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Toxicity of some prevalent organic chemicals to tadpoles and comparison with toxicity to fish based on mode of toxic action

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ABSTRACT

Although mode of action (MOA) plays a key role in the understanding of the toxic mechanism of chemicals, the MOAs of class-based compounds to tadpoles have not been investigated. To explore the MOAs, acute toxicity (expressed as log $1/LC_{50}$) to *Rana chensinensis* tadpoles were determined and molecular descriptors were calculated. Quantitative structure-activity relationship (QSAR) showed that toxicity to tadpoles is closely related to the chemical octanol/water partition coefficient (log K_{OW}), energy of the lowest unoccupied molecular orbital (E_{LUMO}), and number of hydrogen bond donors and acceptors (NH_{DA}), representing the bio-uptake potential in tadpoles, the electrophilicity and hydrogen bonding capacity with target site(s), respectively. Comparison of the toxicity values between tadpoles and fish revealed that there were no significant differences for the overlapping compounds (average residual = 0.29 between tadpole and fish toxicity) with P values of interspecies correlation substantially less than 0.001. Classification of MOAs for the class-based compounds based on the excess toxicity calculated from toxicity ratio suggested that baseline, less inert compounds and some reactive or specifically-acting compounds share same MOAs between tadpoles and fish. Fish and tadpoles can serve as surrogates for each other in the safety evaluation of organic pollutants.

1. Introduction

More than 100,000 synthetic chemicals are used in consumer products and a great portion of these organic chemicals have been discharged into the environment. Some of these organic pollutants have considerable toxic effect and can cause serious influence on ecological safety and human health. Comprehensive safety evaluations to human and environment are required for the substances from manufacturers or importers (Hartung and Rovida, 2009; Taylor et al., 2014). The evaluation of toxic effects of organic compounds has become a critical component of pollution control. Risk assessment can help us not only to understand the ecological risk of compounds, but also to formulate environmental assessment and management standards for organic pollutants.

In order to evaluate the hazard and risk of chemicals, the experimental toxicity data of these substances to aquatic ecosystems are required. Amphibians, as one of widely used model organisms, are sensitive to both aquatic and terrestrial environmental factors because of their unshelled eggs, highly permeable skin, and exposure to aquatic environments at two different early life stages embryos and larvae (Quaranta et al., 2009; Babini et al., 2015; Wang et al., 2015). The number of amphibian population had fallen faster than birds and mammals because of the serious pollution of their living environment and 32% of the amphibian species around the world were unequivocally threatened with extinction (Stuart et al., 2004). The research showed that widely used atrazine has significant interference with the survival and behavior of *R. pipiens* and *Xenopus laevis* embryos and larvae (Diana et al., 2000; Allran and Karasov, 2001). Recent studies revealed that ten agricultural fungicides had the strong lethality and teratogenicity on *Xenopus tropicalis* embryos (Li et al., 2016). Seven s-triazine herbicides can induce significant retardation in growth and development at higher concentrations (0.2–1.0 mg/L), and some of them can induce similar effects even at lower concentrations (0.02–0.1 mg/L) while each showing a linear dose-response (Saka et al., 2017). The tadpoles of all three species (Chiricahua Leopard Frogs, Northern Leopard Frogs and American Bullfrogs) exposed to piscicidal concentrations of 5% rotenone showed that the time to tail resorption at both life stages was nearly doubled in comparison with that of controls (Alvarez et al., 2017).

Although the knowledge of eco-toxicological effects plays a fundamental role for the environmental risk assessment of chemicals, very little information is currently available for the majority of these

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compounds, particularly to tadpoles. The toxicity test is costly, time-consuming, technically difficult, and ethically questionable. To fill the data gap and reduce the animal tests, the alternative in silico techniques like quantitative structure–activity relationship (QSAR) are applied, as recommended by the European legislation REACH (https://ec.europa.eu/commission/index_en) (Cassani et al., 2013). For examples, linear solvation energy relationship (LSER) equation have been proposed and used to predict the narcotic or the lethal effect of chemical compounds to tadpoles (Abraham and Rafols, 1995). They found that the most important descriptors are the solute hydrogen bond basicity and the solute volume. This model assumes that narcotic and lethal effects are related to the partition of a solute between two phases (Fernández-Pumarega et al., 2015). Response-surface analyses using hydrophobic and electrophilic descriptors for 51 structurally diverse benzene derivatives revealed that acute toxicity to *Rana japonica* tadpoles is closely related to the octanol/water partition coefficient (log K_{OW}) which represents the partitioning of each toxicant into the biophase and the energy of the lowest unoccupied molecular orbital (E_{LUMO}) as measurement of the ability of a molecule to gain electrons and characterizes electrophilicity (Huang et al., 2003). This indicates that some of these compounds are electrophiles that may form irreversible covalent bonds with amino acid protein residues and act as reactive mode of action (Enoch et al., 2011).

The assignment of a compound to a particular MOA is particularly important for risk assessment and QSAR model development as this knowledge is used to predict target molecular pathways and can reveal key endpoints for environmental monitoring (Wang et al., 2017). Some methods have been used to classify the MOAs of the compounds according to the survival, reproduction and growth of an aquatic species (McKim et al., 1987; Russom et al., 1997; Von der Ohe et al., 2005; Nendza et al., 2014). At present, the most widely used method is Verhaar's proposal, classifying compounds into four MOAs (i.e. inert/baseline compounds (class 1), less inert compounds (class 2), reactive compounds (class 3) and specifically-acting compounds (class 4)) based on the structure of compounds (Verhaar et al., 1992). The potential effect of baseline or less inert compounds is completely dependent on hydrophobicity and act as narcotic MOA, and a good log K_{OW} single parameter model can be established (Li et al., 2015). Reactive chemicals and specifically-acting chemicals may form irreversible covalent bonds or specific interaction with amino acid protein residues, leading to significantly higher toxicity than baseline compounds (Neuwoehner et al., 2010; Schwöbel et al., 2011; Schramm et al., 2011). Comparison on the MOAs of four environmental species showed that majority of baseline compounds identified from fish toxicity can also be classified as baseline compounds to other aquatic species. They share same MOA and do not exhibit excess toxicity (Li et al., 2018). However, a few classes with excess toxicity to one species exhibit narcotic toxicity to another species, indicating they may have different MOAs between species. This achievement was based on the study on the toxicity to fish, *Daphnia magna*, *Tetrahymena pyriformis*, and *Vibrio fischeri*. No investigation has been carried out on toxicity to tadpoles as compared to other species.

Rana chensinensis (*R. chensinensis*) is a native amphibian species living in the Changbai Mountain and Songhua River throughout the agricultural landscapes in China and was used as a model animal for evaluating adverse chemicals effect to amphibians (Li et al., 2014). Although it is a popular species in China, only a few studies have been reported on the toxicity to the tadpoles (Yang et al., 2005; Chai et al., 2016). No study was carried out on the interspecies correlation and MOA comparison between tadpoles and fish. In this paper, 96 h acute toxicity to *R. chensinensis* was tested and 12 h acute toxicity to *Rana japonica* tadpoles was collected from literature. These toxicity data were used to investigate the fish-tadpole interspecies correlation and MOAs of compounds. The objectives of the present paper were: (1) To explore the acute toxicity of the tested compounds on *R. chensinensis* tadpoles obtained from different exposure periods; (2) To investigate chemical MOAs and interaction between chemicals and biological molecule in tadpoles through QSAR model development; (3) To compare toxicity data between *R. chensinensis* and *R. japonica* tadpoles and investigate interspecies correlation between tadpoles and fish; (4) To compare the MOAs between tadpoles and fish based on the excess toxicity calculated from the toxicity ratio for class-based compounds.

2. Materials and methods

2.1. Toxicity data to tadpoles

Two toxicity datasets to tadpoles were used in this study. One is to *R. Chensinensis* tadpoles determined in our lab. Another is to *Rana japonica* tadpoles obtained from a reference (Huang et al., 2003). The studied 61 compounds encompass a wide range of well-characterized molecular structures with different chemical domains of Verhaar scheme (Verhaar et al., 1992), including 10 baseline compounds (chloro or bromo substituted benzenes), 13 less inert compounds (substituted phenols and anilines), 21 nitro substituted benzenes identified in part as reactive compounds, 3 pesticides and some compounds with unknown MOAs.

The *R. Chensinensis* tadpoles employed for toxicity tests were taken from naturally fertilized egg masses of the Chinese brown frogs (*R. chensinensis*). They were purchased from Wangqi frog farm of Jilin province in the Northeast of China and cultured in laboratory by aerated tap water at 20 ± 2 °C, with a photoperiod of 14 h daylight and 10 h darkness. New born tadpoles ate the egg's membrane and no extra food was given to these tadpoles before toxicity tests. Over 48 h old tadpoles were used for the toxicity tests. Acute toxicity tests of organic chemicals to tadpoles were conducted with a semi-static method from 24 to 96 h, according to the OECD Guideline (OECD guideline 231, 2009). The temperature of the test chambers was maintained at 20 ± 2 °C, pH 6.8–7.0 and salinity 1.8–1.9 g/L. Each compound was tested at five concentrations with 10 tadpoles in 2 L test water. Three replicates were performed for each chemical concentration. One blank and one control containing the solubilizing agent (0.1% DMSO) were run in addition to the test series. Semi-static procedures, three fourths of the test-solution manually exchanged daily, were used to maintain the constant concentrations of the compound and water quality. The number of live tadpoles was counted after 24, 48, 72 and 96 h, respectively. The dead tadpoles were removed during the toxicity tests. The 50% lethal concentrations to tadpoles (LC_{50}, mol/L) were calculated from the dose-response relationships using the least squares regression analysis by the MINITAB software (version 14) for different exposure periods. The acute toxicity data to tadpoles (*R. Chensinensis*) within 24, 48, 72 and 96 h exposure periods are listed in Table S1 of Supplementary material, for the 18 tested compounds.

The toxicity data of the 50% lethal concentrations within 12 h exposure period to *Rana japonica* tadpoles (*R. japonica*) were taken from a reference (Huang et al., 2003). Naturally fertilized egg masses of the frogs were collected from a single pound in Nanjing, a city in the South of China, and the toxicity tests were carried out by the same research group. It contains 51 benzene derivatives, including halogenated benzenes, substituted phenols and dinitrobenzenes. The 51 benzene derivatives reported were structurally and mechanistically diverse, with some compounds having well established mechanisms of action. These compounds were classified into 10 groups based on the structure and functional groups substituted on the benzene. The details of the classification, together with SMILES, CAS and calculated descriptors for each compound, can be found in Table S2 of Supplementary material.

2.2. Toxicity data to fathead minnow (Pimephales promelas)

The toxicity data expressed by LC_{50} (mol/L), the concentration required to kill 50% of fish within 96 h for 578 compounds, were taken from several references (Russom et al., 1997; Yuan et al., 2007; Papa