Chlorinated Paraffins: improved understanding of their bioaccumulation and toxicity in *Daphnia magna*

Mafalda Castro

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Abstract

Chlorinated paraffins (CPs) are industrial chemicals, mainly used as flame retardants, plasticizers and metal cutting fluids. Their production has reached historically high levels in the last decade, with an annual production exceeding one million tonnes. In 2017, short chain chlorinated paraffins (SCCPs) were regulated due to their Persistent, Bioaccumulative and Toxic (PBT) properties, while medium and long chain chlorinated paraffins (MC and LCCPs) were suggested as alternatives. The high hydrophobicity of CPs, which complicates bioaccumulation and aquatic toxicity testing, has hindered proper hazard identification by regulatory authorities. This project was initiated in response to the insufficient understanding of bioaccumulative and toxicological properties of these chemicals, which have even surpassed the environmental levels of legacy Persistent Organic Pollutants (POPs) in certain regions.

The research presented in this thesis, contributes to filling these knowledge gaps by adapting methods for reliable bioaccumulation and aquatic toxicity assessment. In **Paper I**, passive dosing, traditionally used for other highly hydrophobic compounds, was adapted and validated for CPs. SC, MC and LCCPs partitioned from silicone into water and, when the crustacean *Daphnia magna* was introduced into the test system, the CPs were observed to be effectively taken up by the test organism. This passive-dosing approach was further used in **Paper II**, to investigate the bioconcentration and bioaccumulation potential in *D. magna*. All tested CPs were found to bioaccumulate in daphnids, including highly hydrophobic, long chained CP congeners. The two most bioaccumulative CPs in **Paper II** (CP-52 and Huels70C) were thereafter used in a chronic toxicity study (**Paper III**) and significantly decreased population growth and disrupted fatty acid metabolism of *D. magna*. Finally, in **Paper IV,** liposome-mediated delivery of chemicals to aquatic biota was adapted for the first time for organic contaminants, including CPs. This approach yielded stable body burdens of the tested chemicals in *D. magna* and allowed for kinetic and toxicity assessments.

Overall, two alternative bioaccumulation and aquatic toxicity testing methods were successfully adapted for technically challenging (industrial) chemicals. These methods allowed the determination of endpoints of scientific and regulatory interest, such as the high bioaccumulation and toxicity potential of CPs, but were also used to demonstrate their metabolic disruption potential in small crustaceans.

Keywords: *chlorinated paraffins, POPs, bioaccumulation, toxicity, ecotoxicology, aquatic toxicology, daphnia magna, liposomes, passive dosing, hydrophobic, mixtures.*

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Department of Environmental Science

Stockholm University, 106 91 Stockholm