

Endocrine Disrupting Effects of Degraded Polystyrene (Styrene Trimer) in the Ocean Including Bone Metabolism and Degradation of Styrene Trimer by Marine Bacteria

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Summary

Background: It has been believed that microplastics are persistent and do not degrade in the ocean, which is a low-temperature environment. However, it has been reported that styrene oligomers (styrene monomer, styrene dimer: 2,4-diphenyl-1-butene, and styrene trimers: 2,4,6-triphenyl-1-hexene) are actually present in the ocean, especially styrene trimer in high concentrations. Therefore, it is highly likely that microplastics are degraded to styrene oligomers and that these styrene oligomers seem to be affecting marine organisms. Furthermore, high purity styrene oligomers are expensive and commercially difficult to obtain. Therefore, very few studies have investigated the toxic effects of styrene oligomers, and the results have been inconsistent due to the low purity of the synthesized products. On the other hand, we have investigated the effects of chemicals such as bisphenol-A and tributyltin on bone metabolism using fish scales as a bone model and reported that these chemicals have effects on bone metabolism in fish. In the present study, we investigated the effects of styrene oligomers on bone metabolism using goldfish scales. Furthermore, in this study, we conducted experiments to screen for marine bacteria capable of degrading styrene oligomers.

Methods: In the present study, we first examined the estrogen-like activity of styrene oligomers by reporter assay with estrogen receptors in tilapia. Next, the effects of styrene oligomers on bone metabolism were examined, using the scales of goldfish (*Carassius auratus*) as a material. That is, styrene oligomers (10 mg/L, 100 mg/L, 1mg/L) were added to the culture medium, and their effects on osteoblasts and osteoclasts in the scales were compared with those of estrogen. In addition, styrene oligomers were administered to goldfish, and their effects on blood calcium levels were also evaluated. On the other hand, to investigate the degradation of styrene oligomers by marine bacteria, we examined marine bacteria that can utilize styrene oligomers by culturing them on 1/2 seawater/agar plates containing only styrene oligomers.

Results and Discussion: As a result of reporter assay with estrogen receptor (ERα), only styrene trimer showed activity. Therefore, in the following experiments, we focused only on the styrene trimer to examine its effects on bone metabolism. The results of the assay using scales showed that styrene trimer increased osteoblast and osteoclast marker enzyme activities in the same manner as estrogen. Styrene trimer also increased the expression of osteoblast and osteoclast marker genes. In addition, the administration of styrene trimer to goldfish increased

the calcium concentration in the blood, confirming its bone resorption effect (activation of osteoclasts). Since it is well known that estrogen also has a bone resorption effect by increasing the calcium concentration in the blood, it was found that styrene trimers have estrogen-like effects *in vivo* as well as *in vitro*. Next, we screened marine bacteria, using 1/2 seawater-agar plates containing styrene oligomers (10 mg/L). The results indicated that some marine bacteria may capitalize on styrene oligomers