

# Inclusion of metabolism in toxicity predictions comparing *in silico* with *in vivo*

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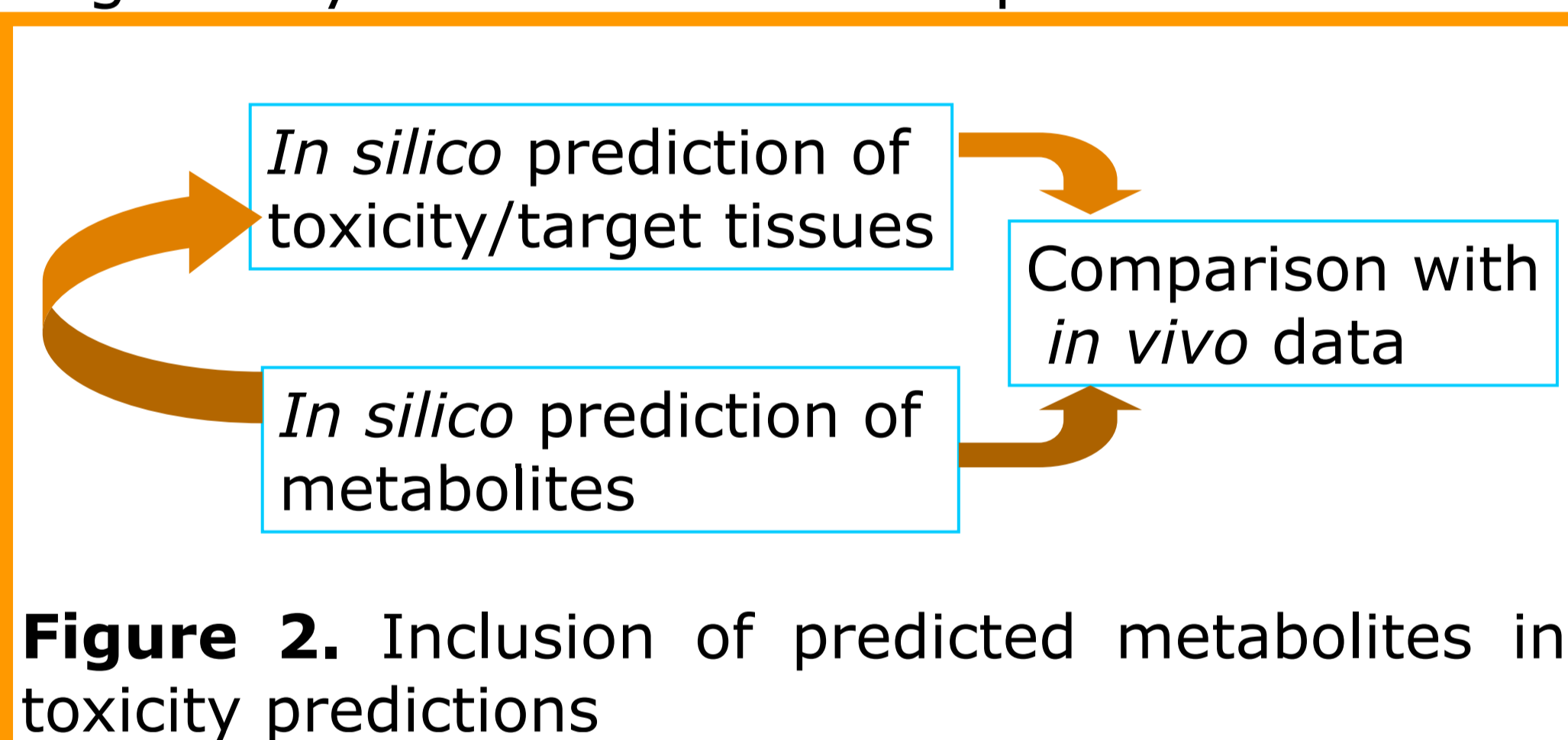
Universiteit Utrecht

## Introduction and aim

This research was conducted within a larger programme to investigate the use of non-animal data (*in silico* and mammalian *in vitro* data on kinetics and toxicity) for predicting human risk. In the absence of animal data, a first step could be the use of *in silico* predictions to direct the testing strategy for new substances. Here we compare the output of software for the generation of metabolism and toxicity predictions with the observed *in vivo* metabolism and toxicity, to investigate whether the inclusion of (predicted) metabolites improves toxicity predictions.

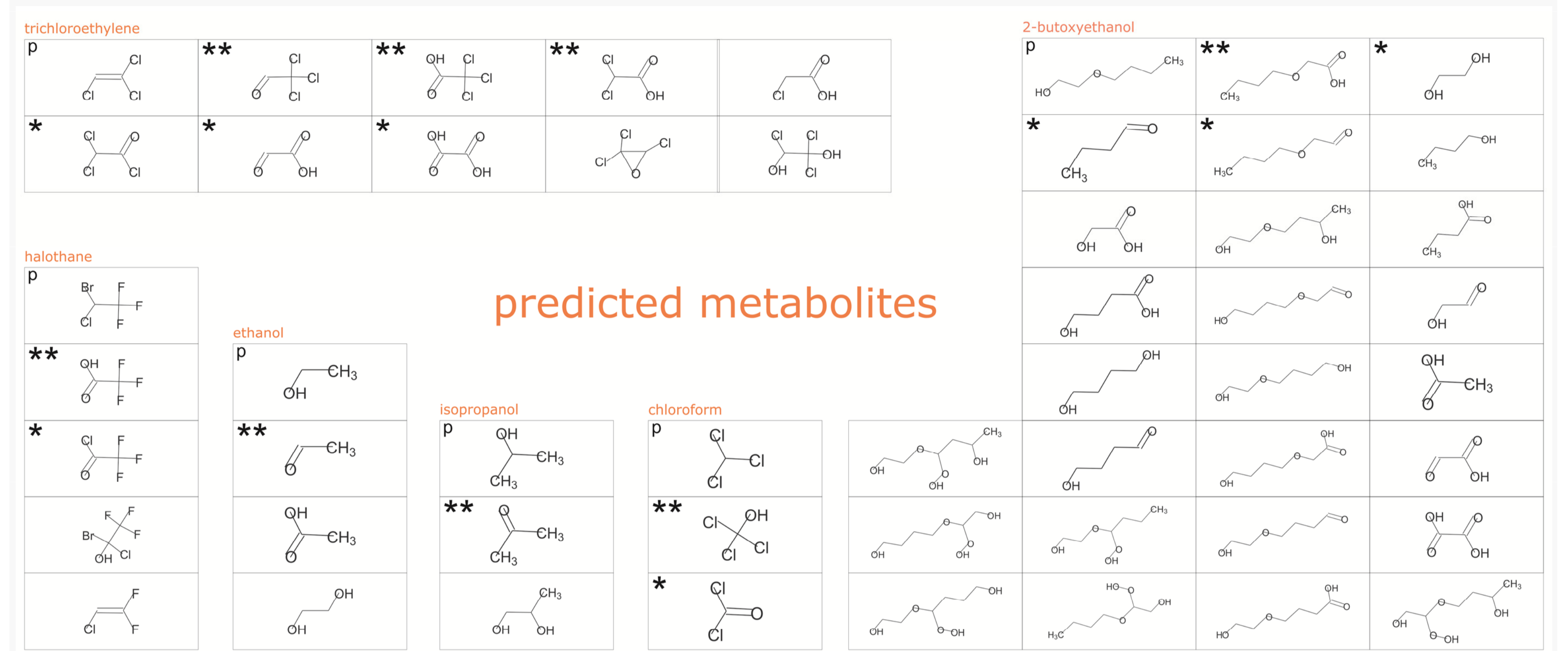
## Methods

Metabolites were predicted using METEOR (Lhasa Ltd, UK) and the OECD QSAR Toolbox. Alerts for toxicity of solvents and predicted phase I metabolites were generated using the knowledge-based systems DEREK (Lhasa Ltd), the OECD QSAR Toolbox and quantitative structure-activity relation (QSAR)-based TOPKAT® (Accelrys). *In vivo* toxicity data were obtained from North American and EU regulatory risk assessment reports.



**Figure 2.** Inclusion of predicted metabolites in toxicity predictions

**Figure 1.** Predicted metabolites for the investigated solvents. The parent chemical is indicated with a 'p', and metabolites that are observed *in vivo* are marked with an \* (metabolites critical for toxicity: \*\*).



**Table 3.** *In vivo* toxicity and toxicity alerts when including predicted metabolites.

	<i>in vivo</i> toxicity	(Additional) alerts		
		no metabolites	all metabolites	<i>in vivo</i> metabolites
2-butoxyethanol	skin* nervous system* hepatotox. - nephrotox. respiratory system*	carcinogen.	skin sens./irrit. teratogen. hepatotox. developmental tox.	skin sens./irrit. developmental tox.
ethanol	nervous system* carcinogenicity* developmental tox. hepatotox.	skin sens. teratogen. developmental tox.	skin irrit. carcinogen.	skin irrit. carcinogen.
isopropanol	nervous system* respiratory system gastrointestinal system	teratogen. developmental tox. carcinogen.	skin irrit.	skin irrit.
halothane	nervous system* cardiovascular system* developmental tox. hepatotox.	hepatotox. carcinogen.	skin sens. nephrotox. developmental tox.	skin sens. developmental tox.
chloroform	nervous system* hepatotox.* carcinogenicity* nephrotox.	carcinogen. hepatotox. skin irrit.	skin sens. developmental tox.	skin sens. developmental tox.
trichloroethylene	nervous system* carcinogenicity* cardiovascular system nephrotox. - hepatotox.	carcinogen. nephrotox. hepatotox.	skin sens./irrit. developmental tox.	skin sens./irrit. developmental tox.

**Conclusion** The number of false predictions for solvents was not reduced when including predicted metabolites. The number of false positives clearly indicates that human risk assessment cannot be based on these *in silico* prediction. However, the **number of false negatives is reduced**. Based on these findings it is recommended to include metabolite formation in toxicity predictions to prevent the postponement of potential toxicants in prioritization for experimental toxicity testing.

**Table 2.** Toxicity alerts for the investigated solvents by DEREK (D), TOPKAT (T) and the OECD Toolbox (O). It is indicated with an (\*) if the *in silico* predictions were based on the presence of the chemical of interest within D or T database, or the chemical being the structural alert. It is indicated with (#) if a D prediction is based on the presence of the chemical of interest in the software database, but other example chemicals were also included for the prediction. It was indicated with (^) if the information for a T prediction was outside the optimal prediction space limits, or if not all atomic fragments were covered. The applicability of the alert for the parent chemical (by 'p') and (number of) metabolites is indicated after each alert. Abbreviations: sensitization: sens.; -genicity: gen.; irritation: irrit.; -toxicity: tox.). Alerts unique for predicted metabolites are marked in a blue font.

<p><b>2-butoxyethanol</b> D: aldehyde (skin sens.;7) D: aldehyde precursor (skin sens.;6) D: alkyl alcohol (teratogen.;1) D: alkyl aldehyde (carcinogen.;1) D: organic peroxide (hepatotox.;6) D: short chain alkyl carboxylic acid (carcinogen.;2) T: (carcinogen.;p+11) T: (developmental tox.;9) T: (skin sens.;4) O: aldehyde (skin irrit.;7) O: aliphatic acids and (met)acrylic acids (skin irrit.;2) O: H acceptor path3 H acceptor (genotox.;p+18) O: simple aldehyde (mutagen./carcinogen.;7)</p> <p><b>ethanol</b> D: aldehyde (skin sens.;1) D: alkyl alcohol (teratogen.;p#) D: alkyl aldehyde (carcinogen.;1) D: short chain alkyl carboxylic acid (carcinogen.;1) T: (carcinogen.;2) T: (developmental tox.;p+2) T: (skin sens.;p+1) O: aldehyde (skin irrit.;1) O: aliphatic acids and (met)acrylic acids (skin irrit.;1) O: H acceptor path3 H acceptor (genotox.;1) O: simple aldehyde (mutagen./carcinogen.;1)</p>	<p><b>isopropanol</b> D: alkyl alcohol (teratogen.;p#) T: (developmental tox.;p+2) T: (carcinogen.;p+1) O: H acceptor path3 H acceptor (genotox.;1) O: ketones (skin irrit.;1)</p> <p><b>halothane</b> D: carboxylic acid halide (skin sens.;1) D: halothane or analogue (hepatotox.;p#) D: halogenated alkene (carcinogen.;1)/(nephrotox.;1) D: halogenated hydrocarbon (hepatotox.;1) T: (carcinogen.;1) T: (developmental tox.;1) T: (skin sens.;1) O: acyl halide (genotox./mutagen./carcinogen.;1) O: aliphatic halogen (genotox./mutagen./carcinogen.;p+1)</p> <p><b>chloroform</b> D: di-/polyhalogenated alkane or cycloalkane (carcinogen.;p) D: carboxylic acid halide (skin sens.;1) D: halogenated hydrocarbon (hepatotox.;p#) T: (carcinogen.;p+2) T: (developmental tox.;1) O: acyl halide (genotox./mutagen./carcinogen.;1) O: aliphatic halogen (genotox./mutagen./carcinogen.;p) O: halogenated alkane (skin irrit.;p)</p>	<p><b>trichloroethylene</b> D: aldehyde (skin sens.;2) D: alkylating agent (carcinogen.;3) D: alpha chloro ether (carcinogen.;1) D: carboxylic acid halide (skin sens.;1) D: di- to halogenated alkane (carcinogen.;4) D: epoxide (developmental tox.;1)/(carcinogen.;1)/(skin sens.;1) D: haloalkane (skin sens.;3) D: halogenated alkene (carcinogen.;p)/(nephrotox.;p#) D: halogenated hydrocarbon (hepatotox.;p+2) T: (carcinogen.;p+2) T: (developmental tox.;5) T: (skin sens.;1) O: acyl halide (genotox./mutagen./carcinogen.;1) O: aldehydes (skin irrit.;2) O: aliphatic alpha-halogen-esters (skin irrit.;2) O: aliphatic halogen (genotox./mutagen./carcinogen.;p+4) O: epoxides-aziridines (genotox./mutagen./carcinogen.;1) O: H acceptor path3 H acceptor (genotox./mutagen./carcinogen.;3) O: simple aldehyde (genotox./mutagen./carcinogen.;2)</p>
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