Comparative study of mechanism of action of allyl alcohols for different endpoints

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Abstract: *α,β-Unsaturated alcohols, i.e. allyl alcohols are typical proelectrophiles which can cause acute and human health effects. In the present work are presented researches for possible mechanism of action of a group of allyl alcohols for different endpoints (acute toxicity (aquatic and terrestrial), skin sensitisation and mutagenicity). The aim is to form chemical categories which will allow to apply a method as read-across to fill data gaps. These are 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 met.*

Key words: *Allyl alcohols, Mechanism of Action, Acute Toxicity, Skin Sensitisation, Mutagenicity*

INTRODUCTION

Alpha, Beta-Unsaturated alcohols includes allyl and propargyl alcohols that are considered typical proelectrophiles [7, 8, 19] since their metabolically activated oxidation yields the corresponding alpha, beta-unsaturated aldehydes or ketones. The latter may further undergo Michael-type addition reaction [7] due to the activated double or triple bond. In agreement with this assumption, a predominantly reactive mode of toxic action has been established only for primary and secondary alpha, beta-unsaturated alcohols [8]:

Scheme 1 Mechanism of allyl alcohol as proelectrophile

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 [5].

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 [13].

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 [6]. Such groupings can be achieved by consideration of close structural analogues 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" [1].

The aim of this study was to examine the reactivity of allyl alcohols and to form reactive categories for different endpoints (acute toxicity (aquatic and terrestrial), skin sensitisation and mutagenicity).

MATERIALS AND METHODS

A list of allyl alcohols (Name and Structure) considered in the present study is provided in Table 1.

*EcoSAR classification***.** EcoSAR software is a user-friendly computer programme developed and routinely applied by the US EPA for predicting aquatic toxicity to fish, daphnids and algae [4]. This software was used for grouping of the chemicals (Table 1).

Log P. Data for the logarithm of the 1-octanol-water partition coefficient (log P) were obtained from the KOWWIN software [18]. Where possible measured log P values were verified and used in preference to calculated values (Table 1).

Acute aquatict toxicity data (Pimephales promelas). The 96h fathead minnow *(Pimephales promelas)* mortality (LC_{50}) data were extracted from the US EPA MED-Duluth Fathead Minnow Database [12]. The lethal concentration was expressed in mmol/l, and the values were then expressed as – log ($1/LC_{50}$) (Table 1).

Acute aquatict toxicity data (Tetrahymena pyriformis). Toxicity values to *Tetrahymena pyriformis* for allyl alcohols were obtained from the literature [14] and reported in Table 1. Population growth impairment was assessed after 40h with the common ciliate *T. pyriformis*.

Acute terrestrial toxicity data. The experimental data for rat and mouse (oral LD_{50} values) were collected from the literature [20] (Table 1).

Baseline models. In this study several models were used for non-polar compounds to aquatic and terrestrial species to determine the acute toxicity of allyl alcohols (Table 1).

Baseline model (saturated alcohols and ketones) of *Tetrahymena pyriformis* [3]:

$\log(1/IGC_{50}) = 0.78*\log P - 2.01$ (1)

$n = 87$ $R^2 = 0.96$ $s = 0.20$ $F = 2131$

Baseline model of *Pimephales promelas* [16]:

$\log(1/ LC_{50}) = 0.87*\log P - 1.76$ (2)

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

Baseline model (saturated alcohols and ketones) of Rat (oral) [7]:

$\log(1/LD_{50}) = 0.805*\log P - 0.971*\log(0.0807*10^{\log P}+1) + 0,984$ (3)

$n = 54$ $R^2 = 0.824$ $s = 0.208$ $F = 35.3$

Baseline model (saturated ketones) of Mouse (oral) [17]:

$\log(1/LD_{50}) = 0.557*\log P - 0.908*\log(0.049*10^{\log P}+1) + 1,201$ (4)

$n = 13$ $R^2 = 0.961$ $s = 0.0758$ $F = 36.5$

Excess toxicity. The property – excess toxicity – was used to define the toxicity of chemicals (reactive or nonrective) [7]. The extent of excess toxicity was determined as the toxic ratio (TR), which was calculated by the following equations 5-6 [7, 9]:

TR = log(1/Endpoint)exp – log (1/Endpoint)calc (5)

or

TR = (predicted baseline toxicity) / (observed toxicity) (6)

Skin sensitisation. Data for the local lymph node assay (LLNA) were taken from the database collated by Roberts et al [11]. The allyl alcohols in this study represent only weak sensitisers.

Mutagenicity. The mutagenicity (*Salmonella typhimurium* (TA100-S9) strain) data were retrieved from the literature [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 [11].

Allyl alcohols are typical proelectrophiles. A probable mechanism of their action for different endpoints is shown on Scheme 1.

RESULTS AND DISCUSSION

In a group of allyl alcohols was studied the reactivity to different toxicities and possible mechanism of action within and between the endpoint(s). These chemicals often contain specific structural fragments responsible for their mechanism of action [11]. 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 [12]. The excess toxicity (TR) of some compounds is demonstrated clearly in Table 1 where toxicity is observed to be not related to hydrophobicity and clearly in excess of baseline toxicity.

In this group of allyl alcohols may be found primary (compounds 1-3,5-6 in Table 1) and secondary (compound 4) allylic alcohols. Their oxidation via alcohol dehydrogenase yields the corresponding α,β-unsaturated aldehyde or ketone Michael-type acceptor which can act as a powerful electrophile. If the alcohol is tertiary (compound 7), such metabolic transformation is not possible and such compounds act solely by a narcosis mechanism for the acute toxicity (aquatic and terrestrial) and unreactive for skin sensitisation.

Table 1 Experimental values of allyl alcohols for different endpoints

№	Name	Chemical category	log P	Exp. TA100-S9, [rev/ µmol]	Exp. EC3 [%]	Exp. *T.pyriformis* log (1/ IGC_{50}), [mmol/l]; TR	Exp. *P.promelas* log (1/ LC_{50}), [mmol/l]; TR	Exp. Rat oral log(1/ LD_{50}), [mmol/ kg]; TR	Exp. Mouse oral log(1/ LD_{50}), [mmol/ kg]; TR
1	2-Propenol	Vinyl/Allyl alcohols	0.17[a]	750		-1.918; -0.031	2.259; 3.871	64; 10.74	96; 11.22
2	2-Butenol	Vinyl/Allyl alcohols	0.63[b]	<1		1.472; 0.057		793; 2.11	
3	2-Methyl-2-propen-1-ol	Vinyl/Allyl alcohols	0.76[b]	<1		-1.663; -0.236			
4	3-Buten-2-ol	Vinyl/Allyl alcohols	0.63[b]	1.5		-1.053; 0.476			
5	3-Phenyl-2-propen-1-ol	Vinyl/Allyl alcohols	1.95[a]	0	21 (Weak)	-0.080; 0.419		2000; 3.11	2675; 2.12
6	3,7-Dimethyl-1,6-octadien-3-ol	Vinyl/Allyl alcohols	3.56[a]	0	26 (Weak)			3600; 1.22	
7	3,7-Dimethyl-2,6-octadien-1-ol	Vinyl/Allyl alcohols	2.97[a]	0	30 (Weak)			2790; 1.95	

[a]Experimetal value of log P; [b]Calculated value of log P.

The excess toxicity of allyl alcohols for *Tetrahymena pyriformis*, *Pimephales promelas*, Rat and Mouse (oral) was evaluated and some differences were shown. Because proelectrophile potency is metabolism based, it has the potential to be both structure dependent and subject to species variability [15].

Toxicity studies with *Pimephales promelas*, Rat and Mouse (oral) found that primary and secondary allylic alcohols were more toxic than *Tetrahymena pyriformis* (Table 1). Therefore, metabolic processes (i.e. enzyme activity), which govern the proelectrophilic mechanism of toxic action, can be species specific.

A probable mechanism for compounds 5 and 6 (Table 1) for skin sensitisation is indergoes activation to form α,β-unsaturated aldehyde (Michael-type acceptor) and compound 7 is unreactive [10]. Therefore, a possible mechanism of action of primary and secondary allylic alcohols is given on Scheme 1 and tertiary one is unreactive for skin sensitisation.

Allyl alcohol and its derivatives show a distinct direct mutagenic effect. The same activation mechanism of formation of an aldehyde (ketone) from the respective alcohol by ADH should be expected to apply with allyl alcohol and its derivatives for the mutagenicity (Scheme 1). Suprisingly, however, these compounds exert only limited direct mutagenic effects; the homologues with larger substituents (compounds 5-7 (Table 1)) are inactive. The reasons may be different. An explanation for this lower activity might be the peculiar substrate specificity of ADH in the sense that alkyl substitutions render the molecules much less suitable for this type of biotransformation [2] or some steric factors associated with Michael-type acceptor. However, for these assumptions need confirmation by more specific investigations.

CONCLUSIONS

In principle, the allyl alcohols are typical proelectrophiles but the alkyl substituents in allyl alcohols (primary, secondary and tertiary), the differences in protocols, the metabolic capacity of species (*Tetrahymena pyriformis*, *Pimephales promelas*, Rat and Mouse) and other factors may change activity them for the different endpoints.

Analysis of the relationships between the structure of a group of allyl alcohols and different endpoints (acute toxicity (aquatic and terrestrial), skin sensitisation and mutagenicity) may be allowed to form chemical categories which will allow to apply the read-across method to fill the missing data.

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Докладът е рецензиран.