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Assessment of aquatic experimental versus predicted and extrapolated chronic toxicity data of four structural analogues

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ABSTRACT

The present study was developed to assess the chronic toxicity predictions and extrapolations for a set of chlorinated anilines (aniline (AN), 4-chloroaniline (CA), 3,5-dichloroaniline (DCA) and 2,3,4-trichloroaniline (TCA)). *Daphnia magna* 21 d chronic experimental data was compared to the chronic toxicity predictions made by the US EPA ECOSAR QSAR tools and to acute-to-chronic extrapolations. Additionally, Species Sensitivity Distributions (SSDs) were constructed to assess the chronic toxicity variability among different species and to investigate the acute versus chronic toxicity in a multi-species context.

Since chlorinated anilines are structural analogues with a designated polar narcotic mode of action, similar toxicity responses were assumed. However, rather large interchemical and interspecies differences in toxicity were observed. Compared to the other three test compounds, TCA exposure had a significantly larger impact on growth and reproduction of *D. magna*. Furthermore, this study illustrated that QSARs or a fixed ACR are not able to account for these interchemical and interspecies differences. Consequently, ECOSAR was found to be inadequate to predict the chronic toxicity of the anilines and the use of a fixed ACR (of 10) led to under of certain species. The experimental ACRs determined in *D. magna* were substantially different among the four aromatic amines (ACR of 32 for AN, 16.9 for CA, 5.7 for DCA and 60.8 for TCA). Furthermore, the SSDs illustrated that *Danio rerio* was rather insensitive to AN in comparison to another fish species, *Phimphales promelas*. It was therefore suggested that available toxicity data should be used in an integrative multi-species way, rather than using individual-based toxicity extrapolations. In this way, a relevant overview of the differences in species sensitivity is given, which in turn can serve as the basis for acute to chronic extrapolations.

1. Introduction

The REACH (Registration, Evaluation and Authorization of Chemicals) legislation (EC, 2006) aims at improved knowledge on the properties and use of individual chemical substances in order to assess and manage their associated human and environmental toxicity risks. Alternative prediction, extrapolation and modeling approaches are regarded as useful tools to fulfil the extensive data needs of REACH in a cost-efficient manner. Moreover, they are compliant with the 3R principles which represent the refinement, reduction and replacement of animal toxicity tests (Hengstler et al., 2006; Schaafsma et al., 2009).

Quantitative structure activity relationships (QSARs) are one of the most commonly used alternative methods that predict the (eco)toxic potential of a particular chemical for different test species. In these approaches, chemical similarities within a group of structural analogues are considered to go hand in hand with a similar (and therefore predictable) biological outcome (Bradbury et al., 2003; Netzeva et al., 2007). These biological outcomes are typical acute and chronic aquatic toxicity data expressed as EC/LC_{50} and NOEC/LOEC values. QSARs are considered to be particularly robust for compounds that elicit their toxicity via a baseline or narcotic mode of action (Oberg, 2004; Zvinavashe et al., 2009). For these compounds, toxicity is assumed to be highly predictable based on the intrinsic relationship with the respective water–octanol partitioning coefficient, $\log K_{ow}$ (Vaal et al., 1997; Ramos et al., 1998), indicating that an increased $\log K_{ow}$ is indicative of a higher toxic potency.

Consequently, acute (eco)toxicity QSARs (for non-polar and polar narcotic compounds) have been used frequently over the last decades (Zvinavashe et al., 2009). In contrast, the availability of chronic toxicity QSAR models is limited. When measured chronic toxicity data is limited or unavailable, acute toxicity responses for a certain compound in a particular species are often used to predict the chronic toxicity. These predictions mostly rely on predefined or fixed acute-to-chronic ratios (ACRs) which are typically defined as the ratio of the EC/LC_{50} over the NOEC or LOEC of a

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chronic test (Worth et al., 2004; Ahlers et al., 2006; Raimondo et al., 2007).

In environmental risk assessment (ERA), it is however not one particular species or a set of standardized test organisms that needs protection, the ultimate levels of concern are populations, communities and ecosystems. Furthermore, it is important to acknowledge that different species can respond differently to a compound at a given concentration and exposure time (Posthuma et al., 2002). In order to account for these specific variations in toxicity, species sensitivity distributions (SSDs) can be constructed. In these distributions, all the available (either acute or chronic) toxicity data are integrated in order to predict a single toxicity value below which only an (acceptable) small percentage of the (aquatic) species, populations and habitats is affected. This improved data usage in the SSDs entails higher ecological relevance, in contrast to the traditional quotient and extrapolation factor approaches (Newman et al., 2000; Wheeler et al., 2002).

Although, it is postulated that the toxicity of polar narcotics is predictable by means of QSARs models (based on the intrinsic relationship with the $\log K_{ow}$), in our previous acute toxicity study (Dom et al., 2010) with a set of polar narcotic compounds (chlorinated anilines: aniline (AN), 4-chloroaniline (CA), 3,5-dichloroaniline (DCA) and 2,3,4-trichloroaniline (TCA)) this linear dependency between measured toxicity and $\log K_{ow}$ proved to be (very) different among different aquatic test species. Moreover, an inverse relationship was observed for *Daphnia magna* i.e. the acute toxicity decreased rather than increased with increasing $\log K_{ow}$ (Dom et al., 2010). Furthermore, it has been described in literature that daphnids and fish have a very similar susceptibility towards simple aromatic polar narcotic compounds (Marchini et al., 1993). In contrast, rather large interspecies variations in acute toxicity were observed. *D. magna* was identified to be very sensitive to acute (chlorinated) aniline exposure, whereas the same compounds were not toxic to *Danio rerio* within the concentration range tested (Dom et al., 2010). Given that the multi-species acute toxicity study illustrated rather large inconsistencies in acute toxicity predictions among the different test species, it was postulated that chronic toxicity QSAR prediction and extrapolation based on fixed assessment factors would even be more problematic.

Consequently, this present study was performed in order to evaluate these chronic toxicity predictions and extrapolations for the defined set of chlorinated anilines. *D. magna* was selected as test organisms because it was identified in the acute toxicity experiments as one of the most sensitive species to chlorinated anilines (Dom et al., 2010), Chronic 21 d reproduction experiments assessing life history characteristics (survival growth and reproduction) were performed with *D. magna*. These experimental data was compared to the results of QSAR predictions generated by means of the US EPA QSAR tool, ECOSAR (ECOlogical Structure Activity Relationship) and to the results of fixed acute-to-chronic extrapolations. Finally, SSDs were constructed based on the available chronic toxicity data in order to evaluate the chronic (chlorinated) aniline toxicity and the acute versus chronic toxicity in a multi species context.

2. Materials and methods

2.1. Test species – culturing conditions

Single clone *D. magna* (clone K6) cultures were held in 1 L glass recipients filled with aerated and biologically filtered tap water, each containing 20–25 individuals. The temperature in the temperature and light controlled chamber (Type WT15′/+5DU-WB, Weiss Technik, Liedekerke, Belgium) was maintained at 20 ± 1 °C with a photoperiod of 14 h light/10 h dark throughout culturing and exposure experiments. Three times weekly the water was renewed and the daphnids were fed a mixture of *Pseudokirchneriella subcapitata* and *Chlamydomonas reinhardtii* in a 3/1 ratio (4×10^5 cells mL^{-1}).

2.2. Chemicals

Aniline (99%) and 4-chloroaniline (98%) were purchased from Sigma Aldrich (Bornem, Belgium). 3,5-dichloroaniline (98%) and 2,3,4-trichloroaniline (>98%) were respectively purchased from Acros (Geel, Belgium) and TCI Europe (Antwerp, Belgium) (more information in Table 1A). Note that all reported concentrations are nominal concentrations.

2.3. Experimental part

2.3.1. Preliminary 96 h acute immobilization tests

In order to determine the chronic exposure concentrations for the four respective test compounds, acute 96 h immobilization tests with *D. magna* neonates (<24 h) were performed. The daphnids were fed the same algae mixture as throughout the culturing. During all exposures neonates were kept at a density of 10 organisms/50 mL reconstituted OECD water ($CaCl_2{\cdot}2H_2O$, 2 mM; $MgSO_4{\cdot}7H_2O$, 500 μM; $NaHCO_3$, 771 μM; KCl, 77.1 μM; water hardness, 250 mg $CaCO_3$; pH 7.8–8.2; OECD guideline 203, Annex 2). Exposures were conducted in triplicate. Daphnids were exposed to different treatments: control, (solvent (ethanol) control in the case of trichloroaniline) and the toxicant in a ½ dilution nominal concentration range; 0.0468–3 mg L^{-1} aniline, 0.010–0.640 mg L^{-1} 4-chloroaniline, 0.075–4.800 mg L^{-1} 3,5-dichloroaniline and 0.1875–12 mg L^{-1} 2,3,4-trichloroaniline. Test media were renewed after 48 h. Daphnids were fed with the renewal of the test media. In order to allow comparison of the chronic responses among the four structural analogues, acute equitoxic concentrations were determined, i.e. every chemical was chronically tested at the same level of acute toxicity using every chemical's respective acute (96 h) effect concentrations. Five exposure concentrations were selected for the 21 d chronic toxicity experiments: 96 h effect concentrations (EC_1, EC_{10} and EC_{50}) (determined using the US EPA Probit analyses software), the $EC_1/2$ and the concentration between the EC1 and EC10 (Table 1B).

2.3.2. Chronic 21 d assessment of life history characteristics

Chronic *D. magna* toxicity tests were performed, starting with neonates (<24 h old), for each respective compound using five exposure concentrations (Table 1) and a blank. Every other day test media were freshly made, renewed and daphnids were fed the same algae mixture as throughout the culturing. In order to assess survival and reproduction, 10 daphnids were placed individually in 50 mL of standardized OECD test water ($CaCl_2{\cdot}2H_2O$, 2 mM; $MgSO_4{\cdot}7H_2O$, 500 μM; $NaHCO_3$, 771 μM; KCl, 77.1 μM; water hardness, 250 mg $CaCO_3$; pH 7.8–8.2; OECD guideline 203, Annex 2) for each treatment. Every other day, age-specific survival and age-specific reproduction were recorded. Furthermore, age at maturity (i.e. age at presence of eggs in the brood pouch) and age at reproduction were monitored. With the available survival and reproduction data, the intrinsic rate of natural increase (r_m) was calculated for all conditions:

$$\sum_{x=0}^{21} l_x \cdot m_x \cdot e^{-r_m \cdot x} = 1$$

This formula integrates survival (l_x) and reproductive success represented by the amount of neonates produced (m) at different points in time (x). Since this parameter combines lethal and sublethal parameters into one measure that is informative of the population growth rate, it is suggested to be a very valuable parameter in ERA (Walthall and Stark, 1997).