Abstract

Microorganisms are ubiquitous and present in animal microbiomes, particulates, and colonizable surfaces of test systems. From an ecotoxicological perspective, they are metabolically active biological compartments that respond to test conditions, including test substances. In exposure experiments, microorganisms can both alleviate toxicity via, for example, biotransformation, and reinforce the adverse effects via, for example, disrupted microbiome-host interactions. Acknowledging these interactions is essential for a mechanistic understanding of results in effect studies and developing assays towards ecologically relevant hazard assessment. Therefore, there is increasing attention toward "microbiome aware ecotoxicology" in recent years, focusing mostly on test organism microbiomes.

I studied how microorganisms present in systems designed for acute and chronic toxicity assays with *Daphnia magna* affect the test outcome. The experimental studies showed that bacteria introduced in the system intentionally (as a part of the experimental design; Papers I, II, and III) or unintentionally (with the microbiome of the test animals; Paper IV) responded to the test substances and mediated the exposure for the target species. In these studies, we employed the emerging contaminants ciprofloxacin (an antibiotic drug; Paper I) and various fossil-based polymers (microplastic; Papers II, III, and IV), representing a microbiome-disrupting and a biofilm-promoting type of substances, respectively.

In Paper I, we hypothesized that exposure to antibiotics would primarily target the daphnid microbiome with downstream effects on the host fitness. To test this hypothesis, we chronically exposed daphnids to ciprofloxacin, which resulted in decreased microbiome diversity. However, contrary to our hypothesis, there were significant stimulatory effects on the host fitness and antioxidant production, due to the direct pro-oxidative ciprofloxacin effects on the host. Although the microbiome was not directly involved in the growth-related responses to the ciprofloxacin exposure, the microbiome's alterations suggest that exposure to any antimicrobials, which – unlike ciprofloxacin – do not stimulate antioxidant production, would result in gut dysbiosis with possible adverse effects on the host.

Further, we hypothesized that in assays with particulate test materials, such as microplastic, bacterial biofilms increase particle aggregation, affecting exposure levels. This hypothesis was tested using *D. magna* exposed to a mixture of kaolin clay and polystyrene with and without biofilm (Paper II). We found that biofilm significantly decreased the adverse effects exerted by particulates directly, most likely, by providing nutrition for the daphnids, and indirectly, by inducing particle aggregation. In Paper III, we compared biofilm communities established on the plastic (polyethylene, polypropylene, and polystyrene) *vs.* non-plastic (cellulose and glass) substrates. The biofilm communities on the plastic were significantly different from those on the non-plastic materials; hence, microplastic contribution to the suspended solids in the exposure can drive the biofilm community composition in the system. Finally, in Paper IV, we found that in a closed system designed to evaluate microplastic effects on *D. magna*, bacteria originated from the daphnid microbiome colonize particulates and affect their aggregation and animal survival. Together, these findings

suggest that chemical exposure (Paper I), the microbiome of the test animal (Paper IV), the composition of the suspended solids (SS) (Papers II and IV), and their surface properties (Paper III) contribute to the diversity and abundance of the biofilm in the test system, which can affect the test outcome. Thus, the microbiome reacts to and interacts with contaminants within a test system, which calls for the appreciation of these interactions when interpreting the results as well as new developments toward standardization of the bacterial component in (eco)toxicity assays with eukaryotic test species.