Session: Alternative Approaches to Animal Testing for Ecotoxicity Assessments: Exploring New And Novel Approaches (P)
Poster, Exhibition Hall, ID MO067
Monday, May 27th, 2019, 8:30 AM

# Comparison of metabolism of fragrance chemicals in rainbow trout liver S9 sub-cellular fractions and in the invertebrate *Hyalella azteca*

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Bioaccumulation in aquatic species is a critical endpoint in the regulatory assessment of chemicals which usually involves the determination of the bioconcentration factor (BCF) in fish. Two assays measuring *in vitro* intrinsic clearance rates of chemicals have been recently adopted as new OECD test guidelines (TGs) using hepatocytes or liver S9 sub-cellular fractions from rainbow trout to refine BCF predictions. Bioconcentration studies in the invertebrate *Hyalella azteca* had been recently proposed as an alternative non-vertebrate *in vivo* screening assay. One of the major uncertainties using an invertebrate as a surrogate for fish bioaccumulation may be encountered due to a different and limited metabolic capability compared to vertebrates. The goal of this study was to compare the metabolites formed for 3 different fragrance chemicals in rainbow trout assessed by the RT-S9 *in vitro* assay and in parallel *H. azteca*. BCF studies were performed with 3 different fragrance chemicals in a flow-through system with *H. azteca*. Concentrations of the parent chemicals in water and in the animal were determined by GCMS analysis. Additional samples were prepared from exposed animals at two time points during the exposure phase for metabolite identification. Substrate depletion assays for the same chemicals were performed using RT-S9 following the new OECD TG319B. All samples were purified by SPE and metabolites determined using GC-MS and LC-MS-analysis. For all 3 fragrance chemicals hydroxylations as Phase I reactions followed by the formation of Phase II conjugates were observed both for RT-S9 and *H. azteca*. However, different kinds of Phase II conjugates were found for the two organisms. In RT-S9 conjugates with glucuronic acid were identified as major Phase II metabolites, whereas in *H. azteca* glucose conjugates were detected. Additionally, conjugates with sulfate or sulfate plus glucose were found in the invertebrate. Interestingly, a different preference in biotransformation for the different isomers of one of the fragrance chemicals was observed in *H. azteca* compared to RTS9. Comparable metabolic routes, i.e. hydroxylation of hydrophobic chemicals followed by conjugation reactions using glucuronic acid or glucose and sulfate were observed for 3 fragrance chemicals in rainbow trout assessed in RT-S9 and in *H. azteca* indicating a high metabolic capacity which may render this invertebrate a suitable model for BCF studies as a novel screening approach.