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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Abstract Number 3670
Poster Number P155

Introduction

- Chemicals that enter the body can be metabolically activated through enzymatic transformation.
- Enzyme activity varies across human populations due to inter-individual 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 potential impact of pathway-related population variability.
- In this project, we developed a generalized modular workflow (Fig. 1) to incorporate pathway-related variability for a range of enzymes across human populations into PB-K models.
- This poster presents the workflow, describes data sources, and provides a case study demonstration.

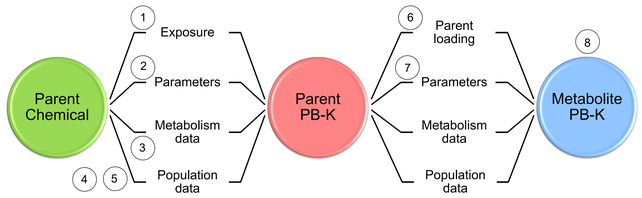

Figure 1: Generalized modular workflow. *Numbers correspond to descriptions in Workflow section below.*

Workflow

1. Parent chemical dose, amenable to scaling, 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 the Open (Quantitative) Structure-activity/property Relationship App (OPERA) QSAR models (Mansouri et al. 2018).
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 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 from OPERA also 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.

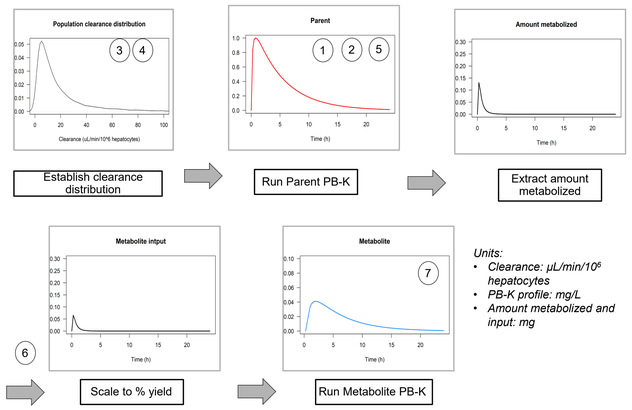

Figure 2: Example workflow for a single run for a parent chemical and metabolite.

Case Study Methods

- The workflow was used to evaluate the metabolism of 10 case study chemicals (Table 1).
- One round of metabolism was simulated from the CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 enzyme suites in the ADMET Predictor database.
- An exposure of 1 mg/kg oral dose was assumed. PB-K input parameters for each chemical and its metabolites were predicted with OPERA v2.8.
- Monte Carlo simulation (n=5,000) was used to estimate effects of population variability using EFSA data; analyses focused on the 95% interval of results.
- PB-K models were used to predict plasma profiles and maximum concentration (Cmax) distributions.

Table1 : Physicochemical properties for case study chemicals and their metabolites. These properties are predicted by OPERA v2.8 and used as input parameters for the httk v2.2.1 models (Pearce et al. 2017).

Parent DTXSID	Parent Name	Chemical	% Yield	Clint	fu	LogP	pKa	LogHL
DTXSID50769190	Rimsulfurondesulfon	Parent		4.23	0.04	1.96	6.66	-10.88
		M1	9	7.23	0.04	1.47	7.48	-10.82
		M2	41	6.26	0.05	1.89	8.73	-10.8
		M3	38	1.93	0.34	1.73	6.15	-10.89
		M4	12	4.07	0.03	1.63	8.4	-10.69
DTXSID40279339	2-(methylamino)fluoren-9-one	Parent		91.83	0.02	2.36	11.61	-8.94
		M1	76	12.39	0.01	2.41	NA	-9.35
		M2	3	12.39	0.01	2.41	NA	-9.35
		M3	13	12.39	0.01	2.41	NA	-9.35
		M4	7	12.39	0.01	2.41	NA	-9.35
DTXSID00532896	1-(2H-1,3-Benzodioxol-5-yl)-3-(4-methoxyphenyl)propane-1,3-dione	Parent		33.11	0.02	3.29	NA	-10.06
		M1	18	9.09	0.01	2.54	5.08	-10.6
		M2	82	49.05	0.03	2.47	8.4	-8.67
DTXSID80875354	Tioclomarol	Parent		22.74	0.02	4.06	5.79	-11.18
		M1	40	8	0.07	2.76	5.99	-11.18
		M2	28	58.01	0.03	3.42	NA	-8.29
		M3	27	18.91	0.06	4.01	6.1	-10.94
		M4	6	10.68	0.06	3.85	6.67	-10.94
DTXSID00210268	Morpholine	Parent		11.42	0.21	1.36	5.52	-7.87
		M1	11	13.01	0.36	1.4	3.89	-10.15
		M2	27	16.76	0.33	1.3	5.57	-7.95
		M3	42	60.88	0.37	1.3	7.03	-7.95
		M4	20	11.22	0.33	1.31	6.31	-10.11

Parent DTXSID	Parent Name	Chemical	% Yield	Clint	fu	LogP	pKa	LogHL
DTXSID80862527	1,3-Diphenylpropane	Parent		23.32	0.03	3.44	NA	-3.5
		M1	84	26.21	0.04	3.79	10.68	-5.63
		M2	16	18.52	0.04	3.06	11.83	-5.77
DTXSID10372043	5-(4-tert-Butylphenyl)-4-[4-(propan-2-yl)phenyl]-2,4-dihydro-3H-1,2,7	Parent		17.9	0.01	4.76	3.2	-7.75
		M1	11	20.23	0.01	5.09	33.55	-6.9
		M2	33	11.94	0.02	3.18	2.15	-9.05
		M3	43	21.53	0.02	2.85	3.81	-7.78
		M4	7	12.64	0.06	3.18	3.68	-7.69
DTXSID10274053	4-(7-Methyloctyl)phenol	Parent		26.39	0.03	5.62	11.38	-4.97
		M1	5	22.3	0.01	4.73	11.12	-5.41
		M2	30	24.21	0.03	3.76	NA	-5.43
		M3	21	30.87	0.01	4.07	10.73	-5.41
		M4	44	31.05	0.04	4.02	11	-5.41
DTXSID90470632	3-Chloro-1-(4-nitrophenyl)-5,6-dihydropyridin-2(1H)-one	Parent		3.89	0.28	2.93	0.92	-7.6
		M1	5	9.65	0.32	1.77	NA	-9.19
		M2	61	66.62	0.16	3.22	-0.69	-7.62
		M3	34	24.65	0.34	1.64	-0.9	-8.8
DTXSID00274042	2-Dodecylphenol	Parent		33.37	0.04	7.49	10.38	-5.22
		M1	4	19.45	0.04	7.03	10.27	-7.03
		M2	24	45.35	0.04	5.05	NA	-5.81
		M3	10	19.06	0.04	7.13	10.4	-7
		M4	21	30.52	0.03	5.93	NA	-5.89
		M5	20	16.04	0.09	6.62	9.3	-6.98

Abbreviations: Clint: intrinsic clearance (µL/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

Case Study Results: Plasma Profiles for Select Chemicals

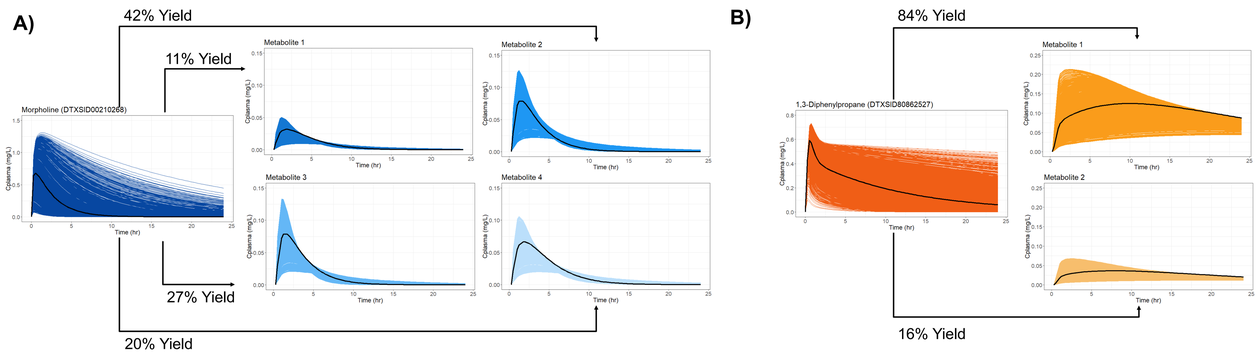

Figure 3: Plasma profiles for A) morpholine and B) 1,3-diphenylpropane. *Black lines show PB-K simulations with OPERA-predicted parameters and colored lines show results of Monte Carlo sampling to represent population variability based on metabolic enzyme activity. The % yield of each metabolite is given.*

Case Study Results: Plasma Cmax for Parent and Metabolite Chermicals

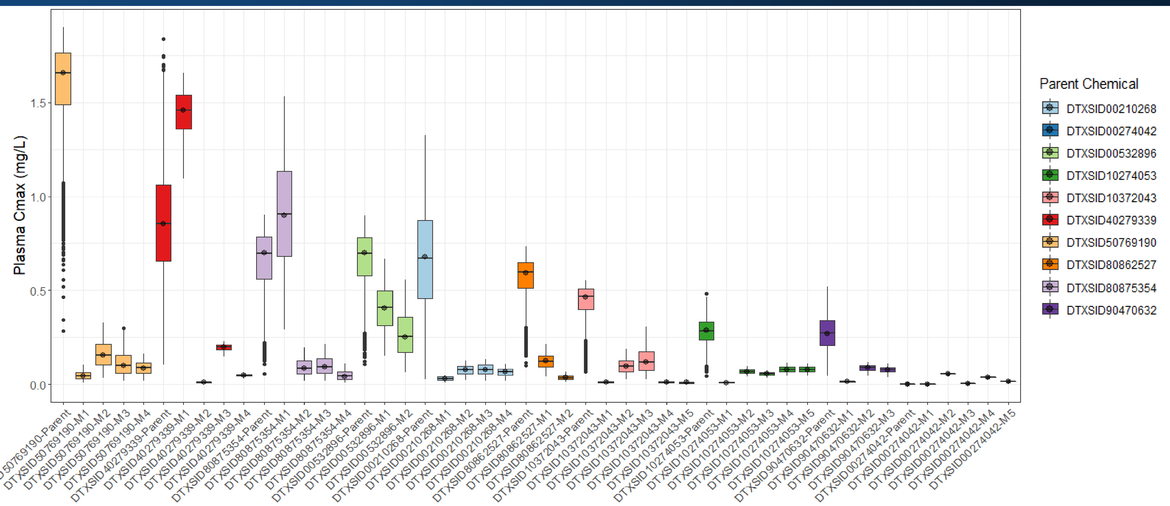

Figure 4: Summary of Monte Carlo sampling results for parent chemicals and their metabolites using Cmax*. Boxplots show minimum, maximum, interquartile range, and median, while transparent circles show results of PB-K simulations with OPERA-predicted parameters.*

Results Summary

- Each parent chemical had two to five metabolites with varying ranges in percent yield.
- These preliminary results demonstrate how both parent chemical and metabolite kinetics impact internal exposure.
  - The plasma concentration profile for 1,3-diphenylpropane (Fig 2B) shows that the higher percent yield metabolite, Metabolite 1 (84% yield), generally had a higher concentration over time than Metabolite 2 (16% yield). This results in a higher potential Cmax for Metabolite 1 over Metabolite 2 (Fig 3).
  - Some chemicals do not show such a dramatic difference. Morpholine's Metabolite 3 (42% yield) resulted in Cmax only 1% greater than Metabolite 2 (27% yield) (Fig 3), which may be due to differences in intrinsic clearance.
- Most case study chemicals have a higher Cmax for parents than metabolites.
  - Some chemicals, like 2-(methylamino)fluoren-9-one and tioclomarol, have a higher simulated Cmax for the metabolites. These parent compounds typically have high intrinsic clearance rates compared to metabolites.

Discussion and Conclusion

- The NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) has developed a workflow to integrate metabolite predictions into PB-K models. Monte Carlo simulations are used to estimate the ranges of internal exposure using uncertainties in metabolic clearance rages.
- The workflow is modular, producing both parent chemical and metabolite tissue predictions.
- Quantifying the range of tissue concentrations resulting from metabolic pathway variability facilitates more health-protective risk assessment for susceptible population groups.
- The case study was limited to a small set of CYP450 enzymes to correspond with metabolite prediction capabilities.
- FUTURE GOALS: This workflow will be implemented for a set of approximately 1 million parent chemicals and their metabolites available in ADMET Predictor. The predictions will be integrated into the Integrated Chemical Environment (ICE; https://ice.ntp.niehs.nih.gov).
- Models to predict toxicological endpoints, (e.g., endocrine disruption, acute toxicity), will be applied across parents and metabolites.

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Acknowledgments

This project was funded with federal funds from the National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN273201500010C.

We thank Catherine Sprankle, Inotiv, for editorial input.

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