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Comparison of the dietary bioavailability of copper sulphate and copper oxide nanomaterials in *ex vivo* gut sacs of rainbow trout: effects of low pH and amino acids in the lumen†



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Abstract

Diet is an important exposure pathway of engineered nanomaterials (ENMs) in fish, but the long duration of *in vivo* toxicity tests is a barrier to hazard assessment. This study used *ex vivo* gut sacs from rainbow trout (*Oncorhynchus mykiss*) to compare the bioaccumulation of Cu from CuO ENMs with CuSO₄ in the tissue layers of the gut after short-term (4 h) exposure. The effect of gut lumen conditions such as pH and the presence of amino acids on the behaviour and dissolution of the CuO ENMs was explored and how amino acids (cysteine, histidine) altered bioaccumulation in the gut sacs. Exposure to either CuSO₄ or CuO ENMs at pH 7.8 resulted in Cu accumulation in the mucosa of gut sacs prepared from the stomach, anterior-, mid- and posterior-regions of the gut when compared to saline only controls. In contrast, only CuSO₄ accumulated in the underlying muscularis which suggests transepithelial transport of CuO ENMs may be limited. Dialysis experiments showed that at pH 2, typical of the stomach, more than 90% of the Cu from the CuO ENMs became dissolved suggesting that the particulate is transformed to dissolved metal for most of the gastrointestinal tract. Amino acids greatly increased ENM dissolution, and in the gut sacs, L-cysteine caused elevated Cu accumulation in the muscularis after exposure to CuO ENMs at pH 7.8. In conclusion, CuO ENMs have lower bioavailability than CuSO₄ in gut sacs, but dissolution of ENMs in the gut can lead to dissolved Cu accumulation in fish.