

Kinetic of microplastics and their effects on behavior and cholinergic nerve system in Japanese medaka (*Oryzias latipes*)

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Title : Kinetic of microplastics and their effects on behavior and cholinergic nerve system in Japanese medaka (*Oryzias latipes*)
(メダカにおけるマイクロプラスチックの体内動態とコリン作動神経系への影響)

Category : Kou

Thesis Summary

Microplastics (MPs) whose particle size is < 5 mm are considered an emerging and hazardous environmental pollutant because of their stable chemical properties and long-term ecological existence. Through a series of migrations and transformations in the marine environment, microplastics are ingested by aquatic organisms and subsequently bio-accumulated and connected with the marine food chain, ultimately posing a severe threat to the survival of marine life.

MPs and its related impacts on aquatic species have drawn worldwide attention. However, the knowledge of the kinetics of MPs in fish still needs to be updated. Firstly, we conducted exposure and depuration tests of spherical fluorescent-labeled polyethylene (PE) and polystyrene (PS) MPs in 106 particles/L (PS 2- μ m, PS 20- μ m, PE 20- μ m) and 104 particles/L (PE 200- μ m) concentrations using Japanese medaka (*Oryzias latipes*). The distribution and concentration of MPs in medaka were determined in situ after tissue clearance. During the 14-day exposure, MPs were mainly detected in the intestines of fish. The bioconcentration factor (BCF, L/kg) for MPs in medaka was estimated as 74.4 (200- μ m PE), 25.7 (20- μ m PE), 16.8 (20- μ m PS), and 139.9 (2- μ m PS). MPs were eliminated from the fish body within five days of depuration, but 2- μ m PS-MPs could still be detected in the intestine at the 10-day depuration phase. These suggested 2- μ m MPs may pose risks to aquatic species due to their relatively higher BCF and the potential for long-term persistence in the body.

Thus, the second experiment was performed to verify the residual of 2- μ m MP in the fish body and its distribution. Exposure and depuration tests of 2- μ m spherical fluorescent-labeled PS under 106 particles/L concentration in Japanese medaka were conducted. The distribution and concentration of MPs in medaka were determined in situ after tissue-transparency treatment. During the exposure period (4-day exposure, 8-day exposure, 18-day exposure), MPs were mainly accumulated in the intestine. After seven days of depuration, $10.1 \times 10^3 \pm 4.9 \times 10^3$, $17.4 \times 10^3 \pm 11.4 \times 10^3$, $128 \times 10^3 \pm 46.4 \times 10^3$ particles/g of 2- μ m MPs were detected in the intestine of fish. From the histological treatment and counting, $10.1 \times 10^3 \pm 4.9 \times 10^3$, $17.4 \times 10^3 \pm 11.4 \times 10^3$, $128 \times 10^3 \pm 46.4 \times 10^3$ particles/g of 2- μ m MPs were detected in the intestine of fish. Our results showed the positive exposure-time-dependent number of 2- μ m MPs that remained in the medaka intestine after depuration, and 2- μ m MPs particles were found in the cavity of the medaka's intestinal tract near the intestine wall, which were suspected to be embedded in the intestinal lumen.

Further study focused on MPs' effect on fish behavior and its mechanism. We examined the effects of exposure to 2- μ m spherical fluorescent PS-MP (0.1 mg/L, 2.5×10^7 particles/L) on the behavior and the cholinergic nerve system-related gene expression; acetylcholinesterase (AChE) and choline acetyltransferase (CAT) in brain and gut of Japanese medaka. We found that shoaling behavior tended to be inhibited by the exposure of PS-MP for 14 days. The average frequency at which medaka left the shoaling area was 7.6 ± 3.2 in the exposed group and 3.6 ± 3.2 in the control group ($p=0.25$). Although there was no significant change in those parameters, we observed that over-excited or over-depressed behavior occurred in the exposed group. The qPCR results showed that the expression of AChE in the brain increased by 1.7-fold ($p=0.05$) in the exposure group, while there was no increase in gut CAT expression. Results showed fish exposed to 2- μ m PS-MP would cause social behavior disorders, the changes in expression of genes involved in behavior in the gut and brain of the fish.

In conclusion, our results show that exposure to 2- μ m PS-MPs concentrated and remained in the fish's intestinal tract, disrupting the cholinergic nerve system and affecting fish behavior.