Interfacial rheology of lung surfactant: experiments & modelling to explore disruption of breathing by aerosolised compounds

NATIONAL RESEARCH CENTRE
FOR THE WORKING ENVIRONMENT

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Contents

- Context: Assuring inhalation Safety
- Lung Surfactant Rheology
- Experimental Setup – Constrained Droplet Surfactometer
- Experimental Results
- Modelling/Theory
- Conclusions & Future Work

Context :

Assuring safety of consumer products

Assuring inhalation safety: Inhalation exposure depends on product type and habits & practices

Several consumer goods products can lead to an unintentional inhalation exposure :

Can we safely use x% of ingredient y in product z?

Household cleaning products

Hairsprays (pump and aerosol)

Shampoos

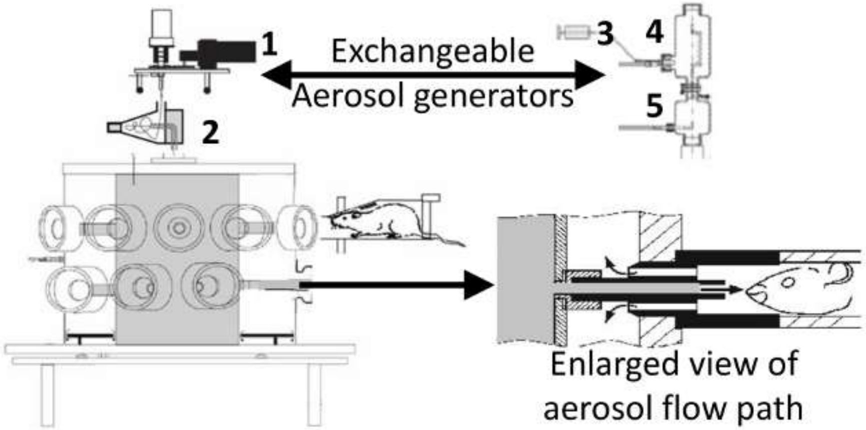
Anti-perspirant/ deodorant aerosols

Need for robust safety assessment of ingredients in consumer products

Assuring inhalation safety without animal testing

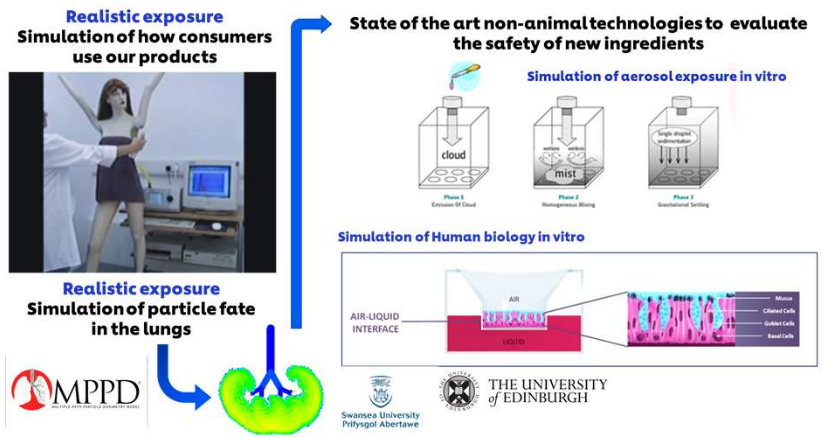
'Traditional' Risk Assessment → 'Next Generation' Risk Assessment

Historically risk assessment of ingredients (xenobiotic) in aerosols and sprays formulations relied on animal tests **in rats exposed to aerosols** for 28 or 90-days, 6h/day



Philips et al. Journ. Vis. Experiments 2017

based on advances in <u>human</u> biology and in vitro/computational modelling



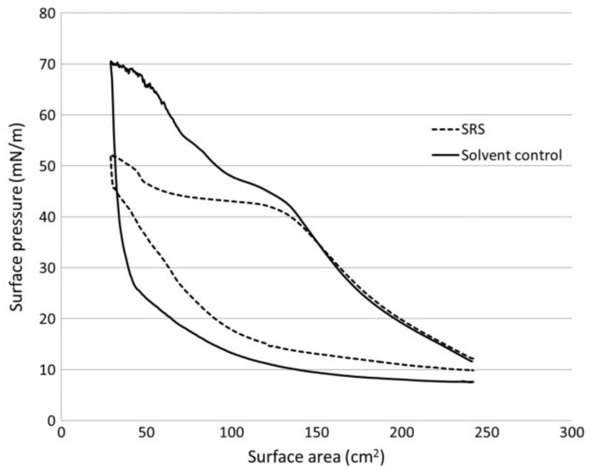
SEAC Inhalation Safety Science

Example from other industry sector; Consumer harm from lung surfactant inhibition

Aerosols of alkylsiloxane polymers produced by US tile coating company used in waterproofing were recalled from sale after they caused hospitalisation.

Eric Lipton, New York Times, 2007

Injury was shown to be caused by interactions between polymer and lung surfactant



Duch et al, Clin. Tox. 2014

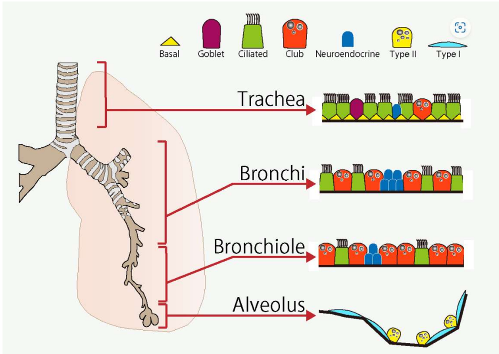
Testing strategy needs to be developed to understand and protect consumers in case of adverse interactions between novel products and lung surfactant

Lung Surfactant

Respiratory System Rheology

Epithelial Tissues

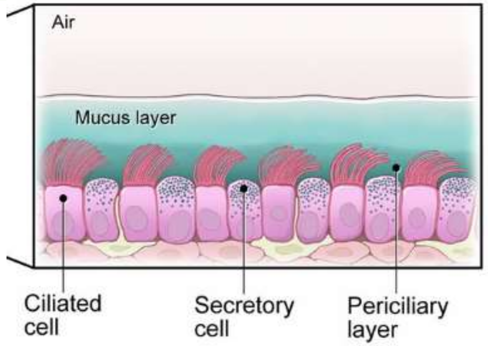
Multiple cell types undergoing stretching and compression



Morimoto, MMCHDPH, 2020

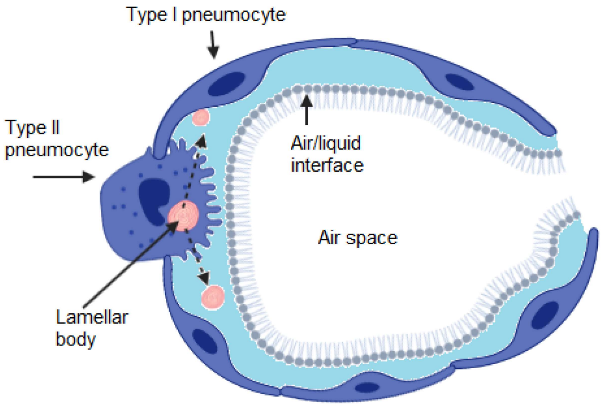
Lung Mucus

Flows along upper airway to remove pathogens



Dicky. PNAS 2018

Lung Surfactant



Dziura et al. Symmetry 2021

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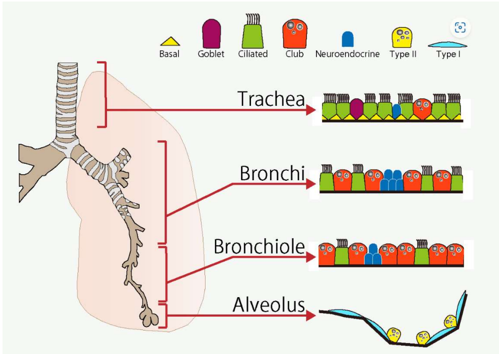

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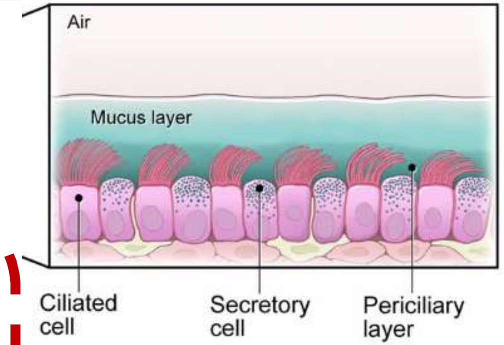

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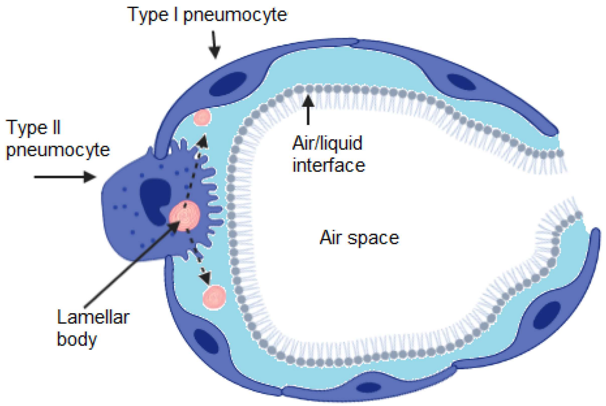


Dzjura et al. Symmetry 2021

Lung Surfactant

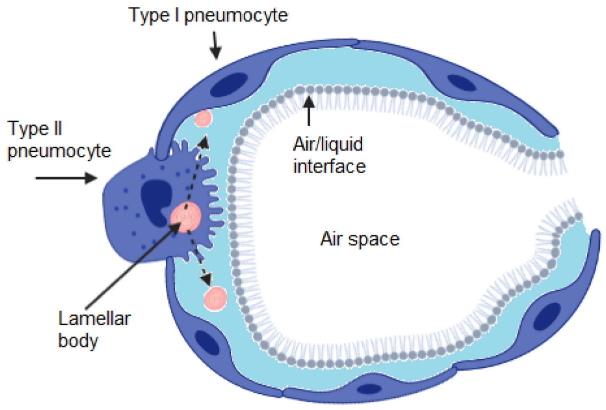
~80-90% Phospholipids

~10% Surfactant Proteins

Surfactant monolayers form at air/liquid Interface within alveoli

Laplace Pressure

$$\Delta p = \frac{2\gamma}{R}$$



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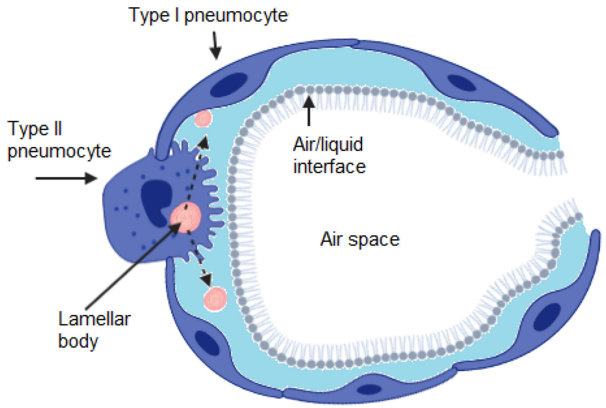


Dziura et al. Symmetry 2021

$$\Delta p = \frac{2\gamma}{R}$$

During Breathing the alveoli expand and contract over time

$$\frac{\partial \Delta p}{\partial R} = -\frac{2\gamma}{R^2} + \frac{2}{R}\frac{\partial \gamma}{\partial R} = \frac{2}{R}\left(-\gamma + \frac{\partial \gamma}{\partial \ln A}\right)$$

$$\gamma = \gamma_0 - \Pi$$

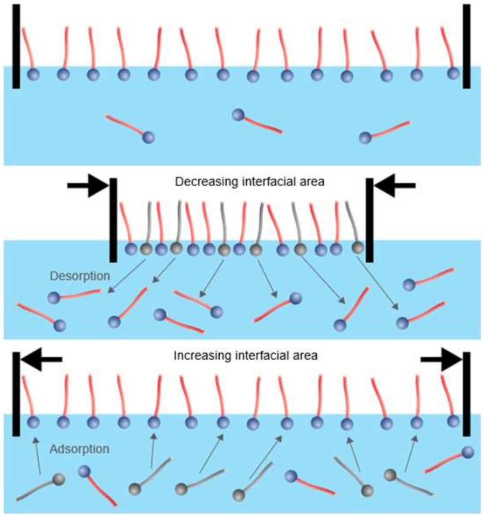
$E = -\frac{\partial \Pi}{\partial \ln A}$ where the dilational elasticity of the lung surfactant

Surfactant Dilational Rheology

Surface Pressure $\Pi(\Gamma)$

Surface Concentration Γ

As surface expands and contracts molecules migrate between bulk and surface



dataphysics-instruments.com

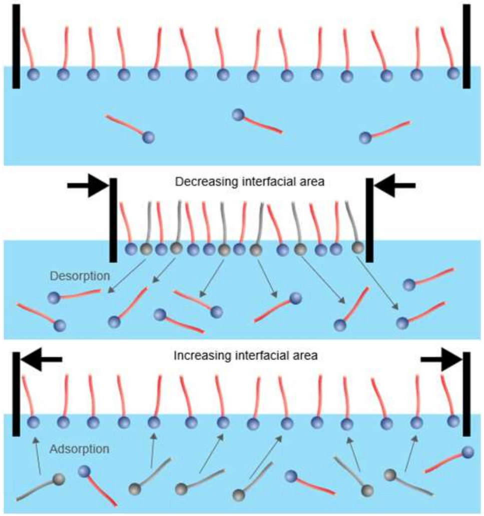
Surfactant Dilational Rheology

Surface Pressure $\Pi(\Gamma)$

Surface Concentration Γ

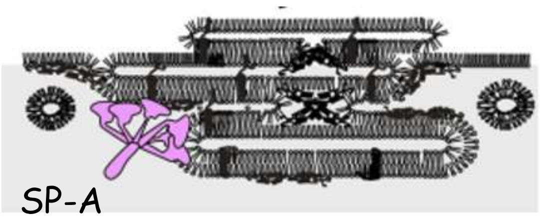
As surface expands and contracts molecules migrate between bulk and surface

Surfactant Proteins modify the process by forming subsurface structures



Increases the rate at which the surfactants re-adsorb during inhalation

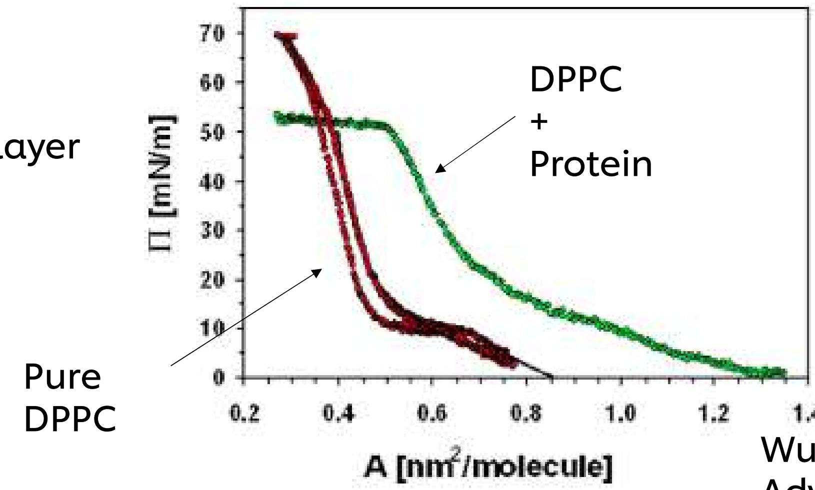
dataphysics-instruments.com



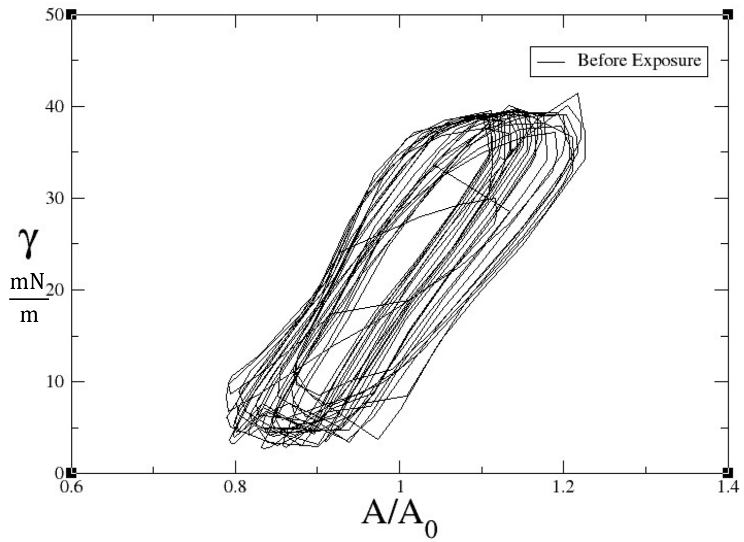
Blanco & Perez-Gil, European Journal of Pharmacology 568 (2007)

Formation of three dimensional structures increases elasticity of monolayer

Without lung surfactant to modify the elasticity of alveoli collapse during exhalation as seen
In pre-term infants



Wustneck et al.
Adv. Coll. Surf. Sci.2006



Unilever

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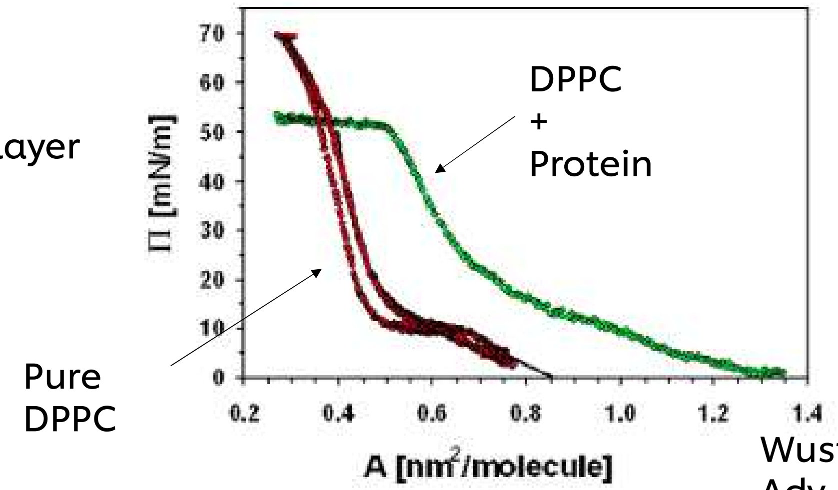
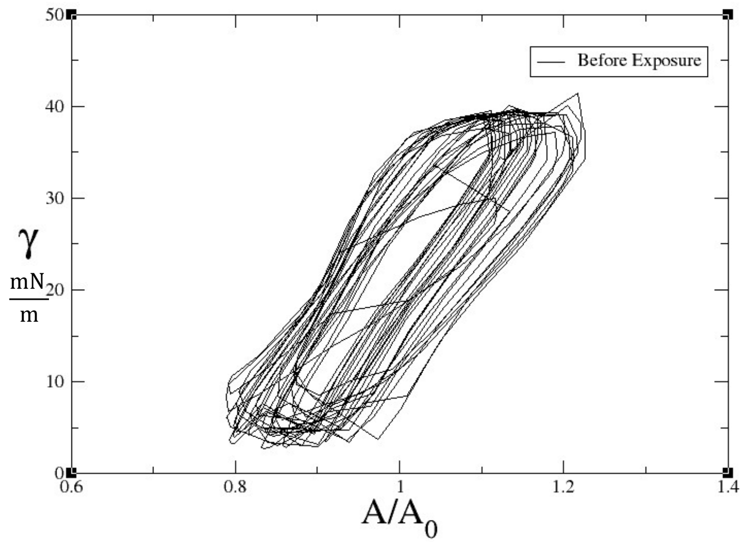


Wustneck et al.
Adv. Coll. Surf. Sci.2006

Can the disruption of breathing due to aerosolised compounds be due to modifications of the Lung surfactant rheology?

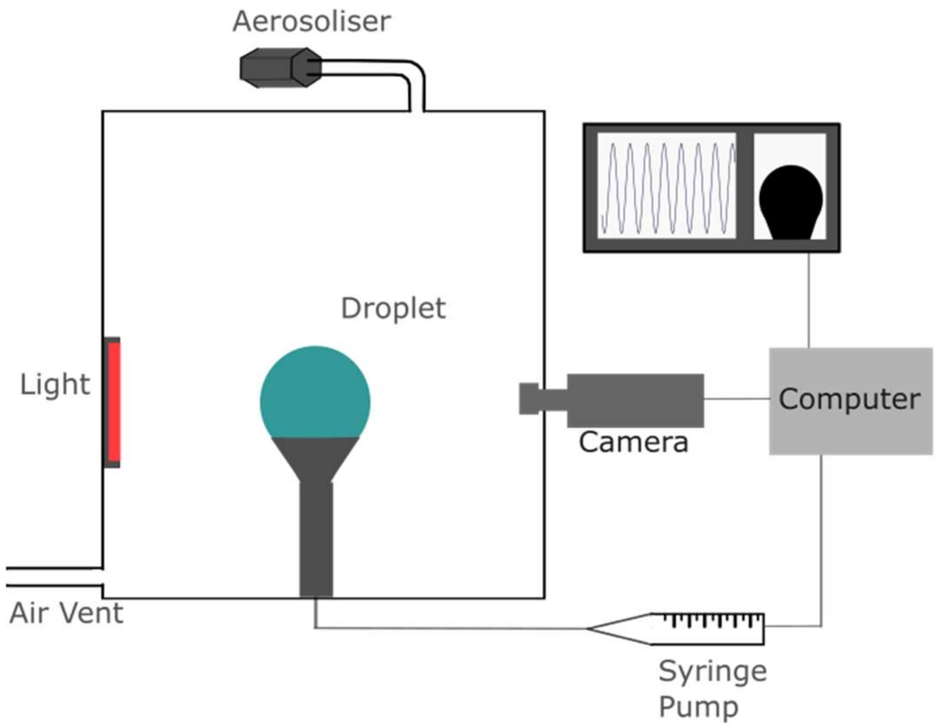


Experiments

Experiments

Solution of model lung surfactant (Curosurf®) prepared at fixed concentration

Droplet size is cycled at fixed rate with 20% amplitude



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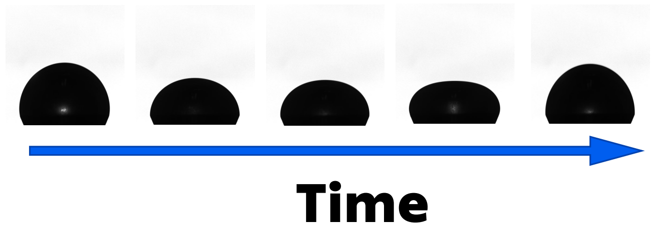
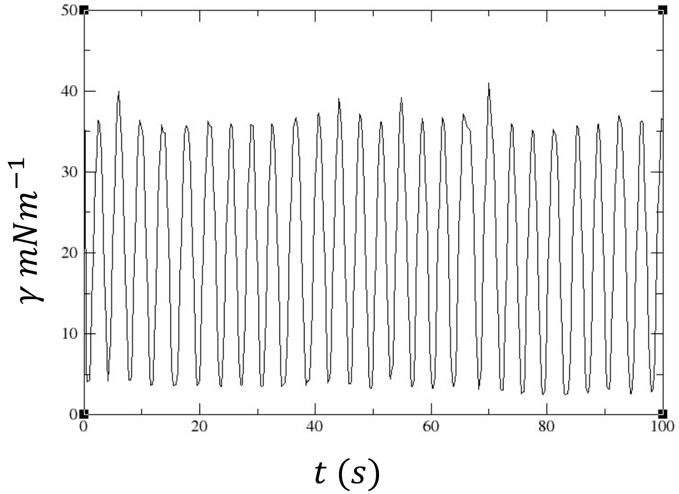
Images of droplet are processed
To measure surface tension





Experiments

Solution of model lung surfactant (Curosurf®) prepared at fixed concentration

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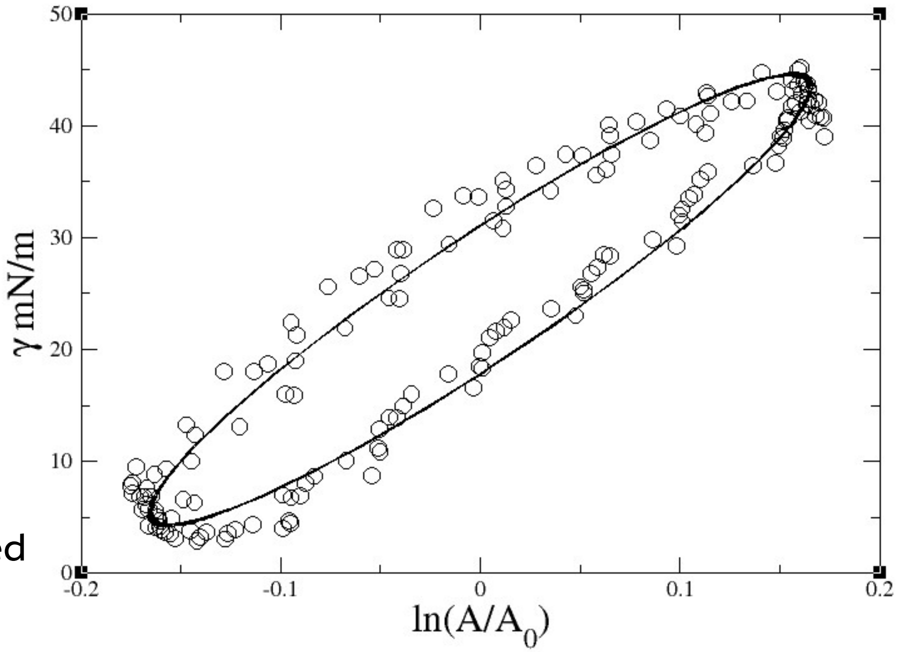
Images of droplet are processed To measure surface tension

Complex dilation modulus obtained via Fourier Transform

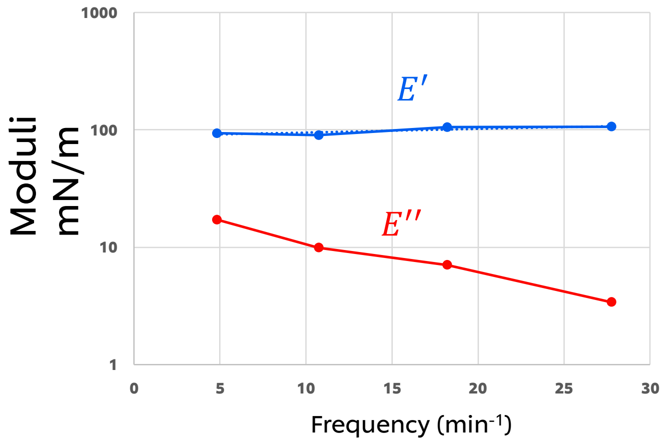


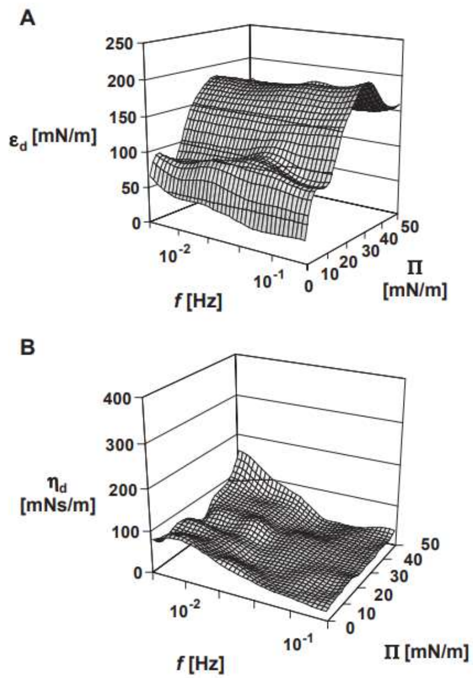
$$E^* = E' + iE'' = {}^{\mathfrak{F}(\gamma)}/_{\mathfrak{F}(\ln A/A_0)}$$

Solid Line $\gamma^2 - 2E' \ln A/A_0\, \gamma + \ln A/A_0{}^2 \left(E'^2 + E''^2\right) = E''^2 \ln A_{max}/A_0{}^2$

Experiments – Base Rheology

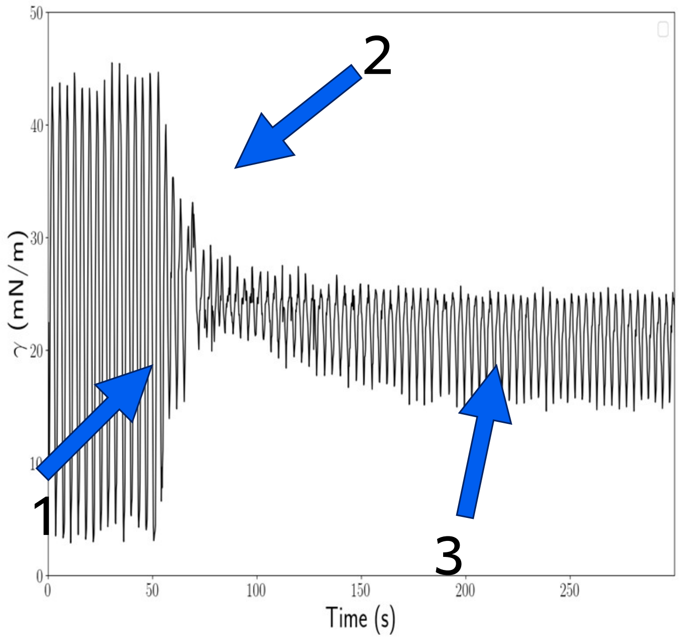
Storage and loss moduli show reasonable agreement with literature values within range of typical human breathing frequencies





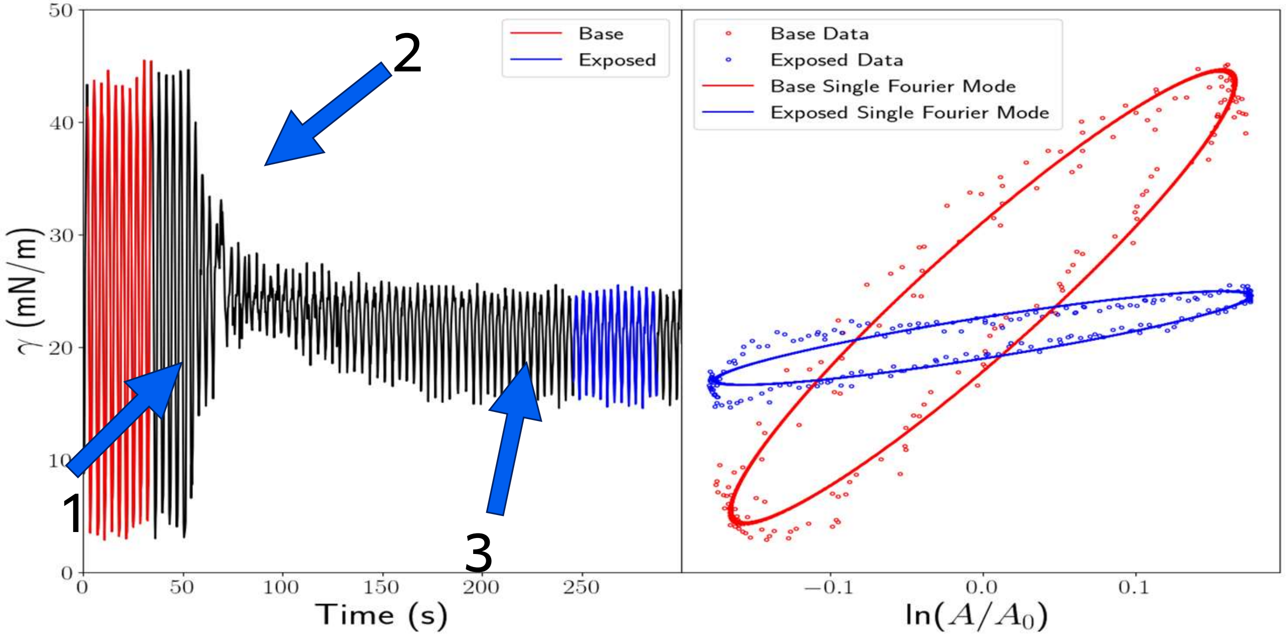
Wuestnec et al. Adv. Coll. & Surf.2005)

Experiments – (Alkylsiloxane Polymer)



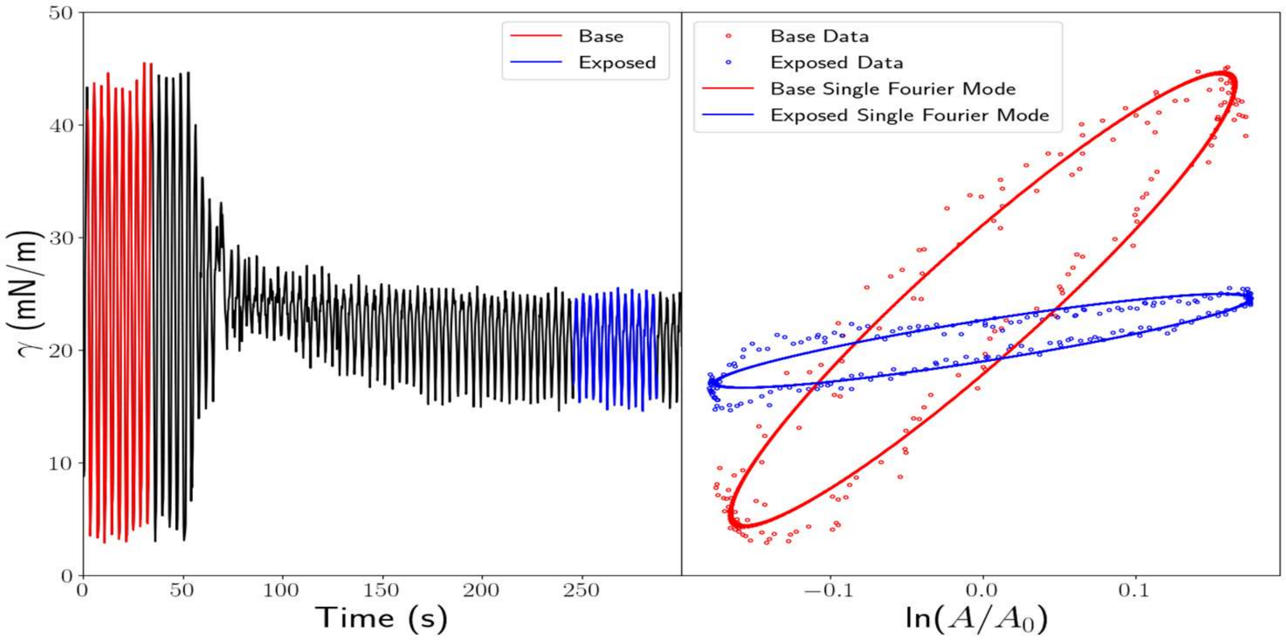
1. Base Response established before infusion
2. Infusion begins at 40s
3. New response appears after short time

Experiments – (Alkylsiloxane Polymer)



1. Base Response established before aerosol infusion
2. Infusion begins at 40s
3. New response appears after short time

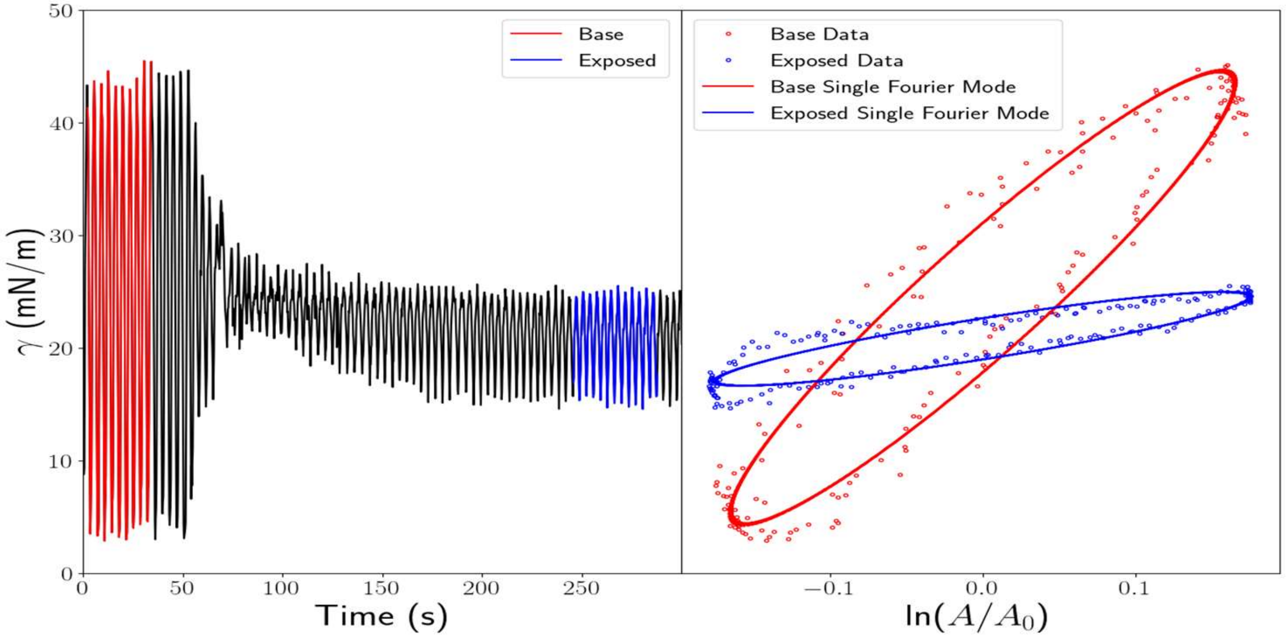
Experiments – (Alkylsiloxane Polymer)



Lissajous curves show significant decreases in elasticity following introduction of xenobiotic

Despite continuous infusion, new steady response is observed

Experiments – (Alkylsiloxane Polymer)



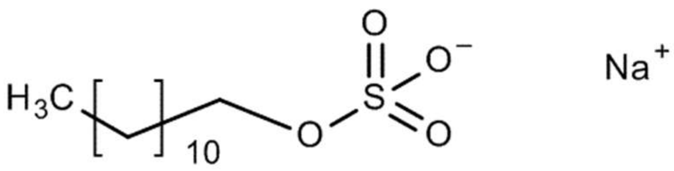
Lissajous curves show significant decreases in elasticity following introduction of xenobiotic

Despite continuous infusion, new steady response is observed

Now to study other compounds

Experiments

Sodium Dodecyl Sulfate (SDS)

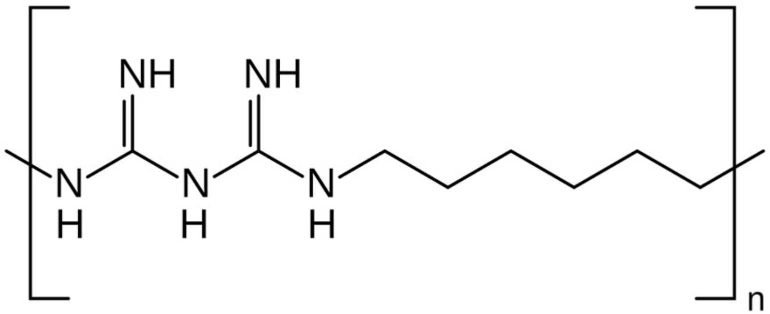


Description: Anionic Surfactant

Toxicology: Known irritant but acceptable for use below known effect levels

Commercial use: Cleaning Products

Polyhexanide (PHMB)



Description: Amphiphilic Polymer

Toxicology: Not suitable for aerosol use

Commercial use: Disinfectant

Experiments

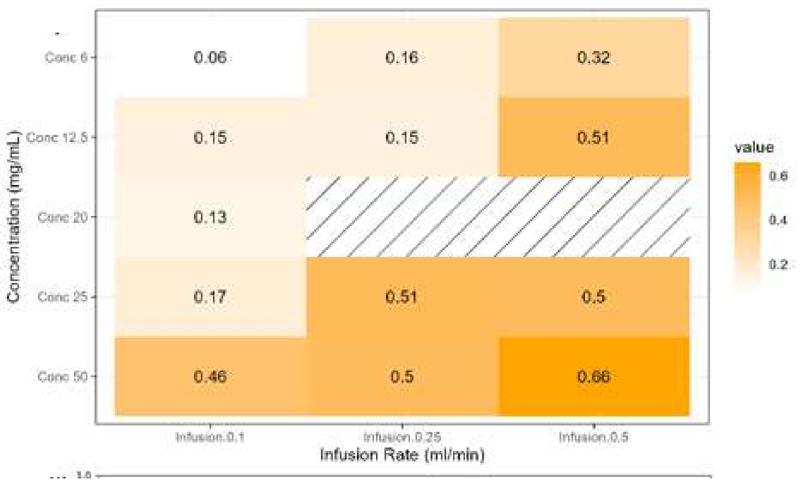
Quantifying change in rheology

$$|E^*| = \sqrt{E'^2 + E''^2}$$

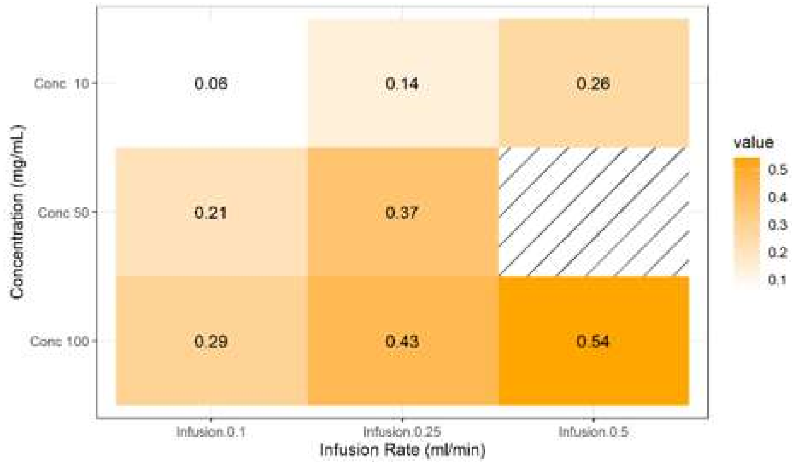
$$\Delta\tilde{E} = \frac{|E^*_{post}| - |E^*_{pre}|}{|E^*_{pre}|}$$

Different concentrations and Infusion rates confirm dose rate hypothesis

SDS



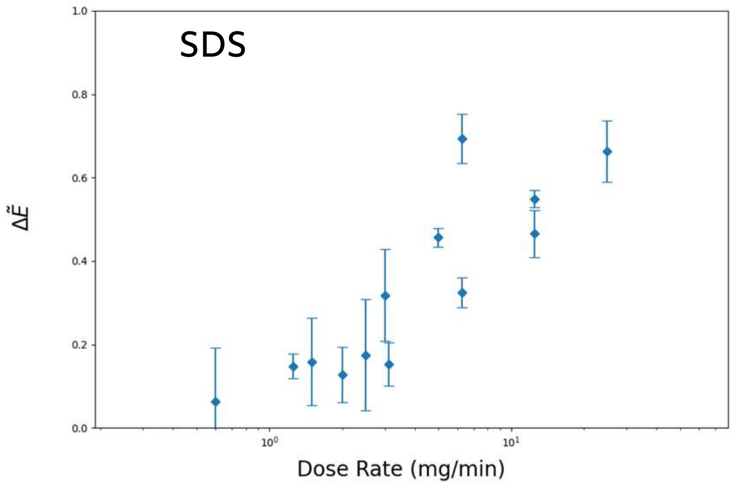
Polyhexanide

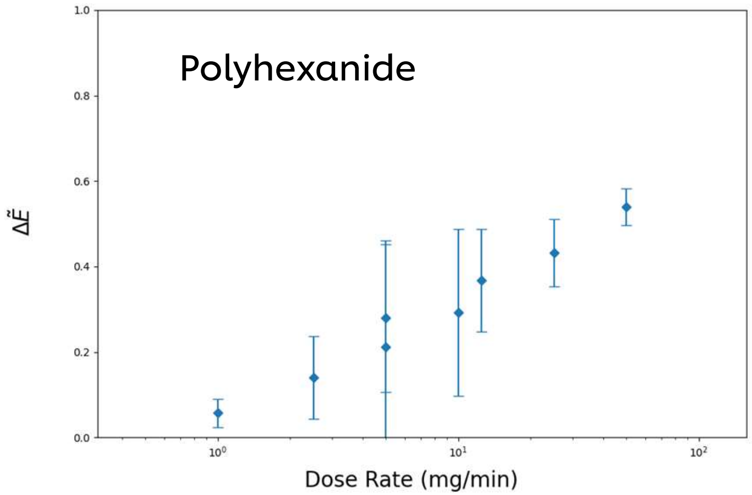


Experiments

In both cases we see reasonable
Curve collapse

Dose Rate=Concentration Infusion Rate





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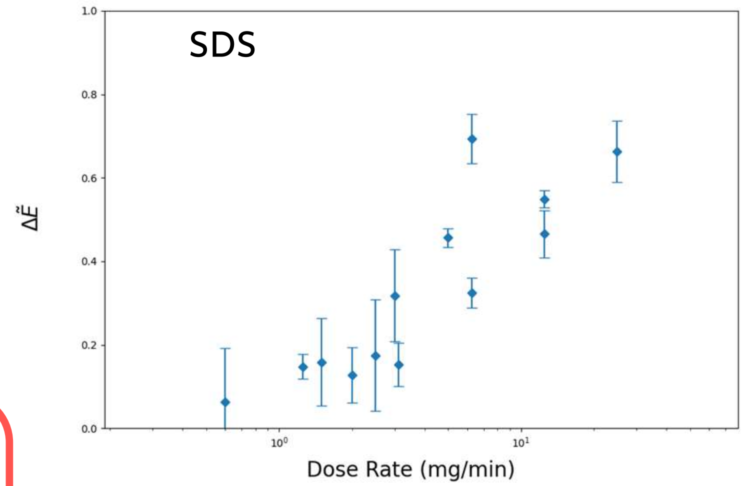
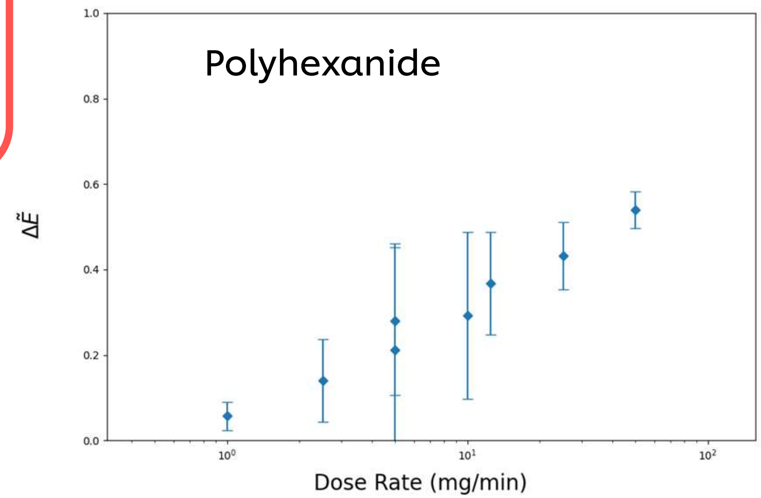
Dose Rate=Concentration ×Infusion Rate

"This suggests that the *dose rate* rather than the *total inhaled dose* of substance is critical for the toxic effect."
Duch et al. Clin. Toxicol. 2014

Effect also seen in vivo suggests that
In vitro method is capturing key
factors for predicting human safety





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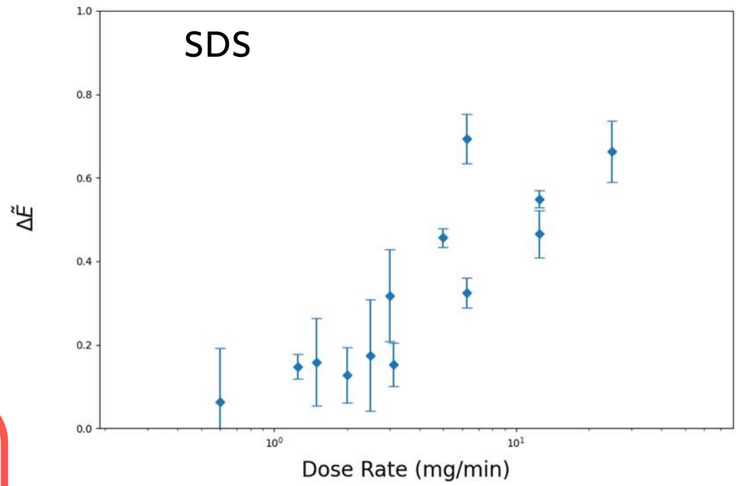
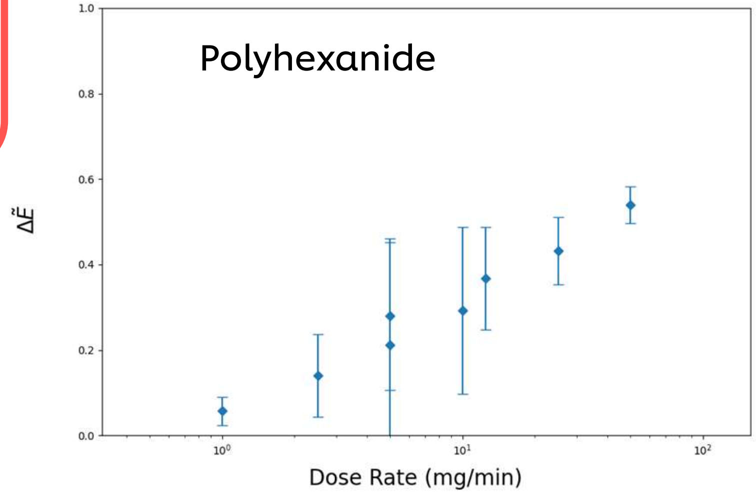
Can we now understand this in terms of surfactant physics?

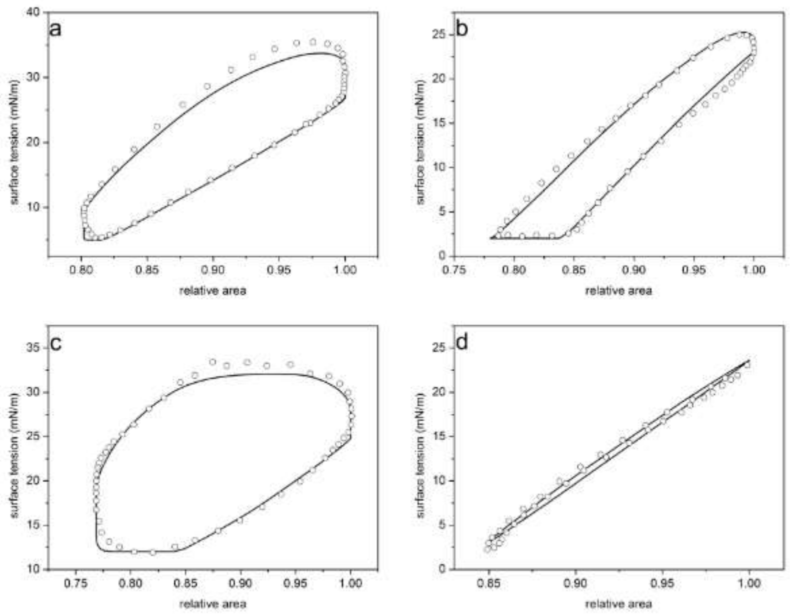




Modelling

Modelling Lung Surfactant

Several models have been developed
To simulate the dynamics of lung
surfactant



Models tend to be quite complex to
account for different regimes/pressures
with many fitting parameters

Our goal here is to construct a minimal
model that can be used to understand how
aerosolised compounds are interfering with
lung surfactant function.

Bouchoris & Bontozoglou
C&S:A. 2021

Modelling - Volmer isotherm

Assume that lung surfactant on surface behaves as a two dimensional gas with surface concentration Γ and migrates between bulk and surface continuously

Surface Tension $\gamma = \gamma_0 - k_B\, T\, \Pi(\Gamma)$

Surface Pressure $\Pi(\Gamma) = m\frac{\Gamma_\infty \Gamma}{\Gamma_\infty - \Gamma}$

Total Surfactant Flux $Q = \frac{d(\Gamma A)}{dt}$

Rate of change surfactant concentration $\frac{d\Gamma}{dt} = k_a C(\Gamma_\infty - \Gamma) - k_d\, \Gamma e^{\frac{\xi(\Gamma)}{k_B T}} - \frac{\Gamma}{A}\frac{dA}{dt}$

Non-local Interactions $\xi(\Gamma) = k_B\, T\, \frac{m\Gamma}{\Gamma_\infty - \Gamma}$

Rate constants k_a, k_d

Empirical Scaling Parameter m

Maximum Concentration $\Gamma_\infty = 50\ \text{Å}^{-2}$

Kralchevsky et al.
Handbook of Surfactant Science
2008

Unilever

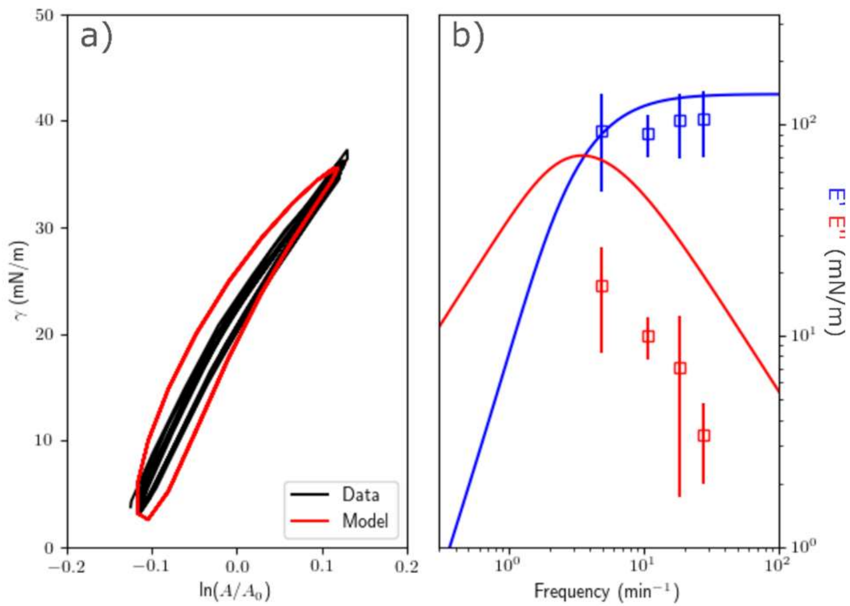
Model – Base Rheology

$$\gamma = \gamma_0 - k_B\, T\, \Pi(\Gamma)$$

$$\Pi(\Gamma) = \frac{m\,\Gamma_\infty \Gamma}{\Gamma_\infty - \Gamma}$$

$$\frac{d\Gamma}{dt} = k_a C(\Gamma_\infty - \Gamma) - k_d\, \Gamma e^{\frac{\xi(\Gamma)}{k_B T}} - \frac{\Gamma}{A}\frac{dA}{dt}$$

$$\xi(\Gamma) = k_B\, T\, \frac{m\Gamma}{\Gamma_\infty - \Gamma}$$



Model parameters are fit to Lissajous curve for single frequency

Model fails to capture both elastic and viscous behaviour exactly but does capture salient features of unexposed lung surfactant viscoelasticity

Unilever

Model – Xenobiotic Effects

Assume that the aerosolised compound is introduced at fixed rate $\dot{\Gamma}_x$

Desorption rate of compound $k_{x,d}$

Non-local Interaction xenobiotic parameter β_x

$\dot{\Gamma}_x = \alpha \times$ Dose Rate

These three parameters are used to fit our model for each chemical studied

$$\gamma = \gamma_0 - k_B\, T\, \Gamma_\infty\, \Pi(\Gamma, \Gamma_x)$$

$$\Pi(\Gamma, \Gamma_x) = m\frac{\Gamma + \Gamma_x}{\Gamma_\infty - \Gamma - \Gamma_x} + \frac{\beta_x}{k_B\, T}(\Gamma_x/\Gamma_\infty)^2$$

$$\frac{d\Gamma}{dt} = k_a C(\Gamma_\infty - \Gamma - \Gamma_x) - k_d\, \Gamma e^{\frac{\xi(\Gamma,\Gamma_x)}{k_B T}} - \frac{\Gamma}{A}\frac{dA}{dt}$$

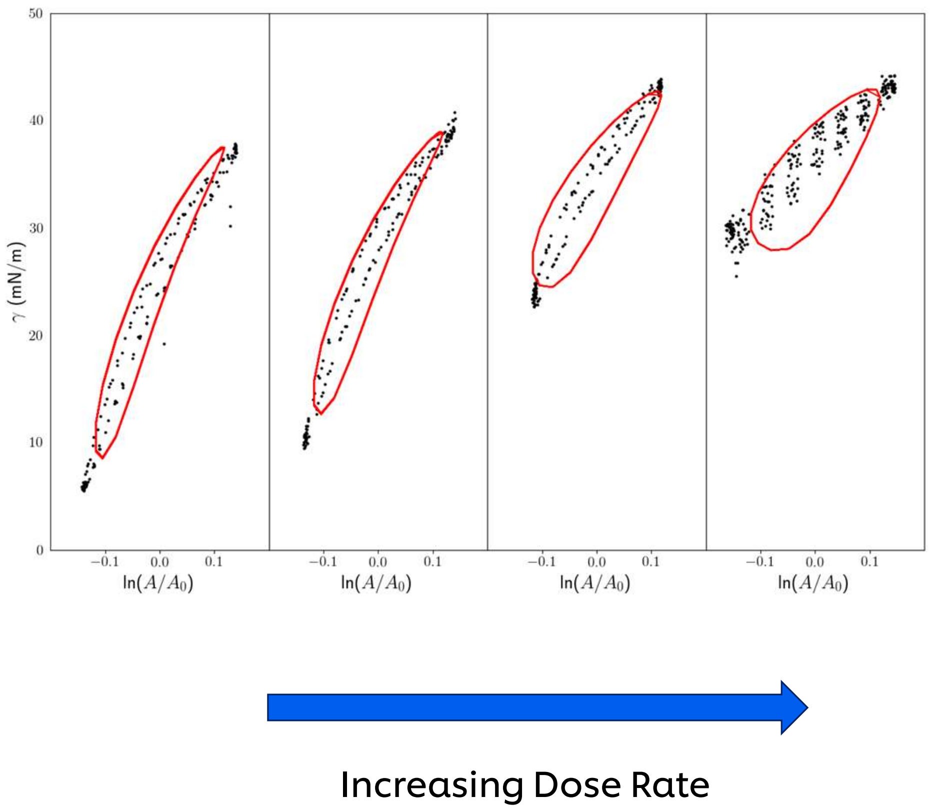
$$\frac{d\Gamma_x}{dt} = \dot{\Gamma}_x - k_{x,d}\, \Gamma_x\, e^{\frac{\xi(\Gamma,\Gamma_x)}{k_B T}} - \frac{\Gamma_x}{A}\frac{dA}{dt}$$

$$\xi(\Gamma, \Gamma_x) = k_B\, Tm\frac{\Gamma + \Gamma_x}{\Gamma_\infty - \Gamma - \Gamma_x} + \beta_x \Gamma_x/\Gamma_\infty$$

Unilever

Modelling – Xenobiotic Effects

Model Accurately reproduces observed change in Lissajous curves with increasing dose rate

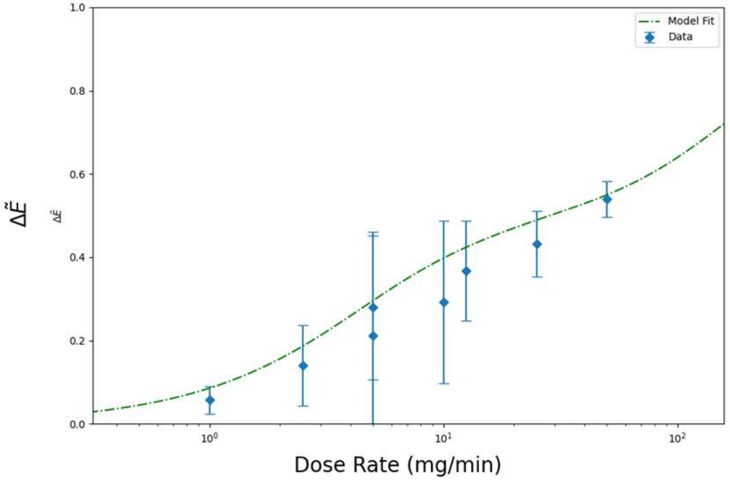


Modelling – Xenobiotic Effects

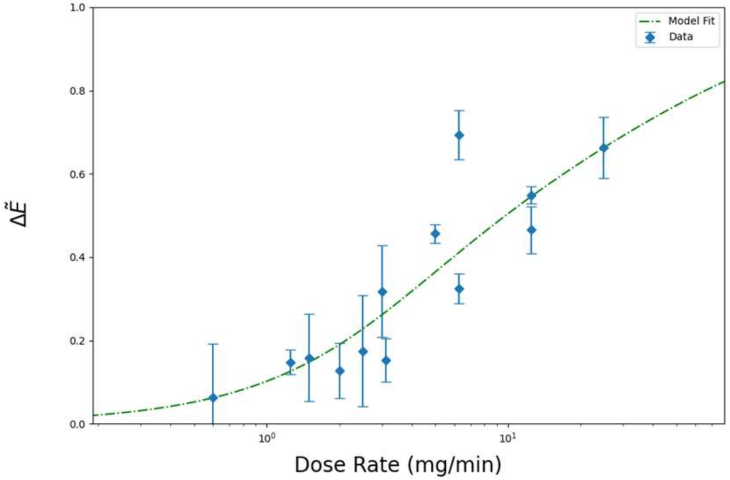
Model Accurately reproduces observed change in Lissajous curves with increasing dose rate

Model also reproduces observed change in dilational modulus for both Polyhexane and SDS

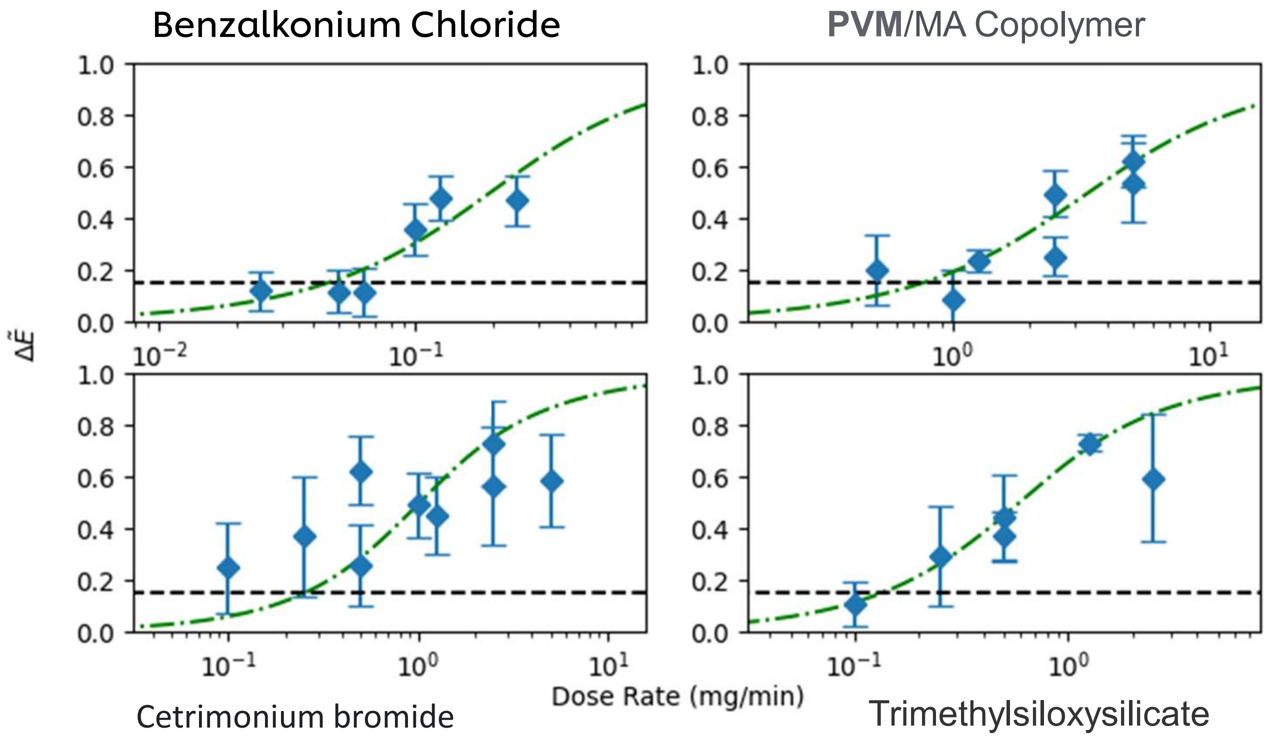
Polyhexane



SDS



Modelling – Xenobiotic Effects



Model can be successfully fit to observed change in rheology across all chemicals studied

Measured relative potencies of each chemical encouragingly agree with literature

Suggests that the mechanism for inhibition is generic and well captured by model

(Larsen et al. B&C P&T 2012)

Unilever

Conclusions

- Inhibition of function of lung surfactant function demonstrated to be linked to compound altering dilational rheology
- In vitro study reproduces dose rate dependence/potencies seen in literature
- Modelling successfully fits all data from experiments suggesting mechanism is generic
- Results of this study act as a very encouraging example of how in vitro experiments and modelling can be used for assuring safety without animal testing

Future Work

- What determines the relative potency of each chemical?
- Extend the modelling to include effects of multilayer structures

Acknowledgements

Thank you & Questions?