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Development of a general baseline toxicity QSAR model for the fish embryo acute toxicity test

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HIGHLIGHTS

- Common baseline toxicity QSAR for fish embryo LC_{50} was established.
- ILC_{50} and La_{50} analysis corroborated the robustness of baseline toxicity QSAR.
- Compounds with specific or reactive MOA converged towards baseline toxicity with increasing hydrophobicity.
- For compounds with log K_{lipw} < 4 the TR approach can be used to classify compounds.

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ABSTRACT

Fish embryos have become a popular model in ecotoxicology and toxicology. The fish embryo acute toxicity test (FET) with the zebrafish embryo was recently adopted by the OECD as technical guideline TG 236 and a large database of concentrations causing 50% lethality (LC_{50}) is available in the literature. Quantitative Structure-Activity Relationships (QSARs) of baseline toxicity (also called narcosis) are helpful to estimate the minimum toxicity of chemicals to be tested and to identify excess toxicity in existing data sets. Here, we analyzed an existing fish embryo toxicity database and established a QSAR for fish embryo LC_{50} using chemicals that were independently classified to act according to the non-specific mode of action of baseline toxicity. The octanol-water partition coefficient K_{ow} is commonly applied to discriminate between non-polar and polar narcotics. Replacing the K_{ow} by the liposome-water partition coefficient K_{lipw} yielded a common QSAR for polar and non-polar baseline toxicants. This developed baseline toxicity QSAR was applied to compare the final mode of action (MOA) assignment of 132 chemicals. Further, we included the analysis of internal lethal concentration (ILC_{50}) and chemical activity (La_{50}) as complementary approaches to evaluate the robustness of the FET baseline toxicity. The analysis of the FET dataset revealed that specifically acting and reactive chemicals converged towards the baseline toxicity QSAR with increasing hydrophobicity. The developed FET baseline toxicity QSAR can be used to identify specifically acting or reactive compounds by determination of the toxic ratio and in combination with appropriate endpoints to infer the MOA for chemicals.

1. Introduction

The fish embryo system as a model in toxicology and ecotoxicology has received increasing attention, because it has the potential to be used as an alternative to adult fish toxicity testing (Embry et al., 2010; Halder et al., 2010; Belanger et al., 2013). In particular the zebrafish embryo test (FET) is already being used as a toxicity screening tool for a wide range of chemicals (Padilla et al., 2012; Truong et al., 2014). Acute toxicity in fish embryos correlates very well with acute toxicity in adults (Knöbel et al., 2012; Belanger et al., 2013). Median lethal concentrations for 50% of fish embryos (LC_{50}) were compiled in a database (Scholz et al., 2014). A recent

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update of the database contains the entries from 2054 studies representing 1480 chemical compounds (Scholz et al., 2016). 98% of the study entries were generated with embryos of the zebrafish. Test protocols comprised static, semi-static and flow through studies using diverse exposure vessels including glass vials and 24-well plastic plates. Exposure durations ranged from 24 to 120 h post fertilization (hpf) starting from fertilization or 24 hpf. The fish embryo toxicity test (FET) was recently adopted as OECD test guideline TG 236 (OECD, 2013). According to OECD TG 236, newly fertilized zebrafish embryos should be exposed for a total of 96 h to the test compound. During the exposure period lethality is recorded every 24 h and the LC_{50} is derived from lethality after 96 h exposure.

Baseline toxicity, also called narcosis, can be described as the minimal toxicity of an organic chemical and any reactive or specific effect would increase the toxicity leading to lower LC_{50} values than expected for baseline toxicity (Könemann, 1981; Veith et al., 1983; van Wezel and Opperhuizen, 1995). The mechanism of narcosis likely results from non-specific reversible disturbance of membrane functioning and integrity as a result of partitioning of chemicals into biological membranes (van Wezel and Opperhuizen, 1995). This disturbance on the cellular level results in decreased activity, loss of equilibrium and can finally lead to death of the organism. Further, it has been shown for narcotic acting chemicals that an increase in lethality strongly correlates with an increase in the chemical's hydrophobicity. To estimate the baseline or minimal toxicity of an organic compound, quantitative structure-activity relationship (QSAR) can be used. The comparison of observed and estimated baseline toxicity can be used as an indicator of a specific non-narcotic mode of action (MOA).

QSARs have been developed to predict the toxicity in an organism based on physicochemical properties of the chemical. The quantitative assessment of baseline toxicity in aquatic organisms exposed to organic compounds were examined for a variety of species (Bradbury et al., 2003), but so far not for zebrafish embryo. Established QSAR models of baseline toxicity are mostly based on the octanol-water partition coefficient log K_{ow}, simply by a linear relationship between the log LC_{50} and log K_{ow} (Bradbury et al., 2003). However, the use of log K_{ow} as descriptor resulted in two distinctly different QSARs for non-polar and polar narcotics (Verhaar et al., 1992). It was subsequently demonstrated that this difference is not due to a different mechanism of toxicity of non-polar and polar narcotics but due to the limitation of K_{ow} as a descriptor (Vaes et al., 1998b). The differentiation between non-polar and polar narcotics QSARs disappeared when K_{ow} was replaced by a better surrogate of biological membranes, the liposome-water partition coefficient K_{lipw} (Vaes et al., 1998b). The application of K_{lipw} allowed the development of a single baseline toxicity QSAR valid for non-polar and polar chemicals also for other biological endpoints (Escher and Schwarzenbach, 2002). However, given the limited availability of K_{lipw} data, it has remained practice to keep K_{ow} and to differentiate between non-polar and polar narcotics. But more recently, poly-parameter linear free-energy relationships (PP-LFER) became available for a class-independent prediction of K_{lipw} (Endo et al., 2011). This progress has not yet translated into a wider application of K_{lipw}-based baseline toxicity QSARs.

In addition to this correlation between exposure concentrations and the hydrophobicity of narcotic compounds, which indicates that the biological membrane is the target site, the internal lethal concentration (ILC) of the organism can be considered as a useful indicator for the acute internal toxicity of chemicals (Escher and Hermens, 2002). The ILC, also called critical body residue (CBR) or lethal body burden (LBB), is based on the observation that the bioconcentration factors (BCF) and LC_{50} for baseline toxicants are inversely related to each other (McCarty, 1986; McCarty et al., 1992; Sijm et al., 1993). The ILC_{50}, which refers to lethality for 50% of the test animals, for baseline toxicants in aquatic organisms is more or less constant and occurs at concentrations of 40–160 mmol per L of membrane (van Wezel and Opperhuizen, 1995; Escher and Schwarzenbach, 2002). Independent of the lipid content differences between organisms, it has been proposed that narcotic toxicity is initiated within a relatively narrow range of chemical activities (Mackay et al., 2009). The chemical activity quantifies the energetic level of a substance relative to saturation (Reichenberg and Mayer, 2006; Thomas et al., 2015). The activity required to cause acute narcotic toxicity is generally in the range of 0.01–0.1 (Mackay et al., 2009; Thomas et al., 2015).

For the development of QSAR models and the application of the chemical activity framework the MOA classification and grouping of chemicals is an important step. A wide suite of empirical and rule-based categorizations for determining the chemical MOA in aquatic organisms is available (Könemann, 1981; McKim et al., 1987; Verhaar et al., 1992; Russom et al., 1997). A measure of the specificity of the effect of a compound is the toxic ratio (TR), which is the quotient of the effect concentration calculated with a baseline toxicity QSAR and the experimental effect concentration (Verhaar et al., 1992). The TR provides guidance whether a compound acts according to baseline toxicity ($TR < 10$) or a reactive or specific mode of toxic action ($TR \geq 10$). The higher the TR, the more intrinsically potent is a chemical (Verhaar et al., 1992).

The present study aims to develop a common baseline toxicity QSAR for non-polar and polar narcotics in fish embryos based on log K_{lipw} using a subset of 14 chemicals with experimental K_{lipw} by using fish embryo toxicity data. We further validated the FET baseline toxicity QSAR with LC_{50} of the second subset consisting of 19 chemicals by using predicted K_{lipw}. Further, we obtained the ILC_{50} and La_{50} values of the FET dataset and determined toxic ratios (TR) for three complementary approaches and compared these to the chemical grouping used in this study.

2. Material and methods

2.1. Fish embryo toxicity LC_{50} values

The FET LC_{50} values were taken from an established FET database (Scholz et al., 2014), which has been recently updated. The full FET database is available at http://echa.europa.eu/documents/10162/13562/annex2_fet_en.xlsx. The database includes fish embryo acute toxicity information for diverse chemical structures and corresponding modes of actions. In this study we considered neutral organic compounds and organic acids and bases that were predominately (>99%) neutral at pH7 and which were in the range of $-1 < \log K_{lipw} < 5$ (Table S1). FET studies of questionable reliability were identified by applying quality criteria that were considered as most influential for the determination of the LC_{50} in the fish embryo test. Only studies with organic compounds were included that fulfill the following criteria:

(1) utilization of zebrafish embryos,
(2) exposure starting between 0 and 8 h post fertilization (hpf) and recording of effects at 96 or 120 hpf, and
(3) LC_{50} value below the water solubility of the test compound.

If for a given compound more than one quality-checked LC_{50} value was available, the geometric mean was calculated (Table S1 and S2).