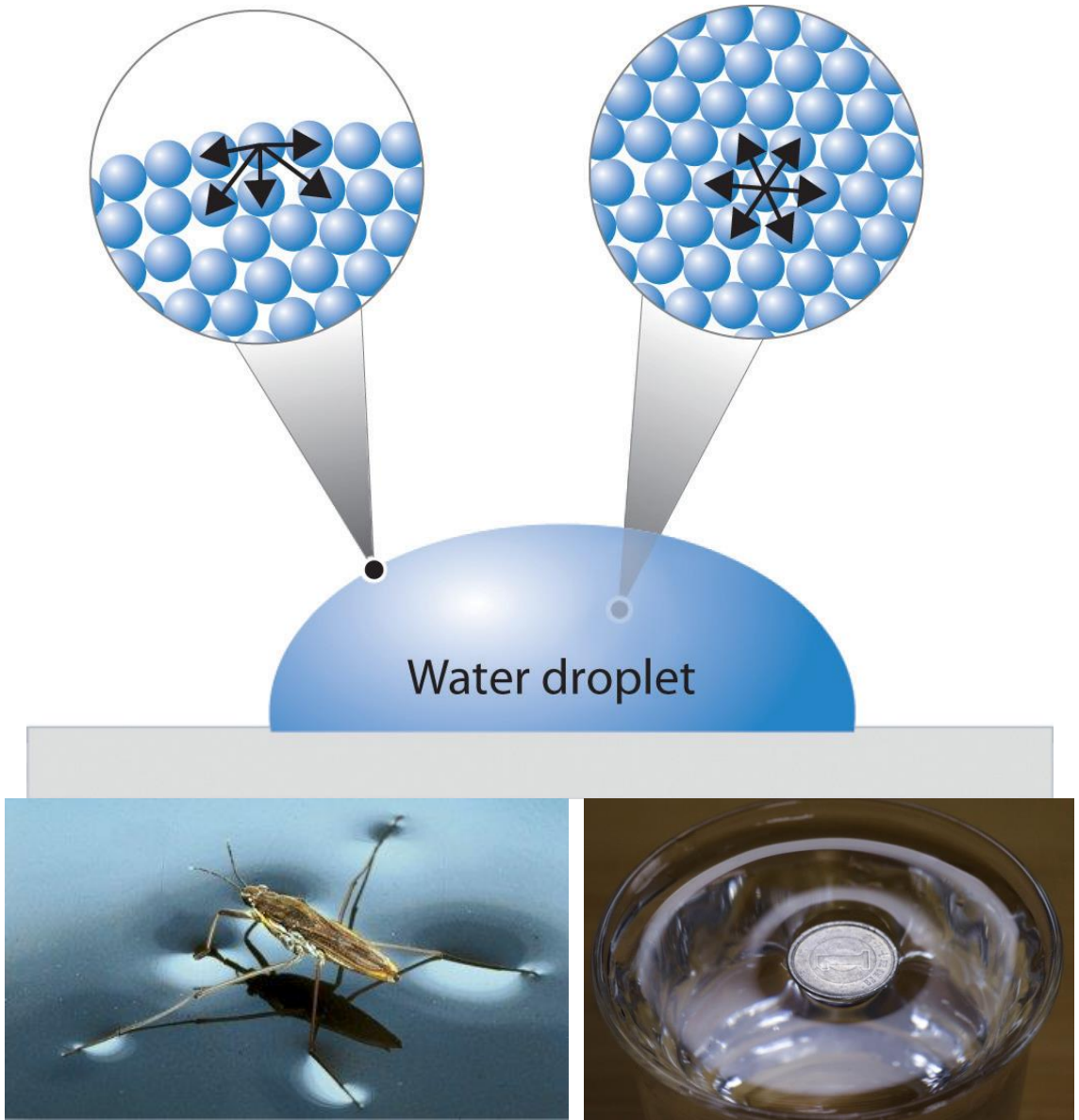
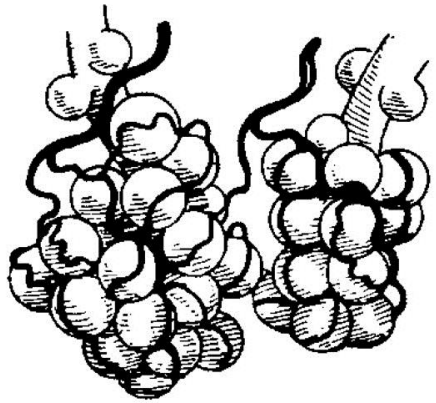
http://usmle.biochemistryformedics.com/respiratory-distress-syndrome-case-discussion

Lung surfactant regulates surface tension

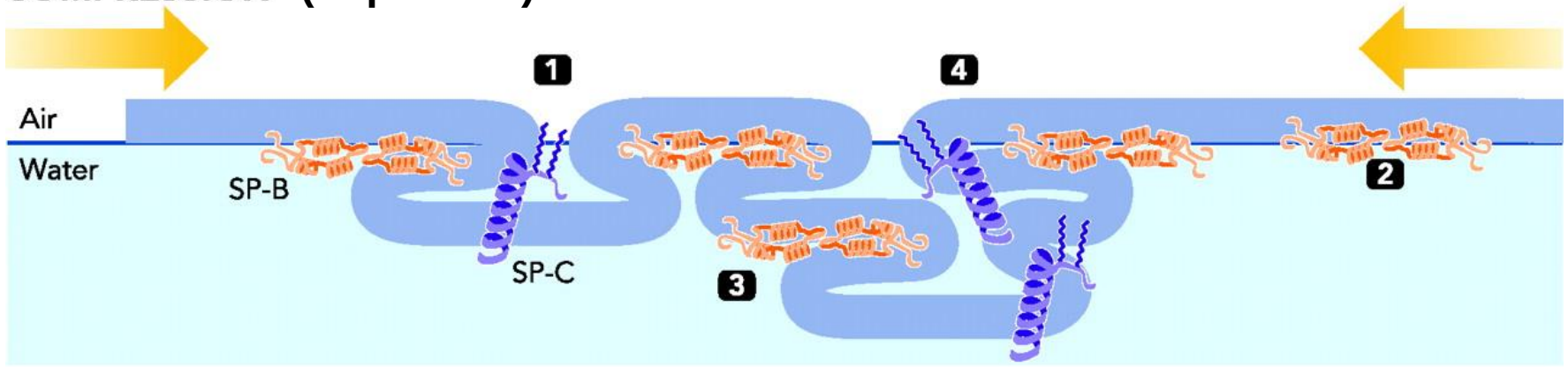


Low surface tension
Normal alveoli

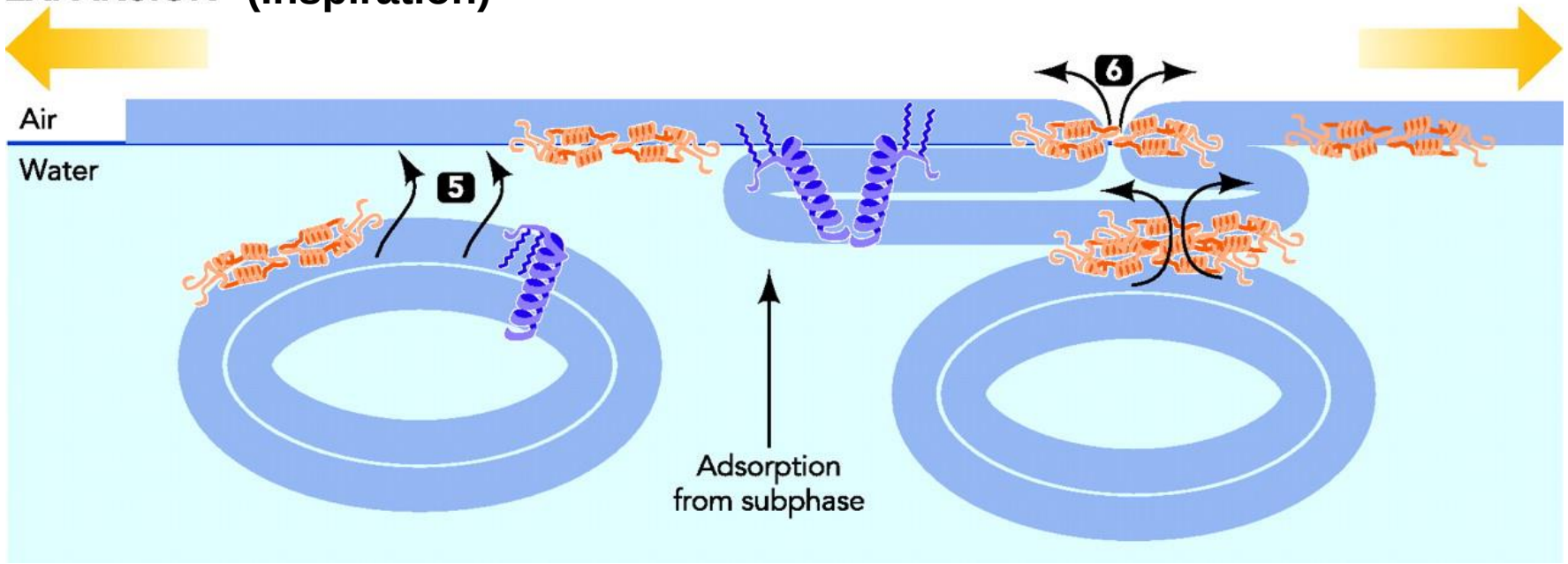


High surface tension
Collapsed alveoli

COMPRESSION (Expiration)



EXPANSION (Inspiration)



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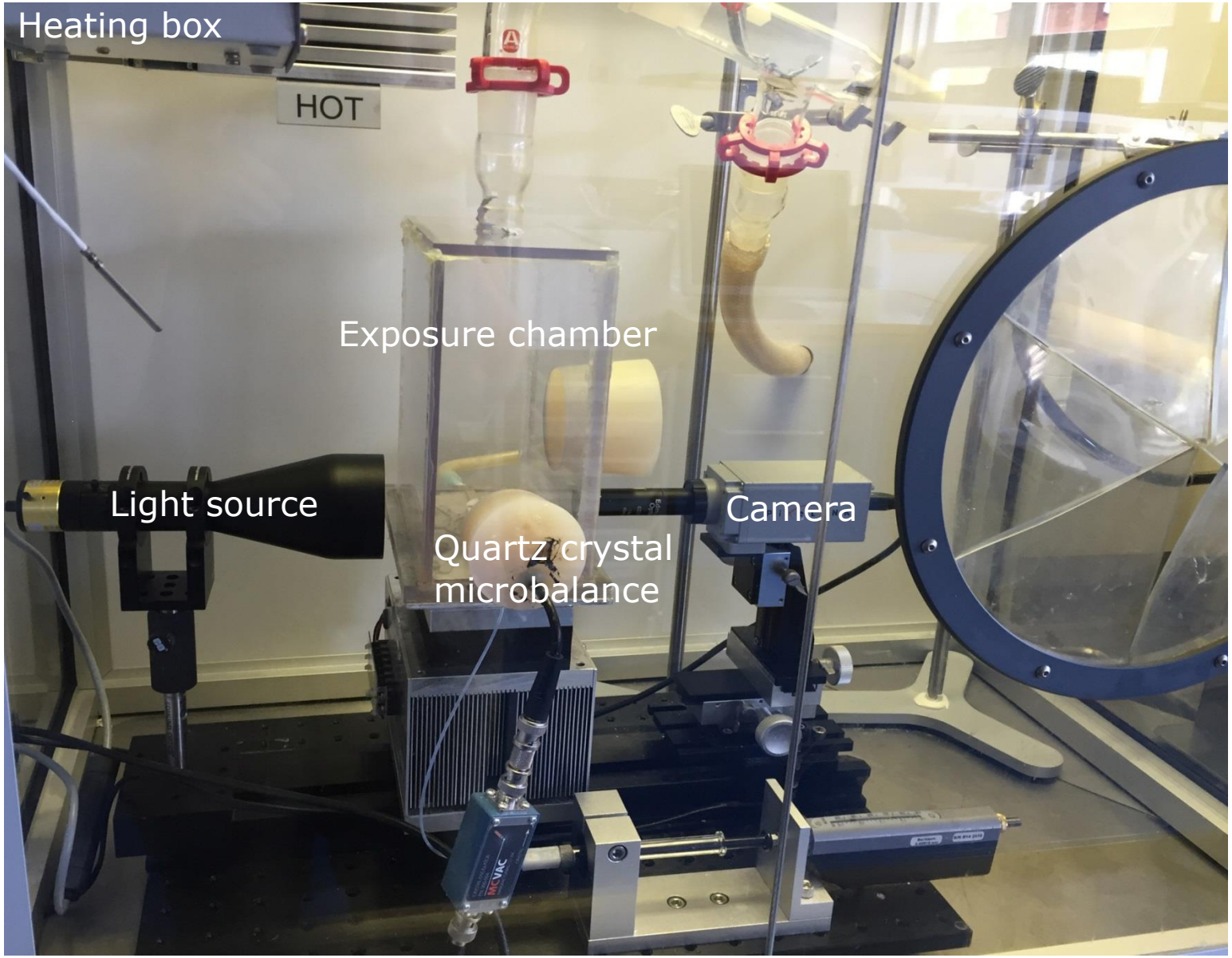
Perez-Gil & Weaver, Physiology, 2010

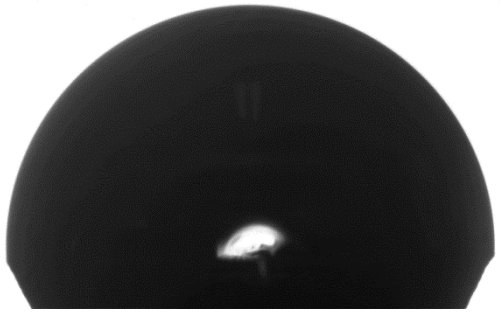
Constrained drop surfactometer



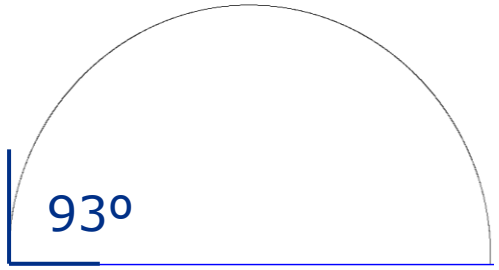
- Measures surface tension
- Temperature control
- Flow through for realistic exposure
- Compatible with different aerosol generators
- Fast, easy, and low cost test

Drop shape

- The contact angle and the shape can be used to calculate the surface tension (ADSA, Zuo *et al.* 2004)



70 mN/m



Water

Lung surfactant

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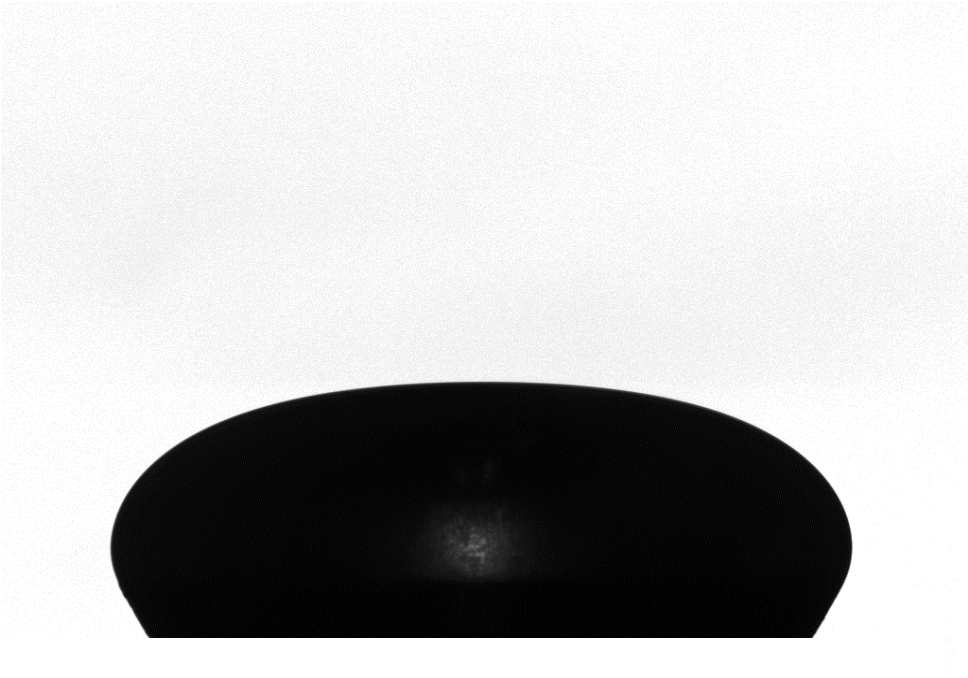

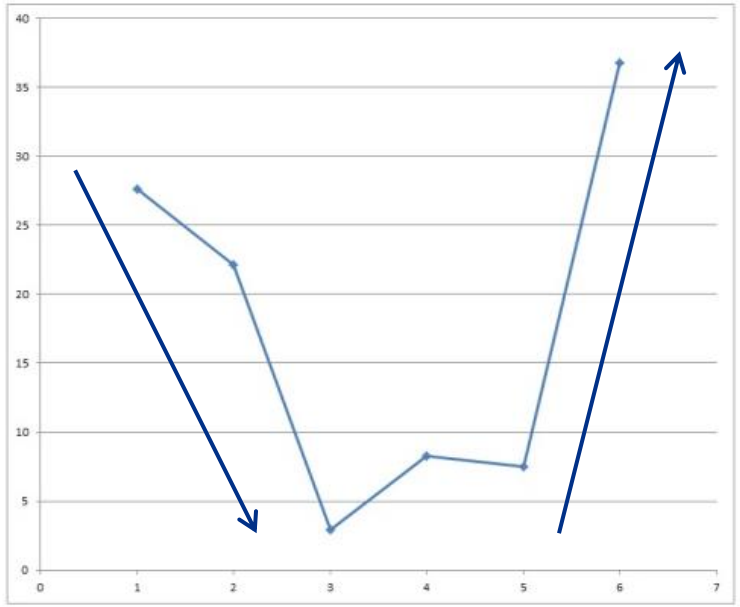

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Alternative integrated testing strategy

- Lung surfactant function is a point-of-entry measure
- Lung surfactant should be added to air-liquid interface cell-based assays for more realistic exposure scenarios
- Lung surfactant function can only be assessed in a dynamic assay, thus this should be done separately
- Disruption of lung surfactant function can be included in an IATA to determine the potential for causing acute inhalation toxicity

Applicability domain

- Impregnation products
  - Frequently used both by professionals and by consumers
  - Make surfaces water and dirt repellant -> easy to clean
  - Cause acute inhalation toxicity regularly

- Inhaled pharmaceuticals (marketed formulations Sørli et al 2015) (excipients Sørli et al 2018, in review)
  - Treatment for lung disease are inhaled and can potentially interact with lung surfactant

- Nano particles can reach deep into the lungs and interact with the lung surfactant

Future work and next steps

- Enhancers in biopharmaceuticals (Sørli et al, paper in review)
- Testing of:
  - Nanoparticles in EU project SmartNanoTox
  - Per-fluorinated compounds in collaboration with The Norwegian Institute of Public Health
  - Surveillance of problematic impregnation or spray products by the Danish poison center and the Danish EPA
  - Cleaning products in spray form
  - Chemicals with a known reported LD50 after inhalation
- Plan to submit the Pre-submission form to EURL-ECVAM

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References

- The need for an alternative test
  - Da Silva and Sørli, Applied In Vitro Toxicology (2018)
- Description of the method
  - Sørli et al, Am. J. Respir. Cell Mol. Biol (2015)
- Use of the CDS to measure LS function
  - **Impregnation product** and LS interaction

    Sørli et al. Altex (2018)
  - **Inhaled pharmaceuticals** and LS interaction

    Sørli et al, Am. J. Respir. Cell Mol. Biol (2015)

    Sørli et al, in review (2018)
  - **Nanoparticle** LS interaction

    Valle et al, ACS nano (2015)

    Valle et al, Acs Sustainable Chemistry & Engineering (2014)

    Chen et al, Journal of Environmental Sciences (2017)

    Hu et al, Nanoscale (2013)

    Yang et al, Small Methods (2018)
  - **Mouse inhalation bioassay** and impregnation products

    Larsen et al, Toxicology letters (2014)

    Nørgaard et al, Toxicol.Sci. (2010)

    Nørgaard et al, Toxicol.Sci. (2014)

    Duch et al, Clin. Toxicol. (Phila) (2014)

    Sørli et al, Altex (2015)