Implementation of Detoxification Pathways in TIMES *in vivo* Genotoxicity Models

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Introduction

Genetic damage in living organisms may often be prevented by procedures which impede metabolically-generated reactive species from ever reaching the DNA in target cells. One of these impediments is substrate channeling where the metabolic products of one enzyme is passed directly to another enzyme without its release to the cytosol or other bodily fluids, thus preventing DNA exposure.

Goal

The aim of this work is to demonstrate the significant difference between *in vitro* and *in vivo* systems through the identification of detoxification pathways organized in *in vivo* detoxification batteries.

The concept

❖ Basic difference between *in vitro* and *in vivo* genotoxicity effects is due to the following:

- ➢ *In vitro* (S9) generated metabolites are freely available to interact with DNA and/or proteins thus causing positive genotoxicity effects.
- ➢ *In vivo* generated metabolites are organized as a result of enzyme-catalyzed substrate channeling which prevents their potential positive genotoxicity effect on macromolecules.
- ➢ The role of *Phase II* reactions is highly pronounced *in vivo* as compared to the *in vitro* environment.

❖ The substrate channeling and pronounced *Phase II* reactions may potentially explain the *in vivo* detoxification of chemicals which could otherwise lead to a positive result *in vitro*.

Metabolic activation and detoxification

❖ Metabolic activation of ethyl benzene leads to the formation of DNA and/or protein active metabolites (yellow) if substrate channeling is not accounted for.

❖ Identification of *in vivo* detoxification pathways is based on expertly defined sequences of metabolic transformations presumably involved in the detoxification of *in vitro* positive chemicals:

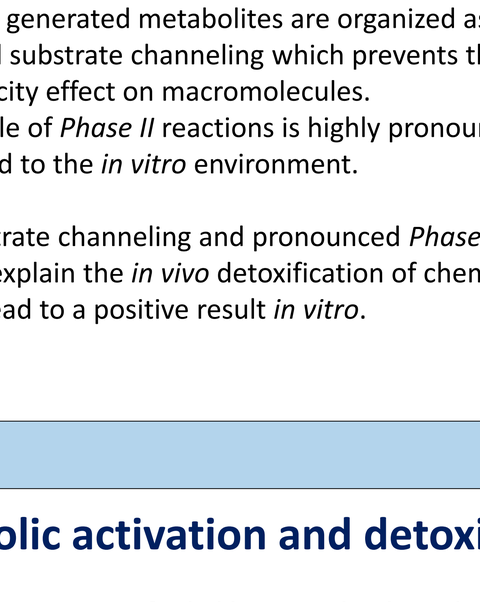

Figure 1. Metabolic activation and detoxification of Ethyl benzene

This poster could be downloaded from : **http://oasis-lmc.org/news/events/qsar2014.aspx**

Battery of detoxification pathways

Identified *in vivo* detoxification pathways are organized in detoxification batteries. Each detoxification battery consists of:

- ➢ a local training set of chemicals belonging to same chemical class
- ➢ sequences of metabolic transformations presumably involved in detoxification of these chemicals.

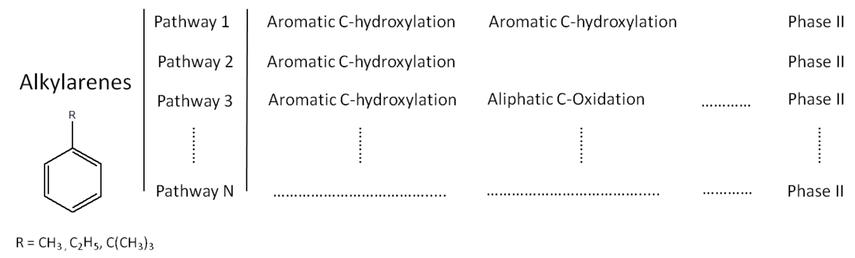

Figure 2. Detoxification battery of Alkyl arenes

Reliability of detoxification batteries

The significance of the defined *in vivo* detoxification pathways is assessed for each battery by assessing its reliability. The reliability estimate is based on the performance (sensitivity and specificity) of each detoxification battery:

# chem in a battery	Obs. or predicted *In vitro* data	Obs. *In vivo* data	Applications of detoxification pathways	Predicted *In vivo* data
1	positive	negative	yes	negative
2	positive	negative	yes	negative
3	positive	negative	yes	negative
4	positive	negative	yes	negative
5	positive	negative	yes	negative
6	positive	negative	yes	negative
7	positive	negative	no	positive
8	positive	positive	no	positive
9	positive	positive	no	positive
10	positive	positive	no	positive

Figure 3. Reliability of detoxification battery of Alkyl arenes

❖ Sensitivity of the *in vivo* detoxification battery is 86% (6 out of 7 correct applications).
❖ Specificity of the *in vivo* detoxification battery is 100% (3 correct non-applications out of 3).

Conclusions

❖ The significant difference between *in vitro* and *in vivo* metabolism could be used to build mechanistically justifiable *in vivo* detoxification pathways.
❖ *In vivo* detoxification pathways include sequences of metabolic transformations presumably involved in the detoxification of *in vitro* positive chemicals.
❖ Lack of information about *in vivo* substrate channeling effect necessitated the use of an empirically-based approach for identifying detoxification pathways.
❖ The multitude of *in vivo* detoxification pathways associated with chemicals belonging to same chemical classes are organized into detoxification batteries.
❖ Reliability of *in vivo* detoxification battery is estimated based on its performance on the local training set chemicals used to derive the battery.

Acknowledgments

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