Using *In Vitro* Structural Alerts For Chromosome Damage To Predict *In Vivo* Activity And Direct Future Testing

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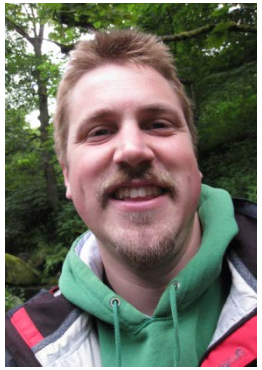
Introductions



- Senior Scientist in knowledge team at Lhasa
- 7 years working for the company
- Started writing Derek alerts for endpoint of mutagenicity
- Have some experience of other endpoints (teratogenicity, hepatotoxicity)
- Moved on to writing/updating *in vitro* chromosome damage alerts
- Project work with National Institute of Health Sciences (NIHS) of Japan led me into *in vivo* genotoxicity work and target organ endpoints
- More recently having been giving scientific support in the development of our statistical mutagenicity prediction system Sarah Nexus

Outline

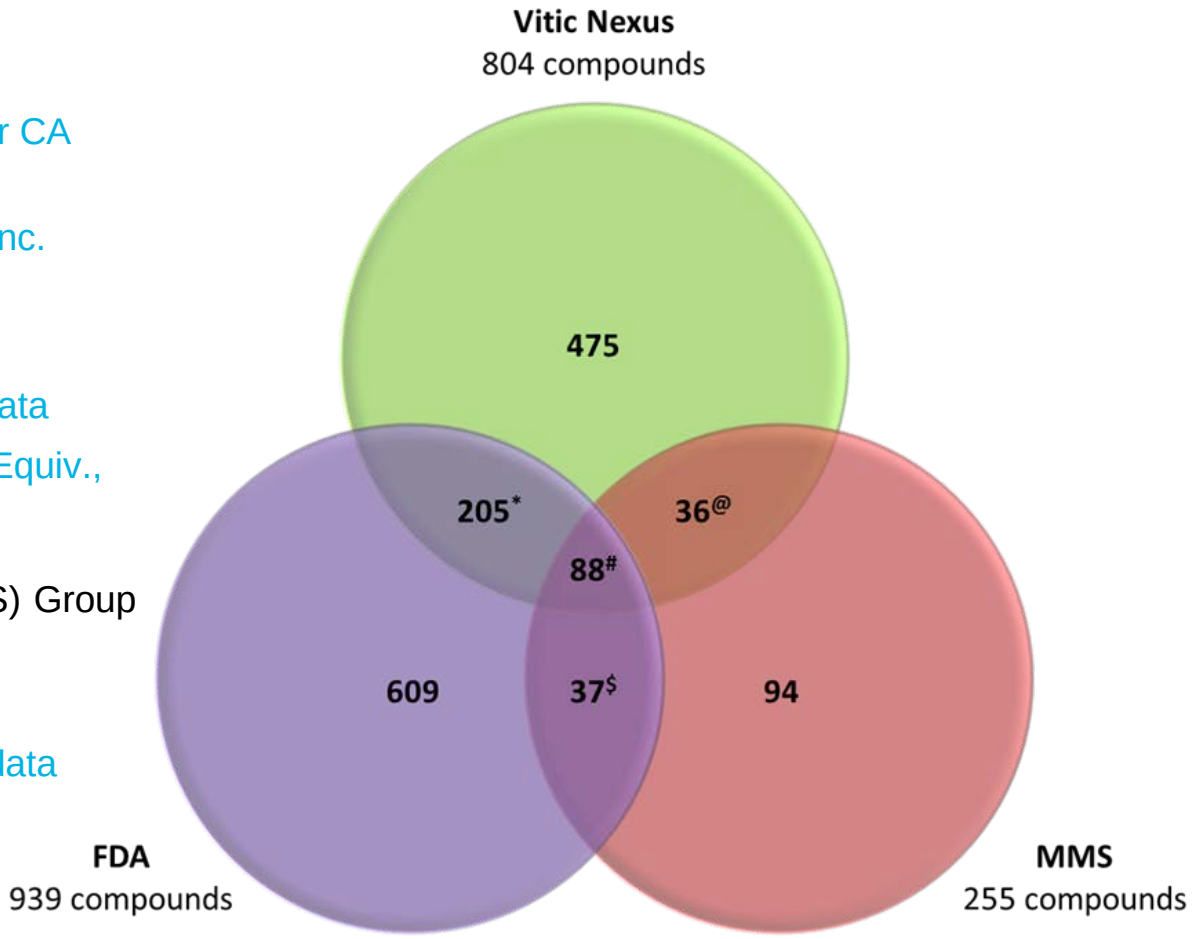
- Background of our work with the National Institute of Health Sciences of Japan (NIHS) and our work on the endpoint of chromosome damage
- Some pitfalls in the relationship between *in vitro* and *in vivo* chromosome damage activity
- Our reasons for approaching the development of the *in vivo* chromosome damage endpoint in the way we did
- The method behind our approach and the results achieved so far
- Some specific examples highlighting the advantages of this approach to modelling these data
- Conclusions

Lhasa Collaborative Project With The NIHS

2003	Genotoxicity feasibility study
2003 - 2006	Genotoxicity (Ames test) project
2006	Hepatotoxicity endpoint development
2007	Nephrotoxicity endpoint development
2007 – 2014	Repeat dose toxicity project Genotoxicity project

In vivo data available for mining

- FDA Data Set
  - 939 compounds with in vivo MN or CA data
  - 288 Pos, 625 Neg, 13 Equiv., 13 Inc.
- Vitic Nexus Data Set
  - 804 compounds with in vivo MN data
  - 241 Pos., 503 Neg., 3 Wpos., 25 Equiv., 13 Inc.
- Mammalian Mutagenicity Study (MMS) Group Data Set
  - 255 Compounds with in vivo MN data
  - 112 Pos., 129 Neg., 14 Inc.
- Combined Data Set
  - 1461 Compounds with in vivo MN or CA data
  - 484 Pos., 977 Neg.



Call agreement between each data set is: #53% have three calls the same, 43% two calls the same, 3% all disagree; @69% calls agree, 31% disagree; *82% agree, 18% disagree; $68% agree, 32% disagree

Modelling Considerations

Practicalities

- Reasonable size data set but skewed towards negatives.
- The binary overall call may be too simplistic.
- Expert-based approach to mining data would be time consuming

Requirements

- An overall prediction for *in vivo* chromosome damage
- Information on mechanism leading to toxicity. Is this mechanism relevant to my use-case?
- Information on protocol dependent activity. What is the best protocol to highlight any potential hazard posed by this substance?

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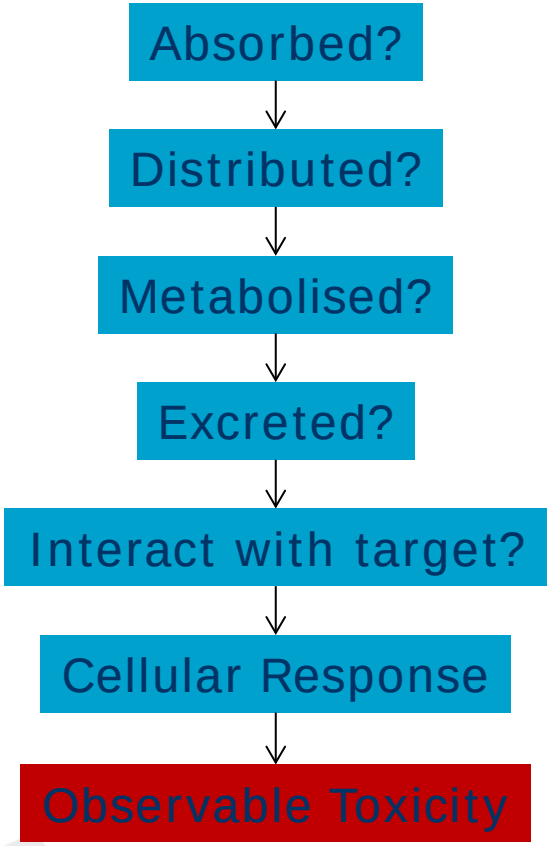
How *in vitro* activity correlates with *in vivo*?

Predicting *in vivo* chromosome damage actually requires the synthesis of several models. This reflects the steps involved in observing toxicity:



Does it get to a sensitive tissue?

Does it require metabolic activation?
If so, is it metabolically activated?
Is there metabolic deactivation?

Does it form DNA adducts?
Does it inhibit topoisomerases, tubulin polymerisation, etc.?

Does this result in chromosome break?

Are chromosomal aberrations or micronuclei observed?

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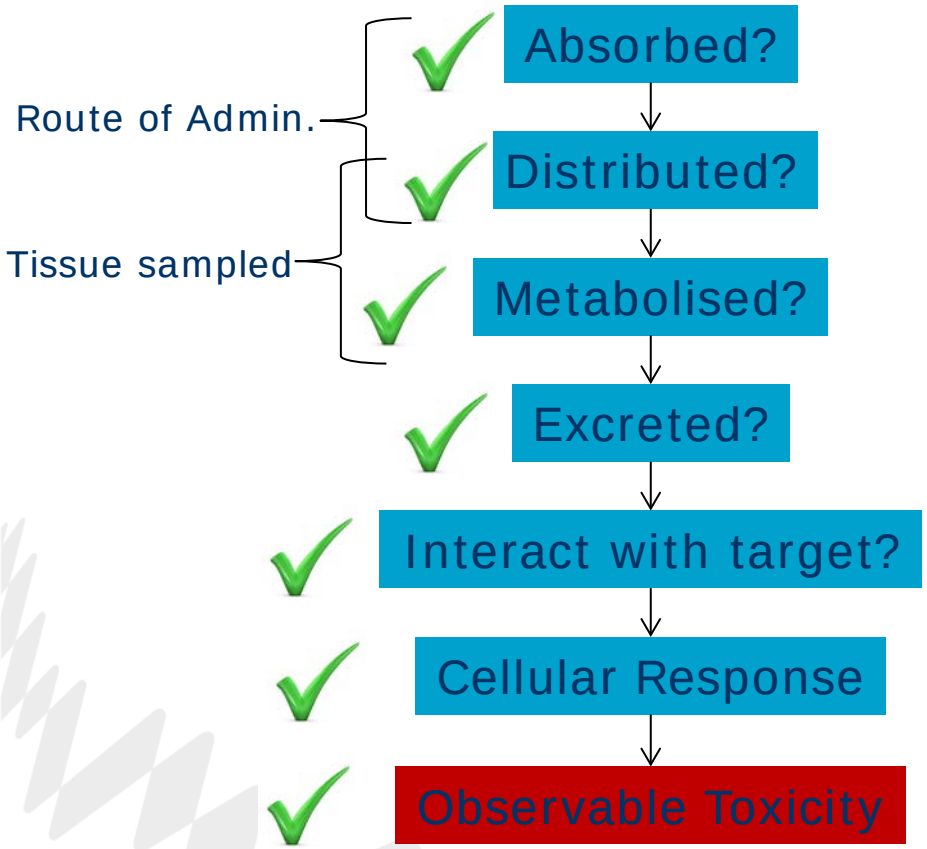

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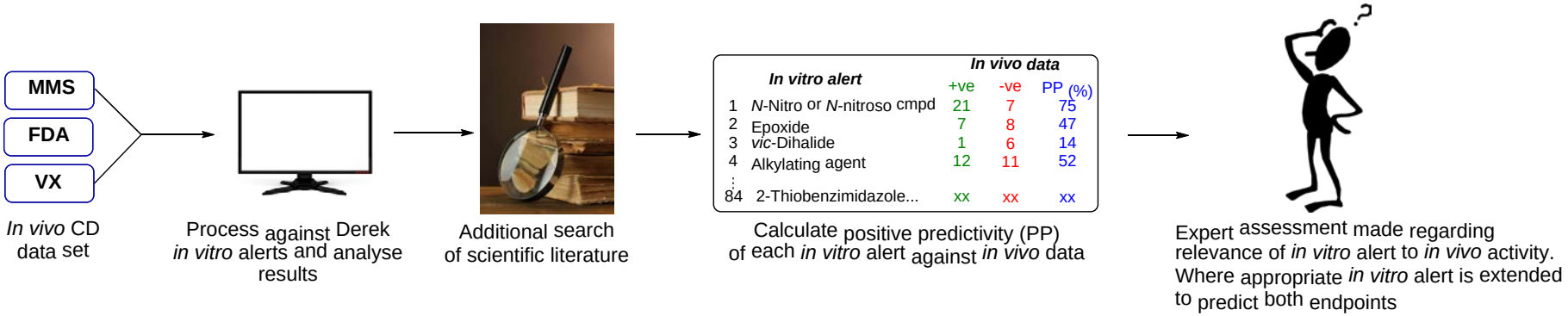
How *in vitro* activity correlates with *in vivo*?

- The OECD guidelines relating to *in vivo* erythrocyte micronucleus testing highlight these concerns
- Specifically, the fact that the test substance or reactive metabolite reach the tissue being tested should be assessed and discussed
- All these variables make it difficult to decide which protocol will be most appropriate for a particular chemical
- This is where our work can help

7. If there is evidence that the test substance, or a reactive metabolite, will not reach the target tissue, it is not appropriate to use this test.

31. The likelihood that the test substance or its metabolites reach the general circulation or specifically the target tissue (e.g. systemic toxicity) should be discussed.

In Vitro To *In Vivo* Alert Extensions



Predictivity	Action
Good	Extend *in vitro* alert to *in vivo* endpoint
Poor, but with rationale	New *in vivo* alert or extend *in vitro* alert with caveats
Poor, no rationale	Alert not extended, comments updated

In Vitro To *In Vivo* Alert Extensions

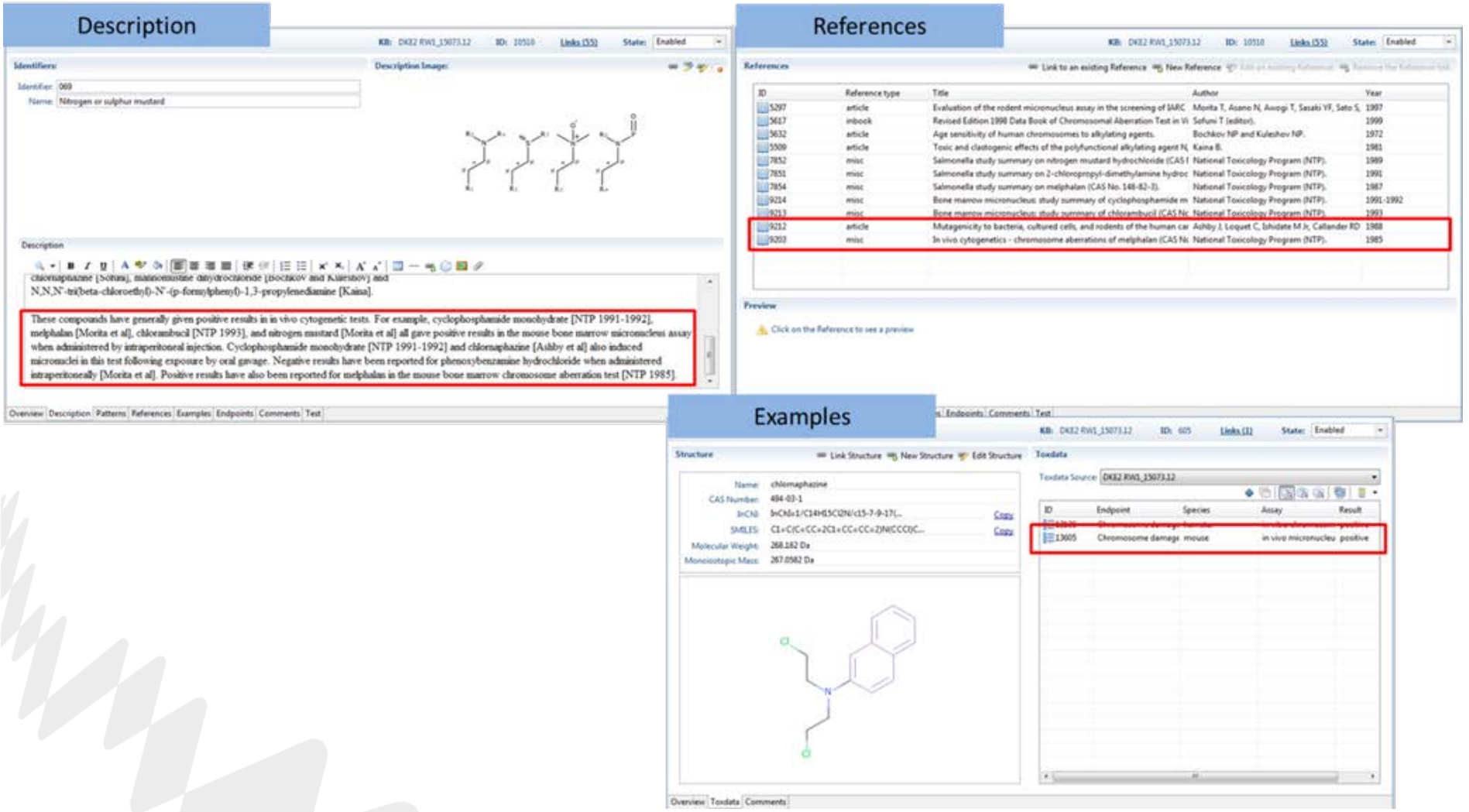
- 19 *in vitro* alerts extended to predict *in vivo* activity
  - 9 alerts predict at the reasoning level of plausible
  - 10 alerts predict at the reasoning level of equivocal

- 4 new *in vivo* chromosome damage alerts implemented to cover a sub-class of *in vitro* alert

- 9 *in vitro* alerts could not be extended to the endpoint of *in vivo* chromosome damage but the comments were updated to reflect findings

- (7 existing *in vivo* chromosome damage alerts)

Knowledge Presented With Alerts



In vivo chromosome damage progress

DfW13:

Exp \ *Predicted*	+ve	-ve
+ve	4	108
-ve	0	130

Sensitivity: 4/112 = **4%**

Specificity: 130/130 = **100%**

Balanced Accuracy: **52%**

VS

DX 2014:

Exp \ *Predicted*	+ve	-ve
+ve	45	67
-ve	28	102

45/112 = **40%**

102/130 = **79%**

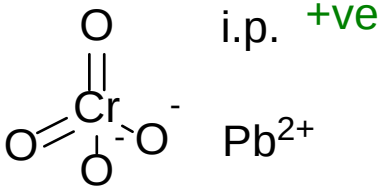
60%

EXAMPLES

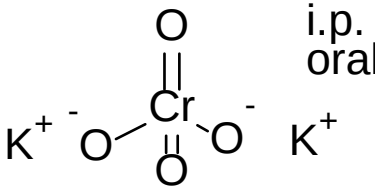
Route of Administration specific *in vivo* activity

Chromium compounds may display route of admin. specific activity

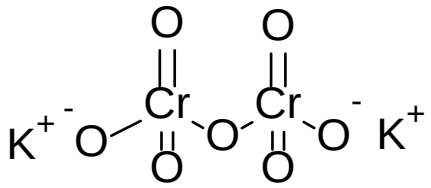
Rodent BM MN test:



lead chromate



potassium chromate



potassium dichromate

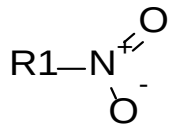
Key Info

i.p. is the most sensitive route of admin to test in vivo activity of chromium compounds

Tissue specific *in vivo* activity

Aromatic nitro compounds may display tissue-specific activity

In vitro alert for aromatic nitro compounds

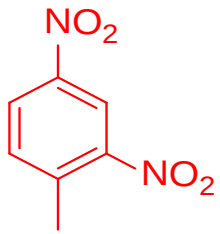


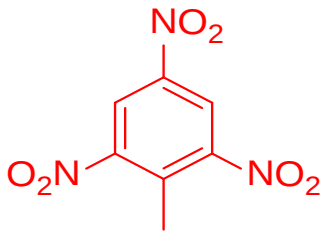
In vivo data:

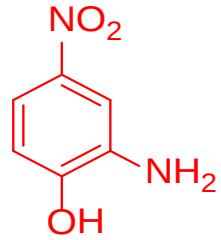
	+ve	-ve	PP
BM:	4	9	31%
Liver:	3	0	100%

Examples:

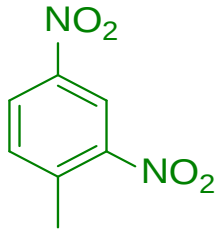
BM: -ve

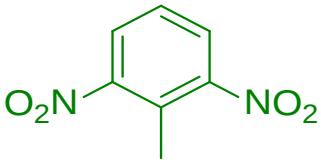


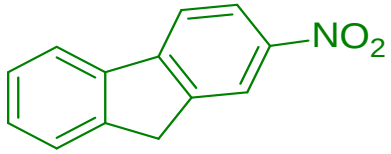


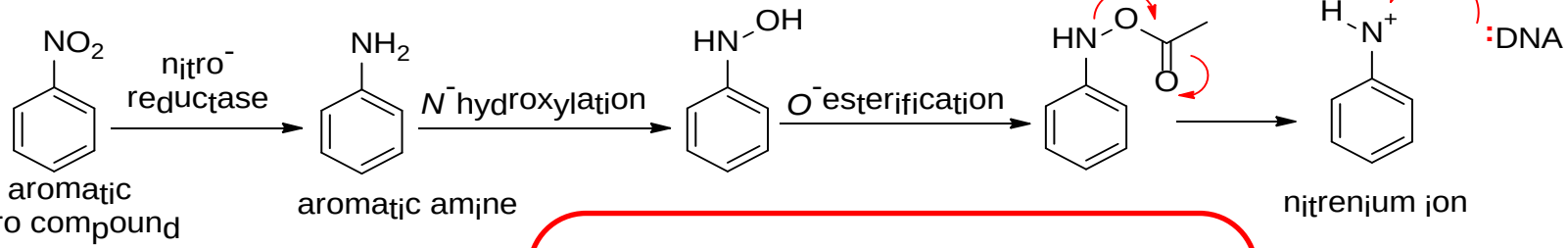


Liver: +ve









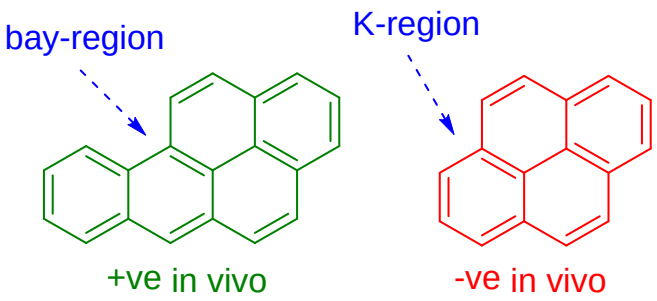
Key Info

The liver is a sensitive tissue to test in vivo activity of aromatic nitro compounds

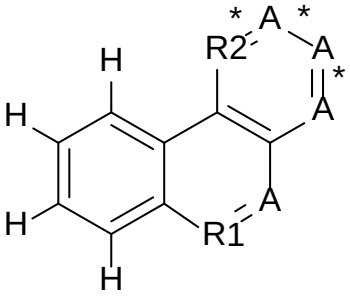
Sub-class specific *in vivo* activity

Modified SAR for *in vivo* activity of PAHs

Examples:



In vivo alert for PAHs



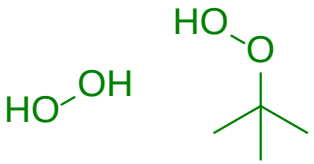
A = any atom; R1 = N, NH^+, C-H;
R2 = N, NH^+, C(fused), C-H,
C-Me, C-Et, C-OH, $C\text{-}O^-$, C-OMe
* = at least one of these bonds must be fused to another aromatic ring.

Key Info

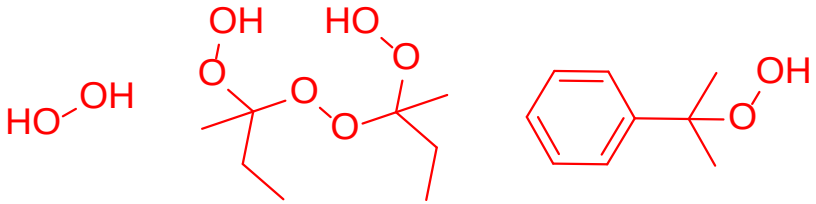
PAHs with a bay-region are more likely to show in vivo activity

In vivo negative classes

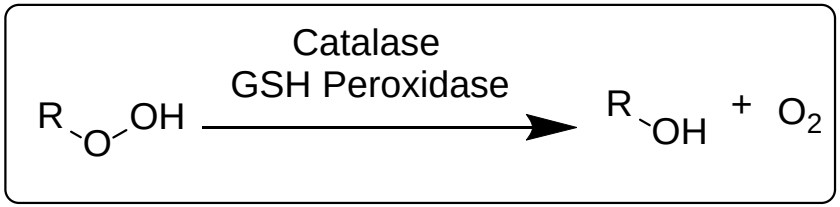
Hydroperoxides are not active *in vivo* in BM or PB



+ve in vitro



-ve in vivo (BM, PB)



Key Info

In vitro activity of aliphatic hydroperoxides is not reflected in vivo

Conclusions and Future Directions

- Sought to improve predictivity of an expert alert system for endpoint of *in vivo* chromosome damage
- Method employed to do this quickly using existing knowledge already captured in the system
- Increased sensitivity from 4% to 40% against MMS data set in relatively short time
- This work has already produced a lot of predictions which will be very useful to an expert user when deciding the next step in genotoxicity testing and in selecting the most appropriate and sensitive protocol for *in vivo* testing
- This technique is reaching its limits and new approaches are now being explored

Acknowledgements

- Dr Honma and Dr Hirose from the NIHS
- Dr Hamada and Dr Morita
- Steve Canipa, Will Drewe and Richard Williams





Publication

http://mutage.oxfordjournals.org/content/early/2015/07/02/mutage.gev047.short?rss=1

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