Investigation of Critical Body Residues and Modes of Toxic Action Based on Injection and Aquatic Exposure in Fish

Yang Wen & Limin Su & Weichao Qin & Yuanhui Zhao & Judith C. Madden & Fabian P. Steinmetz & Mark T. D. Cronin

Abstract The internal concentration represented by the critical body residue (CBR) is an ideal indicator to reflect the intrinsic toxicity of a chemical. Whilst some studies have been performed on CBR, the effect of exposure route on internal toxicity has not been investigated for fish. In this paper, acute toxicity data to fish comprising LC_{50} and LD_{50} values were used to investigate CBR. The results showed that exposure route can significantly affect the internal concentration. LD_{50} and CBR calculated from LC_{50} and BCF both vary independently of hydrophobicity as expressed by log K_{ow}; conversely, LC_{50} is related to log K_{ow}. A poor relationship was observed between LC_{50} and LD_{50}, but the relationship can be improved significantly by introduction of log K_{ow} because log CBR is positively related to log LD_{50}. The parallel relationship of log CBR-log K_{ow} and log LD_{50}-log K_{ow} indicates that LD_{50} does not reflect the actual internal concentration. The average LD_{50} is close to the average CBR for less inert and reactive compounds, but greater than the average CBR for baseline compounds. This difference is due to the lipid fraction being the major storage site for most of the baseline compounds. Investigation on the calculated and observed CBRs shows that calculated CBRs are close to observed CBRs for most of compounds. However, systemic deviations of calculated CBRs have been observed for some compounds. The reasons for these systemic deviations may be attributed to BCF, equilibrium time and experimental error of LC_{50}. These factors are important and should be considered in the calculation of CBRs.

Keywords Critical body residue · Exposure route · Bioconcentration factor · Hydrophobicity · Fish

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1 Introduction

Global industrialisation has resulted in a large number of organic pollutants entering the aquatic environment. These organic pollutants can be accumulated in aquatic organisms following several exposure routes, such as oral, inhalation, injection (through in vivo testing) or dermal exposure, as well as from the food chain. The exposure routes play an important role in assessing the internal concentration. If the concentration of organic pollutants in an organism exceeds the critical body residue, the result to the organism may be lethality. The critical body residue (CBR) is defined as the concentration expressed in moles per kg body weight (mol/kg), which exerts a specific toxic effect such as

death or reduction in growth (McCarty 1987). Barron et al. (2002) questioned the utility of CBR approach for assessing toxicant effects in aquatic biota. The conclusion of this review was that large variability existed among species and toxicants when tissue concentrations were used as the dose metric and that variability was not reduced over that observed for external exposure concentrations. Thus, the study of CBRs based on different exposure routes is useful for the evaluation of toxic mechanisms of organic chemicals to aquatic organisms.

The modes of toxic action play an important role in the assessment of the ecotoxicity of organic compounds. The Verhaar classification scheme is well recognised as a means to classify compounds acting by baseline (or non-polar) and less inert (or polar) narcosis, as well as reactive and specific mechanisms (Verhaar et al. 1992). This scheme has been updated by Enoch et al. (2008). The Verhaar scheme represents a well-established decision tree constructed using a series of structural alerts designed to enable simple organic compounds to be assigned to one of four categories. Chemicals acting by baseline are those that are not reactive when considering overall acute effects and that do not interact with specific receptors in an organism. These chemicals act non-specifically on the cell membranes, and therefore, their toxicity can well be predicted from their octanol/water partition coefficient (K_{ow}) for a number of species (Cronin and Dearden 1995; Dearden et al. 2000; Su et al. 2012). Less inert chemicals are slightly more toxic than baseline toxicity and are commonly identified as possessing hydrogen bond donor acidity on an aromatic ring. Reactive chemicals can react covalently and unselectively with nucleophilic sites commonly found in biomolecules or are metabolised into more toxic species. In principle, the toxicity of reactive compounds is difficult to model, especially when different reaction mechanisms are considered. Specifically acting chemicals exhibit toxicity due to (specific) interactions with certain receptor molecules (specific or receptor toxicity). A series of structural rules which aimed to classify compounds according to modes of toxic action have been reported historically in the literature (Hermens 1990; von der Ohe et al. 2005; Enoch et al. 2008, 2011) and were the basis of those defined by Verhaar et al. (1992).

Target site concentration is an ideal indicator to reflect the intrinsic toxicity of a chemical. Chemicals acting by a lethal narcosis mechanism achieve their effect once a critical concentration, or critical volume, has been reached within some bio-phase site of action in the organism. However, target site concentrations of organic compounds are difficult to obtain directly. As a surrogate, the total concentration in an organism that elicits a critical effect, termed the CBR, has been used. Early studies used measurements of bioconcentration factor (BCF) and the external concentration causing 50 % lethality (LC_{50}) to estimate the residue concentration for 50 % mortality. Estimating the body residue associated with a toxic biological response from QSARs for toxicity and bioconcentration appears to be reasonably successful for neutral narcotic organic chemicals (McCarty 1987; McCarty et al. 1992; Meador 2006). McCarty et al. (1992) first reported that the body residue values for organic compounds vary across different modes of toxic action. For narcotic compounds (both baseline and less inert chemicals), the CBR for a toxic effect such as lethality or growth inhibition is constant, independent of either compound or exposure time (McCarty et al. 1993; Van Wezel and Opperhuizen 1995; Meador et al. 2008). The baseline compounds cause mortality within a very narrow range of whole body tissue concentrations (2–8 mmol/g wet weight or about 50 mmol/g lipid) in small aquatic organisms; the range for less inert compounds is also narrow, but lower (0.6–2 mmol/g) (Meador et al. 2011). However, the CBR for reactive compounds is different. These compounds are either receptor mediated or involve a direct chemical reaction with a biological substrate (macromolecule).

With the advent of CBR as the dose metric, it becomes possible to focus on the internal dose required to produce toxicity in aquatic organisms. However, the internal toxicity is directly related to the exposure route. The exposure route influences toxicokinetic properties (i.e. absorption, distribution, metabolism, elimination) and thus toxicity (Klaassen and Rozman 1991). Although acute toxicity to rat and rainbow trout was compared from different exposure routes, the relationships between toxic effect and exposure routes have not been investigated systematically and reported in the literature. The comparison of acute toxicity within and between rat and rainbow trout over various exposure routes shows that they are likely to be based on similar toxicokinetics and interspecies correlations were good, when matched on exposure routes between trout and rats (Delistraty et al. 1998). Wolf et al. (2004) observed that after establishing a lethal narcosis mechanism through the inhalation exposure route, a compound's

oral toxicity can be reliably estimated. The toxicity related with the exposure routes has been observed by many people (Klaassen and Rozman 1991; Delistraty 1999, 2000).

Although studies on CBR for narcotic compounds were reported in the literature (McCarty et al. 1992, 1993; Barron et al. 1997), the data are limited, and there is a lack of information regarding how the exposure route affects the concentration of the biologically active material at the site of action. By comparing the CBRs to same species for organic compounds with different modes of toxic action and following different exposure routes, the mechanism of toxic action of organic compounds would be investigated. In this study, LC_{50} toxicity data to fish for 965 compounds, LD_{50} to fish for 51 compounds and CBR to fish for 33 compounds previously reported in the literature and database were investigated. The aims of the current study were as follows: first, to identify the modes of toxic action of organic compounds according to updated Verhaar classification scheme for the compounds obtained; second, to study the relationship between LC_{50}, LD_{50} and descriptor for hydrophobicity; third, to investigate the CBR to fish by comparing different exposure routes; fourth, to discuss the factors influencing the accuracy of calculated CBR based on BCF, equilibrium time and experimental error.

2 Materials and Methods

2.1 Biological Data

The acute toxicity data expressed by the concentration required to kill 50 % of fish within 96 h (LC_{50}) were retrieved from several literature studies and databases as described herein. The LC_{50} values to the guppy (*Poecilia reticulata*) and rainbow trout (*Oncorhynchus mykiss*) were taken from Raevsky et al. (2008, 2009). The LC_{50} values to fathead minnow (*Pimephales promelas*) were taken from Russom et al. (1997), Yuan et al. (2007), Papa et al. (2005), Eroglu et al. (2007) and Raevsky et al. (2008, 2009). The LC_{50} values to Medaka (*Oryzias latipes*) were taken from the Japanese CHRIP (Chemical Risk Information Platform: http://www.safe.nite.go.jp/english/db.html) database. A total of 965 organic compounds were collated in this study. The value of LC_{50} for each compound was expressed as log $1/LC_{50}$ in mmol/L according to standard QSAR practices. The 965 compounds were classified into different classes/

homologues based on their functional groups. The details of classification, together with CAS numbers and descriptors calculated for each compound, are reported in Table S1 of Supplementary material.

The toxicity data expressed by the dose required to kill 50 % of fish (LD_{50}) were taken from the most extensive, publicly available data compilation, the Environmental Residue Effects Database (ERED, http://el.erdc.usace.army.mil/ered/). The LD_{50} toxicity data to fish in this database consisted of 938 records for 161 compounds from 164 references. Only data with intraperitoneal injection/exposure were collected, providing toxicity data for 51 compounds. The value of LD_{50} for each compound was expressed as log LD_{50} mmol/kg (mmol per kg of fish body weight). The details of name, together with CAS and descriptors calculated for each compound, are reported in Table S2 of Supplementary material.

Thirty-three CBRs from the aqueous exposure route were obtained from Environmental Residue Effects Database (ERED). Where possible, the CBR was confirmed to be at 50 % response levels. Most data apply to whole body residues, but a few organ levels were also included. The value of CBR for each compound was expressed as log CBR mmol/kg (mmol per kg of fish body weight). The details of the name, together with CAS and descriptors calculated for each compound, are reported in Table S3 of Supplementary material.

2.2 Fish Bioconcentration Factor (BCF)

The log BCF values were estimated from the log BCF-log K_{ow} relationship (see Eq. 1). This equation was used to estimate the log BCF values for the compounds with log K_{ow} in the range of 1−7 using the BCFBAF program in EPI Suite software version 4.0 (http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm). Appendix E of EPI Suite software lists all correction factors used by BCFBAF.

$$\log BCF = 0.660 \log K_{ow} - 0.333 + \text{Correction factors} \quad (1)$$

2.3 Molecular Descriptors and Statistical Analysis

The logarithm of the octanol/water partition coefficient (log K_{ow}) was obtained from the KOWWIN program in

EPI Suite software version 4.0. Where possible, measured log K_{ow} values were used in preference to calculated values. The other 19 descriptors representing different physico-chemical properties were calculated for all the studied compounds. These descriptors represent molecular size, solubility, polarity, degree of ionization, flexibility, hydrogen bonding acidity and basicity. They are molecular weight (MW), distribution coefficient (D), Abraham descriptors (E, S, A, B, V), solubility, acid and base pKa value, fraction of unionized (F_0), positive (F_+), negative (F_-), zwitterionic ($F_\pm$) forms at given pH. The descriptors were calculated using software of ACD/Labs suite (Advanced Chemistry Development, Inc., http://www.acdlabs.com).

Regression analysis was performed using the Minitab software (version 14). For each regression analysis, the following statistics were recorded: number of observations used in the analysis (N), coefficient of determination adjusted for degrees of freedom (R^2), standard error of the estimate (S) and Fisher's criterion (F). The models were evaluated using the average error (AE=$\sum$(Obs−Pred)/n), the average absolute error (AAE=$\sum$|Obs−Pred|/n), the root-mean squared error (RMSE=($\sum$(Obs−Pred)^2/n)$^{1/2}$), where вObs^ is the observed CBR value and вPred^ is the predicted CBR value from LC_{50} and BCF.

2.4 Assignment of Mode of Action

An in-house KNIME Workflow was used to identify protein reactive compounds (Enoch et al. 2011). Depending on the classification given by the KNIME Workflow, the organic compounds were assigned to each mode of action using a manual implementation of the Verhaar et al. (1992) scheme. The toxicity categories assigned in this study, based on the Verhaar categorisation system for organic compounds, were class 1 baseline chemicals, class 2 less inert chemicals, class 3 reactive chemicals, class 4 specifically acting chemicals, class 5 any chemicals not in classes 1–4 is classified as вno decision can be made about this chemical^.

3 Results

3.1 Modes of Toxic Action

The Verhaar classification scheme is a well-established decision tree constructed using a series of structural alerts and physico-chemical properties designed to enable simple organic chemicals to be assigned to one of four modes of toxic action based categories (Verhaar et al. 1992). Figure 1 shows the histogram of modes of toxic action assigned to 965 organic compounds with LC_{50} values and 51 compounds with LD_{50} values. According to the Verhaar classification scheme, 447 of the 965 compounds (46.3 %) fall into class 1, 247 of the 965 compounds (25.6 %) fall into class 2, 215 of the 965 compounds (22.3 %) fall into class 3, 27 of the 965 compounds (2.8 %) fall in the class 4 and 29 of the 965 compounds (3 %) could not be assigned and hence fall into class 5. The results of the profiling according to protein reactivity support these findings. Most of the organic compounds were assigned to be classes 1 and 2 (Fig. 1). The classifications, based on the Verhaar classification scheme, will be used in the following QSAR studies. Because the toxic mechanisms of classes 4 and 5 are complex, especially for class 5 chemicals which exhibit unknown mode of toxic action, classes 4 and 5 are excluded in this study.

3.2 Relationships Between LC_{50} or LD_{50} and log K_{ow}

Regression analyses between log1/LC_{50} or log1/LD_{50} and log K_{ow} were performed, and the results are recorded in Table 1. Model 1 in Table 1 shows a significant relationship, but expected poor statistical fit (R^2=0.47) between log1/LC_{50} and log K_{ow} for all 965 compounds. It is well known that K_{ow} is an important parameter to predict acute toxicity for various compounds over a wide range of biological systems. The correlation
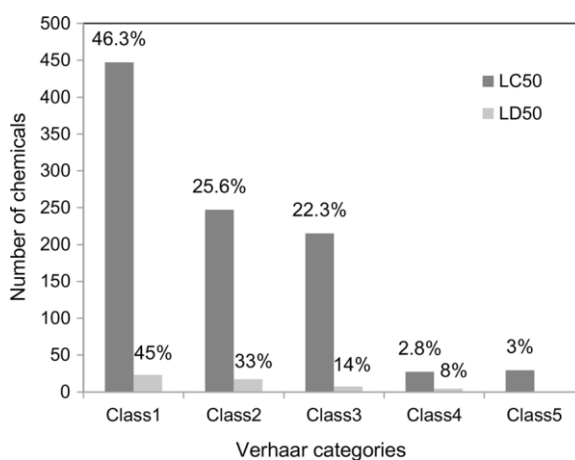

Fig. 1 Verhaar classification for the compounds with 965 LC_{50} and 51 LD_{50} values, respectively

Table 1 Simple and multiple linear regressions for fish acute toxicity

Equation no.	Regression equations	M	N	R^2	S	F
1	$\log 1/LC_{50}=0.486 \log K_{ow} - 0.112$	A	965	0.47	0.94	856
2	$\log 1/LC_{50}=0.622 \log K_{ow} - 0.929$	1	447	0.72	0.70	1126
3	$\log 1/LC_{50}=0.609 \log K_{ow} - 0.472$	2	247	0.77	0.50	823
4	$\log 1/LD_{50}=0.219 \log K_{ow} - 1.10$	A	51	0.16	0.96	9
5	$\log 1/LD_{50}=0.178 \log K_{ow} - 1.78$	1	23	0.62	0.30	34
6	$\log 1/LD_{50}=0.278 \log K_{ow} - 0.916$	2	17	0.22	0.59	4
7	$\log 1/LC_{50}=0.8 \log 1/LD_{50}+1.64$	A	37	0.20	1.19	9
8	$\log 1/LC_{50}=3.04 \log 1/LD_{50}+4.55$	1	19	0.61	1.08	27
9	$\log 1/LC_{50}=0.883 \log 1/LD_{50}+1.48$	2	15	0.34	0.71	7
10	$\log 1/LC_{50}=0.498 \log 1/LD_{50}+0.672 \log K_{ow} - 0.332$	A	37	0.82	0.58	77
11	$\log 1/LC_{50}=0.382 \log 1/LD_{50}+0.847 \log K_{ow} - 1.12$	1	19	0.99	0.22	510
12	$\log 1/LC_{50}=-0.086 \log 1/LD_{50}+0.738 \log K_{ow} - 0.682$	2	15	0.96	0.19	131

M chemical classification. *A* all compounds; *1* baseline compounds (or non-polar narcotics); *2* less inert compounds (or polar narcotics). *N* number of observations used in model; R^2 determination coefficient; *S* standard error of estimate; *F* Fisher's criterion. Unit of $\log 1/LC_{50}$: mmol/L; unit of $\log 1/LD_{50}$: mmol/kg

between $\log 1/LC_{50}$ and $\log K_{ow}$ for narcotic compounds is particularly significant (Könemann 1981; Veith and Broderius 1987; Dearden et al. 2000). Better relationships were obtained by restricting the data set to baseline and less inert compounds (models 2 and 3). The slopes and intercepts of models 2 and 3 indicate that the toxicity for less inert compounds is relatively higher than that for baseline compounds.

Table 1 also shows the relationships between $\log 1/LD_{50}$ and $\log K_{ow}$. The results show that the correlation for all 51 compounds was very poor; R^2 value was only 0.16 (model 4). The linear regression analysis between $\log 1/LD_{50}$ and $\log K_{ow}$ was also performed for baseline and less inert compounds. Although there is a relationship between $\log 1/LD_{50}$ and $\log K_{ow}$ with $R^2=0.62$ for baseline compounds (model 5), the regression equation was not as significant as compared with the model 2. No correlation was found for less inert compounds (model 6).

3.3 Correlations of Acute Toxicity LD_{50} and LC_{50}

The simplest approach to investigate the relationship between toxicities within species is by using correlation analysis. Models 7, 8 and 9 are the relationships between $\log 1/LC_{50}$ and $\log 1/LD_{50}$ for all compounds, baseline and less inert compounds, respectively. The correlations are poor not only for all the compounds, but also for baseline and less inert compounds (Table 1). Only about 20 % of the variance in $\log 1/LC_{50}$ could be

accounted by $\log 1/LD_{50}$ alone for all compounds. However, the coefficients of determination can be significantly improved following the inclusion of $\log K_{ow}$, especially for baseline and less inert compounds. The regression results are listed in Table 1 as models 10, 11 and 12, respectively.

3.4 CBR Calculated from LC_{50} and LD_{50}

Although LC_{50} and LD_{50} are all acute toxicity data, different toxic effects were observed in these toxicity endpoints to same species. LD_{50} is defined as a single dose of a chemical that kills 50 % of fish within a certain time by intraperitoneal injection, whereas LC_{50} is defined as the aqueous concentration of a chemical that kills 50 % of fish within a certain time through gill and skin exposure. LC_{50} is the external effect concentration of a chemical. On the other hand, LD_{50} is the internal effective dose by intraperitoneal injection. There is a growing acceptance that, where possible, the internal concentration or CBR should be used as the indicator of toxicity. To investigate the effect of exposure routes on the toxicity endpoints (LC_{50} and LD_{50}), the CBRs were calculated from the LC_{50} and BCF described by using Eq. (2).

$$CBR = LC_{50} \times BCF \qquad (2)$$

The CBRs associated with 50 % mortality were calculated using Eq. (2). Figure 2 shows the plot of log

Fig. 2 Plot of log CBR against log K_{ow} for 965 organic compounds. *Straight line 1* average value of log CBR (0.69) for baseline compounds; *straight line 2* average value of log CBR (0.30) for less inert compounds. *Group 4* alcohol and alcohol-ethers; *group 11* primary mono-alcohols; *group 13* amine-alcohols; *group 14* diamines and polyamines; *group 15* nitriles; *group 19* disulphides; *group 30* anilines; *group 43* pyridines
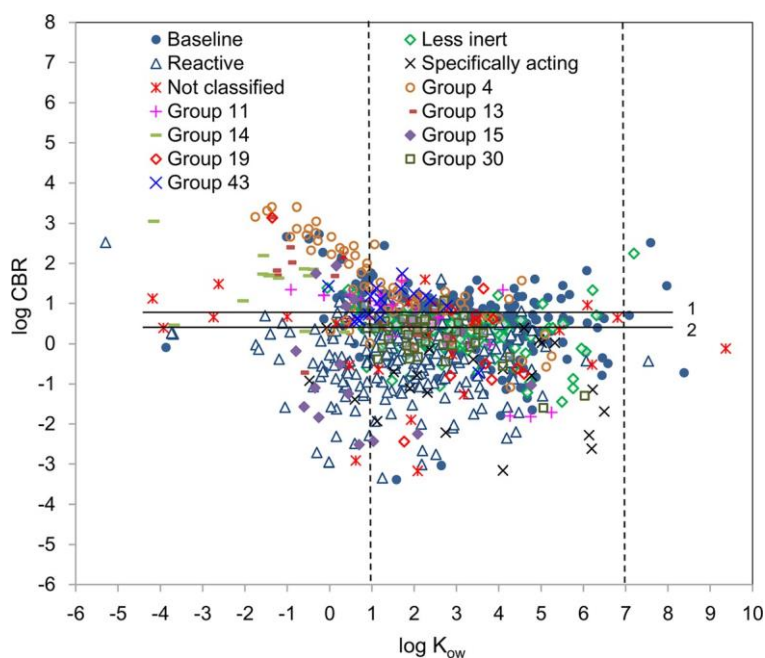
CBR against log K_{ow} for all 965 compounds. Log CBR is independent of hydrophobicity as described by log K_{ow} and varies in a narrow range for baseline and less inert compounds. The average log CBR is 0.69 for baseline, 0.30 for less inert and −0.49 for reactive compounds (Table 2). As expected, the CBR is higher for baseline and less inert compounds. Reactive compounds exhibit higher toxicity with very low log CBR. In addition, for the baseline and less inert compounds with $1 < \log K_{ow} < 7$, the log CBRs are approximately constant with the means of 0.48 and 0.20, respectively. However, for the narcotic compounds with log $K_{ow} > 7$ and log $K_{ow} < 1$, the log CBRs are higher than the average values of baseline and less inert compounds (Fig. 2). Examination of means of log CBR for each group shows that there are systematic biases for some groups. For example, the average of log CBRs for alcohols and alcohol-ethers are higher than the baseline average value; the average of log

CBRs for primary mono-amines, amine-alcohols, diamines, polyamines and pyridines are higher than the less inert and reactive average value (groups 4, 11, 13, 14, 43). On the other hand, some compounds such as the nitriles, disulphides and anilines (groups 15, 19, 30) exhibit lower CBRs than the average of log CBRs for baseline, less inert and reactive compounds, respectively.

Compared to LC_{50}, LD_{50} (intraperitoneal injection) is related directly to internal concentration. Figure 3 shows the plot of log LD_{50} and log K_{ow} for the 51 compounds. Log LD_{50} does not vary significantly with increasing hydrophobicity. The average log LD_{50} is 1.29 for baseline compounds, 0.23 for less inert compounds and −0.52 for reactive compounds (Table 2). The average LD_{50} is greatest for baseline compounds followed by less inert and reactive compounds. The trends are similar with the CBR calculated from LC_{50} and BCF. Comparison of CBRs and LD_{50} shows that the average

Table 2 Averages of internal toxicity for different exposure routes

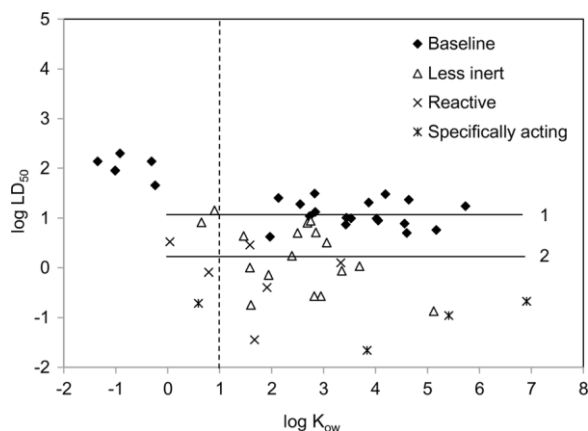| Mode of toxic action	N	Average log CBR (mmol/kg)	SD	N	Average log LD_{50} (mmol/kg)	SD
Baseline compound	447	0.69	0.88	23	1.29	0.48
Less inert compound	247	0.30	0.61	17	0.23	0.65
Reactive compound	215	−0.49	0.91	7	−0.52	1.20

N number of observations, SD standard error of estimate

Fig. 3 Plot of log LD_{50} against log K_{ow} for 51 organic compounds. *Straight line 1* average value of log LD_{50} (1.09) for baseline compounds with log $K_{ow}>1$; *straight line 2* average value log LD_{50} (0.23) for less inert compounds

log LD_{50} is higher than the average log CBR for baseline compounds, but is close to the average log CBR for less inert and reactive compounds (Table 2). For the narcotic compounds with log $K_{ow}<1$, the log LD_{50} is higher than the average value (Fig. 3). At the same time, for the baseline compounds with log $K_{ow}>1$, the log LD_{50} values are approximately constant with a mean log LD_{50} value of 1.09. There is a difference of 0.20 log units compared with the average log LD_{50} for all baseline compounds.

Examination of LC_{50} and LD_{50} shows that 37 compounds are in both data sets. The average residual (AE) and average absolute residual (AAE) of internal concentration between LD_{50} and CBR were 0.26 and 0.61, respectively, for the 37 overlapping compounds. Log CBR-log K_{ow} and log LD_{50}-log K_{ow} relationships are parallel. The relationship between CBR and LD_{50} for the 37 overlapping compounds can be expressed in Eq. (3):

$$\log\ CBR\ ¼\ 0{:}64\log LD_{50} \qquad \text{ð3Þ}$$

4 Discussion

4.1 Relationship Between log $1/LC_{50}$ or log $1/LD_{50}$ and Hydrophobicity

If steady state has been reached between water and lipid phase of the organism and no biotransformation is considered in the toxicity test, the internal concentration of a compound exposed to a fish can be related to LC_{50} by BCF (Maeder et al. 2004), and the relationship among LC_{50}, CBR and BCF can be derived from Eq. (2) and expressed by the following equation:

$$\log\ CBR\ ¼\ \log ð LC_{50}\times BCF Þ\ ¼\ \log BCF - \log 1{=}LC_{50}$$
$$\text{ð4Þ}$$

It is generally recognized that organic chemical hydrophobicity is the principal driving force of bioconcentration. The most simple and common method for estimating bioconcentration potential consists of establishing correlations between BCF values and hydrophobicity (K_{ow}) of organic chemicals. The majority of these relationships have been obtained from linear regression models between log BCF and log K_{ow} (Veith et al. 1979; Meylan et al. 1999; Pavan et al. 2008). Introducing the log BCF-log K_{ow} relationship into Eq. (4), a relationship between toxicity and hydrophobicity as log K_{ow} is obtained:

$$\log 1{=}LC_{50}\ ¼\ -\log CBR Þ\ \log BCF\ ¼\ -\log CBR Þ\ a\log K_{ow} Þ\ b$$
$$\text{ð5Þ}$$

Equation (5) is the theoretical relationship between log $1/LC_{50}$ and log K_{ow}. For narcotic compounds, the CBRs vary in a narrow range (Fig. 2), and toxicity should be linearly related with the hydrophobicity. For reactive compounds, the CBRs are much lower than those of narcotic compounds, and log $1/LC_{50}$ is expected to have a poor relationship with log K_{ow}. This theory explains why the relationships between log $1/LC_{50}$ and log K_{ow} for baseline and less inert compounds are individually better than that for all compounds (models 1−3 in Table 1).

In contrast to the relationship of LC_{50} and BCF, the relationship between LD_{50} and K_{ow} is not as strong. LD_{50} does not vary significantly with hydrophobicity, and parallel lines were observed between log LD_{50} and log K_{ow} for baseline and less inert compounds (Fig. 3). The difference in the relationships between log $1/LC_{50}$ or log $1/LD_{50}$ and log K_{ow} is due to the different exposure routes. LC_{50} and LD_{50} were obtained from the bioaccumulation of chemicals in aquatic organism and intraperitoneal injection, respectively. The intraperitoneal injection delivers the compound to target sites by blood. This exposure route of intraperitoneal injection is a process of distribution from abdomen and different from the process of a compound partitioning from water into the lipid compartment of an organism. Thus, the toxicity by intraperitoneal injection is not related to

hydrophobicity. It explains why a poor relationship was observed between log $1/LD_{50}$ and log K_{ow} (model 4 in Table 1).

4.2 Relationship Between LC_{50} and LD_{50}

The relationships between LC_{50} and LD_{50} have been described from the models 7–9 with low coefficients of determination. Inclusion of log K_{ow} can significantly improve the correlations (models 10–12). This phenomenon is clearly due to the different exposure routes because log K_{ow} is the descriptor commonly used to parameterize BCF. As mentioned above, there is a linear relationship between log CBR and log LD_{50} expressed as log CBR=0.64 log LD_{50}. Introducing this equation into Eq. (5), the relationship between log $1/LC_{50}$ and log LD_{50} with hydrophobicity is obtained (Eq. (6)).

$$\log 1=LC_{50} = -0.64\log\ LD_{50} + a\ \log\ K_{ow} + b \qquad (6)$$

Equation (6) can be used to explain why the coefficients of determination increase after the introduction of log K_{ow} into the relationships between log $1/LC_{50}$ and log $1/LD_{50}$ (models 10–12 in Table 1).

4.3 CBRs of Baseline, Less Inert and Reactive Chemicals

The CBR reflects the intrinsic toxicity of a chemical and plays an important role in the study of the toxic effect. However, the observed CBRs are limited in the literature. Therefore, calculated CBRs, as an alternative to observed CBRs, of chemicals in fish are commonly used in the study of internal concentration. In order to examine whether or not the calculated CBRs are close to the measured CBRs, the experimental CBRs were compared with the CBRs calculated from LC_{50} and BCF. Inspection of the absolute residuals between measured and calculated CBRs for 21 compounds reveals that 71 % of compounds have a residual less than 0.5 log unit. The AE, AAE and RMSE of the 21 compounds are −0.25, 0.47 and 0.68, respectively. The maximum difference for the CBR values occurs for 2,3,5-trichlorophenol, with an absolute residual of 1.80 log unit. The large residual for this compound may be attributed to the ionisation, leading to an erroneous calculated BCF value. Although some differences were observed between calculated and measured CBRs, no significant difference

was observed for most of the compounds. This indicates that the CBRs calculated from LC_{50} and BCF can reflect the critical concentrations for the organic compounds in fish.

In theory, LD_{50}s obtained from intraperitoneal injection to fish should be close to the CBRs. The results in this study show that LD_{50}s are close to the average CBRs for less inert and reactive compounds, but higher than the average CBR for baseline compounds. This difference may be due to the greater hydrophobicity of the baseline compounds. Most of these baseline compounds are highly hydrophobic compounds with non-polar functional groups. The lipid fraction is the major storage site, and these compounds accumulate easily in lipid fraction after intraperitoneal injection. This will result in an uneven distribution throughout the body of the fish and lower concentration in the target site, leading to the LD_{50} higher than CBR. Conversely, lipid fraction is not the major storage site for some compounds such as hydrophilic compounds, including some of the less inert and reactive compounds, which can be distributed easily to the target site through the blood and interact with the macromolecules. Therefore, no great difference was observed between the LD_{50} and CBR for less inert and reactive compounds.

4.4 The Factors Affecting the Accuracy of Calculated CBRs

Although the CBR is an ideal indicator to reflect the internal concentration of a chemical, some factors, such as the BCF, equilibrium time and experimental error of LC_{50}, can contribute to the accuracy of calculating CBRs. This may result in the difference between CBRs calculated from LC_{50} and BCF and observed CBRs.

The accuracy of BCF is one of the issues that influence the accuracy of calculated CBRs. The calculated CBRs shows that log CBR values vary in a narrow range for the narcotic compounds with $1<\log K_{ow}<7$. However, the CBRs of compounds with log $K_{ow}<1$ or log $K_{ow}>7$ were greater than the average CBRs (Fig. 2). Equation (1) used to predict BCF is relatively accurate for compounds with log K_{ow} in the range of 1–7, but not for the compounds with log $K_{ow}<1$ or log $K_{ow}>7$ (BCFBAF program in EPI Suite software). Although correction factors were introduced into the linear model between log BCF and log K_{ow} (Eq. (1)) to improve the accuracy of predicted BCF, the errors were quite high

for the compounds with log K_{ow}<1 or >7, resulting in larger errors for the CBRs calculated from Eq. (3) for highly hydrophilic compounds (e.g. log K_{ow}<1) and hydrophobic compounds (i.e. log K_{ow}>7). For example, for groups including alcohols and alcohols-ethers (group 4), primary mono-amines (group 11), amine-alcohols (group 13), diamines and polyamines (group 14), pyridines (group 43), some compounds have log K_{ow} values less than 1.0. The reason is attributed to that highly hydrophilic and hydrophobic compound uptake from other tissues/organs and chemical bioavailability in water plays a much more important role than lipid content. In addition, metabolism is another factor that can contribute to the variability of BCF. It reduces the BCFs of metabolically active compounds. There are a number of substances which have been shown to rapidly transform in solution. The hydroxyphenols, aminophenols (group 29) and diamines (group 14) can be oxidized easily to benzoquinones in water. Metabolism can result in the decrease of concentration for the parent compound, leading to a bias in the bioconcentration. The log BCF was over-estimated from the model for these compounds. The CBRs calculated from LC_{50} and BCF for these compounds were significantly higher than the average value.

Equilibrium time is another factor that can affect the accuracy of calculated CBRs. In principle, the CBR should be estimated from the bioconcentration ratio (BCR) at 96 h in fish, rather than the BCF. However, these BCR values are scarce and arduous to measure for compounds with a wide range of structures. Therefore, BCF, instead of BCR, was used to estimate CBR in Eq. (2). However, the BCFs reported in the literature were the experimental values measured in fish at steady state, rather than at the 96 h (the time for the 96 h-LC_{50} for fish toxicity). To reach equilibrium, the uptake times from water of highly hydrophobic chemicals can be longer than 96 h (Mackay and Fraser 2000). The steady-state assumption of Eq. (2) might not be fulfilled for hydrophobic compounds, which have slower bioconcentration kinetics. This has the effect of increasing these CBR values, especially for highly hydrophobic compounds (e.g. for chemicals with log K_{ow}>7), which require longer equilibrium times to reach equilibrium. That may be another principle cause of overestimating the CBRs from BCFs for highly hydrophobic compounds.

The high experimental error of LC_{50} will influence on CBR. The reliability of the LC_{50} values used in this study is very important. Inspection of LC_{50} data shows that there are 192 overlapping compounds in the four data sets of LC_{50} compiled and 46 compounds have exactly the same values. The average absolute error of log $1/LC_{50}$ for the remaining 146 chemicals between the maxima and minima is 0.30. The maximum difference in log $1/LC_{50}$ is 1.50 log units (diethylamine) (Fig. 4). Experimental uncertainty is a possible explanation for the difference in toxicity. As often stated in the scientific literature, toxicity data for organic chemicals in aquatic organisms should be reliable. Such data should ideally be obtained from well-standardised assays, with a clear and unambiguous endpoint. High-quality data will have lower experimental error associated with them (Cronin and Schultz 2003). However, these variables are difficult, or impossible, to control. Examination of the LC_{50} data obtained reveals that there are some compounds

Fig. 4 Histogram of absolute residuals of log $1/LC_{50}$ for 146 compounds
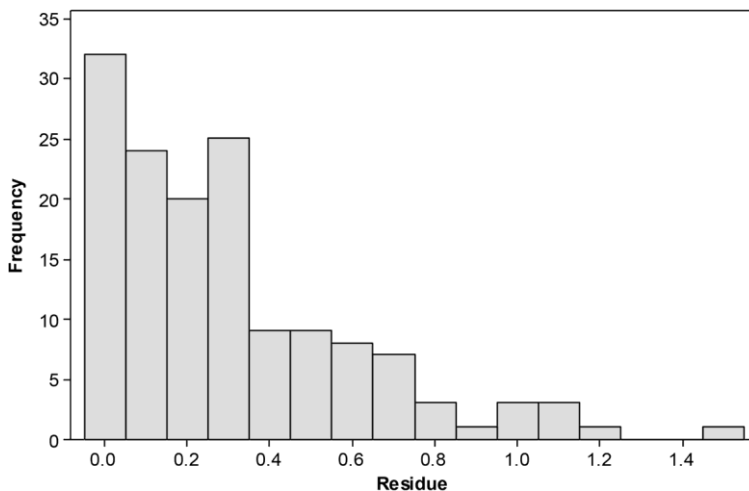
with significant differences in toxicity. The test species, test condition, exposure concentration and determination method of toxicity may result in the difference in measured LC_{50}. Some log CBRs in nitriles (group 15) are lower than the average log CBR of reactive compounds. These chemicals can undergo hydrolysis to the amide or carboxylic acid, leading to measured LC_{50} with large experimental error. It is not clear why some of the log CBR data for disulphides (group 19) and anilines (group 30) are lower than the mean. More studies are needed to study the toxic mechanism for these compounds.

5 Conclusions

The relationships between toxicity and hydrophobicity have been investigated with an emphasis on narcotic compounds. There were significant relationships between log $1/LC_{50}$ and log K_{ow} for baseline and less inert compounds. Conversely, LD_{50} does not vary significantly with hydrophobicity. For baseline compounds with log $K_{ow} > 1$, the log LD_{50} values are approximately constant with a mean value of 1.09. The relationship between LC_{50} and LD_{50} was poor, but statistical fit is improved significantly after the inclusion of log K_{ow}. This phenomenon is clearly due to the different exposure routes since log K_{ow} is the descriptor commonly used to parameterize BCF. LC_{50} (external concentration) and LD_{50} (internal concentration) endpoints can be representative of different exposure routes. To compare the similarities and differences between LC_{50} and LD_{50}, internal concentration expressed as CBR was used in the study. A parallel relationship has been observed between log CBR-log K_{ow} and log LD_{50}-log K_{ow}. The average LD_{50} is close to the average CBR for less inert and reactive compounds, but higher than the average CBR for baseline compounds. The difference is due to the lipid fraction being the major storage site for most of the baseline compounds, and they accumulate easily in lipid fraction after intraperitoneal injection. Although there is a close relationship between LD_{50} and CBR, the LD_{50} cannot reflect actual CBR. The CBRs calculated for different modes of toxic action based on LC_{50} and BCF for 965 organic compounds show that the CBRs vary in a narrow range for baseline and less inert compounds with 1<log K_{ow} <7. Reactive compounds exhibit high toxicity with low log CBR. Although no significant differences

were observed between calculated and observed CBRs for most of the compounds, some differences have been observed for calculated CBRs. The greatest errors were found for alcohols, amino alcohols, diamines and polyamines, pyridines, nitrils, disulphides, some highly hydrophilic and hydrophobic compounds. The reason may be due to BCF, equilibrium time and experimental error of LC_{50}. These factors are important and should be considered in reliable CBR calculation.

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