**Systematic analysis of fish embryo toxicity (FET) data to define its applicability domain for toxicity testing**

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Toxicity data is required for the registration and authorization of industrial chemicals, plant protection products, biocides, pharmaceuticals, and feed additives. There is a strong societal demand for replacing animal experiments conducted for safety assessment of chemicals. Fish embryos until the onset of independent feeding are considered as nonprotected life stages by the current European Directive Directive 2010/63/EU on the protection of animals used for scientific purposes and are therefore considered as an alternative to the testing of (juvenile or adult) animals. Fish embryos, in particular zebrafish embryos, offer the possibility to perform large-scale, high-throughput analyses and are already used as a screening model in various applications. Nevertheless, concerns of limitations in the application domain and/or predictive capacity for specific compounds have been raised (e.g. differences in kinetics and/or limitations in metabolic capacity). Here we focus on the use of fish embryos for acute toxicity testing. Recently, a guideline for the 96 h fish embryo acute toxicity test (FET) has been adopted by OECD (OECD TG 236) and this provides an opportunity to reduce acute fish toxicity testing for regulatory purposes. The acute fish toxicity test (AFT) is usually conducted according to the OECD testing guideline (TG) 203 and represents the most commonly conducted vertebrate test for environmental hazard and risk assessment. The end point assessed in the AFT is mortality resulting in severe suffering and distress of the test animals. The fish embryo toxicity test has been proposed as an alternative for the acute fish toxicity test but concerns have been raised on its predictivity given that a few compounds exhibited only a weak acute toxicity in the fish embryo. Furthermore, the identification of mechanisms leading to a deviating toxicity is crucial to improve the application domain, reduce the probability for false negatives and increase acceptance of the FET for regulatory applications, e.g. for REACH. Therefore, we have established a database of published fish embryo LC50 values, which actually comprises data of 1337 compounds.

We performed a systematic analysis of the dataset, i.e. distribution of FET LC50 in relation to the octanol-water partition coefficient log *Kow*; the liposome-water distribution ratio at the pH of the experiment, log *Dlipw*; and AFT data. Thereby we identified potential outliers and we analysed the compound uptake of zebrafish embryos for 6 outliers. Based on the outlier analysis and identifications of boundaries and limitations we discuss the applicability domain of the FET and whether it is needed and possible to restrict the application domain. This discussion include a consideration of the overall sensitivity and potential uncertainty by e.g. analysis of the order of magnitude by which outliers fail from predicting the acute fish toxicity. The results are of high relevance for other applications of the fish embryo model that are presently discussed, such as the prediction of fish chronic toxicity, developmental/reproductive toxicity, mammalian acute toxicity and teratogenicity. Thus, it contributes to the 3Rs beyond environmental risk assessment and is of impact for various industrial sectors including the chemical, cosmetic and pharmaceutical industry.