# Design Project - SIE 2024

## The fate of phathalates in lungs : desorption from microplastics and enzymatic degradration

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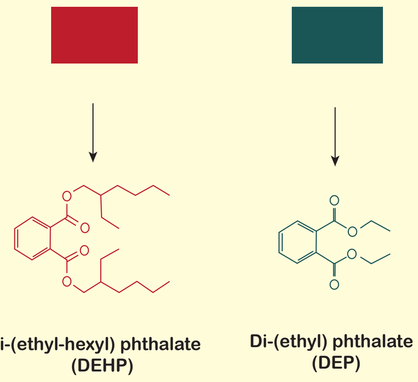
**Context :** Aside from pollution issues, plastics are targetted for their adverse effects on health. The main concern is related to the additives added during polymerisation, like phthalates. Not bonded to the polymers, phthalates can diffuse within the matrix and leach to the environment. Then, they can absorb onto particle surfaces, including dust and microplastics. The latter can be inhaled, introducing phthalates in the lung system. Over time, phthalates desorb from the particles surface and considered as intruders, they are hydrolysed by lung enzymes. Therefore, characterising desorption and hydrolysis is crucial to understand phthalates fate in lungs and the subsequent impacts on human health.

### Phathalate esters




- Additives included to increase plastic flexibility, transparency and durability (plasticizers)
- Endrocine disrupters and carcinogenic

### Lung fluid


- Simulated lung fluid = Proteins + Lipids + Salts + Organic acids + Ultra pure H2O
- T= 37°C - pH = 7.2-4
- Physical defense

### Enzymes

- Carboxyl esterases
- Catalyze hydrolysis of carboxylic esters

*R-COO-R' + H2O → R-OH + R'-COOH*

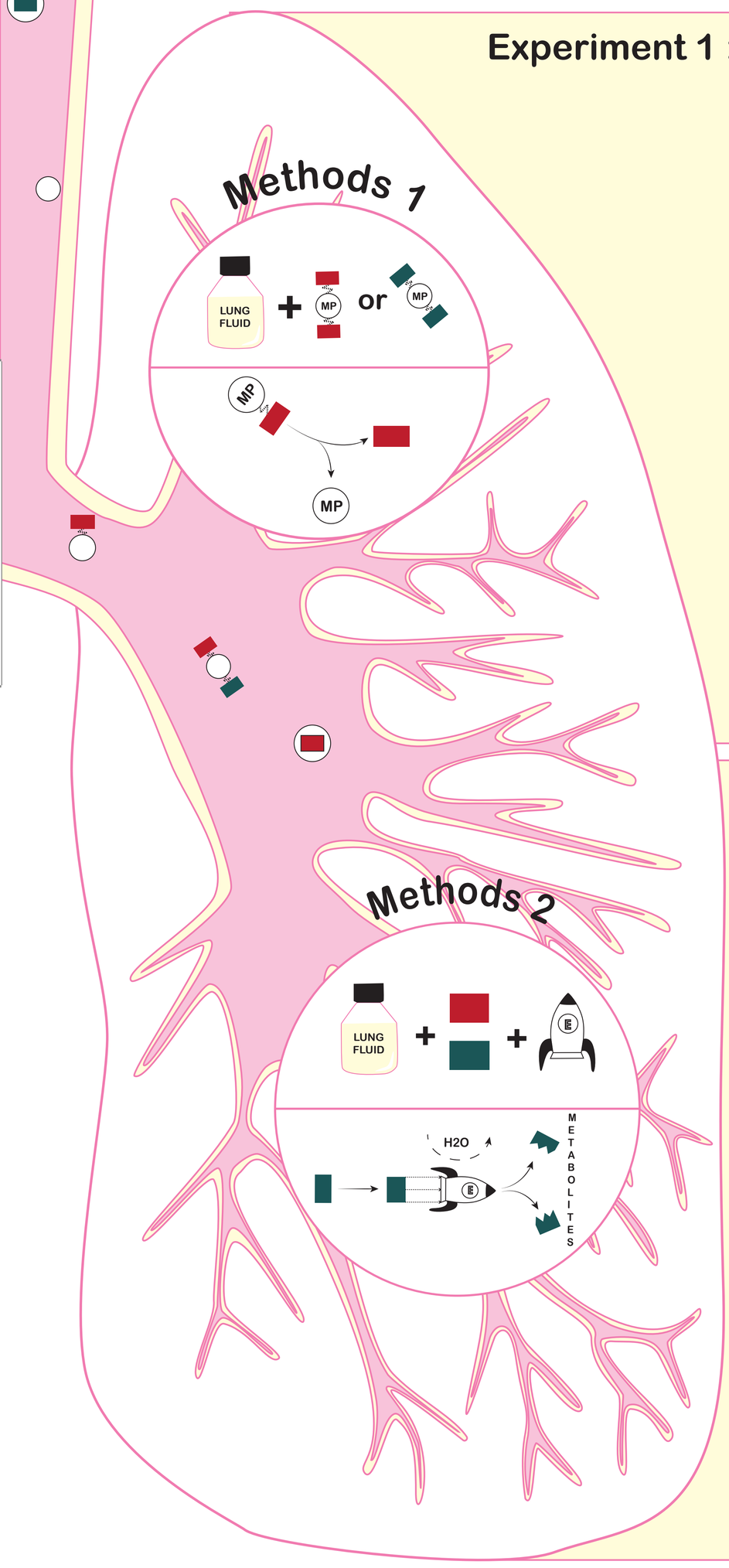
- Biological defense

### Microplastics


- Polypropylene beads
- Size < 5 mm
- Hydrophobic
- Causing inflammation in lungs
- Carrier of phthalates

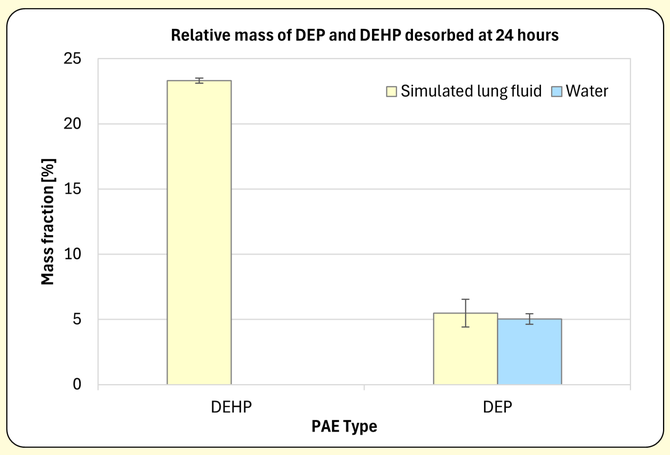
## Goals of the study

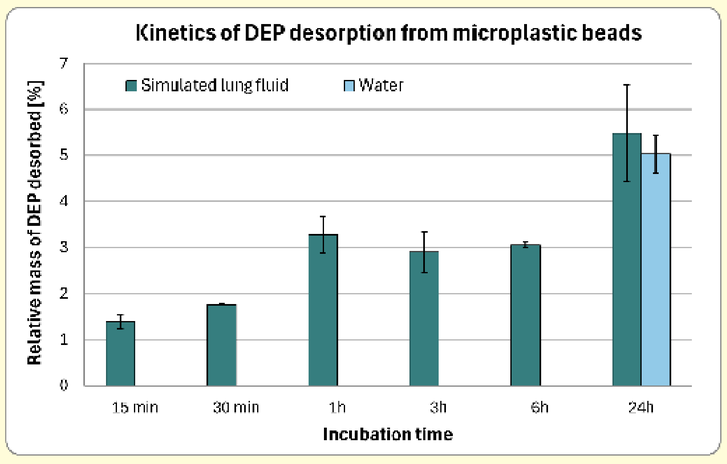
- Using *in vitro* experiments, this study aims at answering the following questions :
- After microplastic inhalation :
  ***How are DEP and DEHP desorbing from the MP beads into artificial lung fluid ?***
- Once desorbed, :
  ***How fast are they hydrolysed under carboxyl esterases catalysis in lung fluids?***
  ***What are the metabolites formed ?***




## Experiment 1 : Desorption kinetics of DEP and DEHP in artificial lung fluid and water

Methods
- A preliminary step includes artificial adsorption of DEP or DEHP onto MPs surface.
- Then, enriched DEP-MPs or DEHP-MPs are incubated in simulated lung fluid - Similar incubation in water as reference.
- Finally, phthalate desorption is quantified using liquid-liquid extractions and gas chromatography-mass spectrometry (GC-MS) analysis.



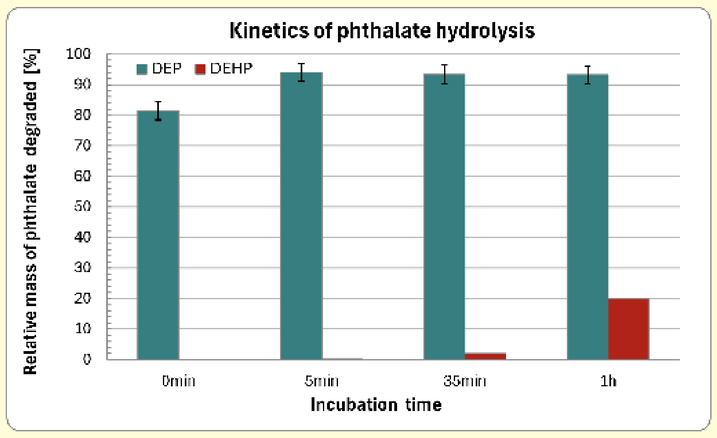



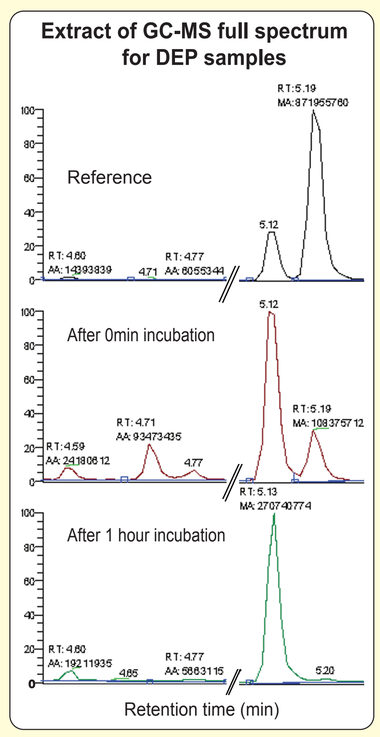

Results :
- The mass of DEP desorbed increases with incubation time. On the contrary, the desorption speed decreases as the reaction proceeds and reaches equilibrium.
- DEP desorption is slighly greater (+0.5%) in simulated lung fluid than in water. This affinity is explained by DEP low hydrophobicity.
- Comparing the two phthalates, DEHP desorbs 4 times more than DEP. Again, this is due to DEP lower hydrophobicity.

## Experiment 2 : Kinetics of DEP and DEHP hydrolysis

Methods :
- Free phthalates are incubated in simulated lung fluid containing carboxyl esterases. References were done in similar solutions, but without enzymes.
- Then, samples are extracted using liquid-liquid extractions and centrifugation.
- The kinetics of degradation is obtained by GC-MS analysis, focusing on DEP or DEHP masses.
- The research of metabolites is performed by scanning the full mass spectrum in GC-MS analysis.

One expects the formation of phthalate monoesters and alcoholic compounds.




Results :
- DEP is hydrolysed much faster than DEHP : carboxyl esterase has a higher affinity for DEP. This trend is due to DEP smaller size, reaching more easily enzymes active sites.
- The degradation speed is proportional to DEP and DEHP concentration.
- Regarding DEP full spectrum, consumption (5.19 min peak) and production processes (4.17 min peak) are observed. The area of the peak at 5.13 min is not changing over time and should correspond to the internal standard.
- Each peak is associated with m/z masses but could not be linked to a unique compound.

**Conclusion :** Desorption and degradation of phthalates are relevant processes happening in simulated lung fluid. By comparing the two reactions speeds, one can infer that phthalates residence time in lungs is short. Though, the metabolites formed, especially for DEHP, might be more toxic than the mother compounds. The next step should focus on metabolites occurence and behaviour in lung fluid. Finally, the experiments presented here are approximations of the true lung conditions and confirmation of the results with in vivo samples is needed.

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