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

IOMC

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

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Environment Directorate

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

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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/).

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

Background

1. The OECD (Q)SAR Application Toolbox has six work modules, which are used in a work flow with the goal of filling data gaps through the use of the chemical category. The Toolbox modules include: 1) Chemical Input; 2) Profiling; 3) Endpoints; 4) Category Definition; 5) Filling Data Gaps, and 6) Report. To build a category or to perform a simple analogue approach, the user goes through these modules sequentially. However, Category Definition and Profiling are the critical steps in the workflow. While the Toolbox provides many ways to set up a category, defining a category based on similar mechanisms or modes of action of its members is the most appropriate.

2. The careful use of expert judgment to define the boundaries of a chemical category is crucial to the reliable application of the Toolbox to estimate values for untested chemicals. Formal definitions of which chemicals should be included in a category and conversely which chemicals should be excluded, a well defined applicability domain is essential for reliable estimates of missing values. The expert judgment forming the category should be described in a transparent manner in order that the category can be evaluated by others.

3. Experience from using the Toolbox has shown a common mechanism of action to be a critical factor in deciding what chemicals would be expected to be members of a category. Variations in chemical structure can affect both toxicokinetics (uptake and bioavailability) and toxicodynamics (e.g. interactions with receptors and enzymes). Two-D structural alerts (SAs) in the form of mechanistic profilers have proven to be useful in identifying a chemical category for filling data gaps. For example, having an amino group substituted on an aromatic system is relevant to enzymatic transformation to the hydroxylamine derivative and the hazard endpoint carcinogenicity.

4. The current DNA binding categorization scheme (DNA-binding profiler) used in the latest version of the Toolbox (Version 1.1) is based on the model developed by the Laboratory of Mathematical Chemistry and donated to the proof of concept version of the Toolbox. The scheme includes 19 categories with each category being defined by SAs that are a necessary condition for a chemical to covalently interact with DNA. Definition of these alerts was justified by their interaction mechanisms with DNA, found in the literature. This classification scheme is particularly relevant for genotoxic hazards.

5. The Benigni/Bossa rulebase for mutagenicity and carcinogenicity was developed as a module (plug-in) to the Toxtree software. The SAs from the Benigni/Bossa rulebase were included as a profiler in the Toolbox version 1.1. This list of SAs is derived from empirical data much of which has a mechanistic basis for genotoxicity, but includes also a number of SAs for nongenotoxic carcinogens.

6. While there is much overlap between these two profilers, the current DNA-profiler is based in large part on an understanding of organic chemical reactions, while the Benigni/Bossa profiler is based on experimental evidence. Experience using the Toolbox has shown using these two profilers in tandem provide a transparent, mechanistic basis for categorization with a high level of confidence.

7. The OECD (Q)SAR Application Toolbox is a stand-alone system intended to facilitate the formation of chemical categories and filling data gaps. The first version of the Toolbox released in March 2008 is already helpful to member countries and other stakeholders in forming categories and using existing data to fill data gaps. Phase 2 of the development of the Toolbox started in November 2008 and the aim is to ensure that the categories approach works uniformly for all discrete organic chemicals and for all regulatory endpoints. The 42nd Joint Meeting agreed that the main work item in the phase 2 project will be to gather and maintain additional categorisation methods [ENV/JM(2008)7]

Workshop

8. The expert consultation was held on 20 October 2009 in Paris at OECD headquarters. The agenda is outlined in Annex 1.

9. The consultation was attended by experts from Canada, the Czech Republic, Denmark, Germany, Italy, Japan, Sweden, the United States, the European Commission, BIAC, ICAPO. The list of the participants is attached to this document as Annex 2. The expert consultation was chaired by the OECD Secretariat.

Scope and Objectives

10. The Secretariat presented the scope and objectives of the expert consultation for setting the scene. The stated scope of the expert consultation was to evaluate a revised set of SAs for estimating covalent DNA-binding.

11. The objectives of the expert consultation were to:

- get an overview of the revised organic chemistry-based mechanistic SAs for identifying DNA binders, the literature on which they are based, and how it is envisioned they may be used in the Toolbox;
- get a review from experts of the proposed structural rules for DNA-binding;
- get a recommendation from the OECD member countries on the proposed SAs and accompanying documentation, in particular if they should be implemented in the Toolbox.

12. The Secretariat indicated that the importance of alert-based expert systems (so-called profilers) in the Toolbox is to allow for the formation of toxicologically meaningful categories. Such a category means that all the chemicals falling within it can be assessed when only a few members are tested. This enables transparent and defensible categories to be formed. Version 1 of the Toolbox only contains a relatively small number of profilers many of which are incomplete. Incorporation of new and better profilers is seen as being essential to add new functionalities to the Toolbox. The better the profiler, the better and more precise the category. It is important to note that in the Toolbox profilers are not be used to predict adverse effects. Rather, the profilers are used to group chemicals to allow for read-across using existing experimental results.

Preparation for the Expert Consultation

13. A scientific re-evaluation of known SAs for covalent DNA binding was undertaken by Liverpool John Moores University (LJMU) as part of the development of a new profiler for the OECD QSAR Application Toolbox, financed by the European Chemicals Agency. The focus of this work was SAs for DNA-binding based on organic chemical reactions, which were extracted from the literature. The work plan identified five issues, which included:

A. Identification of the scientific literature detailing SAs for DNA binding and mutagenicity.
B. Analysis of the identified alerts and the rationalization of the associated mechanistic chemistry.
C. Identification of mitigating factors that may alter DNA-binding.
D. Construction of clear and concise documentation related to each alert including; name and pictorial representation of the alert, as well as sections detailing the mechanistic chemistry that leads the alert being able to covalently bind to DNA, any mitigating factors that should be considered as part of the alert, and references that support the mechanistic chemistry information.
E. Associated confidence in the suggested alerts.

In preparations for the expert consulation LJMU perpared a consultation document entitled: "**Re-evaluation of Structural Alerts for the Binding of Molecules to DNA and the Development of a Comprehensive Profiler of Alerts**", which is reported in Annex 3. The consultation document has a number of supplementary information, which is compiled in Annex 4. In addition, the LJMU presented an overview of the alerts, which is reported in Annex 5.

Preparatory Work by Three Experts

14. Three expert reviewers were selected by the Secretariat. These were Dr Romualdo Benigni of the Instituto Superiore di Sanita, Italy, Dr Yintak Woo from the US-EPA, and Dr Makoto Hayashi from Japan. The reviewers were provided with the consultation documents.

15. In an effort to provide guidance to the reviewers, a series of questions were drafted by the OECD Secretariat and submitted to the reviewers. These questions are as follows.

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.

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

16. The review report of Dr. Benigni is reported in Annex 6.

17. The review report of Dr. Woo is reported in Annex 7.

18. The review report of Dr. Hayashi is reported in Annex 8.

19. Comments provided by participants from Canada, France and BIAC are compiled in Annex 9.

Proceedings of the Expert Consultations

20. The Consultations was conducted as described in the agenda reported in Annex 1. Briefly, the LJMU presented an overview of the SAs, which was followed by the reviews of Drs. Benigni, Woo, and Hayashi. LJMU then provided clarification and response to the reviews. The clarifications were followed by a general discussion by all participants.

Outcome of the Expert Consultation

21. Summary responses to the six questions asked of the reviewers and agreed upon by the expert consultation are as follows:

1) Regarding the completeness of the literature search the meeting agreed with the reviewers that while it would be better to have a broader primary literature search, the contractors did a good job of identifying the currently available relevant reviews of covalent DNA-binding and their mechanistic interpretation.
2) Regarding the adequacy and completeness of the SAs for forming categories based on mechanism of covalent DNA-binding the meeting agreed with the reviewers that in general the list of 56 SAs presented by the contractors were a significant improvement over the SAs listed in version 1.0 of the Toolbox. Noted exceptions were the missing alerts referred to by Dr Woo. The meeting furthermore agreed with the suggestions from the contractor that these new alerts be added to their proposed series forming an expanded series of 63 SAs.
3) Regarding the adequacy of the mitigating factors, the meeting agreed with the reviewers that the covalent DNA-binding profiler with its basis on chemical mechanism should project binding in the broader or more generic sense. The meeting furthermore agreed that mitigating factors are important and their usage should be considered further (e.g. in a subcategorization profiler).
4) Regarding the documentation of the SAs the meeting agreed with the reviewers that the documentation as presented is easy to follow, and consistent with other profilers in the Toolbox, but that it may need to be improved for some SAs (e.g. consistency, level of detail, in chemico/in vitro/in vivo basis). The meeting agreed that this information would be a welcome addition to the Toolbox. The meeting furthermore agreed with the suggestions from the contractor to review the documentation for each SA with the understanding that the level of detail know varies from one alert to another.
5) Regarding the confidence noted for each SA the meeting agreed with the reviewers that the confidence is related to our knowledge of organic chemistry and not reported biological effects and this needs to be stated.

6) Regarding further suggestions for improvements the meeting agreed with the reviewers that there needs to be a clearer explanation and distinction noted between terms such as genotoxicity, mutagenicity and DNA-binding specifically noting that covalent DNA-binding is causing primary DNA damage and potentially leading to mutation. The meeting further agreed with the reviewers that the high overlap between the Benigni & Bossa SAs and the newly proposed DNA-binding SAs means that a strategy in using the different profilers in the Toolbox needs to be developed.

22. The Conclusions and Recommendations from the expert consultation are as follows:

1) While not all covalent DNA-binding SAs have been identified the new proposed DNA-binding profiler is an improvement over the current version.
2) The OECD is encouraged to expand this work to include the recommendations of the reviewers.
3) The tool is useful to build chemical categories.
4) It is recommended that the SAs should be implemented in the OECD (Q)SAR Application Toolbox.
5) Further discussions are necessary on how to best use the Benigni & Bossa profiler and the newly proposed covalent DNA-binding profiler within the Toolbox and how to use the mitigating factors.
6) Further guidance is necessary on how to improve the confidence in the read-across approach for biological endpoints (mutagenicity and carcinogenicity), using all the relevant profilers and the relevant experimental data in the Toolbox databases.

ANNEX 1:
AGENDA OF THE EXPERT CONSULTATION ON SCIENTIFIC AND REGULATORY EVALUATION OF ORGANIC CHEMISTRY MECHANISM-BASED STRUCTURAL ALERTS FOR THE IDENTIFICATION OF DNA BINDING CHEMICALS

held at the Room 7, OECD Conference Center, Paris, France

20 October 2009

The meeting starts at 09h30 and closes at 17h30 on Tuesday, 20 October 2009.

09h30 **1 Opening and the adoption of the agenda (10min)**
The meeting will be opened by the OECD Secretariat. The Secretariat will explain the purpose of the Expert Consultation and housekeeping items. The Secretariat will also confirm that the participants have all meeting documents. The meeting participants will briefly introduce themselves to the meeting (Tour de Table). The Consultation participants will be asked to approve the agenda, and discuss changes in meeting papers and scheduling of the agenda items if necessary.

09h40 **2 Background information (5min)**
The Secretariat will explain the history and rational for the project leading to this Expert Consultation. The Consultation participants will be invited to take note of this activity.

09h45 **3 Overview of the Revised DNA-binding Profiler (45min)**
The OECD Secretariat will ask Drs Mark Cronin and Steve Enoch of Liverpool John Moores University to present a detailed overview of the Organic Chemistry Mechanism-Based Structural Alerts for the Identification of DNA binding Chemicals. The consultation participants will be invited to take note of this activity and ask questions as appropriate.

10h30 **4 First review of the Revised DNA-binding Profiler (30min)**
The Secretariat will ask Dr Romualdo Benigni of the Instituto Superiore di Sanita, Italy to present his review of the alerts described by Drs Cronin and Enoch. The consultation participants will be invited to take note of this review and ask questions as appropriate.

11h00 *Coffee Break (20min)*

11h20 **5 Second review of the Revised DNA-binding Profiler (30min)**
The Secretariat will ask Dr Yintak Woo from the Risk Assessment Division, Office of Pollution Prevention & Toxics, US-EPA to present his review of the alerts described by Drs Cronin and Enoch. The consultation participants will be invited to take note of this review and ask questions as appropriate.

11h50 **6 Third review of the of the Revised DNA-binding Profiler (30min)**
The Secretariat will ask Dr Makoto Hayashi from the National Institute of Technology and Evaluation and the Biosafety Research Institutes Japan to present his review of the alerts described by Drs Cronin and Enoch. The consultation participants will be invited to take note of this review and ask questions as appropriate.

12h20 *Lunch Break (90min)*

13h50 **7 Clarification and Responses to the Review's Comments (30min)**
The Secretariat will ask Drs Cronin and Enoch to provide clarification of the alerts and respond to the reviews. The consultation participants will be invited to take note of this activity.

14h20 **8 Discussion of the Organic Chemistry Mechanism-Based Structural Alerts for the Identification of DNA binding Chemicals (90min)**
The OECD Secretariat will invite the participants to discuss the alerts and comment as appropriate.

15h50 *Coffee Break (30min)*

16h20 **9 Initial Finding of the Consultation (40min)**
The OECD Secretariat will present the initial findings of the consultation. The consultation participants will be invited to provide comments as appropriate.

17h00 **10 Any Other Issues Relating to the Consultation (30min)**
The Secretariat will consider any other issues related to the consultation raised by the participants.

17h30 *Meeting adjourns*

ANNEX 2: LIST OF PARTICIPANTS

Canada/Canada

Mark BONNELL — *Senior Science Advisor*
Environment Canada

Dr. Sunil KULKARNI — *Scientific Evaluator, Risk Assessment*
Risk Assessment Bureau
Health Canada

Czech Republic/République Tchèque

Dr. Marian RUCKI — *Expert*
National Institute of Public Health

Mr. Milon TICHY — *Expert*
Centre of Occupational Health
National Institute of Public Health

Denmark/Danemark

Mr. Jay NIEMELA — *Department of Toxicology and Risk Assessment*
Danish Institute for Food and Veterinary Research

France/France

Mme Brigitte MOLINIER — *Service de sécurité d'évaluatiuon des médicaments*
Sanofi aventis

Mme Veronique THYBAUD — *Dpt. Sécurité du Médicament*
Sanofi-Aventis

Germany/Allemagne

Dr. Ulrike BERNAUER — *Safety of products and formulations Toxikologie der Chemikalien*
Federal Institute for Risk Assessment (BfR)

Italy/Italie

Mr. Romualdo BENIGNI — *Experimental and Computational Carcinogenesis*

Japan/Japon

Dr. Makoto HAYASHI
Technical Adviser
Safety Assessment Division, Chemical Management Center
National Institute of Technology and Evaluation

Dr. Atsushi ONO
Senior Researcher
Division of Risk Assessment
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Dr. Hiroaki SHIRAISHI
Director
Research Center for Environmental Risk
National Institute for Environmental Studies

Netherlands/Pays-Bas

Dr. Emiel RORIJE
SEC
RIVM-National Institute for Public Health and the Environment

Poland/Pologne

Mr. Jacek CIESLA
Expert of the Ministry of Health, Senior Specialist, IT Manager
Dept for Risk Assessment
Bureau for Chemical Substances and Preparations

Sweden/Suède

Dr. Jörgen HENRIKSSON
Principal Scientific Officer
Swedish Chemicals Agency (KEMI)

United States/États-Unis

Kelly MAYO
Chemist
US Environmental Protection Agency

Yintak WOO
Senior Toxicologist
US Environmental Protection Agency

EC/CE

Dr. Tatiana NETZEVA	*Senior Scientific Officer* *Unit C2 - Registration* *European Chemicals Agency -ECHA*
Doris HIRMANN	*Scientific Officer* *Unit for Scientific IT Tools* *European Chemicals Agency*
Mrs. Rositsa SERAFIMOVA	*JRC ISPRA* *European Commission*

Business and Industry Advisory Committee (BIAC)/Comité consultatif économique et industriel (BIAC)

Dr. Joseph DULKA	*Global Regulatory Ecotoxicologist* *Crop Protection* *DuPont*

International Council on Animal Protection in OECD Programmes/International Council on Animal Protection in OECD Programmes

Alexander TROPSHA	*Director* *Laboratory for Molecular Modeling*

OECD/OCDE

Mr. Kees VAN LEEUWEN	*TNO Quality of Life*
Dr. Mark CRONIN	*School of Pharmacy and Chemistry* *Liverpool John Moores University*
M. Bob DIDERICH	*Principal Administrator* *ENV/EHS* *OECD*
Dr. Sabcho DIMITROV	*Laboratory of Mathematical Chemistry (LMC)* *University "Prof. Assen Zlatarov"*
Steven ENOCH	*Post-Doctoral Research Fellow* *Liverpool John Moores University*
Dr. Philip JUDSON	*Scientific Advisor* *Lhasa Limited*

Professor Ovanes MEKENYAN	*Laboratory of Mathematical Chemistry (LMC), Bourgas University*
Mr. Michihiro OI	*Adminstrator, (Q)SARs and Exposure Assessment ENV/EHS OECD*
Mr. Todor PAVLOV	*Laboratory of Mathematical Chemistry (LMC), Bourgas University*
Mr. Terry SCHULTZ	*Administrator, (Q)SARs ENV/EHS OECD*
Mr. Dave WATSON	*Chief Executive Officer Lhasa Limited*

ANNEX 3:
A REPORT ON RE-EVALUATION OF STRUCTURAL ALERTS FOR THE BINDING OF MOLECULES TO DNA AND THE DEVELOPMENT OF A COMPREHENSIVE PROFILER OF ALERTS

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Executive Summary

This report describes the development of a new profiler compiling mechanistic organic chemistry fragments (in the form of structural alerts) for the binding of organic compounds to DNA. It is intended that the new profiler will replace the current DNA binding profiler that is available in version 1.1.01 of the OECD (Q)SAR Application Toolbox. The profiler was created following the mapping of existing structural alerts for genotoxicity. The mapping was performed to achieve maximum overlap and usability whilst restricting redundancy in the alerts. A total of 56 new or re-defined alerts have been created; of these all but two are supported by mechanistic information and meta data. The alerts cross six broad organic chemistry mechanisms and represent the most comprehensive listing of structural alerts for DNA binding currently available.

1. Introduction

A mutagen is a physical or chemical agent that changes the genetic material (usually DNA) of an organism and thus increases the frequency of mutations above the natural background level. There are many sources of mutagens in the environment, both natural and man-made. Mutation is, of course, a natural process which promotes evolution. It can also be a harmful process resulting in deleterious effects such as cancer.

Exposure to chemical substances is one route of mutagenesis (i.e. the production of unwanted mutagenic events). There is therefore a desire to assess chemical substances to determine their mutagenic potential. There are a number of methods to determine this potential, both *in vitro* and *in vivo*. However, there is an increasing appreciation of the costs (financial and animal use) associated with such testing, particularly with regard to the regulatory assessment. Thus alternatives are being sought to rapidly screen compounds for their mutagenic potential. There would also be great utility of such techniques to develop new compounds more rapidly and efficiently.

In order to develop alternatives, there must be an appreciation of the mechanism (i.e. biochemical effect) of the chemicals at the molecular level. In terms of genotoxic mechanisms of mutagenicity, a primary requirement is for an electron deficient molecular fragment or molecule to form a covalent bond with an electron rich site on DNA, thus forming a DNA adduct. These nucleophile-electrophile interactions are well understood in chemistry and are commonly associated with genotoxic compounds.

A key technique to develop non-test methods to assess the genotoxic potential of chemicals are the *in silico* approaches. These attempt to relate the chemical and / or structural properties to a molecule to its activity. The techniques include the development of (quantitative) structure-activity relationships ((Q)SARs) and the formation of categories to facilitate read-across. The possibility of relating structure to mutagenic activity has been explored for several decades. As well as developing "traditional" QSARs (i.e. statistical techniques relating activity to molecular descriptors), there has been a keen interest in developing so-called "structural alerts". These are essentially molecular fragments that are known, or thought to, be related to the effect in question. Thus, over twenty years ago Ashby and Tennant published their "supermolecule" summarising the state of the art of structural alerts at that time. The philosophy of such an approach is clear and elegant: it allows structural fragments associated with mutagenicity to be defined and related to a (organic chemistry) mechanism of action. Structural alerts have been used for many years to identify potentially mutagenic molecules i.e. if the target molecule contains the alert is may be mutagenic. An added bonus has been their more recent use to assist in the development of chemical categories or groupings which may assist in the filling of data gaps for regulatory purposes. Such collections, or compilations, of structural alerts can be thought of as profilers for this effect and if associated with a defined chemical grouping are easily coded into a computational format for further application.

2. Aims

This report details the recent scientific efforts to update the DNA binding profiler within OECD QSAR Application Toolbox V1.1.07 (referred to throughout as 'the OECD Toolbox'). The alerts for the new profiler are referred to throughout as 'The Updated DNA Binding Profiler'. The aims of this analysis can be summarised as follows:

- To review the current scientific knowledge relating to genotoxic DNA binding structural alerts, where a structural alert relates to a fragment of a molecule able to covalently bind to DNA.
- To map the existing structural alerts in terms of their relationships with mechanistic organic chemistry.
- To undertake an analysis of the underlying mechanistic chemistry for each alert.
- To compile a new, and complete (at the time of writing) set of DNA binding structural alerts.

It is important to state that no attempt has been made to validate the newly suggested genotoxic alert compilation against toxicological data (although the mechanisms associated with the alerts have been suggested in at least one peer-reviewed literature source to be capable of reacting with DNA).

3. Existing approaches to model DNA binding

A number of previous research studies have been undertaken in an effort to model, through *in silico* means, DNA binding (using mutagenicity and carcinogenicity data). These efforts have ranged from statistically-derived global modelling approaches, through to so-called expert systems, and the definition of mechanistically derived structural alerts. These methods can be considered as a continuous spectrum with increasing mechanistic interpretability and transparency (Figure 3.1). The following very general conclusions can be drawn from the analysis of the modelling approaches. A full evaluation of individual approaches according the OECD Principles for the Validation of (Q)SARs is outside the scope of this report. However, the approaches are considered according to their mechanistic relevance (i.e. can the model be interpreted with regard to the mechanism of action to which it relates). Another key factor is the transparency i.e. can the user of the structural alert quickly and rationally obtain the mechanistic relevance. Whilst these are by no means the only criteria for evaluating an *in silico* model, it is key to the development of meaningful structural alerts and their compilation into a profiler suitable for category formation.

- Statistically derived global models are the least mechanistically interpretable. However, in some cases they can be used in the derivation of new mechanistic information about the causes of toxicity.
- Mechanistic alerts are at the opposite end of the spectrum and are based on the current scientific knowledge regarding the ability of chemicals to bind covalently to DNA.
- Expert systems fall somewhere in-between these two extremes with systems being constructed using either a mixture of mechanistic alerts and statistical models or favouring one end of the spectrum.

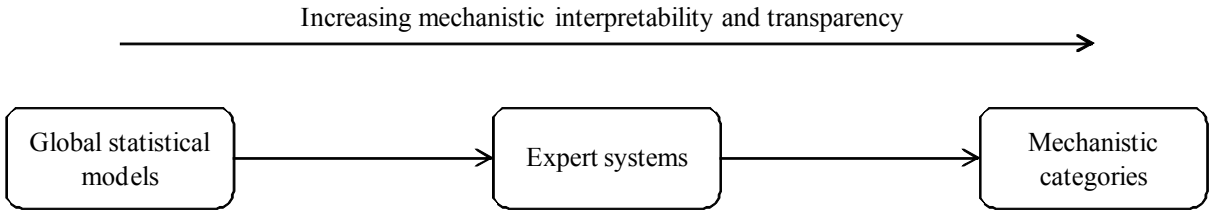


Figure 3.1: Schematic showing the relationship between modelling approach and mechanistic interpretability and transparency

The various previous approaches for the modelling of DNA binding, along with the type of methodology employed are summarised in Table 3.1. The advantages and disadvantages of the three methodologies (global statistical models, expert systems, and mechanistic categories) are summarised in Table 3.2.

Software	Methodology	Further information
CAESAR	Global statistical	http://www.caesar-project.eu/software/index.htm
PASS	Global statistical	http://195.178.207.233/PASS/index.html
Topkat	Global statistical	http://accelrys.com/products/discovery-studio/toxicology/
LAZAR	Global statistical	http://lazar.in-silico.de
MultiCase	Global statistical	http://www.multicase.com/
Hazard Expert Pro	Expert system	http://www.compudrug.com/
DEREK for Windows	Expert system	http://www.lhasalimited.org
Toxtree	Expert system	http://ecb.jrc.ec.europa.eu/qsar/qsar-tools/index.php?c=TOXTREE
OncoLogic	Expert system	http://www.epa.gov/oppt/newchems/tools/oncologic.htm
OECD QSAR Application Toolbox	Mechanistic categories	http://www.oecd.org/document/23/0,3343,en_2649_34379_33957015_1_1_1_1,00.html

Table 3.1: Summary of previous efforts in the modelling of DNA binding

Modelling approach	Advantages	Disadvantages
Statistics derived global	Useful when mechanism of action is unknown. Good for screening large numbers of chemicals.	Can be difficult to interpret the models' predictions. Models are frequently not transparent to the end-user. Applicability domain can be difficult to define.
Expert systems	Can be mechanistically based. Good for screening large numbers of chemicals.	Limited (although well defined) applicability domain. Not always transparent or mechanistically based
Mechanistic alerts	Derived from knowledge of the underlying mechanism of action. Useful for defining chemical categories. Interpretable and transparent.	Each alert has a limited (although well defined) applicability domain.

Table 3.2: Advantages and disadvantages of the differing approaches to modelling DNA binding

The regulatory application of such methods is still relatively limited in the European Union, in contrast the United States Environment Protection Agency (US EPA) has utilised the OncoLogic expert system to aid decision making. The OncoLogic system aims to predict carcinogenesis of fibres, metals, polymers and (importantly in terms of the current work) 48 classes of organic chemicals. The system contains a decision tree which utilises a number of rules including structural alerts for known genotoxic chemicals. This places the OncoLogic expert system firmly towards the mechanistic end of the spectrum shown (Figure 3.1). Importantly, the alerts are used in conjunction with other rules (such as structural features that modulate activity) to define a risk level. It is this ability to define a risk level (and thus automatically performs a level of structure-activity relationship analysis) that makes the OncoLogic system an 'expert system'. The resulting output from analysis being a mechanistically evaluated statement of the potential carcinogenic potential of the chemical of interest.

In the European Union the main focus towards the development of predictive DNA binding software has been the OECD (Q)SAR Application Toolbox (the OECD Toolbox). This software aims to allow an end-user to develop mechanistic categories within which (Q)SAR and trend analysis can be performed. Such analyses are aimed at allowing data gaps to be filled in a chemical (or a series of chemicals) toxicological profiles, thus reducing the animal burden within the REACH legislation (*1*). The Toolbox contains a number of profilers enable chemicals to be grouped into categories using mechanistic information (other methods such as similarity are also encoded). The current DNA profiler within the OECD Toolbox contains 22 genotoxic structural alerts derived from the TIMES software (*2-4*).

4. Electrophilic mechanistic chemistry

It is important to realise that, with perhaps the exception of the global statistical modelling methods, all of the modelling methods discussed above are ultimately attempting to predict the ability of a chemical to bind covalently to DNA. If one specifically considers the mechanistically based models then an understanding of how the alerts relate to the underlying chemistry is extremely important. It is has been known for several decades that in order for a chemical to be genotoxic it must be (or can be metabolised to) an electrophile (*5, 6*). In the simplest terms this means that an electron rich nucleophilic centre on DNA attacks an electron deficient electrophilic centre on an exogenous chemical resulting in the formation of a

new chemical bond and a DNA-chemical adduct. This adduct formation is considered to be an initiation process of mutagenesis.

As an example, acrolein (structure shown in Figure 4.1) is a well-known mutagen. One can comprehend the underlying chemistry of this event by consideration of the chemical structure and likely reactivity of acrolein. In acrolein the terminal carbon atom (the so-called β-carbon) of the alkene is electron deficient due to the presence of the carbonyl group (which contains an electronegative oxygen atom that 'pulls' electron density towards itself). This makes this β-carbon electrophilic and thus susceptible to nucleophilic attack (by which one means attack by a nucleophile such as DNA) and thus capable of forming a covalent bond. One can thus define the fragment (based on the mechanism) required to make the β-carbon electrophilic as a structural alert (Figure 4.2).

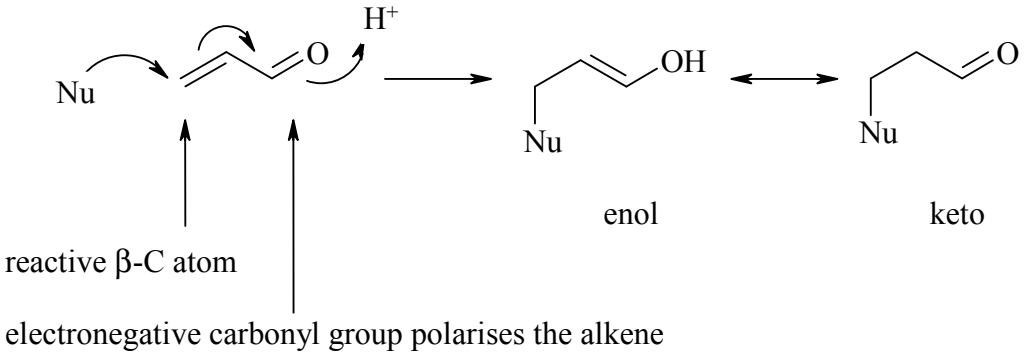
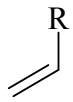


Figure 4.1: Schematic of a nucleophile attacking the electrophile acrolein resulting in a DNA-chemical adduct (Nu = biological nucleophile)



R = aldehyde, ketone, or ester

Figure 4.2: Structural alert corresponding to polarised α,β-unsaturated carbonyl compounds

Organic chemistry is replete with such definitions for how, and when, atoms within molecules can be activated to make them susceptible to nucleophilic attack, these definitions can be summarised into mechanistic reactivity domains (*7*). This idea of electrophilic fragments within molecules being responsible for DNA binding was first discussed by Ashby and Tennant over twenty years ago and led to the definition of the 'supermolecule' which illustrated a range of electrophilic fragments in one chemical (Figure 4.3).

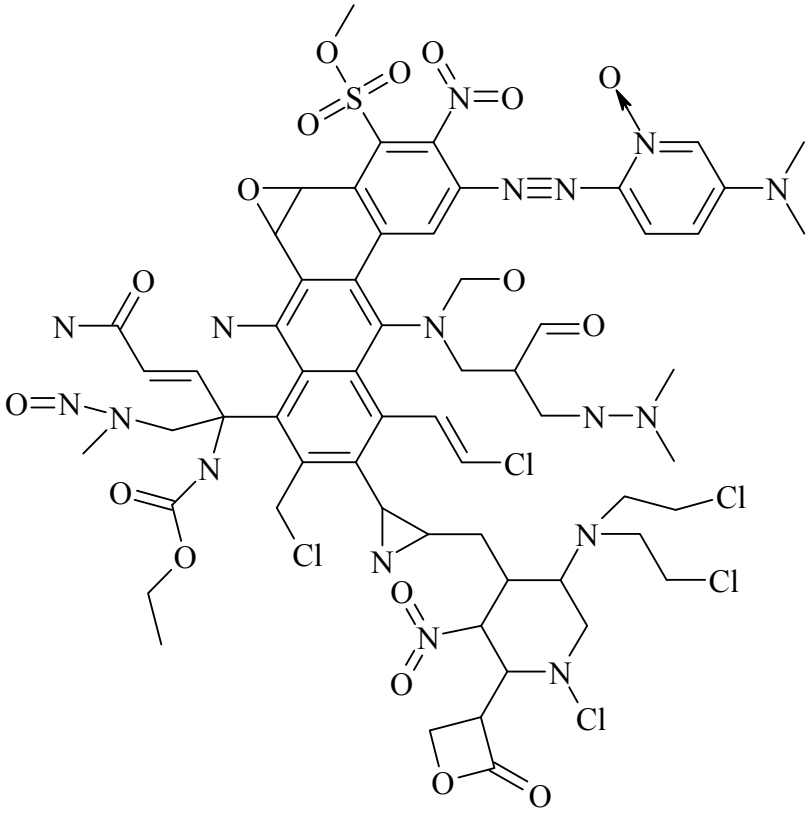


Figure 4.3: Ashby and Tennant's supermolecule

The nucleophilic centres within a DNA molecule are the nitrogen atoms within the purine and pyrimidine bases as these bases have lone pairs of electrons and thus are electron rich. It is the presence of these lone pairs that make these nitrogen atoms nucleophilic, and thus capable of undergoing nucleophilic addition reactions with electrophilic exogenous chemicals (Figure 4.4).

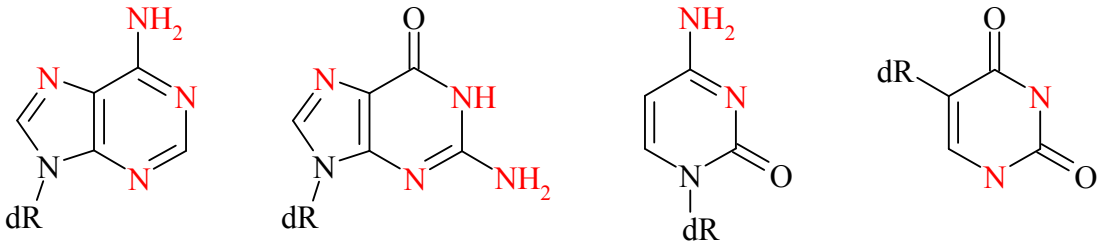


Figure 4.4: Purine and pyrimidine bases of DNA, potential nucleophilic nitrogen atoms are shown in red (dR = deoxyribose phosphate fragment)

5. Structural alert literature sources

Six literature compilations of structural alerts (*2-4, 11-13*) have been considered in detail in this analysis and have been subsequently utilised in development of The Updated DNA Binding Profiler. Importantly, these six literature sources covered previous (*14*) compilations of structural alerts (for example the compilation of Ashby and Tennant (*6*)). Three of these literature sources formed the basis of the original set of structural alerts currently available in The Toolbox (*2-4*). The alerts detailed below are all listed in the supplementary information: 'Literature alert compilations' – supplied as a separate .pdf file.

5.1 Toolbox alerts (TB)

Background

22 structural alerts are present in the OECD Toolbox ver 1.1.01. These alerts have been derived as part of a number of studies using a combination of TIMES and the COREPA (Common Reactivity Parameters) approach to QSAR modelling (*2-4*). This methodology involved the use of a decision tree to model DNA binding. One of the steps within the decision tree utilised COREPA constructed QSAR models to model DNA binding within predefined mechanistic domains. These mechanistic domains were defined using structural alerts (which included those previously suggested by Ashby (*15*)) based on the knowledge of the electrophilic interactions responsible for covalent bond formation. In addition, a number of metabolic transformations were also included.

Critical assessment

Given that this set of alerts features in the current DNA profiler within The Toolbox it is perhaps understandable that they conform to the need for clear mechanistic transparency. However, the number of alerts appears limited compared to other alert compilations.

5.2 Benigni / Bossa alerts (BB)

Background

A recent compilation of 33 structural alerts for DNA binding was derived as part of the development of a module for the freely available Toxtree software (for the availability of this software please refer to Table 3.1). This work identified structural alerts based on an analysis of the historical compilations of structural alerts. In addition, a validation exercise was performed on the new alert compilation (the so-called Benigni-Bossa alerts) using a large carcinogenicity database (*11*). The validation exercise showed the Benigni-Bossa alert compilation could correctly identify around 80 % of carcinogens.

Critical assessment

This alert compilation represents the most comprehensive and recent review of the mutagenicity and carcinogenicity data for industrial chemicals. Thus is should be considered as the central set of alerts within the current study. However, detailed mechanistic chemistry is lacking for the alerts and thus the end-user may not be clear on the rationale for a given alert.

5.3 Kazius alerts (KS)

Background

This literature source contains 29 alerts derived by a statistical analysis of an Ames mutagenicity database of 4337 chemicals (*12*). The dataset was made up of 2401 mutagens and 1936 non-mutagens tested in any of the possible bacterial strains (including data gathered utilising the S9 fraction for metabolism). The development of the structural alerts consisted of the development of an algorithm to fragment the dataset into a wide range of potential toxicophores. These potential toxicophores were then subjected to a statistical analysis in order to select those able to distinguish mutagens from non-mutagens. Importantly, no a prior mechanistic knowledge was utilised to select the either the initial set of toxicophores or those used in the final classification model. The resulting set of 29 alerts was shown to have a classification error of about 15 % which is in keeping with the experimental error of the Ames assay.

Critical assessment

These 29 alerts were not developed from a mechanistic standpoint, with the authors of the study clearly stating that it was not their intention to provide mechanistic rational for the resulting data. However, the inspection of the resulting alerts shows that they are related to well-defined electrophilic (or pro-electrophilic) chemical fragments. Thus, they provide useful mechanistic information regarding potential mechanisms of DNA binding.

5.4 Kalgutkar alerts (KR)

Background

The final literature source utilised was not a traditional compilation of structural alerts (i.e. no substructures were listed as reactive fragments), instead it is an extensive literature review of the bio-activation pathways for common organic functional groups (*13*). This source provides an extensive number of activation pathways that can convert unreactive organic chemicals into electrophilic species drawn from extensive literature mainly related to data from the drug discovery environment. Clearly, such activation pathways are likely to be important in DNA binding. The information within this compilation was converted into 25 structural alerts that had been implicated in idiosyncratic drug toxicity potentially associated with DNA binding.

Critical assessment

The information contained within this literature source (and the alerts derived from it) is potential the most important additional data available. This is because these data potentially expand the types of alerts and associated mechanistic knowledge by making use of data from drug toxicity studies. Such data have not been widely utilised in the development of previous structural alert compilations (which have been derived using industrial chemicals).

5.5 Alert overlap

As discussed, one can consider the Benigni / Bossa alerts as the most comprehensive set currently published into the literature. Given that the Benigni / Bossa rule base has already been implemented into the OECD Toolbox (ver 1.1.01) it is important to illustrate how structural alerts within the new mechanistic alert DNA profiler will differ. Two important areas are currently lacking in the Benigni / Bossa rule base the first being the availability of supporting mechanistic chemistry for each of the alerts and the second being the consideration of potential DNA binding from studies in idiosyncratic drug toxicity. The first of these issues is a significant part of the current work plan and will form the meta data for each of the alerts (discussed later in this report). The second can be highlighted by considering the overlap between the four alert compilations discussed above (Figure 5.1).

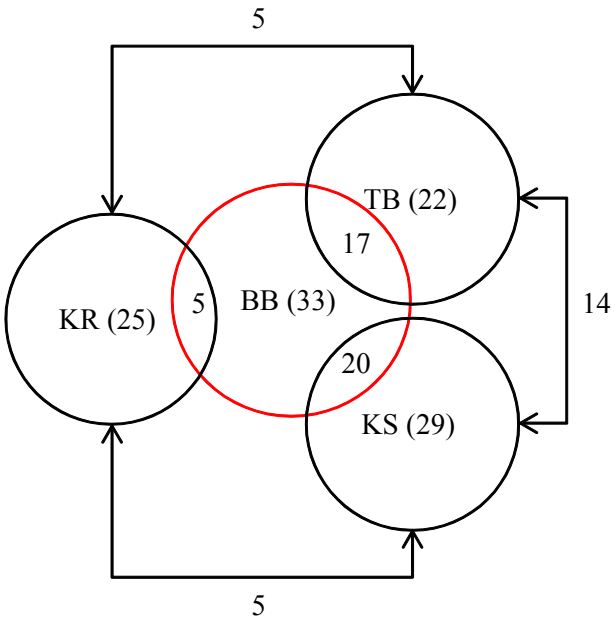


Figure 5.1: Alert overlap between the four literature compilations considered in the current work plan

The Venn diagram clearly shows that the 33 alerts of the Benigni / Bossa (BB) rule base cover the majority of the same areas of chemistry as the Kazius (KS) and Toolbox (TB) alerts. In contrast, the overlap between the Kalgutkar (KR) and Benigni / Bossa (BB) alerts is significantly less with the BB alerts only containing five of the KR alerts. This is perhaps unsurprising considering that the KR alerts have been derived from data not (necessarily) utilised in the development of the BB alerts (which were developed from mutagenicity and carcinogenicity data exclusively). This illustrates the potential for the development of new alerts based on the additional information contained within the KR set of alerts.

It is important to distinguish the rationale behind the new alerts that will be part of The Updated DNA Binding Profiler from the alerts utilised in previous systems, especially the Benigni / Bossa compilation (which are currently the most comprehensive compilation). Such compilations have utilised large toxicological databases in order to select alerts based on their frequent occurrence and ability to distinguish mutagens (or carcinogens) from non-mutagens (or non-carcinogens). Thus, these alerts compilations have been developed with the aim of predicting toxicity rather than for developing mechanistic categories.

Despite this, such alert compilations usually have a sound mechanistic basis, although discussion of the mechanisms is lacking.

In contrast to this approach the primary criteria for alert inclusion in The Updated DNA Profiler is the presence of a potential electrophilic mechanism related to DNA binding. This method has been employed in order to allow categories to be developed about alerts based on a clear mechanistic rationale. The aim being that (Q)SAR or trend analysis within the category enables the toxicity to be predicted. Importantly, the new alerts are not utilised as a predictor of toxicity.

6. Mechanistic alerts

The previous section has outlined briefly the importance of considering the underlying chemistry associated with DNA binding. It is clear that the fundamental step in this type of toxicity is the covalent bond formed between nucleophile and electrophile. The mechanistic importance of this chemical reaction makes the mechanistic alert approach the natural choice for The Updated DNA Profiler. The development of the profiler has a number of key advantages, these being;

1. The use of mechanistic chemistry based alerts enables transparent category formation. The use of mechanistic classes (usually as defined by the presence of common reactive functional group) has enabled a number of QSAR models to be developed for DNA binding (*8, 9*). In addition, the mechanistic domain approach has been shown to be successful in the prediction of other endpoints in which electrophilic reaction chemistry is important such as skin sensitisation (*10*).
2. Mechanistic alert based profilers enable the prioritising of chemicals for assessment using a transparent methodology.
3. A mechanistic alert based profiler is in keeping with the OECD Principles for the Validation of (Q)SARs and thus any trend analysis and / or QSAR developed within a category is more likely to accepted in a regulatory environment.
4. Such a profiler is easy to implement into the current OECD Toolbox architecture.

Thus, the aim of the current work has been to define the mechanistic chemistry associated with previously published structural alerts. The following section details the six literature sources that have been utilised in the development of The Updated DNA Binding Profiler.

7. Alert development methodology

In addition to reviewing the current structural alert compilations, this study also aimed to develop the reporting of the associated mechanistic chemistry. This information is needed for the meta data that the end user of the OECD Toolbox will have access to it in order to support the development of a given chemical category. Given this requirement a clear protocol was required in order to allow the mechanistic chemistry and meta data to be developed in a consistent and transparent manner. Importantly, the assessment of the mechanistic domain overlap between corresponding structural alerts in the literature compilations also needed to be investigated. This analysis is required in order to ensure that for a given alert in the new mechanistic alert DNA profiler that the maximum mechanistic information (and thus domain) was extracted.

7.1 Alert evaluation protocol

The initial stage in the development of the new set of DNA binding alerts involved detailing the mechanistic chemistry associated with the Benigni-Bossa (BB) set of alerts. The BB set was chosen for this analysis as they cover the greatest area of chemistry and have the maximum overlap with the other three alert compilations (Figure 5.1). The mechanistic chemistry was detailed as follows:

- Establish a clear mechanism or mechanisms for each alert. The mechanism (or mechanisms) needed to be supported by peer-reviewed literature.
- Mechanisms were documented as a schematic indicating how the alert was (or could be) metabolised into a potential electrophile (capable of DNA binding).
- Assign the mechanism (or mechanisms) to a mechanistic domain. The potential domains being: Michael acceptor, acylation, Schiff base formation, S_NAr, S_N (which covers both S_N2 and S_N1 mechanisms as they are frequently closely related), and radical mechanisms. A final domain was created for alerts in which the literature suggested that a clear mechanism could not be established (Unclear).
- No information regarding potential DNA adducts was documented (in many instances this type of information is lacking in the literature).
- No toxicological data associated with a given alert were recorded as The Updated DNA Profiler is based on potential mechanisms of action and these data will be available to the user in the OECD Toolbox.

Importantly the development of the mechanistic chemistry information during the alert evaluation and alert mapping procedures was not intended to define specific DNA adducts that might be formed by the covalent reaction (one of the reasons being the numerous adduct possibilities that exist for many of the alerts). Instead the mechanistic information is intended to outline how the alert can act as a direct electrophile or how it can be converted into an electrophile. The mechanistic information was intended to allow the user to clearly understand the chemistry, and thus the mechanistic rationale, that defines the chemical category.

A final important consideration within the mechanistic chemistry framework is the inclusion of potential metabolic activation. In the current profiler, known metabolic activation pathways have been included within each alert. Extensive metabolic pathways are not included with only relevant routes being detailed (as defined by the literature associated with the mechanism for the alert). Importantly, separate alerts have been generated for metabolically related chemicals if both pro-electrophiles and the corresponding electrophile can exist separately, for example hydroquinones and quinones. The rationale being that the metabolic conversion could be the rate determining step in the overall toxicity.

7.2 Alert mapping protocol

An additional important step in the development of the new set of alerts for The Updated DNA Binding Profiler was the process of mapping the four sets of literature alerts (as discussed in Sections 5.1 – 5.4) onto one another. This process ensured that subsequent analysis of the underlying mechanistic chemistry was undertaken on a non-redundant set of new alerts. The process undertaken can be summarised as a flow chart (Figure 7.1).

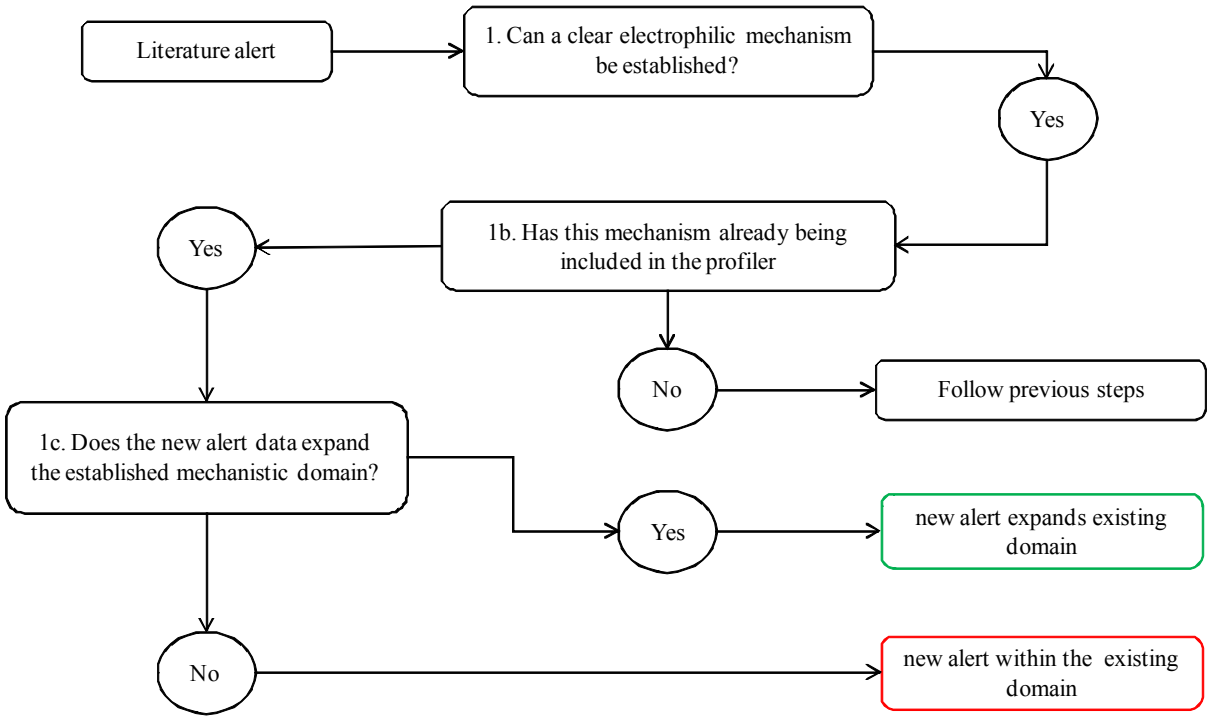
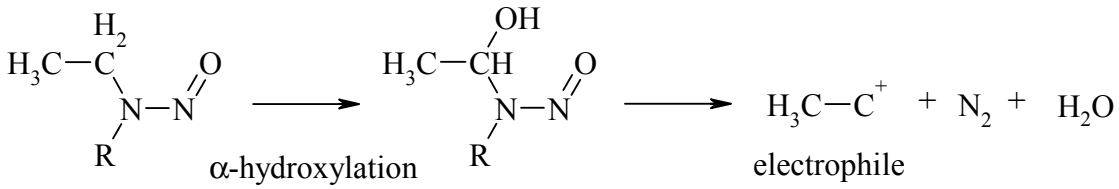
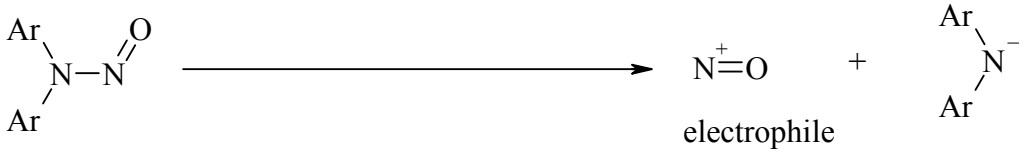


Figure 7.1: Flow chart detailing the process of alert mapping (the follow previous steps refers to the bullet points above)

As a simple example of the alert mapping process consider the alerts TB16, TB22, BB23, and KS5 (Tables 2-5). These four alerts all relate to the structural features that control the ability of aliphatic nitroso compounds to bind covalently to DNA. An initial mechanistic analysis of the TB16 alert reveals the possibility of two S_N1 mechanisms, either an alkylation or nitrosation depending on the substitution pattern (Figure 7.2).



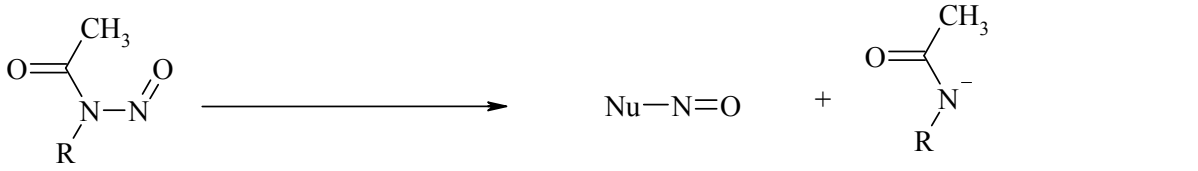
1. alkylation



2. nitrosation

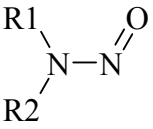
Figure 7.2: Mechanism for nitroso alkylation or nitrosation

Analysis of the remaining three alerts using the mapping procedure (Figure 7.1) results in an appreciation that two of the alerts do not expand the mechanistic domain of TB16 (BB23 and KS5). However, the alert TB22 does expand the mechanistic domain by covering an additional area of (related) chemical space covering the possibility of nitrosation via an S_N2 mechanism (Figure 7.3) (*18*). It is important to realise that this nitrosation mechanism can be considered as mechanistically equivalent to the S_N1 mechanism detailed previously (Figure 7.2). This is because it is difficult to state categorically that the nitrosation of DNA occurs after dissociation (making the mechanism purely S_N1). Such mechanisms are better considered as a mixture of S_N1 and S_N2 in which both occur simultaneously, with it being the exact substituents that control the relative contributions from each mechanism (this illustrates why the these two mechanistic domains have been grouped together under the S_N mechanistic domain). The result of this mechanistic analysis and the mapping procedure is the new alert for nitroso (Figure 7.4).



DNA adduct formed by direct attack on the nitroso group
Nu = biological nucleophile

Figure 7.3: Nitrosation S_N2 mechanism



R1 = any carbon
R2 = alkyl C, aryl C, C=O, C=N, S=O

Figure 7.4: New alert developed for nitroso

Having established the potential mechanisms relating to the four nitroso alerts detailed in the literature sources, the alert is then assigned to the appropriate mechanistic domain. In the case of nitroso the reported mechanisms are all within the S_N domain (Alert SN09 in the supporting information PDF file 'Aliphatic Nucleophilic Substitution Mechanistic Domain'). In the above example (and in the majority of cases) the potential mechanisms can all be assigned to a single mechanistic domain. However, it is important to realise that for three of the alerts the mechanistic chemistry analysis resulted in potential mechanisms that were in different mechanistic domains. In these cases the alert was included in both domains (Table 7.1).

Alert	Mechanistic domains	Alert numbers
Benzylamines	Acylation / Schiff base	AC07 / SB05
Polycyclic aromatic hydrocarbons	Michael addition / S_N	MA12 / SN14
Thiophenes	Michael addition / S_N	MA11 / SN01

Table 7.1: Alerts for which mechanisms belong to more than a single mechanistic domain

7.3 Alert summary

The above analysis resulted in 56 alerts, for 54 of these alerts a clear mechanism (or mechanisms) could be found in the literature, however, in the case of two alerts (azides and aromatic ring N-oxides) no definitive mechanism could be identified. These alerts were assigned to the mechanistic domains as shown (Table 7.2). (The mechanism domain alert number refers to the appropriate alert in the supplementary information meta data files. The first two letters correspond to the mechanism: AC = acylation, MA = Michael addition, SB = Schiff base, SN = aliphatic nucleophilic substitution (S_N), RD = radical, UN = Unclear). Table 7.3 shows the number of alerts per domain.

Alert name	Mechanism domain alert number
Acyl halide	AC01
Isocyanates	AC02
Thiazolidinediones	AC03
Formamides	AC04
Sulfonylureas	AC05
1,1-Dihaloalkanes	AC06
Alpha-beta-unsaturated carbonyls	MA01
Quinones	MA02
Hydroquinones	MA03
Quinone methides	MA04
Alkyl phenols	MA05
Furans	MA06
3-Methylindole	MA07
Methylenedioxyphenyl	MA08
5-Alkoxyindoles	MA09
Arenes	MA10
Thiophenes	MA11 / SN01
Diazonium	RD01
Imides	RD02
N-methylol derivatives	SB01
Aliphatic aldehydes	SB02
Alpha-dicarbonyl	SB03
Thiazoles	SB04
Benzylamines	SB05 / AC07
Hydrazine	SN02
Aliphatic N-Nitro	SN03
Triazines	SN04
Aromatic azo	SN05
Aromatic amine	SN06
Protected aromatic amines	SN07
Aromatic nitro	SN08
Aromatic nitroso	SN09
Aromatic N-hydroxylamines	SN10
Ureides	SN11
Diazo	SN12
Ester aromatic hydroxylamine	SN13
Polycyclic aromatic hydrocarbons (PAHs)	MA12 / SN14

Aliphatic nitroso	SN15
Mustards	SN16
1,2-Dihaloalkanes	SN17
Monohalo alkenes	SN18
Coumarins	SN19
Alkyl nitrate	SN20
Alkyl carbamates	SN21
Epoxides	SN22
Aliphatic halide	SN23
Lactones	SN24
Sultones	SN25
Sulfonic esters	SN26
Phosphonic esters	SN27
N-acyloxy-N-alkoxyamides	SN28
Thioureas	SN29
Aliphatic tertiary amines	SN30
Polarised alkenes	SN31
Azides	UN01
Aromatic ring N-oxides	UN02

Table 7.2: List of the new alerts resulting from the analysis of the literature sources

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

Table 7.3: The number of alerts per mechanistic domain

7.4 Mitigating factors

The presence of mitigating factors associated with an alert are also defined within the mechanistic chemistry analysis. In the current analysis a mitigating factor is only considered as part of the alert if it has been shown to completely abolish DNA binding activity. This is in keeping with other definitions of the mitigating factors and ensures that such factors are transparent and (importantly) easy to encode computationally into the final alert. In addition, defining mitigating factors that modulate activity is made more difficult for DNA binding given the binary nature of the biological assay from which many of the alerts have been drawn. Mitigating factors can be (approximately) divided into three classes: steric, electronic, and detoxifying (Table 7.4). Importantly, it is frequently difficult to definitively assign the mechanism of mitigation. For example, the presence of a sulphate groups on an aromatic amine is known to remove activity it has been suggested that this could be due to increased detoxification or the electronic effect of the sulphate group reducing the ability of the amine to be metabolised into the electrophilic nitrenium ion. Given this difficulty no attempt has been made to suggest mechanisms for mitigating factors.

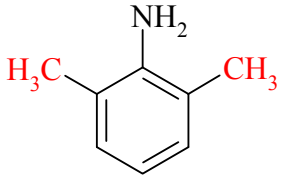
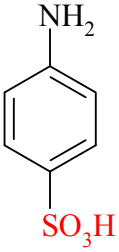
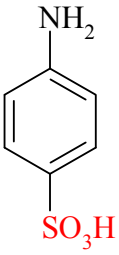
Mitigating factor	Example inactive chemical
Steric	
Electronic	
Detoxifying	

Table 7.4: Classes of potential mitigating factors for aromatic amines (mitigating factor in red)

7.5 The meta data

The mechanistic chemistry information contained within the meta data files is designed to be as transparent and simple as possible, with, as discussed, the idea being to help the user understand the chemistry (and associated mitigating / metabolic factors) behind each alert. The meta data are not designed to highlight any examples of toxicological data associated with the alerts as this information can be accessed by running the profiler through the databases contained within The Toolbox. The meta data details the structural alert, the mechanism (or mechanisms) involved in covalent DNA binding, known mitigating factors, and the literature sources from which the mechanistic information has been drawn. The meta data contain the following information (under the shown headings):

- 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 mitigating factors

Importantly the meta data do not contain any of the following information:

- Information about the types of DNA adducts formed
- Results of toxicological testing (this can be found by running the profiler though databases within The Toolbox

The resulting meta data for each alert have been compiled into mechanistic domain specific meta data files (see supplementary information). These files contain all the meta data for alerts associated with a given mechanistic domain. As discussed if an alert had potential mechanisms across more than a single mechanistic domain then it was assigned to both domains (although only the mechanism associated with the specific mechanistic domain was included i.e. for the benzylamine alert the acylation mechanistic chemistry was only reported in the acylation meta data).

8. Conclusions

This report has detailed the development of The Updated DNA Binding Profiler in which previously published lists of structural alerts have been utilised as its basis. These alert compilations have been subjected to an analysis in order to place the information contained within the literature alerts into a mechanistic chemistry framework (in which knowledge of metabolism and potential mitigating factors are included). It is this mechanistic chemistry that is used as the basis for chemical category formation when utilising the new profiler, and thus the associated meta data. In addition, the inclusion of data from a comprehensive review of idiosyncratic drug toxicity has expanded the domain of The Updated DNA Binding Profiler to include potentially DNA binding fragments not covered in previous structural alert compilations. The result of the analysis undertaken to fulfil the deliverable being the development of 56 DNA binding structural alerts, supported by mechanistic chemistry and references to the scientific literature.

9. Supplementary information

This report has a number of files associated with it these are as follows (all of which can be found within the zip file accompanying this report, see **Annex.4**.):

- A .pdf file containing the full listings of the literature alerts utilised in the development of the new alerts
    - Literature Alert Compilations
- A .pdf file containing the full listing by mechanistic domain of the new alerts entitled (each mechanistic domain is tabulated separately):
    - New Alert Compilation by Mechanistic Domain
- Separate .pdf files for the meta data for each of the mechanistic domains. These are entitled:
    - Acylation Mechanistic Domain
    - Aliphatic Nucleophilic Substitution Mechanistic Domain
    - Michael Addition Mechanistic Domain

- Radical Mechanistic Domain
- Schiff Base Mechanistic Domain
- Unclear Mechanistic Domain

10. References

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ANNEX 4: SUPPLEMENTARY INFORMARION TO THE REPORT

1. Literature Alert Compilations

Alert Num	Alert name
TB1	aromatic N-acetoxy
TB2	aromatic N-acyloxy-N-alkoxyamides
TB3	aliphatic epoxides, aziridiens, epoxyethers
TB4	alpha, beta unsaturated aldehdyes
TB5	aromatic amines
TB6	aromatic N-hydroxylamines
TB7	azo
TB8	beta-lactone
TB9	polycyclic aromatic hydrocarbons
TB10	1,2-dihaloethanes
TB11	Mustards
TB12	N mustards
TB13	haloalkanes (labile halogen)
TB14	Hydrazine
TB15	nitro
TB16	nitrosoamines
TB17	Nitrosoarenes
TB18	phosphates
TB19	Quinines
TB20	sulphonates, sulphate
TB21	Ureides
TB22	Nitrosoureas

Table 1: Current Toolbox (TB) DNA binding alerts

Alert num	Alert name
BB1	acyl halide
BB2	alkyl or benzyl of sulphonic acid
BB3	alkyl or benzyl phosphonic acid
BB4	N-methylol derivatives
BB5	Monohaloalkene
BB6	S or N mustards
BB7	propiolactones
BB8	Propiosultones
BB9	expoxides, aziridines
BB10	aliphatic halogen
BB11	alkyl nitrite
BB12	alpha, beta unsaturated carbonyls
BB13	aldehyde
BB14	Quinines
BB15	Hydrazine
BB16	aliphatic azo and azoxy
BB17	isocyanate and isothiocyanate
BB18	alkyl carbamate and thiocarbamate
BB19	thiocarbonyl (non genotoxic)
BB20	PAH (3 or more rings)
BB21	HPAH (3 or more rings)
BB22	Poly halogenated cycloalkanes (non genotoxic)
BB23	alkyl and aryl N-nitroso
BB24	Triazine
BB25	azide
BB26	aliphatic N-nitro
BB27	alpha, beta unsaturated aliphatic alkoxy
BB28	aromatic nitroso
BB29	aromatic ring N-oxide
BB30	nitro aromatic
BB31	primary aromatic amine, hydroxyl amine and esters
BB32	aromatic amine mono and dialkylamine
BB33	aromatic N-acyl amine
BB34	aromatic diazo
BB35	coumarins and furocoumarins
BB36	halogenated benzene (non genotoxic)
BB37	halogenated PAH (non genotoxic)
BB38	halogenated dibenzodioxins (non genotoxic)

Table 2: Benigni / Bossa (BB) DNA binding alerts

Alert num	Alert name
KS1	specific-arom-nitro
KS2	specific-arom-amine
KS3	aromatic-nitroso
KS4	alkyl-nitrite
KS5	Nitrosamine
KS6	Epoxide
KS7	Aziridine
KS8	Azide
KS9	Diazo
KS10	Triazene
KS11	aromatic-azo
KS12	unsubstituted-heteroatom-bonded-heteroatom
KS13	aromatic-hydroxylamine
KS14	aliphatic-halide
KS15	carboxylic-acid-halide
KS16	nitrogen-or-sulfur-mustard
KS17	bay-region-in-polycyclic-aromatic-hydrocarbons
KS18	K-region-in-polycyclic-aromatic-hydrocarbons
KS19	polycyclic-aromatic-system
KS20	sulfonate-bonded-carbon-(alkyl-alkane-sulfonate-or-dialkyl-sulfate)
KS21	aliphatic-N-nitro
KS22	alpha,beta-unsaturated-aldehyde-(including-R-carbonyl-aldehyde)
KS23	Diazonium
KS24	beta-propiolactone
KS25	alpha,beta-unsaturated-alkoxy-group
KS26	1-aryl-2-monoalkyl-hydrazine
KS27	aromatic-methylamine
KS28	ester-derivative-of-aromatic-hydroxylamine
KS29	polycyclic-planar-system

Table 3: Kazius (KS) DNA binding alerts

Alert num	Alert name
KR1	aromatic amine
KR2	hydrazine / hydrazide
KR3	Nitroarenes
KR4	fused azaheterocycles
KR5	aliphatic tertiary amines
KR6	Benzylamines
KR7	Formamides
KR8	Imides
KR9	Sulfonylureas
KR10	Thioureas
KR11	Quinines
KR12	quinone-methides
KR13	Methylenedioxyphenyl
KR14	5-alkoxyindoles
KR15	3-methylindole
KR16	Furan
KR17	Thiophenes
KR18	Thiazole
KR19	Thiazolidinediones
KR20	Arenes
KR21	Bromobenzene
KR22	alpha, beta-unsaturated carbonyl
KR23	alkyl halides
KR24	Hydroquinones
KR25	o- or p-alkylphenols

Table 4: Kalgutkar (KR) DNA binding alerts

2. New Alert Compilation by Mechanistic Domain

Alert name	Mechanism domain number	TB alert	BB alert	KS alert	KR alert	Mitigating factors
acyl halide	AC01		BB1	KS15		Y
Isocyanates	AC02		BB17			N
Thiazolidinediones	AC03				KR19	N
Formamides	AC04				KR7	N
Sulfonylureas	AC05				KR9	N
1,1-dihaloalkanes	AC06				KR23	N
Benzylamines	AC07 (also SB05)				KR6	N

Table 1: New alerts compiled for the acylation domain (TB alert, BB alert, KS alert, and KR alert columns indicate where the alerts have been mapped from)

Alert name	Mechanism domain number	TB alert	BB alert	KS alert	KR alert	Mitigating factors
alpha-beta-unsaturated carbonyls	MA01	TB4	BB12	K22	KR22	N
Quinines	MA02	TB19	BB14		KR11	N
Hydroquinones	MA03				KR24	N
quinone methides	MA04				KR12	N
alkyl phenols	MA05				KR25	N
Furans	MA06				KR16	N
3-methylindole	MA07				KR15	N
methylenedioxyphenyl	MA08				KR13	N
5-alkoxyindoles	MA09				KR14	N
Arenes	MA10				KR20, KR21	N
Thiophenes	MA11 (also SN01)				KR17	N
PAHs	MA12 (also SN14)	TB9	BB20, BB21	KS17, KS18, KS19, KS29		N

Table 2: New alerts compiled for the Michael addition domain (TB alert, BB alert, KS alert, and KR alert columns indicate where the alerts have been mapped from)

Alert name	Mechanism domain number	TB alert	BB alert	KS alert	KR alert	Mitigating factors
diazonium	RD01			KS23		N
imides	RD02				KR8	N

Table 3: New alerts compiled for the radical domain (TB alert, BB alert, KS alert, and KR alert columns indicate where the alerts have been mapped from)

Alert name	Mechanism domain number	TB alert	BB alert	KS alert	KR alert	Mitigating factors
N-methylol derivatives	SB01		BB4			N
aliphatic aldehydes	SB02		BB13			Y
alpha-dicarbonyl	SB03					N
Thiazoles	SB04				KR18	N
Benzylamines	SB05 (also AC07)				KR6	N

Table 4: New alerts compiled for the Schiff base domain (TB alert, BB alert, KS alert, and KR alert columns indicate where the alerts have been mapped from)

Alert name	Mechanism domain number	TB alert	BB alert	KS alert	KR alert	Mitigating factors
Thiophenes	SN01 (also MA11)				KR17	N
Hydrazine	SN02	TB14	BB15	KS26	KR2	N
aliphatic N-Nitro	SN03		BB26	KS21		N
Triazines	SN04		BB24	KS10		N
aromatic azo	SN05	TB7	BB34	KS11		Y
aromatic amine	SN06	TB5	BB31, BB32, BB33	KS2, KS27	KR1	Y
protected aromatic amines	SN07					
aromatic nitro	SN08	TB15	BB30	KS1	KR3	Y
aromatic nitroso	SN09	TB17	BB28	KS3		Y
aromatic N-hydroxylamines	SN10	TB6	BB31	KS12, KS13		Y
Ureides	SN11	TB21				Y
Diazo	SN12		BB16	KS9		N
ester aromatic hydroxylamine	SN13	TB1		KS28		Y
PAHs	SN14 (also MA12)	TB9	BB20, BB21	KS17, KS18, KS19, KS29		N
Nitroso	SN15	TB16, TB22	BB23	KS5		N
Mustards	SN16	TB11, TB12	BB6	KS16		N
1,2-dihaloalkanes	SN17	TB10				N
monohalo alkenes	SN18		BB5			N
Coumarins	SN19		BB35			N
alkyl nitrate	SN20		BB11	KS4		N
alkyl carbamates	SN21		BB18			N
Epoxides	SN22	TB3	BB9	KS6,KS7		N
aliphatic halide	SN23	TB13	BB10	KS14		N
Lactones	SN24	TB8	BB7	KS24		N
Sultones	SN25	TB20	BB8			N
sulfonic esters	SN26	TB20	BB2	KS20		Y
phosphonic esters	SN27	TB18	BB3			Y
N-acyloxy-N-alkoxyamides	SN28	TB2				N
Thioureas	SN29				KR10	N

aliphatic tertiary amines	SN30				KR5	N
polarised alkenes	SN31		BB27	KS25		N

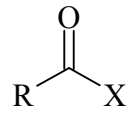
Table 5: New alerts compiled for the aliphatic nucleophilic substitution domain (TB alert, BB alert, KS alert, and KR alert columns indicate where the alerts have been mapped from)

Alert name	Mechanism domain number	TB alert	BB alert	KS alert	KR alert	Mitigating factors
Azides	UN01		BB25	KS8		Y
aromatic ring N-oxides	UN02		BB29			N

Table 6: New alerts compiled for the unclear domain (TB alert, BB alert, KS alert, and KR alert columns indicate where the alerts have been mapped from)

3. Meta Data for Acylation Mechanistic Domain

Alert AC01: Acyl halide

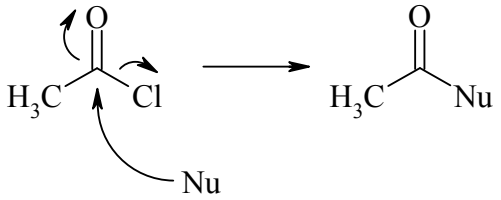


X = F, Cl, Br, I

R = alkyl, aryl

Mechanism

An acylation mechanism has been suggested to be responsible for the ability of acyl halides to bind to DNA macromolecules (Benigni et al 2008, Kazius et al 2005).



Nu = biological nucleophile

Mitigating factors

- No mitigating factors have been reported

References

Benigni et al (2008) Mutation Research 659, 248-261
Kazius et al (2005) Journal Medicinal Chemistry 48, 312-320

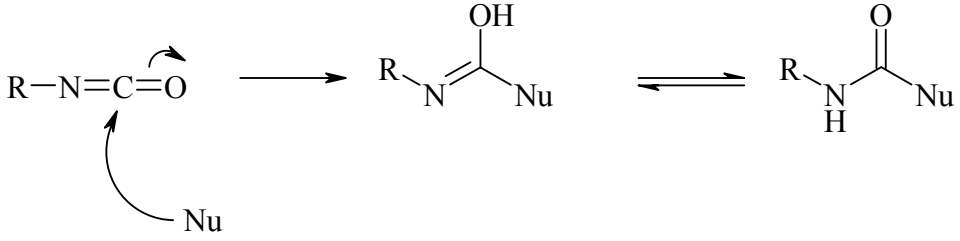
Alert AC02: Isocyanate and Isothiocyanate

R—N=C=O R—N=C=S

R = alkyl, aryl, H

Mechanism

Isocyanates and isothiocyanates are thought to bind to DNA via an acylation mechanism (Bayerbach et al 2006).



Nu = biological nucleophile

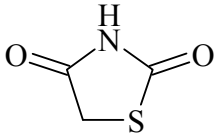
Mitigating factors

- No mitigating factors have been identified

References

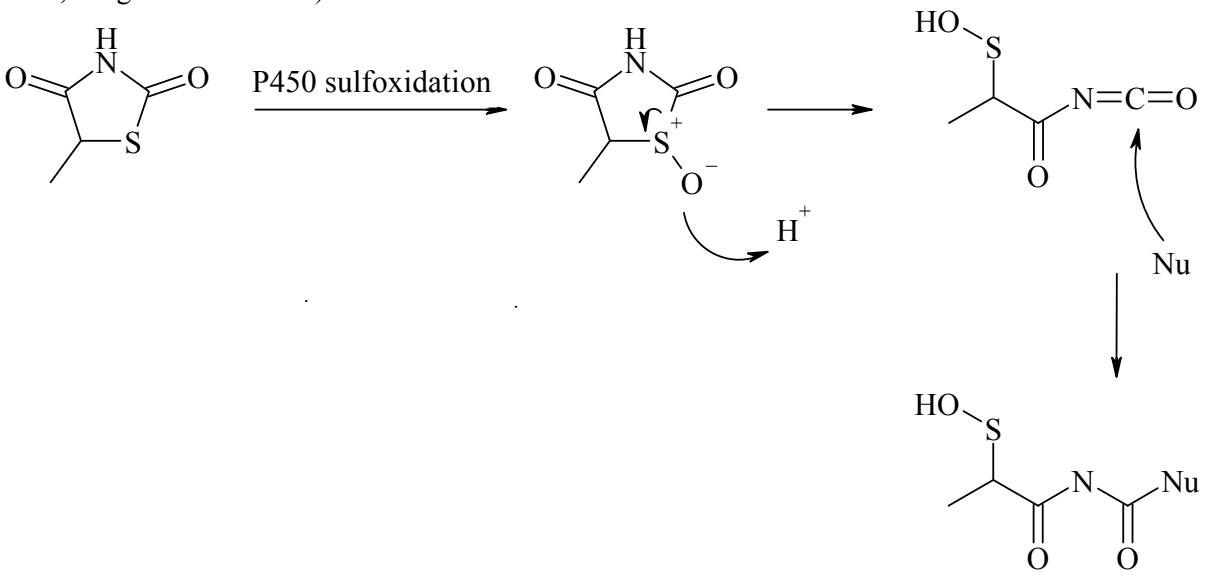
Bayerbach et al (2006) Chemical Research in Toxicology, 19, p1611-1618

Alert AC03: Thiazolidinediones



Mechanism

The most likely mechanism for DNA binding that has been suggested involves a P450 mediated sulfoxidation. This reactive intermediate species then undergoes ring scission to produce an isocycanate. This isocyanate undergoes an acylation mechanism with a biological nucleophile such as DNA (Bedir et al 2008, Kalgutkar et al 2005)



Nu = biological nucleophile

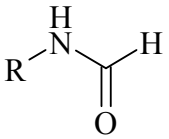
Mitigating factors

- No mitigating factors have been reported

References

Bedir et al (2008) Environmental and Molecular Mutagenesis, 49, p185-191
Kalgutkar et al (2005) Current Drug Metabolism, 6, p161-225

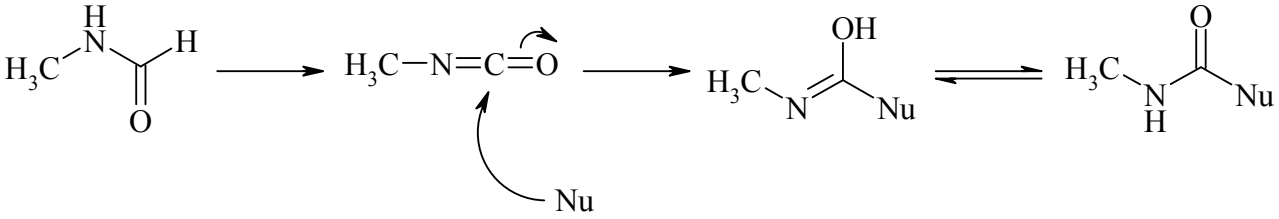
Alert AC04: Formamides



R = alkyl, aryl

Mechanism

Formamides have been suggested to be metabolised by P450 into reactive isocyanate species (Kalgutkar et al 2005). Isocyanates have been shown to be able to covalently bind to DNA via an acylation mechanism (Bayerbach et al 2006).



Nu = biological nucleophile

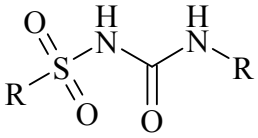
Mitigating factors

- No mitigating factors have been reported

References

Bayerbach et al (2006) Chemical Research in Toxicology, 19, p1611-1618
Kalgutkar et al (2005) Current Drug Metabolism, 6, p161-225

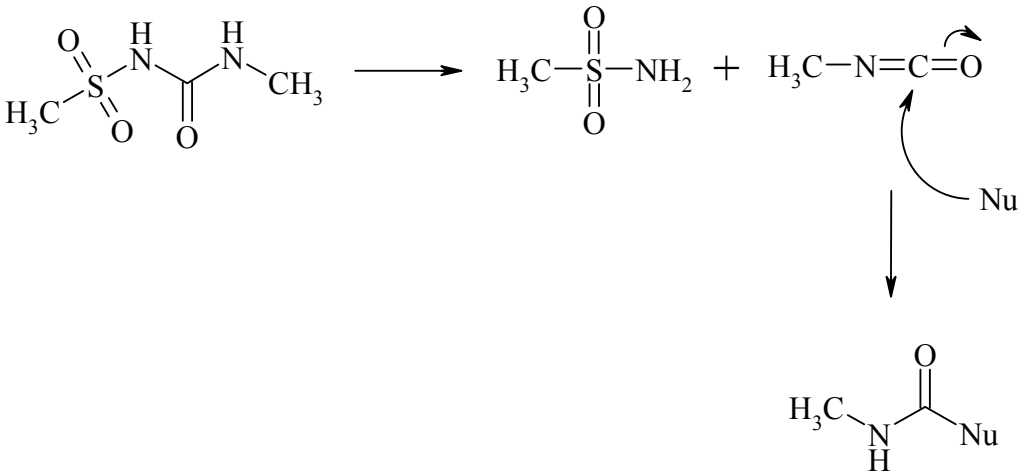
Alert AC05: Sulfonylureas



R = alkyl, aryl

Mechanism

Sulfonylureas have been suggested to be metabolised via amide bond cleavage to produce reactive isocyanate species (Kalgutkar et al 2005). Isocycnates have been demonstrated to covalently bind to DNA via a acylation mechanism (Bayerbach et al 2006).



Nu = biological nucleophile

Mitigating factors

- No mitigating factors have been reported

References

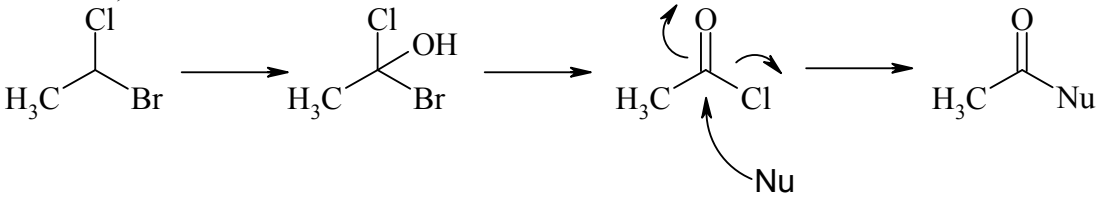
Bayerbach et al (2006) Chemical Research in Toxicology, 19, p1611-1618
Kalgutkar et al (2005) Current Drug Metabolism, 6, p161-225

Alert AC06: 1,1-Dihaloalkanes

R = any
X = halide

Mechanism

P450 mediated oxidative dehalogenation into an acyl halide has been suggested to be responsible for the toxicity of 1,1-dihaloalkanes. The acyl halide is able to bind DNA via an acylation mechanism (Kalgutkar et al 2005).



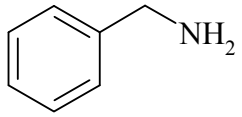
Nu = biological nucleophile

Mitigating factors

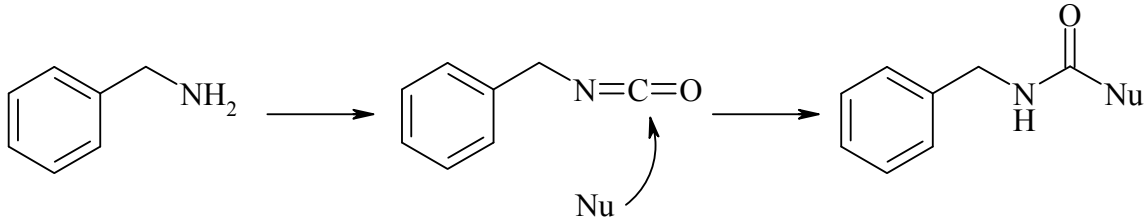
- No mitigating factors have been identified

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

Alert AC07: Benzylamines



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



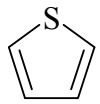
Nu = biological nucleophile

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

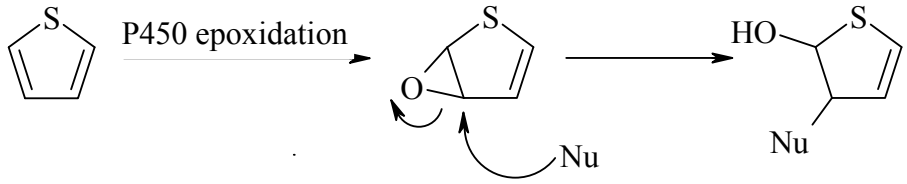
4. Meta Data for Aliphatic Nucleophilic Substitution Mechanistic Domain

Alert SN01: Thiophenes



Mechanism

An S_N2 mechanism involving P450 epoxidation followed by an ring opening reaction has been suggested to lead to DNA alkylation (Kalgutkar et al 2005, Mosier et al 2003)..



Nu = biological nucleophile

Mitigating factors

- No mitigating factors have been reported

References

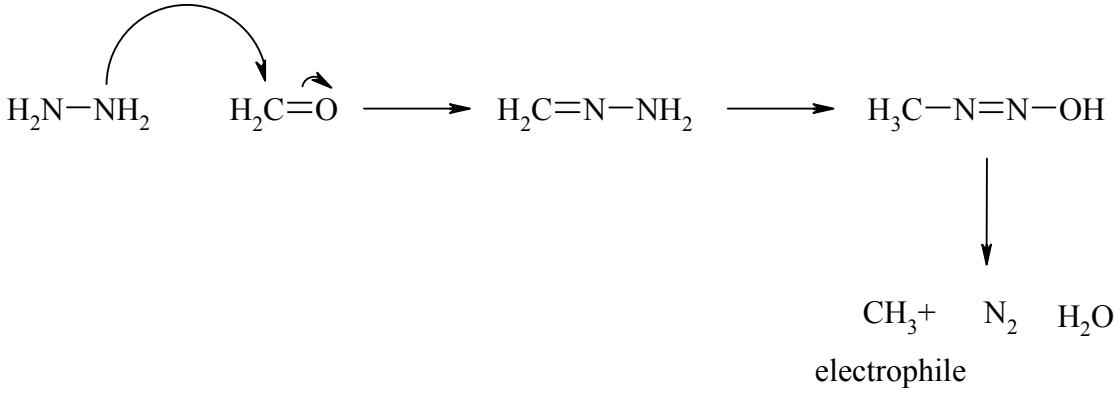
Kalgutkar et al (2005) Current Drug Metabolism, 6, p161-225
Mosier et al (2003) Chemical Research in Toxicology, 16, p721-732

Alert SN02: Hydrazine

R = alkyl, aryl

Mechanism

Formaldehyde hydrozone formation via a Schiff base mechanism followed by DNA alkylation via an S_N1 mechanism has been suggested to lead to DNA alkylation (FitzGerald et al 1996, Lambert et al 1988)



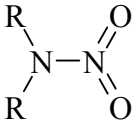
Mitigating factors

- No mitigating factors have been reported

References

FitzGerald et al (1996) Carcinogenesis, 17, 2703-2709
Lambert et al (1988) Carcinogenesis, 9, 65-70

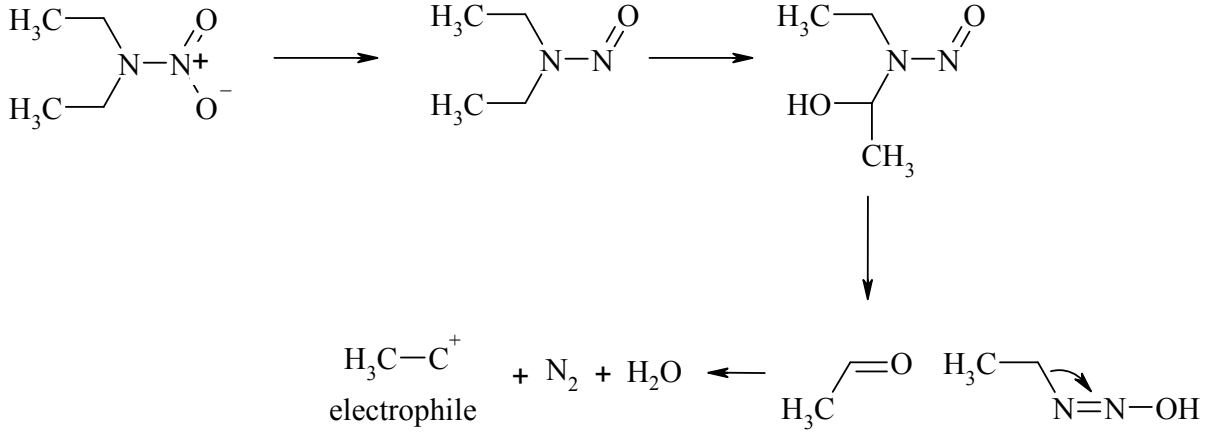
Alert SN03: Aliphatic N-Nitro



R = aliphatic C

Mechanism

This alert has been suggested to be important in DNA binding in several previous alert compilations (Benigni et al 2008, Kazius et al 2005). Despite this inclusion in alert compilations no further mechanistic studies regarding the DNA adducts can be found in the literature. However, the most plausible mechanism involves reduction of the nitro group to a nitroso and then formation of a carbenium ion resulting in DNA binding via an S_N1 mechanism (via the same mechanism as the nitroso alert).



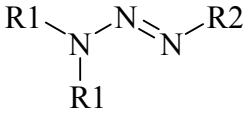
Mitigating factors

- No mitigating factors have been reported

References

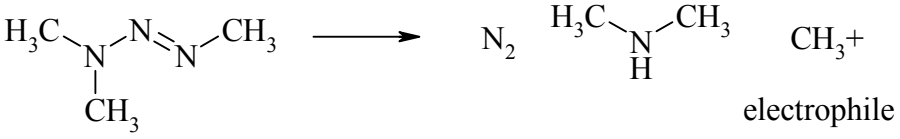
Benigni et al (2008) Mutation Research, 659, p248-261
Kazius et al (2005) Journal of Medicinal Chemistry, 48, p312-320

Alert SN04: Triazines



R1 = alkyl, aryl, hydrogen
R2 = alkyl

Mechanism
Triazines in which R2 is an alkyl group have been suggested to alkylate DNA via an S_N1 mechanism (after the production of a carbocation) (Preussmann et al 1970, Kroeger-Koepke et al 1991).

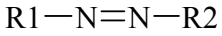


Mitigating factors

- No mitigating factors have been reported

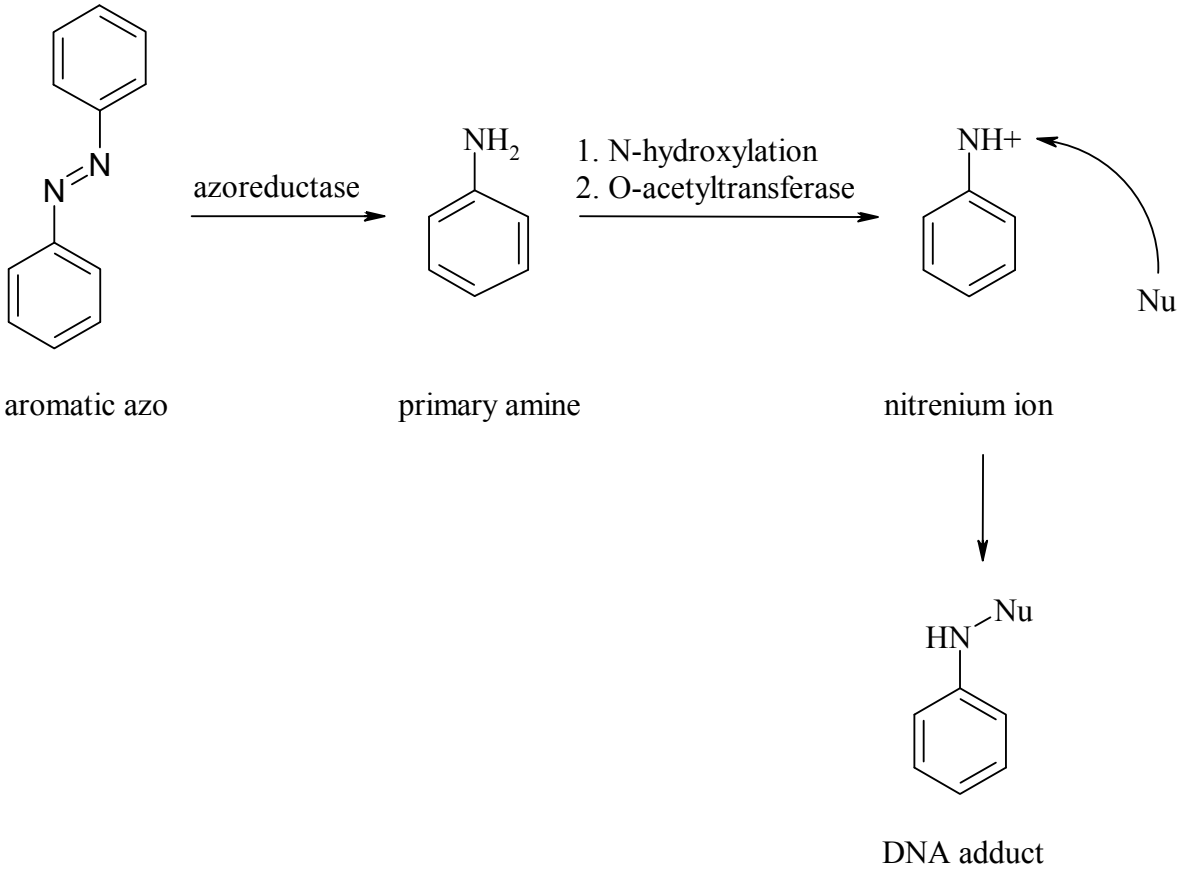
References
Kroeger-Koepke et al (1991) Chemical Research in Toxicology 4, 334-340
Preussmann et al (1970) Biochemical Pharmacology 19, 1505-1508

Alert SN05: Aromatic azo



R1 = aromatic
R2 = any

Mechanism
The most likely mechanism is phase 1 metabolism of the azo via azoreductase producing an aromatic amine which then undergoes metabolism into the DNA reactive (via an S_N1 mechanism) nitrenium ion (Kalgutkar et al 2005, Moller et al 2000).



Nu = biological nucleophile

Mitigating factors

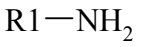
The following mitigating factors have been identified (Benigni et al 2008)

- The presence of sulphate (SO_3H) groups in any ring position. This group probably causes increased solubility and thus leads to increased detoxification.
- Substituents in the 2 and 6 position on the benzene ring. This causes steric hindrance which presumably prevents the initial metabolism step thus preventing the formation of the nitrenium ion.
- The presence of a carboxylate group (CO_2H) in the 2 position. This could be due to the formation of a six membered intramolecular hydrogen bonded ring which inhibits the initial metabolic transformation.

References

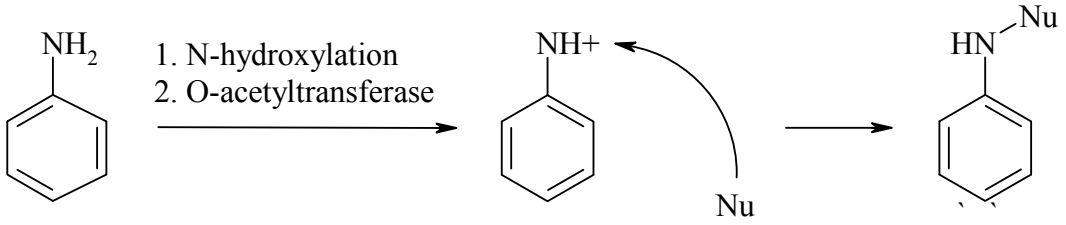
Benigni et al (2008) Mutation Research, 659, p248-261
Kalgutkar et al (2005) Current Drug Metabolism, 6, p161-225
Moller et al (2000) Mutation Research, 462, p13-30

Alert SN06: Primary aromatic amine



R1 = aromatic
R2 = any combination of methyl, ethyl, hydrogen

Mechanism
Primary aromatic amines undergo metabolism to a reactive nitrenium ion. This ion can bind to DNA via an S_N1 mechanism (Kalgutkar et al 2005, Jones et al 2003).



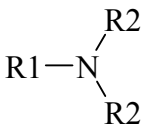
primary amine nitrenium ion DNA adduct

Mitigating factors
The following mitigating factors have been identified (Benigni et al 2008)

- The presence of sulphate (SO_3H) groups in any ring position. This group probably causes increased solubility and thus leads to increased detoxification.
- Substituents in the 2 and 6 position on the benzene ring. This causes steric hindrance which presumably prevents the initial metabolism step thus preventing the formation of the nitrenium ion.
- The presence of a carboxylate group (CO_2H) in the 2 position. This could be due to the formation of a six membered intramolecular hydrogen bonded ring which inhibits the initial metabolic transformation.

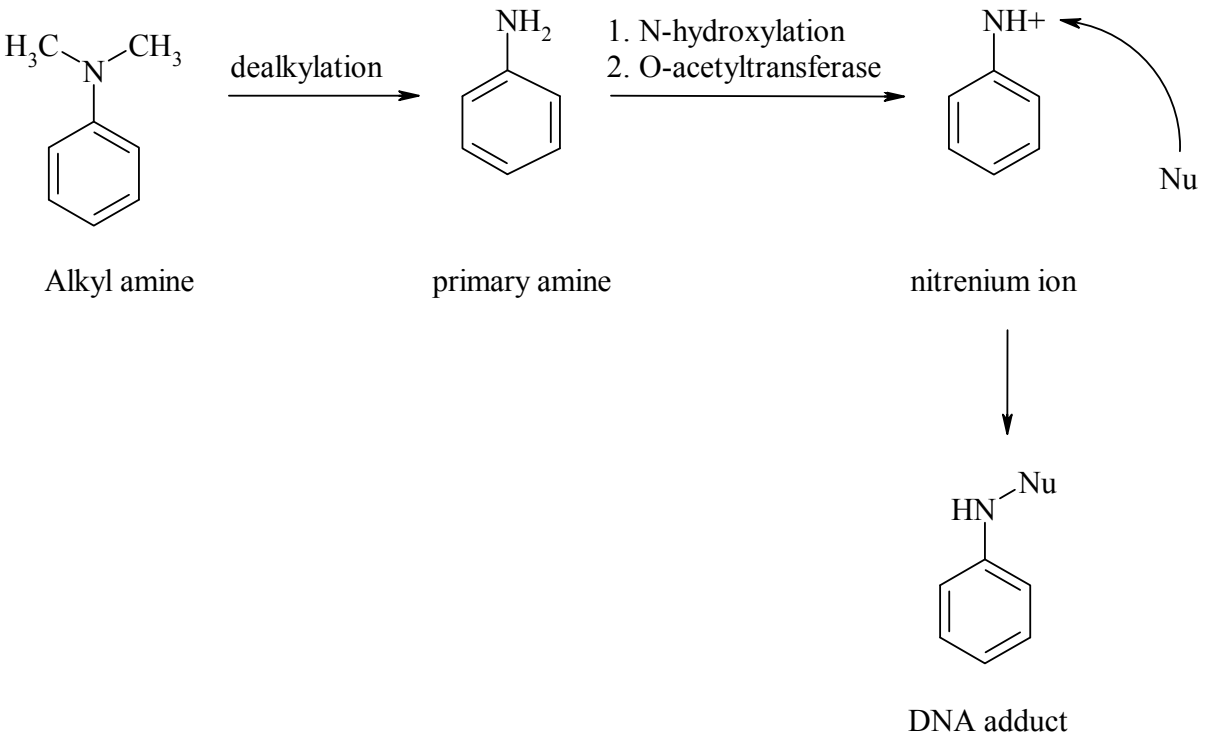
References
Benigni et al (2008) Mutation Research, 659, p248-261
Kalgutkar et al (2005) Current Drug Metabolism, 6, p161-225
Jones et al (2003) Chemical Research in Toxicology, 16, p1251-1263

Alert SN07: Protected aromatic amine



R1 = aromatic
R2 = any combination of methyl, ethyl

Mechanism
Protected secondary and tertiary aromatic amines (methyl and ethyl) undergo metabolism to a reactive nitrenium ion. This ion can bind to DNA via an S_N1 mechanism (Kalgutkar et al 2005, Jones et al 2003).



Mitigating factors

The following mitigating factors have been identified (Benigni et al 2008)

- The presence of sulphate (SO_3H) groups in any ring position. This group probably causes increased solubility and thus leads to increased detoxification.
- Substituents in the 2 and 6 position on the benzene ring. This causes steric hindrance which presumably prevents the initial metabolism step thus preventing the formation of the nitrenium ion.
- The presence of a carboxylate group (CO_2H) in the 2 position. This could be due to the formation of a six membered intramolecular hydrogen bonded ring which inhibits the initial metabolic transformation.

References

Benigni et al (2008) Mutation Research, 659, p248-261
Kalgutkar et al (2005) Current Drug Metabolism, 6, p161-225
Jones et al (2003) Chemical Research in Toxicology, 16, p1251-1263

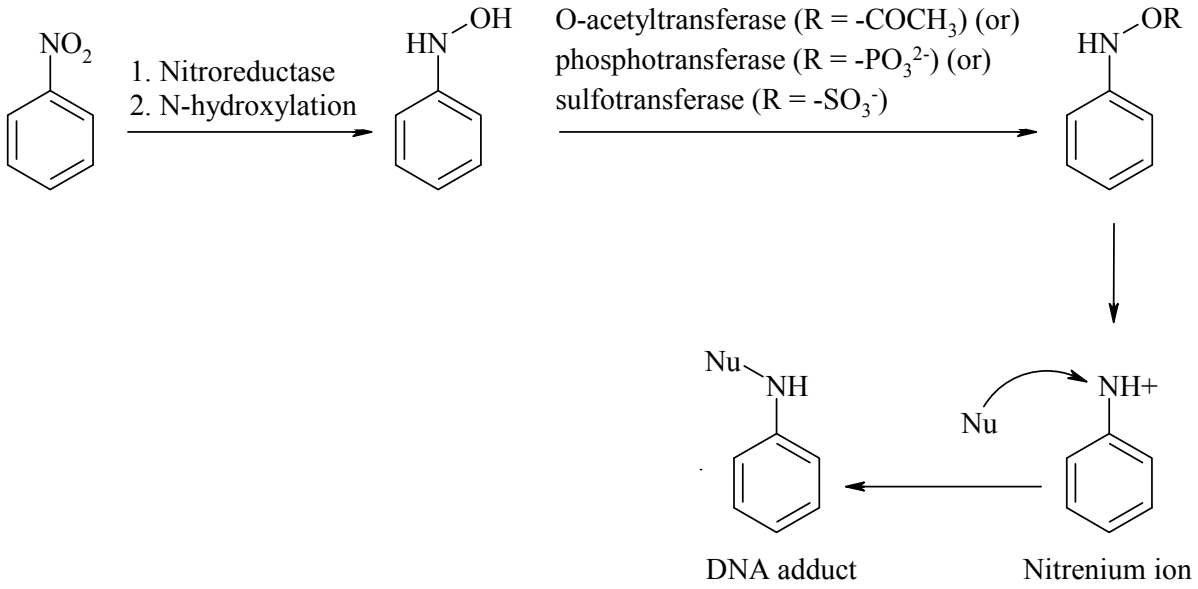
Alert SN08: Aromatic nitro

$R—NO_2$

R = aromatic

Mechanism

Aromatic nitro groups are metabolised into a N-hydroxylated intermediate which subsequently undergoes either acetyl-, phospo- or sulfotransferase. This species then produces the electrophilic nitrenium ion which is capable of reacting with DNA via an S_N1 mechanism (Kalgutkar 2005, Arlt et al 2003, Bieler et al 2003, Fu 1990).



Nu = biological nucleophile

Mitigating factors

The following mitigating factors have been identified (Benigni et al 2008).

- The presence of sulphate (SO_3H) groups in any ring position. This group probably causes increased solubility and thus leads to increased detoxification.
- Substituents in the 2 and 6 position on the benzene ring. This causes steric hindrance which presumably prevents the initial metabolism step thus preventing the formation of the nitrenium ion.
- The presence of a carboxylate group (CO_2H) in the 2 position. This could be due to the formation of a six membered intramolecular hydrogen bonded ring which inhibits the initial metabolic transformation.

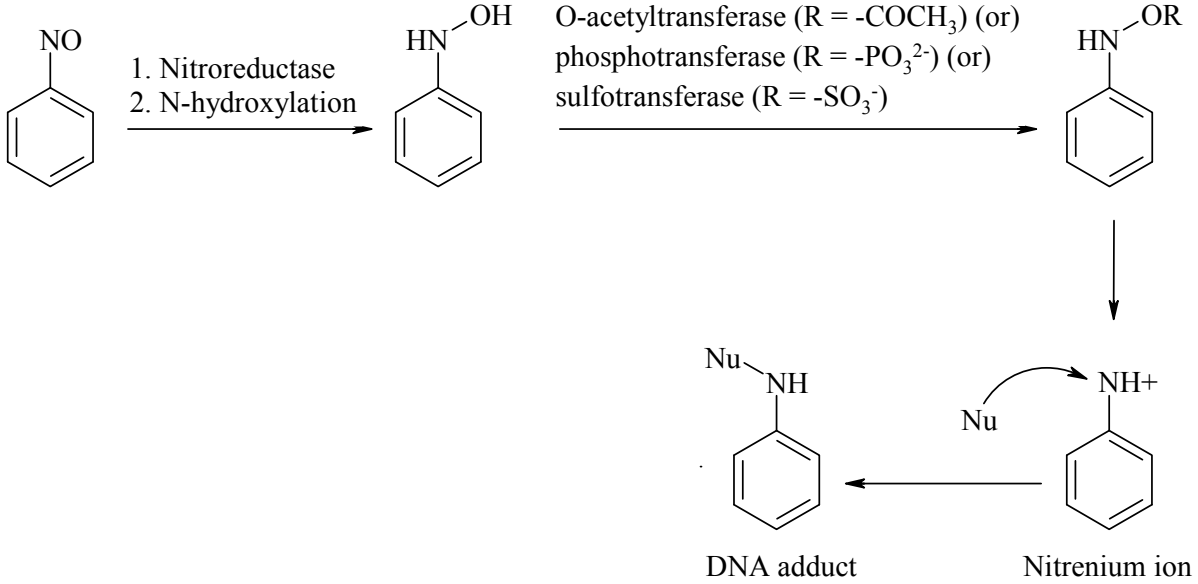
References

Arlt et al (2003) Biochemical and Biophysical Research Communications, 300, p107-114
Benigni et al (2008) Mutation Research, 659, p248-261
Bieler et al (2003) Cancer Letters, 200, p9-18
Fu (1990) Drug Metabolism Reviews, 22, p209-268
Kalgutkar (2005) Current Drug Metabolism, 6, p161-225

Alert SN09: Aromatic nitroso

R—NO
R = aromatic

Mechanism
Aromatic nitroso compounds are reduced and then hydroxylated to an N-hydroxylamine intermediate. This species is then further metabolised by one of three potential transferases, which themselves produce the reactive nitrenium ion which can bind DNA via an S_N1 mechanism (Kalgutkaer 2005).



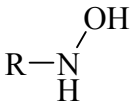
Nu = biological nucleophile

Mitigating factors
The following mitigating factors have been identified (Benigni et al 2008).

- The presence of sulphate (SO_3H) groups in any ring position. This group probably causes increased solubility and thus leads to increased detoxification.
- Substituents in the 2 and 6 position on the benzene ring. This causes steric hindrance which presumably prevents the initial metabolism step thus preventing the formation of the nitrenium ion.
- The presence of a carboxylate group (CO_2H) in the 2 position. This could be due to the formation of a six membered intramolecular hydrogen bonded ring which inhibits the initial metabolic transformation.

References
Benigni et al (2008) Mutation Research, 659, p248-261
Kalgutkar (2005) Current Drug Metabolism, 6, p161-225

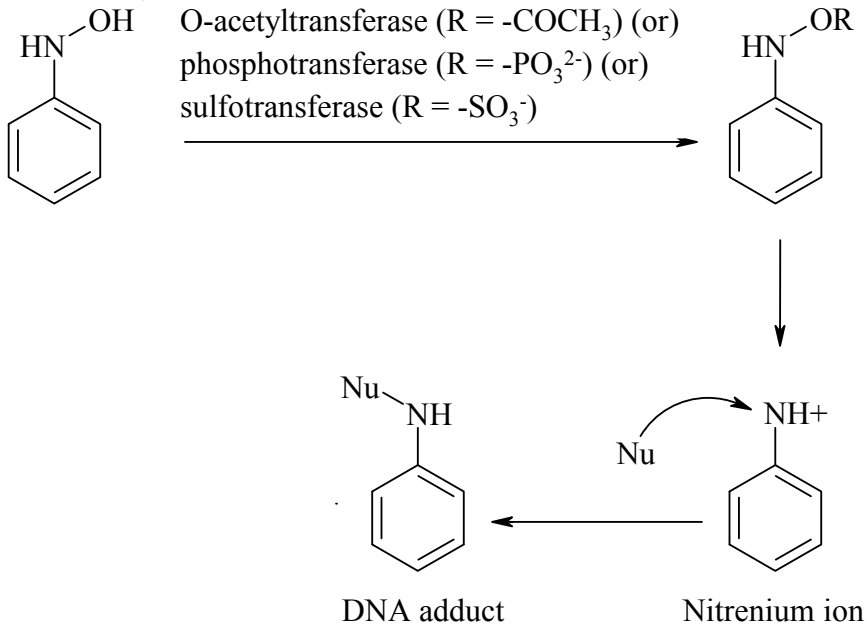
Alert SN10: Aromatic N-hydroxylamines



R = aromatic

Mechanism

Aromatic N-hydroxylated groups are metabolised by either acetyl-, phospo- or sulfotransferase. These species then produce the electrophilic nitrenium ion which is capable of reacting with DNA via an S_N1 mechanism (Kalgutkar 2005).



Nu = biological nucleophile

Mitigating factors

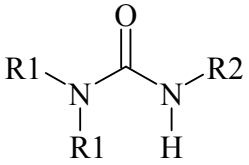
The following mitigating factors have been identified (Benigni et al 2008).

- The presence of sulphate (SO_3H) groups in any ring position. This group probably causes increased solubility and thus leads to increased detoxification.
- Substituents in the 2 and 6 position on the benzene ring. This causes steric hindrance which presumably prevents the initial metabolism step thus preventing the formation of the nitrenium ion.
- The presence of a carboxylate group (CO_2H) in the 2 position. This could be due to the formation of a six membered intramolecular hydrogen bonded ring which inhibits the initial metabolic transformation.

References

Benigni et al (2008) Mutation Research, 659, p248-261
Kalgutkar (2005) Current Drug Metabolism, 6, p161-225

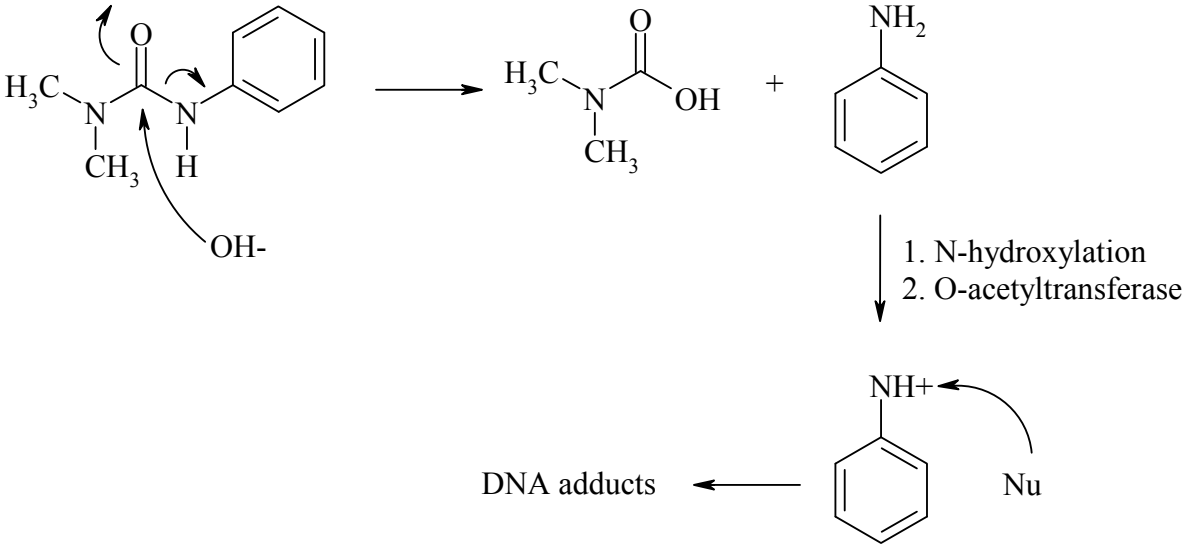
Alert SN11: Ureides



R1 = any
R2 = aromatic carbon

Mechanism

Hydrolysis of the amide bond to produce an aromatic amine moiety has been suggested to be responsible for the toxicity of chemicals containing this alert. The formation of the nitrenium ion results in DNA binding via an S_N1 mechanism (Guengerich et al 1997).



Nu = biological nucleophile

Mitigating factors

Given the toxicity of this alert depends on the production of an aromatic amine the mitigating factors that are part of that alert apply to this one (Benigni et al 2008). These being:

- The presence of sulphate (SO_3H) groups in any ring position. This group probably causes increased solubility and thus leads to increased detoxification.
- Substituents in the 2 and 6 position on the benzene ring. This causes steric hindrance which presumably prevents the initial metabolism step thus preventing the formation of the nitrenium ion.
- The presence of a carboxylate group (CO_2H) in the 2 position. This could be due to the formation of a six membered intramolecular hydrogen bonded ring which inhibits the initial metabolic transformation.

References

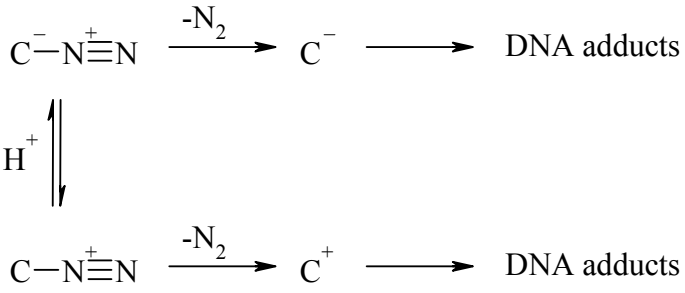
Benigni et al (2008) Mutation Research, 659, p248-261
Guengerich et al (1997) Drug Metabolism and Disposition, 25, p1234-1241

Alert SN12: Diazo

(either isomer is acceptable)

Mechanism

Two possible mechanisms have been suggested that can lead to DNA adducts and cleavage (Arya et al 1995). Both mechanisms produce a reactive carbon ion as shown (and can be considered S_N1 type mechanisms).



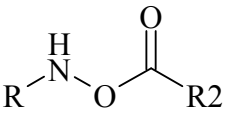
Mitigating factors

- No mitigating factors have been reported

References

Arya et al (1995) Journal of Organic Chemistry, 60, p3268-3269

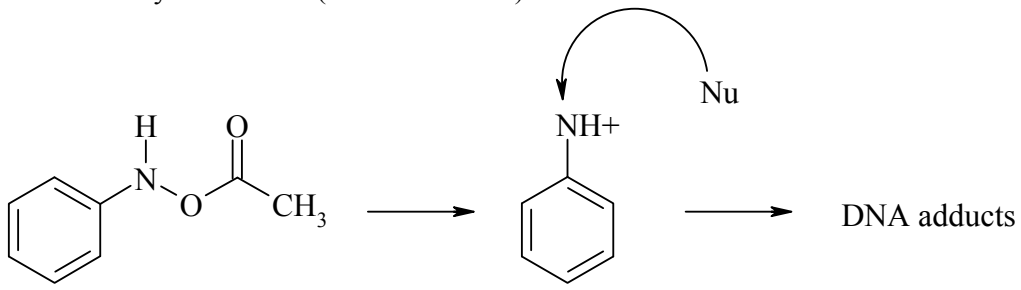
Alert SN13: Ester aromatic hydroxylamine



R = aromatic
R2 = alkyl

Mechanism

Desterification to produce a reactive nitrenium ion capable of reacting with DNA via an S_N1 mechanism is the most likely mechanism (Jones et al 2003).



Nu = biological nucleophile

Mitigating factors

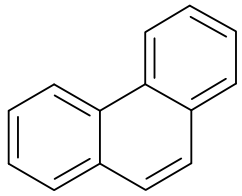
The following mitigating factors have been identified (Benigni et al 2008)

- The presence of sulphate (SO_3H) groups in any ring position. This group probably causes increased solubility and thus leads to increased detoxification.
- Substituents in the 2 and 6 position on the benzene ring. This causes steric hindrance which presumably prevents the initial metabolism step thus preventing the formation of the nitrenium ion.
- The presence of a carboxylate group (CO_2H) in the 2 position. This could be due to the formation of a six membered intramolecular hydrogen bonded ring which inhibits the initial metabolic transformation.

References

Benigni et al (2008) Mutation Research, 659, p248-261
Jones et al (2003) Chemical Research in Toxicology, 16, p1251-1263

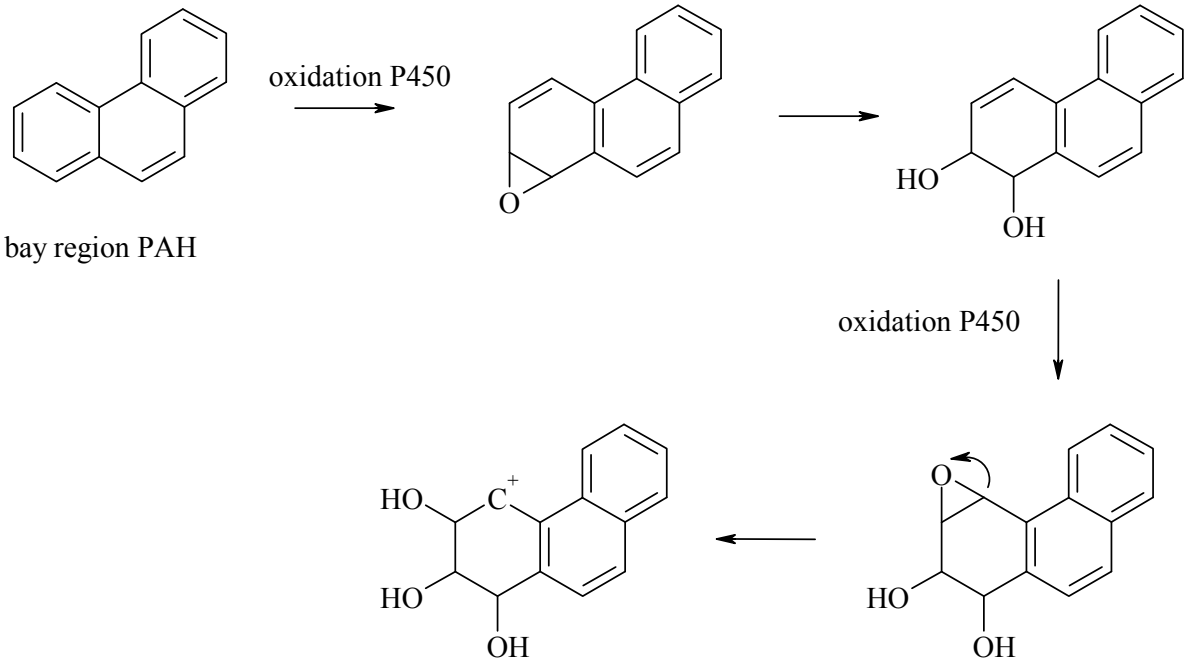
Alert SN14: polycyclic (PAHs) and heterocyclic (HACs) aromatic hydrocarbons



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

Mechanism

Bay region PAHs and HACs undergo P450 mediated oxidation to produce a reactive carbenium ion. Alkylation then occurs via an S_N1 mechanism (Desler et al 2009, Xue et al 2005)



Nu = biological nucleophile

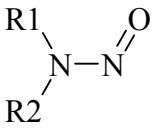
Mitigating factors

- 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

Alert SN15: Nitroso

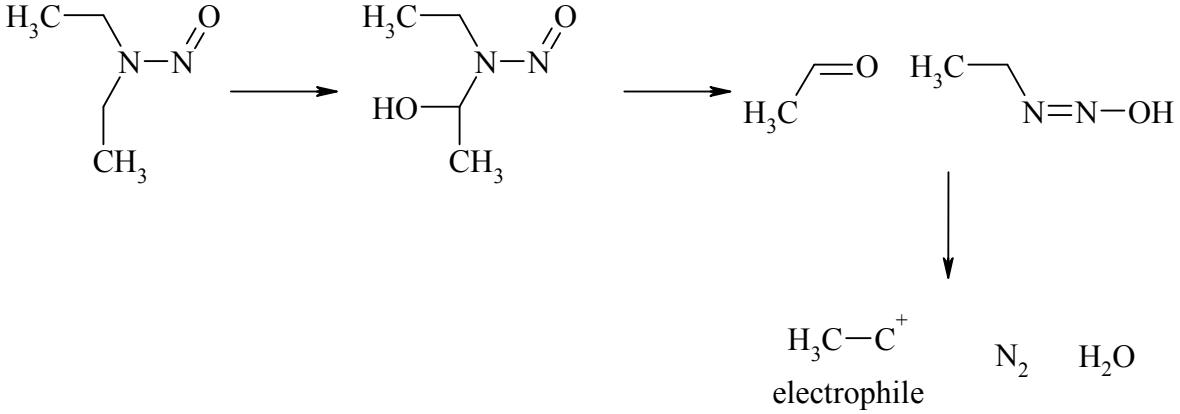


R1 = any carbon
R2 = alkyl C, aryl C, C=O, C=N, S=O

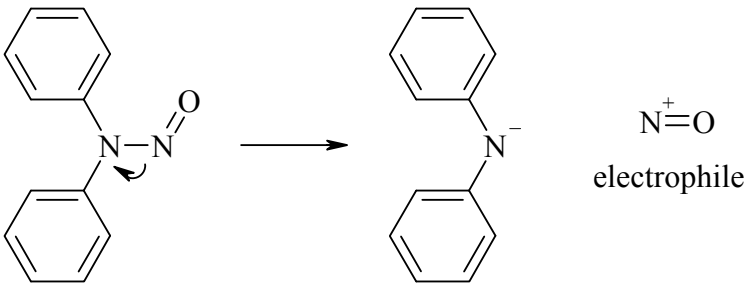
Mechanism

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

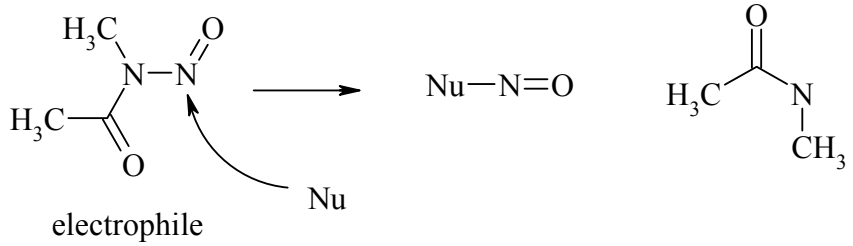
Mechanism 1: At least one alkyl R group: α-hydroxylation producing a carbenium ion leading to an S_N1 alkylation reaction



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



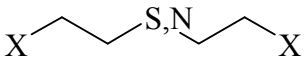
Mitigating factors

- 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

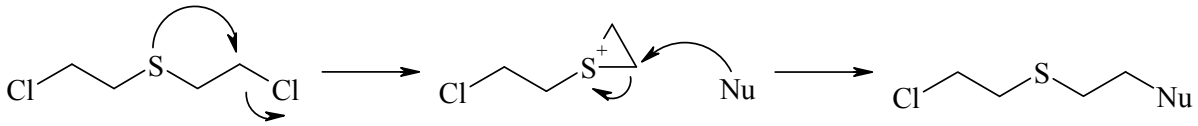
Alert SN16: Mustards



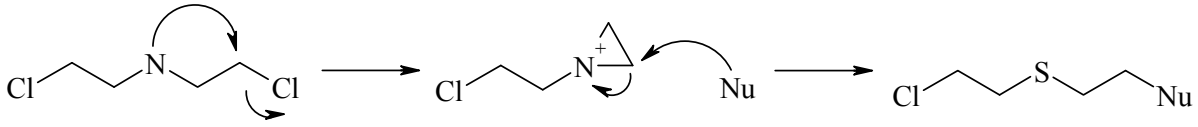
X = F, Cl, Br, I

Mechanism

Mustards have been suggested to undergo an intra-molecular cyclisation to form an electrophilic reactive episulfonium ion. The episulfonium ion is then susceptible to S_N2 attack by biological nucleophiles (Noll et al 2006, Smith et al 1995).



electrophile: episulfonium ion



electrophile: aziridinium ion DNA adducts

Nu = biological nucleophile.

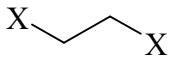
Mitigating factors

- No mitigating factors have been reported

References

Noll et al (2006) Chemical Reviews, 106, 277-301
Smith et al (1995) Journal of the American Academy of Dermatology, 32, 765-776

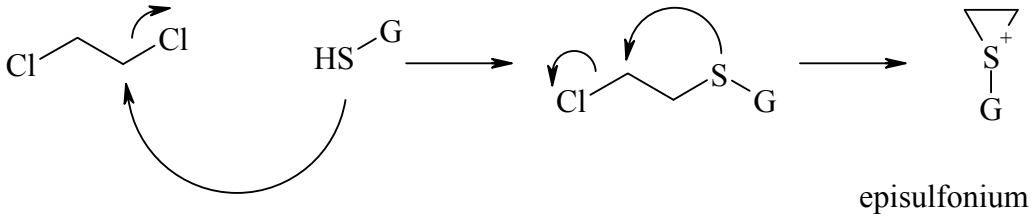
Alert SN17: 1,2-Dihaloalkane



X = F, Cl, Br, I

Mechanism

It has been suggested that 1,2-dihaloalkanes undergo an initial attack by glutathione followed by internal cyclisation resulting in the formation of a reactive episulfonium ion. This ion can then undergo an S_N2 type ring opening reaction (Granville et al 2005).



episulfonium ion

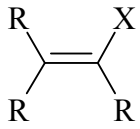
Mitigating factors

- No mitigating factors have been identified

References

Granville et al (2005) Mutation Research, 572, 98-112

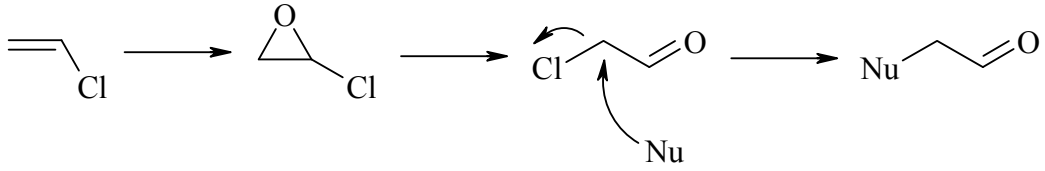
Alert SN18: Monohalo alkenes



R = alkyl, aryl, hydrogen

X = F, Cl, Br, I

Mechanism
It has been suggested that monohalo alkenes are metabolised by CYP P450 initially into halo epoxides. These chemical can either directly bind to DNA or can undergo rapid rearrangement to an equally DNA reactive halo aldehyde (binding via an S_N2 mechanism) (Dogliotti 2006).

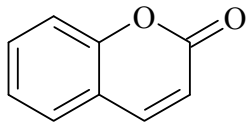


Nu = biological nucleophile

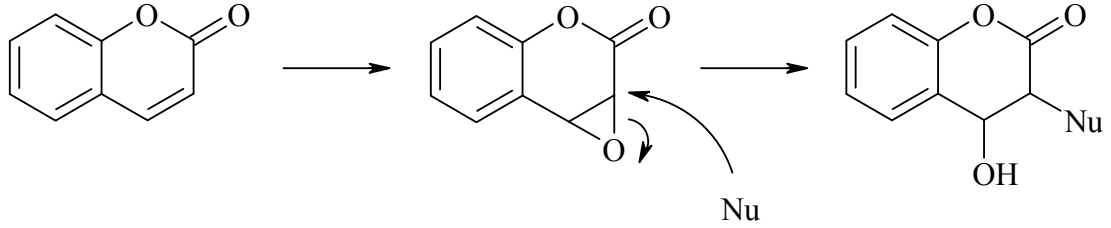
Mitigating factors
- No mitigating factors have been reported

References
Dogliotti (2006) Annali dell'Istituto superiore di sanità, 42, 163-169

Alert SN19: Coumarins



Mechanism
Epoxidation to coumarin-3,4-epoxide has been suggested as the primary route of toxicity. The epoxidated species can undergo covalent reaction via an S_N2 mechanism with biological nucleophiles such as DNA.

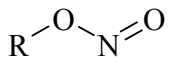


Nu = biological nucleophile

Mitigating factors
- No mitigating factors have been reported

References
Lake (1999) Food and Chemical Toxicology 37, 423-453

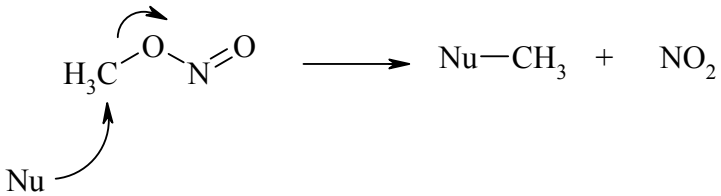
Alert SN20: Alkyl nitrate



R = alkyl

Mechanism

It has been suggested that the most likely mechanism of action is an S_N2 alkylation with the loss of the NO_2 group. An alternate nitrosation mechanism cannot be ruled out (Wild et al 1983). There is limited literature evidence for either mechanism.



Nu = biological nucleophile

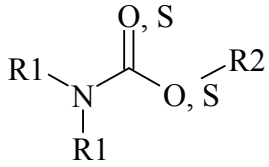
Mitigating factors

- No mitigating factors have been reported

References

Wild et al (1983) Food and Chemical Toxicology 21, 707-719

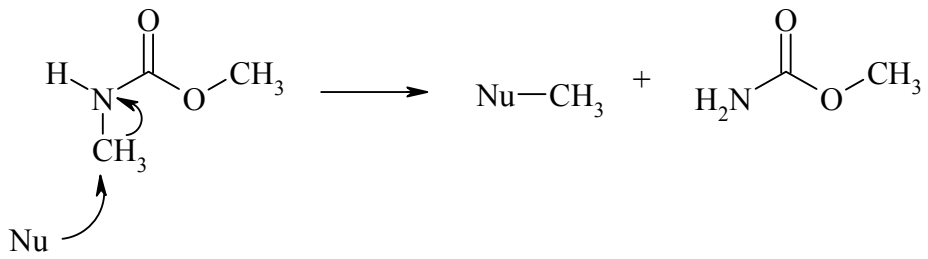
Alert SN21: Alkyl carbamates



R1 = at least one must be alkyl
R2 = alkyl, aryl

Mechanism

The most likely mechanism has been suggested to be an S_N2 alkylation reaction (Pound et al 1976a, 1976b).



Nu = biological nucleophile

Mitigating factors

- No mitigating factors have been reported

References

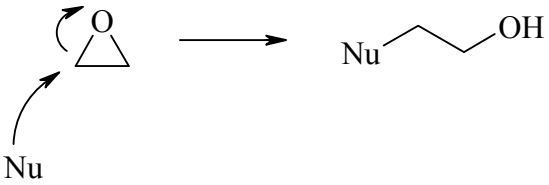
Pound et al (1976a) Cancer research 36, 1101-1107
Pound et al (1976b) Chemico-Biological Interactions 14, 149-163

Alert SN22: Epoxides

X = O, N

Mechanism

Alkylation occurs via an S_N2 ring opening mechanism (Sawatari et al 2001).



Nu = biological nucleophile

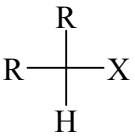
Mitigating factors

- No mitigating factors have been reported

References

Sawatari et al (2001) Industrial Health, 39, p341-345

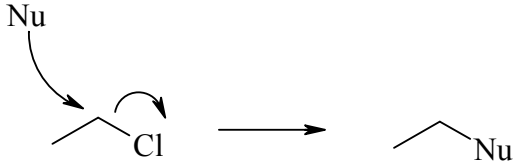
Alert SN23: Aliphatic halide



X = F, Cl, Br, I
R = alkyl, hydrogen

Mechanism

An S_N2 mechanism has been proposed as the primary method of DNA alkylation (Sobol et al 2007).



Nu = biological nucleophile

Note: 1,2-dihaloalkanes are excluded as they react via an episulfonium ion

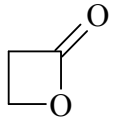
Mitigating factors

- No mitigating factors have been reported

References

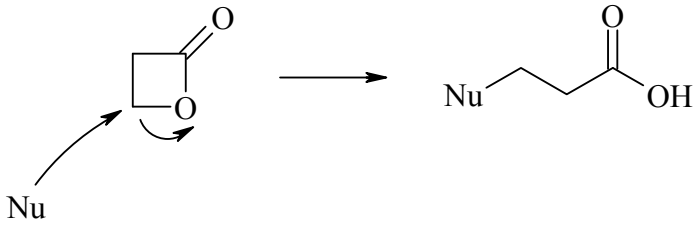
Sobol et al (2007) Mutation Research 633, 80-94

Alert SN24: Lactones



Mechanism

An S_N2 ring opening mechanism has been suggested to result in DNA binding (Gomez-Bombarelli et al 2008, Hemminki et al 1981).



Nu = biological nucleophile

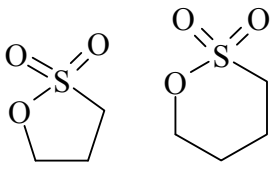
Mitigating factors

- No mitigating factors have been reported

References

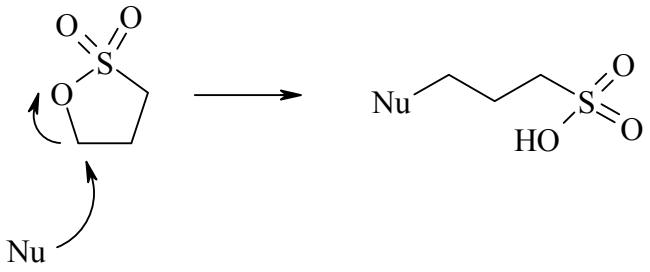
Gomez-Bombarelli et al (2008) Chemical Research in Toxicology, 21, p1964-1969
Hemminki et al (1981) Chemico-Biological Interactions, 34, p323-331

Alert SN25: Sultones



Mechanism

A ring opening S_N2 mechanism has been suggested to be responsible for DNA binding (Osterman-Golkar et al 1976).



Nu = biological nucleophile

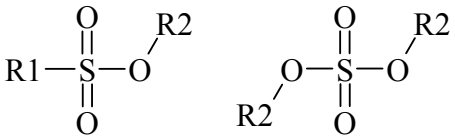
Mitigating factors

- No mitigating factors have been reported

References

Benigni et al (2008) Mutation Research, 649, p248-261
Osterman-Golkar et al (1976) Chemico-Biological Interactions, 14, p195-202

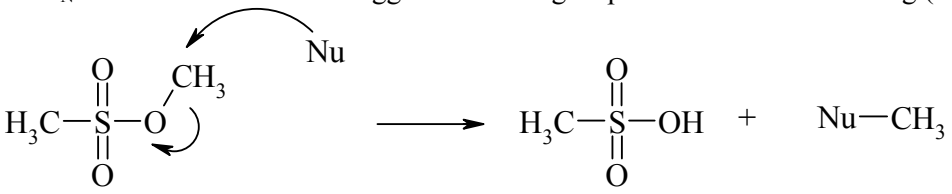
Alert SN26: Sulfonic esters



(alkyl sulfonate) (alkyl sulfate)
R1 ≠ OH, SH, O^-, S^-
R2 = alkyl

Mechanism

An S_N2 mechanism has been suggested as being responsible for DNA binding (Swanson et al 1978).



Nu = biological nucleophile

Mitigating factors

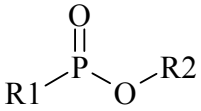
The following mitigating factors have been identified (Benigni et al 2008)

- The presence of an ionisable hydroxyl group in place of one (or more) of the esters removes activity.
- Alkyl chains must not be greater than 4 carbons.

References

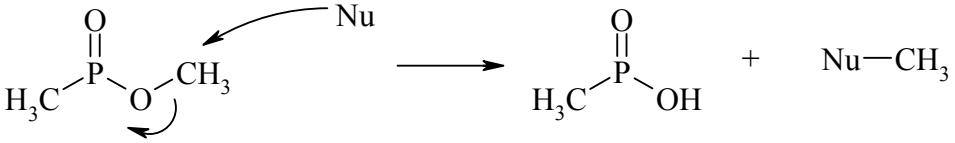
Benigni et al (2008) Mutation Research, 649, p248-261
Swanson et al (1978) The Biochemical Journal, 171, p575-587

Alert SN27: Phosphonic esters



R1 ≠ OH, SH, O^-, S^-
R2 = alkyl

Mechanism
An S_N2 mechanism analogous to that for the sulfonic esters is the most likely route to DNA binding.



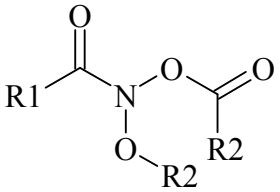
Nu = biological nucleophile

Mitigating factors
The following mitigating factors have been suggested (Benigni et al 2008).

- The presence of an ionisable hydroxyl group in place of one (or more) of the esters.
- Alkyl chains must not be greater than 4 carbons.

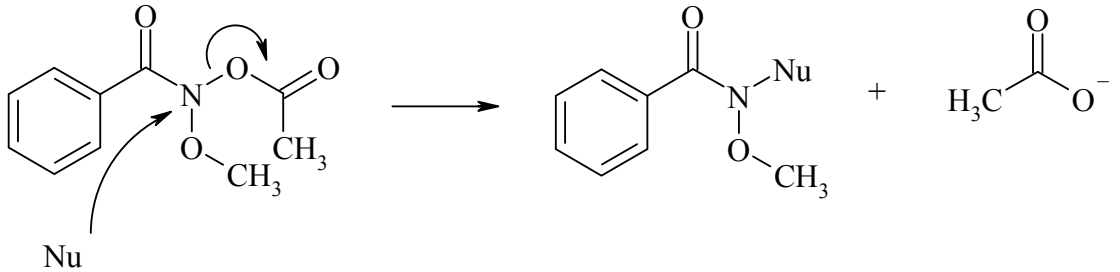
References
Benigni et al (2008) Mutation Research, 649, p248-261

Alert SN28: N-acyloxy-N-alkoxyamides



R1 = aromatic
R2 = alkyl

Mechanism
An S_N2 mechanism has been suggested to be responsible for the alkylation of DNA (Andrews et al 2006, Banks et al 2003, Bonin et al 2001).



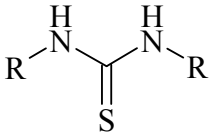
Nu = biological nucleophile

Mitigating factors

- No mitigating factors have been identified

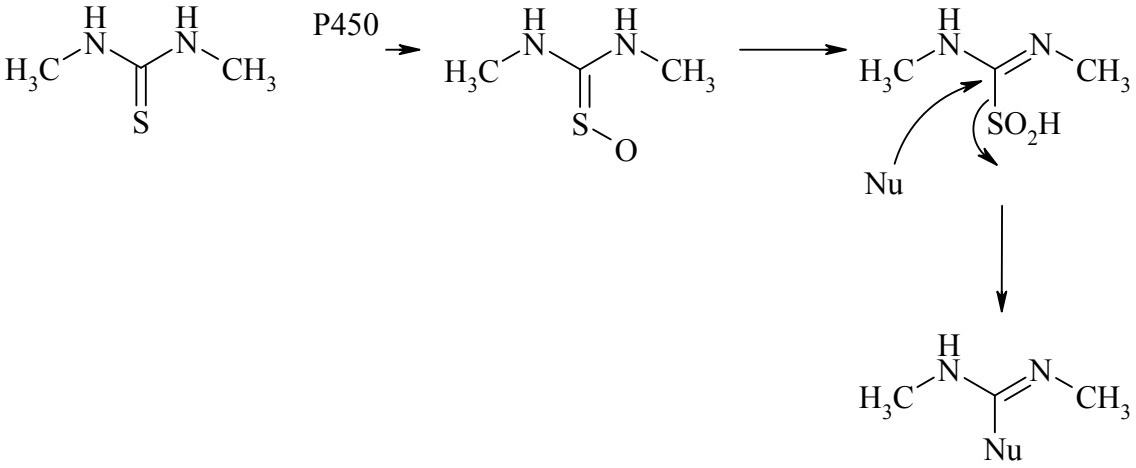
References
Andrews et al (2006) Mutation Research, 605, p51-62
Banks et al (2003) Organic Biomolecular Chemistry, 1, p2238-2246
Bonin et al (2001) Mutation Research, 494, p115-134

Alert SN29: Thioureas



R = alkyl, aryl

Mechanism
Thioureas have been suggested to be sulfoxidated by P450 resulting in the production of electrophilic sulfinic acids (Kalgutkar et al 2005). These species are capable of reacting with biological nucleophiles via an S_N2 type mechanism.



Nu = biological nucleophile

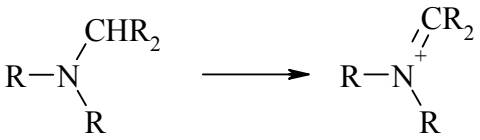
Mitigating factors
- No mitigating factors have been reported

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

Alert SN30: Aliphatic tertiary amines

R = aliphatic C (one of the aliphatic C attached to the nitrogen must contain a C-H)
The cyclic aliphatic ring system can be any size above n = 3 (i.e. not aziridine)

Mechanism
P450 metabolism to a reactive iminium species has been suggested as a potential pathway to DNA adducts (Kalgutkar et al 2005).



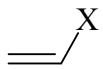
iminium ion (electrophile)

Mitigating factors

- At least one of the aliphatic carbon atoms attached to the nitrogen must contain a C-H in order for formation of the iminium ion

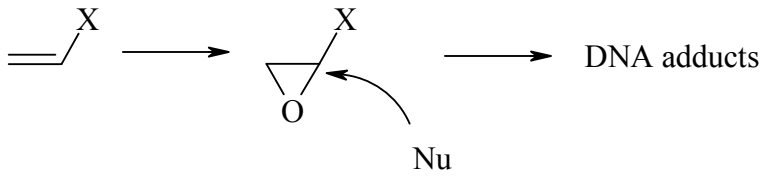
References
Kalgutkar et al (2005) Current Drug Metabolism, 6, p161-225

Alert SN31: Polarised alkenes



X = OC, halide

Mechanism
P450 mediated epoxidation of the alkene leads to a reactive epoxide species capable of alkylating DNA via an S_N2 mechanism (Rannug et al 1976).



Nu = biological nucleophile

Mitigating factors

- No mitigating factors have been reported

References
Rannug et al (2005) Chemical-Biological Interactions, 12, p251-263