

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

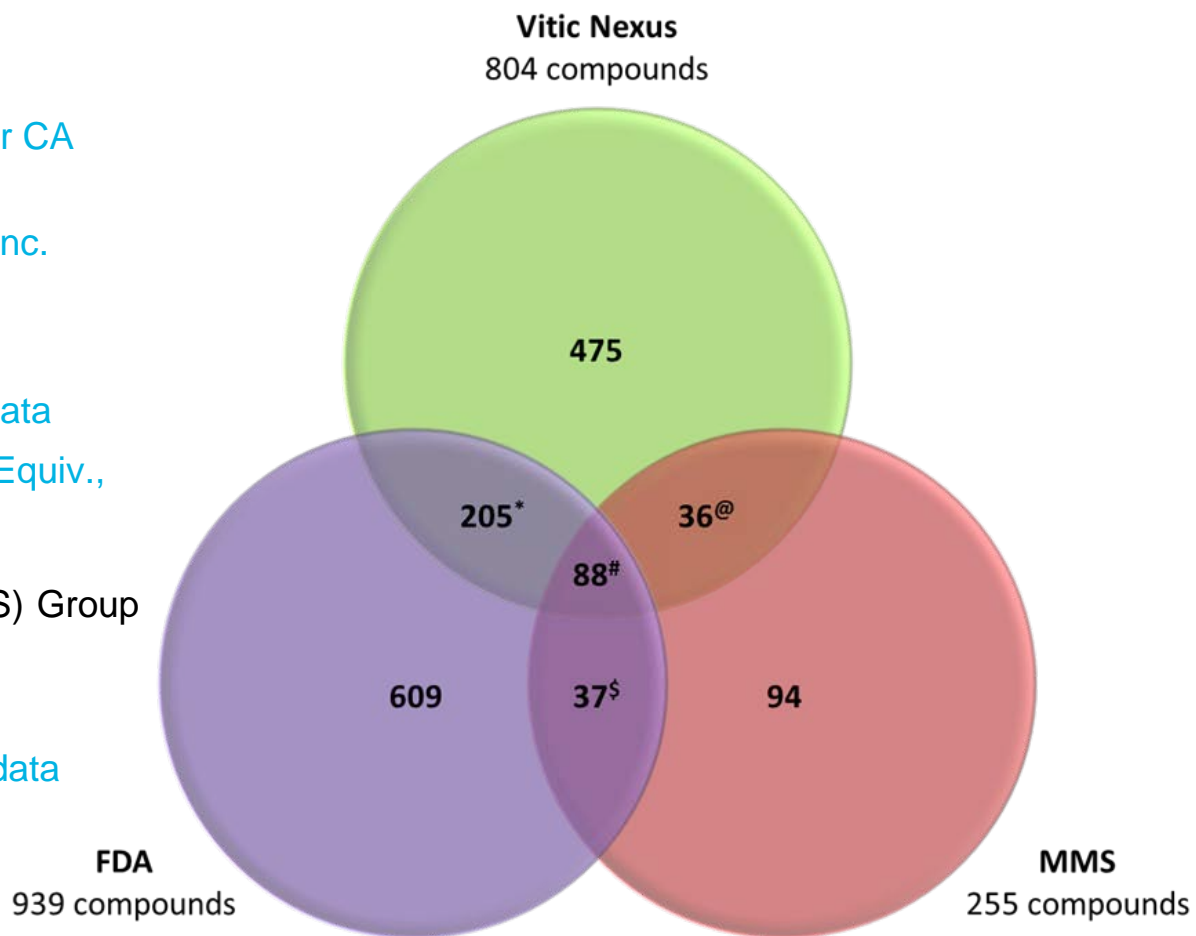
2006 Hepatotoxicity endpoint development

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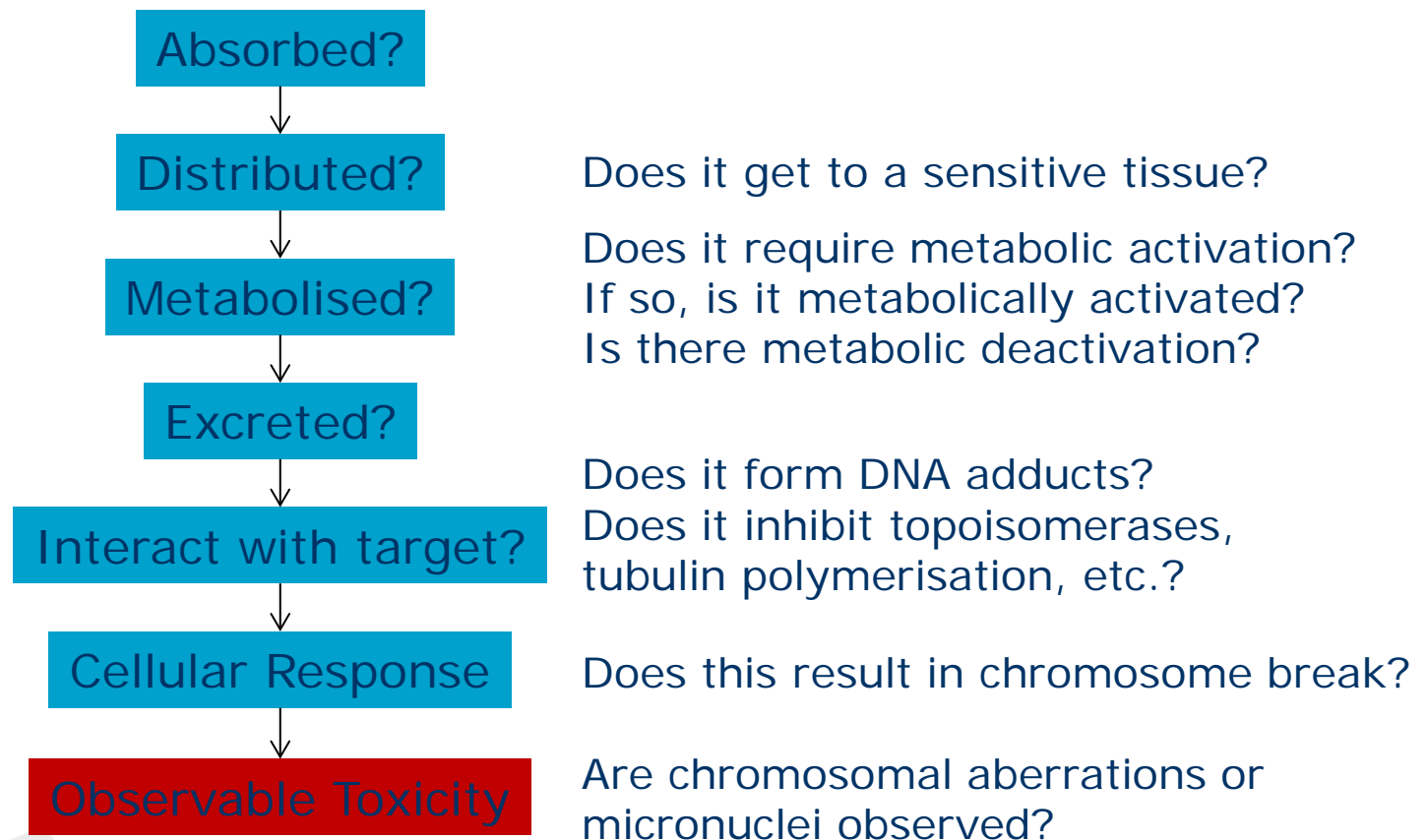
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How *in vitro* activity correlates with *in vivo*?

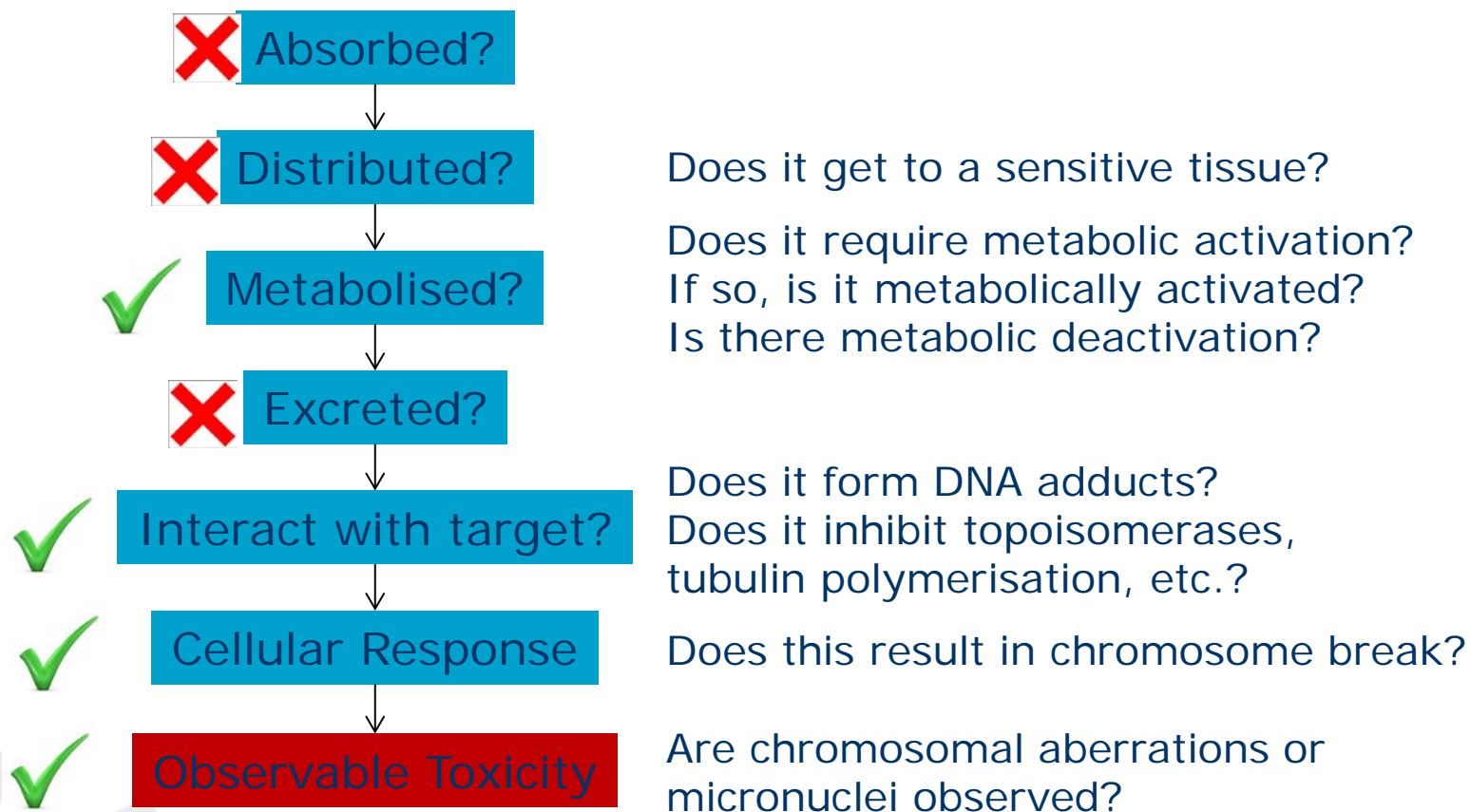
Predicting *in vivo* chromosome damage actually requires the synthesis of several models. This reflects the steps involved in observing toxicity:



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

A structural alert looks simple, but is actually a synthesis of several models. This reflects the steps involved in observing toxicity:

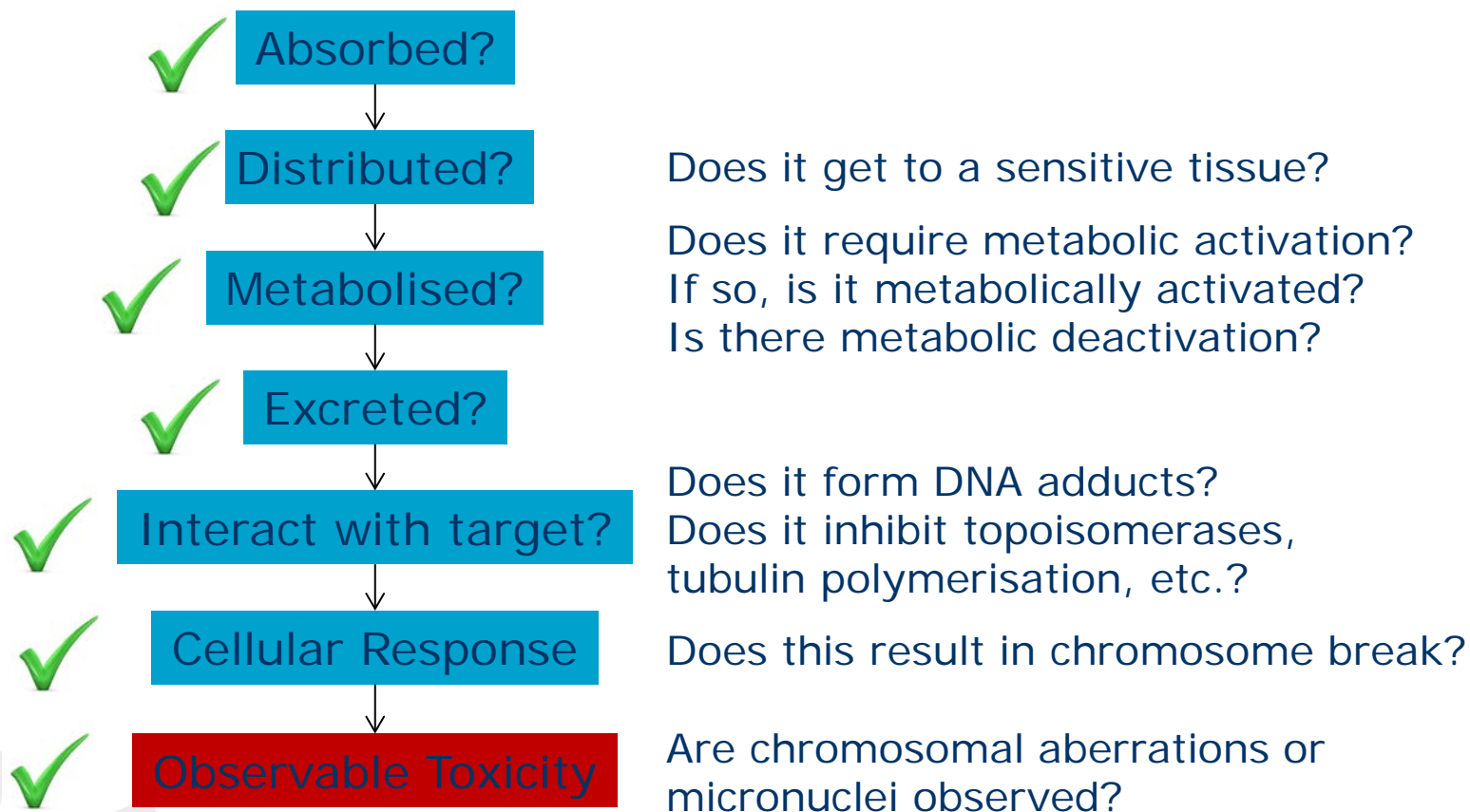
In vitro Chromosome Damage



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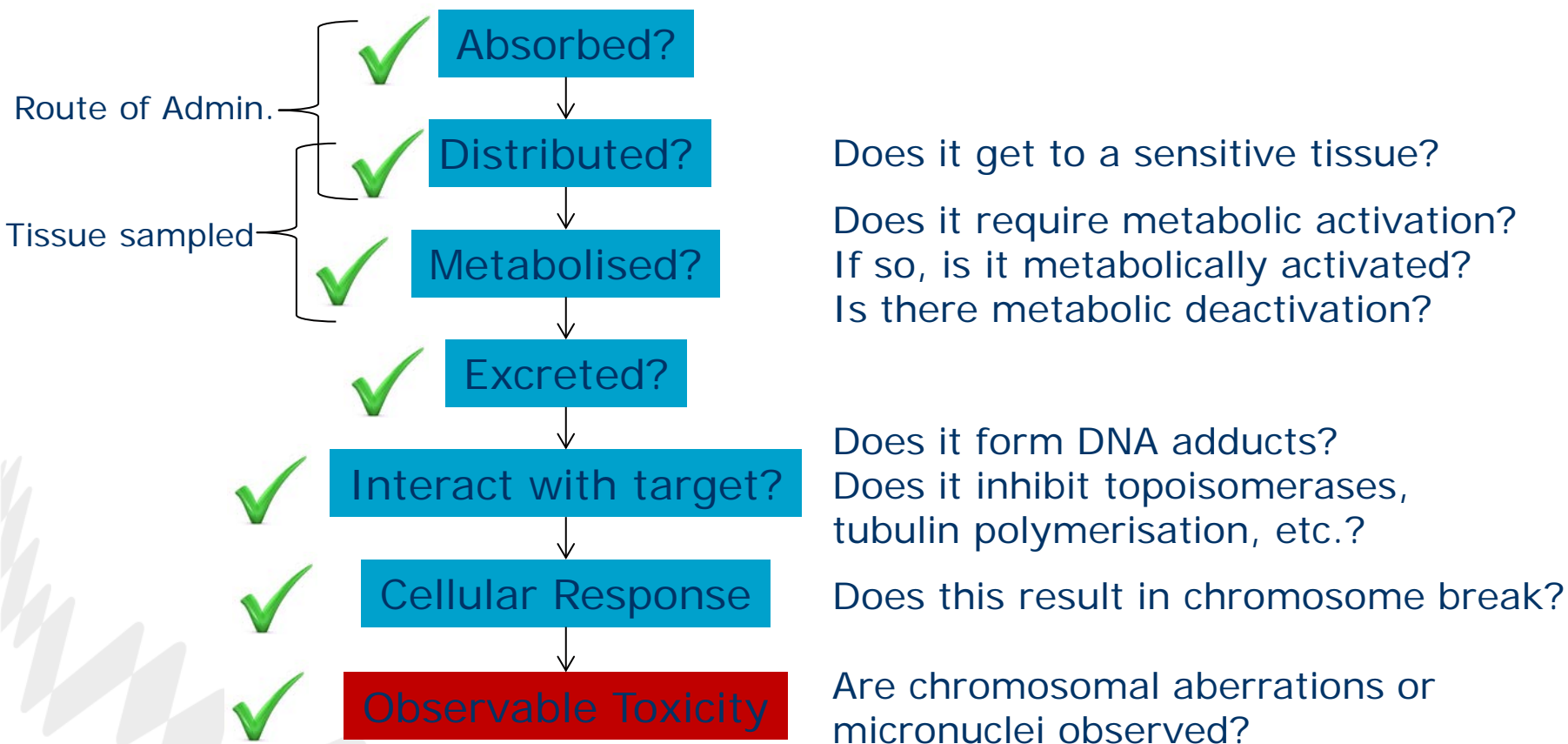
In vivo Chromosome Damage



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In vivo Chromosome Damage



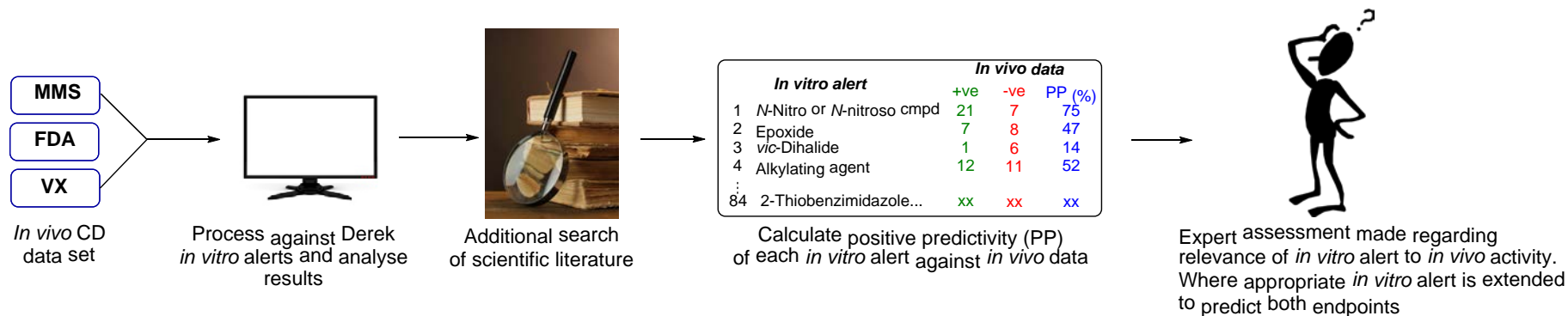
How *in vitro* activity correlates with *in vivo*?

- 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 <i>in vitro</i> alert to <i>in vivo</i> endpoint |
| Poor, but with rationale | New <i>in vivo</i> alert or extend <i>in vitro</i> alert with caveats |
| Poor, no rationale | Alert not extended, comments updated |

In Vitro To In Vivo Alert Extensions

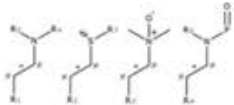
- 19 *in vitro* alerts extended to predict *in vivo* activity
 - 9 alerts predict at the reasoning level of plausible
 - 10 alerts predict at the reasoning level of equivocal
- 4 new *in vivo* chromosome damage alerts implemented to cover a sub-class of *in vitro* alert
- 9 *in vitro* alerts could not 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

Description

KB: DK12 RW1_15073.12 ID: 10510 Links (55) State: Enabled

Identifiers: 069
Name: Nitrogen or sulphur mustard



Description

chlorophazine [Sohns], misonosine dihydrochloride [Bochkov and Kuleshov] and N,N,N'-tri(beta-chloroethyl)-N-(p-formylphenyl)-1,3-propylenediamine [Kaina].

These compounds have generally given positive results in *in vivo* cytogenetic tests. For example, cyclophosphamide monohydrate [NTP 1991-1992], melphalan [Morita et al], chlorambucil [NTP 1993], and nitrogen mustard [Morita et al] all gave positive results in the mouse bone marrow micronucleus assay when administered by intraperitoneal injection. Cyclophosphamide monohydrate [NTP 1991-1992] and chlorambucil [Ashby et al] also induced micronuclei in this test following exposure by oral gavage. Negative results have been reported for phenoxylbenzamine hydrochloride when administered intraperitoneally [Morita et al]. Positive results have also been reported for melphalan in the mouse bone marrow chromosome aberration test [NTP 1985].

Overview Description Patterns References Examples Endpoints Comments Test

References

KB: DK12 RW1_15073.12 ID: 10510 Links (55) State: Enabled

References

| ID | Reference type | Title | Author | Year |
|------|----------------|---|--|-----------|
| 5297 | article | Evaluation of the rodent micronucleus assay in the screening of IARC | Morita T, Atano N, Awogi T, Sasaki YF, Sato S | 1997 |
| 5617 | inbook | Revised Edition 1999 Data Book of Chromosomal Aberration Test in Vi | Sofuni T (editor) | 1999 |
| 5632 | article | Age sensitivity of human chromosomes to alkylating agents. | Bochkov NP and Kuleshov NP. | 1972 |
| 5509 | article | Toxic and clastogenic effects of the polyfunctional alkylating agent N | Kaina B. | 1981 |
| 7852 | misc | Salmonella study summary on nitrogen mustard hydrochloride (CAS 1 | National Toxicology Program (NTP) | 1989 |
| 7851 | misc | Salmonella study summary on 2-chloropropyl-dimethylamine hydroc | National Toxicology Program (NTP) | 1991 |
| 7854 | misc | Salmonella study summary on melphalan (CAS No. 148-82-3). | National Toxicology Program (NTP) | 1987 |
| 9214 | misc | Bone marrow micronucleus study summary of cyclophosphamide m | National Toxicology Program (NTP) | 1991-1992 |
| 9213 | misc | Bone marrow micronucleus study summary of chlorambucil (CAS No | National Toxicology Program (NTP) | 1993 |
| 9212 | article | Mutagenicity to bacteria, cultured cells, and rodents of the human ca | Ashby J, Loquet C, Ishidate M Jr, Callander RD | 1988 |
| 9203 | misc | <i>In vivo</i> cytogenetics - chromosome aberrations of melphalan (CAS No | National Toxicology Program (NTP) | 1985 |

Preview

Click on the Reference to see a preview

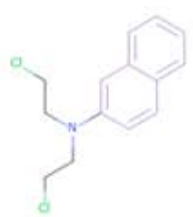
Overview Description Patterns References Examples Endpoints Comments Test

Examples

KB: DK12 RW1_15073.12 ID: 605 Links (11) State: Enabled

Structure

Name: chlorambucil
CAS Number: 494-03-1
InChI: InChI=1/C14H15Cl2N/c15-7-9-17L
SMILES: ClC=C(CC=CC1=CC=CC=C1)CC1=CC=CC=C1
Molecular Weight: 268.182 Da
Monoisotopic Mass: 267.0982 Da



Toxdata

Toxdata Source: DK12 RW1_15073.12

| ID | Endpoint | Species | Assay | Result |
|-------|-------------------|---------|----------------------|----------|
| 11300 | Chromosome damage | human | in vivo micronucleus | positive |
| 11305 | Chromosome damage | mouse | in vivo micronucleus | positive |

Overview Toxdata Comments

In vivo chromosome damage progress

DfW13:

| | | <i>Predicted</i> | |
|------------|-----|------------------|-----|
| | | +ve | -ve |
| <i>Exp</i> | +ve | 4 | 108 |
| | -ve | 0 | 130 |

Sensitivity:

$$4/112 = 4\%$$

Specificity:

$$130/130 = 100\%$$

Balanced Accuracy:

52%

VS

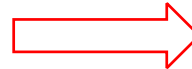
DX 2014:

| | | <i>Predicted</i> | |
|------------|-----|------------------|-----|
| | | +ve | -ve |
| <i>Exp</i> | +ve | 45 | 67 |
| | -ve | 28 | 102 |

$$45/112 = 40\%$$

$$102/130 = 79\%$$

60%



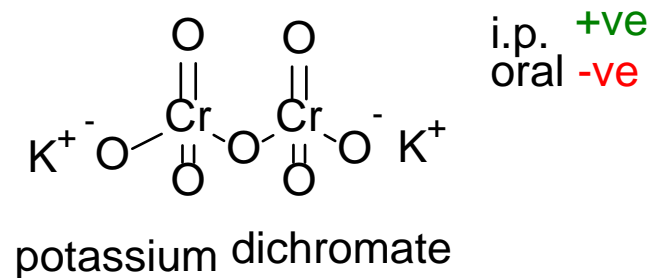
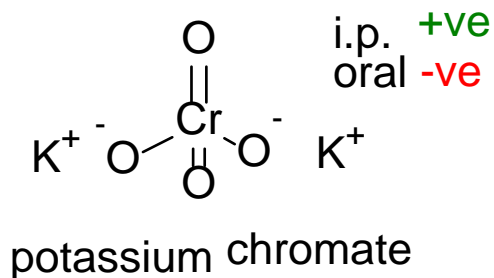
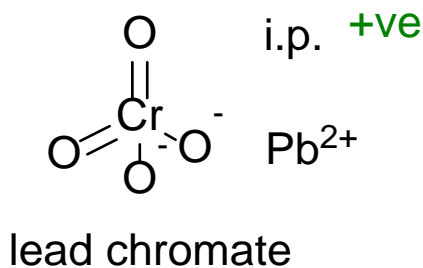


EXAMPLES

Route of Administration specific *in vivo* activity

Chromium compounds may display route of admin. specific activity

Rodent BM MN test:



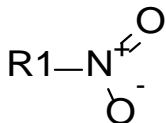
Key Info

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

Tissue specific *in vivo* activity

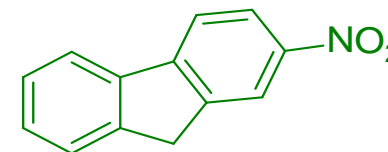
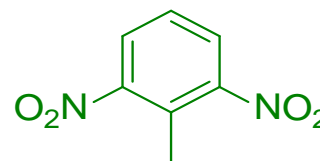
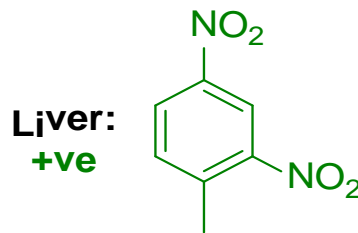
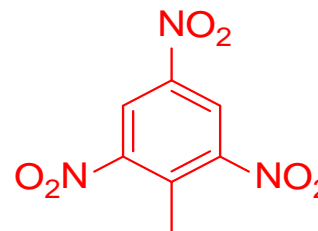
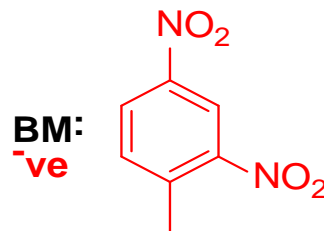
Aromatic nitro compounds may display tissue-specific activity

In vitro alert for aromatic nitro compounds

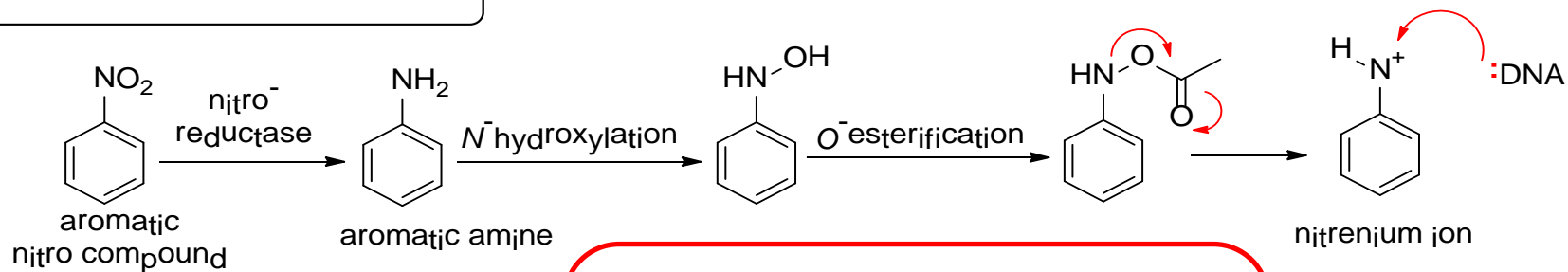


R1 = C (aromatic)

Examples:



| <i>In vivo</i> data: | | | |
|----------------------|-----|-----|------|
| | +ve | -ve | PP |
| BM: | 4 | 9 | 31% |
| Liver: | 3 | 0 | 100% |



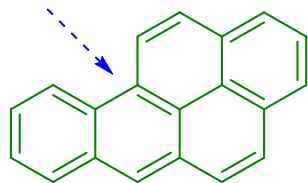
Key Info
The liver is a sensitive tissue to test *in vivo* activity of aromatic nitro compounds

Sub-class specific *in vivo* activity

Modified SAR for *in vivo* activity of PAHs

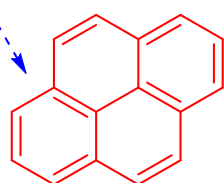
Examples:

bay-region



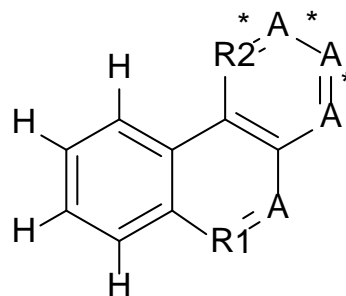
+ve in vivo

K-region



-ve in vivo

In vivo alert for PAHs

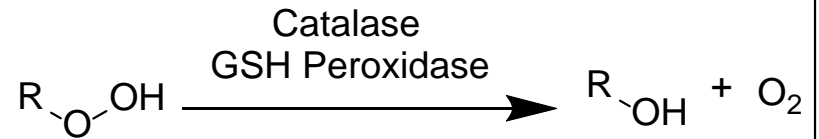
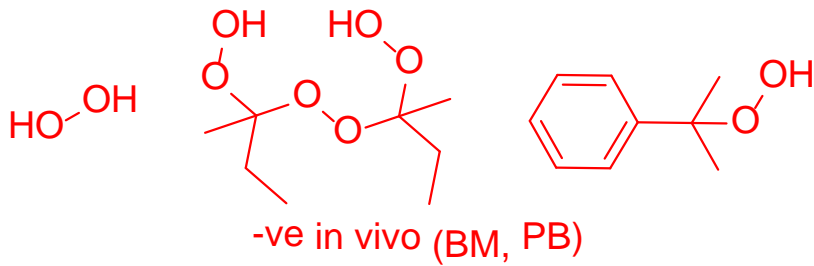
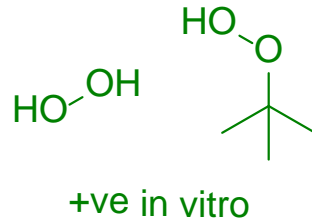


A = any atom; R1 = N, NH⁺, C-H;
R2 = N, NH⁺, C(fused), C-H,
C-Me, C-Et, C-OH, C-O, C-OMe
* = at least one of these bonds must
be fused to another aromatic ring.

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



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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