Ecotoxicological hazard assessment of persistent, water-soluble polymers

Summary

Persistent, water-soluble polymers ("liquid plastics") are produced in huge amounts and may pose a yet unrecognized threat for aquatic ecosystems. The project will provide robust ecotoxicity data facilitating a sound environmental hazard and risk assessment for these chemicals.

Current state of research

Synthetic polymers, commonly referred to as plastics, are an integral part of modern societies and contribute significantly to technological progress. Due to their versatile properties, world production has increased exponentially in the last 70 years and comprised a total of 8300 million tons (Mt) from 1950 to 2015 (Geyer et al. 2017). In 2016, 322 Mt of plastics were produced worldwide with a share of 64 mT in Europe alone (Bellasi et al. 2020). 4900 Mt (= 77% of the total plastic waste) have so far been disposed without proper treatment on landfills or discharged directly into the environment (Geyer et al. 2017) where they remain for decades due to their persistence, fragmenting slowly into microplastic (MP) particles (Barnes et al. 2009). In particular, the environmental impact of MPs with a size <5 mm has been intensively investigated in the last years, initially in the marine environment, and in recent years also in freshwater ecosystems. The Aquatic Ecotoxicology Department at Goethe University has made major contributions to this research within the framework of an EU, four BMBF and one BMV project, in which it was shown that freshwater organisms ingest MPs in the digestive tract, but also quickly excrete it again. No uptake into the surrounding tissue occurred in any of the species examined. Negative effects on the organisms only occurred at very high concentrations, which were several orders of magnitude higher than the maximum concentrations measured in the environment. However, numerous substances leached out of the MP which, in addition to high basic toxicity, also showed specific effects such as modulating the endocrine system, triggering oxidative stress or being mutagenic (e.g., Jekel et al. 2020, Schür et al. 2020, Weber et al. 2020, Zimmermann et al. 2020a, b, Klein et al. 2021a).

While negative ecotoxicological effects of microplastics are rather unlikely at current exposure levels, another group of synthetic polymers has recently come into the scientific focus, the persistent water-soluble polymers (PWPs) (Arp & Knutsen 2020). These include, for example, polyacrylamide (PAM) and polycarboxylates, which are used, inter alia, in cosmetics, detergents and cleaning agents, with around 50,000 tons discharged into wastewater in Germany each year (Bertling et al. 2018). There are also other applications for PWPs. For example, PAM is used as a flocculant in wastewater treatment, in paper production, as an agricultural soil conditioner and in oil and gas production, including fracking (Arp & Knutsen 2020). These polymers remain in the water cycle for a long time and are therefore widely distributed due to their good water solubility. As polymers, they are currently not subject to the European chemicals regulation under REACH, which explains the tremendous lack of ecotoxicological data yet. So far, their ecotoxicological relevance is expected to be low, but this assessment is mainly based on short-term studies (acute tests) and ignores that some PWPs will degrade to smaller, even more mobile polymers, in addition to oligomers, monomers, and other chemical byproducts. The example of PAM and its monomer acrylamide, a very potent neurotoxicant, underlines the potential ecotoxicological relevance of these compounds. In industrial wastewater, PAM concentrations of up to 1000 mg/L have been reported (Xiong et al. 2018) which are acutely toxic to freshwater organisms (Arp & Knutsen 2020). The long-term effects of PWPs and their potential degradation products, are insufficiently investigated, hampering a reliable assessment of their ecotoxicological hazards, which however, is a prerequisite for a sound environmental risk assessment.

<u>Research plan</u>

The **scientific aim** of the project is to contribute to an ecotoxicological hazard evaluation of PWPs by extensively characterizing the effects of selected polymers on aquatic organisms. The main focus is on chronic effects in long-term tests with representatives of aquatic key taxa, including life cycle and multi-generation studies, and specific mechanisms of action using *in vitro* assays. As test items those PWPs are examined which, due to their use in personal care products, detergents and cleaning agents, end up in the wastewater and are therefore particularly relevant for the protection of aquatic ecosystems. In addition to polyacrylamides (CAS no. 9003-05-8), this also includes various polycarboxylates, which are mainly used in phosphate-free washing and dishwashing detergents. The investigations include both the parent substances and their transformation and degradation products, which are formed during weathering experiments under UV-C and UV-A / B irradiation in the laboratory under controlled conditions (Klein et al. 2021b).

The investigation program for PWPs comprises the following standardized test methods:

- *In vivo* tests:
    - Chronic growth inhibition test (7 d) with *Lemna* spec. (OECD guideline 221)
    - Sediment/water toxicity test (28 d) *Lumbriculus variegatus* (OECD guideline 225)
    - Chronic reproduction test with *Potamopyrgus antipodarum* (OECD guideline 242)
    - Acute test (48 h) and chronic reproduction (21 d) test with *Daphnia magna* (OECD guidelines 202 and 211)
    - Multigeneration test with *Daphnia magna* (Schür et al. 2020)
    - A chronic test with *Hyalella azteca* (Report RM/33, Environment and Climate Change Canada)
    - cute test (48 h) with *Chironomus riparius* (OECD guideline 235)
    - Chronic sediment/water toxicity test (28 d) with *Chironomus riparius* (spiked sediment or spiked water, OECD guidelines 218 and 219
    - Sediment/water life-cycle test (42 d) with *Chironomus riparius* (OECD guideline 233)
    - Fish embryo toxicity (FET) test (96 h) with *Danio rerio* (OECD guideline 236)
- *In vitro* assays:
    - Basic or cytotoxicity test with *Aliivibrio fischeri* (Microtox assay, ISO guideline 11348:2007)
    - Antioxidant-response-element- (ARE-) assay to assess oxidative stress using AREc32cells with luciferase as reporter-gene
    - Recombinant yeast (*Saccharomyces cerevisiae*) reporter-gene assays for receptor-mediated endocrine activity (ISO guideline 19040-1:2018):
        - YES – yeast estrogen assay for estrogenic activity
        - YAS – yeast androgen assay for androgenic activity
        - YAES – yeast anti-estrogen assay for anti-estrogenic activity
        - YAAS – yeast anti-androgen assay for anti-androgenic activity
        - YDS – yeast dioxin assay for dioxin-like activity
    - T-screen for the detection of thyroid hormone system impairment (GH3 cells reflecting thyroid dependent cell proliferation)
    - Ames fluctuation test for mutagenicity with *Salmonella typhimurium* (OECD guideline 471, ISO guideline 11350:2012) using strains TA 98, TA 100, YG 7108, YG 1041 and YG 1042, each with and without S9 mix

With this work programme, another essential goal of the project, the **further advancement of Dr. Olcay Hisar's profile** can be reached. The application of state-of-the-art techniques in the ecotoxicological hazard and risk assessment of substances will qualify Dr. Hisar for professional activities in the European academic sector as well as outside academia, for example in public authorities, industry and in the consulting sector.

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