



# Simulating Metabolism of Xenobiotic Chemicals as an Indicator of Toxicity

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research & development

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## Abstract

EPA is faced with long lists of chemicals that need to be assessed for hazard, and a gap in evaluating chemical risk is accounting for metabolic activation resulting in increased toxicity. The goals of this project are to develop a capability to forecast the metabolism of xenobiotic chemicals, to predict the most likely formed metabolites, and to interface that information with toxic effect models. Results will identify metabolites of equal or greater toxicity than the parent chemical. An existing metabolism simulator is being refined by focusing on reactions leading to increased toxicity; the toxic effect endpoint considered is endocrine disruption mediated by direct chemical binding to the estrogen receptor (ER). The principal reactions under investigation, ring oxidation and O-dealkylation, are those that result in hydroxylated metabolites predicted to bind the ER with greater affinity than the parent chemical. Chemicals for study were selected from an EPA concern list and include chemicals predicted to be non-estrogenic as parent but for which forecasted metabolites are predicted to bind ER. Model results will be used to prioritize chemicals for further study.

Simulator performance will be enhanced by collecting chemical metabolism maps from the published literature and determined from *in vitro* rat hepatic microsomes experiments. Newly acquired maps (and transformations) will be used to re-train the metabolism simulator and improve reliability estimates. Metabolites formed in rat microsomes will also be assessed in fish liver tissue slices. Liver slices from male fish that produce vitellogenin (Vtg) when exposed to xenobiotics will be used to study chemical bioactivation to ER-active forms. Data from these studies are used to improve the metabolic simulator and prioritize chemicals for testing that have the potential to be bioactivated to more toxic species. Metabolism data for training and improvement of the simulator will be stored and accessed using a database manager software under development. The database software is capable of search functions, depiction of metabolic maps, and provides access via structures to metabolism information and associated data collected from EPA's Office of Pesticide Programs (OPP). The database will be used by OPP risk assessors to increase efficiency of chemical data access and performance of risk assessments. In its simplest mode, the database will furnish curated structures of chemicals/pesticides and their metabolites suitable for searches in other databases and provide metabolic maps plus tabulations of amounts of metabolites and other parameters. In a more advanced mode, the database will allow the risk assessor to perform searches for specific compounds and toxicophores and identify metabolite commonalities and differences across pesticides and species.

Finally, prioritized chemical lists (based upon predicted toxic effects of parent chemical and metabolites) with transformation reliability estimates will be provided to OPP/TS for chemical evaluations (risk assessments) and ranking for toxicity testing. This research will expand the knowledge-base of metabolic pathways and transformation products for important groups of toxic chemicals and demonstrate an approach that integrates metabolism simulation with toxic effects modeling.

## Science Question

**Issue:** Methods and tools are needed to prioritize chemicals for toxicity testing and hazard assessment.  
**Approach:** Use computational advances to develop a simulator of metabolism for identification of chemical metabolites; link input to toxic effects model to target metabolites of greater toxic potential than the parent chemical.

## Research Goals

The proposed research will develop a capability for forecasting the metabolism of xenobiotic chemicals of EPA interest, to predict what chemical metabolites are the most likely to be formed, and to interface that information with toxic effect models that predict chemical binding to the estrogen receptor (ER), a well-recognized pathway of toxicity leading to endocrine disruption. This project will (a) illustrate the importance of considering metabolic activation in toxic effects modeling to predict not only parent chemical toxic potential but to identify chemical metabolites of equal or greater potential toxicity than the parent chemical, and (b) demonstrate an approach to provide this capability for large chemical lists of risk assessment concern.

## Metabolism Database Manager

General database (METAPATH) program display

List of available maps within a database - file folders, which upon expansion detail the "tree view" hierarchical list of metabolites affiliated with a particular parent chemical.

Map view editing and export tools

Metabolic map display depicting parent chemical and metabolites

Two-dimensional (2D) structure of either the parent or any metabolite. The chemical displayed is chosen from the tree view (upper left) or selected in a structure from the map view (upper right). A right-hand mouse click on the structure produces a pop-up box which allows for 2D depiction options, the ability to Copy the SMILES, or Save the picture as a graphic image.

Abbreviated systems, showing the connectivity map for parent and metabolites.

Searching the Database by sub-structure, species, dose, etc.

Create a Search Query by species (e.g., metabolites measured in rat (O1), type of administered dose (oral gavage) (Q2), and a hypothesized metabolite or structure (e.g., 1,2-dioxin-like (Q3)). The three clauses Q1, Q2 and Q3 are tied together with "AND" logic, with a double-click activated the search. "SEARCH RESULTS" are displayed in a pop-up window (below). Searches may be as simple or complex as the user desires, but they can always be saved and reloaded for future use.

The gathered list of SEARCH RESULTS will correspond to the selected structure and the links on the original start up page of the program. The blue highlighted cursors will move its one scrolls through the search results listings.

Within a database of dozens of maps, 3 metabolites were found that satisfy the search criteria, i.e., a triazole metabolite found in rats dosed by oral gavage, and the entire metabolic tree. The user may scroll through the listed results.

RESULTS may be viewed with respect to transformations of parent.

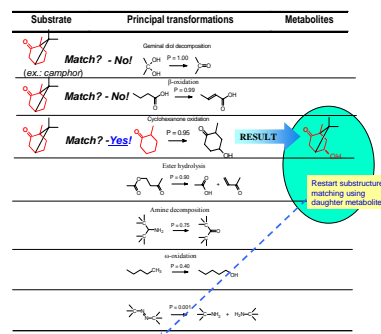
## Metabolism Simulator

Metabolic Simulator General Approach

Probabilistic Knowledge-Based Expert System for Forecasting Metabolism of Organic Chemicals

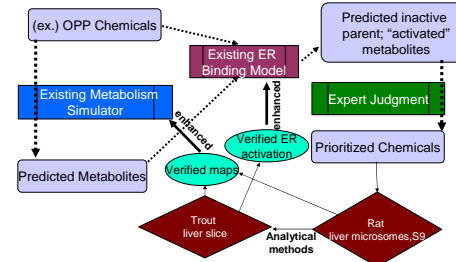
- Utilizes a library of functional-group transformations targeting a specific system (environment, tissue, etc).
- Literature-derived, experimentally determined metabolic maps for diverse chemicals are used for the model training data.
- Each functional-group transformation is assigned a "probability" of the reaction occurring. These probabilities are derived based upon how well measured data is simulated.
- Most probable transformations are then applied to determine the dominant daughter, granddaughter, great-granddaughter product from a parent compound with an unknown metabolic pathway.

Substrate matching engine applying a hierarchically ordered list of metabolic transformations (mammalian liver)



\*\*The current simulator contains a library of > 350 transformation reactions, representing major transformation types including aromatic C-hydroxylation, alpha-oxidation, arene epoxide formation, oxidative N-dealkylation, dehalogenation, ester and amide hydrolysis, and oxidative dealkylations, to name a few. The training set of data for development of the metabolic simulator consists of >340 metabolic maps of xenobiotic compounds.

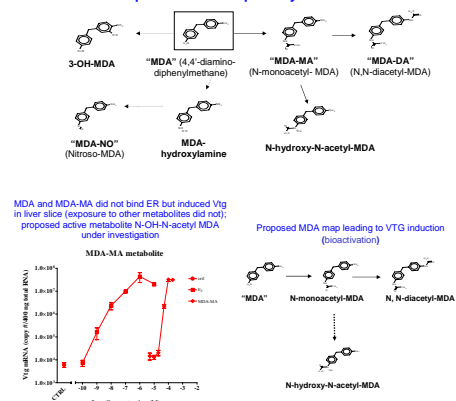
## Project Goal: Enhance Metabolic Simulator for Assessing EPA Regulatory Lists



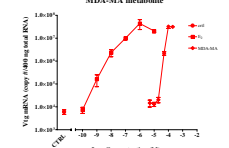
## Experimental Metabolism Studies: Enhance Metabolic Simulator

Approach: select chemicals; determine metabolic map; determine bioactivation

Proposed metabolic pathway for MDA



MDA and MDA-MA did not bind ER but induced VTG in liver slice (exposure to other metabolites did not); proposed active metabolite N-OH-N-acetyl-MDA under investigation



## Results/Conclusions

- Provide a capability for predicting bioactive metabolites from parents.
- Rank chemicals for *in vitro* or *in vivo* screening and toxicity testing based upon predicted toxic effects
- Develop searchable metabolism database to identify toxic chemicals and metabolites for risk assessment
- Provide linkage of effects based toxicity models with metabolism simulations.

## Impact and Outcomes

The development of a metabolic simulator for prediction of chemical metabolic maps integrated with a quantitative structure-activity relationship (QSAR) toxic effects model will provide effects-based prioritization of chemicals (parent compound and metabolites) relevant to EPA Program Offices. One of the primary objectives of the CompTox Program and this proposal is the development of computational tools for prioritization of chemicals for toxicity testing with the goal of minimizing dependence on test animals.

## Future Directions

- Continue development and expansion of the liver metabolism database, especially for chemicals and transformation reactions underrepresented in the current database.
- Finalize development of the search and data input functions of the metabolism database manager (MetaPath).
- Enhance the performance of the existing metabolic simulator by incorporating reliable metabolism data and expansion of relevant transformation reactions.
- Continue laboratory experiments for chemicals and chemical transformations underrepresented in the metabolism database to verify maps/metabolites forecasted by the simulator and evidence for enhanced estrogenicity.

## References

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Schmieder, P., Tapper, M., Denny, J., Kolanczyk, R., Sheedy, B., Henry, T., and Veith, G. (2004). Use of trout liver slices to enhance mechanistic interpretation of ER binding for cost-effective prioritization of chemicals within large inventories. Environ Sci Tech. 38, 6333-6342.

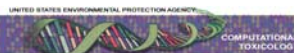
## Partners

Bourgas University, Bourgas, Bulgaria

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US EPA Office of Pesticides Program (HED and EFED)

## Long Term Goal II



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