

## Summary of the BMBF-funded project InnoMat.Life

InnoMat.Life studied selected innovative materials during their life cycle and assessed material properties, release and exposure as well as potential hazards for humans and the environment. Three innovative material classes were selected: (nano-)fibres; polydisperse polymer particles for additive manufacturing and materials with complex composition and/ or morphology. InnoMat.Life build on existing knowledge primarily from nanosafety research and tested for instance the applicability/ transferability of existing test methods. To avoid testing each and every material variant individually, InnoMat.Life developed criteria catalogues to describe similarity to establish grouping approaches as an overall project goal. InnoMat.Life therefore delivered important contributions to the risk assessment of the selected innovative materials.

The following results were achieved:

### **(1) (Nano-)fibres**

The fibre principle describes a well characterised morphological principle according to which fibres with specific physico-chemical properties (so-called WHO-fibres) may cause severe human health hazards upon inhalation, e.g. fibroses, lung tumors or mesothelioma.

Nanofibres are in particular challenging. Thin fibres are not stiff enough, can coil up and will not show a characteristic fibre mode-of-action. However, currently there are no reliable methods to determine rigidity. Moreover, most of the previous projects focused on one specific type of nanofibers, carbon nanotubes (CNTs). InnoMat.Life build on the existing knowledge from projects such as the BMBF-funded project nanoGRAVUR (Project ID 03XP0002) or the EU project GRACIOUS (Grant Agreement No 760840) but for the first time a much broader selection of fibres was investigated, including different nanofibers.

Extensive tests on dustiness were performed. In addition, a new method to generate fibre aerosols with a high fraction of individual fibres was developed. This was coupled to a newly established approach allowing for size classification, which is in particular relevant for in vitro testing strategies. Furthermore, a protocol was worked out that allows fibre deposition from aerosols for in vitro investigations whereby firstly the fibres are deposited and afterwards the cell culture is performed to investigate toxicity mechanisms. These methods will allow to investigate fibre toxicity in vitro in a much better and more targeted manner in future.

Proteomics methods were applied to detect substantial changes in the proteome of cell cultures after the application of SiC and TiO<sub>2</sub> fibres. Moreover, transformation of fibres in biological media were investigated in a systematic manner for the first time. Some silver fibres were shown to get thinner in their diameters thereby altering in rigidity.

InnoMat.Life also systematically studied possible implications of fibre morphology in ecotoxicity showing that fibres may elicit specific effects in algae but not in daphnia. The observed effect was related to the formation of retiform agglomerates. However, the influence of fibre length and diameters could not yet be fully resolved.

InnoMat.Life delivered important contributions for the establishment of a fibre testing and assessment strategy. In particular several important methods could be established, which will be used in follow-up projects (e.g. EU HARMLESS).

## **(2) Polydisperse polymer particles for additive manufacturing (3D printing)**

Many polymers are considered biocompatible such that they can be grouped together following the OECD Polymers of Low Concern concept. To which extent this concept could be transferred to polymer particles and which criteria would be needed was unclear at the start of the InnoMat.Life project. We focused on polymer particles for innovative applications, specifically additive manufacturing. However, the investigations also have a relevance for polymer particles found in the environment, so called micro- and nanoplastic. Most previous research focused primarily on polystyrene particles. InnoMat.Life for the first time systematically investigated a larger selection of different polymer particles, established several useful methods and thereby delivered an important contribution to the risk assessment.

All InnoMat.Life polymer particles were specifically synthesized for the project and were relevant for additive manufacturing. They had a broad size distribution from 10 µm to 500 µm. In addition, a few commercially available polymer particles and particles from recycled tires were considered, which partially had a smaller size distribution. The investigated materials showed no obvious toxicity in any of the assays. Only PA-6 particles showed mild inflammatory effects in one cell model. Therefore, all investigated polymer particles were grouped in one category in analogy to the OECD Polymers of Low Concern concept. In addition, more detailed investigations were performed, e.g. for biodegradation in compost, UV-aging and fragmentation into smaller particles and for the carrier hypothesis. The carrier hypothesis postulates that the particles act as transport vehicles for toxic environmental pollutants. The extensive studies conducted in InnoMat.Life showed that all investigated polymer particles could bind the selected pollutants very efficiently and at least partially can also release them. However, other transport ways seem to be more relevant in the overall view.

InnoMat.Life established many useful methods, which partially are taken up by follow-up projects such as for instance the EU project POLYRISK (Grant Agreement No 964766). Moreover, extensive data sets were generated for different polymer particles.

## **(3) Materials with complex composition and/or morphology**

This material class was in particular challenging. On the one hand our investigations focused on characterizing a potential impact of the morphology on the hazards. For this purpose selected materials in different forms were studied (e.g. titanium dioxide particles versus cubes versus fibres). However, only the fibre morphology showed a distinct effect. On the other hand different hybrid metal particles for additive manufacturing were investigated. No specific effects were detected. In addition, workplace measurements showed that only post processing of 3D-printed parts led to a significant particle release, which in sum was not considered critical. This demonstrates that occupational safety is given for professional 3D printing. Based on the results and the investigated criteria an overarching grouping scheme for more complex materials was established. Follow-up projects, specifically the EU project HARMLESS (Grant Agreement No 953183), will take up this concept and will further develop it. In addition the InnoMat.Life scheme was already presented at the OECD WPMN Steering Group Advanced Materials.

**Grouping with respect to environmental effects** The two selected environmental organisms algae and daphnia differed with respect to the toxicity of the investigated materials. Only materials, which could release toxic ions, showed a toxicity in daphnia. Therefore, most of the initially formulated grouping hypotheses were rejected for daphnia. For algae, in addition the

agglomeration potential was a main criterion to determine toxicity for materials that do not release toxic ions. The agglomeration potential was influenced by the size and morphology of the materials. For algae most of the initial grouping hypotheses could be accepted or further developed. Based on the results it was possible to develop a comprehensive grouping scheme for environmental hazards for both organisms, which is applicable also for innovative materials.

### Publications:

- 1) Broßell D., Meyer-Plath A., Kaempf K., Plitzko S., Wohlleben W., Stahlmecke B., Wiemann M., and Haase A. (2020): A human risk banding scheme for high aspect-ratio materials. In: Synthetic Nano- and Microfibers. Wetsus.nl. ISBN: 978-1-71663-242-6
- 2) Pfohl P., Roth C., Meyer L., Heinemeyer U., Gruending T., Lang C., Nestle N., Hofmann T., Wohlleben W., and Jessl S. (2021): Microplastic extraction protocols can impact the polymer structure. In: Microplastics and Nanoplastics 1:8. <https://doi.org/10.1186/s43591-021-00009-9>
- 3) Pfohl P., Wagner M., Meyer L., Domercq P., Praetorius A., Hüffer T., Hofmann T., Wohlleben W. (2022): Environmental Degradation of Microplastics: How to Measure Fragmentation Rates to Secondary Micro- and Nanoplastic Fragments and Dissociation into Dissolved Organics. In: Environmental Science & Technology 56(16):11323-34. <https://doi.org/10.1021/acs.est.2c01228>
- 4) Hund-Rinke K, Broßell D., Eilebrecht S., Schlich K., Schlinkert R., Steska T., Wolf C., Kühnel D. (2022): Prioritising nano- and microparticles - identification of physicochemical properties relevant for toxicity to *Raphidocelis subcapitata* and *Daphnia magna*. In: Environmental Sciences Europe 34:116; <https://doi.org/10.1186/s12302-022-00695-z>
- 5) Pfohl P., Bahl D., Rückel M., Wagner M., Meyer L., Bolduan P., Battagliari G., Hüffer T., Zumstein M., Hofmann T., Wohlleben W.. (2022) Effect of Polymer Properties on the Biodegradation of Polyurethane Microplastics, In: Environmental Science & Technology, <https://pubs.acs.org/doi/abs/10.1021/acs.est.2c05602>
- 6) Emecheta EE, Borda Borda D, Pfohl PM, Wohlleben W, Hutzler C, Haase A, Roloff A A Comparative Investigation of the Sorption of Polycyclic Aromatic Hydrocarbons to Various Polydisperse Micro- and Nanoplastics using a Novel Third-Phase Partition Method. In: Microplastics and Nanoplastics 2, 29 (2022). <https://doi.org/10.1186/s43591-022-00049-9>