Optimisation of aquatic acute toxicity testing for regulatory purposes

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Introduction

The quality of *in vivo* test results is an important issue for the development of alternative testing methods, which are increasingly being used for regulatory assessment of chemicals, for e.g. quantitative structure-activity relationships (QSARs). However, our investigation of the variability of toxicity information in databases showed significant variability of LC50 fish acute toxicity test results reaching for several orders of magnitude, which represents a problem in developing alternative methods. Differences in test results may be influenced by the physical test conditions (water temperature, pH, and hardness), chemical properties of the test agents (e.g. solubility), other technical factors (different testing protocols used, measurement error etc.) and natural biological factors (e.g., the choice of test species or the life stage of the test species, species and life stage differences in sensitivity). Our recommendations were firstly, to optimize testing protocols by restricting the choice for factors that may cause data variability and secondly, to improve the recording of test results and conditions into the databases. Rainbow trout (*Oncorhynchus mykiss*) showed to be a good representative species as it is one of the most sensitive fish species and already the most frequently used in the regulatory toxicity testing.

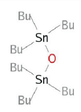
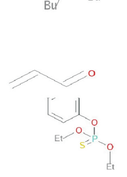
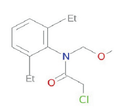
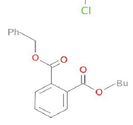
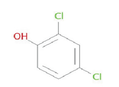
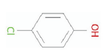
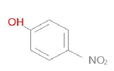
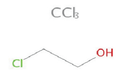
The aim of the present study was to compare the acute toxicity data for *O. mykiss* with the data for any tested fish species extracted from the US EPA ECOTOX database in order to see the differences in LC50 variability and the impact on classification and labelling. Furthermore, median LC50 values of these results were compared to predicted QSAR values.

Materials & Methods

Acute toxicity LC50 test results were extracted from the widely used and publicly available US EPA ECOTOX database (US EPA, 1995). For QSAR predictions inorganic compounds, inorganic salt, mixtures, organo-metallic compound or duplicates were eliminated. At the end, 12 substances with at least ten LC50 values available for all fish species were selected yielding a total of 348 records. For *O. mykiss* 60 records were available for those substances.

The QSAR values were obtained using DEMETRA, TOPKAT v 6.1 and four LogP based models for narcosis and polar narcosis. To perform the prediction with the LogP based models MlogP (calculated using Dragon v 5.5) was used . The other QSAR values were obtained using DEMETRA and TOPKAT v 6.1 and LogP based models. Calculated values were compared to median LC50 values (mg l^{-1}) of measured data recorded in ECOTOX database for any fish species and for *O. mykiss* where possible (for two substances for *O. mykiss* single available LC50 test result were used). The chemical names and the SMILES codes were generated using ECOSAR v 0.99 for EPI SUITE v 3 or ChemIDplus. The evaluation of the models was done by verification of the model training set, chemical domain and by errors of the predictions. Chemical domain assessment for the LogP based model was derived from the Verhaar classification scheme.

Table 1. **Median *in vivo* LC50 test results and DEMETRA predictions [mg l^{-1}] for 12 substances selected from ECOTOX database. LC50 results [mg l^{-1}] are log transformed.**

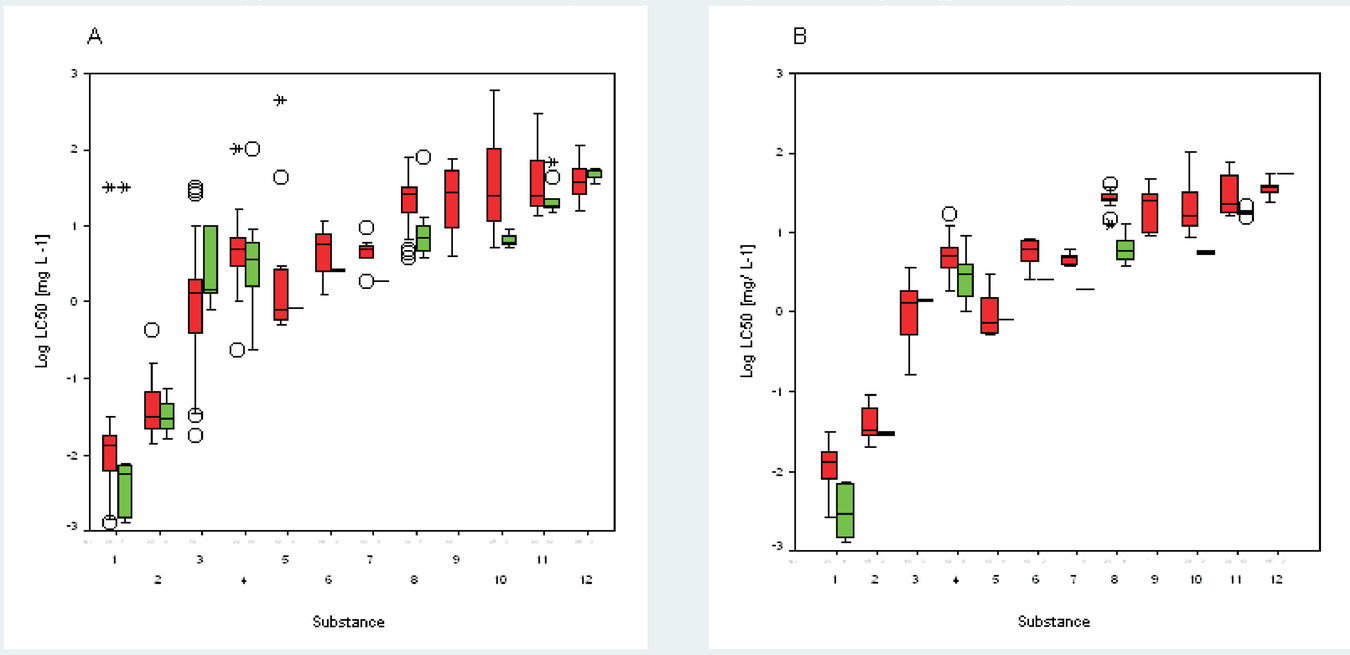
	CAS	Chemical name	Structure	Chemical class	Verhaar classification (OECD toolbox, Toxtree 1.51)	Median LC50 [mg l^{-1}]	No. of LC50 results	Median LC50 [mg l^{-1}]	No. of LC50 results	LC50 [mg l^{-1}] predicted by DEMETRA
						All fish species		*O. mykiss*		
1	56-35-9	Hexabutylditin		Neutral Organics	4	-1,89	28	-2,25	7	-1,51
2	107-02-8	Acrylaldehyde		Aldehydes	3	-1,49	22	-1,52	4	1,7
3	56-38-2	Parathion		Esters	5	0,11	79	0,16	9	0,06
4	15972-60-8	Alachlor		Haloacetamides	3,5	0,7	24	0,56	11	0,53
5	85-68-7	Benzyl butyl phtalate		Esters	3,5	-0,1	12	-0.09*	1	-0,02
6	120-83-2	Phenol, 2,4-dichloro-		Phenols	2	0,76	16	0,42	2	-0,05
7	106-48-9	Phenol, 4-chloro-		Phenols	2	0,69	10	0.28*	1	0,56
8	100-02-7	4-nitrophenol		Phenols	2	1,43	34	0,85	7	1,01
9	100-42-5	Benzene, ethenyl-		Neutral Organics	1	1,43	12	/**	/	0,92
10	71-43-2	Benzene		Neutral Organics	1	1,39	45	0,77	3	1,04
11	67-66-3	Methane, trichloro-		Neutral Organics	1	1,4	40	1,26	12	-0,68
12	107-07-3	Ethanol, 2-chloro-		Neutral Organics	3	1,58	26	1,73	3	1,74

*** single LC50 test result [mg l^{-1}] available ** no test result available**

Results & Discussion

Median values of LC50 test results for all fish species compared with median or single measured values for *O. mykiss* were within the same logarithmic class, meaning that there would be no impact on classification and labelling, when using only rainbow trout test results. In most of the cases LC50 values for *O. mykiss* were lower than median LC50 values for all fish species, showing that *O. mykiss* is amongst the most sensitive species (Figure 1).

Figure 1. **Box plots of all available LC50 fish acute toxicity test results (A) and for the results withouth LC50 values outside the mean value +/- 1 standard deviation (B) for 12 substances for all fish species (red colour) and for *O. mykiss* (green colour).**



Although the assessment was done only on 12 compounds, it is possible to see that the predictions from all models were close to median LC50 values of measured data. Preliminary results showed that DEMETRA calculation model can provide several approximations very near to experimental values compared to other methods (Table 1). Only two compounds (CAS 107-02-8, 67-66-3) were outside the chemical domain of the DEMETRA model and were mispredicted. Also for TOPKAT two compounds are outside the chemical domain of the model but software predictions are biased because 8 out of 9 compounds were already in the dataset used to develop the model. The chemicals that can be reliably predicted by the LogP based models are only three for Verhaar class 1 and three for class 2 (out of twelve). The performances of these four models are similarly good as DEMETRA or TOPKAT when compounds belongs to class 1 and 2 but DEMETRA and TOPKAT are generalist models.

The evaluation of the error of the prediction for compounds in chemical domain, where mean error and mean absolute error were considered, also showed that DEMETRA model seems to be the best prediction model (Figure 2, 3). Table 1 shows the prediction of DEMETRA model. The approximations are better for *O. mykiss* than for any fish.

Figure 2. **Comparison of Lc50 values prediction between different QSAR models. Only compounds in the applicability domain of each model were taken into consideration. The R^2 for DEMETRA and TOPKAT were also indicated.**

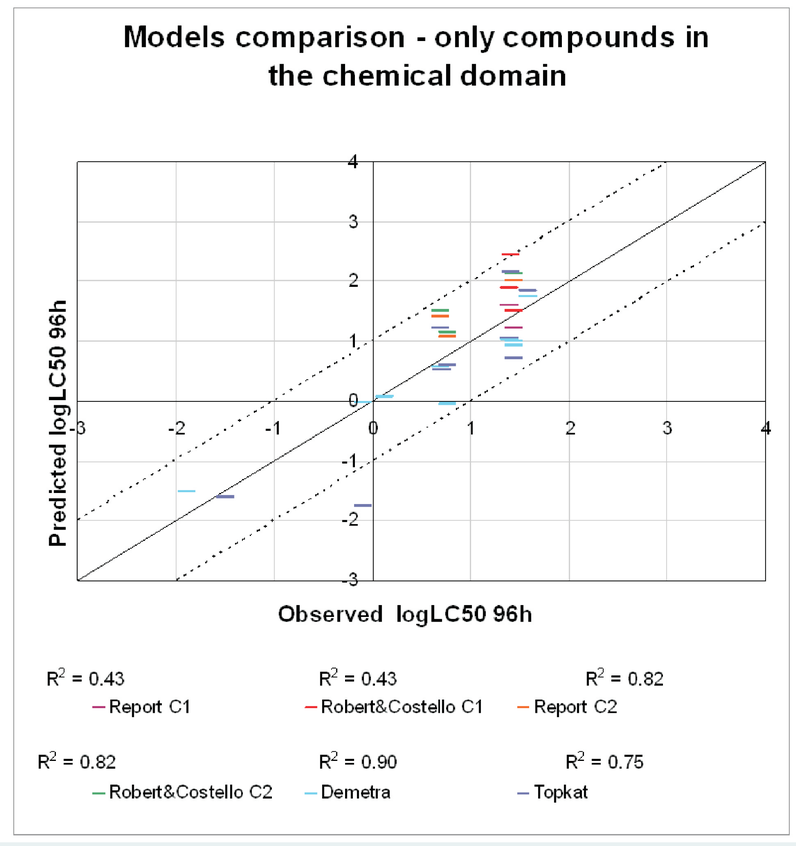
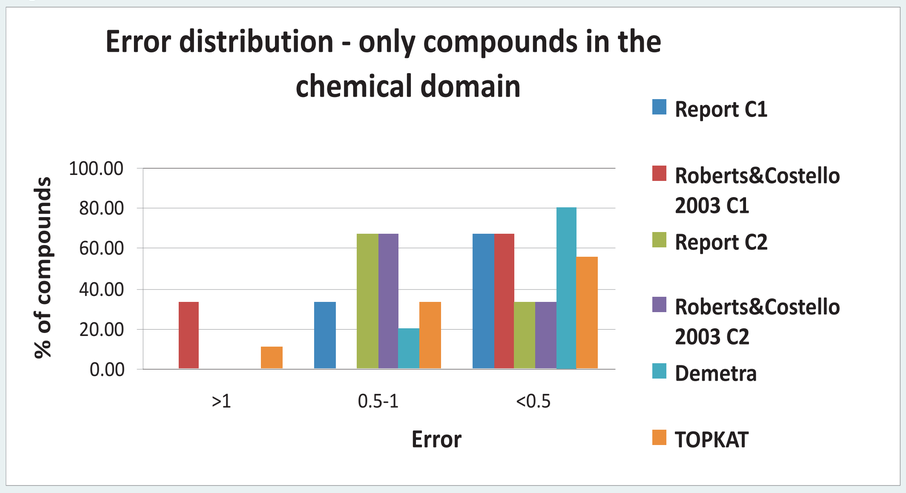


Figure 3. **Error distribution for substances in the chemical domain of QSAR models.**



Conclusions

Quantitative structure-activity relationship (QSAR) models are expected to play an important role in reducing the number of animals used for toxicity testing according to new European Union chemical regulation REACH (Registration, Evaluation, and Authorization of Chemicals). The development of alternative models requires reliable and qualitative *in vivo* toxicity data. For this purpose optimised testing protocols and rigorous quality control of data entries into the databases are required. The comparison of LC50 test results for all fish species tested and *O. mykiss* showed that tests with rainbow trout alone give representative results for classification and labelling purpose. Any additional information on other species toxicity is valuable on a case by case basis in risk assessment.

QSAR calculations can provide a good prediction of the acute toxicity depending on the model used. QSAR models showed to be very useful in predicting fish acute toxicity and therefore are recommended to be used in new testing strategies for chemical testing.

Acknowledgement

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