Vector Effects of Microplastics on Polycyclic Aromatic Hydrocarbons and Chlorobenzenes in Japanese Medaka, Oryzias latipes

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Title　：Vector Effects of Microplastics on Polycyclic Aromatic Hydrocarbons and Chlorobenzenes in Japanese Medaka, *Oryzias latipes*
（メダカ *Oryzias latipes* におけるマイクロプラスチックの多環芳香族炭化水素およびクロロベンゼン蓄積に対するベクター効果）

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Thesis Summary

Microplastics (MPs) have become a great environmental issue of concern due to their widespread presence and pollution in a variety of water bodies. Besides MPs, there is evidence that other contaminants like hydrophobic organic chemicals (HOCs) are detected in water bodies and MPs have been assumed to absorb HOCs from water and can act as a potential carrier to aquatic inhabitants. MPs as a vector for bioaccumulation of several HOCs have been reported in some studies for example anthracene (Ant) and polychlorinated biphenyl (PCB). Some studies declined the commonly believed hypothesis and argued that MPs have no significant contribution to the enhancement in the accumulation of co-contaminants from water, hence, the vector effects of MPs still remain debatable. Moreover, studies on the bioaccumulation of Ant, phenanthrene (Phe), and polychlorinated benzenes (CBs) in fish with different polymer types, concentrations, and sizes of MPs are still lacking. So, the objective of this thesis is to examine the possibility of MPs with various polymer types, sizes, and concentrations on bioaccumulation of two types of polycyclic aromatic hydrocarbons (PAHs; Ant and Phe) and four CBs (1,3,5 trichlorobenzene, tetrachlorobenzene, pentachlorobenzene, and hexachlorobenzene; Tri, Tetra, Penta, and Hexa CB) in fish, Japanese medaka, (*Oryzias latipes*).

To demonstrate the vector effects of polyethylene microplastics (PE-MP) on the accumulation of Phe in medaka, we exposed fish with only Phe (0.2 mg/L) and Phe (0.2 mg/L)+PE-MP (6 mg/L) and also a control group (free of either contaminants). The study consisted of 10 days of uptake and 4 days of excretion (maintained in clean dechlorinated water) periods. Phe and PE-MP concentration in water and fish was measured on day 6, 9 and 10, 11, 12, 13, 14 respectively, and one-compartment model analyses were performed to evaluate the vector effects of PE-MP. Phe was measured by the HPLC system. MP concentrations in fish and water were quantified using the All-in-one fluorescence microscope (BZ-X810; Keyence, Osaka, Japan). Phe was absorbed by PE-MP and bioaccumulation of Phe in medaka occurred. The one-compartment model analysis clearly showed that PE-MP acted as a vector for the accumulation of Phe in fish. Vector effects of MPs on Ant; another PAH , have been documented in earlier studies (Qiu et al., 2020; Takai et al., 2023) and our findings are consistent with that of. In a separate study, to reveal the concentration effect of PE-MP on the accumulation of Ant, we exposed medaka for 10 days in Ant (0.2 mg/L) and three co-exposure groups having Ant (0.2 mg/L) plus 0.6, 6, and 60 mg/L PE-MP and allowed the fish to excrete for 4 days. We also set up a control group having no contaminants. Similar to the first experiment, the concentration of Ant in water and fish was measured on day 1, 3, 6, 9 and 4, 7, 10, 10(+8h), 11, 12, and 14 respectively. Vector effects were also determined with a one-compartment model in each concentration group of PE-MP. As a result, the vector effect of PE-MP on Ant was observed but the strength of vector effects was not concentration-dependent. This might be caused by saturation of absorption sites of MPs for Ant.

In another study, we co-exposed medaka in the only CBs water exposed and CBs+PS-MP groups for 7 days of uptake and 3 days of depuration periods. We used a mixer of four kinds of chlorobenzenes (Tri, Tetra, Penta, and Hexa CB) at 10, 10, 10, 8 μg/L respectively with two different sizes of polystyrene microplastics (10-μm PS-MP at 0.1 mg/L and 45-μm PS-MP at 0.45 mg/L). MPs and CBs in water and fish were measured on day 7, 8, 9 and 10. CBs in water and fish bodies were measured using the GC-MS system. We carried out another co-exposed test with 75-425-μm aged fragmented PE-MP and four CBs like the previous experiment. We co-exposed medaka to the same concentration of CBs as the above study but with different concentrations of fragmented PE-MP such as CBs+0.1 mg/L and CBs+1 mg/L groups. Incase of CBs, both sphere and fragmented MPs did not show any vector role for the accumulation of CBs in fish. Also, no vector effects were observed in either PS-MP or PE-MP for CBs accumulation in medaka. The reason may be no or less desorption of CBs to PE-MP in the gut and only water phase CBs accumulated in the fish body. However, strongly absorbed molecules were reported as low or desorption-resistant and subsequent low or no contribution of MPs in the accumulation of chemicals in the fish body (no vector effects).

In conclusion, MPs have a potential role as a vector for hydrophobic chemicals. However, this might depend on the nature of the chemical: LogKow and hydrogen bonding andπ-π interaction, van der-Waals force and halogen bonds, etc. The risk of microplastic when coexisting with a mixture of HOCs should be clear for protecting the ecosystem. MP pollution and co-occurrence of hydrophobic chemicals in nature are increasing day by day, so we think that further studies with different concentrations of chemicals and several polymer types, kinds, sizes, etc. may be carried out to reach a clear consensus about MPs as a potential carrier for enhanced bioaccumulation in a real-world situation.