
SAR and QSAR in Environmental Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gsar20

# Development of a chronic fish toxicity model for predicting sub-lethal NOEC values for non-polar narcotics

T.J. Austin[a] & C.V. Eadsforth

[a] Shell Risk Science Team, Shell Health, Manchester, UK

Published online: 17 Mar 2014.

To cite this article: T.J. Austin & C.V. Eadsforth (2014) Development of a chronic fish toxicity model for predicting sub-lethal NOEC values for non-polar narcotics, SAR and QSAR in Environmental Research, 25:2, 147-160, DOI: 10.1080/1062936X.2013.871577

To link to this article: http://dx.doi.org/10.1080/1062936X.2013.871577

*SAR and QSAR in Environmental Research*, 2014
Vol. 25, No. 2, 147–160, http://dx.doi.org/10.1080/1062936X.2013.871577

Taylor & Francis
Taylor & Francis Group

# Development of a chronic fish toxicity model for predicting sub-lethal NOEC values for non-polar narcotics

T.J. Austin and C.V. Eadsforth

*Shell Risk Science Team, Shell Health, Manchester, UK*

(*Received 6 September 2013; in final form 4 October 2013*)

To comply with the REACH (Registration, Evaluation, Authorisation and restriction of Chemicals) regulations, the generation of chronic fish toxicity data is required for chemicals produced or imported within or into the EU in quantities greater than 100 tonnes per year. This comes at a great cost to industry and consumers alike and requires the sacrifice of many vertebrates. In acknowledgment of these issues the REACH regulations encourage the use of non-testing methods (NTM). These include read-across, weight-of-evidence and QSAR (quantitative structure–activity relationship) techniques. There are many QSAR tools available to generate predictive values for a number of physico-chemical properties, as well as human and environmental health end points; however, close analysis of the currently available chronic fish models identified room for improvement in both the selection of data used and in its application in model creation. In light of this a model was developed using only sub-lethal no-observed-effect concentration (NOEC) end-point data according to best practice QSAR development. Only the lowest value was taken for each compound, in line with the conservative approach taken by the European Chemicals Agency (ECHA). The model developed meets the Organisation for Economic Co-operation and Development (OECD) principles, has strong internal and external validation statistics, and can reliably predict sub-lethal chronic NOEC values for fish within its defined applicability domain.

**Keywords:** QSAR; fish; toxicity; chronic; NOEC; non-polar; narcosis



## 1. Introduction

The EU regulation, REACH, came into force on June 1st 2007. REACH (Registration, Evaluation, Authorisation and restriction of Chemicals) requires the registration of all chemicals manufactured and imported into the EU economic region above a certain tonnage. The information requirements for the registration and authorisation of a substance vary according to the tonnage manufactured/imported per year: the higher the tonnage, the more detailed the information that is required. Where chemicals are imported or manufactured at over 100 tonnes per year, chronic toxicity testing on fish is required under the REACH legislation [1]. The generation of such information comes at great cost to industry and the (eco) toxicity tests which generate information to protect human and environmental health require the sacrifice of many vertebrates.

In acknowledgment of these issues the REACH regulations encourage the use of non-testing methods (NTM). These include read-across, weight-of-evidence and QSAR (quantitative structure–activity relationship) techniques. QSARs are mathematical models that relate a quantitative parameter associated with a chemical's structure with a quantitative measurable parameter e.g. its toxicity. REACH article 25 stipulates the obligation of both the

*Corresponding author. Email: tom.austin@shell.com

registrant and regulatory body (European Chemicals Agency – ECHA) to perform or require vertebrate testing only as a last resort, and to consider all other options beforehand [1]. QSARs can be used (along with the physico-chemical properties of a substance) to help determine whether a compound has the potential to cause human or environmental health hazards and therefore whether further vertebrate testing is required. Where testing does not appear scientifically necessary, QSARs can be used as a replacement for regulatory purposes; however, this can only be done if they are in accordance with conditions laid out in Annex XI of the REACH regulations [1] which states:

> 'Results obtained from valid qualitative or quantitative structure–activity relationship models ((Q) SARs) may indicate the presence or absence of a certain dangerous property. Results of (Q)SARs may be used instead of testing when the following conditions are met:
>
> – results are derived from a (Q)SAR model whose scientific validity has been established,
>
> – the substance falls within the applicability domain of the (Q)SAR model,
>
> – results are adequate for the purpose of classification and labelling and/or risk assessment, and
>
> – adequate and reliable documentation of the applied method is provided.'

QSARs are most often used in weight-of-evidence, read-across or category approaches, usually in conjunction with experimental data that are available for related compounds. In cases of read-across, QSARs may be used to fill data gaps in between compounds with experimental data which show a clear trend. Alternatively they may be used as part of a weight-of-evidence approach with other independent data sources to help justify the waiving of experimental data. QSARs are therefore a very important tool for meeting EU regulatory goals, whilst reducing the number of sacrificial vertebrates and costs to industry.

There are many QSAR tools available to generate predictive values for a number of physico-chemical properties, human and environmental health end points. Models which may be used to predict chronic toxicity to fish for compounds which act via non-polar narcosis (i.e. neutral organics), include ECOSAR™ (http://www.epa.gov/oppt/newchems/tools/21ecosar.htm) and QSARCHE (http://www.arche-consulting.be/organics-toolbox/qsarche-model/) [2]. ECOSAR™ is included in the Organisation for Economic Co-operation and Development (OECD) QSAR toolbox (http://www.qsartoolbox.org/), its inclusion implying its adherence to OECD QSAR principles [3].

Dearden et al. [4] have identified 21 types of error which commonly occur in the creation of QSAR models which can hinder their conformity to the OECD principles. Many of these errors are only apparent upon detailed inspection of the QSAR models which on the face of it would appear to align with the principles laid out by the OECD [3]. ECOSAR™ and QSARCHE both contain common errors identified by Dearden et al. [4]. An error common to both models is that there is a replication of compounds within the training data set. Including multiple data points for one compound will alter the resultant model algorithm and therefore the predictions made using it. Additionally, statistics which represent the robustness and predictivity of the model, e.g. the leave-many-out internally cross-validated correlation coefficient ($Q^2_{LMO}$), will be altered and may be misleading as to the model's quality.

Looked at individually, these two models could also be improved in other areas. The ECOSAR™ chronic fish toxicity model is based on a ChV or 'chronic' value. Within the guidance

document this ChV value 'is defined as the geometric mean of the no-observed-effect concentration (NOEC) and the lowest-observed-effect concentration (LOEC)' [5]. Upon inspection of the data, however, it can be seen that the end points consist of a mixture of NOEC, LOEC and MATC (maximum acceptable toxicant concentration) values. Additionally, these values are taken forward in work to refine this QSAR by de Haas et al. [6]. In contrast, the QSARCHE model uses only NOEC values; however, these cover end points for growth/reproduction and mortality. The mortality end-point values will always be significantly higher than the more sensitive growth/reproduction values and will likely provide a less conservative model. Including these different end points also increases what will already be a large variation between experimental values (because of the different test methods and test species used). The QSARCHE training-set data also includes other inadequate data, including data generated using subchronic tests as well as data for an acid and product mixture which should not be included in the model. Many of these inclusions happen because of misleading information given in data sources; this is discussed later. In light of these findings, as well as the confidential nature of some data included in ECOSAR$^{TM}$, here it was deemed necessary to carry out a separate and thorough data-mining exercise including more stringent data-inclusion criteria.

Neutral organic compounds exert toxicity via non-polar narcosis or 'baseline toxicity' and tend to be relatively hydrophobic [7,8]. The neutral organic class toxicity has a strong relationship with the the octanol-water partition coefficient ($K_{ow}$) parameter and includes chemicals such as aliphatic and aromatic hydrocarbons, alcohols, alkenes, alkyl and aryl halides, cyanates, ethers, ketones, sulfides and disulfides [5].

The aim of this work was to create a QSAR for the chronic toxicity of neutral organic compounds (that act via non-polar narcosis), based, as far as possible, on best QSAR practice.

## 2. Methods

### 2.1 *Data gathering and processing*

Classifying chemicals for chronic toxicity under the CLP regulation (the EU's implementation of the United Nation's Globally Harmonised System for classification and labelling) and amendments hitherto [9,10], is carried out by assessing chronic NOEC or $EC_{10}$ values. When considering environmental risk, the REACH regulations take a conservative approach [1]. Where there are multiple valid toxicity values for an end point, the most conservative (worst case) is always used; additionally, if there is a lack of data across trophic levels, assessment factors may be applied to compensate for the uncertainty. In this vein, and considering the inherent uncertainty in QSAR predictions, it was decided that a conservative approach would be taken and only NOEC data would be gathered and included into the QSAR training set as opposed to LOEC or MATC values. Best practice in QSAR development dictates that only one value should be included for each compound within the training set [4]. To ensure the most conservative model, only the most sensitive end points were included for each substance (growth/reproduction) and mortality NOECs were filtered out at an early stage.

Chronic fish NOEC data were obtained from the following sources:

- The EPA's ECOTOX database (http://cfpub.epa.gov/ecotox/advanced_query.htm).
- The ECETOC database downloaded through the OECD QSAR Toolbox (http://www.oecd.org/env/ehs/risk-assessment/theoecdqsartoolbox.htm).
- The ECHA dossier dissemination portal (http://echa.europa.eu/information-on-chemicals/registered-substances).

The ECOTOX database was searched for chronic data using its inbuilt advanced query function. The ECETOC database was downloaded through the OECD QSAR Toolbox and the data were filtered in Excel™. The ECHA dossier dissemination portal does not have an advanced search feature, therefore this database was searched using the eChemPortal (http://www.echemportal.org/echemportal/propertysearch/treeselect_input.action?queryID=PROQ2cpy). A high amount of replication was observed between the ECOTOX and ECETOC databases.

The ECHA dossier dissemination portal was searched for dossiers containing experimental studies scoring 1 or 2 on the Klimisch scale. The Klimisch scoring system is the method of choice for assessing the suitability of test data for REACH compliance purposes [11]. Studies given Klimisch scores of 1 and 2 whilst not necessarily being carried out under official GLP (Good Laboratory Practice), are deemed reliable enough to be used for chemical regulatory classifications and have therefore been seen as an acceptable data standard for QSAR development.

Results were only carried forward from the original search if they were acceptable in a regulatory context i.e. if OECD 210, 215, EPA OPP 72-4 or 72-5 methods (or equivalent) were followed. Equivalent methods were only accepted if the test protocol was scientifically sound and had an exposure duration of at least 21 days. Test data on all freshwater fish species were included. Where possible, a thorough analysis of the publication cited was carried out to assess the suitability of the data for inclusion in the QSAR. In a small number of cases this was not possible where data were obtained from an ECHA dossier and limited details of the study were divulged; expert judgment on their suitability was used in these cases. An emphasis was placed on checking the source material cited by each database. As seen in other QSAR work, normally carried out under strict time constraints in the case of industry, it is very easy to presume database values are reliable; however, the examination of other QSAR work such as QSARCHE [2] shows that extreme care must be taken when using experimental data found in databases such as ECETOC and ECOTOX.

Inspection of the QSARCHE Verhaar class 1 training set led to the identification of a number of inadequate NOEC values which should be removed from the model. A NOEC value of 0.110 mmol/L (98 mg/L) for propylene glycol (CAS 57-56-6) was taken from the ECETOC database and included in the QSARCHE training set. Inspection of the literature source [12] revealed that this value was in fact for a mixture containing propylene glycol and the NOEC value for pure propylene glycol was much higher (less toxic). The higher NOEC value was given as a less than (<) value (an effect was seen at the lowest concentration measured) and was not deemed appropriate for inclusion in the current study; however, the $EC_{25}$ of the formulated mixture was a factor of 60 lower than that of pure propylene glycol and therefore it is clear the additives within drove the toxicity during this test. Additionally, the test used in this study was based on Weber et al. [13], a short-term test method used to estimate chronic toxicity. Other issues with the QSARCHE training set exist e.g. the inclusion of Aroclor (CAS 12672-29-6), a mixture, which should not be included in the training set. Additionally, other sub-chronic test data values have been included e.g. for octan-1-ol (CAS 111-87-5). In this study every effort was made to ensure only true chronic studies were included for single substances of high purity.

Experimental log $K_{ow}$ values for each compound were taken from either their ECHA dossier or the ECOSAR™ (v1.11) PhysProp database. Additionally, the molecular weight of each compound was obtained by using the batch-processing option available in ECOSAR™ (v1.11). The log $K_{ow}$ parameter was used as the only descriptor for the model, with the

response parameter being the log of the experimental NOEC value in mmol/L (NOEC (mg/L)/ molecular weight).

### 2.2 *Toxicity mode of action and chemical classification*

Based on previous work to create an acute QSAR for chemicals that act via non-polar narcosis [14], the Verhaar scheme (modified) within Toxtree v2.5.0 (http://toxtree.sourceforge.net/), was used to place each compound into a class (1–5) based on mode of action. The Verhaar scheme [15] is a decision tree which categorises a compound using various structural alerts. It was updated and modified by Enoch et al. [16]. It classifies chemicals into one of five categories as follows:

- Class 1 (narcosis or baseline toxicity)
- Class 2 (less inert compounds or polar narcotics)
- Class 3 (chemicals that demonstrate unspecific reactivity)
- Class 4 (compounds and groups of compounds acting by a specific mechanism)
- Class 5 (chemicals that cannot be classified as belonging to classes 1, 2 or 3 and that are not known to act by a specific mechanism)

Enoch et al. [16] emphasise that the Verhaar scheme is a work in progress and is therefore not 100% accurate. It is, however, a good indicator of a chemical's mode of action. Chronic data were only carried forward for compounds that were classified as 1 using the Verhaar (modified) scheme. Compounds were additionally processed using ECOSAR™ (v1.11). ECOSAR™ has an inbuilt function which allows it to class chemicals into groups such as neutral organics. The majority of compounds classed into the neutral organics group act via non-polar narcosis, however, this computerised system is also not 100% accurate [17,18]. In addition to being classed as one by the Verhaar scheme, chemicals also had to be classed into neutral organics by ECOSAR™.

### 2.3 *Model creation and statistical model validation*

Model processing and statistical analysis were performed using Minitab 16 Statistical Software (http://www.minitab.com/en-us/products/minitab/). Potential outliers within the preliminary dataset were identified using regression analysis to identify data points with a high normalized residual value (>2). These data points were subject to further investigation to check whether they were indeed outliers. To allow external validation of the model the data were divided into a training set and a test set. Dearden et al. [4] discuss the pitfalls that can occur in this process and suggest a rational approach be adopted; additionally, a 2:1 training to test set ratio is recommended. Here, the full data set was ordered by log $K_{ow}$ from low to high. Then, going from low to high, every third compound was taken and placed separately for the test set (excluded from model creation). This method allowed the created model to be tested across the full range of log $K_{ow}$ values and ensured the resulting external validation parameters were not unfairly dependent on the test-set selection. The lowest and highest log $K_{ow}$ compounds were left in the training set each time in order to keep the largest applicability domain possible, therefore the last compound selected for the test set was the second to last rather than the last.

The final model was generated in Minitab using a fitted line plot ($x$ = log $K_{ow}$, $y$ = log NOEC (mmol/L)). The statistics used to characterize and validate the model were based on those recommended by OECD guidance [19] and Dearden et al. [4]. The standard and

adjusted coefficients of determination ($r^2$ and $r^2_{adj}$), leave-one-out internally cross-validated correlation coefficient ($Q^2_{LOO}$), the Fisher statistic (and associated $p$ values), and the standard error of the estimate ($S_{est}$) were all calculated during regression analysis. Additionally, a variety of residual distribution plots were created to examine the goodness of model fit and if any systemic error was apparent.

To provide external validation of the model, NOEC predictions were made using the model algorithm for the test-set compounds. These values were then correlated against the experimental NOEC values for these compounds to generate an $r^2_{ext}$ value. The $r^2_{ext}$ value obtained indicates the external predictive powers of the model.

## 3. Results and discussion

### 3.1 *Model creation*

Tables 1 and 2 display the data obtained during the data search divided into a training set ($n$ = 19) and test set ($n$ = 10). The model generated using the training set is of the equation:

$$\log \text{NOEC (mmol/L)} = 0.711 - 0.914 \log K_{ow}$$

The goodness of fit, as measured by the coefficient of determination ($r^2$) and adjusted for degrees of freedom ($r^2_{adj}$), robustness, as measured by internal cross validation ($Q^2_{LOO}$), and the standard error of the estimate ($S_{est}$) were used as measures of internal performance as per the OECD principles [19]. Like the $r^2$ value, the $r^2_{adj}$ value shows how well the model fits the training-set data 'adjusted' for the number of independent variables. The $r^2_{adj}$ value can therefore be used as a comparative value between models with different training-set sizes. As shown in Table 3, the chronic toxicity model has a high $r^2_{adj}$ value of 0.9 with an $S_{est}$ of 0.38. This demonstrates that the model represents the training set very well and there is a strong relationship between chronic toxicity and log $K_{ow}$ (Figure 1). Additionally the Fisher and associated $p$ values were 165 and < 0.001 respectively; it can therefore be concluded that this correlation did not occur by chance. Residual plots are included for the model regression (Figure 2); these demonstrate that there is no systematic error in the model which would be an indicator of poor descriptor choice.

The $Q^2_{LOO}$ value indicates a model's robustness and how much it is affected by small changes in the training set.

The $Q^2_{LOO}$ value is calculated by systematically creating a series of models by removing each observation from the data set once. Each model is then used to predict the value that has been removed giving an indication of how well the overall model may predict new observations. The closer these values are to one, the better; values of above 0.5 are generally considered acceptable [20,21]. The model created in this study achieved a $Q^2_{LOO}$ of 0.88; this shows that the model is robust and indicates good external predictivity, although external validation is now generally accepted as being the best way to truly validate a model [4].

To test the predictive powers of the model algorithm, predictions were made for compounds included in the test set (Table 2). Entering each compound's log $K_{ow}$ into the equation, a series of predicted values were obtained. These values were plotted against the experimental values found for these compounds (Figure 3). Residual plots are included for the model validation in Figure 4. An $r^2_{ext}$ value of 0.885 was obtained, indicating that the model created has a very strong predictive capability. Additionally, the standard error of prediction ($S_{pre}$) was low at 0.41 log units; this value is not much higher than the typical

Table 1. Training-set data.

| CAS number | Chemical name | Molecular weight | Source (database) | Reference (if given) | Species tested | Observed duration (days) | log $K_{ow}$ (experimental) | NOEC (mg/L) | NOEC (mmol/L) | log NOEC |
|---|---|---|---|---|---|---|---|---|---|---|
| 109-99-9 | Tetrahydrofuran | 72.11 | ECOTOX | [23] | *Pimephales promelas* | 33 | 0.46 | 216.00 | 3.00 | 0.48 |
| 122-99-6 | 2-Phenoxyethanol | 138.17 | ECHA | | *Pimephales promelas* | 34 | 1.16 | 51.30 | 0.37 | –0.43 |
| 75-09-2 | Dichloromethane | 84.93 | ECHA | [24] | *Pimephales promelas* | 28 | 1.25 | 83.00 | 0.98 | –0.01 |
| 107-06-2 | 1,2-Dichloroethane | 98.96 | ECOTOX | [25] | *Pimephales promelas* | 32 | 1.48 | 29.00 | 0.29 | –0.53 |
| 79-00-5 | 1,1,2-Trichloroethane | 133.41 | ECOTOX | [25] | *Pimephales promelas* | 32 | 1.89 | 6.00 | 0.04 | –1.35 |
| 78-87-5 | 1,2-Dichloropropane | 112.99 | ECHA | | *Pimephales promelas* | 32 | 1.98 | 6.00 | 0.05 | –1.27 |
| 150-78-7 | 1,4-Dimethoxybenzene | 138.17 | ECOTOX | [23] | *Pimephales promelas* | 31 | 2.04 | 16.60 | 0.12 | –0.92 |
| 79-34-5 | 1,1,2,2-Tetrachloroethane | 167.85 | ECOTOX | [25] | *Pimephales promelas* | 32 | 2.39 | 1.40 | 0.01 | –2.08 |
| 108-88-3 | Toluene | 92.14 | ECHA | | *Oncorhynchus kisutch* | 40 | 2.73 | 1.39 | 0.02 | –1.82 |
| 822-86-6 | *trans*-1,2-Dichlorocyclohexane | 153.05 | ECOTOX | [23] | *Pimephales promelas* | 31 | 3.21 | 0.61 | 0.00 | –2.40 |
| 76-01-7 | Pentachloroethane | 202.3 | ECOTOX | [25] | *Pimephales promelas* | 32 | 3.22 | 0.90 | 0.00 | –2.35 |
| 103-50-4 | 1,1'-[oxybis(methylene)] dibenzene | 198.27 | ECHA | | *Oryzias latipes* | 21 | 3.31 | 0.48 | 0.00 | –2.62 |
| 106-43-4 | 1-Chloro-4-methylbenzene | 126.59 | ECOTOX | [26] | *Danio rerio* | 28 | 3.33 | 3.40 | 0.03 | –1.57 |

(*Continued*)

Table 1. (*Continued*).

| CAS number | Chemical name | Molecular weight | Source (database) | Reference (if given) | Species tested | Observed duration (days) | log $K_{ow}$ (experimental) | NOEC (mg/L) | NOEC (mmol/L) | log NOEC |
|---|---|---|---|---|---|---|---|---|---|---|
| 541-73-1 | 1,3-Dichlorobenzene | 147 | ECOTOX | [25] | *Pimephales promelas* | 32 | 3.53 | 1.00 | 0.01 | –2.17 |
| 95-75-0 | 3,4-Dichlorotoluene | 161.03 | ECOTOX | [23] | *Pimephales promelas* | 31 | 3.95 | 0.08 | 0.00 | –3.31 |
| 120-82-1 | 1,2,4-Trichlorobenzene | 181.45 | ECHA | | Oryzias latipes | 21 | 4.02 | 0.26 | 0.00 | –2.84 |
| 87-61-6 | 1,2,3-Trichlorobenzene | 181.45 | ECOTOX | [26] | Danio rerio | 28 | 4.05 | 0.25 | 0.00 | –2.86 |
| 85-01-8 | Phenanthrene | 178.24 | ECOTOX | [27] | Oncorhynchus mykiss | 60 | 4.46 | 0.02 | 0.00 | –3.97 |
| 1222-05-5 | 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-γ-2-benzopyran | 258.41 | ECHA | [28] | Pimephales promelas | 36 | 5.3 | 0.068 | 0.00 | –3.58 |

Table 2. Test-set data.

| CAS number | Chemical name | Molecular weight | Source (database) | Reference (if given) | Species tested | Observed duration (days) | log $K_{ow}$ (experimental) | NOEC (mg/L) | NOEC (mmol/L) | log NOEC (experimental) | log NOEC (predicted) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1634-04-4 | Propane, 2-methoxy-2-methyl- | 88.15 | ECHA | | *Pimephales promelas* | 31 | 0.94 | 299 | 3.39 | 0.53 | –0.15 |
| 108-10-1 | 4-Methyl-2-pentanone | 100.16 | ECOTOX | [23] | *Pimephales promelas* | 31 | 1.31 | 57 | 0.57 | –0.24 | –0.49 |
| 106-93-4 | 1,2-Dibromoethane | 187.86 | ECETOC | [29] | *Oryzias latipes* | 28 | 1.96 | 5.81 | 0.03 | –1.51 | –1.08 |
| 71-43-2 | Benzene | 78.11 | ECHA | | *Pimephales promelas* | 32 | 2.13 | 0.80 | 0.01 | –1.99 | –1.24 |
| 108-90-7 | Chlorobenzene | 112.56 | ECHA | [26] | *Danio rerio* | 28 | 2.84 | 4.80 | 0.04 | –1.37 | –1.88 |
| 91-20-3 | Naphthalene | 128.18 | ECHA | [30] | *Oncorhynchus kisutch* | 40 | 3.30 | 0.37 | 0.00 | –2.54 | –2.30 |
| 106-46-7 | 1,4-Dichlorobenzene | 147 | ECOTOX | [25] | *Pimephales promelas* | 32 | 3.44 | 0.57 | 0.00 | –2.42 | –2.43 |
| 92-52-4 | Biphenyl | 154.21 | ECHA | | *Oncorhynchus mykiss* | 87 | 4.01 | 0.23 | 0.00 | –2.83 | –2.95 |
| 67-72-1 | Hexachloroethane | 236.74 | ECOTOX | [25] | *Pimephales promelas* | 32 | 4.14 | 0.07 | 0.00 | –3.54 | –3.07 |
| 634-66-2 | 1,2,3,4-Tetrachlorobenzene | 215.89 | ECOTOX | [26] | *Danio rerio* | 28 | 4.60 | 0.10 | 0.00 | –3.33 | –3.49 |

Table 3. Statistical parameters of the currently available chronic models.

| Model | $r^2$ | $r^2_{adj}$ | $Q^2_{LOO}$ | $S_{est}$ (log units) | Fisher statistic (internal) | $p$ | $r^2_{ext}$ | $S_{pre}$ (log units) |
|---|---|---|---|---|---|---|---|---|
| New model | 0.91 | 0.90 | 0.88 | 0.38 | 165 | < 0.001 | 0.89 | 0.41 |
| QSARCHE [2] | 0.76 | 0.76 | 0.75 | N/A | 495 | Significant | 0.73 | N/A |
| de Haas [6] | 0.77 | N/A | 0.76 | N/A | N/A | N/A | 0.84 | N/A |

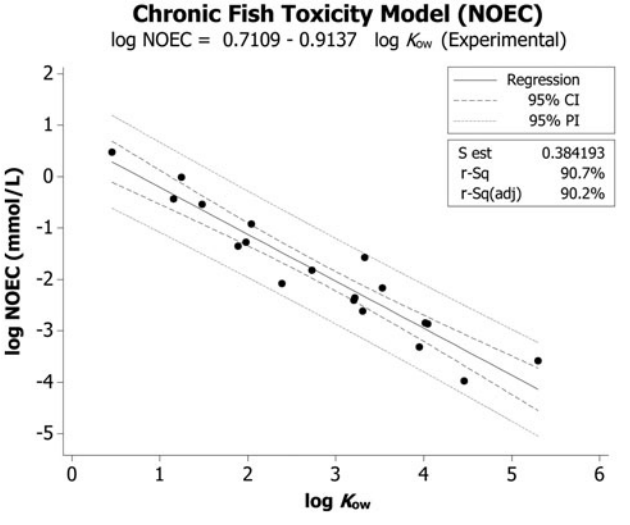


Figure 1. Chronic fish toxicity model (generated from Table 1 data).

experimental error associated with determining the log $K_{ow}$ (0.35 log units) [22]. Considering that there will be a large error associated with each experimental chronic value (varying across a selection of test methods), it would be unrealistic to achieve lower error values based on the data used. This low error value is likely a result of the stringent data-selection criteria and thorough inspection of data sources (where this was possible).

The statistical parameters of this model compare favourably with other works in this area (Table 3). It is of note that the external validation value ($r^2_{ext}$) taken from de Haas et al. [6] is very likely misleadingly high, as five compounds within the training set of their refined ECOSAR$^{TM}$ model are repeated in the test set ($n$ = 23) which reduces the strenuousness of the external test and reduces its significance.

### 3.2 *Model use*

The model created in this study has been shown to predict accurately the chronic toxicity for compounds which fall into its applicability domain. To fall within the applicability domain of this model, compounds must:

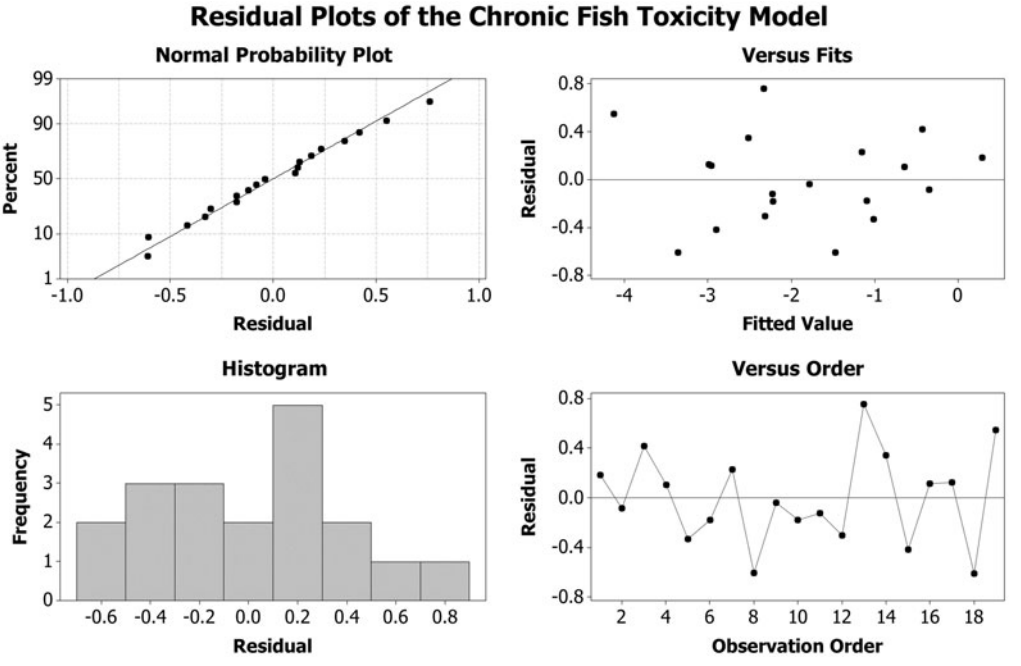


Figure 2. Residual plots and distributions of the chronic fish toxicity model.

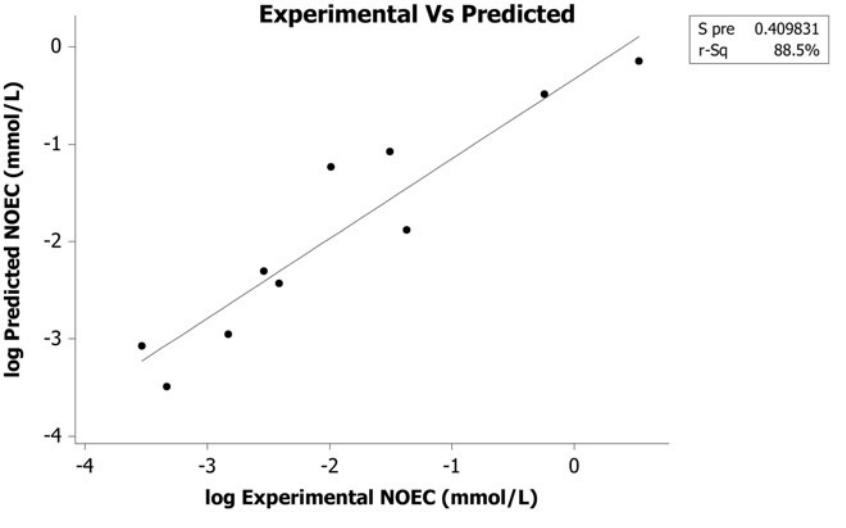


Figure 3. External model validation; experimental *vs.* predicted.

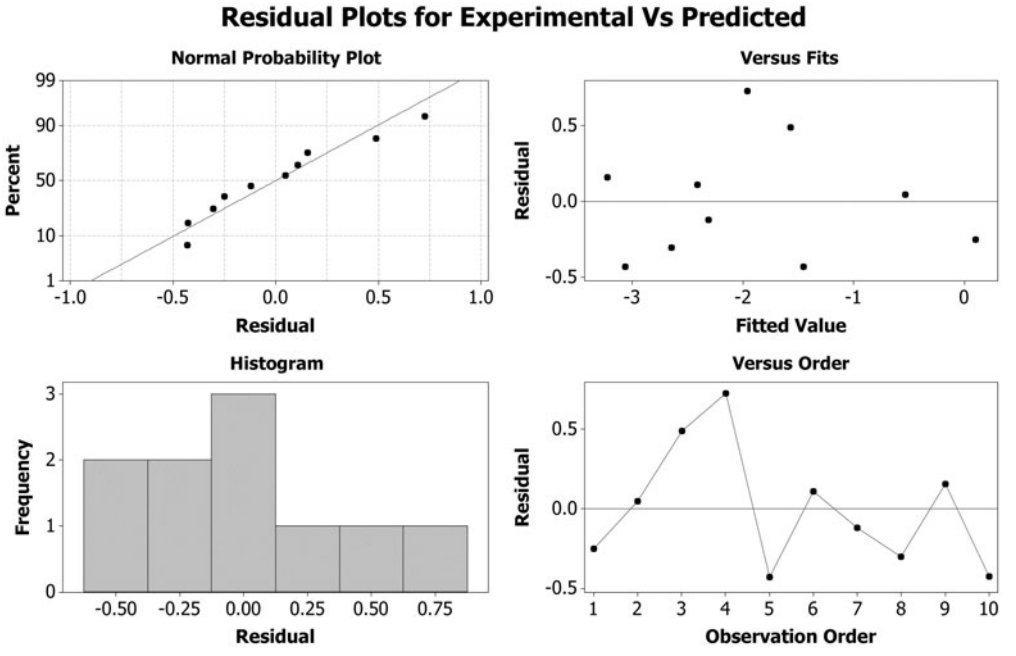


Figure 4. External model validation; experimental *vs.* predicted residual plots.

- exert toxicity via non-polar narcosis.
- have a log $K_{ow}$ value between 0.46 and 5.30.

The model output will be in log mmol/L; to get a chronic toxicity value in mg/L, the user must use anti-log and then multiply this value by the molecular weight of their compound.

As shown in Figure 1, both the confidence interval (CI) and prediction interval (PI) get larger towards the outer limits of the model, in part due to the reduction in data density (as compared to the middle of the model). Based on the relatively simple linear relationship between chronic toxicity and log $K_{ow}$ it is feasible that accurate predictions may be made outside the log $K_{ow}$ range of this model; however, extra care in the use of this value should be taken and, for higher log $K_{ow}$ values, the toxicity value should be checked against the water solubility of the compound to determine whether effects would occur.

## 4. Conclusions

The purpose of this study was to create a conservative chronic-toxicity QSAR with the ability to predict sub-lethal NOEC values of non-polar narcotic compounds with respect to fish. It can be concluded that the model created here, following best practice in QSAR development, is capable of providing reliable predictions within its defined applicability domain. The model can therefore be used to identify chemicals which may be an aquatic environmental hazard with regards to regulatory compliance in lieu of chronic-toxicity screening methods involving vertebrates. The model was created using the most conservative sub-lethal NOEC values and

is therefore more likely to provide conservative and environmentally-protective predictions. In the context of regulatory science this is very important as a conservative/protective approach is consistently applied, even more so where less information is available for a specific compound. To reduce the number of animal tests required to satisfy regulatory bodies, and therefore the cost to industry and consumers, it is increasingly important to increase the reliability and accuracy of QSARs where data allow.

The chronic QSAR created meets the criteria set out by the OECD [3]: the developed QSAR has a defined end point, an unambiguous algorithm, a defined domain of applicability, appropriate measures of goodness of fit, robustness and predictivity and a mechanistic interpretation, as well as adhering to good QSAR development practices discussed by Dearden et al. [4]. In addition, the QSAR compares favourably with other works currently used to generate chronic values.

This work highlights the need for a thorough data inspection, especially when obtaining data from large databases such as ECOTOX.



## References


[1] European Commission, *Regulation (EC) No 1907/2006 of the European Parliament and the Council of 18 December 2006 concerning the registration, evaluation, authorisation and restriction of chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/105/EC and 2000/21/EC*, OJEU. L396, (2006).

[2] L. Claeys, F. Iaccino, C.R. Janssen, P. Van Sprang and F. Verdonck, *Development and validation of a QSAR for chronic narcosis to fish*, Environ. Toxicol. Chem. 32, (2013), pp. 2217–2225.

[3] OECD, *The report from an expert group on (quantitative) structure–activity relationship (Q)SARs) on the principles for the validation of (Q)SARs*, OECD Series on Testing and Assessment No. 49. ENV/JM/MONO 24 (2004).

[4] J.C. Dearden, M.T.D. Cronin, and K.L.E. Kaiser, *How not to develop a quantitative structure–activity or structure–property relationship (QSAR/QSPR)*, SAR QSAR Environ. Res. 20 (2009), pp. 241–266.

[5] K. Mayo-Bean, K. Moran, B. Meylan, and P. Ranslow, *Methodology document for the ECOSAR$^{TM}$ class program*, (2012). Available at http://www.epa.gov/oppt/newchems/tools/ecosartechfinal.pdf.

[6] E.M. De Haas, T. Eikelboom, and T. Bouwman, *Internal and external validation of the long-term QSARs for neutral organics to fish from ECOSAR$^{TM}$*, SAR QSAR Environ. Res. 22, (2011), pp. 545–559.

[7] N. Franks and W. Lieb, *Mechanisms of general anesthesia*, Environ. Health Perspect. 87 (1990), pp. 199–205.

[8] G.D. Veith and S. Broderius, *Rules for distinguishing toxicants that cause types I and type II narcosis syndromes*, Environ. Health Perspect. 87 (1990), pp. 207–211.

[9] European Commission, *Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006*, OJEU. L353 (2008).

[10] European Commission, *Commission Regulation (EU) No 286/2011 of 10 March 2011 amending, for the purposes of its adaptation to technical and scientific progress, Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures*, OJEU. L83/1 (2011), pp. 1–53.

[11] J. Klimisch, M. Andreae, and U. Tillmann, *A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data*, Regul. Toxicol. Pharm. 25 (1997), pp. 1–5.

[12] D.A. Pillard, *Comparative toxicity of formulated glycol deicers and pure ethylene and propylene glycol to* Ceriodaphnia dubia *and* Pimephales promelas, Environ. Toxicol. Chem. 14 (1995), pp. 311–315.
[13] C.I. Weber, W.H. Peltier, T.J. Norberg-King, W.B. Horning, and F.A. Kessler, eds., *Short Term Methods for Estimating Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*, 2nd ed., EPA 600/4 89/001, US Environmental Protection Agency, Cincinnati, OH, 1989.
[14] T.J. Austin, M. Denoyelle, A.N. Chaudry, S. Stradling, and C.V. Eadsforth, *Development of a fish toxicity model for predicting acute LC50 values: ECHA dossier submissions as an experimental data source*, submitted for publication (2013).
[15] H.J.M. Verhaar, C. Van Leeuven and J.L.M. Hermens, *Classifying environmental pollutants. 1: Structure–activity relationships for prediction of aquatic toxicity*, Chemosphere 25 (1992), pp. 471–491.
[16] S.J. Enoch, M. Hewitt, M.T.D. Cronin, S. Azam, and J.C. Madden, *Classification of chemicals according to mechanism of aquatic toxicity: An evaluation of the implementation of the Verhaar scheme in Toxtree*, Chemosphere 73 (2008), pp. 243–248.
[17] J.C. Madden, S.J. Enoch, M. Hewitt, and M.T.D. Cronin, *Pharmaceuticals in the environment: Good practice in predicting acute ecotoxicological effects*, Toxicol. Lett. 185 (2009), pp. 85–101.
[18] P. Reuschenbach, M. Silvani, M. Dammann, D. Warnecke, and T. Knacker, *ECOSARTM model performance with a large test set of industrial chemicals*, Chemosphere 71 (2008), pp. 1986–1995.
[19] OECD, *Guidance document on the validation of (quantitative) structure-activity relationships ((Q) SAR) models*, OECD Environment Health and Safety Publications Series on Testing and Assessment No. 69. ENV/JM/MONO 2 (2007).
[20] P. Gramatica, *Principles of QSAR models validation: Internal and external*, QSAR Comb. Sci. 26 (2007), pp. 694–701.
[21] P.P. Roy, S. Paul, I. Mitra, and K. Roy, *On two novel parameters for validation of predictive QSAR models*, Molecules 14 (2009), pp. 1660–1701.
[22] J.C. Dearden, *Prediction of physicochemical properties, in Computational Toxicology: Volume I*, B. Reisfeld and A.N. Mayeno, eds., Methods in Mol. Biol. 929 (2012), pp. 93–138.
[23] D.J. Call, L.T. Brooke, M.L. Knuth, and S.H. Poirier, *Fish subchronic toxicity prediction model for industrial organic chemicals that produce narcosis*, Environ. Toxicol. Chem. 4 (1985), pp. 335–341.
[24] D.C. Dill, P.G. Murphy, and M.A. Mayes, *Toxicity of methylene chloride to life stages of the fathead minnow*, Bull. Environ. Contam. Toxicol. 39 (1987), pp. 869–876.
[25] K. Ahmad, D. Benoit, L. Brooke, D. Call, A. Carlson, D. Defoe, J. Huot, A. Moriarity, J. Richter, P. Shubet, C. Veith, and C. Walbridge, *Aquatic toxicity tests to characterise the hazard of volatile organic chemicals in water: A toxicity data summary – Parts I and II*, EPA-600/3-84-009 (1984).
[26] C.J. Van Leeuwen, D.M.M. Adema, and J. Hermens, *Quantitative structure–activity relationships for fish early life stage toxicity*, Aquat. Toxicol. 16 (1990), pp. 321–334.
[27] D.R. Passino-Reader, W.H. Berlin, and J.P. Hickey, *Chronic bioassays of rainbow trout fry with compounds representative of contaminants in great lakes fish*, J. Great Lakes Res. 21 (1995), pp. 373–383.
[28] F. Balk and R.A. Ford, *Environmental risk assessment for the polycyclic musks, AHTN and HHCB: II. Effect assessment and risk characterisation*, Toxicol. Lett. 111 (1999), pp. 81–94.
[29] G.W. Holcombe, D.A. Benoit, D.E. Hammermeister, E.N. Leonard, and R.D. Johnson, *Acute and long-term effects of nine chemicals on the Japanese Medaka* (Oryzias latipes), Arch. Environ. Contam. Toxicol. 28 (1995), pp. 287–297.
[30] A. Moles, S. Bates, S.D. Rice, and S. Korn, *Reduced growth of coho salmon fry exposed to two petroleum components, toluene and naphthalene, in fresh water*, Trans. Am. Fish. Soc. 110 (1981), pp. 430–436.