

Integrating In Vitro and Computational Methods into the SSbD Framework

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The development of chemicals and materials under the Safe and Sustainable by Design (SSbD) framework requires assessment strategies that embed safety and sustainability from earliest design stages while reducing reliance on (later stage) animal testing. New Approach Methodologies (NAMs) are central to this shift, yet guidance on how *in vitro* and computational methods can be systematically coupled across the SSbD framework remains scarce.

We present a structured approach that operationally integrates these two method families, mapping mechanistic *in vitro* evidence and predictive computational models to the relevant innovation stages in order to jointly inform hazard, exposure, and sustainability assessment. In this strategy, candidate integrations are ranked by operational maturity, defined through input-output compatibility, standardization, and evidence of use. Mature couplings include *in vitro* hazard data combined with Quantitative Structure-Activity Relationship models, physiologically based pharmacokinetic (PBPK) modelling, and quantitative *in vitro*-to-*in vivo* extrapolation. Less mature couplings, such as advanced 3D and organ-on-chip systems linked to Adverse Outcome Pathway networks or life cycle characterization, mark where harmonization of endpoints and modelling interfaces is still needed.

In the sustainability dimension, we expand the USEtox framework with this *in vitro* information. Within the USEtox framework, characterization factors are calculated as a product of substance-specific fate, exposure, and effect factors. The latter factor is based on toxicological data and is the link between USEtox and NAMs. This is done by deriving an *in vitro* concentration-response relationship for a human-relevant endpoint, allowing to obtain a benchmark concentration as point of departure, and translating this then into a human-equivalent external dose through PBPK-based reverse dosimetry. Such an *in vitro*-derived point of departure allows to replace traditionally required animal data by the USEtox method.

By linking *in vitro* and computational evidence from early hazard screening through to life-cycle impact, this strategy strengthens the consistency between safety and sustainability assessment and supports safer, more sustainable innovation.

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