# OLIMPIC - Overcoming current Limitations In Metabolism Prediction of Industrial Chemicals

Molecular Networks
Inspiring Chemical Discovery

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## Introduction

There is a growing interest in alternative methods for hazard and risk assessment of chemicals, e.g. due to the REACH initiative [1]. Computational methods have to be developed and validated with respect to their suitability for the evaluation of toxicity and risk of chemical compounds. The modeling should also consider the aspects of reactive toxicity and metabolism. In this paper, the development of tiered testing strategies for the prediction of metabolism of industrial chemicals is presented. For this purpose, (Q)SAR prediction models based on *in vitro* and *in vivo* metabolism data are evaluated. A chemoinformatics approach for predicting the metabolism of industrial chemicals is presented.

## Project Description

### Aim

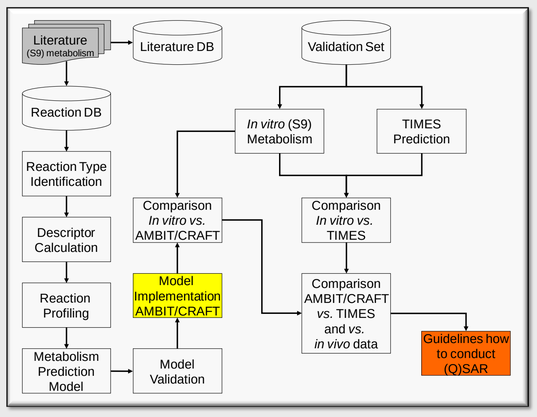
The aim of the OLIMPIC project is to provide improvements in the prediction of metabolism of industrial chemicals in mammals. The holistic investigation will consider *in vivo*, *in vitro* and *in silico* data in order to identify discrepancies between the different methods and to enhance the predictive power of *in silico* models for metabolism.

### Approach

In this poster we will report on the compilation of a standard data set of industrial chemicals from literature, the measurement of S9 *in vitro* metabolism data for additional 30 compounds, the evaluation of the software package TIMES based on the experimental measurements, and initial results for the reactivity modeling. The focus of the investigation is limited to the following compound classes: diketones, quinolines, isoquinolines, triazolopyrimidines, sulfonamides, and organophosphates.

## Workflow

In the figure below the sequence of the individual workpackages is assemebeld to a workflow.




The project has a duration of 24 months. The emphasis during the first twelve months is on the compilation of the training data set, the *in vitro* S9 metabolism measurements of the validation data set, the evaluation of TIMES, and analysis of the reaction types which are relevant for the selected compound classes. The second year will be dedicated to the development and implementation of improved *in silico* prediction methods.

## Current Status and Outlook

- A training set was compiled from literature data.
- Experimental measurements on the metabolism of all 30 compounds in the validation data set were performed.
- The *in silico* prediction of the compounds from the validation data set with the software TIMES were analyzed.
- The chemoinformatics modelling is in progress.

## Acknowledgements

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## References

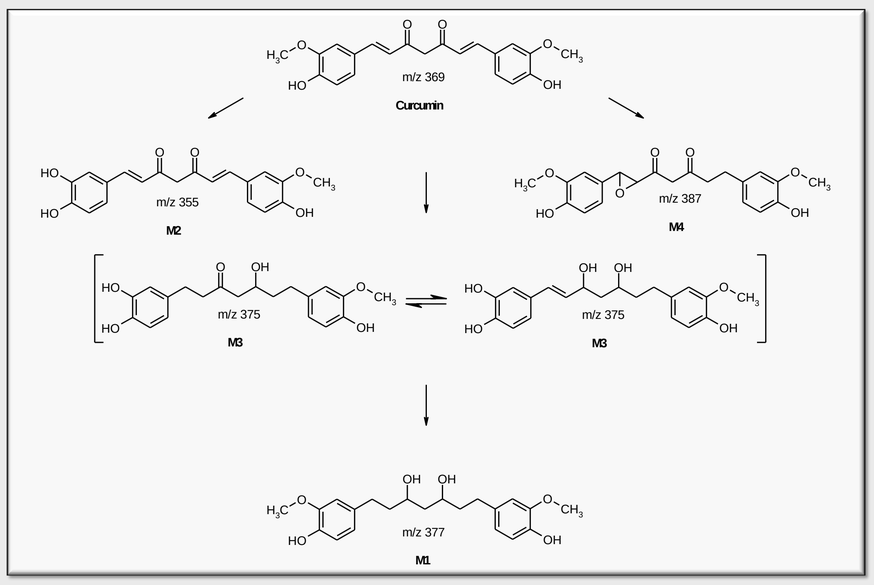
[1] EC 1907/2006, http://ec.europa.eu/environment/chemicals/reach/reach_intro.htm
[2] Tamvakopoulos C, Sofianos ZD, Garbis SD, Pantazis P. Analysis of the in vitro metabolites of diferuloylmethane (curcumin) by liquid chromatography--tandem mass spectrometry on a hybrid quadrupole linear ion trap system: newly identified metabolites. *Eur. J. Drug Metab. Pharmacokinet.* **2007**, *32*, 51-57.

## Experimental S9 Metabolism Measurements

### Experimental setup

Metabolic clearance of curcumin (CAS: 000458-37-7) was performed by standard incubations in rat liver S9-fraction in a cofactor (NADPH) containing buffer system at 37 °C. After incubation proteins of incubates were precipitated by the addition of acetone and the supernatant was analyzed by LC/UV and LC/MS/MS.

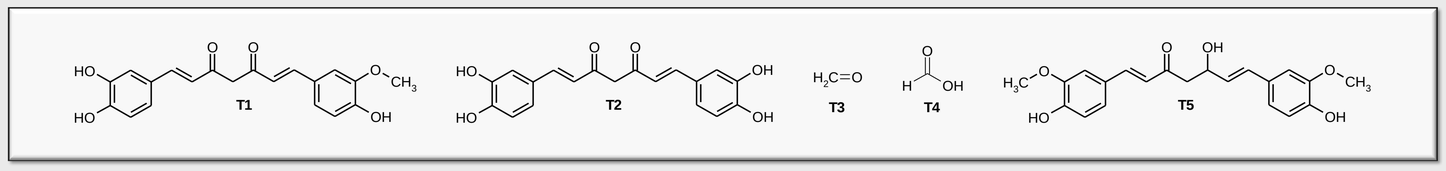
### Metabolic pathway of curcumin in rat liver S9-fraction




## Evaluation of TIMES

OASIS TIMES was used to generate *in silico* the metabolites of curcumin. Curcumin was in the applicability domain of TIMES. TIMES predicts five metabolites of curcumin. Although only the metabolite M2 (S9-fraction incubation), which is equal to T1 (TIMES evaluation) was detected *in vitro* and *in silico*, the principle functionalization reactions were predicted correctly. The metabolite M2 *resp.* T1 was also detected *in vitro* by Tamvakopoulos et al.[2]

### Scheme of curcumin metabolites predicted by TIMES

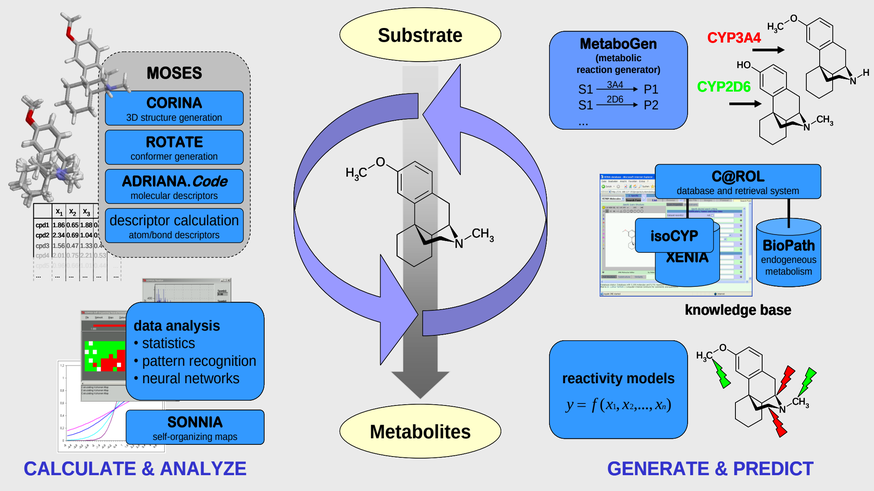



Tamvakopoulos et al. detected in mouse and human liver microsomal preparations also the proposed TIMES metabolites T2 and T5. TIMES did not evaluate the *in vitro* detected metabolites M1, M3 and M4.

## Chemoinformatics Modelling of Metabolism

### Metabolite Prediction

- Prediction has to consider the chemo- and regioselectivity
- Define reaction rules based on the knowledge of human metabolism
- Derive probabilities for each reaction rule from a reaction database
- Apply reaction rules to query compounds and rank the reactions according to their probability




### Metabolite Prediction with the Current Model

- The metabolite M2 resulting from the O-demethylation of a methoxy group attached to an aromatic ring is predicted as principle metabolite with a likelihood of 0.27.
- Glucuronidation or sulphation of the aromatic hydroxy group is predicted with a likelihood of 0.15.

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