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Title: Effects of ingested microplastics with sorbed triclosan on fish gut health and assessment of triclosan bioavailability

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Microplastics (MPs) are plastic particles (5 mm–1 μm) that contaminate aquatic environments and are frequently ingested by organisms. Even if MPs are not absorbed and do not accumulate within internal tissues, trophic transfer of MPs can occur (including transfer to humans), because organisms in higher trophic levels consume the entire prey organism including the gut containing MPs. The gut microbiota of organisms is very important for organism health, and disruption of gut microbiota by ingestion of MPs containing substances with antimicrobial properties is a concern. Triclosan (TCS) is a widely used antimicrobial hydrophobic compound that has been detected sorbed to MPs specifically polyethylene aquatic debris. Exposure of fish to TCS in aqueous phase (even at low doses, <100ng/L) can disrupt fish gut microbiota. The goal of this study was to assess the bioavailability of TCS sorbed to high-density polyethylene (HDPE, 125-250 μm) MPs after 0-5 days of ingestion in rainbow trout *Oncorhynchus mykiss*. After 7 and 14 days, the effects of ingestion of MP with and without with sorbed TCS on gut microbiota and fish immune system response were evaluated. Measurements of target gene expression by quantitative reverse transcriptase PCR (RTqPCR) were used to assess TCS bioavailability and immune system response. The bioavailability of sorbed TCS was assessed by measurement of glutathione S-transferase (GST) expression in liver of fish, and the immune system response was assessed by measurement of the expression of serum amyloid A (SAA) in the liver and Immunoglobulin M (IgM) in the spleen. Alterations in the gut microbiota genetic diversity were assessed by high-throughput paired-end Illumina sequencing. The expression of IgM in the spleen was significantly higher on week 1 on the TCS, HPDE+TCS and HPDE treatments, but only the TCS treatment differed from the control treatments. The expression of SAA in the liver was significantly higher than controls on the second week, but not HDPE nor HDPE+TCS. We anticipate that our results (ongoing data analysis) will contribute to assessments of the risk of MPs exposure in fish and other vertebrates.