InSilicoTox Project

Deliverable 4 (D4)

Report on Reaction Mechanisms in Toxicology

October 2009

European Union Sixth Framework Programme Marie Curie Actions
Project No.: **42328**
Project Acronym: **InSilicoTox**
Project Full Name: ***In Silico*** **Tools to Reduce the Use of Animals in Toxicity Testing for Bioreactive Chemicals**

Deliverable Prepared by

Johannes Schwöbel, Mark Cronin, Yana Koleva, Judith Madden,
School of Pharmacy and Chemistry
Liverpool John Moores University
Byrom Street
Liverpool
L3 3AF
England

Important Information

D4. Report on Reaction Mechanisms in Toxicology

Summary

The toxicity of a compound, related to different modes of action, is linked to the specific chemical reactivity of this compound. Therefore, a mechanistic understanding is needed for a sound assessment and prediction of toxicological and ecotoxicological behaviour.[1] In the framework of the EU regulations Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), risk assessment of industrial chemicals is a very important issue in the upcoming decade.[2]

Depending on the specific target site of a toxicological mode of action, the compound shows a variation in the underlying biochemical mechanism.[3] This comes along with Pearson's Hard and Soft Acid and Bases (HSAB) concept.[4, 5] Molecular interactions between electrophiles and nucleophiles are governed by their properties, which follow hardness and softness patterns in general. Biological nucleophiles, such as the DNA molecule, are predominantly hard chemicals, acting via rather hard oxygen or nitrogen containing moieties. Softness of a nucleophile can be associated with easy oxidisability and low electronegativity, for example thiol residues of peptides and proteins with its sulphur atom. As many environmental pollutants are soft agents, this is a first clue to understand their toxicity. Dissimilar hardness leads to a higher potential energy barrier of reactions between electrophiles and nucleophiles.

As an example for dissimilar reactivity, nitroaromatics do not show any reactivity in skin sensitisation,[6] while they form reactive DNA attacking hydroxylamine intermediates, conducted by the enzyme nitroreductase,[6] but they follow nucleophilic substitution (S_N1 or S_N2) in acute aquatic toxicity.[7] In contrast, α,β-unsaturated carbonyl compounds follow the same mechanism in protein binding

initiating skin sensitisation, in DNA binding, and in acute aquatic toxicity, namely Michael-type addition.[8]

As a promising tool in alternative testing and *in silico* strategies, qualitative QSAR models evaluate the presence or absence of a specific substructure in a molecule, called structural alerts. They imply a specific mechanism connected with a substructure. The substructures should be specific enough for reliable prediction of a particular mechanistic pathway.

A systematic compilation of substructures, following different toxicological modes of action, is provided by this report. For the first time, similarities and dissimilarities in their biochemical mechanisms are assembled systematically in the following table. Namely, the toxicological endpoints are acute aquatic toxicity, skin sensitisation, and mutagenicity. All of them are considered as important endpoints, according to REACH legislation. Briefly, for the ecotoxicological endpoint acute aquatic toxicity,[9] testing submerges species of fish to certain environmental concentrations of a substance as a single dose expose to determine the lethality level. Skin sensitisation is the induction of allergic responses in the skin.[10, 11] Exposure of a hapten to the skin leads to formation of a hapten-protein adduct, following induction of allergen-specific lymphocytes and antibodies, both being ready to respond to the next exposure of the hapten. Agents causing mutagenicity,[12] react either directly with DNA and cause structural changes, or indirectly by cell metabolites that have the direct mutagenic effect. Apart from that, some mutagens may act as base analogues and get inserted into the DNA strand.

Literature

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2. REACH, Regulation (EC) No 1907/2006 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, 93/67/EEC, 93/105/EC and 2000/21/EC. 2006.

3. Borgert, C. J.; Quill, T. F.; McCarty, L. S.; Mason, A. M., Can mode of action predict mixture toxicity for risk assessment? . *Toxicol. Appl. Pharmacol.* **2004,** *201*, 85-96.

4. Pearson, R. G.; Songstad, J., Application of the principle of hard and soft acids and bases to organic chemistry. *J. Am. Chem. Soc.* **1967,** *89*, 1827-1836.

5. Ho, T.-L.; Ho, H. C.; Hamilton, L. D., Biochemical significance of the hard and soft acids and bases principle. *Chem. Biol. Interact.* **1978,** *23*, 65-84.

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7. Roberts, D. W., An analysis of published data on fish toxicity of nitrobenzene and amine derivatives. In *QSAR in Environmental Toxicology*, Kaiser, K. L. E., Ed. Reidel: Dordrecht, The Netherlands, 1987; Vol. II., pp 295-308.

8. Koleva, Y. K.; Madden, J. C.; Cronin, M. T. D., Formation of categories from structure-activity relationships to allow read-across for risk assessment: Toxicity of α,β-unsaturated carbonyl compounds. *Chem. Res. Toxicol.* **2008,** *21*, 2300-2313.

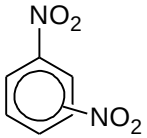
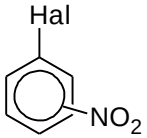
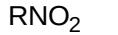
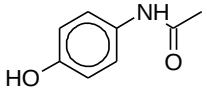
9. Russom, C. L.; Bradbury, S. P.; Broderius, S. J.; Hammermeister, D. E.; Drummond, R. A., Predicting modes of toxic action from chemical structure: acute toxicity in the fathead minnow (*Pimephales promelas*). *Environ. Toxicol. Chem.* **1997,** *16*, 948-967.

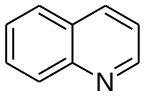
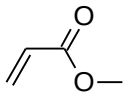
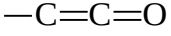
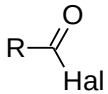
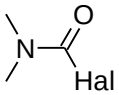
10. Roberts, D. W.; Aptula, A. O.; Patlewicz, G., Electrophilic chemistry related to skin sensitization. Reaction mechanistic applicability domain classification for a published data set of 106 chemicals tested in the mouse local lymph node assay. *Chem. Res. Toxicol.* **2007,** *20*, 40-60.

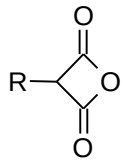
11. Roberts, D. W.; Patlewicz, G.; Kern, P. S.; Gerberick, F.; Kimber, I.; Dearman, R. J.; Ryan, C. A.; Basketter, D. A.; Aptula, A. O., Mechanistic applicability domain classification of a local lymph node assay dataset for skin sensitization. *Chem. Res. Toxicol.* **2007,** *20*, 1019-1030.

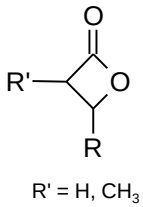
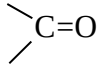
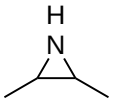
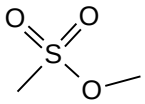
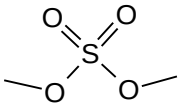
12. Lutz, W. K., Quantitative evaluation of DNA binding data for risk estimation and for classification of direct and indirect carcinogens. *Cancer Res. Clin. Oncol.* **1986,** *112*, 85-91.

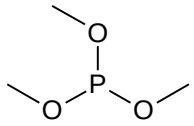
Table 1: *Substructures and associated mechanisms for different endpoints*

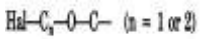
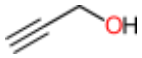
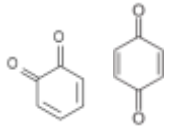
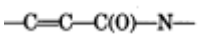
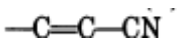
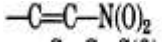
Substructure		Acute Aquatic Toxicity		Skin Sensitisation		Mutagenicity	
Description	**2D Fragment**	**Mechanism**	**Ref.**	**Mechanism**	**Ref.**	**Mechanism**	**Ref.**
Benzene rings, without aniline or phenol substructures, that have two nitro groups on one ring		Various (including S_N1+S_N2)	1, 17-21	No binding	9	Ambiguous mechanisms; Compared models for reduction of compounds by xanthine oxidase (because the crucial activation step for mutagenic activity of these chemicals is the metabolic reduction by one or more cytosolic nitroreductases). Nitroreductase;	63-71, 79 9, 105, 106
Halogenated nitroaromatic		Nucleophilic substitution	9, 29	Nucleophilic substitution of haloaromatics	9	Nitroreductase	9
Aliphatic nitro		Basesurface narcotics	1, 9, 10, 29	No binding	9	Ambiguous mechanisms; Nitroreductase	9
Acetamidophenols		Unknown; (Pro-Michael-type addition)	1, 10, 22	No binding	9	No binding	9

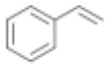
Quinolines		Unknown	1, 10	No binding	9	Unknown; No binding	72, 73
Chlorodiesters				Arylcarboxylate aminolysis	9, 107	No binding	9
Acrylates		Michael-type nucleophilic addition; S_N2	1, 2, 4, 6, 10, 23-27, 57, 58	Michael-type nucleophilic addition;	11, 61	No binding	9
Diesters		Unknown	28				
Ketenes		Acylation	29	No binding	9	No binding	9
Acid halides (Acyl halide)		Acylation	29	Nucleophilic substitution of acyl halides	9, 107	Electrophilic mechanism	9
Dialkyl carbomylchlorides		Acylation	29	Nucleophilic substitution of acyl halides	9	Electrophilic mechanism	9

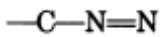
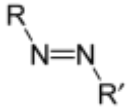
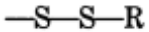
Carboxylic acid anhydrides	 R = CH_3, C_2H_5	Acylation	29	Nucleophilic substitution of cyclic dicarbonyls;	9	No binding	9
Isocyanates	—N=C=O	Acylation	1, 29	Nucleophilic addition to isocyanates	9, 108, 109	No binding	9
Isothiocyanates	—N=C=S	Acylation; Michael-type nucleophilic addition Two mechanisms, which are likely to take place concurrently: (a) addition across the C=N triple bond, and (b) nucleophilic attack at the benzylic sulfur with displace-ment of the cyanide leaving group. In (a), these compounds act as electrophiles, but not as cyanogenic toxicants; in (b), they are both electrophiles and cyanogenic toxicants.	1, 8, 29, 30, 31	Nucleophilic addition to isothiocyanates	9	No binding	9

Lactone (α-, β-, and unsaturated)		Schiff base formation; Strained ring electrophiles: Cyanide release via cyanohydrin-like toxicant	1, 2, 8, 29	Michael-type nucleophilic addition; Nucleophilic heterocycle ring opening	11, 59, 60, 9	Direct alkylating agents	9
Aldehydes		Schiff base formation; Benzaldehydes: unknown	1- 3, 5, 25, 28-29, 32- 35	Schiff base formation	9, 11-13, 62	No binding	9
Epoxides		Alkylation	1, 10, 29, 36-38	Nucleophilic heterocycle ring opening	9	Ambiguous mechanisms; Direct alkylation	76-78, 9
Aziridines		Alkylation	29	Nucleophilic heterocycle ring opening	9	Direct alkylation	9
Sulfonic acid esters		Alkylation	29, 39, 40	Nucleophilic substitution of alkyl sulfonates	9	No binding	9
Sulfuric acid esters		Alkylation	29	Nucleophilic substitution of alkyl sulfates	9	No binding	9

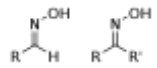
Phosphoric acid esters		Alkylation	29	Nucleophilic substitution of alkyl phosphonates and (thio)phosphates	9	No binding	9
Sulfonyl halide		Miscellaneous reactions	29	Nucleophilic substitution of sulfonyl halides	9	No binding	9
Sulfonium salt		Basesurface narcotics	9	No binding	9	Carbenium and episulfonium ions: act directly on the DNA bases	9
Haloacetamides	Hal—C—C(O)—N—	Nucleophilic substitution	29	Nucleophilic substitution of haloaliphatics	9	Haloalkanes and compounds, containing labile halogen: electrophilic mechanism	9
Haloacetates	Hal—C—C(O)—OH	Nucleophilic substitution	29	Nucleophilic substitution of haloaliphatics	9	Haloalkanes and compounds, containing labile halogen: electrophilic mechanism	9
Haloethyl amines	Hal—C—C—N—	Narcotic amines; Nucleophilic substitution	9, 29	No binding	9	Electrophilic mechanism	9
Haloethyl sulfides	Hal—C—C—S—	Nucleophilic substitution	29	Disulfide formation	9	Electrophilic mechanism	9

Haloethers		Basesurface narcotics; Nucleophilic substitution	9, 29	No binding	9	Electrophilic mechanism	9
Allylic alcohols		Alcohol dehydrogenase activation	1, 8, 10, 41	No binding	9	No binding	9
Propargylic alcohols		Alcohol dehydrogenase activation	1, 10, 42,43, 44-46	No binding	9	No binding	9
Quinones		Michael-type nucleophilic addition	1, 10, 29, 47, 48	Pro- Michael-type acceptors, activated by oxidation (Michael-type nucleophilic addition);	9, 11	No binding	9
Unsaturated amides		Addition to activated carbon-carbon double bond	1, 10, 29	Michael-type nucleophilic addition	9	No binding	9
Allylic cyano group		Various; Addition to activated carbon-carbon double bond	1, 8, 10, 29	No binding	9	No binding	9
Allylic nitro group		Addition to activated carbon-carbon double bond	1, 10, 29	No binding	9	Nitroreductase	9

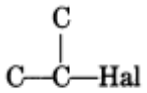
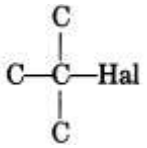
Allylic sulfone group	—C=C—S(O)$_2$—	Addition to activated carbon-carbon double bond	1, 10, 29	No binding	9	No binding	9
Allylic carboxy group	—C=C—C(0)—OH	Addition to activated carbon-carbon double bond	1, 10, 29	No binding	9	No binding	9
Allylic carbonyl group (aldehydes and ketones) ; α,β-Unsaturated carbonyl compounds;	—C=C—C(0)—H —C=C—C(0)—	Michael-type nucleophilic addition; Schiff base formation	1-10	Michael-type nucleophilic addition; Schiff base formation	9, 11, 12, 13	Michael-type nucleophilic addition	9, 14, 15, 16
Styrenes		Basesurface narcotics	1, 10	No binding	9	No binding	9
Allylic halides	—C=C—C—Hal	Nucleophilic substitution	1, 10, 29, 49-51	Nucleophilic substitution of haloaliphatics	9	Electrophilic mechanism	9
Benzylic halides	C(ar)—C—Hal	Nucleophilic substitution	1, 10	Nucleophilic substitution of haloaliphatics	9	Electrophilic mechanism	9

Diazo compounds		Nucleophilic substitution	1, 9, 10, 29	No binding	9	Electrophilic mechanism	9
Aromatic azo				No binding	9	Decomposition of azoarenes to active species (radicals) – Radical mechanism	9
Mustards (Nitrogene mustard)		Narcotic amines	1, 10	No binding	9	Electrophilic mechanism	9
Disulfides		Miscellaneous reactions	1, 10, 29	No binding	9	No binding	9
Sulfenyl halides		Miscellaneous reactions	1, 10, 29	Disulfide formation	9	No binding	9
Peroxides			1, 10	Peroxide free radical decomposition	9	No binding	9
Thiocyanates			1, 10	Disulfide formation	9	No binding	9
Hydrazines			1, 10	No binding	9	Unknown Nucleophilic addition Reactions	74, 9

N-nitroso compounds	Monooxygenase-mediated N-demethylation transformation followed by tauto-merism from p-nitrosoaniline to the corresponding Michael-type acceptor quinoid oxime structure.	1,8, 10	No binding	9	Unknown	104
C-nitroso compounds		1, 10	No binding	9	No binding	9

1) Allylic and propargylic nitriles, or 2) α-halogenated substituted nitriles		Cyanide release via cyanohydrin-like toxicant; The resulting intermediate liberates formaldehyde, a Schiff-base electrophile, and p-chloroaniline on hydrolysis; Two different mechanisms acting concurrently: (a) Michael-type addition across the double bond conjugated to two cyano-groups with covaent bond formation, and (b) corresponding addition of water with ready loss of malononitrile and *o*-chloro-benzaldehyde. Malononitrile can undergo further Metabolic transformation to generate two moles of cyanide; *o*-chlorobenzaldehyde can act as an electrophile by Schiff-base formation; Alkylation, S_N2	1, 10, 29, 52-54	1) No binding; 2) Nucleophilic substitution of haloaliphatics	9	1) No binding; 2) Electrophilic agent	9
Oximes	 aldoxime ketoxime		1, 10	Oxime group condensation	9	No binding	9

β-Halogenated alcohols			1, 10	No binding	9	Electrophilic agent	9
Halogenated acetophenones			1, 10	1. Nucleophilic addition to ketones; 2. Nucleophilic substitution of haloaliphatics	9	Electrophilic agent	9
Pyridiniums ammonium compounds		Various	1, 10, 55, 56	No binding	9	No binding	9
Quaternary ammonium compounds			1, 10	Electrostatic interaction with proteins	9	No binding	9
Diketones		Unknown	1, 2, 8, 10	Schiff base formation; Nucleophilic cycloaddition to diketones	13, 62 9	No binding	9

Primary alkyl monohalide	—C—Hal	Basesurface narcotics; Nucleophilic substitution	9	No binding	9	Ambiguous mechanisms; Electrophilic agent	75, 80, 9
Secondary alkyl monohalide		Basesurface narcotics; Nucleophilic substitution	9	No binding	9	Ambiguous mechanisms; Electrophilic agent;	75, 80, 9
Tertiary alkyl monohalide		Basesurface narcotics; Nucleophilic substitution	9	No binding	9	Ambiguous mechanisms; Electrophilic agent;	75, 80, 9
Alkyl multihalide		Basesurface narcotics	9	No binding	9	Ambiguous mechanisms; Electrophilic agent;	75, 80, 9
Ethylene sulfide				Nucleophilic heterocycle ring opening	9	No binding	9
Cyclopropane				No binding	9	No binding	9
Oxetane		Basesurface narcotics	9	No binding	9	No binding	9
Trimethyleneimine		Basesurface narcotics	9	No binding	9	No binding	9

Aromatic primary amine			No binding	9	ambiguous mechanisms; electrophilic reactants	81-102, 9
Aromatic secondary amine					ambiguous mechanisms; electrophilic reactants	81-102, 9
Aromatic tertiary amine					ambiguous mechanisms; electrophilic reactants	81-102, 9
Aromatic sulfonamide			No binding	9	No binding	9
Aliphatic primary amine	Narcotic amines	9	No binding	9	No binding	9
Hydroxylamine			No binding	9	No binding	9
Pyridine-N-oxide			No binding	9	No binding	9
Diazonium salt			No binding	9	No binding	9
Hydrazone			No binding	9	Nucleophilic addition Reactions	9
Nitryl			No binding	9		
Anilide			No binding	9	No binding	9

Carboxylic acid amide		Basesurface narcotics	9	No binding	9	Unknown	103
Sulfamide				No binding	9	No binding	9
Halogenated aromatic ring	$C_6H_5—Hal$	Basesurface narcotics; Nucleophilic substitution	9	No binding	9	No binding	9
Sulfonic acid				No binding	9	No binding	9

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