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Comparison of modes of action between fish and zebrafish embryo toxicity for baseline, less inert, reactive and specifically-acting compounds

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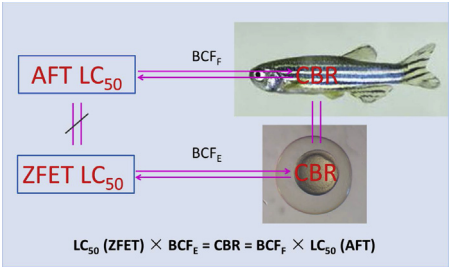
HIGHLIGHTS

- Baseline and less inert compounds share mode of action to fish and embryos.
- Reactive compounds react with target molecules through addition oxidations, or amination.
- Specifically-acting compounds have strong capable of docking with protein molecules.
- Differences of physiological structures between embryos and fish result in differences in excess toxicity.

GRAPHICAL ABSTRACT



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ABSTRACT

The mode of action (MOA) plays a key role in the risk assessment of pollutants in water. Although fish is a key model organism used in the risk assessment of pollutants in water, the MOAs have not been compared between fish and embryo toxicity for classified compounds. In this paper, regression analysis was carried out for fish and embryo toxicities against the calculated molecular descriptors and MOAs were evaluated from toxicity ratio. The toxicity significantly related with the chemical hydrophobicity for baseline and less inert compounds, respectively, indicates that these two classes of compounds share the same MOAs between fish and embryos. Comparison of the toxicity ratios shows that reactive compounds exhibit excess toxicity to both fish and embryos. These compounds can react covalently with biologically target molecules through nucleophilic addition reactions, Michael addition oxidation, or amination. Comparing with baseline, less inert and reactive compounds, many specifically-acting compounds have strong docking capacity with protein molecules. Some specifically-acting compounds, such as fungicides, have very similar toxic effect to both fish and embryos. However, insecticides are more toxic to fish than embryos; herbicides and medications are more toxic to embryos than fish. Differences in the interactions of chemicals with target molecules or bioconcentration potentials between fish and embryos may result in the differences in toxic effects. There are some factors that influence the identification of MOAs, such

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as quality of toxicity data, bioavailability and ionization. These factors should be considered in the identification of MOAs in the risk assessment of organic pollutants.

1. Introduction

With more and more compounds synthesized and used in the agriculture and industry, many chemicals have been discharged into the aquatic environment. It poses a challenge in the risk assessment for the increasing number of toxic substances. Fish as the main consumer in the aquatic environment play an important role in the food chain and the acute fish test is a mandatory component in chemical hazard and risk assessment. The experiments using fish as a biological model have attracted unanimous attention (Russom et al., 1997; Lammer et al., 2009a; Li et al., 2018). Several fish species were recommended as model organisms in the acute toxicity assay, such as fathead minnow, rainbow fish and the guppies (OECD, 1992). In recent years, zebrafish has been used to determine the toxicity of a series of compounds and become a popular model for biomedical research and (eco)toxicology (Strähle et al., 2012; Ducharme et al., 2015).

Although there are many fish toxicity data available in the literature, a large data gap exists for the increasing numbers of organic compounds discharged into the aquatic environment. Also, the acute fish toxicity test is criticized from both ethical and scientific point of views. The use of fish embryos to predict whole fish toxicity has garnered significant interest from researchers to develop high throughput screening assays for chemicals and to reduce the use of aquatic animals in testing (Lammer et al., 2009a; Ali et al., 2011; Scholz et al., 2014). A comparative evaluation of both fish and fish embryo toxicity data for 143 compounds confirms that fish embryo tests (FET) are neither better nor worse than acute fish toxicity tests (AFT) and the fish embryo test is a potential animal alternative for the acute fish toxicity test (Lammer et al., 2009b; Strähle et al., 2012). Statistical analysis of zebrafish embryo and fish toxicity data from different species for 56 compounds reveals that there is a reliable correlation between the fish embryo test and the acute fish test ($\log LC_{50}$ (AFT) = 0.951 $\log LC_{50}$ (FET) + 0.171, $R^2 = 0.85$, where LC_{50} is 50% lethal concentration) (Braunbeck et al., 2005). A very strong correlation has been observed between zebrafish embryo and fish acute toxicity for 27 organic industrial chemicals with a wide range of physicochemical properties and mode of actions (MOAs) (Knöbel et al., 2012). The FET–AFT relationships were not quantitatively different from acute fish–acute fish toxicity relationships for 72 chemicals ($\log LC_{50}$ (FET) = 0.989 $\log LC_{50}$ (AFT) − 0.195, $R^2 = 0.95$) (Belanger et al., 2013), indicating many compounds share same MOAs between fish and embryo toxicity.

The Verhaar's scheme intends to classify organic pollutants into one of four distinct MOA classes: inert, less inert, reactive and specifically–acting compounds (Verhaar et al., 1992). 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 nonpolar narcosis, or called baseline toxicity. Their toxicity is well correlated with hydrophobicity parameterized by the logarithm of the octanol/water partition coefficient ($\log K_{OW}$) (Veith et al., 1983; McKim et al., 1987). The acute toxicity of compounds acting by the nonpolar and polar narcotic mechanism of action can be well predicted. However, it has been challenging to develop models for reactive and specifically-acting compounds with such as diverse range of structures and toxic mechanisms (Cronin, 2017). The determination of MOAs has been recognized as a key limitation in the assessment of chemical toxicity as will assist in class-based predictive modelling of toxicity (Barron et al., 2015). Assignment to a MOA is based not only on the chemical itself, but also on the understanding of interaction between the chemical and the living organism (Li et al., 2015).

Although the MOAs have been well investigated in fish toxicity, a few studies have been carried on the MOAs to fish embryos (Klüver et al., 2016). No comparison has been made on the MOAs between fish and fish embryo toxicity for classified compounds. In this paper, the toxicity data to different fish species and zebrafish embryos were collected for a large number of diverse compounds from different source, respectively. Assessment was carried out on the fish toxicity obtained from different species and the intra- and inter-laboratory reproducibility of zebrafish embryo toxicity test, respectively. The aim of the work was to investigate the relationship of toxicity between fish and zebrafish embryos, and to compare the chemical MOAs identified from acute fish test and fish embryo test, respectively.

2. Materials and methods

2.1. Zebrafish embryo toxicity (ZFET) LC_{50} values

The ZFET LC_{50} (mmol/L) values were taken from a well-established database (http://echa.europa.eu/documents/10162/13562/annex2_fet_en.xlsx) based on extensive review of fish embryo acute toxicity data (Scholz et al., 2014; Klüver et al., 2016). The database including ZFET information for 2036 compounds with diverse chemical structures was classified into six subsets based on: (1) 96–120 h exposure, (2) if no toxicity: baseline toxicity vs. highest tested concentration > 10, (3) exposure duration < 96 h (i.e. 48–72 h), (4) compounds with no toxicity but tested > 10 fold above the baseline concentration, (5) high control variability or LC_{50} could not be calculated due to weaker toxicity at higher concentrations, and (6) inorganic compounds. In this paper, only the ZFET LC_{50} values in subsets (1) and (3) were used in the analysis because subsets (2), (4) and (5) have no ZFET LC_{50} values and subset (6) is inorganic compounds. Inspection of the database showed that many compounds contain multiple ZFET LC_{50} values. These multiple toxicity values will be used for the assessment of the toxicity data quality in the following analysis (see discussion section). After removing compounds with no toxicity data, the remaining 558 compounds were classified into different groups/classes based on their structures and applications as showed Table S1. It contains 218 industrial chemicals, such as alcohols, ethers, asters, amines, substituted benzenes, phenols, anilines and polycyclic aromatic hydrocarbons (PAHs), 185 pesticides (e.g. insecticides, herbicides and fungicides), 70 medications, 22 natural compounds and some other compounds with multiple or unknown applications. The more detail information on the classification of the compounds, together with the toxicity values, chemical names, SMILES and CAS numbers, can be found in Table S1 of Supplementary material. The evaluation of the toxicity data to embryos can also be found in the Supplementary material.