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

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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 KOW. The factors that influence the toxicity ratio calculated from baseline level are discussed in this paper.

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 aMOA 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 KOW) (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). TheMOA 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 non specifically 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 (Tpred) and the observed toxicity (Tobs) (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, LC50 values within a factor of 10 of baseline toxicity (i.e. TR b 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/LC50 (mol/L) for all analyses. The 3363 compounds were classified into different classes/homologues based on chemical functional groups as described in Section 2.3. The averaged toxicity values were used for compounds with multiple values for each individual species. Two groups of chemicals were excluded from the analysis of the data sets, namely charged and organometallic compounds. The toxicity values to the four species, together with names, SMILES and CAS numbers, can be found in Table S1-S4 of Supplementary material.

2.1.1. Fish 50% lethal concentration

Fish acute toxicity values for 965 compounds, for example those taken from the fathead minnow (Pimephales promelas) toxicity database, as well as data for the guppy (Poecilia reticulata), medaka

(Oryzias latipes) and rainbow trout (Oncorhynchusmykiss), were recorded as the concentration causing 50% lethality of a test population (LC50) after 96 h (Li et al., 2015). Inspection of the fish toxicity data in Table S1 of Supplementary material shows that they have very close toxicity value and good interspecies correlations of toxicity between the four fish species. Because limited numbers of toxicity values to fish, a combined toxicity dataset was used in the comparative analysis with other species (Raevsky et al., 2008; Raevsky et al., 2009; Zhang et al., 2010). The log1/LC50 collected fromthe different sources for all the compounds are recorded in Table S1.

2.1.2. Daphnia magna (DM) 50% effective concentration

The toxicity data of 757 chemicals to Daphnia magna were compiled from Li et al. (Li et al., 2015), and had been compiled previously from the Japanese CHRIP database and several other references. The toxicity values were reported either as LC50 (50% lethal concentration in 48 h) or EC50 (50% effective concentration in 48 h). The log1/EC50 collected from different sources for all the compounds are in listed Table S2.

2.1.3. Tetrahymena pyriformis (TP) 50% growth inhibition concentration

The acute toxicity data for 2049 compounds to Tetrahymena pyriformis were expressed as the concentration that causes 50% growth inhibition (IGC50) after 40 or 48 h for 2049 compounds. Very close IGC50 values were observed between the 40 h and 48 h assays for compounds with

comparable data. The log1/IGC50 values collected from different sources for all the compounds are in Table S3.

2.1.4. Vibrio fischeri (VF) 50% bioluminescence inhibition concentration

The concentrations for 1277 compounds causing a 50% inhibition of bioluminescence after 15 or 30 min exposure to Vibrio fischeri (or called Aliivibrio fischeri) expressed as IBC50 were also taken from Li et al. (Li et al., 2015). Comparison of the data in Table S4 shows that the two toxicity endpoints have very close values (Steinmetz et al., 2015). Preference was given to 30 min over 15 min where available. The log1/IBC50 collected from different sources for all the compounds are in Table S4.

2.2. Excess toxicity

In order to evaluate and discriminate compounds exhibiting excess toxicity, the toxicity ratio (or called toxic ratio or toxicity enhancement) between the QSAR-predicted baseline toxicity and experimental toxicity was calculated (Verhaar et al., 1992; von der Ohe et al., 2005; Neuwoehner et al., 2010; Schramm et al., 2011). The toxicity ratio (TR), a descriptor of excess toxicity, was calculated as follows:

TR = Tpred (baseline) / =Tobs (1)

log TR = log 1/Tobs − log 1/Tpred (baseline) = Residual (2)

Chemicals with TR values b 10 may be considered to act by a nonpolar narcosis mechanism. In contrast, chemicals for which TR is ≥10, corresponding to toxicity 10 times above baseline toxicity, were defined as demonstrating excess toxicity due to the possible existence of a reactive, or more specific molecular, mechanism of action (Zhao et al., 1998). The toxicity value used in Eq. (1) is lethal concentration (LC50), effective concentration (EC50), growth inhibition concentration (IGC50), or inhibition concentration of bioluminescence (IBC50) respectively for the species considered. It can be easily converted into the logarithmic form (see Eq. (2)). Thus, compounds were classified as being either baseline or reactive/specifically acting toxicants using the cutoff of log TR= 1. The complete listing of calculated toxicity ratios is available as Supplementary material in Table S5, together with MOA for baseline and reactive or specifically acting compounds predicted by log TR= 1 to the four species.

2.3. Assignment of modes of action (MOAs)

Based on the Verhaar classification scheme implemented in the freely available Toxtree software (version 1.50, https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology/qsar_tools/ toxtree), the MOAs of 3363 compoundswere classified into class 1 (narcosis or baseline toxicity) or class 2 (less inert toxicity), class 3 (unspecific reactivity mechanism), class 4 (specific reactivity mechanism) and class 5 (not possible to classify). In addition,

the MOA assignments were evaluated manually using information derived from a number of key studies (Verhaar et al., 1992; Russom et al., 1997; Enoch et al., 2011; Schwöbel et al., 2011). The evaluations were then combined to assign a specific mode of action to each chemical. The mode of action assessments are compared with the results of Toxtree software. If there was consistency in the classifications fromthe two methods, the mode of action of organic compounds was confirmed. The details of the classification of the MOAs for each compound can be found in Table S5 of Supplementary material. Fig. 1 shows a histogram of the modes of action assigned to 424 organic compounds with toxicity data to fish, 311 organic compounds to DM, 745 organic compounds to TP and 384 organic compounds to VF. These compounds encompass a wide range of well-characterized molecular structures with different chemical domain of the Verhaar scheme (see Table S5).

2.4. Molecular descriptors and statistical analysis

The logarithms of octanol/water partition coefficients (log KOW) were obtained from the KOWWIN program in the EPI Suite version 4.0.Where possible measured log KOW values were used in preference to calculated values. Regression analysis was performed using least squares linear regression with the Minitab software (version 14) between the physicochemical parameters (log KOW) and toxicity data. For each regression analysis, the following descriptive information is provided: number of observations used in the analysis (N), the coefficient of determination (R2), standard error of the estimate (S) and Fisher statistic (F). The species sensitivity was evaluated from the average residual (AR) between the species toxicity endpoints (AR=Σ (Toxicity in species A − Toxicity in species B) / N.

3. Results

3.1. Interspecies relationships for the toxicity of chemicals to the four species considered

Interspecies relationships of toxicity are useful to investigate the similarities and differences in toxic mode of action, for the same compounds, between species (Koleva et al., 2011). For this reason, linear regression analysis between toxicity data to Fish (F), Daphnia magna (DM), Vibrio fischeri (VF) and Tetrahymena pyriformis (TP) was performed. The relationships between these species are reported in Table 1, along with the interspecies coefficients of determination (R2). The interspecies coefficients of determination of 0.72 (Models 1 and 2 in Table 1) indicate significant correlations between the toxicity data to fish with those to DM and TP. Conversely, interspecies relationships between the toxicity to VF and fish, TP, DM or DM and TP are less significant with coefficients of determination ranging from 0.45 to 0.63 (Models 3–6 in Table 1). The overall results (Models 3–6 in Table 1) suggest that there are marked differences between the toxicities to organic chemicals for the four species considered. The elicitation of toxicity involves both the transport of the toxicant to the target site(s) of interaction and interaction between the toxicant and target (Zhang et al., 2010). Good correlations (Models 1 and 2 in Table 1) between the toxicity data indicate that a great proportion of compounds have a similar trend in bio-uptake and similar toxic metabolism between fish and Mor TP fish, they may elicit identical modes of action. Poor correlations indicate that some compounds may act by different toxic modes of action or have substantially different toxicokinetics. The differences in mechanisms between two species could be as a result of different metabolism (e.g. esters are metabolized to reactive compounds in fish but not in TP (Jaworska et al., 1995)) or

different physiology (e.g. antibiotics will be more toxic to the bacterium VF than fish). In addition, significant outliers (i.e. N1) have been observed within these interspecies correlations, as such, it is clear that the toxic effect of a compound is species-dependent (Zhang et al., 2013; Li et al., 2015). The coefficients of determination (R2) between any two of four species to baseline, less inert and reactive compounds are also given in Table 1 (Models 7–24). The relatively significant correlations were found between these four species for baseline compounds (R2 = 0.65–0.93), such as alkanes, alcohols, ketones and their alkane and chlorine derivatives (Table S5), relatively poor correlations were observed for less inert and reactive compounds with R2 = 0.42–0.87 and 0.40– 0.78, respectively. The results suggest that the interspecies correlation is related strongly to the structural characteristics of chemicals. Many baseline compounds may share same mode of toxic action between species, but some less inert and reactive compounds may not and the relative toxicodynamic input may vary between species. It is important to note that baseline narcosis would be essentially identical across different aquatic species. Great correlation should be observed for the toxicity between species for baseline compounds. However, relative poor regression coefficient for the toxicity data of baseline compounds between Daphnia magna and Vibrio fischeri (0.65) suggests that some factors can influence the toxicity values for these baseline compounds. The details will be discussed in the Discussion section.

3.2. Comparison of toxicity sensitivity for the different species

Table 1 lists the regression equations (No. 1–6) for the toxicities to overall compounds between any two of four species. The slopes are either significantly greater or less than one (e.g. <0.7 for Model 5 in Table 1) or the intercepts are greater or less than zero (e.g. N0.80 for Models 5 and 6 in Table 1) for some interspecies correlation equations indicating that the aquatic species (fish, DM, TP and VF) may have different sensitivities to organic compounds. In order to further investigate the differences and similarities in sensitivity between the different species in detail, the average residuals of toxicities (AR) between toxicity values for the compounds with comparable data for different species were calculated (Table 1). AR reflects the difference in overall sensitivity between two species. Comparison of AR values in Table 1 indicates that DM is the most sensitive, and TP is the least sensitive, of the four species. The order of sensitivity of these species is: DM N Fish N VF N TP. The higher the AR values, the greater difference in sensitivity between the two species. The sensitivity of TP is very different to the sensitivity of the other three species with the AR (Fish–TP) = 0.64 and AR (DM– TP) = 0.86 and AR (VF–TP) = 0.65, respectively. The results in this study (Table S5) indicate that there is no single species that is most sensitive to all compounds; species that are very sensitive to one group of compounds might be less so to other groups (Slooff and Canton, 1983; Cairns, 1986; Suter, 1993; Vaal et al., 1997). Inspection of toxicity data of classified compounds in Table S5 shows that the average log1/EC50 values are greater than log 1/LC50 for secondary amino alcohols, anilines (N-alkyl) with fluoro and chloro groups, tertiary amino alcohols, anilides with hydroxy and amino groups, but lower than log 1/LC50 for aldehydes and esters. More classes elicit higher toxicity to the DM which indicating that overall toxicity sensitivity to DM is greater than that to fish. Similar results can be seen from the comparison of toxicities among other species. In order to further investigate the differences and similarities of toxicities between the four species for the classified compounds, a statistical analysis was performed for baseline, less inert and reactive compounds, respectively. The average residuals between toxicities of any two of four species are listed in Table 1. Inspection of the AR values shows that the toxicity sensitivity of baseline compounds to DM, fish and VF is very similar and their average residuals are b0.13 logarithmic units, with AR (DM-fish)=0.05 and AR (DM–

VF)=0.10. A similar situation is observed among the toxicity sensitivity of less inert compounds among DM, fish and VF with AR (VF-fish) = 0.03, AR (DM– VF) = 0.04 and AR (DM-fish) = 0.11. However, the sensitivity of TP is very different from other three species with sensitivity to baseline and less inert compounds to fish, DM and VF being higher than that to TP. Comparison of the AR values of reactive compounds shows that toxicity sensitivity of fish is quite similar to DM with AR (fish - DM) = 0.07. However, great differences in the toxicity sensitivity were observed for VF and TP compared with fish or DM (Table 1). The differences in the toxicity sensitivity between various organisms to a chemical reflect differences in specificity at the site of toxic action, transport, or metabolic transformation (Holmes et al., 1995; Johnson et al., 2000; Koleva et al., 2011).

3.3. Identification of modes of toxic action from the same threshold

Although the analysis of interspecies relationships and toxicity sensitivity suggest that there are similarities and differences among these four species, it is more difficult to compare the differences in the toxic mechanisms from these results. The TR is a very useful tool to discriminate reactive or specifically acting compounds from baseline toxicity (McCarty and Mackay, 1993; Maeder et al., 2004; von der Ohe et al., 2005; Schramm et al., 2011). To calculate the TR, baseline models need to be developed from narcotic compounds for the different species. Since it is well-known that the toxicity is linearly related to log KOW for the baseline compounds to many species (Könemann, 1981; Cronin and Dearden, 1995; Raevsky et al., 2008; Qin et al., 2010). The linear regression analysis between log KOW and the toxicity data to fish, DM, TP and VF was performed. The resultant equations are listed in Table 2. The compounds used to develop the baseline models were selected carefully being simple neutral compounds and widely recognized as baseline compounds i.e. alkanes, alcohols, ketones, ethers, benzenes and their alkyl, fluorine and chlorine derivatives with 0 b log KOW b 7 (Table S5). As described below, some compounds, whilst classified as baseline compounds by Verhaar et al. (Verhaar et al., 1992), including esters, alkenes, cycloalkanes, aliphatic acids and their derivatives, were not selected and used to develop the baseline models, as well as those with residuals (observed – predicted toxicity) N1 or b−1,were excluded from the development of baseline models.

Log TR values for species are listed in Table S5 of Supplementary material with the threshold of log TR ≥ 1 indicating reactive or specifically acting compounds and log TR b 1 indicating baseline compounds. Fig. 1 shows the percentages of compounds with log TR ≥ 1 and log TR b 1 for baseline, less inert and reactive/specifically acting compounds to the four species. Inspection of Fig. 1 shows that number of compounds with log TR ≥ 1 and log TRb 1 is not same between the four species, particularly for TP. Many more compounds have log TR below the threshold for baseline toxicity for TP than that for fish, DM and VF (Fig. 1). It is understandable that reactive or specifically acting compounds may have different toxic mechanisms between species, leading to different excess toxicity for same compounds. However, it is unreasonable for baseline compounds because these compounds should share same toxic mechanism in different species. The different number of baseline compounds identified from the threshold of log TR=1 may be due to the difference in the toxicity sensitivity from different species.

Fig. 2 illustrates the distribution of toxicity for baseline and reactive or specifically acting compounds for the species with different sensitivities, in theory this should be a normal distribution. If same threshold (e.g. log TR = 1) is used for the classification of reactive or specifically acting compounds

from baseline level, the majority the compounds predicted as baseline or reactive acting compounds in another species. However, incorrect classifications can be given for some compounds. For example, some compounds were predicted as baseline for a species with low sensitivity from this threshold, yet predicted as reactive or specifically acting to species with high sensitivity species (Fig. 2) and vice versa. Thus, using the same threshold for species with different sensitivities can result in incorrect classification for some reactive or specifically acting and baseline compounds. In other words, the threshold is species-dependent.

4. Discussion

4.1. Development of the thresholds of excess toxicity for different species

The threshold of log TR=1 for excess toxicity to fish is based on the normal distribution of log TR for the baseline, less inert, reactive and specifically acting compounds (Verhaar et al., 1992; Zhang et al., 2013). In principle, the thresholds of excess toxicity for D. magna, V. fischeri and T. pyriformis should also be developed from the distribution of log TR for the baseline, less inert and reactive or specifically acting compounds. However, the compounds identified as baseline, less inert and reactive or specifically acting compounds in fish may not be the same in other species, particularly for the reactive or specifically acting compounds (see following discussion). To overcome the above problem and obtain comparable thresholds for different species, the baseline compounds with high certainty of acting as non-polar narcotics as described above (see Table S5) were selected and compared. They are alkanes, alcohols, ketones, ethers and benzenes with alkyl, fluorine and chlorine groups. Some compounds identified as baseline compounds from Verhaar scheme were not used the baseline model development, such as log KOW > 7 or < 0 (Table S5). The reason for excluding the compounds will be explained below. Fig. 3 is the plots of toxicities against log KOW for the four species. It shows that the toxicities of baseline compounds were well correlated with log KOW. The baseline models for fish, DM and VF are close to each other with almost identical slopes and intercepts, indicating that baseline compounds have similar species sensitivity and similarity in modes of action for all three species. This conclusion is reinforced from the analysis of AR between toxicity values for the baseline compounds for different species (Table 1). Therefore, the threshold of log TR=1which is based on the distribution of toxicity data to fish can also be used to discriminate reactive or specifically acting compounds from baseline narcotics for DM and VF. Fig. 1 shows the number of compounds with log TR ≥ 1 for baseline compounds for different species. Almost all the compounds identified as baseline compounds from fish toxicity can also identified as baselines from DM and VF toxicity. Slight differences in prediction accuracy may be due to experimental uncertainty as a result of different endpoints obtained from different species.

Conversely, the slope of the baseline model for TP is markedly less than that for fish, DM and VF. This can be seen from the confidence intervals of equation coefficients and intercepts in Table 2. The marked difference in sensitivity in TP was observed as comparing to fish and DM and VF (Table 2 and Fig. 3). This suggests that the threshold of log TR=1 obtained from fish toxicity is not an ideal to identify excess toxicity to TP and the comparable threshold of log TR should be b1. In order to obtain the specific threshold to discriminate excess toxicity from narcotic level compounds for TP, the average absolute residual (AAR) between observed and predicted toxicities for the compounds used to develop baseline models was calculated for fish and TP. If no consideration is given to the

experimental error from different toxicity endpoints for different species, the AAR value, which reflects the deviation in the fit of the baselinemodels, should be positively related to sensitivity. The species with greater sensitivity will have more error in the fit of its baseline model. The AAR should be closely related to the thresholds of log TR between species and can be expressed as following relationship:

$$\frac{log\ TR_F(=1.00)}{AAR_F(=0.25)} = \frac{logTR_{TP}(=0.84)}{AAR_{TP}(=0.21)} \quad (3)$$

Where, AAR for fish (AARF) is equal to 0.25 in log units and AAR for TP (AARTP) is equal to 0.21. The threshold of log TR=1 used for fish suggests that the threshold for log TR for TP should be 0.84. Although this threshold has developed based on the deviation of fits in the baseline models, it can be validated from the classification accuracy for these thresholds. Fig. 1 shows that threshold of log TR= 0.84 can give same classification accuracy with log TR = 1 for baseline compounds in TP toxicity, which is almost same from the threshold of log TR= 1 in fish toxicity. This suggests that the threshold of log TR= 0.84 of TP is comparable to the threshold of fish for the discrimination of reactive or specifically acting compounds from baseline level. Comparing with log TR=1, 18% more compounds (142 over 760) being identified as having excess toxicity using log TR = 0.84. It has marked influence for some classes. For example, 6 of 31 substituted benzaldehyes (class 35) are identified as having excess toxicity from log TR = 0.84 as comparing with 2 of 31 from log TR= 1. It is worth noting that not all reactive or specifically acting compounds have log TR greater than thresholds (Table S6). This can be seen from the distribution of log TR for reactive or specifically acting compounds in Fig. 2 (Verhaar et al., 1992). The interaction of reactive or specifically acting compounds with biological macromolecules may not have much more toxic contribution than that of baseline compounds, resulting in log TR less than one (Zhang et al., 2013). It is reason why not all the reactive compounds do not exhibit excess toxicity with log TR > 1.

4.2. Classification of modes of action with different thresholds for different species

4.2.1. Baseline compounds

The toxicity ratios calculated by Eq. (2) are listed in Table S5 in Supplementary material for all the compounds and the four species. The summary statistics for the compounds identified with different MOAs are listed in Table S6 of Supplementary material. Because of the limited number of compounds, some classes are not listed in Table S6, however, the full results are available in Table S5 of Supplementary material. Inspection of the numbers of compounds with log TR ≥ 1 or 0.84 reveals that majority of baseline compounds have log TR less than the threshold of log TR = 1 for fish, DM and VF or log TR= 0.84 for TP. Only a small number of previously identified baseline compounds have log TR greater than these thresholds for the four species. Experimental error is a possible cause for these outliers. The large number of compounds with log TR less than the thresholds suggests that these baseline compounds share the same MOA among four species. They are characterized as neutral compounds with simple and unreactive structures acting via the “non-polar narcosis” mechanism for the four species (Raevsky et al., 2008).

4.2.2. Less inert compounds

The statistics in Table S6 shows that many of less inert compounds have log TR below than the thresholds. However, more less inert compounds have log TR values higher than the thresholds as compared to baseline compounds for all the four species (15, 23, 26 and 35% for fish, DM, TP and VF, respectively). The less inert compounds are mainly "polar narcotics" and include substituted phenols and anilines (Verhaar et al., 1992; Raevsky et al., 2009). It is important to note that less inert compounds exhibit higher toxicity to the lower species (TP and VF) as compared to the higher species (fish and DM). A greater number of less inert compounds with log TR above the thresholds were observed for TP and VF than fish and DM. This may be attributed to the differences in the physiology between species, resulting in differences in bio-uptake and clearance, thus leading to different potency between species. For instance, compounds that share the same MO Abetween species will demonstrate comparable effects related to bio-uptake potential. Hydrophobicity is the main driving force for the bio-uptake of neutral compounds with a linear relationship between bioconcentration factor (BCF) and hydrophobicity for many hydrophobic compounds (Meylan et al., 1999). However, for hydrophilic compounds in the lower species there is a higher relative volume fraction of water than the higher level organisms. These hydrophilic compounds may more easily pass through a cell membrane than the outer covering (e.g. skin, exoskeleton) of higher organisms and thus have a higher observed bioconcentration factors in lower species, leading to a greater number of less inert compounds with log TR greater than the thresholds for TP and VF than fish and DM species.

4.2.3. Reactive or specifically acting compounds

Table S6 lists the compounds with log TR greater than the thresholds (i.e. 1 or 0.84). Whilst some are identified as being reactive with well established MOAs, the MOA of others is less obvious. Inspection of the numbers of compounds with log TR greater than the thresholds shows that most of the classes with excess toxicity to one species also exhibit excess toxicity to other species (e.g. Classes 10, 18, 19, 25, 27, 30, 31, 36, 44, 45, 46, 47, 51 and 53). A number of compounds with log TR greater than the thresholds are observed in these classes. These compounds are reactive or specifically interact with the biomacromolecules at the target site(s) and show excess toxicity to these species. There is, however, a small number of classes with excess toxicity to one species but which do not exhibit excess toxicity to other species. For example, some β-halogenated alcohols (class 4) show excess toxicity to fish, DM and TP, but not to VF. Nitrates (class 23) exhibit strong toxicity to VF, but not to TP. Alcohol and alkoxy-substituted benzenes are relatively more toxic to VF than that to fish, DM and TP (class 34). These compounds may share different MOAs among these species.

Inspection of the log KOW values reveals that the hydrophilic compounds are relatively more toxic than the hydrophobic compounds (classes 20, 21, 22 and 31) i.e. compounds with log KOW b 0 have log TR significantly greater than the thresholds. There are two possible reasons for this excess toxicity. Firstly, they are reactive or specifically acting compounds with enhanced toxicity. It is well-known that the reactive mechanism includes the formation of covalent bonds between an electron-poor (electrophilic) substrate and a biological electron-rich (nucleophilic) target molecule, especially biological macromolecules such as nucleic acids and proteins (Enoch et al., 2011). The hydrophilic

chemicals considered are small and intrinsically reactive interacting unspecifically with biomolecules or certain receptor molecules through Schiff base formation, bi-molecular nucleophilic substitution (SN2), acylation and aromatic nucleophilic substitution (SNAr) reactions (Hermens, 1990; Lipnick, 1991; Aptula and Roberts, 2006; Böhme et al., 2016). Secondly, the apparent or observed bioconcentration potential of such compounds is under-estimated from hydrophobicity (Meylan et al., 1999). The theoretical basis of discrimination of excess toxicity from baseline is the linear relationship between bioconcentration factor (log BCF) and log KOW (Li et al., 2015), resulting in linear relationship between toxicities and log KOW(Table S6). However, the apparent log BCF is not linearly related with log KOW for highly hydrophilic compounds, and log KOW is not a surrogate for BCF for the highly hydrophilic compounds. Comparing with the lipid phase in fish, aqueous-phase fraction is more important for quantifying their concentration in the organism. The under-estimated log BCF indicates the under-estimated toxicity from the baseline level calculated from log KOW, leading to over-calculated log TR values from Eq. 2 for these hydrophilic compounds.

The statistics in Table S6 shows that some ionizable compounds have relatively high toxicity with log TR N 1 or 0.84 (classes 17, 18, 19 and 40). For example, a number of aliphatic diacids exhibit excess toxicity to all four species and benzoic acids to VF. In principle, excess toxicity expressed as log TR is only applicable for neutral compounds, rather than for the ionizable compounds, as the baseline models are based on the linear relationship between toxicities and log KOW of the neutral species. Therefore, the log KOW values are markedly over-estimated for ionizable compounds (Li et al., 2016).Over-estimated log KOW values indicate over-estimated baseline toxicity and thus ionizable compounds should have lower log TR values calculated by Eq. 2. However, the excess toxicity calculated by log KOW in neutral species for these ionizable compounds suggests that they are very toxic and reactive or specifically acting compounds for these species. Some of them are well-known antifungal or being used for antiseptic disinfection in the pharmaceutical and food industries.

4.3. The factors that influence the toxicity ratios calculated from the baseline level

It is noteworthy that LC50, EC50, IGC50 and IBC50 are the external critical concentration, rather than the internal critical concentration (or called critical body residue, CBR). The toxicity sensitivity calculated in the paper is relative sensitivity of organisms toward toxicant stress, rather than the absolute sensitivity (Schüürmann et al., 1996; Blaschke et al., 2010). Therefore, several factors can affect the toxic effect and result in the differences in the toxicity sensitivity or toxicity ratios to the four species.

Firstly, there are four toxicity endpoints used in the evaluation of toxicity to fish, D. magna, T. pyriformis and V. fischeri, i.e. mortality, immobilisation, growth and bioluminescence, respectively. The inhibition of bioluminescence to V. fischeri may be closely related with mortality and immobilisation, resulting in a similar sensitivity between fish, D. magna and V. fischeri. However, as compared with the other three endpoints, the rate of growth is not a sensitive response to TP, resulting in poor sensitivity (Li et al., 2015). It is based on population density measured spectrophotometrically at 540 nm after 40-h. This density based approach may not be a sensitive assay to T. pyriformis.

Secondly, the differences of species sensitivity can be attributed to the differences in physiology between species. In theory, the tissues and organs of fish and D. magna can restrict transport of some compounds. On the other hand, TP and VF do not have the outer barrier when the compounds

were absorbed. The bacteria as unicellular organisms are known to have a peptidoglycan polymer outside their plasma membrane that forms an important part of their cell wall. These differences in physiology can lead to different toxicity among the four different species.

Thirdly, experimental error is possible for the observed toxicities less than that predicted from baseline models. In theory, baseline toxicity is the minimum toxicity that compounds exhibit, as such the log TR values calculated from Eq. 2 should be close to, or greater than, zero for all compounds. However, inspection of Table S5 in Supplementary material shows that there are marked deviations for some classes of compounds with log TR values significantly less than the thresholds (i.e.−1 for fish, DM and VF or −0.84 for TP). Sorption to vial walls or extracellular material, can affect the toxicity values, leading to different classification from excess toxicity. Furthermore, the LC, EC, IGC and IBC values are coming from different experiments that most likely are conducted with different numbers of treatments, replicates and individuals per replicate. The observed toxicity difference between species might be driven by the different exposure durations. This means there is a different level of uncertainty behind the data compared to another arising from the different tests and the different models used to actually derive the end value. Inspection of the toxicity values in Tables S1 to S5 shows that the TP toxicity data collected in this paper were tested in a single laboratory by a single, reliable and robust method (Cronin et al., 2002). On the other hand, fish, DM and VF toxicity data used in this paper were compiled from different laboratories and the quality of these data is often not known. The volatilization to the lab air can also affect the prediction of reactive or specifically acting compounds from baseline level. Inspection of the data in Table S5 of Supplementary material shows that nominal toxicity values of some highly volatile compounds are markedly less than the predicted values with log TR b 1. The exposure loss can affect the slope and intercept of the narcosis regression equations, resulting in wrong classification of the MOAs by using the toxicity ratio (Blaschke et al., 2010; Schramm et al., 2011). Therefore, these volatile compounds need to be excluded from the baseline model development in Table 2.

Fourthly, the highly hydrophobic compounds, especially compounds with log KOW N 7 need to be considered. These highly hydrophobic compounds have very low solubility or limited bioavailability (Meylan et al., 1999). The dissolved concentrations in water are considerably lower than the nominal concentrations for these compounds, resulting in a lack of bioavailability in water. A bilinear relationship was observed between log BCF and log KOW for the highly hydrophobic compounds. It explains why most of these compounds exhibit lower toxicity than expected for fish, DM and VF species (no data available for TP). The compounds with long chains (Class 1 in Table S6) were identified as having very poor capability to penetrate the epidermal membranes to enter aquatic organisms, resulting in poor bioconcentration and leading to the outliers with log TR significantly b−1 (Su et al., 2014). The polycyclic aromatic hydrocarbons (PAHs, class 56 in Table S6) also have low toxicity because of low solubility. A quite number of outliers were observed for VF and but no outlier was observed from the limited number of data for other species.

Fifthly, ionization is another factor that can affect the toxicity to different species. A number of aliphatic carboxylic acids (class 17 in Table S6) have very low toxicity to VF. These ionizable compounds exhibit markedly low toxicity because of their ionization in water. As discussed above, the log KOW values used for the calculation of log TR are for the neutral species of chemicals, rather than for the ionised species. The log KOW values are greatly over-estimated for ionizable compounds, resulting in over-predicted toxicity from the baseline model and leading to log TR markedly b −1 (Li et al., 2016).

5. Conclusions

The baseline models for toxicity to fish, DM and VF are similar to each other with almost identical slopes and intercepts confirming similar species sensitivity and mode of action for these species. The sensitivity of TP to baseline compounds was shown to be different from the other three species. Therefore, whilst the threshold of log TR = 1 based on the distribution of toxicity data to fish can also be used to discriminate reactive or specifically acting compounds from baseline narcotics for DM and VF, a log TR = 0.84 for TP is proposed. The summary statistics of log TR of fish, DM, TP and VF show that majority of baseline compounds have log TR below these thresholds. More less inert compounds with log TR greater than the thresholds were observed for TP and VF than fish and DM. This may be attributed to the differences in the physiology between species, resulting in differences in bio-uptake and thus leading to different toxic effects between species. Most of reactive or specifically acting chemicals exhibiting excess toxicity (log TR N threshold) to one species also show excess toxicity to the other three species. Slight differences in toxic effects for some compounds may be attributed to the under- or over- estimation of log KOW and differences physiology. These compounds may have different MOAs between these species. Hydrophilic compounds were shown to be relatively more toxic than the hydrophobic compounds and exhibit higher excess toxicity due to their intrinsic high reactivity and the under-estimation of their bio-concentration potential from log KOW. Perhaps surprisingly, most ionised compounds have high toxicity with log TR greater than one; however, some ionised compounds still had lower log TRs due to the over-estimated of log KOW for these compounds. It is also possible that some observed toxicities are less than that predicted from baseline models for different species due to experimental error, the size of the molecules, high hydrophobicity and the degree of ionization. The results fromthis investigation suggest that species-specific thresholds perform well to classify baseline, less inert and reactive/specifically acting compounds. Supplementary data to this article can be found online at https://doi. org/10.1016/j.scitotenv.2017.10.308.

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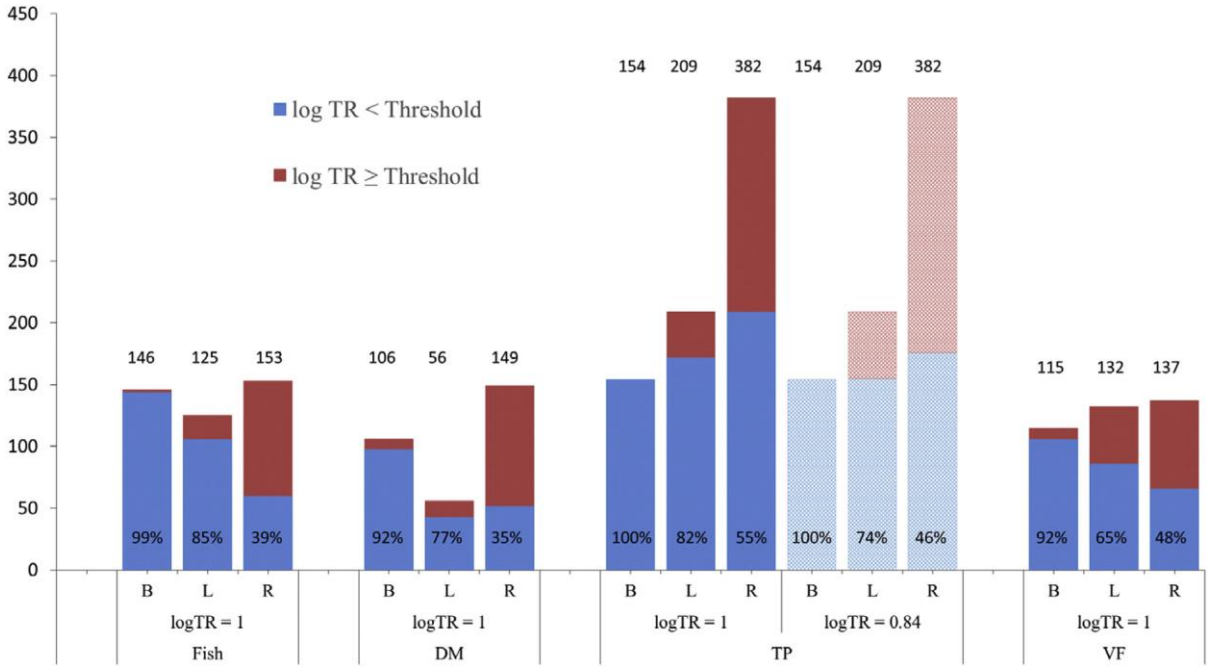

Fig. 1. The histogram of number of compounds with empirical modes of toxic action and classification accuracy (%) from different thresholds for four species (B: Baseline compounds; L: less inert compounds; R: Reactive or specifically acting compounds; DM: Daphnia magna; TP: Tetrahymena pyriformis; VF: Vibrio fischeri; log TR=1: Classification accuracy from threshold of log TR = 1 for the four species, respectively; log TR= 0.84: Classification accuracy from the threshold of log TR = 0.84 for Tetrahymena pyriformis).

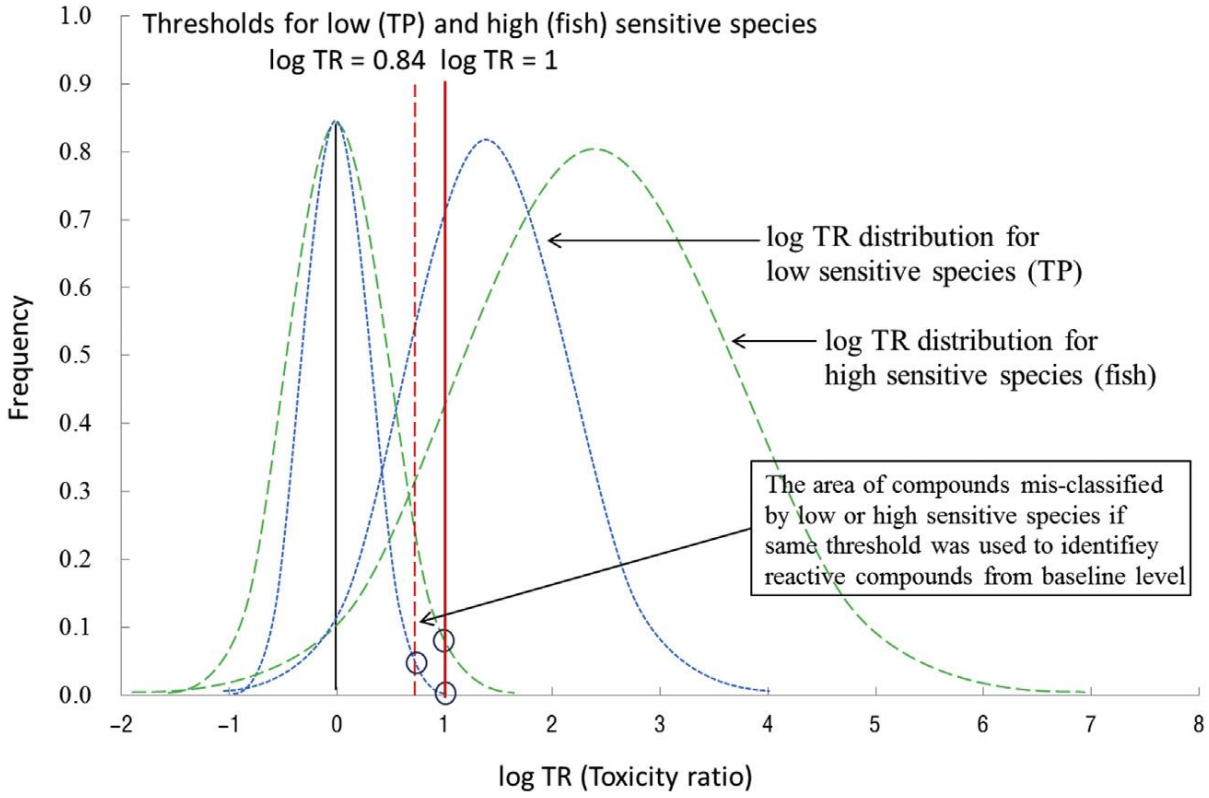

Fig. 2. Normal distributions of log TR for baseline (left) and reactive (right) compounds for two species with different toxicity sensitivity. O is the cross-points of thresholds with log TR distribution curves (Frequency log TR−2), where σ is the standard error of log TR and μ is the averaged log TR for baseline and reactive compounds, respectively). log TR = 1: Threshold for fish, Daphnia magna or Vibrio fischeri. Log TR = 0.84: Threshold for Tetrahymena pyriformis.

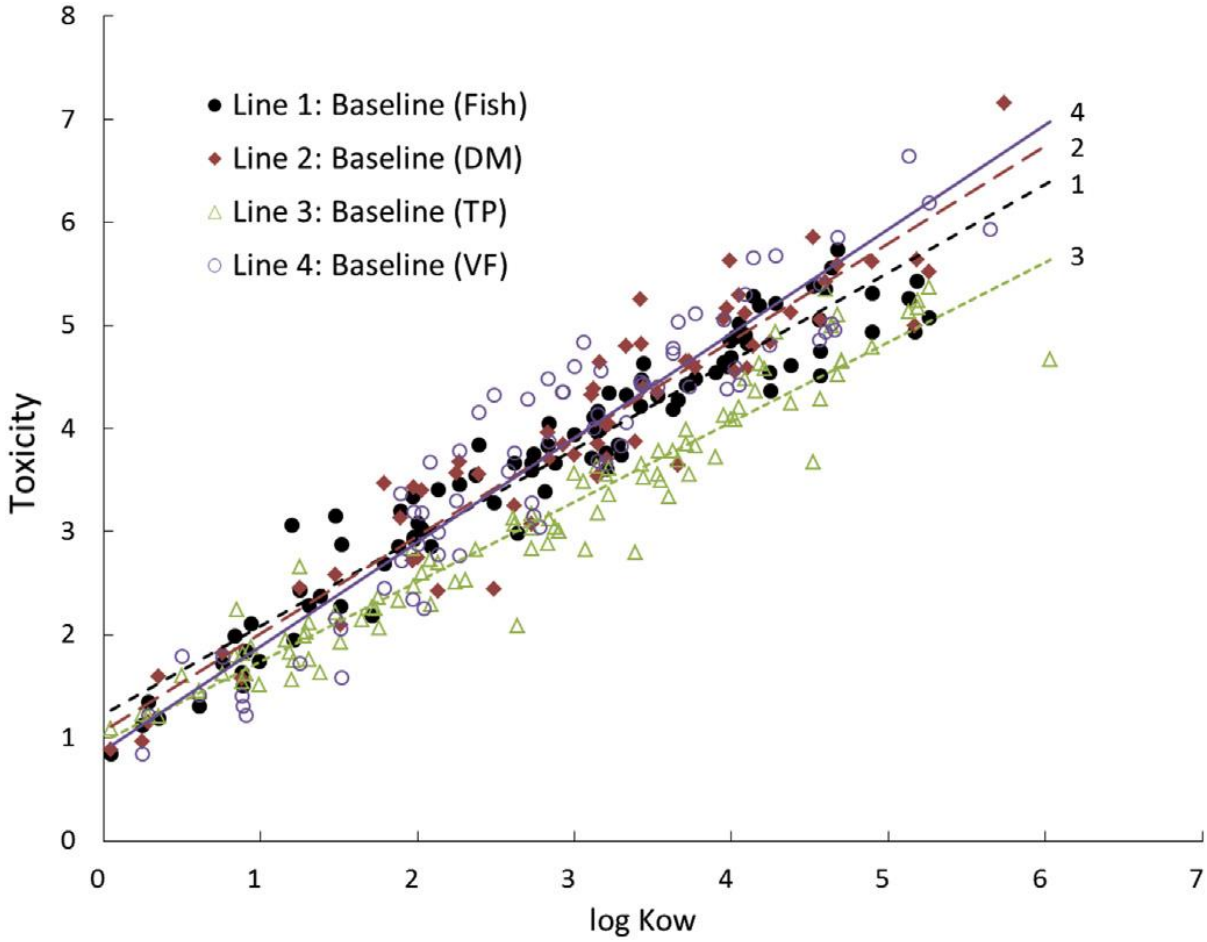

Fig. 3. Plots of toxicity (log 1/LC50, log1/EC50, log 1/IGC50 and log 1/IBC50) for fish, DM, TP and VF, respectively, against log KOW for baseline compounds.

Table 1

Interspecies relationships of toxicity for four species for all, baseline, less inert and reactive compounds, respectively.

No Species A – B Interspecies correlation N R2 AR

Overall compounds

1 Fish–DM log1/EC50 = 0.891 log 1/LC50 + 0.659 467 0.72 −0.19

2 Fish–TP log1/IGC50 = 0.752 log 1/LC50 + 0.326 478 0.72 0.64

3 Fish–VF log1/IBC50 = 0.771 log 1/LC50 + 0.790 304 0.54 0.11

4 DM–TP log1/IGC50 = 0.703 log 1/EC50 + 0.361 287 0.63 0.86

5 DM–VF log1/IBC50 = 0.611 log 1/EC50 + 1.272 294 0.45 0.37

6 TP–VF log1/IBC50 = 0.947 log 1/IGC50 + 0.828 556 0.63 −0.65

Baseline compounds

7 Fish–DM log1/EC50 = 0.996 log 1/LC50 + 0.065 92 0.83 −0.05

8 Fish–TP log1/IGC50 = 0.855 log 1/LC50 + 0.015 71 0.93 0.48

9 Fish–VF log1/IBC50 = 0.992 log 1/LC50−0.100 72 0.73 0.13

10 DM–TP log1/IGC50 = 0.742 log 1/EC50 + 0.523 42 0.81 0.34

11 DM–VF log1/IBC50 = 0.883 log 1/EC50 + 0.310 63 0.65 0.10

12 TP–VF log1/IBC50 = 1.148 log 1/IGC50 + 0.022 64 0.79 −0.47

Less inert compounds

13 Fish–DM log1/EC50 = 0.966 log 1/LC50 + 0.270 52 0.87 −0.11

14 Fish–TP log1/IGC50 = 0.779 log 1/LC50 + 0.373 102 0.69 0.52

15 Fish–VF log1/IBC50 = 0.885 log 1/LC50 + 0.497 75 0.50 −0.03

16 DM–TP log1/IGC50 = 0.652 log 1/EC50 + 0.800 45 0.47 0.76

17 DM–VF log1/IBC50 = 0.836 log 1/EC50 + 0.668 37 0.58 0.04

18 TP–VF log1/IBC50 = 0.630log 1/IGC50 + 1.920 112 0.42 −0.64

Reactive compounds

19 Fish–DM log1/EC50 = 0.910 log 1/LC50 + 0.345 67 0.66 0.07

20 Fish–TP log1/IGC50 = 0.702 log 1/LC50 + 0.214 79 0.59 1.04

21 Fish–VF log1/IBC50 = 0.770 log 1/LC50 + 0.221 26 0.54 0.82

22 DM–TP log1/IGC50 = 0.895 log 1/EC50−0.351 49 0.73 0.79

23 DM–VF log1/IBC50 = 0.511log 1/EC50 + 1.545 42 0.40 0.70

24 TP–VF log1/IBC50 = 1.088 log 1/IGC50 + 0.184 69 0.78 −0.49

DM: D. magna, TP: T. pyriformis, VF: V. fischeri. N: Number of overlapping compounds between any two of four species. AR=Σ(Toxicity in species A – Toxicity in species B)/N. R2: Coefficient of determination.

Table 2

Baseline models for the four species.

No.	Species	Models	N	R2	S	F
1	Fish	log 1/LC50 = 0.858(±0.048)log KOW + 1.22 (±0.15)	94	0.93	0.31	1268
2	DM	log 1/EC50 = 0.946 (±0.081)log KOW + 1.06(±0.27)	63	0.90	0.42	543
3	TP	log 1/IGC50 = 0.775(±0.042) log KOW + 0.96 (±0.13)	100	0.93	0.30	1369
4	VF	log 1/IBC50 = 1.01 (±0.08) log KOW + 0.87(±0.27)	73	0.89	0.46	558