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Number 120

REPORT OF THE EXPERT CONSULTATION ON SCIENTIFIC AND REGULATORY EVALUATION OF ORGANIC CHEMISTRY MECHANISM-BASED STRUCTURAL ALERTS FOR THE IDENTIFICATION OF DNA BINDING CHEMICALS

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Series on Testing and Assessment

No. 120 PART 2

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IOMC

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among FAO, ILO, UNEP, UNIDO, UNITAR, WHO and OECD

Environment Directorate

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

Paris 2010

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No. 73, *Report of the Validation of the Rat Hershberger Assay: Phase 3: Coded Testing of Androgen Agonists, Androgen Antagonists and Negative Reference Chemicals by Multiple Laboratories. Surgical Castrate Model Protocol (2007)*

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The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 31 industrialised countries in North America, Europe and the Asia and Pacific region, as well as the European Commission, meet to co-ordinate and harmonise policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD's work is carried out by more than 200 specialised committees and working groups composed of member country delegates. Observers from several countries with special status at the OECD, and from interested international organisations, attend many of the OECD's workshops and other meetings. Committees and working groups are served by the OECD Secretariat, located in Paris, France, which is organised into directorates and divisions.

The Environment, Health and Safety Division publishes free-of-charge documents in ten different series: **Testing and Assessment**; **Good Laboratory Practice and Compliance Monitoring**; **Pesticides and Biocides**; **Risk Management**; **Harmonisation of Regulatory Oversight in Biotechnology**; **Safety of Novel Foods and Feeds; Chemical Accidents; Pollutant Release and Transfer Registers; Emission Scenario Documents; and the Safety of Manufactured Nanomaterials.** More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (http://www.oecd.org/ehs/).

This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organizations.

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The participating organisations are FAO, ILO, OECD, UNEP, UNIDO, UNITAR and WHO. The World Bank and UNDP are observers. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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FOREWORD

This document is a report of the expert consultation held on 20 October 2009 with the aim to evaluate a set of structual alerts for estimating covalent binding of chemicals with DNA. This consultation was held based on a key recommendation from the OECD Workshop on Structural Alerts for the OECD (Q)SAR Application Toolbox held in May 2008 [see ENV/JM/MONO(2009)4]. The resulting set of alerts will be implemented in version 2.0 of the OECD (Q)SAR Application Toolbox, which is to be released in 2010.

This document is published on the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology of the OECD.

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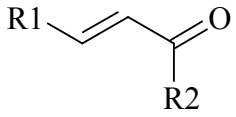
5. Meta Data for Michael Addition Mechanistic Domain

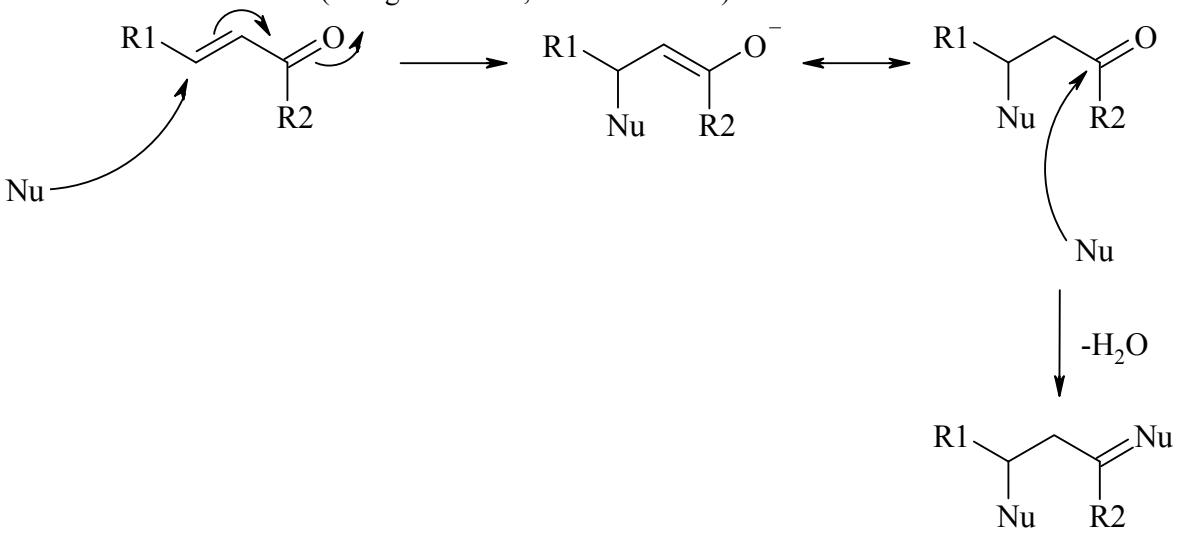
Alert MA01: α, β-Unsaturated carbonyls



R1 = alkyl, hydrogen,
R2 = hydrogen, alkyl, alkoxy

Mechanism

An initial Michael addition mechanism has been suggested to be primarily responsible for the ability of these chemicals to alkylate DNA. A subsequent Schiff base reaction at the carbonyl can result in cross linked DNA adducts (Wang et al 2009, Stein et al 2006).



Nu = biological nucleophile

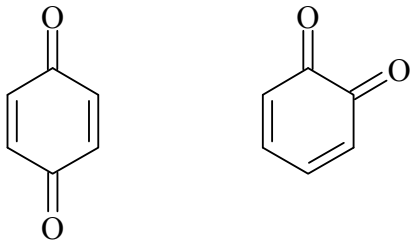
Mitigating factors

- No mitigating factors have been reported

References

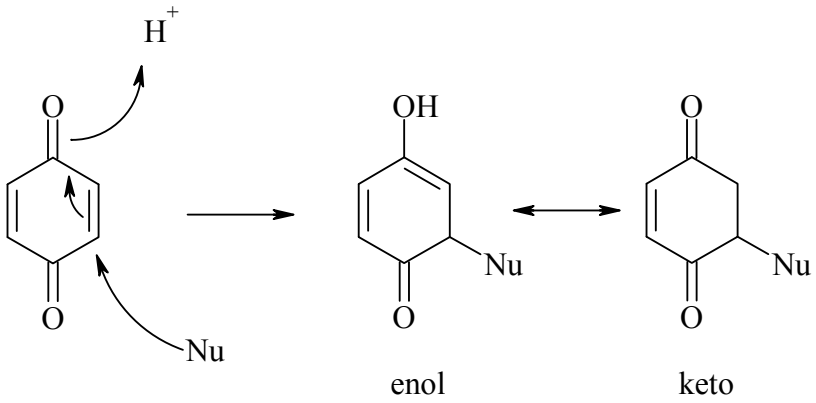
Stein et al (2006) Mutation Research, 608, p1-7
Wang et al (2009) Chemical Research in Toxicology, 22, p511-517

Alert MA02: Quinones



Mechanism

A Michael addition mechanism has been suggested result in a range of DNA adducts (Bolton et al 2008, Stack et al 2008, Saeed et al 2007).



Nu = biological nucleophile
N.B. the Michael addition product exists as a mixture of tautomers (enol and keto forms)

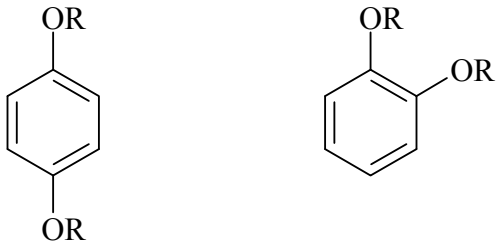
Mitigating factors

- No mitigating factors have been reported

References

Bolton et al (2008) Chemical Research in Toxicology, 21, p93-101
Saeed et al (2007) Chemico-Biological Interactions, 165, p175-188
Stack et al (2008) Chemical Research in Toxicology, 21, p1415-1425

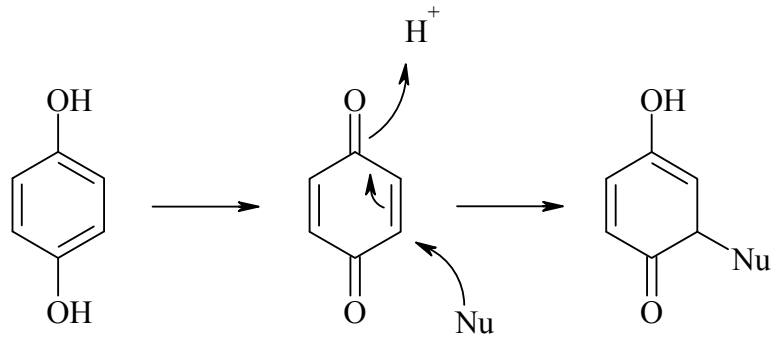
Alert MA03: Hydroquinones



R = hydrogen, methyl

Mechanism

Hydroquinones have been shown to be oxidised to quinones which can then bind to DNA via a Michael addition mechanism (McGregor 2007, Kalgutkar 2005). Methoxy quinones undergo demethylation to produce the corresponding hydroquinone.



Nu = biological nucleophile

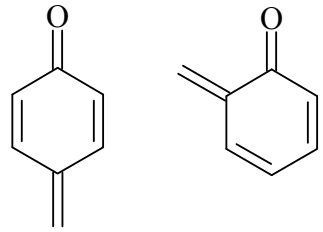
Mitigating factors

- No mitigating factors have been reported

References

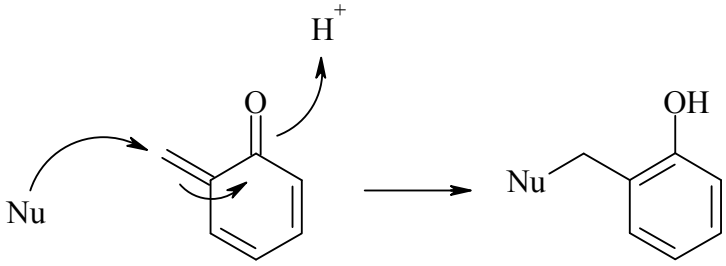
McGregor (2007) Critical Reviews in Toxicology, 37, p887-914
Kalgutkar (2005) Current Drug Metabolism, 6, p161-225

Alert MA04: Quinone-methides



Mechanism

A Michael type addition mechanism has been shown to lead to alkylation of DNA (Zhou et al 2007, Weinert et al 2006 and Kalgutkar 2005).



Nu = biological nucleophile

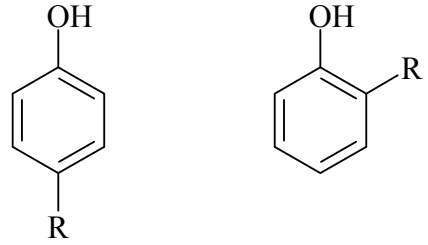
Mitigating factors

- No mitigating factors have been identified

References

Kalgutker (2005) Current Drug Metabolism, 6, p161-225
Weinert et al (2006) Journal of the American Chemical Society, 128, p11940-11947
Zhou et al (2007) Chemical Research in Toxicology, 20, p1069-1074

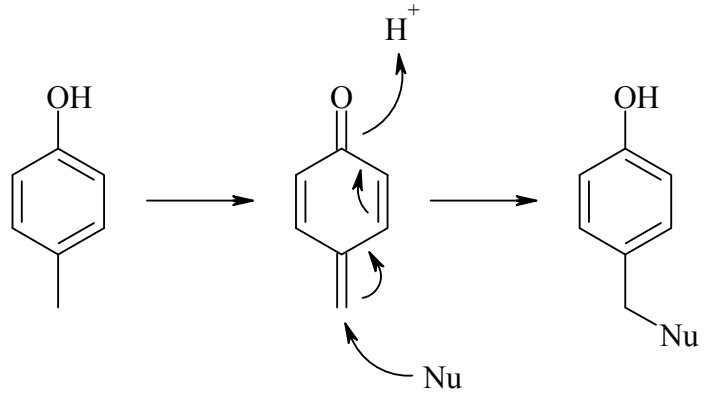
Alert MA05: Alkyl phenols



R = alkyl

Mechanism

Oxidation by cytochrome P450 to a quinone methide followed by Michael addition has been suggested to be the primary route of DNA binding (Gaikwad et al 2003, 2001, Thompson et al 1995a, 1995b).



Nu = biological nucleophile

Mitigating factors

- No mitigating factors have been identified

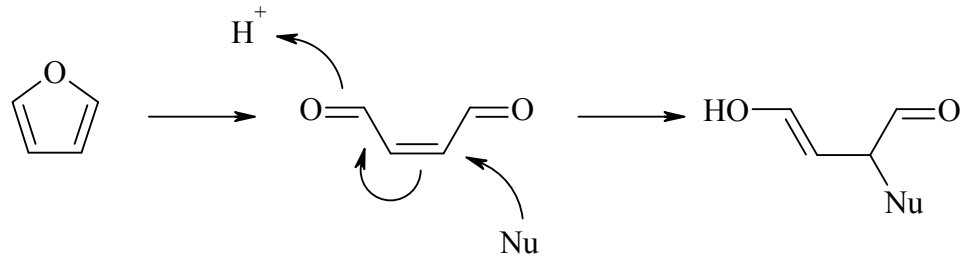
References

Gaikwad et al (2003) Chemico-Biological Interactions, 145, p149-158
Gaikwad et al (2001) Chemico-Biological Interactions, 138, p217-229
Thompson et al (1995a) Chemical Research in Toxicology, 8, p55-60
Thompson et al (1995b) Chemical Research in Toxicology, p323-327

Alert MA06: Furans

Mechanism

A cytochrome P450 mediated ring opening reaction producing a reactive dial capable of undergoing Michael addition has been proposed (Kellert et al 2008, Peterson et al 2006, Kalgutkar 2005).



Nu = biological nucleophile

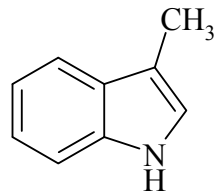
Mitigating factors

- No mitigating factors have been reported

References

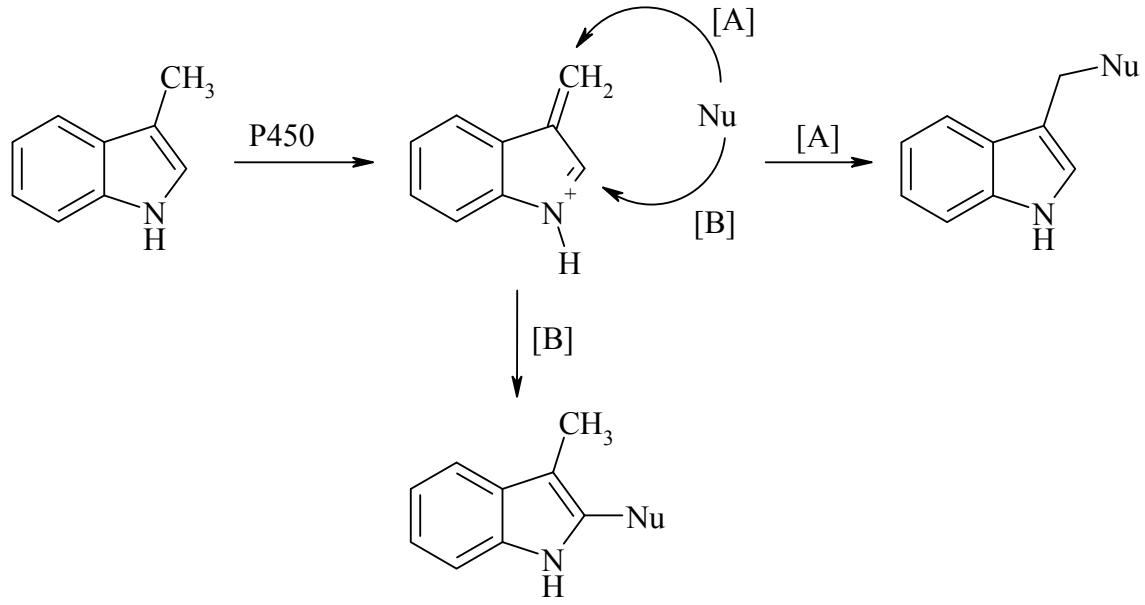
Kalgutkar (2005) Current Drug Metabolism, 6, p161-225
Kellert et al (2008) Mutation Research, 657, p127-132
Peterson (2006) Drug Metabolism Reviews, 38, p615-626

Alert MA07: 3-Methylindole derivatives



Mechanism

P450 dehydrogenation results in an imine-methide intermediate capable of undergoing Michael addition with biological nucleophiles (Kalgutkar et al 2005, Regal et al 2001).



Nu = biological nucleophile

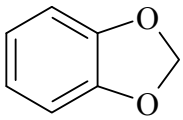
Mitigating factors

- No mitigating factors have been reported

References

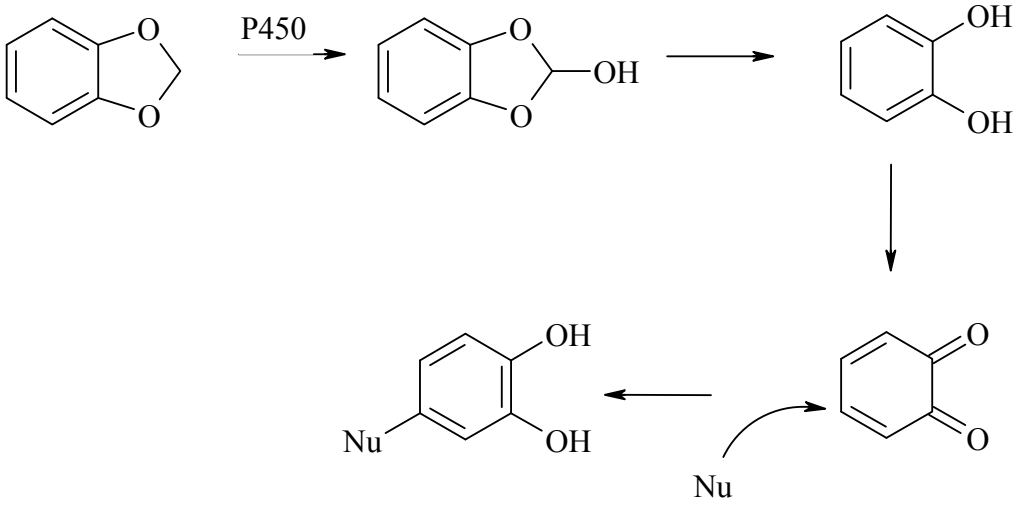
Kalgutkar et al (2005) Current Drug Metabolism, 6, p161-225
Regal et al (2001) Chemical Research in Toxicology, 14, p1014-1024

Alert MA08: Methylenedioxyphenyl



Mechanism

Methylene dioxyphenyl is metabolised by P450 into an ortho substituted phenol (Kalgutkar et al 2005, Beaumont et al 1996). Ortho-substituted phenols can then be further metabolised into quinones which are capable of DNA binding via Michael addition (Bolton et al 2008, Stack et al 2008, Saeed et al 2007).



Nu = biological nucleophile

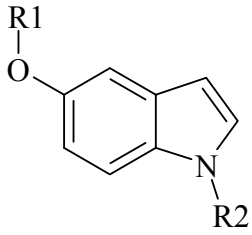
Mitigating factors

- No mitigating factors have been reported

References

Beaumont et al (1996) Xenobiotica, 26, p459-471
Bolton et al (2008) Chemical Research in Toxicology, 21, p93-101
Kalgutkar et al (2005) Current Drug Metabolism, 6, p161-225
Saeed et al (2007) Chemico-Biological Interactions, 165, p175-188
Stack et al (2008) Chemical Research in Toxicology, 21, p1415-1425

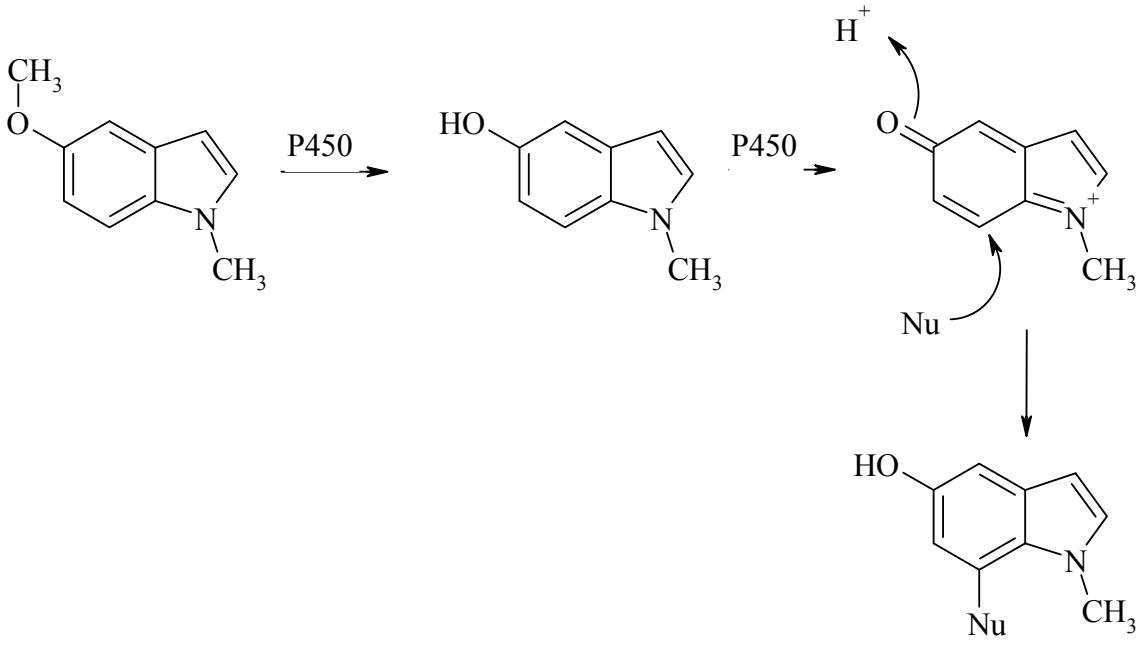
Alert MA09: 5-Alkoxyindoles



R1 = Methyl, Hydrogen
R2 = any

Mechanism

A P450 mediated mechanism producing a quinone type species has been suggested as the primary route of toxicity. This species can then react with biological nucleophiles via a Michael addition mechanism (Kalgutkar et al 2005).



Nu = biological nucleophile

Mitigating factors

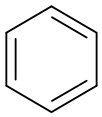
The following mitigating factor has been identified

- R1 must be methyl or hydrogen in order to be oxidised to the quinone.

References

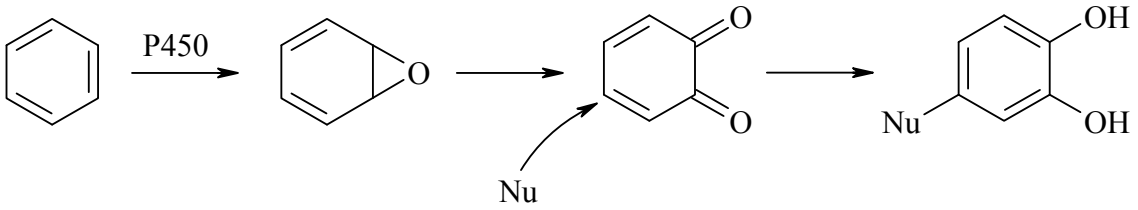
Kalgutkar et al (2005) Current Drug Metabolism, 6, p161-225

Alert MA10: Arenes



Mechanism

A P450 mediated epoxidation followed by conversion to a reactive quinone has been postulated as the primary cause of benzene derivatives ability to bind to biological nucleophiles (via a Michael addition mechanism) (Ishihama et al 2008, Saghir et al 2009).



Nu = biological nucleophile

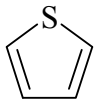
Mitigating factors

- No mitigating factors have been reported

References

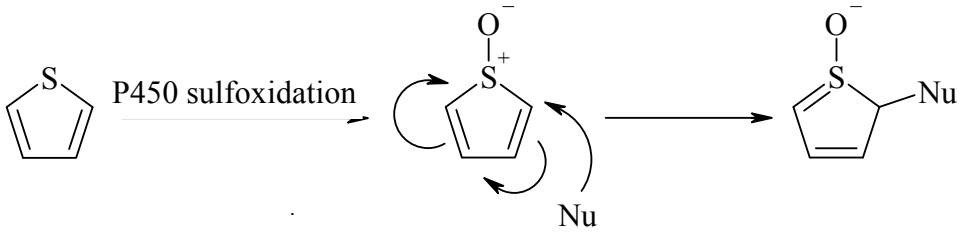
Ishihama et al (2008) Toxicology in Vitro, 22, p1861-1868
Saghir et al (2009) Toxicological Sciences, 107, p352-366

Alert MA11: Thiophenes



Mechanism

A P450 mediated sulfoxidation followed by Michael type addition has been suggested as a potential mechanism leading to DNA alkylation (Kalgutkar et al 2005, Mosier et al 2003).



Nu = biological nucleophile

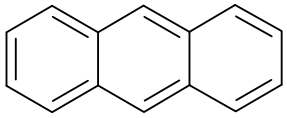
Mitigating factors

- No mitigating factors have been reported

References

Kalgutkar et al (2005) Current Drug Metabolism, 6, p161-225
Mosier et al (2003) Chemical Research in Toxicology, 16, p721-732

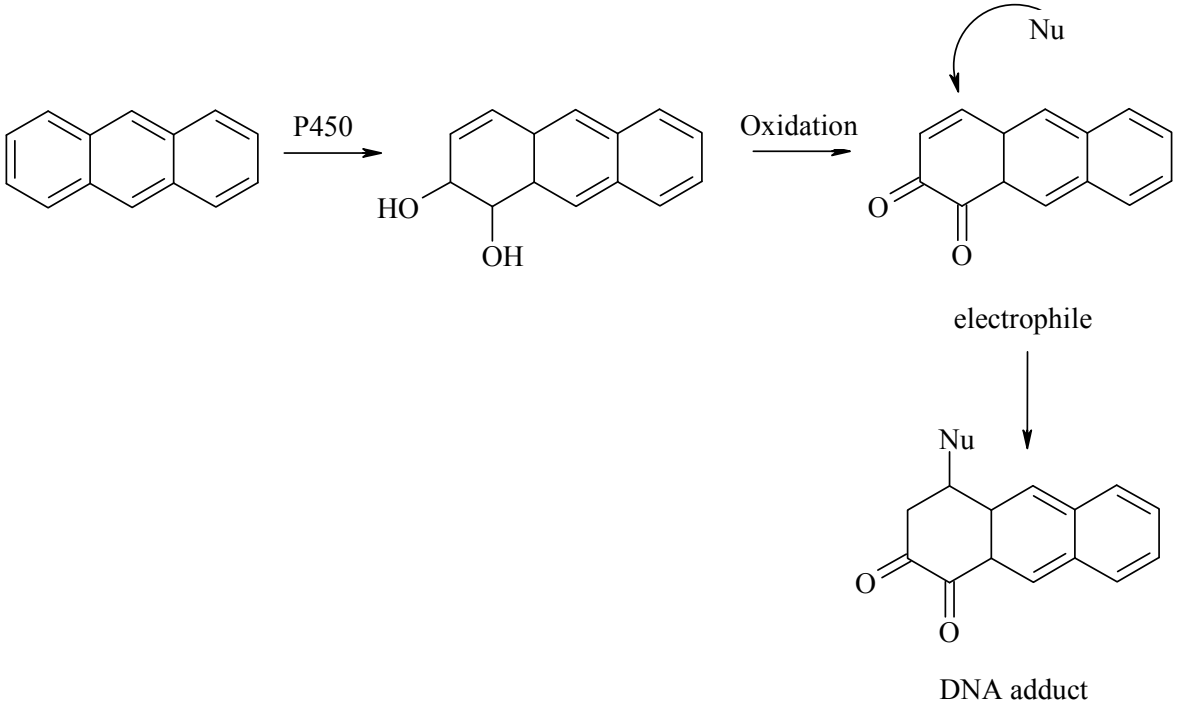
MA12: Alert: polycyclic (PAHs) and heterocyclic (HACs) aromatic hydrocarbons



(any C in the above structures can be substituted for N)

Mechanism

PAHs and HACs without bay region can undergo oxidation to quinone like species (Desler et al 2009, Xue et al 2005). These quinones are then susceptible to Michael addition reactions (Nu = biological nucleophile).



Nu = biological nucleophile

Mitigating factors

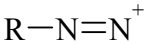
- No mitigating factors have been reported

References

Desler et al (2009) Chemico-Biological Interactions, 177, 212-217
Xue et al (2005) Toxicology and Applied Pharmacology, 206, 73-93

6. Meta Data for Radical Mechanistic Domain

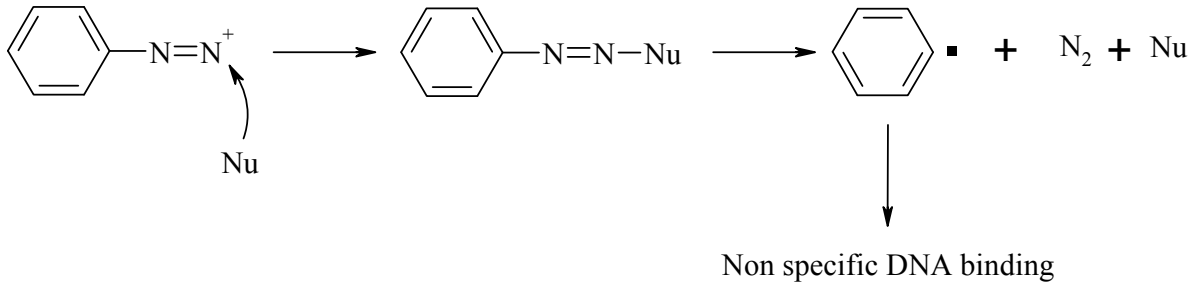
Alert RD01: Diazonium



R = aryl

Mechanism

A mechanism involving the loss of N_2 to produce a reactive radical species that can alkylate DNA via a radical mechanism has been proposed (Laufer et al 2002).



Nu = biological nucleophile

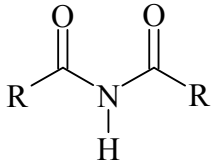
Mitigating factors

- No mitigating factors have been reported

References

Laufer et al (2002) Journal of the American Chemical Society, 124, p1854-1855

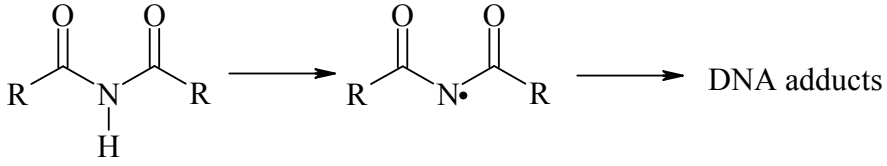
Alert RD02: Imides



R = any

Mechanism

The formation of an imide radical has been postulated as being capable of reacting with biological nucleophiles (Kalgutkar et al 2005).



Mitigating factors

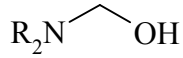
- No mitigating factors have been reported

References

Kalgutkar et al (2005) Current Drug Metabolism, 6, p161-225

7. Meta Data for Schiff Base Mechanistic Domain

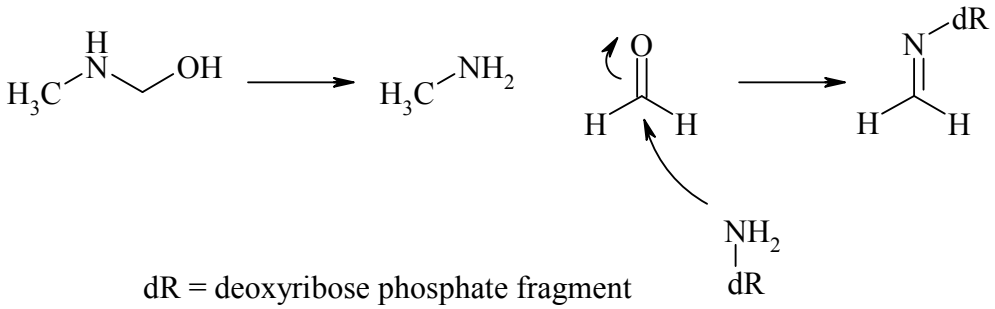
Alert SB01: N-methylol derivatives



R = alkyl, aryl, H

Mechanism

N-methylol derivatives have been suggested to be genotoxic via hydrolysis into formaldehyde (Ashby et al 1985). Formaldehyde then undergoes DNA binding via a Schiff base reaction (Cheng et al 2003).



dR = deoxyribose phosphate fragment

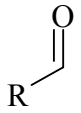
Mitigating factors

- No mitigating factors have been reported

References

Ashby et al (1985) Mutation Research, 156, 19-32
Cheng et al (2003) Chemical Research in Toxicology, 16, 145-152

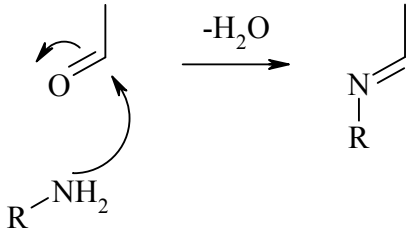
Alert SB02: Aliphatic aldehydes



R = akyl C, H

Mechanism

Aliphatic aldehydes undergo Schiff base formation (Garcia et al 2009, Hecht et al 2001).



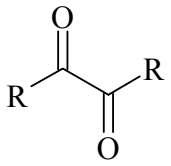
R = DNA chain

Mitigating factors
The following mitigating factors have been identified

- Aromatic aldehydes are not reactive enough due to the carbonyl electrons being involved in the aromatic ring systems. At least a single aliphatic atom is required between the carbonyl group and any aromatic system for activity.

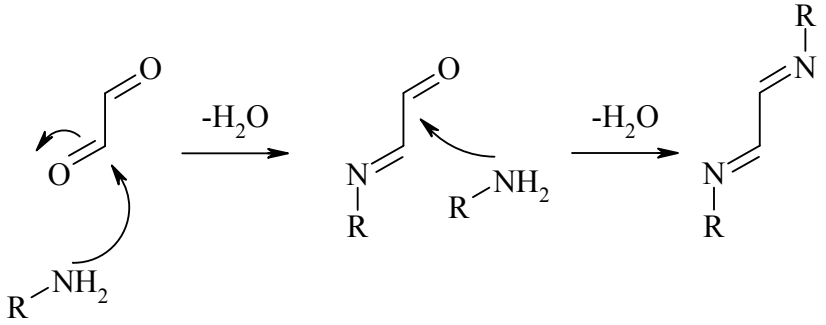
References
Cheng et al (2003) Chemical Research in Toxicology, 16, 145-152
Garcia et al (2009) Mutation Research, 662, 3-9

Alert SB03: Alpha-dicarbonyl



R = alkyl, aryl, hydrogen

Mechanism
A multi step Schiff base mechanism has been proposed (Mellado et al 1994, Dorado et al 1992).



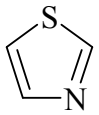
R = DNA chain

Mitigating factors

- No mitigating factors have been identified

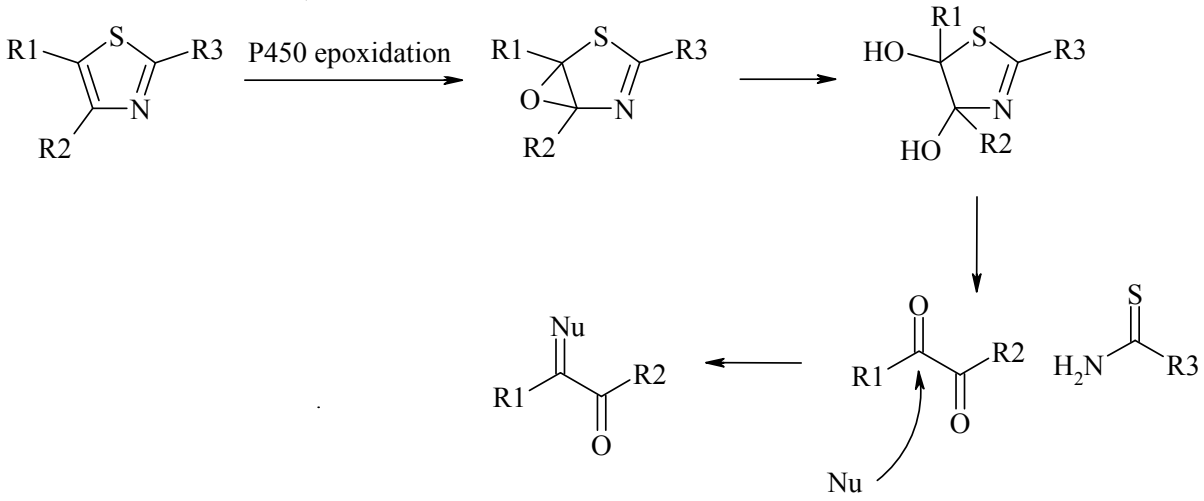
References
Dorado et al (1992) Mutation Research, 269, p301-306
Mellado et al (1994) Mutation Research, 304, p261-264

Alert SB04: Thiazoles



Mechanism

Epoxidation followed by ring scission have been suggested to produce potentially toxic α,β-unsaturated carbonyl metabolites which can bind DNA via a Schiff base mechanism (Kalgutkar et al 2005, Mizutani et al 1994).



Nu = biological nucleophile
R1, R2, R3 = alkyl, aryl, hydrogen

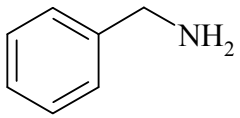
Mitigating factors

- No mitigating factors have been reported

References

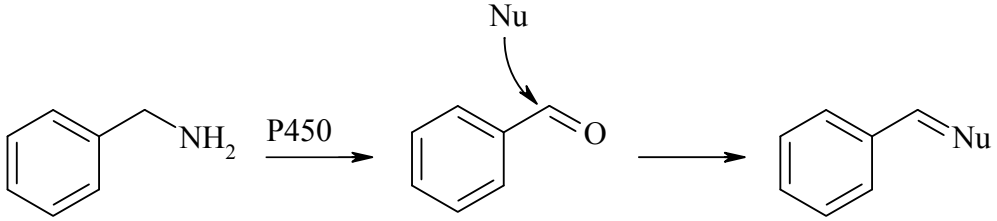
Kalgutkar et al (2005) Current Drug metabolism, 6, p161-225
Mizutani et al (1994) Drug Metabolism and Disposition, 22, p750-755

Alert SB05: Benzylamines



Mechanism

Benzylamines have been shown to be metabolised into several reactive species capable of covalently binding to biological nucleophiles via a Schiff base mechanism (Kalgutkar et al 2005, Mutlib et al 2002).



Mitigating factors

- No mitigating factors have been reported

References

Kalgutkar et al (2005) Current Drug Metabolism, 6, p161-225
Mutlib et al (2002) Chemical Research in Toxicology, 15, p1190-1207

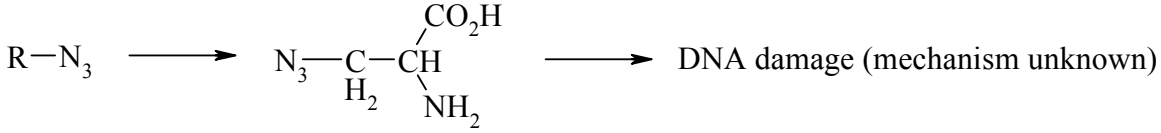
8. Meta Data for Unclear Mechanistic Domain

Alert UN01: Azides

N=[N+]=[N-]

Mechanism

It has been suggested that azides are metabolised into L-azidoalanine, however the precise mechanism resulting in DNA binding is unknown (Sadiq et al 2000,Owais et al 1988).



azide azidoanaline

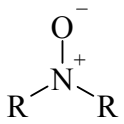
Mitigating factors

- No mitigating factors have been reported.

References

Sadiq et al (2000) Mutation Research, 469, p253-257
Owais et al (1988) Mutation Research, 197, p313-323

Alert UN02: Aromatic ring N oxides



R = aromatic ring system

Mechanism

Aromatic ring N-oxides have been reported as being genotoxic (Benigni et al 2008). However, no clear mechanism has been reported in the literature.

Mitigating factors

- None have been reported

References

Benigni et al (2008) Mutation Research, 659, p248-261

ANNEX 5: PRESENTATION BY LIVERPOOL JOHN MOORES UNIVERSITY

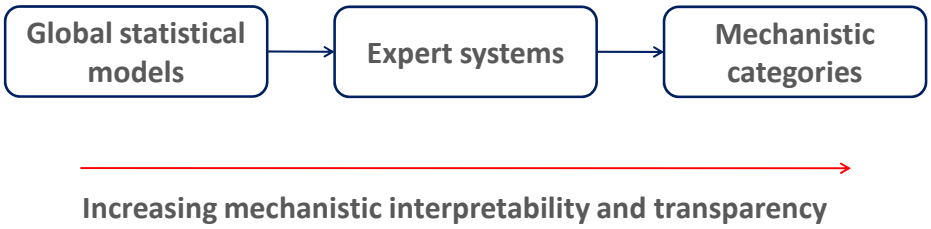
Re-evaluation of Structural Alerts for the Binding of Molecules to DNA and the Development of a Comprehensive Profiler of Alerts (Deliverable D2.10)

Steven Enoch and Mark Cronin
School of Pharmacy and Chemistry
Liverpool John Moores University

Aims of Deliverable 2.10

- **Review currently published structural alert compilations**
- **Perform a mechanistic chemistry analysis of these alerts to generate the 'meta data'**
- **Compile a new set of alerts for the OECD Toolbox**
- **No validation was to be undertaken**

QSAR Modelling Approaches



Global Statistical: MultiCASE

- Biophores and biophobes are automatically detected from a training set of chemicals
- These are structural fragments are then related to DNA binding using statistics
- The resulting global model can then be used to predict the DNA binding of new compounds
- Potential mechanisms are rationalised *after* modelling
- However, biophores and biophobes are sometimes, but not always related to the electrophilic chemistry

Global Statistical: TOPKAT

- QSARs developed from literature and regulatory data
- Based on statistical techniques such as discriminant analysis
- 2-D descriptors are utilised
- No real mechanistic understanding can be placed on the models

Expert Systems: Derek / OncoLogic / TIMES

- Mechanistic knowledge derived from the toxicological literature
- Systems give an indication of likely toxicity based on a decision tree approach utilising mechanistic knowledge and physico-chemical parameters
- End-user cannot develop their own (Q)SAR or undertake trend analysis using the embedded mechanistic information
- Rules from TIMES form the basis of the current Toolbox DNA Binding Profiler

Mechanistic Categories: (Q)SAR

- Numerous (Q)SARs have been developed for mechanistically related chemicals
- Quantitative predictions of DNA binding
- Well defined applicability domain
- Development of the mechanistic categories allows the end-user to develop their own (Q)SAR using previous derived expert knowledge
- Require and can be supported by literature data to give a basis to the prediction and support the mechanism

Expert Systems vs. Categories

- Expert systems
  - Good for screening large numbers of chemicals
  - Limited applicability domain (or complete lack of)
  - Not all systems are transparent
  - Potentially expensive for the end-user
- Categories (and (Q)SAR)
  - Transparent methodology (important for regulatory use)
  - Mechanistically relevant
  - They are not limited to specific end-points - instead they relate to the underlying chemistry
  - Easily implemented into the current Toolbox architecture
  - Can be supplemented by literature / meta data

Electrophilic Reaction Chemistry

- Genotoxic chemicals are electrophiles (or can be metabolised into electrophiles)
- DNA contains nucleophilic centres
- Thus, principles derived from organic chemistry are essential in mechanistic category formation
- Structural alerts can be utilised to relate the chemistry to DNA binding

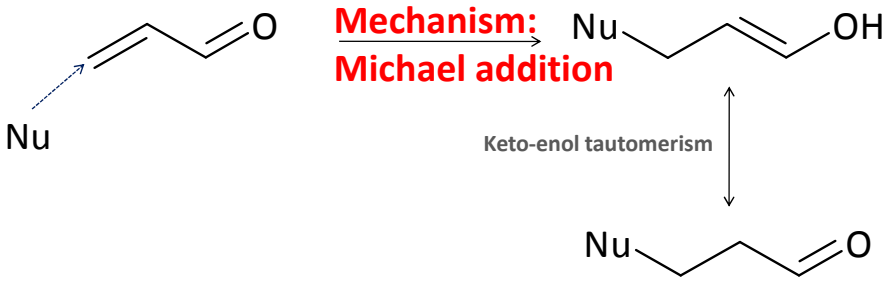
Electrophilic Reaction Chemistry

Mechanism? → DNA binding

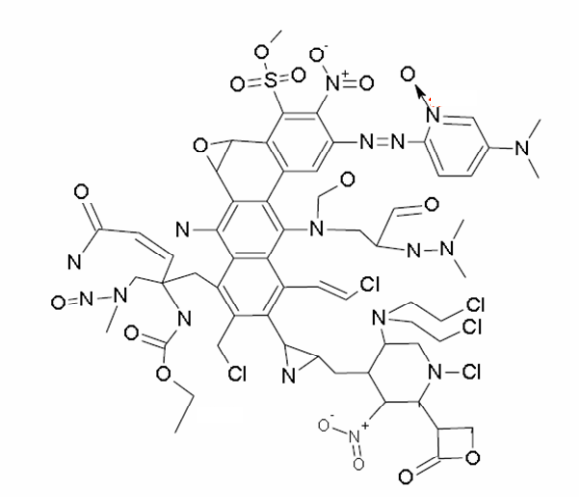
Electronegative carbonyl polarises the alkene

Electrophilic carbon atom

Electrophilic Reaction Chemistry



The Ashby and Tennant Supermolecule



Mechanistic Structural Alerts

- Mechanistic chemistry enables transparent category formation
- QSAR / read-across more likely to be successful within mechanistic domains
- Such analyses in keeping with OECD QSAR validation principles
- The new profiler is easier to implement into the current OECD Toolbox software architecture

Literature Structural Alert Compilations

- 19 genotoxicity alerts (Ashby and Tennant 1988)
- 48 chemical classes present in OncoLogic (Woo et al 1995)
- 29 mutagenicity alerts (Kazius et al 2005)
- 17 chromosomal aberration alerts related to DNA binding (Mekenyan et al 2007)
- 15 mutagenicity alerts (Serafimova et al 2007)
- 33 DNA binding alerts (Benigni / Bossa 2008)
- 28 alerts compiled from a review of bioactivation pathways (Kalgutkar et al 2005)

OECD Toolbox Alerts (TB)

- 22 structural alerts
- Derived from expert analysis of the mechanistic chemistry responsible for DNA adduct formation – originally from the TIMES system
- Analysis involved mutagenicity and chromosomal aberration data – relates CA to DNA binding
- Analysis also highlighted 30 potential metabolic activation pathways

Benigni / Bossa (BB)

- 33 genotoxic structural alerts
- Derived from expert analysis of previous structural alert lists including the list of Ashby and Tennant
- Analysis utilised mutagenicity and carcinogenicity data
- Alerts were validated showing an overall accuracy of 78 % for mutagenicity

Kazius et al (KS)

- 29 structural alerts
- Derived using a statistical analysis of a database of 4337 mutagenicity test results
- Alerts were validated using an external dataset and showed an overall accuracy of 82 %

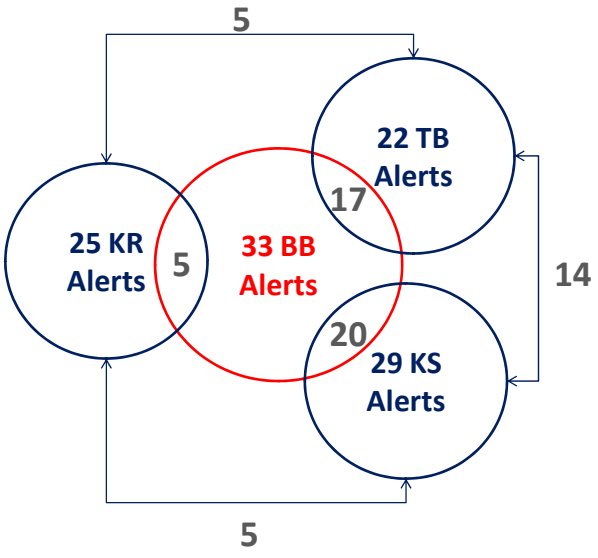
Kalgutkar et al (KS)

- Not a compilation of alerts as such, instead a review of bioactivation pathways
- Review not specifically aimed at genotoxicity rather at rationalising idiosyncratic adverse drug reactions
- However, many of these reactions are related to DNA binding
- Analysis of the review suggested 25 potential structural alerts related to organic functional groups

Literature Alerts 'Applicability Domain'

- Benigni / Bossa alerts are currently the most comprehensive compilation
- However, no detailed mechanistic chemistry analysis exists to complement these alerts
- Also, inclusion of potentially important data from idiosyncratic drug toxicity is lacking

Literature Alerts 'Applicability Domain'



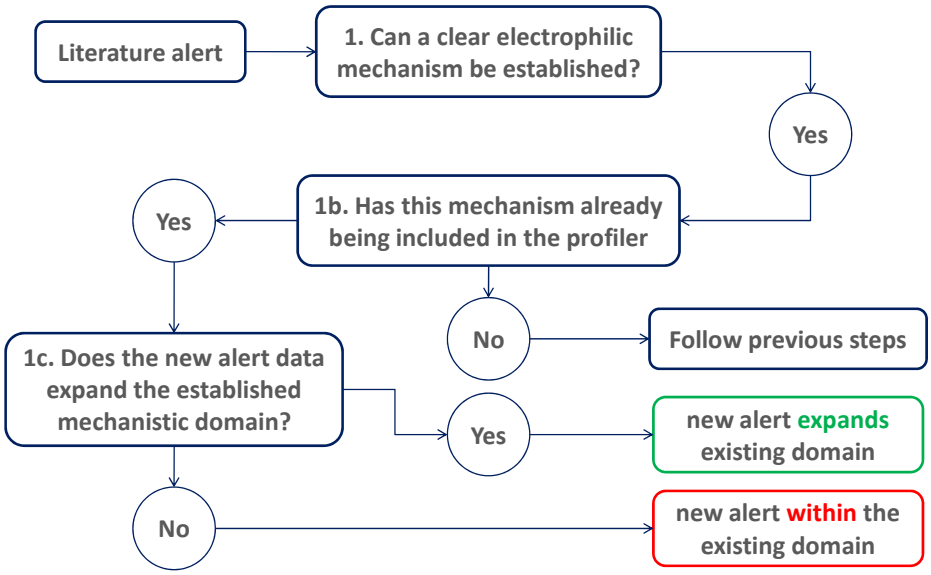
Methodology – Documented Information

- Establish a clear mechanism(s) for each literature alert
- Schematically document the mechanism
- Assign the mechanism to a mechanistic domain
  - Michael acceptor
  - Schiff base formation
  - S_NAr
  - S_N (which covers S_N1 and S_N2 mechanisms)
  - Radical
  - Unclear

Methodology – Information Not Documented

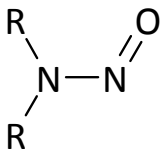
- Potential DNA adducts
- Toxicological data associated with each alert / mechanism
- Documented information is there to aid the user understand the chemistry behind the mechanism and thus the potential category

Alert Mapping



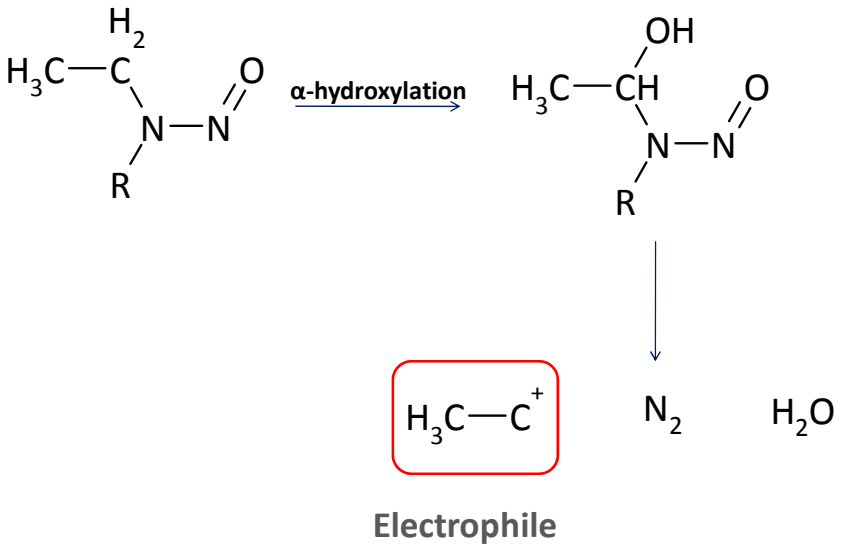
Development of Alert G01: nitroso

- TB16 alert for nitroso (R = alkyl, aryl)

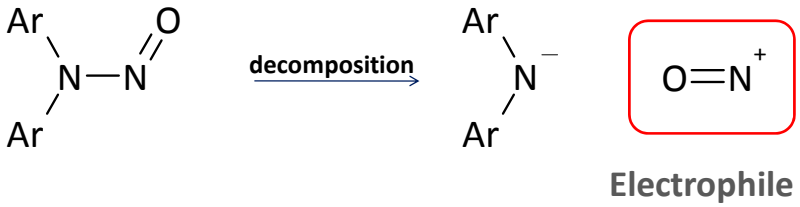


- Literature shows two potential S_N1 mechanisms depending on exact substitution

S_N1 Alkylation Mechanism

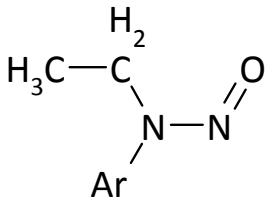


S_N1 Nitrosation Mechanism



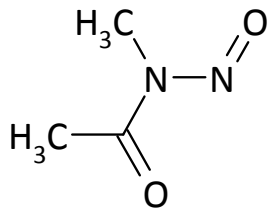
Development of Alert G01: nitroso

- TB16 has two potential S_N1 mechanisms
- Do any of the other alerts expand the alert domain?
- For example, do we know what mechanism is involved in?

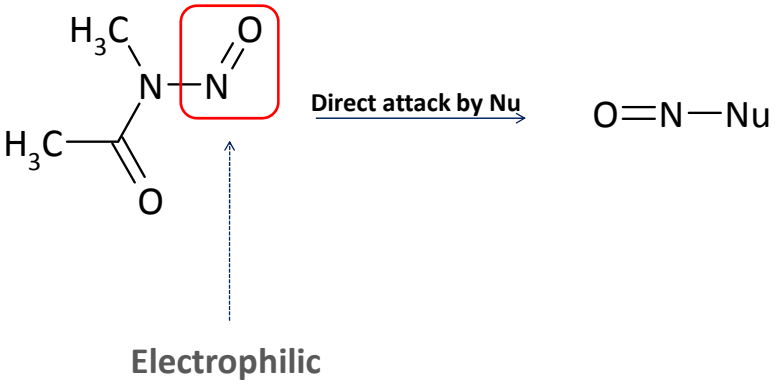


Development of Alert G01: nitroso

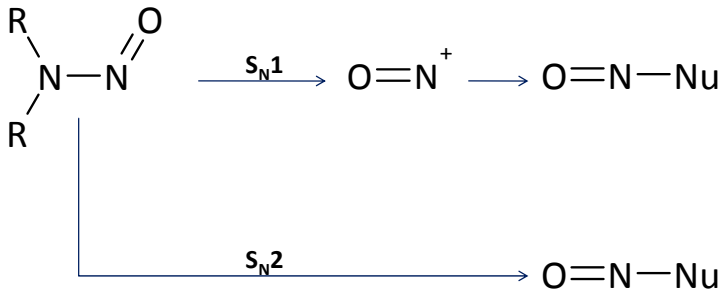
- Three other alerts cover the same area of chemistry
- Two of them **do not** expand the mechanistic domain
- However, the alert TB22 **does** expand the mechanistic domain



S_N2 Nitrosation Mechanism



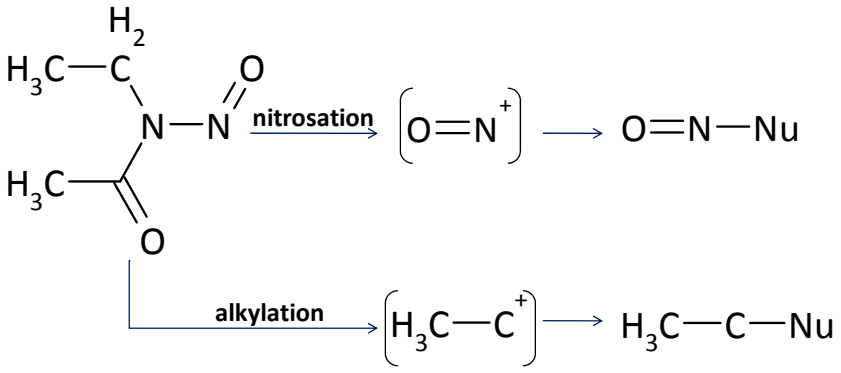
S_N1 vs. S_N2 Nitrosation Mechanism



R = aryl and / or electron withdrawing group

Importantly the same competition process occurs for the alkylation mechanism

Alkylation vs. Nitrosation Mechanisms



Development of Alert G01: nitroso

- **Two mechanisms are possible for nitroso compounds (depending on the nature of the R groups)**
- **An alkylation and nitrosation (both via a mixture of S_N1 and S_N2 mechanisms)**
- **Therefore the single nitroso alert SN15 is developed**
- **Alert is assigned to the S_N mechanistic domain**

Alert Development

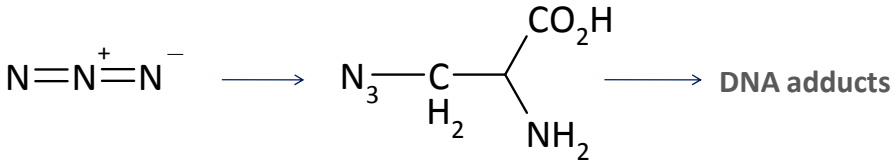
- **Mapping results in alerts being grouped together based on related mechanistic chemistry**
- **However, metabolically related chemicals generally have separate alerts in the profiler**
- **This being due to fact the metabolic step can be rate determining**

Alert Summary

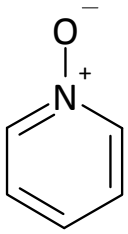
- **56 structural alerts for DNA binding**
- **54 supported by mechanistic chemistry from the literature**

Mechanistic domain	Number of alerts
Acylation (AC)	7
Michael addition (MA)	12
Schiff base (SB)	5
S_N (SN)	31
Radical (RD)	2
Unclear (UN)	2

Alerts with Unclear Mechanisms

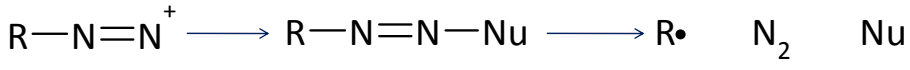


azide azidoaniline

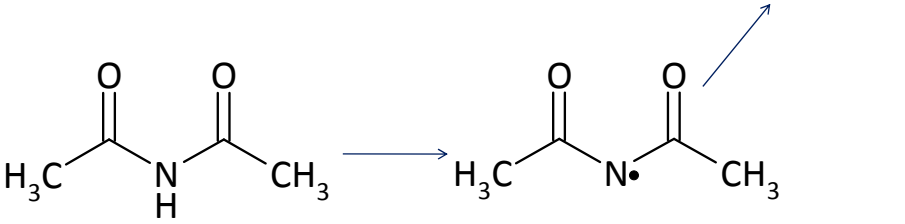


aromatic ring N-oxides

Alerts with Radical Mechanisms



Diazonium (R = aryl, Nu = nucleophile)

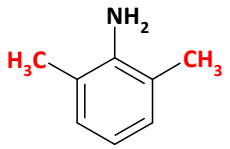
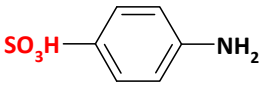
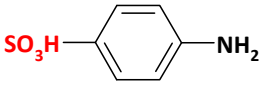


Imides

Mitigating Factors

- 12 alerts have been reported to have mitigating factors
- Such factors were only included in the profiler if they had been shown to completely remove toxicological activity
- Modulating factors were not encoded within the current alerts

Mitigating Factors

Mitigating factor	Example inactive chemical
Steric	
Electronic	
Detoxifying	

The 'Meta Data'

- Alert domain: the structural features that define the alert
- Mechanism: the electrophilic reaction chemistry
- Mitigating factors: structural features that remove activity
- References: literature supporting the mechanism and the mitigating factors

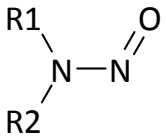
The 'Meta Data'

- The 'meta data' does not include:
  - Information about the types of DNA adducts formed
  - Results of toxicological testing
- Alert meta data grouped by mechanistic domains
- An alert can appear in more than a single domain

Alert: nitroso

R1 = any carbon

R2 = alkyl C, aryl C, C=O, C=N, S=O



Mechanism

- **Several mechanisms are possible depending on substitution at R1 and R2 (Wang et al 2007, Wang et al 2002, Cooper et al 2000). These can be summarised as follows (next slides):**

Mitigating factors

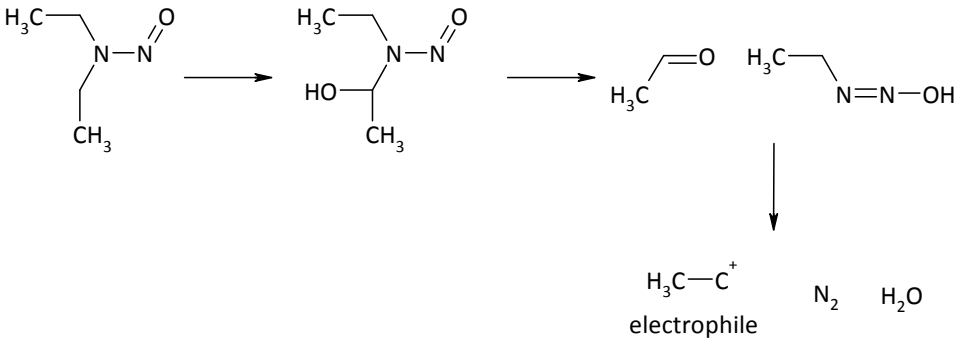
- **No mitigating factors have been reported**

References

- **Cooper et al (2000) Mutation Research, 454, 45-52**
- **Wang et al (2007) Chemical Research in Toxicology 20, 625-633**
- **Wang et al (2002) Chemical Reviews 102, 1091-1134**

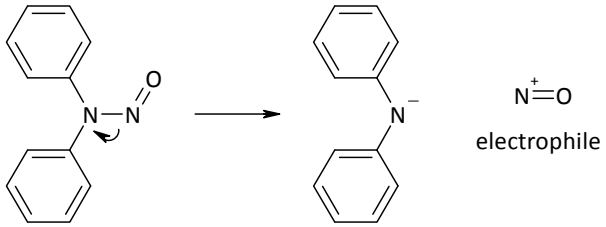
Mechanism

- ***Mechanism 1*: At least one alkyl R group: α-hydroxylation producing a carbenium ion leading to an S_N1 alkylation reaction**

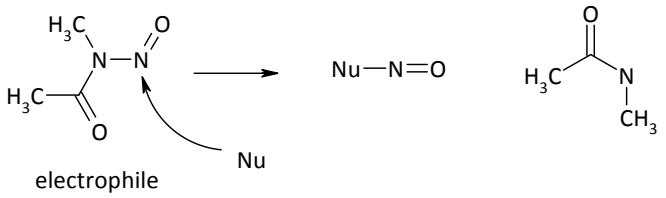


Mechanism

- ***Mechanism 2*: Both R groups aryl: N-N=O bond cleavage leading to nitrosation via an S_N1 mechanism**



- ***Mechanism 3*: One R group C=O, C=N, S=O, or aryl: direct S_N2 attack by a biological nucleophile (Nu) leading to nitrosation**



Use of the Alerts in the Toolbox

- **The new profiler will allow for the formation of categories on the basis of mechanistic chemistry, supported by meta data**
- **The categories will allow for read-across**
- **To be used along side Benigni-Bossa rules which are conservative; these rules are broader - both profilers will be in Toolbox**
- **The Benigni-Bossa rules are intended to predict genotoxicity; they are not intended to form categories**
- **Up to user to decide which to use**

Conclusions

- D2.10 aimed to develop a comprehensive listing of structural alerts based on current literature knowledge
- This has resulted in 56 alerts for DNA binding
- These alerts are supported by mechanistic chemistry
- This mechanistic information forms the 'meta data'
- No validation has been performed

Future Work

- Coding of the Alerts for the Toolbox
- Input of meta data information
- Checking of functionality of alerts
- Evaluation of performance of the alerts

ANNEX 6: REVIEW REPORT BY DR BENIGNI

The Updated DNA binding profiler: comments

Romualdo Benigni

Istituto Superiore di Sanita'
Rome Italy

The Updated DNA binding profiler

• Focus on covalent DNA binding

• One of the main pathways to DNA damage, possibly followed by mutation (including gene mutation and chromosomal effects) and cancer

• Mutations may also arise from non-covalent DNA binding (e.g., intercalation 4), and other phenomena (e.g., unbalanced pool of nucleotides, interaction with proteins such as in mitotic spindle)

Main information sources (besides old DNA binding profiler)

	Tailored on
• **Toxtree rulebase for mut/canc (SA_BB)** (mechanistic)	Biological endpoint (Canc / Mut)
• **Kazius et al., 2005** (data mining plus mechanistic)	Biological endpoint (Mut)
• **Kalgutkar et al., 2005** (mechanistic)	Bioactivation

Results:

The Updated DNA binding profiler (SA_DNA_cov)

and

Classification of SAs into six broad organic chemistry mechanisms

- Michael acceptor
- Acylation
- Schiff base formation
- SNAr
- SN (SN2 and SN1)
- Radical mechanisms

Specific Queries for the Reviewers:

• **Query 1.** Please comment on the completeness of literature reviewed. Please indicate any additional literature, which you feel would further clarify or support SAs for DNA-binding.
• **Query 2.** Please comment on the adequacy and completeness of the SAs for forming categories based on mechanisms of DNA-binding.
• **Query 3.** Please comment on the adequacy of the mitigating factors, affecting either toxicokinetics or toxicodynamics, which alter DNA-binding ability.
• **Query 4.** Please comment on documentation associated with each alert. In particular is the rationalization complete yet easy to follow.
• **Query 5.** Please comment on the associated confidence noted for each alert, especially for those alerts where you feel the confidence may be misstated.
• **Question 6.** Please make any further suggestions for improvements in presenting the SAs and their underlying rationale.

Specific Queries for the Reviewers:

• **Query 1.** Please comment on the **completeness of literature** reviewed. Please indicate any additional literature, which you feel would further clarify or support SAs for DNA-binding.

• **Query 2.** Please comment on the **adequacy and completeness of the SAs** for forming categories based on mechanisms of DNA-binding.

• **Query 4.** Please comment on **documentation** associated with each alert. In particular is the rationalization complete yet easy to follow.

OK to all

Specific Queries for the Reviewers:

• **Query 3.** Please comment on the adequacy of the **mitigating factors**, affecting either toxicokinetics or toxicodynamics, which alter DNA-binding ability.

• **Query 5.** Please comment on the associated **confidence** noted for each alert, especially for those alerts where you feel the confidence may be misstated.

• **Question 6.** Please make any further **suggestions for improvements** in presenting the SAs and their underlying rationale.

Put into context

Main issue

Large **overlap:**

out of **56** SAs in **SA_DNA_cov, 38** SAs already in **SA_BB**

How to get the best out of the simultaneous presence of two related profilers in Toolbox ?

Coverage of SAs

SA_DNA_cov	**Covalent DNA binding**	SA_BB
	NonCovalent DNA binding	SA_BB
	NonGenotoxic	SA_BB

SA_BB covers a wider range than SA_DNA_cov

Place in toxicity pathway:

SA_DNA_cov: potential for **chemical reaction** (initial steps)
SA_BB: potential for **biological endpoint**

• **SA_BB** (with modulating factors) more biologically focused, less generic with respect to **SA_DNA_cov**

In addition

• Six **organic chemistry mechanisms** more generic in respect to **SA_DNA_cov**

Proposal: Build a hierarchy in Toolbox profiling

The less generic the shared profile, the higher the similarity (score?) of two chemicals

Organic chemistry mechanisms class More generic

SA_DNA_cov

SA_BB Less generic

Specific Queries for the Reviewers:

Query 3. *Please comment on the adequacy of the mitigating factors, affecting either toxicokinetics or toxicodynamics, which alter DNA-binding ability*.

SA_DNA_cov express reactivity potential in a very broad sense, mitigating factors not necessary

Query 5. *Please comment on the associated confidence noted for each alert, especially for those alerts where you feel the confidence may be misstated.*

Confidence related to organic chemistry knowledge, not to reported biological effects.

Question 6. Please make any further suggestions for improvements in presenting the SAs and their underlying rationale

Pages 3 – 4 Definition of mutagenicity, etc.., not accurate.
The expression: “Genotoxic mechanisms of mutagenicity” sounds weird. “Genotoxic” and “mutagenic” are largely overlapping terms.

In addition, mutagenicity / genotoxicity include various types of genetic damage, i.e., gene mutations, chromosomal effects (both structural and numerical), all deriving from a range of damages to biological macromolecules (not only covalent binding to DNA by electrophiles). This is overlooked in the report.

Question 6. Please make any further suggestions for improvements in presenting the SAs and their underlying rationale

Pages 6 – 8 Definition and classification of (Q)SAR approaches quite debatable. Perhaps, such a detailed classification is not necessary here.

For example (page 6), the distinction between: a) Statistically derived models; b) Mechanistic alerts; and c) Expert systems, leaves out the extremely important (and quite well represented) category of mechanistically-based QSARs in Hansch-style [1].

In addition, this description does not consider the fact that e.g., Toxtree includes both SAs and mechanistic QSARs in the same module, and that expert systems such as Oncologic and Toxtree are completely

mechanistically based. Please also note that the legend to Table 3.2. is not correct: the approaches presented are not aimed at modeling DNA binding, but rather toxicological endpoints.

Question 6. Please make any further suggestions for improvements in presenting the SAs and their underlying rationale

Page 11. Not correct. Electrophiles react with both N and O atoms in DNA bases, and usually the O-adducts are much more hazardous than the N-adducts 5.

Page 13. SA_BB agrees with mutagenicity around 78%, and with carcinogenicity around 70% [2].

Page 13 KS Alerts. My reading of the paper is that both mechanistic knowledge and data mining were used in combination to identify / validate the alerts. A paper based only on data mining is [3].

Page 17 Paragraph 1. The QSARs referenced were not aimed at modeling DNA binding, but mutagenicity and carcinogenicity. A more comprehensive reference is [1].

Reference List

1. Benigni,R., *Chem.Revs.* 2005, 105, 1767.

2. Benigni,R.; Bossa, C., *Mutat.Res.Revs.* 2008, 659, 248.

3. Kazius,J.; Nijssen, S.; Kok, J.; Back, T.; Ijzerman, A. P., *J.Chem.Inf.Model.* 2006, 46, 597.

4. Snyder,R.D.; Ewing, D.; Hendry, L. B., *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*. 2006, 609 (1), 47.

5. Vogel,E.W.; Nivard, M. J. M., *Mutation Research*. 1994, 305, 13.

ANNEX 7: REVIEW REPORT BY DR WOO

OECD Expert Consultation on DNA Binders
October 20, 2009 Meeting Comments

Yin-tak Woo, Ph.D., DABT
Risk Assessment Division (7403M)
Office of Pollution Prevention & Toxics
U.S. Environmental Protection Agency
Washington, DC 20460

Disclaimer: Scientific views expressed are solely those of the presenter and do not necessarily reflect those of the Agency

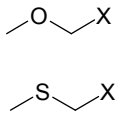
Overview of the evaluation

- SAs for DNA binding can significantly enhance the mechanistic support of the QSAR toolbox
- Coverage extensive but some more are needed
- Mitigating factors need expansion for some SAs
- Documentation for some SAs needs improvement
- Confidence evaluation may need more support
- Suggestions for improvement of current version
- Suggestions for future consideration

Additional SAs/category for consideration

- α-Haloether/thioether (e.g., BCME direct acting; 1,1-dihaloalkane via GSH conjugation)
- Alkenylbenzene (e.g., safrole; estragole)
- Pyrrolic esters (e.g., ridelliine; lasiocarpine) giving rise to DHP or dehydroretronecine
- Furocoumarin (e.g., psoralen)
- Carbamoylating agent (e.g., DMCC)
- Additional MA (e.g., acrylates?, quinoneimine?)
- Arylating agents (e.g., 2,4-dinitrochlorobenzene)

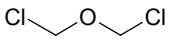
Alpha-Halo-ethers/thioethers.



X = F, Cl, Br, I

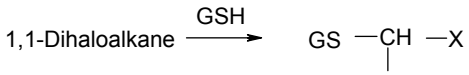
Direct-Acting:

Bis-(chloromethyl)ether, one of the most potent human carcinogen (via inhalation). Alkylation and crosslinking. Oxonium ion may play a role.



Indirect-acting

1,1-Dihaloalkanes (any halogens except difluoro) vis GSH conjugation



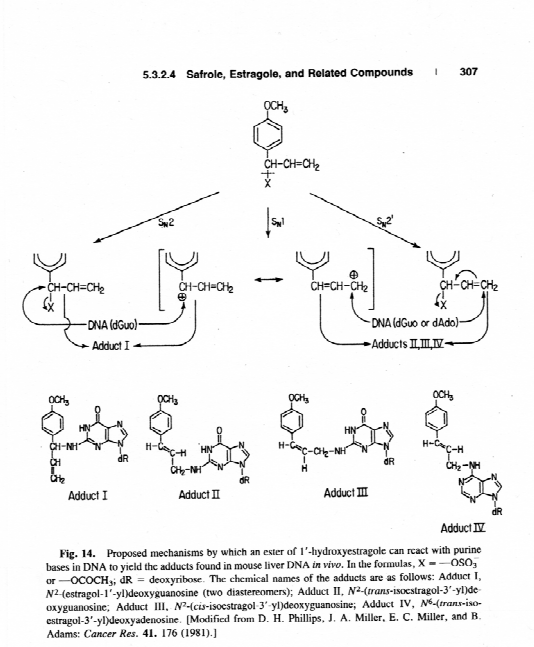
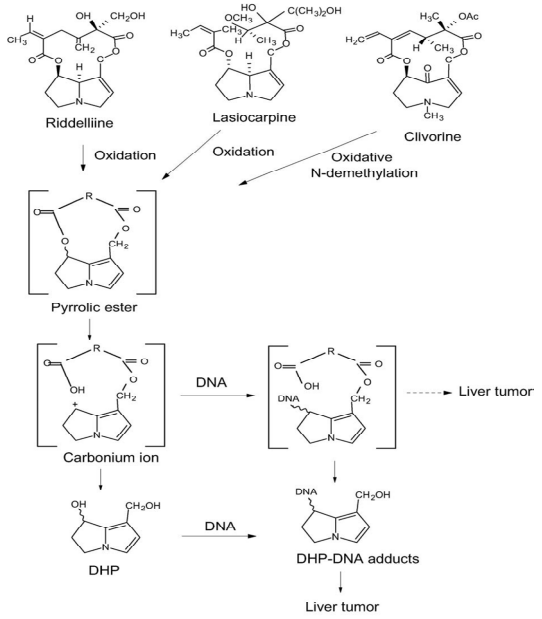


Fig. 14. Proposed mechanisms by which an ester of 1′-hydroxyestragole can react with purine bases in DNA to yield the adducts found in mouse liver DNA *in vivo*. In the formulas, X = —OSO_3 or —$OCOCH_3$; dR = deoxyribose. The chemical names of the adducts are as follows: Adduct I, N^2-(estragol-1′-yl)deoxyguanosine (two diastereomers); Adduct II, N^2-(*trans*-isoestragol-3′-yl)deoxyguanosine; Adduct III, N^2-(*cis*-isoestragol-3′-yl)deoxyguanosine; Adduct IV, N^6-(*trans*-isoestragol-3′-yl)deoxyadenosine. [Modified from D. H. Phillips, J. A. Miller, E. C. Miller, and B. Adams: *Cancer Res.* **41**, 176 (1981).]

Source: Woo, YT et al. "Chemical Induction of Cancer" Vol. IIIC, p.307, 1988



Yan et al. (2008) Tox. Ind. Hlth. 24, 181; Xia et al. (2008) Tox. Lett. 178, 77; Fu et al. (2004) Drug Metab. Rev. 26,1.

DHP = (+/-) 6,7-Dihydro-7-hydroxy-1-hydroxymethyl-5H-pyrrolizine (also known as dehydoretronecine)

Known carcinogens, *in vivo* DNA adduct data with good correlation to carcinogenic potency

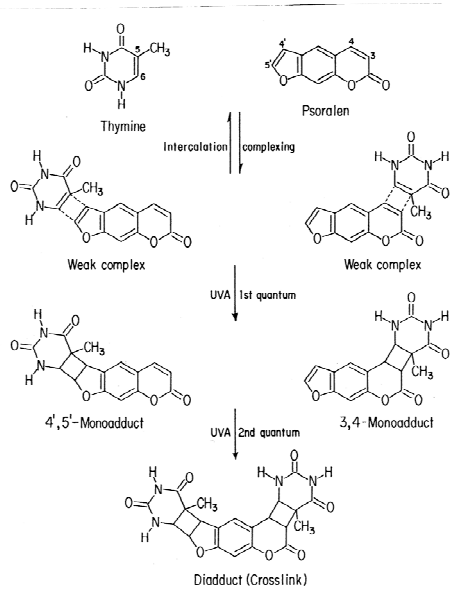


Fig. 17. Proposed mechanism for photobinding of psoralen to DNA. The first step is believed to involve weak, intercalation complexing between psoralen and pyrimidine (e.g., thymine) residues in DNA. Upon UVA irradiation, a C_4-cycloaddition reaction takes place, resulting in the formation of 3,4- or 4',5'-monoadduct. The addition of a second molecule of pyrimidine to either monoadduct gives rise to diadduct (crosslink). [Modified from B. R. Scott, M. A. Pathak, and G. R. Mohn: *Mutat. Res.* **39**, 29 (1976).]

Source: Woo, YT et al. "Chemical Induction of Cancer" Vol. IIIC, p.344, 1988

Additional SAs/category for consideration

- α-Haloether/thioether (e.g., BCME direct acting; 1,1-dihaloalkane via GSH conjugation)
- Alkenylbenzene (e.g., safrole; estragole)
- Pyrrolic esters (e.g., ridelliine; lasiocarpine) giving rise to DHP or dehydroretronecine
- Furocoumarin (e.g., psoralen)
- Carbamoylating agent (e.g., DMCC)
- Additional MA (e.g., acrylates?, quinoneimine?)
- Arylating agents (e.g., 2,4-dinitrochlorobenzene)

Some thoughts on Mitigating Factors

- Current version somewhat limited
- Reactivity (e.g., size of R as alkylator?)
- Stability (e.g., direct-acting acylators or α-halo-ethers/thioethers via inhalation only?)
- Resonance stabilization (e.g., aryl vs. alkyl)
- Metabolic activation (e.g., steric hindrance) vs. detoxification (e.g., sulfonate)
- Toxicodynamics issues: adduct stability, critical (promutagenic) binding, persistence
- Enhancing factors for higher concern?

Some thoughts on SA documentation

- Acyl alerts: direct vs indirect; exposure scenario; include carbamoyl chloride (e.g., DMCC)
- MA alerts: MA5 alkylphenol R=methyl only; include Acrylates?, Quinoneimine/diimine
- SB alerts: SB3 α-Dicarbonyl should be α,β-Dicarbonyl
- The “Unclear” and “Radical” alerts seems to be quite hypothetical

Some thoughts on SN alerts documentation

- SN2 applies to unsubstituted hydrazine only?
- SN4 “Triazines” should be “Triazenes”. Note: extensive info available for aryldialkyltriazenes (CIC vol.IIIA)
- SN11 “Ureides” should really be phenylureas
- SN12 “Diazo” should really be “Diazoalkane”
- SN14 not clear; applicable to 4 to 6 ring PAHs? For heterocyclic PAHs, a heteroatom in wrong place may kill bay region activation
- SN15 nitrosamines R2 needs clarification; α-OH probably the major mechanism
- SN16 substantial carrier effect for mustard (e.g., uridine much higher)

Some thoughts on SN alerts documentation

- SN17/23 haloalkanes not reactive if *all X*=fluoro due to strong C-F bond in the absence of activating groups
- SN18 epoxidation hindered if large alkyl/aryl
- SN21 disagree; extensive studies showing importance of the alkyl group in alkyl carbamate (NTP, Millers studies)
- SN22 how far does the epoxide extend to?
- SN26 should rename Dialkyl sulfate and alkyl alkanesulfonate; (add alkyl tosylate?)
- SN27 phosphonates much weaker than trialkyl phosphate; boundary of R?
- SN28,29 seem hypothetical; thiourea mainly hormonal

Some thoughts on Confidence

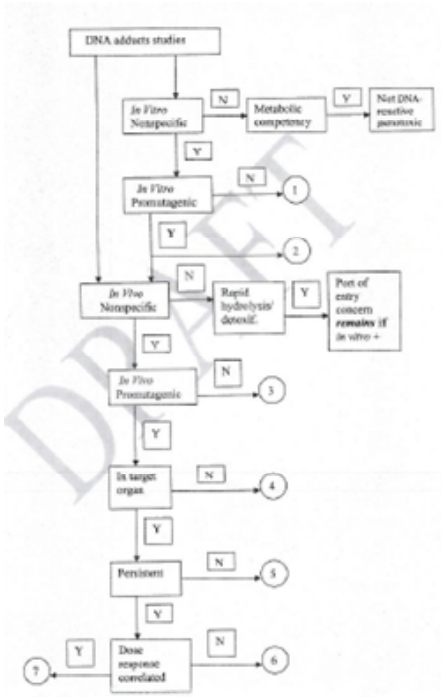
- Currently briefly discussed
- Degree of confidence (probable/well established vs. possible/hypothetical)
- Screening for hazard vs. assessing potential risk
- Tolerance for false positive/negative
- Supportive evidence/documentation (e.g.,DNA adduct data/analysis, representative mutagen/carcinogen, SAR)
- Regulatory acceptance

Suggestions for improving current version

- Add some more SAs, especially potent/known carcinogens
- More boundaries for mitigating factors
- Review/edit SA documentation
- Consider expansion of supportive evidence for confidence

Suggestions for future consideration

- Conditions/factors affecting formation
- Stability of reactive species ability to reach DNA
- Organic chemistry of adduct stability/pesistence (e.g, hard vs soft; propensity to critical binding; polyfunctionality)
- Systematic supportive evidence for confidence
- Biological significance and predictive values of DNA adducts



Predictive Value of Various DNA Adducts Studies

Level	Predictive Value
1	DNA-reactive with possible carcinogenic potential
2	DNA-reactive with probable carcinogenic potential
3	Biomarker of exposure. Internal dosimeter. DNA-reactive with possible carcinogenic potential
4	Biomarker of exposure. Internal dosimeter. DNA-reactive with probable carcinogenic potential
5	All of the above + Possible verification of MOA.
6	All of the above + Probable verification of MOA.
7	All of the above + Possible use in low dose extrapolation.

ANNEX 8: REVIREW REPORT BY DR HAYASHI

nite

Paris
October 20, 2009

OECD Expert Consultation on DNA-Binding

Makoto Hayashi, D.Sc.
National Institute of Technology and Evaluation
and
Biosafety Research Institute

nite

Specific Queries for the Reviewers

- Query 1. Please comment on the completeness of literature reviewed. Please indicate any additional literature, which you feel would further clarify or support SAs for DNA-binding.
- Query 2. Please comment on the adequacy and completeness of the SAs for forming categories based on mechanisms of DNA-binding.
- Query 3. Please comment on the adequacy of the mitigating factors, affecting either toxicokinetics or toxicodynamics, which alter DNA-binding ability.
- Query 4. Please comment on documentation associated with each alert. In particular is the rationalization complete yet easy to follow.
- Query 5. Please comment on the associated confidence noted for each alert, especially for those alerts where you feel the confidence may be misstated.
- Question 6. Please make any further suggestions for improvements in presenting the SAs and their underlying rationale.

nite

Query 1

- Literatures cited look OK, however, I feel it is important that more literatures studied DNA-binding in *in vivo* assays should be cited and reviewed. Because it is critical to understand the ability of DNA-binding at the site of the body that is targeted, *e.g.*, for carcinogenicity or heritable effects of the chemical to the next generations.

Makoto HAYASHI / 2009

3

nite

Query 2

- It is important to understand the mechanisms of DNA-binding as a potential of activities of the chemical that induce genotoxicity resulting mutation.
- It is also adequate to use DNA-binding ability as a characteristic of chemicals for making category.
- It should, however, be careful to interpret the binding ability in correlation to other toxicological events.
- Intercalation into base-pairs?

Makoto HAYASHI / 2009

4

nite

Query 3

- No additional comment.
- This is also very important but we would like to know it is really occurred not only naked DNA or bacterial DNA but also in the body of eukaryote.
- In the case of bacterial mutagenicity, it was not easy to improve performance introducing modification factors including mitigation factors, e.g., aromatic amine.

Makoto HAYASHI / 2009

5

nite

Query 6-1

- It looks also important to make clear definition between genotoxicity and mutagenicity to avoid misunderstanding. DNA-binding is genotoxic event but not mutagenic one. Widely used definition is those defined in the UK COM guidance, i.e., mutagenicity was defined as gene mutation and chromosomal aberration, while genotoxicity was defined more widely meaning events of chemicals including DNA-binding, DNA strand breaks, which can be detected by the comet assay, sister chromatid exchanges, etc.

Makoto HAYASHI / 2009

6

nite

Query 6-2

- As I mentioned above, the ability of DNA-binding is very important characteristic of the chemicals to be assed their safety. It is genotoxic event but very first step of mutagenicity and it will follow by DNA repair and majority of them will be repaired to the normal. Also, I think there are big differences among DNA-binding between a chemical and DNA as an organic chemical polymer, a chemical and bacterial DNA, a chemical and, e.g., mammalian cells in culture, and a chemical and cells of organs in anima body.

Makoto HAYASHI / 2009

7

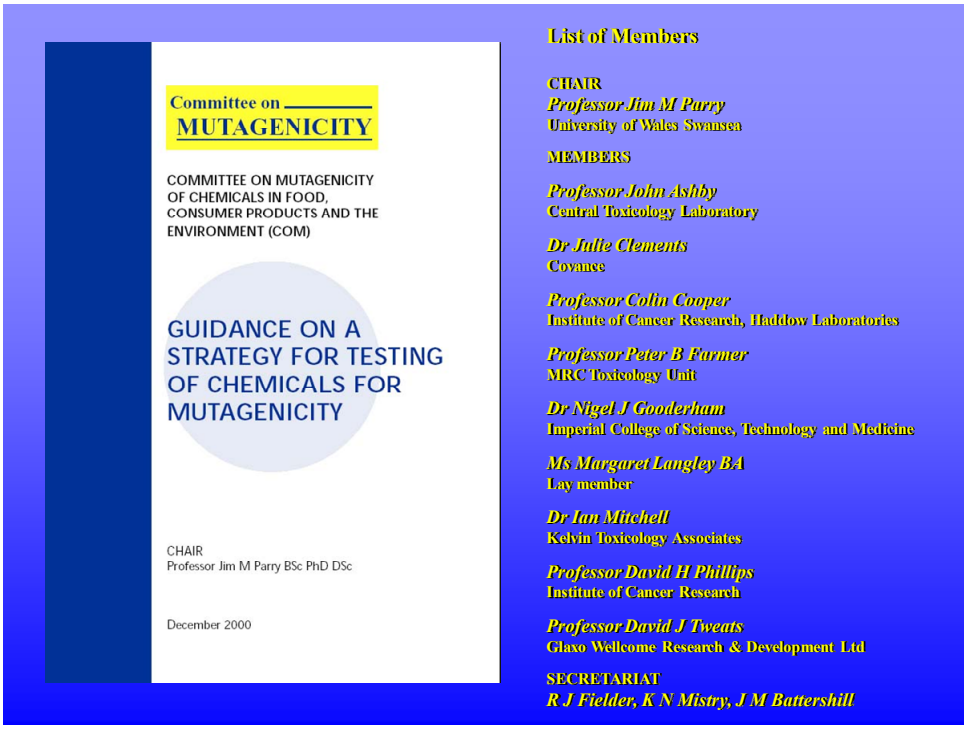
nite

Query 6-3

- The actual existing situation of DNA is completely different from the chemical DNA. In the mammalian cells, DNA is surrounded by many proteins, e.g., histons, and packed by holding. I think this is a factor to make false evaluation. The biological mechanisms are essential as well as chemical mechanisms for DNA-binding.

Makoto HAYASHI / 2009

8


Committee on
MUTAGENICITY
COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COM)
GUIDANCE ON A STRATEGY FOR TESTING OF CHEMICALS FOR MUTAGENICITY
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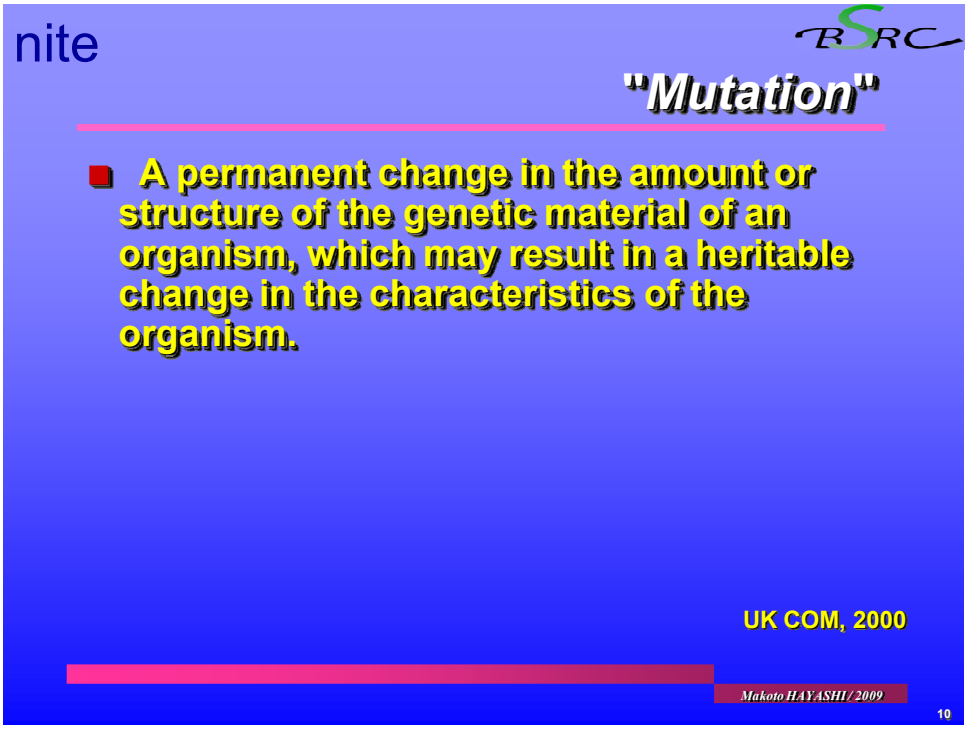
Lay member
Dr Ian Mitchell
Kelvin Toxicology Associates
Professor David H Phillips
Institute of Cancer Research
Professor David J Tweats
Glaxo Wellcome Research & Development Ltd
SECRETARIAT
R J Fielder, K N Mistry, J M Battershill


nite
BSRC
"Mutation"
A permanent change in the amount or structure of the genetic material of an organism, which may result in a heritable change in the characteristics of the organism.
UK COM, 2000
Makoto HAYASHI / 2009
10

nite

RSRC

"Genotoxicity"

Genotoxic refers to agents which interact with the DNA and/or the cellular apparatus which regulates the fidelity of the genome, *e.g.*, the spindle apparatus, and enzymes such as the topoisomerases. It is a broad term that includes mutation as well as damage to DNA or production of DNA adducts, by the chemical itself or its metabolites. It includes UDS, SCE, and mitotic recombination.

UK COM, 2000

Makoto HAYASHI / 2009

11

nite

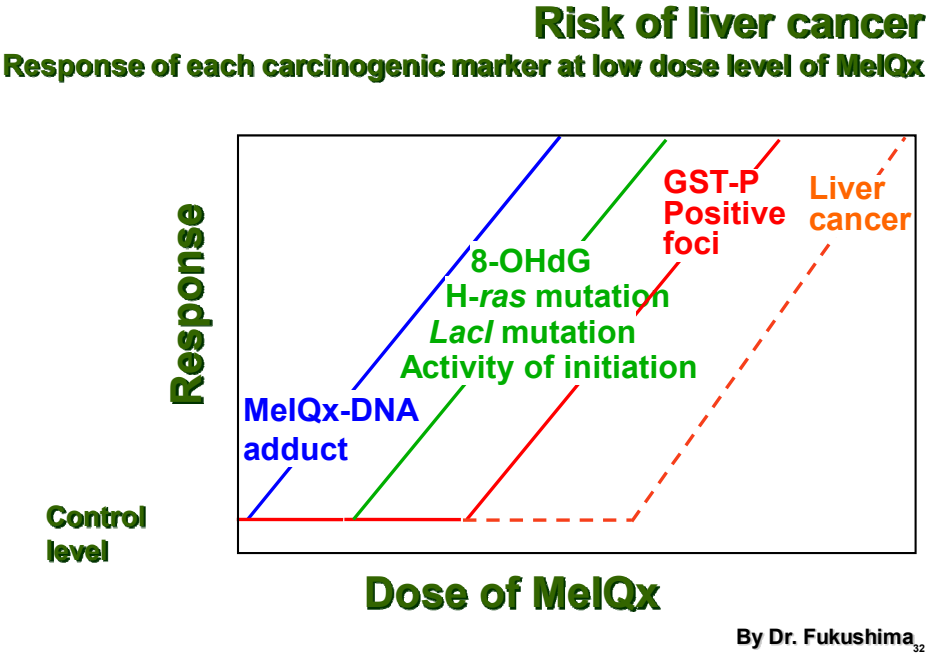
RSRC

Genotoxicity assay

	DNA damage	Gene mutation	Chromosomal aberrations
in vitro	*rec* assay, Comet assay, UDS assay	Ames test MLA	Metaphase analysis, MN, MLA
in vivo	Comet assay, UDS assay	Transgenic animal model	Rodent MN assay

Makoto HAYASHI / 2009

12


Risk of liver cancer
Response of each carcinogenic marker at low dose level of MeIQx
Response
GST-P
Positive
foci
Liver
cancer
8-OHdG
H-ras mutation
LacI mutation
Activity of initiation
MeIQx-DNA
adduct
Control
level
Dose of MeIQx
By Dr. Fukushima32

ANNEX 9: COMMENTS PROVIDED BY PARTICIPANTS OF THE EXPERT CONSULTATION

1. Canada

HEALTH CANADA RESPONSES TO DNA BINDING QUERIES

Query 1. Please comment on the completeness of literature reviewed. Please indicate any additional literature, which you feel would further clarify or support SAs for DNA-binding.

No additional literature was identified that would further clarify or support SAs for DNA binding. Health Canada believes the literature reviewed is thorough and complete.

Query 2. Please comment on the adequacy and completeness of the SAs for forming categories based on mechanisms of DNA-binding.

There are a total of 56 structural alerts for DNA binding all of which are supported by mechanistic chemistry. Although some SAs seem to be redundant, they capture additional steps involved in activation of certain compounds for DNA binding (i.e., metabolic activation (P450 activated)).

Question:

Have O- and S-heterocyclic compounds been examined ? It was not clear from the available SAs. However, it is not certain that there is clear evidence and a mechanism to show that these compounds are genotoxic.

Also, are some SAs repeated in different domains? According to our estimations, there are a total of 59 SAs (7 AC, 12 MA, 5 SB, 31 SN, 2 RD and 2 UN for a total of 59) however, in the text is says there are 56 two of which have no clear mechanism (see slide 34 of DNA binding document)?

Query 3. Please comment on the adequacy of the mitigating factors, affecting either toxicokinetics or toxicodynamics, which alter DNA-binding ability.

We agree with the use of mitigating factors only if they have been shown to completely remove toxicological activity (which the authors clearly state they have done).

However, it may be difficult to completely incorporate modulating factors and it is suggested that these not be used in future iterations of the DNA profiler. Although certain phys-chem properties (i.e., Bay regions for PAHs) will affect the potency, we believe it would be very difficult to quantify such potency and incorporate it into the model.

Query 4. Please comment on documentation associated with each alert. In particular is the rationalization complete yet easy to follow.

For each SA, there was a reference to a journal or textbook which was easy to follow, however, it might be useful to have these references summarized by mechanistic domain at the end of the document to make it easier for the reader to access. Other than that, rationalization was easy to follow.

Query 5. Please comment on the associated confidence noted for each alert, especially for those alerts where you feel the confidence may be misstated.

The application of the Uncertain Mechanistic Domain is questionable in our opinion. The purpose of the Toolbox is to identify clear structural alerts with a clear chemical mechanism. Having an uncertain Domain does not add to the understanding of mechanism and might confuse the user.

Question 6. Please make any further suggestions for improvements in presenting the SAs and their underlying rationale.

This is a general overall comment:

In the presentation, the authors state that both profilers will be in the Toolbox, and that it is up to the author to decide which to use. Hypothetically speaking, how will the Toolbox handle conflicting SAs and results from each DNA profiler ? Is there a preferred profiler here ? Some guidance is suggested to avoid confusion for users.

2. France

OECD Expert Meeting on DNA-binding Structural Alerts

Preliminary comments

sanofi aventis
Because health matters

Context

- Pharmaceutical companies use in silico tools to predict genotoxicity:
  - For occupational safety
  - For genotoxic impurities
  - For metabolites
  - For discovery
- Multi-tool approach using commercial products, e.g.,:
  - DEREK
  - Multicase
  - Leadscope
- Regulatory authorities also use this multi-tool approach.
- We recommend to check that the analysis done with OECD tool is consistent with the regulatory accepted existing ones.
- We support the development of a freely available SAR in silico tool. Very promising tool, and good starting point.

2

sanofi aventis
Because health matters

Comments on Objectives

- Alerts identify potential interaction with nucleophilic cellular components
  - Need to differentiate if the main interactions are with DNA or proteins (e.g. hepatotoxins)
- Need to clarify if the tool considers both mutagenic activity and potential induction of chromosome damage via other mechanisms. Other tools generally also predict effects on chromosome, some being more indirect effects.

3

sanofi aventis
Because health matters

Description of the alerts

- Consistency in the description reaction could be improved.
- Recommendation to make sure that for each reaction:
  - Electron transfers are indicated.
  - Structure of final DNA adduct is described.
  - All final reaction products (including "leaving groups") are described.
  - Enzymatic reactions, and if possible enzyme names, are indicated in the reaction, when appropriate.
  - When multiple steps are needed, all reactions are properly described.
  - DNA, i.e. "Nu", appears in all reactions. Clarify why sometimes specific part of nucleotides (e.g. dR-NH2) are mentioned.

4

sanofi aventis
Because health matters

Description of the alerts

- The format of the paragraph providing information on each reaction should be as much as possible consistent. Some are very "light" while the others are quite detailed.
- More literature (e.g. examples) references might be useful.
- It might be useful to list the different synonyms that could be found in the literature for the alert names (structures) e.g. in an additional column in the "supplementary information: new alert compilation by mechanistic domain".
- Information on the predictive value or accuracy of the alerts might be useful (comparison with assay results).
  - Like a comparison with external public datasets (e.g., dataset from Zeiger (NTP), Kirkland, etc.) to provide the following information to each alert: positive tested/total tested=% predictivity.

5

sanofi aventis
Because health matters

Comparison with other in silico tools, e.g. DEREK

- As compared to DEREK ~15-20% alerts are different (mostly present in DEREK and missing in OECD tool).
- Some DEREK alerts are missing, mainly because DEREK also consist of alerts identified by the user group as results of proprietary data analysis (e.g. alerts for chromosome damage).
- Similarly, some alerts have been removed from DEREK, because mitigating factors have indicated that the alerts are finally not biologically relevant. For the same reasons, more mitigating factors are reported in DEREK as compared to the OECD descriptions.
- In addition, some alerts should be valid for proteins but may be not relevant for DNA. It is particularly true for metabolites formed after liver metabolic activation. The compounds could be hepatotoxins but not mutagens.

6

sanofi aventis
Because health matters

Concerns and points to consider

- The OECD tool might be oversensitive, i.e., as was DEREK 10-15 years ago, because key mitigating factors might be missing.
- There would be a need to validate the alerts by looking at actual genotoxicity data, i.e., how often is the genotoxic/mutagenic activity confirms in genetic toxicity assays. Could be useful additional information.
- Contrarily, some OECD alerts might be missing in the other tools. Close collaboration might be useful and advisable.
- The regulatory authorities today use at least in the pharmaceutical area DEREK/Multicase/Leadscope, etc. It would be necessary to carefully compare the OECD tool with those models, in order to make sure that similar results would be obtained and ensure consistency.

7

sanofi aventis
Because health matters

3. BIAC

New DNA binding alerts proposed for the OECD Toolbox

QUERY 1
Please comment on the completeness of the literature reviewed. Please indicate any additional literature which you feel would further clarify for supporting Structure Alerts (SAs) for DNA binding.
Uncomfortable with the use of the term DNA binding *per se*. Further comments on this issue are provided in answer to Query 6 below.
In terms of completeness of the literature reviewed – appears OK. Since LHASA Ltd are one of the contractors to the OECD Toolbox, it would be interesting to know whether they contributed to the evaluation if at all in terms of providing "mechanistic insight" from their own alerts for carcinogenicity and mutagenicity. Clearly Derek for Windows has an extensive compilation, some of which must be in the public domain but might not necessarily have been published as a compilation.

QUERY 2
Please comment on the adequacy and completeness of the SAs for forming categories based on the mechanisms of DNA binding.
OK based on the literature review conducted. Will there be any additional efforts made in extending the coverage to new alerts for other chemical groups in the future? To what extent do the alerts now included in the revised set cover the breadth of chemicals within the inventories included in the Toolbox at present? Are there any plans to seek out new data sources to extract out potential new alerts? Is there anything that could be mined from the newly planned databases to be added into the Toolbox (Document 7-1; specifically datasets 10-12), 13-15), 18-19), 24))?

QUERY 3
Please comment on the adequacy of the mitigating factors, affecting either toxicokinetics or toxicodynamics which alter DNA binding ability.
The authors state that the alerts they have described are solely for the grouping of substances into categories in accordance with common mechanisms. It is unclear what the purposes of these mitigating factors represent – factors which preclude a given reaction pathway from occurring? or factors that diminish the toxicity that might be expressed? If it is the latter, then it is even unclearer how these alerts might be used in practice in terms of how they relate to the existing Benigni-Bossa alerts for instance. Further comments are provided under Query 6.

QUERY 4
Please comment on documentation associated with each alert. In particular is the rationalisation complete yet easy to follow?
The rationalisation is brief but interpretable. The documentation for the present DNA binding alerts within the Toolbox (TB) are more extensively described with a larger number of references. Presumably the new revised alerts supersede the present TB alerts, in which case, this documentation will cease to remain? Could the Secretariat comment on this point? (also discussed in Query 6)

QUERY 5
Please comment on the associated confidence noted for each alert, especially for those alerts where you feel the confidence may be misstated.
Since the alerts as described are for the grouping of substances into categories on the basis of mechanistic domains and not for the prediction of toxicity per se as so stated by the authors on page

16; associated confidence does not appear to be relevant. Surely any confidence is thus associated with the organic chemistry principles upon which these mechanistic domains are based?

QUERY 6

Please make any further suggestions for improvements in presenting the SAs and their underlying rationale.

- Welcome the efforts of the OECD and the contractors in attempting to refine and improve existing profilers within the Toolbox.

Have a number of questions and comments in relation to the DNA binding report and how the work will be implemented into the next release of the Toolbox.

- Some rewording and revision in the introduction of the report is merited. There is a strange mixing of terms such as genotoxic, mutagenicity, DNA binding etc. Genotoxic mechanism of mutagenicity does sound odd since genotoxicity is defined within the REACH guidance as a broader term covering mutagenicity. Perhaps the REACH Technical Guidance text could be used to summarise and define the various terms to put into clearer context the scope of the alerts here evaluated. The Wikipedia, which appears to be where the proposed definition was taken, is not sufficiently comprehensive.
- The DNA profiler within the Toolbox is taken from TIMES and underpinned largely by Ames mutagenicity data. The chemistry reaction schemes rationalise the effects largely on the basis of an electrophilic-nucleophilic interaction between chemical and DNA.

- The Ashby Tennant structural alerts were developed for carcinogenicity based on a knowledge and understanding of genotoxic carcinogenicity. These alerts were also found to apply to mutagenicity in bacteria. These alerts have been extensively evaluated by Benigni and others - good correlations have been observed between these alerts and Ames data. Therefore, the Ashby Tennant alerts are not "alerts for DNA binding" *per se*.
- The nucleophiles site in DNA are not restricted to nitrogen, oxygen sites also feature – this should be corrected on page 11.
- Existing approaches to model DNA binding are described. The terminology of DNA binding appears to be incorrect, based on the examples provided. Oncologic is an expert system to estimate a concern level for carcinogenicity. TOPKAT contains empirical models for the prediction of carcinogenicity (based on FDA and NTP datasets) and Ames mutagenicity. Derek for Windows contains alerts for carcinogenicity, chromosomal aberrations etc None of these are "DNA binding" predictors, and therefore, should not be termed as such.
- Oncologic is used within the US EPA as a screening tool for cancer hazard under PMN. The structural alerts are used in conjunction with other information such as route of exposure. Concern levels might differ dependent on whether the route of exposure is by oral, dermal or inhalation.
- Within the EU, it is perhaps somewhat unfair to say that the OECD Toolbox is the only approach that has been used. Initiatives such as implementing the Benigni-Bossa alerts into Toxtree or releasing the Danish EPA's database of predictions show that there have been efforts to make (Q)SAR approaches for evaluating mutagenicity/carcinogenicity hazard available for wider use. Use of Derek for Windows, for example, has been widely used within EU even if its predictions have not been relied upon solely in any C&L/RA decision making.
- Where do the 22 genotoxic structural alerts within the Toolbox come from? Launching the TB and browsing the DNA binding alerts within v1.1.01 show 18 distinct headers. Inspection of the alerts themselves do match up with the 22 cited with exception to that of "carbenium and episulfonium ions". Realise the latter does not make sense to include as an alerting group but it would be helpful to clarify in the report how the 18 alerts presented are really 22 distinct alerts. e.g. TB15, 17& 22 seem to be grouped under one header as are TB1 & TB2 and there is no separate header for mustards. Some clarification would be helpful.
- Toolbox v1.1.01 is referenced in the document but on page 4, v1.1.07 is referenced? This is confusing to the end reader –is this a typo?

- The COREPA approach is embedded as part of the TIMES platform and the 3D QSARs for Ames mutagenicity are derived using this approach – some clarification might be helpful in the text.
- Alerts such as those currently contained within the TB are arguably supplemented with an extensive discussion of mechanisms. The Benigni-Bossa alerts are described more fully in the JRC User manual that accompanied the Toxtree implementation – the mechanisms are not fully rationalised there but are described in publications that are cited. (Benigni R, Bossa C, Jeliazkova N, Netzeva T & Worth A (2008). The Benigni / Bossa rulebase for mutagenicity and carcinogenicity – a module of Toxtree. European Commission report EUR 23241 EN).
- The premise as understood in this revised alert is to provide a framework to help in the grouping of chemicals into categories. This is very much welcomed as it should enable some "translation" between different alert schemes. With this goal in mind, it would seem to make sense to include a hierarchy of profilers within the Toolbox. The new TB alerts defining the mechanistic domain - a first tier, subcategorisations could then be performed with the specific alerts or with alerts such as the Benigni-Bossa ones.
- It is true to say that the mechanistic domain approach has been successful in rationalising skin sensitisation. However in that respect, it has been successful in facilitating mechanistic read-across, QMM formation and rationalising the potency between substances within the same mechanistic domain.
- It is unclear what is meant by mitigating factors – as discussed in Query 3, if the mechanistic domains provide a means of facilitating grouping by virtue of common mechanism – what do the mitigating factors represent? Inclusion and exclusion rules pertaining to the ability of a chemical reacting via a pathway or the modulation of the mutagenic effect?
- Envisage implementation of the revised alerts to take the form of a hierarchy. The mechanistic domain forming the first tier – since these domains have been successful for sensitisation – it would make some sense downstream to use the domain as pragmatic first step grouping approaches on the basis of organic chemistry principles. Is a substance in the overall domain of Michael acceptors. A second tier ("subcategorisation" profiler) could be used to identify the specific alert groups highlighting the overall chemical reaction. The third tier of alerts would be those that are more customised to predict mutagenic and/or carcinogenic effects i.e. Benigni-Bossa alerts. From the description it is unclear what is envisaged as the implementation and whether the alerts presented here will supersede the Benigni-Bossa alerts or not..and if not what guidance will be provided in terms of training an end-user to use these profilers sensibly.
- Supporting documentation provided describes a "superlist" of DNA binding alerts – will this list update and replace the present TB compilation? If so the existing and extensive documentation for the 18 alerts now available will be superseded by less substantive information? Perhaps the OECD could comment and clarify.