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PREDICTION OF α,β-UNSATURATED ALDEHYDES FROM STRUCTURE FOR ACUTE AQUATIC TOXICITY

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ABSTRACT

Chemical category formation and the use of read-across to fill data gaps are seen as crucial methods for the risk assessment of chemicals under the REACH legislation. Such methods are especially important if the goal of reducing the number of experimental animals used in toxicological testing is going to be met. One of the crucial steps in the development of a chemical category is the definition of the applicability domain of the category in terms of the types of chemicals that should be included in the category. The aim of this study was to form a “category” of α,β-unsaturated aldehydes, assumed to act by a common mechanism of action (Michael-type nucleophilic addition). This toxicologically and mechanistically important category was formed on the premise of quantitative structure-activity relationships. The acute aquatic toxicities to *Pimephales promelas* of compounds within the category were obtained in an effort to investigate approaches for read-across. The results indicate that a category for prediction can be formed that allows structural information and boundaries to be elucidated.

Keywords: Acute aquatic toxicity, α,β-unsaturated aldehydes, Michael-type nucleophilic addition, electrophilicity index

INTRODUCTION

There is an increased interest in predicting toxicological effects from chemical structure for many reasons. For new chemicals this will optimize the product development process by eliminating toxic compounds early. For existing compounds, these approaches enable the prioritization of potentially harmful compounds [1]. The formation of toxicological and chemical reactivity domains, and (quantitative) structure-activity relationships (SARs and QSARs) will decrease costs and reduce animal using for chemical risk assessment. In the framework of the new European Union (EU) regulation Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), risk assessment of industrial chemicals is a very important issue in the upcoming decade [2].

The use of computational ‘*in silico*’ techniques to predict toxicity varies in sophistication from the relatively simplistic approach of forming chemical groupings

(category formation) to the more complex development of SARs (qualitative identification of chemical (sub-)structures with the potential of being reactive or toxic) and QSARs (quantitative prediction of relative reactivity or toxicity). There is a rich diversity of *in silico* techniques, however, it is generally acknowledged that a mechanistic basis to developing models allows for easier interpretation and provides greater confidence to the user [3].

Recently, there has been a growth of interest in forming groups of compounds (called categories) with common structural features presumed to be associated with a common mechanism of action [4]. Such groupings can be achieved by consideration of close structural analogs or can be formed using knowledge of the chemistry underpinning the mechanistic basis. If a robust grouping or category can be formed, interpolation of effects can take place – a process called "read-across" [5].

The aim of this study was to determine the usefulness and domain of applicability of electrophilicity index (ω) of α,β-unsaturated aldehydes in predicting the toxicity within the Michael addition mechanism for acute aquatic toxicity using read-across.

MATERIALS AND METHODS

A listing of α,β-unsaturated aldehydes considered in the present study for which are given Name, found experimental values for acute aquatic toxicity in *Pimephales promelas*, electrophilicity index (ω), and known or predicted mechanism are provided. Read-across predictions of the chemicals are presented in Table 1.

Acute aquatict toxicity data. The 96h fathead minnow *(Pimephales promelas)* mortality (LC_{50}) data were extracted from the US EPA MED-Duluth Fathead Minnow Database [6]. The lethal concentration was expressed in mmol/l, and the values were then expressed as - log ($1/LC_{50}$).

EcoSAR software. EcoSAR is a user-friendly computer programme developed and routinely applied by the US EPA for predicting aquatic toxicity to fish, daphnids and algae [7]. This software was used for grouping of the chemicals.

Log P. Data for the logarithm of the 1-octanol-water partition coefficient (log P) were obtained from the KOWWIN software [8]. Where possible measured log P values were verified and used in preference to calculated values.

Excess toxicity. Aldehydes are compounds of special interest as they are often found to have toxicity in excess of baseline. This property - excess toxicity - was used to define the reactive toxicity of these chemicals [9]. The nonpolar narcosis baseline QSAR model for the fish *Pimephales promelas* is [10]:

$$\log(1/LC_{50}) = 0.87 \log P - 1.76 \quad (1)$$

where n= 70, R^2 = 0.95, q^2 = 0.94

and the extent of excess toxicity was determined as the toxic ratio (TR), which was calculated by the following equation [11]:

$$TR = \log (1/LC_{50})exp - \log (1/LC_{50})calc \quad (2)$$

Mechanistic category. Reactive electrophilic chemicals fall naturally into several mechanistic domains based on classic organic reaction chemistry. The major

domains are Michael type acceptor, S_NAr, S_N1, S_N2, Schiff base formation, and acyl transfer [12]. Of these, Michael type addition is proving to be important in toxicity and is well-studied. The basic criteria for a compound to be a Michael type acceptor is summarized in Scheme 1.

Characteristic: double or triple bond where X = electron withdrawing substituent.

Scheme 1. *Michael-type addition reaction*

Computational chemical calculation. All calculation on chemical structure were performed using the Gaussian03 package of programs utilizing the B3LYP/6-31G(d) level of theory [13].

Electrophilicity index (ω). The global electrophilicity parameter (ω) was then calculated for each optimized chemicals as shown by eqs 3–5. The index is derived from chemical potential (μ) and chemical hardness (η), which in turn have been shown to be related to the energies of the highest molecular orbital and the lowest unoccupied orbital (eqs 4 and 5) [14]:

$$\text{Electrophilicity index } (\omega) = \mu^2/2\eta \quad (3)$$

in which

$$\mu = (E_{HOMO} + E_{LUMO})/2 \quad (4)$$

$$\eta = E_{LUMO} - E_{HOMO} \quad (5)$$

where E_{HOMO} and E_{LUMO} are the one-electron energies of the highest occupied and lowest unoccupied molecular orbitals respectively.

Mechanism-based read-across predictions. Read-across predictions for the chemicals were made using the following methodologies (Table 1) [4, 15].

RESULTS AND DISCUSSION

α,*β*-Unsaturated aldehydes belong to the Michael type acceptor mechanistic domain. These compounds are a group that contains a number of relatively reactive organic compounds that are characterized by the presence of a polarized carbon-oxygen double bond. Those that possess a double bond between carbons 2 and 3 (α and β) are conjugated with the carbonyl group, the β-carbon is positively polarized and become the preferred site of nucleophilic attack. These chemicals often contain specific structural fragments responsible for their mechanism of action [12]. There are several modes of action for acute aquatic toxicity. For the reactive mode(s) of toxic action, where toxicity is observed to be in excess of narcosis, the mechanism is reaction chemistry-based, involving covalent modification of proteins [6]. The excess toxicity of these compounds is demonstrated clearly in Figure 1 where toxicity is observed to be not related to hydrophobicity and clearly in excess of baseline toxicity.

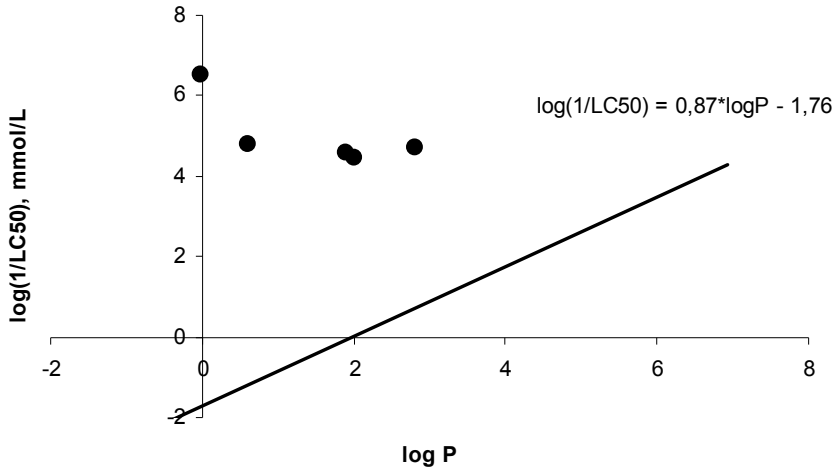

Figure 1. *Plot of toxicity to Pimephales promelas vs log P for α,β-unsaturated aldehydes showing baseline toxicity. Baseline (non-polar) model (log(1/LC$_{50}$)=0.87logP – 1.76)- – , α,β-unsaturated aldehydes (excess toxicity) – ●*

According to McFarland (1970) [16], toxicity is the result of the penetration of a toxicant into the biophases and the interaction of the toxicant with the site of action, but key to the use of computational methods is the ability to group chemicals by mechanistic domains and then to model the key molecular events leading to a toxic effect [17]. In terms of acute aquatic toxicity, it is understood that chemicals as Michael acceptors in which chemical reactivity are the key events leading to a response. A recent study has proposed the utility of an electrophilicity index (ω) to predict the rates of Michael addition reactions within similar chemical classes [14].

In this study, the mechanistically relevant electrophilicity index (ω) was used to rank chemicals in the mechanistic domain based on their electrophilicity, with the rationale being that chemicals that have similar ω values should have approximately similar acute toxic potentials (Table 1). The electrophilic ranking of the chemicals in the Table 1 reveals the ability of ω to offer the expected ordering in terms of electrophilicity of series of related chemicals (within the Michael acceptor domain). The range of electrophilic index (ω) of Michael acceptor mechanistic domain is from 2.252 to 1.187.

Table 1. *Experimental and predicted values of α,β-unsaturated aldehydes for Michael addition domain.*

№	Name	Chemical category	Known or predicted mechanism of action	Exp. log (1/LC$_{50}$), [mmol/l]	Pred. log (1/LC$_{50}$), [mmol/l]	TR	ω, [eV]
1	3-Phenyl-2-propenal	Aldehydes	Michael addition	1.59	NP	1.70	2.252
2	3-(2-Methoxyphenyl)-2-propenal	Aldehydes	Pred. Michael addition		1.532		1.924
3	2-Propenal	Aldehydes	Michael addition	3.518	1.741	5.29	1.843

4	2-Methyl-2-propenal	Aldehydes	Pred. Michael addition		2.251		1.706
5	2-Butenal	Aldehydes	Michael addition	1.81	1.485	3.05	1.658
6	2-Hexyl-3-phenyl-2-propenal	Aldehydes	Pred. Michael addition		1.481		1.635
7	2-(Phenylmethylene) heptanal	Aldehydes	Pred. Michael addition		1.480		1.629
8	2-Methyl-3-phenyl-2-propenal	Aldehydes	Pred. Michael addition		1.476		1.608
9	3-(4-(Dimethylamino) phenyl)-2-propenal	Aldehydes	Michael addition	1.473	1.664	1.49	1.590
10	(1,3,3-Trimethylindolin-2-ylidene) acetaldehyde	Aldehydes	Michael addition	1.71	NP	1.03	1.187

In the present work, we use a selected series of Michael acceptors, which toxicity were predicted by the read-across methodology. Read-across has been suggested as a useful method for making toxicological predictions within a given mechanism of action for a particular endpoint [15]. This methodology relies on the principle that similar chemicals should have similar toxic effects for a given endpoint, with predictions being made by selecting a number of close 'neighbors' (whose activity is known) and then making an averaged (or weighted average) prediction.

Mechanism-based read-across of Michael acceptor mechanistic domain by electrophilic index (ω) for acute toxicity prediction were possible for 8 (measured and nonmeasured) of the 10 chemicals in the study. It is clear that the LC_{50} prediction by electrophilic index for the majority of the chemicals are in relatively good agreement with experimentally observed values for Michael acceptor mechanistic domain.

CONCLUSION

The formation of a category (or class) of chemicals allowing for read-across to occur is simple but an extremely transparent and powerful technique for filling data gaps in toxicological databases. α,β-Unsaturated aldehydes are in the Michael-type mechanistic domain and has demonstrated that the electrophilic index (ω) can be used to rank a series of direct-acting Michael acceptors. The study has also demonstrated the ability of ω to be used, within carefully considered mechanistic applicability domains, to perform mechanism-based read-across to predict acute aquatic toxicity (LC_{50}) values.

REFERENCES

1. Cronin, M. T. D., and D.J. Livingstone, *Predicting Chemical Toxicity and Fate*. CRC Press: Boca Raton, FL, USA, 2004.
2. Schaafsma, G., et al., *Regulatory Toxicology & Pharmacology*, 2009**,** 53, 70-80.
3. Freidig, A.P, J.L.M. Hermens, *Quant. Struct. Act. Rel.*, 2001, 19, 547-553.
4. Koleva, Y. K., J. C. Madden, M.T.D. Cronin, *Chem. Res. Toxicol.*, 2008, 21, 2300-2312.
5. Diderich, R., Tools for category formation and read-across overview of the OECD (Q)SAR application toolbox. In *In Silico Toxicology: Principles and Applications*, Cronin, M. T. D.; Madden, J. C., Eds. RSC: Cambridge, 2010.
6. Russom, C. L., S.P Bradbury, S. J. Broderius, D. E. Hammermeister, and R. A. Drummond, *Environ. Toxicol. Chem.,* 1997, 16, 948–967.
7. EPA website: http://www.epa.gov/oppt/newchems/tools/21ecosar.htm
8. US EPA, KOWWIN; software available at http://www.epa.gov/oppt/exposure/pubs/episuite.htm
9. Lipnick, R. L., *Science of the Total Environment*, 1991, 109, 131–153.
10. Schultz, T. W., G. D. Sinks, and A. P. Bearden, QSAR in aquatic toxicology: A mechanism of action approach comparing toxic potency to *Pimephales promelas*, *Tetrahymena pyriformis*, and *Vibrio fischeri*. In *Comparati*V*e QSAR* (Devillers, J., Ed.) 51-109, Taylor and Francis, New York, 1998.
11. Nendza, M., and M. Müller, *SAR and QSAR in Environmental Research*, 2007, 18, 155–168.
12. Roberts, D. W., G. Patlewicz, P. S. Kern, F. Gerberick, I. Kimber, R. J. Dearman, C. A. Ryan, D. A. Basketter, A. O. Aptula, *Chemical Research in Toxicology*, 2007, 20, 1019-1030.
13. Frisch, M., et al., *Gaussian03*, Revision B.04, Gaussian, Inc., Wallingford, CT, 2003.
14. Domingo, L. R., et al., *Tetrahedron*, 2004, 60, 6585-6591.
15. Roberts, D., et al., *Chemical Research in Toxicology*, 2007, 20, 44-60.
16. McFarland, J. W., *Journal of Medicinal Chemistry*, 1970, 13(6), 1192-1196.
17. Schultz, T. W., et al., *SAR QSAR Environ. Res.,* 2006, 17, 413-428.