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Evaluation of a tiered approach for the bioaccumulation assessment of a fragrance substance: *in silico*, *in vitro* assays, invertebrate vs. *in vivo* fish bioconcentration test

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Bioaccumulation is a key end point in environmental hazard and risk assessment, especially for substances with a high octanol water partition coefficient ($\log K_{OW}$). To measure the BioConcentration Factor (BCF), a tiered approach is followed starting from the assessment of the octanol water partitioning coefficient as a measure for lipophilicity, which is often used as surrogate for lipid partitioning up to an experimental BCF value which is considered as the gold standard for fish bioaccumulation assessment. We have applied a series of non-animal tests to predict the BCF values and compared those outcomes to the results from a BCF test in order to validate this alternative approach.

Several fragrances from the tetranorlabdane diterpenoids family, either composed of a single or a mixture of stereoisomers were tested. The $\log K_{OW}$ predicted by QSAR ranges from 4.75 - 5.41 and is > 6.2 when determined by HPLC (OECD 117). The slow stir method (OECD 123) provides a $\log K_{OW}$ of 5.09 which is retained as the reference value. Various structure-activity relationship models were used to predict the fish bioconcentration factor, which ranged from ~ 1000 to ~ 4500, not exceeding the EU criteria for (very) Bioaccumulative substances (vB), however, the structure was mostly outside the applicability domain of the models. Therefore in vitro assays were conducted on rainbow trout S9 fractions and hepatocytes confirming the potential of biotransformation; the refined BCF values calculated with IVIVE extrapolation models were < 1000. In addition the bioaccumulation potential of one isomer was investigated in a flowthrough test on the invertebrate *Hyalella azteca* resulting in a BCF_{SS} or kinetic < 500 L/kg. Finally an experimental fish BCF of ~500 (OECD 305) confirms that the fragrance composed of various isomers is not bioaccumulative, and supports the in vitro biotransformation findings. Histopathological results from toxicological studies showed liver hypertrophy consistent with the increased metabolism associated with detoxification processes.

A tiered weight-of-evidence approach is clearly justified for the current bioaccumulation assessment, confirming that the tests described in the abstract may offer alternatives to animal testing when sufficient and supportive evidence is provided.