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Evaluation of the integrated testing strategy for PNEC derivation under REACH

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ABSTRACT

Species sensitivity evaluation represents an approach to avoid chronic toxicity testing of aquatic vertebrates in accordance with the animal welfare concept of the EU chemicals regulation. In this study a data set of chemicals is analysed for relative species sensitivity between *Daphnia* and fish in chronic testing to evaluate under what condition chronic fish tests can be waived without underestimating the environmental hazard. Chronic fish toxicity is covered in 84% of the evaluated substances by the chronic invertebrate test and an assessment factor of 50. Thus, animal testing can be avoided in environmental hazard assessment for many chemicals. Moreover, it is shown that species sensitivity in chronic testing is associated with species sensitivity in acute testing. The more sensitive species in chronic testing is predicted with a high probability if a species is >5x more sensitive in acute testing. If substances are comparable or more toxic to *Daphnia* in acute testing than to fish chronic fish toxicity is covered by the chronic *Daphnia* test and an assessment factor of 50 in about 95% of the evaluated cases. To provide decision support for the regulation of chemicals a categorization scheme on relative sensitivity comparison is presented.

1. Introduction

With the registration of chemicals under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation (EC) No 1907/2006 manufacturers, importers and downstream users take responsibility for the safe use of their chemicals (EU, 2006). According to the REACH guidance an environmental effects assessment of the aquatic compartment requires a minimum data set that includes results of tests with organisms from three trophic levels: primary producers represented by algae, plant eating animals, represented by invertebrates (i.e. *Daphnia*), and predators, represented by fish (EC, 1996; ECHA, 2008). In particular guideline R.7b provides guidance for the assessment of ecotoxicological endpoints and includes an integrated testing strategy (ITS) (ECHA, 2014). Aquatic short term toxicity studies on the three different trophic levels (algae, invertebrates and fish) are the basis for the ecotoxicological evaluation of a chemical. Depending on the produced or imported quantity of the chemical or when the outcome of the acute testing indicates a risk the REACH Regulation defines each of the experimental data requirements that must be supplied by the registrant for the hazard assessment.

According to REACH standard information requirements, long-term aquatic toxicity testing on invertebrates (preferably *Daphnia*) and fish is required for substances manufactured or imported in quantities of ≥100 tonnes. Long-term studies may be needed at lower tonnages, for instance if the substance is poorly water soluble, the PEClocal/regional is >1/100th of the water solubility or a risk is identified. Based on the animal welfare concept and to avoid animal experiments (Art. 13, Art. 25), the REACH Regulation (EC 1907/2006) still provides options to avoid the chronic fish test. According to Article 25 the study of vertebrates is only considered as a last resort in the data collection. Article 13 suggests alternative methods such as (Q)SAR and read across approaches (EU, 2006). Moreover, the ITS of the guideline R.7b provides options to assess the hazards of substances while taking into account the animal welfare concept by considering relative species sensitivity to avoid animal tests (ECHA, 2014). To evaluate chronic data requirements the possibilities for the prediction of relative species sensitivities from experimental data of

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standard studies should be checked. Hence, species sensitivity represents a key descriptor to evaluated chronic data requirements in this context. Accordingly, chronic fish testing is not necessary if there is compelling evidence that fish is at least a factor of about 10 less sensitive than other trophic levels (R.7b, page 55). Furthermore, chronic testing of fish can be avoided if information is available that *Daphnia* is likely to be more sensitive than fish (R.7b, page 55). A testing proposal for a long-term toxicity study on *Daphnia* should be prepared. Fish testing may not be necessary, if the PEC/PNEC is <1 based on the *Daphnia* long-term toxicity study (R.7b, page 57) (ECHA, 2014). Here, the question arises whether chronic toxicity of fish can be adequately derived from the chronic *Daphnia* test and whether testing needs can be estimated from acute species sensitivity between *Daphnia* and fish. In particular, a problem may arise for specific mechanisms of chronic toxicity in fish which may not be covered by the acute test on fish or chronic invertebrate tests.

Previously, correlation between *Daphnia* and fish toxicity has been evaluated for acute toxicity data (ECETOC, 2003; Hutchinson et al., 2003; Weyers et al., 2000), and for chronic/sub-chronic data (ECETOC, 2003). An evaluation of the New chemicals database further indicated that fish represent the most sensitive trophic level in about 15% of the cases in acute testing whereas Daphnia was in about 30% of the cases the more sensitive species (Jeram et al., 2005). However, an evaluation that investigates whether chronic fish toxicity may be extrapolated from chronic *Daphnia* data and whether the more sensitive species in the chronic test setting can be estimated from acute data has been hampered by the still limited number of substances for that acute and chronic data on both *Daphnia* and fish is available.

Based on a data set of 124 chemicals a relative species sensitivity comparison is performed for acute and chronic toxicity data on both trophic levels to provide information whether chronic sensitivity can be estimated from acute data and under what condition chronic fish testing can be avoided in the environmental hazard assessment. The analysis addresses a current data gap and is relevant with regard to the regulation of industrial chemicals. Therefore, a categorization system for acute sensitivity comparison is proposed based on the evaluated data set to estimate chronic testing requirements for regulatory purpose. Within this context the physicochemical properties water solubility and octanol-water partitioning and their predictive value for the hazard assessment of data information requirement are analysed.

2. Material and methods

Data were gathered by using the OECD eChemPortal, and the Information System Chemical Safety database (ICS) of the German Environmental Agency (UBA). The data set generated by the OECD eChemPortal was based on the ECHA dissemination database and holds physicochemical and ecological information. The entries of this database were generated for the chemical registration in the EU under the REACH regulation and were provided by the registrants. The data are assigned to the Klimisch quality score system for reliability assessment and only data with Klimisch 1 or 2 were selected. Data provided by the ICS database comprising a data set of substances notified under the “New Chemicals” legislation (67/548/EEC) was validated by the German UBA. This data set also included the substances previously used for an acute to chronic ratio study (Ahlers et al., 2006). The data provided by industry and ICS is confidential or possess protection claims so that it is not possible to publish the data in detail.

The data set is confined to standardized tests according to the OECD guidelines and met similar test conditions to reduce data variance. Selection criteria were conformity in species, endpoints investigated, test system and test duration. Only substance entries with acute and chronic studies on both *Daphnia* and fish were considered. A substance was considered as one entry to avoid an overrepresentation of well documented substances in the data set.

For long-term tests on fish the Fish Early-Life Stage test (FELS) according or equivalent to OECD 210 or life cycle tests were used considering the NOEC of the endpoint survival/mortality and the sublethal endpoints hatching and survival, length, weight or abnormal behaviour according to the OECD guideline (OECD, 2013). Non-guideline studies were considered only if a well-documented study met the criteria for guideline requirements. Read across, studies on adult fish according to OECD 204, the embryo and sac-fry stages according to OECD 212 as well as the fish juvenile growth test according to OECD 215 were not used (OECD, 1984; OECD, 1998b; OECD, 2000). Fish studies were preferentially based on the recommended species *Pimephales promelas*, *Danio rerio*, *Oncorhynchus mykiss* and *Oryzias latipes* as given in the OECD 210 guidance document (OECD, 2013) (Suppl. Table 1).

Acute fish toxicity was ascertained from the 96 h LC50 of tests performed according to OECD 203 or comparable design (OECD, 2013). If acute data was available for the species of chronic testing the same species was preferentially considered in a first instance to reduce interspecies data variance (66% of the data set). To further consider the broad variety of available data on fish, different fish species in acute and chronic testing were also included (34% of the data set). A differentiation of the result between same and different species is documented (Suppl. Fig.1).

For acute tests on invertebrates studies on *Daphnia* conducted according or equivalent to the OECD Guideline 202 (*Daphnia* acute test) were considered using the 48 h EC50 (OECD, 2004). Chronic invertebrate tests according or equivalent to OECD 211 (*Daphnia* chronic test) were considered using the 21 d NOEC (OECD, 1998a). Thereby, the most sensitive effect value from the endpoints reproduction or mortality (immobilization) was used as recommended in the guideline.

If more than one study was documented for an endpoint of a substance the lowest effect concentration was used as this is usually chosen as key study for risk assessment under REACH. A comparable approach was applied previously (Ahlers et al., 2006) and was followed for data assessment in this study. The applied procedure is expected to be in line with hazard assessment under REACh. However, it should be noted that the choice of the lowest study value is not always taken for risk assessment by submitters as geometric mean or percentile values may represent a better representation of the central tendency for well-studied compounds. The study included a comprehensive data set of different chemical categories and different structural properties such as inorganics, neutral organic, aromatics, esters, phosphate esters, anilins or phenols, for example. The data set covers a log Kow range from −4.7 to 8.2 (Suppl. Table 1). Open ended toxicity values (<1 mg/L, for example) were not included in the data analysis and bioactive agents, such as pesticides, are not taken into account. The data set is further characterized by an analysis of the mode of action (MoA) according to the Verhaar scheme using the implementation in the OECD tool box (http://www.oecd.org/chemicalsafety/risk-assessment/theoecdqsartoolbox.htm) as nonpolar narcotics (MoA1), polar narcotics (MoA2), reactive (MoA3) and specifically acting substances (MoA4) (Suppl. Fig. 2) (Russom et al., 1997; Verhaar et al., 1992). The evaluation shows that specifically acting chemicals according to Verhaar are not covered by the evaluated data set (Suppl. Fig. 2).

In this study species sensitivity refers to sensitivity of fish relative to *Daphnia*. To compare species sensitivity of individual substances in acute and chronic testing the following quotient between the effect value of the *Daphnia* and fish study were derived: