National Institute of Environmental Health Sciences
Division of Translational Toxicology

Integrating Population Enzyme Variability into Physiologically-Based Kinetic Models of Parent Chemicals and Metabolites

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Introduction

- Enzymes can transform chemicals that enter the body into metabolites.
- Enzyme activity varies across human populations due to genetic variability, making some populations potentially more sensitive to effects from parent chemicals or metabolites.
- Physiologically-based kinetic (PB-K) models can help inform risk assessments for parent chemicals and metabolites, but current methods do not fully capture the effects of pathway-related population variability.
- This poster develops and describes a generalized modular workflow (Figure 1) we developed to incorporate pathway-related variability for a range of enzymes across human populations into PB-K models.
- We present the workflow, describe data sources, and provide a case study demonstration.

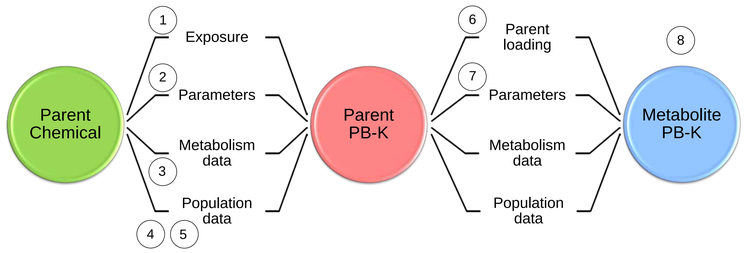
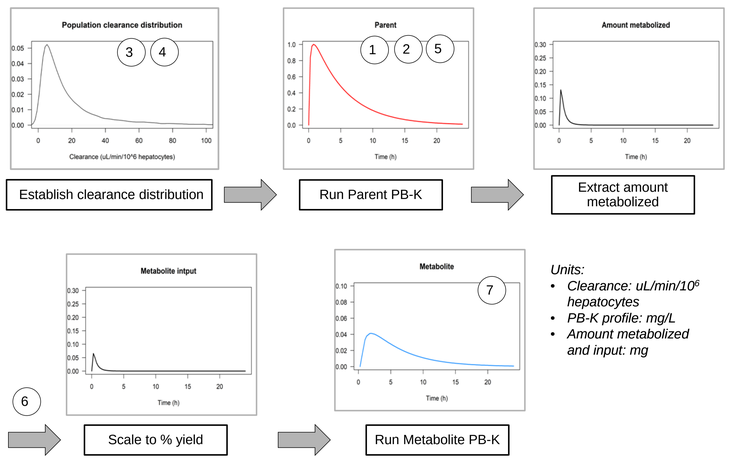


Figure 1: Generalized modular workflow. *Numbers correspond to descriptions in Workflow section below.*

Workflow

1. Parent chemical dose is established.
2. Generalized PB-K models from the U.S. Environmental Protection Agency's httk R package (Pearce et al. 2017) are parameterized using measured data and/or predicted data from quantitative structure-activity relationship (QSAR) models.
3. Information on predicted metabolites, which enzymes contribute to metabolism, and percent yield for each metabolite are obtained from SimulationsPlus ADMET Predictor® (www.simulations-plus.com).
4. Enzyme variability data are obtained from literature reports published by the European Food Safety Agency (EFSA; Darney et al. 2019, 2021).
5. Enzyme variability is integrated into the PB-K model by adjusting the clearance parameters. Monte Carlo sampling (n=10,000) is performed on a lognormal distribution of clearance with coefficient of variation (CV) defined by enzyme CVs from EFSA reports. These enzyme CVs are scaled by relative contribution to metabolism and combined to create a representative value.
6. The amount of parent chemical metabolized is used to create an intravenous dosing time series for each metabolite that is scaled by the metabolite's percent yield.
7. QSAR models predict metabolite PB-K parameters; metabolite PB-K simulations are conducted using the dosing time series as inputs.
8. Parent and metabolite results can be analyzed across the Monte Carlo runs to evaluate the effects of genetic pathway-based variability.



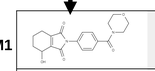
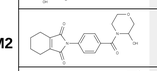
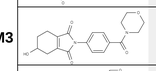
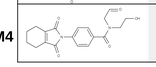
Units:
- *Clearance: uL/min/10^6 hepatocytes*
- *PB-K profile: mg/L*
- *Amount metabolized and input: mg*

Figure 2: Example workflow for a single run for a parent chemical and metabolite.

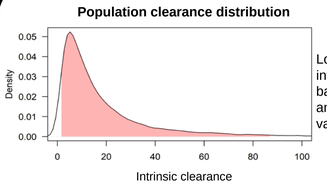
Case Study

- We used the workflow to evaluate metabolism of morpholine (CAS# 61356-09-0) and its metabolites.
- We simulated one round of metabolism from the CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 enzyme suite in the ADMET Predictor database.
- We assumed an exposure of 1 mg/kg oral dose; PB-K input parameters for morpholine and four first round metabolites (M1 through M4 in Figure 3) were predicted with OPERA v2.8 (Mansouri et al. 2018).
- Monte Carlo simulation (n=10,000) was used to estimate effects of population variability using EFSA data; analyses focused on the 95% range of results.
- PB-K models were used to predict plasma profiles and maximum concentration (Cmax) distributions.

Abbreviations:
- *Clint: intrinsic clearance (uL/min/10^6 hepatocytes)*
- *fu: fraction of chemical unbound to plasma protein*
- *LogP: octanol–water partition coefficient*
- *pKa: acid/base dissociation constant*
- *LogHL: Henry's Law*

	Morpholine	Clint	fu	LogP	pKa	LogHL	% Yield
	61356-09-0	11.42	0.21	1.36	5.52	-7.87	
M1		13.01	0.36	1.40	3.89	-10.15	11
M2		16.76	0.33	1.30	5.57	-7.95	27
M3		60.88	0.37	1.30	7.03	-7.95	42
M4		11.22	0.33	1.31	6.31	-10.11	20

98% CYP3A4
2% CYP2D6



Lognormal intrinsic clearance based on QSAR and population variability

Figure 3: Population clearance distribution for morpholine and physiochemical parameters for morpholine and its four metabolites.

Results

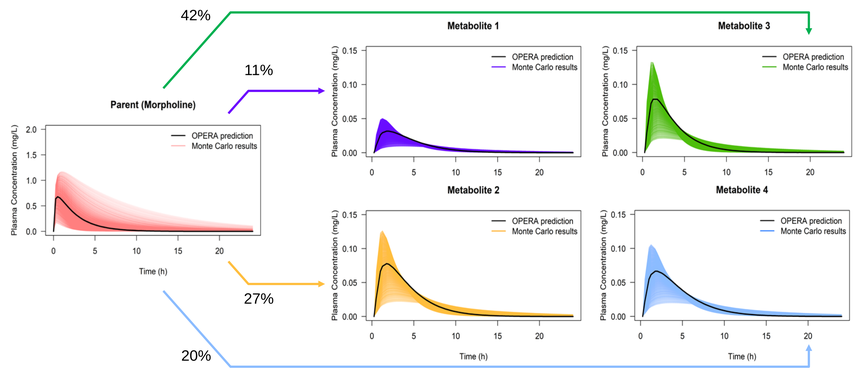


Figure 4: Plasma profiles for morpholine and four metabolites. *Black lines show PB-K simulations with OPERA-predicted parameters, colored lines show predicted effects of population variability.*

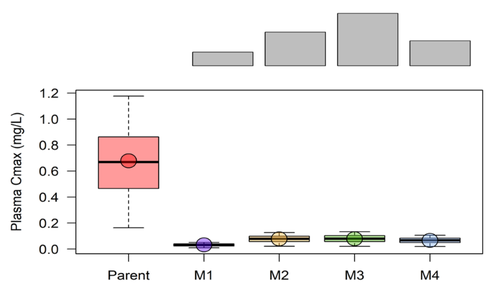


Figure 5: Summary of Monte Carlo results for morpholine and four metabolites using Cmax*. Boxplots show minimum, maximum, interquartile range, and median, while circles show results predicted using OPERA parameters. Gray bar plot on top shows percent yield for each metabolite.*

Results Summary:
- All four metabolites maintained tissue concentrations well below morpholine.
- Metabolite 1 had the lowest percent yield and lowest plasma concentrations.
- Metabolite 3 had the highest percent yield, but metabolites 2, 3, and 4 had similar predicted plasma profiles.
- Higher clearance of Metabolite 3 than other metabolites prevented Metabolite 3 from reaching higher concentrations, despite having the largest input dose.

Discussion

- The workflow integrates metabolite predictions, population variability, QSAR parameter prediction, and high-throughput PB-K models.
- The approach is modular and applicable to multiple rounds of metabolism.
- The case study demonstrates how both parent chemical and metabolite kinetics impact internal concentration.
  - Metabolite 3 (42% yield) resulted in Cmax only 1% greater than Metabolite 2 (27% yield) due to clearance differences.
    - Parameter predictions for morpholine and metabolites were within the applicability domain of OPERA models, but confidence index was low for OPERA Clint predictions for metabolites (< 0.5), and lowest for Metabolite 3 (0.34).
- The case study was limited to a small set of CYP enzymes to correspond with metabolite prediction capabilities.
- Variability within the case study was considered across all demographics.
- The workflow facilitates analysis of subpopulations by modifying enzyme CV inputs to represent a demographic subset.
- Quantifying the range of tissue concentrations resulting from metabolic pathway variability facilitates more health-protective risk assessment for susceptible population groups.
- This workflow will be implemented for a set of approximately 1 million parent chemicals and their metabolites. The predictions will be integrated into the Integrated Chemical Environment (ICE; https://ice.ntp.niehs.nih.gov).

Take Home Messages

- **The NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) has developed a workflow to integrate metabolite predictions and enzyme pathway variability into PB-K models.**
- **The workflow is modular, producing both parent chemical and metabolite tissue predictions.**
- **Tissue concentration variability can inform risk assessments to be protectives of susceptible population groups.**

References

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