Innovative **Materials**: Safety along the **Life** Cycle

# A systematic approach to investigate microplastics hazards with specific consideration of the carrier hypothesis for polycyclic aromatic hydrocarbons (PAHs)

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Investigating possible human health effects of micro- and nanoscaled plastic particles (MNP) is challenging for several reasons. MNPs are complex mixtures of diverse geometries, shapes and sizes that can be based on different polymer types (e.g. polypropylene (PP), polyethylene (PE), polyamide (PA), polyurethane (PU)). Additionally they contain additives (e.g. antioxidants, vulcanizers, pigments) and several contaminants (e.g. remaining traces of catalysts, traces of monomers and oligomers). Moreover, they may act as carriers for environmental persistent organic pollutants (POPs) like polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCB) or others. There is no consensus yet which properties are most important for hazard assessment, or if all solid MNPs should be assessed jointly. Thus, assessing possible hazards of MNPs is a complex endeavor. Within the German BMBF funded project InnoMat.Life (www.innomatlife.de) we investigate a selection of different MNPs, covering different polymers (e.g. PE, PA, PU) with broad size distributions. In a first step ad- and desorption of selected POPs are assessed. In parallel, we conduct in vitro based studies using different cell models (liver and lung cell models). These will guide in vivo studies.

PE has been chosen as a benchmark material. In addition, several types of PA (PA-6, PA-12), PU (4 variants), PMMA (2 variants) and a rubber material obtained from recycled truck tires were included. For assessing the sorption of PAHs, benzo[*a*]pyrene (B[*a*]P) was selected as a lead substance along with dibenzo[*a,l*]pyrene (DB[*a,l*]P) and anthracene (Anth). Firstly, we developed a universally applicable third polymer-phase partitioning (TPP) method, which enabled to quantify the sorption of pollutants on various polymer particles without filtration. We analyzed the sorption of B[a]P for over 20 MNP variants, including a few aged ones. The sorption of Anth and DB[*a,l*]P was also studied for selected variants. The TPP method was validated using the commonly applied batch method. In parallel, we investigated the biocompatibility of the polymer particles in three selected cell models, human intestinal epithelial Caco-2, human liver epithelial HepG2 and rat alveolar macrophages NR8383. In addition, the carrier effects of the MNPs were investigated for B[*a*]P and two heavy metals in HepG2 and Caco-2 cells.

Overall, our work contributes to better understand the human health hazards of polymer particles. The broader aim of our project is to provide criteria for grouping of different polymer particles.

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BMBF InnoMat.Life (FKZ 03XP0216)

# Study Design and Material Selection

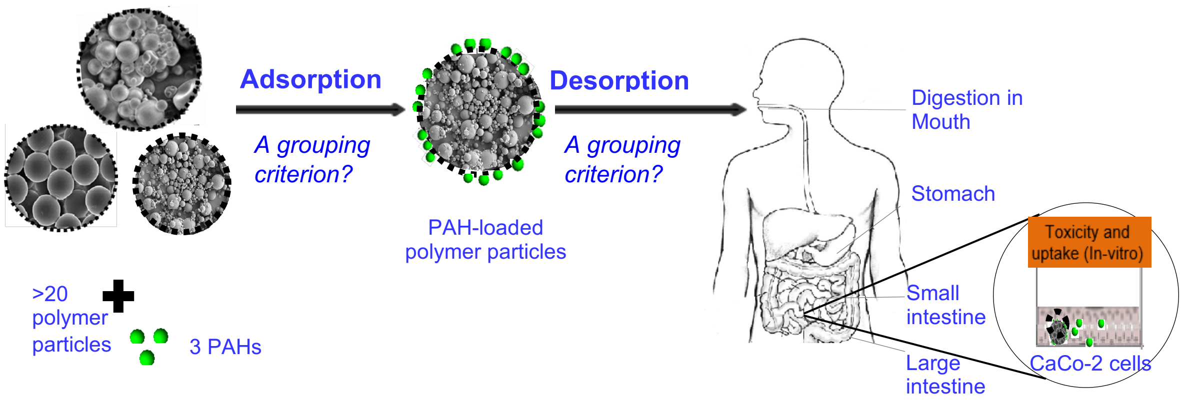


**Figure 1: Schematic study design for investigation of the "carrier hypothesis"**
The "carrier hypothesis" assumes that inert polymer particles could serve as carriers for toxic persistent organic pollutants (POPs) and thereby mediate adverse effects upon uptake into organisms. InnoMat.Life investigates the adsorption and desorption of various POPs for different polymer particles. The toxicity with/ without POPs is assessed in parallel in relevant human relevant cell models.

| Polymer Class | Polymer Variants | Application |
|---|---|---|
| Polyethylene (PE) | Low Density PE_< 80 µm | Benchmark materials |
| | Low Density PE_< 250 µm | Benchmark materials |
| | PE_broad dstribution | Benchmark materials |
| Thermoplastic polyurethane (TPU) | TPU_ester_aromatic | 3D Printing + others |
| | TPU_ester_aliphatic | 3D Printing + others |
| | TPU_ether_aromatic | 3D Printing + others |
| | TPU_ether_aliphatic | 3D Printing + others |
| | TPU_low-melt_aromatic | Binder |
| Polyurethane (PU) | PU foam_< 312 µm | Insulation |
| | PU_binder_arom_1C | Binder |
| | PU_binder_arom_2C | Binder |
| Polyamides (PA) | PA-6_< 10 µm | 3D Printing |
| | PA-6_< 80 µm | 3D Printing |
| | PA-12 _< 80 µm | 3D Printing |
| Poly[methyl methacrylate] (PMMA) | PMMA_monodisperse | Benchmark materials |
| | PMMA_broad distribution | Benchmark materials |
| Rubber | Tire rubber_< 240 µm | Truck tires |

**Table 1: Overview of investigated particles**
PE has been chosen as a benchmark material. In addition, two types of PA (PA-6, PA-12) including two size distributions for PA-6, four types of PU, two types of PMMA and one rubber material obtained from recycled truck tires were considered. Moreover, several polymer particles were additionally investigated after aging (via UV-exposure).

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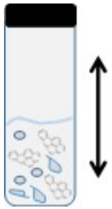
# Adsorption of persistent organic pollutants (POPs)

**Batch Method (based on OECD TG 106)**



- Mixture of polymer particles + PAHs in water
- Shaking (overhead), different time points
- Separation via vacuum filtration
- Analysis of the filtrate

**Third-Phase Partition Method (TPP)**

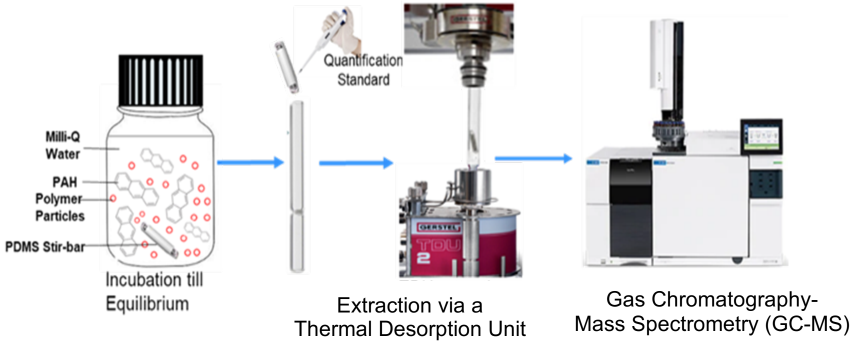


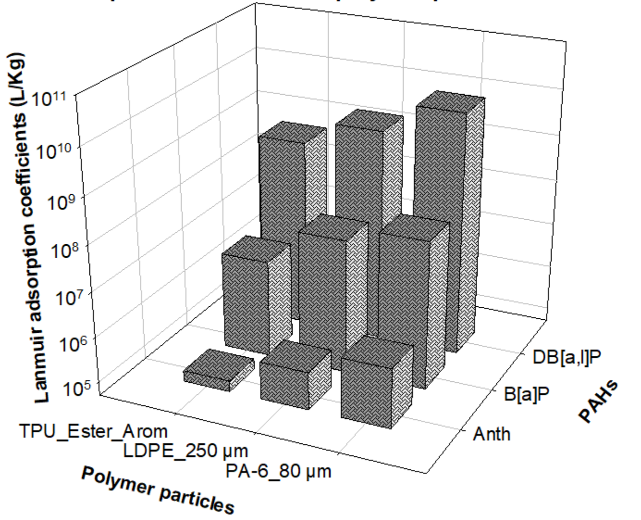

**Figure 2: Overview of methodology to investigate POP adsorption**
Using the conventional batch approach, the polymer particles are incubated with the PAHs in water while continuously shaking. The particles then have to be removed by filtration and PAHs are quantified in the supernatant. The TPP method is based on partitioning of PAHs in a three-phase system consisting of water, the polymeric material in question, and a third phase (a commercially available polydimethylsiloxane (PDMS)-coated stir bar) that is used as a passive sampler. The stir bar can be removed, adsorbed PAH are extracted in a thermal desorption unit (TDU) followed by an online-coupled GC-MS analysis without the need of prior particle removal.

| Name | MW [g/mol] | Toxicity Level | Hydrophobicity |
|---|---|---|---|
| Anthracene | 178 | Low (0.01) | hydrophobic |
| Beno[a]pyrene | 252 | Toxic (1) | very hydrophobic |
| Dibenzo[a,l]pyrene | 302 | Very Toxic (10) | super hydrophobic |

In addition, **polychlorinated biphenyls (PCBs)** and **heavy metals**.

# Adsorption of persistent organic pollutants (POPs)

A)




B)

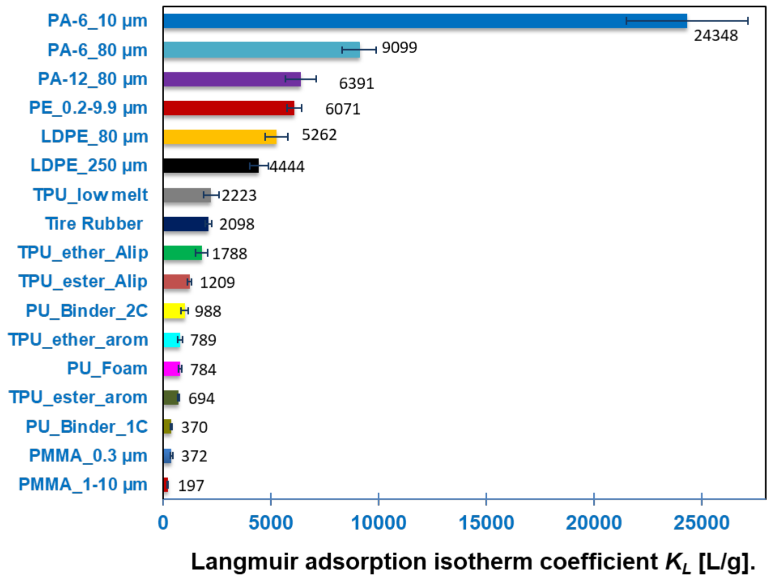



**Figure 3: Selected results for PAH adsorption.** Sorption of Anth, B[*a*]P and DB[*a,l*]P were compared for the three polymers: LDPE_250µm (low-density polyethylene, 250 µm), PA-6_80µm (polyamide-6, 80 µm) and TPU_ester_aromatic (thermoplastic polyurethane block co-polymer containing aromatic esters) showing that adsorption increases with increasing hydrophobicity (A): DB[a,l]P > B[a]P > Anth.
Using the Langmuir model's isotherm constant, the B[*a*]P adsorption for all polymers was ranked (B) showing that mainly the chemical nature of a polymer determines the adsorption with PA and PE showing the highest adsorption and PMMA showing lowest adsorption.

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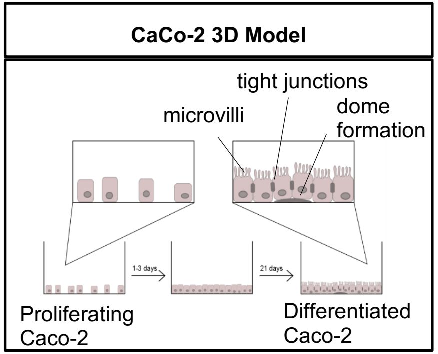
# In vitro toxicity of the polymer particles




First Results for different Polymers

- Not cytotoxic (CaCo-2, HepG2, NR8383)
- Not reactive
- Only tire rubber induced CYP 1A1 on its own

Work in Progress:

- Particle uptake: nile red staining and TEM
- PAH-loaded particles for in-vitro experiments:
  - CYP 1A1 induction
  - Metabolomics

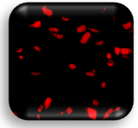

PA-6 inhalable

**Figure 4: Overview on *in vitro* toxicity studies**
Cytotoxicity testing with CTB and MTT assay showed that none of the polymer particles had any impact on differentiated human epithelial CaCo-2 cells. A similar outcome was observed for HepG2 human liver cells and NR83838 rat macrophages. The acellular DCFH-assay confirmed that the polymer particles were non reactive. By assessing the transcript levels using Real-Time PCR only tire rubber particles could induce CYP1A1 on their own. Investigations using the PAH-loaded particles are still ongoing- however, so far we did not find any support for the carrier hypothesis. In parallel we assess particle uptake using Nile red fluorescence labelling as well as TEM analysis.

# UV aging and fragmentation

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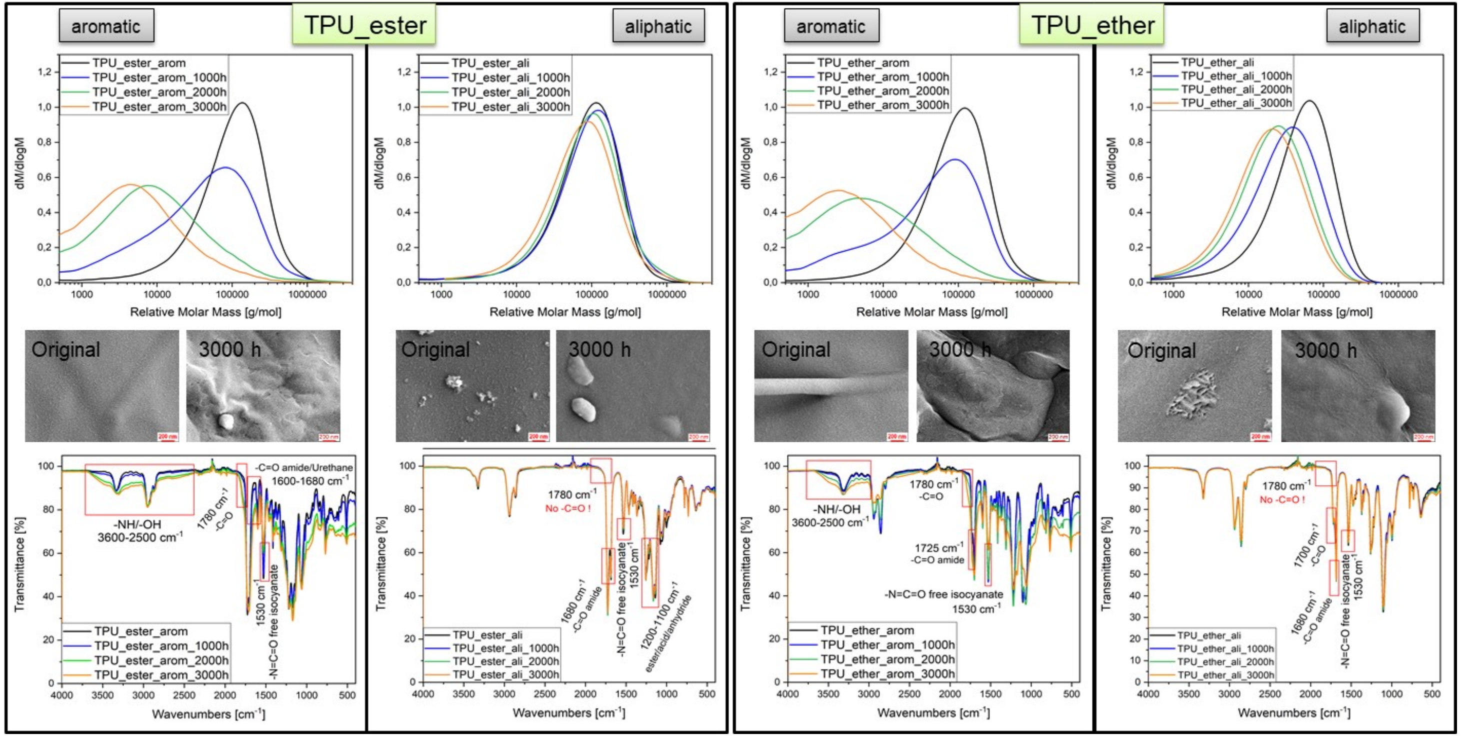


**Figure 5: Aging and Fragmentation Behaviour**
Aromatic TPUs degrade more easily than aliphatic TPUs. TPU ethers are more affected by UV radiation than TPU esters.
The aged polymers showed a decreased adsorption behavior (data not shown).