Testing the Verhaar Rules for Classifying Compounds According to Toxic Mode of Action

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INTRODUCTION

•The REACH legislation allows for the use of non-test data, such as *in silico* predictions, to make risk assessments

•Classifying chemicals into mechanisms of action is crucial for understanding and predicting the toxicity of chemicals

•Classification according to mechanisms is essential for mechanism-based quantitative structure-activity relationships (QSARs) to be developed and used

•Classification schemes also allow historical experimental data to be used for 'read-across' analysis which could be applied to prioritise emerging contaminants on the basis of their predicted mechanism of action (reactivity).

AIM OF THIS INVESTIGATION

•The aim of this investigation was to assess the performances of two schemes for classifying compounds according to the mechansism of action: the Verhaar classification scheme[1] and an in-house implementation of 'Rules for Electrophilicity' [2] for to identify electrophilic fragments.

METHODS

Datasets

•Two datasets of chemicals, previously classified into mechanisms of toxic action, were used in the analysis

•Subsets of compounds known to act by narcotic or electrophilic mechanisms (narcosis:electrophilic) were selected from the two datasets:

- •379 compounds from the fathead minnow dataset (274:94)[3]
- •227 phenols from a *Tetrahymena pyriformis* dataset (173:54)[4]

Classification Schemes

•Compounds were classified using two schemes

- •The Verhaar classification as implemented in the ToxTree software (freely available from http://ecb.jrc.it/qsar/).
  - •Analysis centred on the correct prediction of known narcotic compounds and compounds associated with excess toxicity brought about by electrophilic interactions.
  - •The structural basis of the predictions was assessed to ensure the Verhaar rules were accurate and to make suggestions for improvement
- •'Rules for Electrophilicity' coded in-house from the work of Aptula and Roberts[2]
  - •Developed from extensive analysis of skin sensitisation data
  - •Identifies a number of possible chemical mechanisms responsible for reactive toxicity
  - •Rules identify only electrophilic compounds.
  - •Compounds can be electrophilic or non-electrophilic, however no other mechanism of action can be implied (hence the omission of narcosis classification in Table 1)

Analysis

•Classification schemes were assessed on the ability to identify mechanism of action correctly (as suggested from original data sources)

•Percentage correct classification were reported for narcotic and electrophilic compounds

•Analysis of misclassified compounds to enable improvements to the classification schemes

RESULTS For both datasets, predictions of mechanism of action were made using both schemes. The number and percentage of correctly classified compounds are shown in Table 1.

			Correctly Classifed	
Dataset	Mechanism	Number	Verhaar	Roberts
Phenol	Narcosis	173	76 (43.9)	-
	Electrophilic	54	11 (21.2)	52 (96.3)
Fathead Minnow	Narcosis	275	119 (43.3)	-
	Electrophilic	94	57 (60.6)	49 (52.1)

Table 1. A summary of the predictive ability of the Verharr and 'Rules for Electrophilicity' schemes to predict MOAs for the *T. pyriformis* (Phenol) and fathead minnow datasets.

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DISCUSSION

In silico technologies are important in the prioritisation of emerging contaminants and their use is likely to increase in the future.

Verhaar Scheme:

The Verhaar scheme proves to be successful in correctly predicting the mechanism of action for most narcotic compounds.

Narcotics – some aromatic features were incorrectly assigned. For example, fragments associated with Michael addition (see figure 1 below) are incorrectly assigned to aromatic compounds. This problems also occurred for other mechanisms of action.

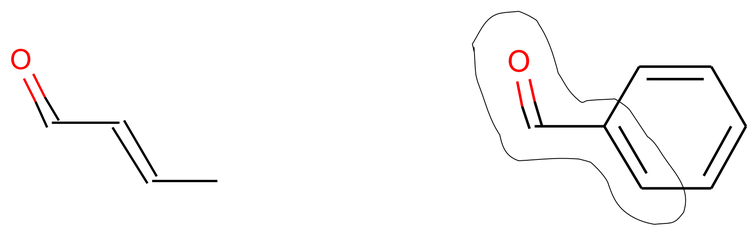


Figure 1. Verhaar scheme incorrectly identifies aromatic structures as being associated with Michael-addition.

Electrophiles – A poorly defined class 2 (narcotic) rule leads to many phenols being incorrectly classified as narcotics :

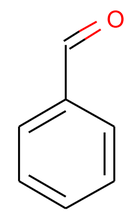
"2.1 be non-or weakly acidic phenols; i.e. phenols with one nitro substituent, and/or one to three chlorine substituents, and/or alkyl substituents"[1]

In-House Coding of 'Rules for Electrophilicity':

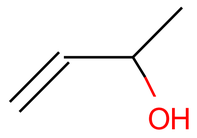
The Electrophilic rules prove to correctly classify the majority of electrophilic compounds.

Narcosis – aliphatic alkyl halides are classified as electrophilic when they should be narcotic in the fish. This is because in skin sensitisation the S_N2 mechanisms is important, however it is not present in the fish.

Electrophiles – aromatic aldehydes are reactive in the fish. There is no rule that covers this in the current scheme. Conjugated secondary alcohols are also reactive in the fish, again there is no rule to cover this.



Aromatic aldehyde



Conjugated secondary alcohol

Both of these missing rules are due to the equivalent mechanisms not being present in the skin data (on which the rules were trained).

CONCLUSIONS

•The schemes were relatively successful at assigning compounds to a particular mechanism

•Neither scheme consistently out-performed the other

•Both schemes studied need revision of several rules to improve their performance

•The Verhaar scheme needs improvement in correctly recognising aromatic fragments as being non-reactive

•Electrophilic rules need extending to include additional reactive fragments known to act in fish but are not important for skin sensitisation.

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