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Data Quality in the Human and Environmental Health Sciences: Using Statistical Confidence Scoring to Improve QSAR/QSPR Modelling

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ABSTRACT:

A greater number of toxicity data are becoming publicly available allowing for *in silico* modelling. However, questions often arise as how to incorporate data quality and how to deal with contradicting data if more than a single datum point is available for the same compound. In this study, two well-known and studied QSAR/QSPR models for skin permeability and aquatic toxicology have been investigated in the context of statistical data quality. In particular, the potential benefits of the incorporation of the statistical Confidence Scoring (CS) approach within modelling and validation. As a result, robust QSAR/QSPR models for the skin permeability coefficient and the toxicity of non-polar narcotics to *Aliivibrio fischeri* assay were created. CS-weighted linear regression for training and CS-weighted root mean square error (RMSE) for validation were statistically superior compared to standard linear regression and standard RMSE. Strategies are proposed as to how to interpret data with high and low CS, as well as how to deal with large datasets containing multiple entries.

KEYWORDS: Data Quality, Confidence Scoring, Weighted Modelling, QSAR/QSPR

1. INTRODUCTION

The assessment of biological, and more specifically toxicological, data quality is crucial for many disciplines. Although the quality of data has no absolute definition, it is strongly associated with attributes such as validity, adequacy (*i.e.* fitness for purpose), reproducibility and reliability.[1] Confidence in the toxicological data, which may be derived in part at least from an assessment of data quality, is of great importance for regulatory bodies which have to make decisions on acceptable limits of chemicals relating to human and environmental exposure. Low, or poor, data quality may also affect the quality of computational models, such as Quantitative Structure-Activity Relationships (QSARs), grouping and read-across, which are relevant both for risk assessment and regulatory decisions.[2-4]

In principle there are two general approaches to assess the quality of biological and toxicological data. The first is based on the assessment of the reported testing information alone. That means data quality is assessed by considering external factors, *e.g.* data and experimental reliability, completeness of documentation and adoption of protocols such as Good Laboratory Practice (GLP). Schemes such as that developed by Klimisch[1] and its formalisation into the ToxRTool (Toxicological data Reliability Assessment Tool) are well known, established and relatively accepted within the scientific community.[1,3] A second approach, where there are multiple and comparable data for the same compound in the same test, is to apply a statistical method. In this case, confidence scores (CS) can be calculated to emphasise data with a high weight of evidence, *i.e.* concordance between two or more independently conducted tests. The CS is the ratio of number of test values (n) and relative standard deviation (RSD) of test results, as defined in Equation 1. Thus, if the same compound was tested independently with the same

assay and the results were comparable, there will be a high CS for this compound and the associated experimental values.[5]

$$CS = \frac{n}{RSD} \quad \text{(Eq. 1)}$$

Examples of calculations of CS are provided in Table 1 representing illustrative scenarios of increasing CS. Compound A is the default (and most common occurrence for a compound with a single experimental value), the CS is 1. Compound B has two relatively divergent data values, differing by an order of magnitude. Clearly there will be greater confidence for the toxicity value than for compound B, but the significant difference in the values introduces some uncertainty, raising CS marginally to 1.73 – in this way there is slightly greater confidence associated with two relatively different values than a single value. More data points are considered for compounds C and D, with increasing precision of the data values. Whilst compound C (n = 4) has more data than compound D (n = 3), the values are more divergent for C (represented by a higher RSD), thus the highest CS is calculated for compound D for which there are three data points, all relatively consistent in the light of the experimental error that might be associated with an experimental test. As such, compound D has the highest CS value.

Table 1: Four examples of compounds with multiple data in the same toxicity test (EC_{50}), along with statistical criteria and CS (cf. supplementary content for data)

Compound	EC_{50} (mol/L)	$\bar{x} \pm SD$[a]	RSD[b]	n[c]	CS[d]
A	10	10 ± n/a	n/a	1	1[e]
B	1 10	5.50 ± 6.36	1.16	2	1.73
C	1 80 50 100	57.75 ± 43.05	0.75	4	5.37
D	1 2 1.4	1.47 ± 0.50	0.34	3	8.74

[a]mean and standard deviation

[b]relative standard deviation

[c]number of data

[d]confidence Score

[e]CS of a compound with n = 1 is defined as 1 is the minimum value

As there is growing interest in techniques such as read-across to fill data gaps for regulatory purposes, and there is increasing accessibility to toxicity data through resources such as the OECD QSAR Toolbox to perform read-across, there are more possibilities to apply approaches such as the confidence scoring to improve the robustness of modelling. In this study the relevance of the statistical CS approach has been assessed with regard to established QSARs for two endpoints, namely skin permeability coefficients and cytotoxicity for which large compilations of historical data are available.

Skin Permeability

There have been many efforts to develop Quantitative Structure-Permeability Relationship (QSPR) models to predict various measures of dermal absorption.[6-11] The most recognised and applied QSPR to predict the skin permeability coefficient (k_p) is that developed by Potts and Guy in 1992 (Eq. 2).[9] They identified the molecular weight (MW), to account for the size of a permeant, and the logarithm of the octanol-water partition coefficient (log K_{OW}), as a descriptor for lipophilicity, as parameters to model k_p following an analysis based on the Flynn data compilation.[12] The mechanistic explanation is that small, lipophilic compounds pass through the *stratum corneum*, the most outer layer of the skin, more easily than larger, more hydrophilic compounds.[9,13]

$$\log k_p \text{ (cm/h)} = -2.7 + 0.71 \log K_{OW} - 0.0061 \text{ MW} \qquad \text{(Eq. 2)}$$

Despite the significance of this model, the quality of data compiled by Flynn from the literature, and hence the robustness of the Potts and Guy QSPR, has been the subject of considerable debate.[14,15] More human *in vitro* k_p data have inevitably become available in the two and half decades since Flynn's seminal publication[14,16-18], thus the QSPR can be reassessed and rebuilt with a greater consideration and understanding of data quality.

Aquatic Toxicology

There are thousands of publically available acute and chronic eco-toxicological data, and a significant proportion are compiled within the US Environmental Protection Agency's (US EPA's) ECOTOX database.[19] Of the ecotoxicological data, those for aquatic species are the most prevalent. Of these, the Microtox assay represents a commonly used and standardised acute aquatic toxicity test, based on the marine bacterium *Aliivibrio fischeri*, with a multitude of

published data. When the photo-luminescent bacteria are exposed to toxicants, the concentration is proportional to the inhibition of light intensity. The negative logarithm of the effective concentration causing 50% light reduction (EC_{50}) is expressed as the pT.[20] Extending the original compilation of Kaiser and Palabrica[20], Steinmetz *et al.*[5] collected a large meta-dataset with 1813 different values for Microtox toxicity. In order to create meaningful QSAR models in aquatic toxicology, there is an application of the well-established relationship between acute toxicity and hydrophobicity for compounds acting by the non-polar narcosis mechanism of action.[21-24] Narcosis mechanisms of action, and non-polar narcosis in particular, are considered to be as a result of membrane perturbation and that specific mechanisms towards endogenous proteins, receptor mediated effects, are not relevant.[25,26] This implies that the toxicity of compounds that are identified as being non-polar narcotic can be well modelled by descriptors for hydrophobicity, *e.g.* log K_{OW}. Steinmetz *et al.*[5] identified a significant proportion of the Microtox toxicity compilation as being capable of acting by the non-polar narcosis mechanism. In addition Steinmetz *et al.*[5] confirmed the findings of Cronin and Schultz[27] that for these compounds the standard exposure times (5, 15 and 30 minutes) had no significant effect on pT, thus enabling global log K_{OW}-derived models (including these three exposure times) to be developed for non-polar narcotics. Consideration of data quality relating to the confidence associated with multiple data for the same chemical, showed that that toxicity data with certain CS thresholds led to more robust QSAR models.[5]

These two examples of historical data compilations are illustrative of the possibilities of applying confidence scoring metrics to historical compilations of toxicity information. There are many open-access resources such as such as ChEMBL[28], PDSP[29], ACToR[30], eChemPortal[31],

TOXNET[32], so the life sciences, and in particular toxicology, has to deal increasingly with large and complex datasets.[33] However, the task of assessing the toxicity data for quality, particularly when contradicting data are present, has not yet been accomplished. Any indication of the quality of data would be very helpful for purposes such as risk assessment, but more crucially for modelling including QSARs and read-across prediction.[3,5]

Therefore, the aim of this study was to investigate how using approaches for statistical data quality, *i.e.* CS, improve the development of QSAR/QSPR models. Specifically, the effect of directly incorporating the CS into the training and testing of the models was considered. To achieve this, the two endpoints described above were chosen for analysis, namely human *in vitro* skin permeability coefficients and the acute toxicity of compounds acting by a non-polar narcotic mechanism of action to *A. fischeri*. The reasons for choosing these endpoints included the fact that there were many historical data of variable and unknown quality, many compounds had been tested multiple times (a pre-requisite of applying the CS) and that there were simple, robust and mechanistically interpretable QSAR models for them. Thus, for both data sets, QSARs were constructed with and without reference to the CS.

2. METHODS

2.1 Data harvest

In vitro skin permeability coefficients (k_p) were collected from the literature by compiling and subsequently merging four of the most comprehensive datasets of human skin k_p values.[14,16-18] All k_p values were converted to a standard unit (cm/h). Duplicate log k_p values (and those within ± 0.01 cm/h) were removed as they are most likely to be derived from the same source. SMILES and InChIKey strings were obtained for each compound from the ChemSpider[34] database. The Flynn dataset contained k_p values for 94 compounds, however, 11 compounds (all substituted steroids) could not be identified with ChemSpider[34] or ChemIDplus[35] and hence no SMILES were available to calculate descriptors. Since the structure of these compounds could not be completely verified they were excluded from subsequent analysis.

The Microtox data compilation from Steinmetz *et al.*[5] was used as resource for the aquatic toxicology dataset. This comprised 1227 compounds for which there were 1813 data points for 5, 15 and 30 minute exposure. Where there were data for different time endpoints, the longest was taken. For modelling all exposure times were combined, since it has been demonstrated that this has no significant effect on the toxicity of non-polar narcotics.[5,27] The EC_{50} values were considered in mmol/L and converted to pT. The SMILES and InChIKeys were obtained from ChemSpider[34]. The structures of all compounds were run through IDEAconsult's Toxtree v2.6.6[36] (mod. Verhaar) and non-polar narcotics were identified as being Class 1 according to the Verhaar scheme.[21]

2.2 Descriptor Generation

Log K_{OW} and molecular weight (MW) were calculated for compounds in both data sets. The SMILES strings were used as the input format for all calculations. Log K_{OW} was calculated with KOWWIN v1.68 within EPI Suite 4.11 (estimated values exclusively).[37] MW was calculated with the CDK node "molecular properties" within KNIME 2.9.[38]

2.3 Calculation of Confidence Scores (CS)

Confidence scores were calculated for the compounds in both data sets with regard to their k_p and EC_{50} values respectively. For compounds with more than a single experimental value, the arithmetic mean ($\overline{x}$), number (n), standard deviation (SD) and relative standard deviation (RSD) were calculated with reference to data in the units stated in Section 2.1 and before logarithmic transformation. A confidence score (CS) was assigned to the arithmetic mean of the experimental values for each compound. Compounds with a single entry (n = 1) were assigned a confidence score of one (CS = 1). For n > 1 the CS was calculated as in Eq. 1.

2.4 Development of QSARs

Uni- and multivariate linear regression was performed on the datasets using R Studio 0.98.501.19.[39] Linear equations were generated and the following statistical, and other, criteria recorded: n (number of data points), S (standard error), R^2_{adj} (coefficient of determination, adjusted for the number of degrees of freedom), t statistics for the descriptors and F statistics for the equation. The regression analysis was performed to develop the QSARs for both datasets with and without weighting. Non-weighted regression analysis and weighted regression analysis was performed by applying CS values as weights in R using lm {stats}. Weighting in linear

regression means that each datum point is associated with a weight. A high weight strengthens, and a low value weakens, the impact of the data point towards the linear regression. In this manner, data for compounds associated with a high confidence score would be more heavily weighted in the regression analysis than compounds with a lower confidence score. Comparison of the statistics of the weighted and unweighted regression analysis provides an indication of whether CS is able to improve the robustness of models.

2.5 Evaluation of the Predictivity of the QSARs/QSPRs

Statistical evaluation of the predictive capability of the CS-weighted QSAR and the CS-weighted QSPR was performed using 10-fold cross-validation, *i.e.* the compounds were ordered by k_p and pT respectively and every 10^{th} compound was removed in turn leading to 10 training and validation sets. After applying the CS-weighted linear regression, the 10 datasets were investigated by the root mean square error (RMSE); predicted (f_i) versus experimental (y_i) values. Additionally the root mean square error adjusted for CS ($RMSE_{CS}$) was calculated (Eq. 3). It is expected that during the validation process, the $RMSE_{CS}$, which incorporates CS-weighting, will be lower than the standard RMSE. As the residuals (f_i - y_i) of the compounds with low CS values are weakened and the residuals of high CS compounds are strengthened, the sum of (squared) errors of the $RMSE_{CS}$ should be reduced in comparison to the conventional RMSE. The R script for $RMSE_{CS}$ cross-validation and the equations are available in the supplementary content.

$$\mathrm{RMSE_{CS}} = \sqrt{\frac{\sum_i \mathrm{CS_i}(\mathrm{f_i}-\mathrm{y_i})^2}{\sum_i \mathrm{CS_i}}} \qquad \text{(Eq. 3)}$$

3. RESULTS

Names of compounds, their InChIKeys, their SMILES strings and all k_p and pT values including references are available for the two datasets in the supplementary content. In addition the R script for $RMSE_{CS}$ cross-validation and a glossary of relevant statistical equations are also available in the supplementary content.

3.1 Data harvest

The compilation of human *in vitro* k_p data resulted in 342 values for 226 different compounds. 55 of these compounds have more than a single k_p value. The log k_p values covered a broad range from -6.10 to 0.16. The structures included in the data set were diverse in terms of physico-chemical properties and structure, *e.g.* solvents, alkaloids, steroids, sugars, nonsteroidal anti-inflammatory drugs *etc*. The solvents, sugars and steroids in particular had many multiple data points. Water, with 13 different data points, had the most k_p values. The range of CS values is from 1 (for single entries) to 76.8 for chlorphenamine (based on two data points). Illustrating the capability of the CS approach, two compounds have moderately high CS values: the synthetic opioid sufentanyl with a CS value of 9.97 (based on two data points) and the cytostatic drug 5-fluorouracil with a CS value of 5.00 (based on four data points).

From the complete dataset of acute toxicity values to *A. fischeri*, comprising 1227 compounds, 203 were identified as potentially acting as non-polar narcotics according to the Verhaar scheme as implemented in Toxtree v2.6.6[36]. A total of 418 different pT values were available for these compounds, with 71 of the 203 compounds having more than a single experimental value. pT values covered a broad range from -4.00 to 4.12. The structures included in the data set were conservative in their structural diversity as they had been selected to represent the non-polar

narcosis domain, including mainly solvents and medium- and long-chained alkanes, partly branched and halogenated, with only a few functional groups, such as hydroxyl- and amino-groups. The compounds investigated have a moderate spread of MW and log K_{OW} and can generally be regarded as lipophilic (cf. Table 2). The CS spread shows the diversity between high confidence compounds, such as methyl isobutyl ketone (CS of 205 with 3 data points) and acetone (CS of 43.7 with 14 entries) and the single entry low confidence compounds (defined as CS = 1).

Table 2: Ranges of properties and CS for the two datasets considered in the analysis

	Human *in vitro* skin permeability coefficients	pT of non-polar narcotics to *A. fischeri*
MW (Da)	18.01 to 764.4	32.04 to 342.4
Log K_{OW}	-6.76 to 8.39	-1.34 to 6.43
CS	1 to 76.8	1 to 205

3.2 Development of QSARs/QSPRs

QSAR/QSPR models were developed using linear regression with the experimental log k_p and pT as the dependent variables and log K_{OW} and MW (for k_p only) as descriptors. Linear regression analysis was performed on both datasets, the resultant QSPRs for skin permeability coefficients based on the Potts and Guy approach (Eq. 4 (unweighted), Eq. 5 (weighted), Fig. 1) and the log K_{OW}-based QSARs for the acute toxicity of non-polar narcotics to *A. fischeri* (Eq. 6 (unweighted), Eq. 7 (weighted), Fig. 2) are reported below.

3.2.1 QSPR: Modelling of skin permeability coefficients

The unweighted QSPR for the dataset of skin permeability coefficients, using the Potts and Guy approach, was:

$$\log k_p = -2.45 + 0.40 \log K_{OW} - 0.0045 MW \quad \text{(Eq. 4)}$$

$n = 226$, $S = 0.82$, $R^2_{adj} = 0.48$, $t_{logKow} = 13.3$, $t_{MW} = -8.97$, $F = 105$

The reanalysis using CS-weighted k_p provided the following, similar, equation with improved statistical fit:

$$\log k_p = -2.51 + 0.50 \log K_{OW} - 0.0051 MW \quad \text{(Eq. 5)}$$

$n = 226$, $S = 1.39$, $R^2_{adj} = 0.61$, $t_{logKow} = 18.7$, $t_{MW} = -9.25$, $F = 177$

Experimental k_p values are plotted against predicted values from Eq. 5 in Figure 1, demonstrating good overall predictivity. In particular, there is a good fit about the line of unity, with a significant trend for compounds with the highest CS (represented by larger circles) to be well predicted, and the significant outliers tending to be compounds with low CS, *i.e.* single data points.

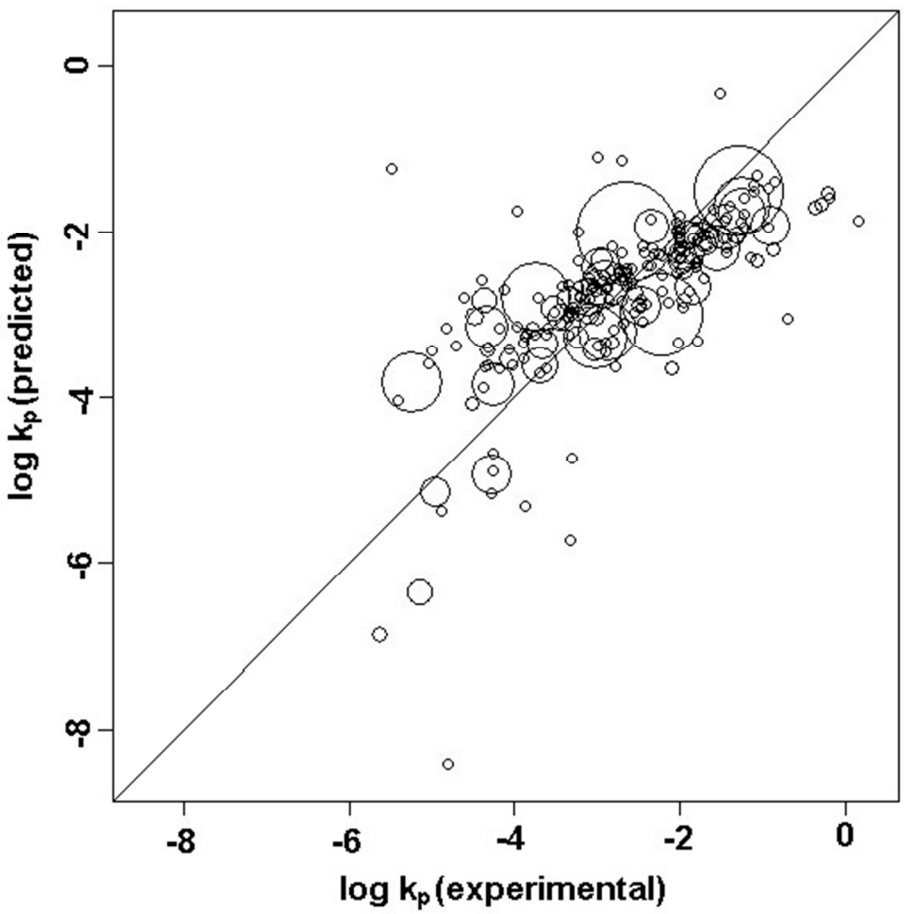

Figure 1: Experimental log k_p versus predicted log k_p from Eq. 5. The area of circles correspond to the CS value; the larger the CS, the greater the area of the circle. The solid line indicates a slope of unity and an intercept of zero.

The QSPR model represented by Eq. 5 was tested using 10-fold cross-validation. The statistical summary is presented in Table 3. Notably the $RMSE_{CS}$ is lower than the RMSE.

Table 3: Statistical summary of 10-fold cross-validation based on Eq. 5 (Skin Permeability)

Training				Test	
Intercept	Log K_{OW}	MW	R^2_{adj}	RMSE	$RMSE_{CS}$
-2.51 ± 0.09	0.497 ± 0.026	-0.0051 ± 0.0004	0.61 ± 0.02	0.83 ± 0.21	0.79 ± 0.21

3.2.2 QSAR: *A. fischeri* non-polar narcosis

The unweighted QSAR for the non-polar narcotics in the Microtox dataset, using a log K_{OW}-based linear regression was:

$$pT = -1.14 + 0.68 \log K_{OW} \quad \text{(Eq. 6)}$$

$n = 203$, $S = 0.95$, $R^2_{adj} = 0.50$, $t_{logKow} = 14.3$, $F = 204$

The reanalysis using CS-weighted pT provided the following equation with improved statistical fit:

$$pT = -1.67 + 0.92 \log K_{OW} \quad \text{(Eq. 7)}$$

$n = 203$, $S = 1.77$, $R^2_{adj} = 0.68$, $t_{logKow} = 20.9$, $F = 478$

Figure 2 demonstrates the relative predictivity of Equation 7. There is a good fit about the line of unity, with a significant trend for compounds with the highest CS (represented by larger circles) to be well predicted, and the significant outliers tending to be compounds with low CS, *i.e.* single values.

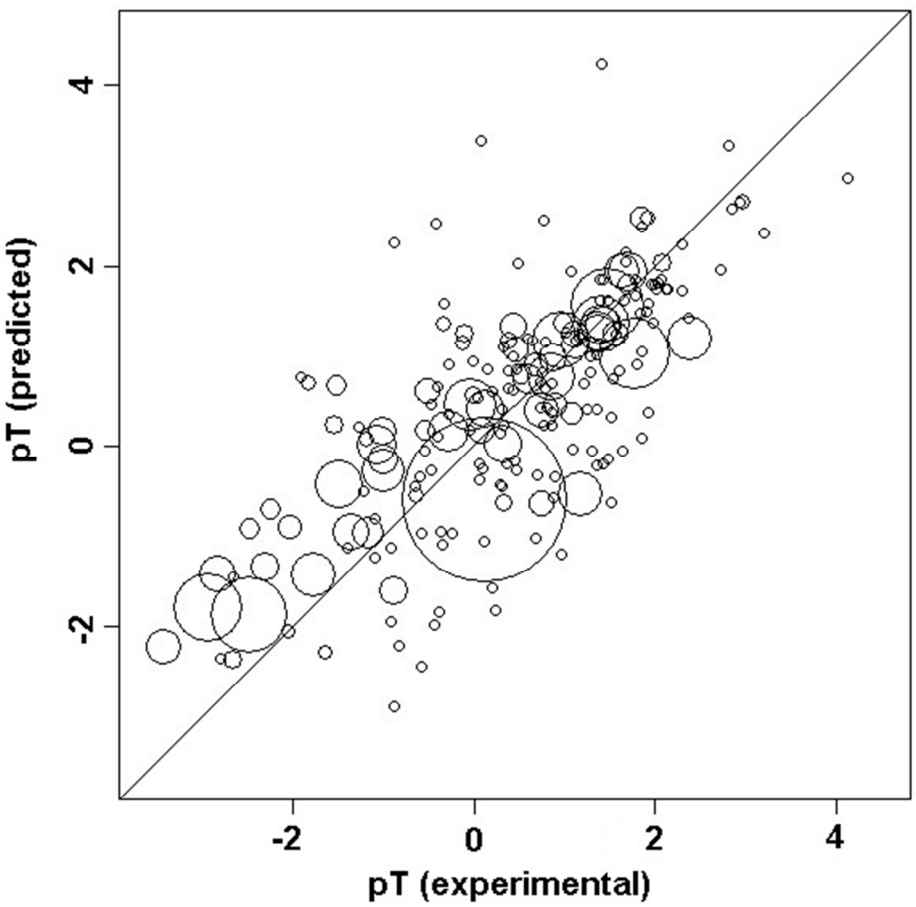

Figure 2: Measured pT versus pT predicted from Eq. 7. The area of circles corresponds to CS value; the larger the CS, the greater the area of the circle. The solid line indicates a slope of unity and an intercept of zero.

The QSAR model Eq. 7 was assessed with 10-fold cross-validation. The summary of the statistics for Eq. 7 is presented in Table 4. The $RMSE_{CS}$ is lower than the RMSE.

Table 4: Statistical summary of 10-fold cross-validation based on Eq. 7 (Aquatic Toxicology)

Training			Test	
Intercept	Log K_{OW}	R_{adj}^2	RMSE	$RMSE_{CS}$
-1.67 ± 0.14	0.92 ± 0.04	0.68 ± 0.03	0.99 ± 0.12	0.87 ± 0.13

4. DISCUSSION

There are many future challenges in human and environmental health sciences which require the use of adequate and reliable data, these include toxicological risk assessment for occupational health and consumer goods. As the quality of toxicological data is variable and often not stated, practical and feasible methods to overcome this issue are crucial to many scientific and regulatory fields. Beside approaches such as Klimisch scoring[1], we suggest a purely statistics-based method to support modelling approaches. It is difficult to determine the extent to which such a statistically-driven approach could be used for regulatory purposes, but neglecting the information multiple data hold for the same substance is not recommended if such data are available.

The aim of this work was not to build new QSAR/QSPR models, but to make two existing models more robust using independent, heterogeneous datasets. The two QSARs and associated datasets chosen are well established. In this study the datasets have been extended by further data harvesting and collection. As part of the data collection activity, multiple data were compiled for the same chemical, thus allowing for the application of the CS approach to determine the reliability of the data. This approach has not been applied formally in the development of QSARs and there are no clear guidelines on how to develop QSARs when multiple data are available for the same chemicals (*i.e.* use of the mean, most conservative value *etc*.). In addition, there appear to be few, if any, attempts to include information such as data quality as a metric or criterion for QSAR development, this being despite it being logical and acknowledged that data quality will affect the robustness of a QSAR.[40] It should also be noted that current means of documenting QSARs provide little opportunity for assessing the quality of data. Therefore approaches that

allow us to identify data quality quantitatively and without subjective bias are of value to develop *in silico* models.

Skin permeability is often assessed by *in vitro* experimental, but also some *in vivo* work is undertaken. *In silico* models are increasingly desirable in areas such as risk assessment where there is a dermal exposure (*e.g.* for cosmetics) and for assessing adverse effects to the skin, *e.g.* skin sensitisation. Since the publication of the Flynn data[12], there have been a number of QSAR analyses of skin permeability coefficients including refinements and extensions to the database.[13] The Potts and Guy approach[9], based on fundamental and mechanistically comprehensible descriptors is one of the more commonly utilised QSAR modelling methodologies. This study has derived a Potts and Guy equation for a larger dataset not only increasing the coverage of the model (*i.e.* greater chemical space) but also incorporating multiple data points for the same chemical and allowing for an assessment of quality through CS. It is noted that published skin permeability coefficients are highly variable, due in no small part to high experimental error arising from the variable nature of the (human) skin utilised and test protocols, *e.g.* use of solvents, enhancers, finite doses *etc*. vehicles, solvents *etc.*[14,15] As such, it is to be expected that models will not have a very significant statistical fit (*i.e.* a high R^2) and this is borne out by many of the published models[9,14], indeed models with significant fit should be treated with some caution as they may be overfitted.

Whilst high statistical fit was not achieved for the skin permeability QSARs, the results show a significant relationship with log k_p and log K_{OW} and MW with both variables demonstrating high t-values. The new QSARs have moderately improved statistical fit as compared to that of Potts and Guy. It should be noted that some values within the Flynn dataset were proven to be

incorrect and would have increased the error in the Potts and Guy QSAR.[15] The novel QSPR model (cf. Eq. 5 and Fig. 1) derived from the skin permeability data has some advantages over the original Potts and Guy[9] model. First of all robustness, due to model development incorporating statistical data quality (cf. Tab. 3); secondly a greater applicability domain due to implementing a dataset with greater chemical diversity (in terms of properties and structure) than Flynn[12]; and thirdly due to the usage of calculated log K_{OW} (whereas the original model used measured values which are more difficult to obtain consistently). Nevertheless the differences between Potts and Guy's Eq. 2 and Eq. 5 are only marginal. It is recognised that there are many limitations to this use of this model. For example it does not predict the effects of mixtures and formulations on the penetration of single compounds, which could be of great importance for risk assessment of products and dermal drug delivery.[41] However, the QSAR approach allows for a "relative" estimation of skin permeability which may be useful to rank compounds, or identify compounds with a high probability of dermal absorption and hence prioritise such compounds in the risk assessment process (*e.g.* for skin sensitisation).

The assessment of effects of chemicals to the bacterium *A. fischeri* (or the Microtox test) is one of the more rapid, cheaper and fundamental measurements of cytotoxicity. Data from the Microtox test show good correlation with higher species, especially for compounds acting by non-specific mechanisms of action such as non-polar narcosis.[42] Thus, if a compound can be identified as being a non-polar narcotic, Microtox data may, if used appropriately and with caution, add further to the weight of evidence associated with a prediction. It is very well established that there is a strong relationship between hydrophobicity, as described by log K_{OW}, and non-polar narcosis for many species.[43,44] This study has expanded the number of chemicals

with data within non-polar narcosis domain for *A. fischeri*, hence expanding the chemical space and extended a previous study.[5] It is of no surprise that the non-polar narcosis data for *A. fischeri* (Microtox) are significantly correlated with log K_{OW}, even if some historical data are obviously of quite poor quality.[5] The QSAR (Eq. 7) is similar to earlier published aquatic toxicology QSAR models, *i.e.* toxicity is increasing linearly with lipophilicity.[5,22-24,43]

Consideration of the QSARs developed in this study shows an improvement in the models when utilising CS-weighted regression. The improvement is both the statistical fit but also the slope for log K_{OW} which approaches one when employing CS-weighting, *i.e.* from 0.68 to 0.90 (cf. Eq. 6 to 7). A slope of one is the theoretical optimum which is commonly associated with models for simple unicellular organisms, *i.e.* the absorption of the compound alone directly into the cellular membrane is responsible for narcosis, whereas in higher organisms other factors such as distribution and clearance become important. The improvements following the application of CS are consistent with the notion that some historical data are of poor quality[45] and demonstrates the utility of an approach such as this when generalistic QSARs are being developed for data sets from various sources and of unknown quality. The importance of the compounds with high CS values can be seen in Fig. 2, when considering that all large CS-circles are close to the line of best prediction. The quantity of data and the incorporation of statistical data quality make a robust equation with an extensive applicability domain – for non-polar narcotics. Clearly this approach could be extended to other data compilations for aquatic acute toxicity.[46]

The identification of compounds acting by the non-polar narcotic mechanism of action is essential to the development of the models. Various approaches have been applied to identify

mechanisms of action including analysis of molecular descriptor space[47], multivariate analysis of mode and mechanism of action space[48], definition of molecular fragments[26] as well as the Verhaar classification scheme that was applied in this study due to its ease of use following coding in the ToxTree software. Due to this definition of the non-polar narcosis domain in the ToxTree software, there appear to be a number of anomalies. For example, aflatoxins are identified by the ToxTree software as being Verhaar Class 1 compounds (non-polar narcotic) but, in reality, they are potent, specifically acting, toxins[5] and therefore do not act as non-polar narcotics, *e.g.* aflatoxin B2 has $pT_{experimental}$ = 1.17 (CS = 15.4) whereas Equation 7 calculates $pT_{predicted}$ = 0.54. This emphasises that continual development is required of decision criteria presented in approaches such as the Verhaar scheme as new knowledge and understanding becomes apparent.

Overall for both data sets, applying CS as a weighting tool improves the training and validation of the QSAR/QSPR models. The improvements are demonstrated as increases in R^2 (Eq. 4 to 5 and Eq. 6 to 7) as a direct result of CS-weighting. Whereas increasing t and F values show improvements in the models as a result of weighting by CS, the S value does not incorporate weights and so only indicates absolute, unweighted error thus it actually increases when the non-weighted regression is compared to the weighted regression. Generally the higher the CS for the data associated with a compound, the greater the evidence is, in terms of similar results for that compound (cf. Fig. 1 and 2). In the validation process, the $RMSE_{CS}$, which incorporates CS-weighting, is lower than the standard RMSE. As residues (f_i - y*i*) of low CS compounds are weakened and residues of high CS compounds are strengthened, the sum of (squared) errors of the $RMSE_{CS}$ becomes lower than in the conventional RMSE. Therefore this

approach could be used even for the validation of models where any metric could be applied to imply confidence, *i.e.* without calculating CS. For example a reversed Klimisch score (4 as the most reliable; 1 the least) could be used as a weight similar to the fuzzy logic approach of Yang *et al.*[49] In the context of validation these weights then determine to what extent residues should have impact on the RMSE.

The CS-weighting approach, whether in model development or validation, is limited by the presence of multiple entries for one compound. Thus, if multiple values are available for the data set, more robust models may potentially be built.[5] This robustness and the associated confidence are helpful in reducing uncertainty and hence increasing acceptance for regulatory decisions. For example in the context of REACH, there is a demand for robust QSAR models to support the toxicological assessment of chemicals. The approach described herein could thus be used to support read-across- and QSAR-based predictions.[50,51]

5. CONCLUSIONS

The assessment of data quality is not trivial. This study has shown that CS provides a means of assessing confidence in data when there are more than a single datum point. The CS scores can be applied to develop QSAR models through the use weighted regression, as demonstrated in this study for historical data compilations with known variability in the quality of the data. Additionally cross-validation with $RMSE_{CS}$ provides a measure of the robustness of an equation utilising metrics (here CS) for weighting.

Note: The authors declare no competing financial interest.

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7. ABBREVIATION

CS:	Confidence score
EC_{50}:	Concentration (in mmol/L) causing 50% of the stated effect
f:	Predicted value
F:	F-value (cf. linear regression)
K_{OW}:	Octanol-water partition coefficient
k_p:	Skin permeability coefficient
n:	Number of data points / test values

InChIKey: International chemical identifier key

MW: Molecular weight (in Da)

pT: Negative decadic logarithm of EC_{50} for toxicity

QSAR: Quantitative structure-activity relationship

QSPR: Quantitative structure-permeability relationship

R^2_{adj}: Coefficient of determination adjusted for degrees of freedom

RMSE: Root mean square error

$RMSE_{CS}$: CS-adjusted RMSE

RSD: Relative standard deviation (also known as coefficient of variation)

S: Standard error (cf. linear regression)

SD: Standard deviation

SMILES: Simplified molecular-input line-entry system

t: t-value (cf. linear regression)

$\bar{x}$: Arithmetic mean

y: Experimental value

8. SUPPLEMENTARY CONTENT

- Microtox and Skin Permeability Data, including statistics glossary
- R-Script for $RMSE_{CS}$ cross-validation

This material is available free of charge via the Internet at http://pubs.acs.org.

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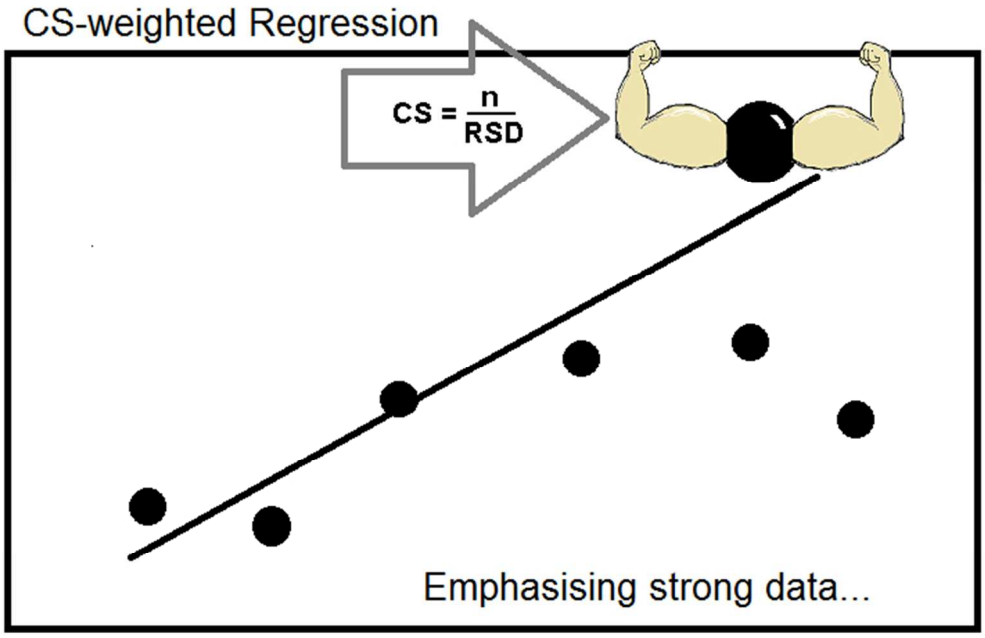

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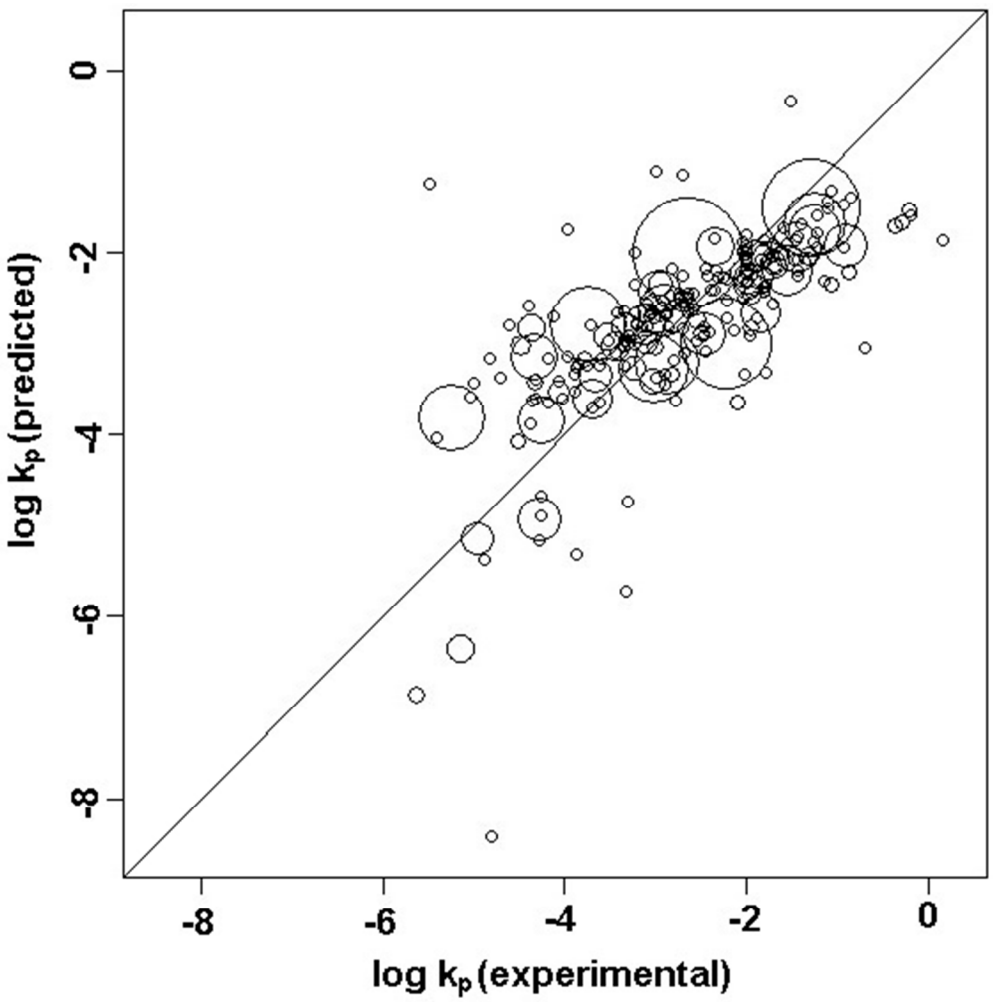

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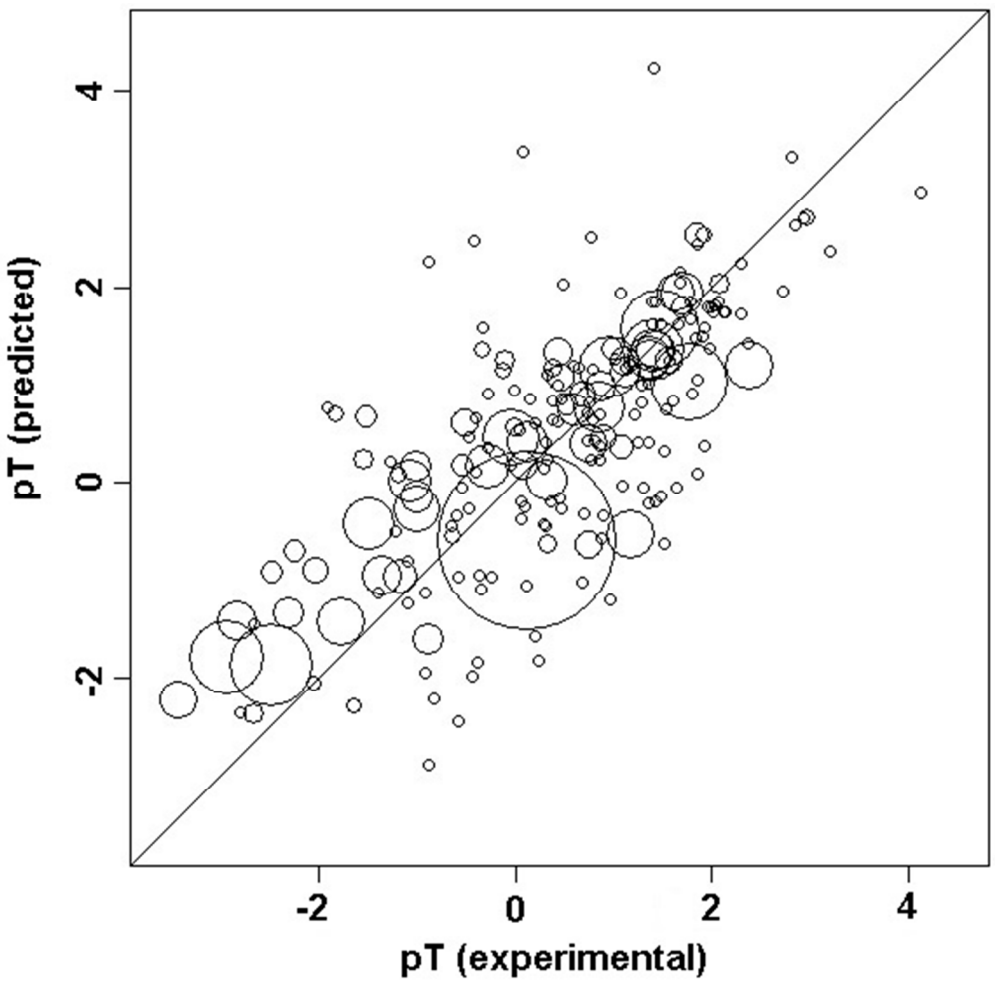

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