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Development of thresholds of excess toxicity for environmental species and their application to identification of modes of acute toxic action

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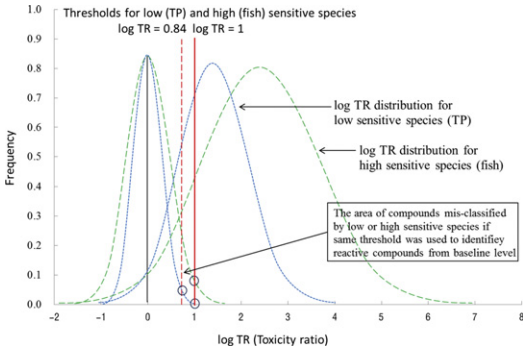
HIGHLIGHTS

- Poor interspecies correlations are observed for reactive chemicals between studied species.
- Thresholds of excess toxicity have been evaluated by comparing with fish toxicity sensitivity.
- Threshold of excess toxicity to *T. pyriformis* is different from fish, *D. magna* and *V. fischeri*.
- The factors that influence toxicity ratio calculated from baseline level are discussed.

GRAPHICAL ABSTRACT



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ABSTRACT

The acute toxicity of organic pollutants to fish, *Daphnia magna*, *Tetrahymena pyriformis*, and *Vibrio fischeri* was investigated. The results indicated that the Toxicity Ratio (TR) threshold of log TR = 1, which has been based on the distribution of toxicity data to fish, can also be used to discriminate reactive or specifically acting compounds from baseline narcotics for *Daphnia magna* and *Vibrio fischeri*. A log TR = 0.84 is proposed for *Tetrahymena pyriformis* following investigation of the relationships between the species sensitivity and the absolute averaged residuals (AAR) between the predicted baseline toxicity and the experimental toxicity. Less inert compounds exhibit relatively higher toxicity to the lower species (*Tetrahymena pyriformis* and *Vibrio fischeri*) than the higher species (fish and *Daphnia magna*). A greater number of less inert compounds with log TR greater than the thresholds was observed for *Tetrahymena pyriformis* and *Vibrio fischeri*. This may be attributed to the hydrophilic compounds which may pass more easily through cell membranes than the skin or exoskeleton of organisms and have higher bioconcentration factors in the lower species, leading to higher toxicity. Most of classes of chemical associated with excess toxicity to one species also exhibited excess toxicity to other species, however, a few classes with excess toxicity to one species exhibiting narcotic toxicity to other species and thus may have different MOAs between species. Some ionizable compounds have log TR much lower than one because of the over-estimated log K_{OW}. The factors that influence the toxicity ratio calculated from baseline level are discussed in this paper.

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1. Introduction

In aquatic toxicology, the ability to determine the mode of action (MOA) for a diverse group of chemicals is a critical part of ecological risk assessment and chemical regulation (Martin et al., 2013; Cronin, 2017). The determination of MOA has been recognized as a key limitation in the assessment of chemical toxicity as it is essential for the development of alternatives to animal testing and will assist in class-based predictive modeling of toxicity (Barron et al., 2015). The limitation is because assignment to a MOA is based not only on chemical structure, but also on the understanding of the interaction between the chemical and the living organism (Li et al., 2015). The Verhaar classification scheme intends to place organic pollutants into one of four distinct MOA classes based on physicochemical properties and structural rules (Verhaar et al., 1992; Verhaar et al., 2000; de Wolf et al., 2005; Barron et al., 2015). The four classes are: (1) inert compounds causing narcosis, (2) less inert more toxic compounds causing polar narcosis, (3) reactive compounds with enhanced toxicity, and (4) specifically acting chemicals/specific or receptor mediated toxicity (Barron et al., 2015). The Verhaar classification scheme has been adapted and extended by a number of workers (Enoch et al., 2008; Ellison et al., 2015; Ellison et al., 2016).

Inert compounds are chemicals that do not interact with specific receptors in an organism. The MOA of such compounds in acute aquatic toxicity is termed narcosis. These chemicals are considered to elicit toxicity by acting non-specifically at the cell membrane (Antczak et al., 2015). Therefore, their toxicity to different species is well predicted from their hydrophobicity, often parameterized by the logarithm of the octanol/water partition coefficient (log K_{OW}) (Cronin and Dearden, 1995; Dearden et al., 2000; Su et al., 2012; Wen et al., 2015) and this toxicity is termed "minimal" or "baseline" toxicity (Cronin, 2017). Less inert chemicals are somewhat more toxic than estimated by baseline toxicity. These compounds, which include phenols and anilines, are commonly characterized as possessing hydrogen bond donor acidity (Verhaar et al., 2000). The MOA of such compounds in acute aquatic toxicity is often termed "polar narcosis" (Schultz et al., 1986; Veith and Broderius, 1990). Reactive and specifically acting chemicals exhibit considerably higher toxicity than predicted from hydrophobicity (i.e. baseline toxicity) alone. Reactive chemicals display an elevated toxicity as these chemicals can react nonspecifically with biomolecules (e.g. through electrophile – nucleophile interactions), or are metabolized into more toxic species (Hermens, 1990; Lipnick, 1991). Specifically acting chemicals exhibit toxicity due to the specific interaction with certain receptor molecules (specific or receptor toxicity), again leading to elevated, or excess toxicity (Ariens, 1986).

It is often difficult to determine the precise mechanism of action of an organic chemical (von der Ohe et al., 2005). Chemicals acting by more specific mechanisms will have toxic potency elevated above this baseline, in other words, they are more potent, in terms of lethality, than would be associated with simple membrane disruption (McKim et al., 1987; Freidig et al., 2007). In order to identify reactive and specifically reactive compounds, the concept of excess toxicity has been employed to discriminate the elevated toxic responses from baseline narcotic effects (Lipnick et al., 1987). Toxicity above that associated with narcosis is defined in terms of "excess toxicity" (TR), which is defined more specifically as the ratio of the toxicity predicted from narcosis (T_{pred}) and the observed toxicity (T_{obs}) (von der Ohe et al., 2005; Sazonovas et al., 2010). Several TR thresholds have been reported in the literature to discriminate excess toxicity to different species (von der Ohe et al., 2005; Koleva et al., 2011; Li et al., 2015), for example, LC_{50} values within a factor of 10 of baseline toxicity (i.e. TR < 10) are classified as being narcotics and the remainder indicate excess toxicity. However, the threshold of TR = 10 used commonly to discriminate excess toxicity from the baseline narcotic level is based on the distribution of fish toxicity data. It should be borne in mind that the reference threshold of excess toxicity used in the fish toxicity may not be appropriate to discriminate reactive chemicals from baseline narcotics for other species. The difference of sensitivity for some species may mean that there could be differences in the cut-off for TR for these other species.

Inter-species variation in sensitivity to toxicants can be substantial, with the most sensitive species being of utmost concern for risk management. These differences in sensitivity between species may result from a number of factors, including variations in physiology, the use of a standardized, arbitrary exposure time for testing, the indiscriminate use of different effect parameters (growth, reproduction, survival), ignorance of sensitive life stages and so on (Roelofs et al., 2003). The effect of species sensitivity on the discrimination of excess toxicity to different species has been investigated (Li et al., 2015). The results show that the MOAs of chemicals is species dependent, with the difference in species sensitivity being one of the most important reasons resulting in the differences in relative inter-species toxicity. Many compounds share the same mode of action to different species, however some may not e.g. as a result of metabolic differences, presence or absences of (de-)toxifying enzymes etc. Thus, the direct application of a scheme developed for one species, e.g. fish, can lead to problems in classification for chemicals to other species, e.g. algae. In addition, differences in physiology, notably those affecting bio-kinetics (e.g. metabolism, clearance etc.) may result in different thresholds (TR) to discriminate excess toxicity from the narcotic effect for different species. Better elucidation of these inter-species effects will greatly increase the accuracy of classification between baseline or less inert and reactive compounds.

Although the influence of species sensitivity on the classification of MOAs has been appreciated to a limited extent, with some analysis of the relationships between the species sensitivity and MOA, little attention has been paid to the theoretical considerations of using different thresholds to discriminate excess toxicity and narcotic effect sensitivity to different aquatic organisms. Thus, in order to improve the accuracy of MOAs predictions, a set of thresholds for different species, which are obtained from specific toxicity data, should be developed to discriminate the MOAs. The objective of the current study was to develop such species-specific thresholds allowing for the better discrimination of acute modes of toxic action for different species. This was achieved by assessing the effect of species sensitivity on classifying different MOAs, comparison and analysis the classification differences of species-specific threshold. In this study, a data matrix of 4995 acute toxicity data for over 3363 compounds was created for four aquatic species (949 toxicity data for fish, 757 for *Daphnia magna*, 2050 for *Tetrahymena pyriformis*, 1239 for *Vibrio fischeri*). The orders of sensitivity for the four species were investigated based on compounds with data to all species and interspecies correlations between the toxicity data of class-based compounds to any two of four species.

2. Materials and methods

2.1. Biological data

A total of 4995 toxicity data for 3363 chemicals to fish, *Daphnia magna*, *Tetrahymena pyriformis* and *Vibrio fischeri* were compiled from a number of sources including several publications and databases. Most toxicity data were taken from Li et al. (Li et al., 2015), with a further toxicity data for 1060 chemicals to *Tetrahymena pyriformis* compiled from Ruusmann and Maran (Ruusmann and Maran, 2013). It is noteworthy that not all the compounds have toxicity data for all the four species (see Tables S1-S5 of Supplementary material). Not all the compounds can be assigned as MOA according to rules from Verhaar scheme for a number of compounds (1579 unclassified compounds in Table S5). It is the reasons why limited numbers of compounds were used in the following analysis.

All the toxicity data were converted into negative of the logarithm of the molar concentration e.g. log $1/LC_{50}$ (mol/L) for all analyses. The 3363 compounds were classified into different classes/homologues based on