2023-LB-A-3023-SOT

Constructing an Enzyme Ontogeny Database to Improve Characterization of Toxicokinetic Lifestage Differences

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Introduction

Certain enzymes facilitate the elimination of specific substrates from the body, including drugs and toxicants. Across life stages, the availability of specific enzymes varies, which greatly impacts the efficiency at which the body can eliminate parent compounds and their metabolites (Fig. 1). Enzyme activity at different life stages (enzyme ontogeny) may also lead to differential metabolic activation of parent compounds, causing life-stage-dependent differences in toxic metabolites.

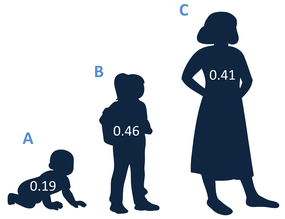


Figure 1. In vitro activity of CYP1A2 enzymes across lifestages (enzyme ontogeny) on imipramine measured in nmol/min/mg: (A) An infant aged 3-12 months, (B) a child aged 5-15 years, and (C) an adult aged 20-50 years. Activity data from Alcorn and McNamara 2002;[3] original data from Berthou et al. 1988.[4]

- Knowledge of the presence, capacity, and activation of specific enzymes at different life stages can help expand understanding and modeling capacity of the toxicokinetic (TK) differences between infants, children, and adults.[1,2]
- Differences in enzymatic expression during development, composition and size of body compartments, and the airflow or circulatory flows among them are changing rapidly, all of which affect metabolism and distribution.
- We present a workflow (Fig. 2) to create a publicly available database containing human data on enzymes for early life stages to support a greater web of knowledge regarding TK differences across life-stages.

Methods

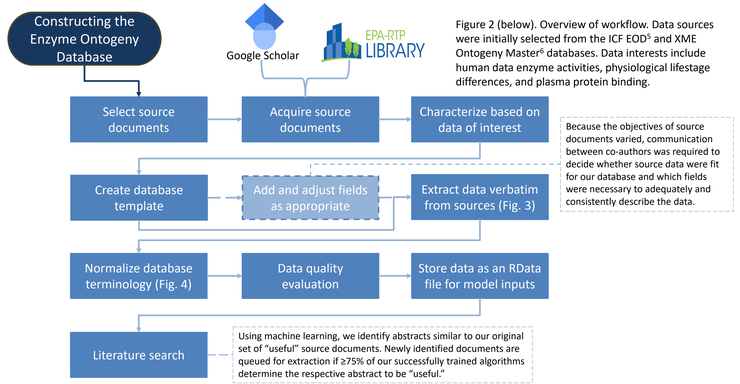


Figure 2 (below). Overview of workflow. Data sources were initially selected from the ICF EOD[5] and XME Ontogeny Master[6] databases. Data interests include human data enzyme activities, physiological lifestage differences, and plasma protein binding.

Methods Cont.

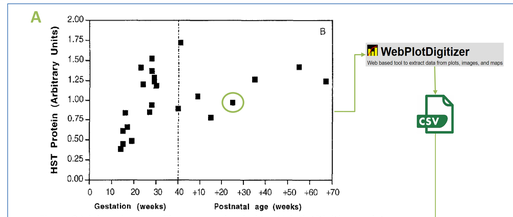


Figure 3A (above). Image files were uploaded to *WebPlotDigitizer:* a data extraction web tool. Data points were collected, and results were exported to a .csv file before importation to the EO database. Screenshot from Hume et al. 1996.[7]

B

Table 2. Summary of PON1 enzymatic activity in children and mothers.

Age (years)	No.	AREase (U/mL) Mean ± SD	AREase (U/mL) Range
0	336	33.1 ± 14.5	3.8–98.0

Figure 3B (above). Data tables were manually extracted. Screenshot from Huen et al. 2009.[8]

C

EGA (days)	Sex	CYP2B6 (pmol/mg)
89	2	7.54

Sex is coded and defined in a metadata document.

Figure 3C (left). Data available in the McCarver et al. 2017[6] database were imported directly. Screenshot of McCarver et al. 2017[6] database; original data from Croom et al. 2009.[9]

	Species	Enzyme	Enzyme normalized	Age	Age Units	Lifestage	Sex	Sample Size	Tissue Prep	Index of Activity	In vitro Parameter	In vitro Measurement	In vitro Measurement Spread	In vitro Measurement Range	In vitro Measurement Units	Probe Substrate
A	Human	HST	SULT2A1	25	weeks	Postnatal			Lung cytosol		HST Protein	0.96819788			Arbitrary units	
B	Human	PON1		0	years			336	Plasma	Substrate turnover	Enzymatic Activity	33.1	14.5	3.8-98.0	U/mL	Phenyl acetate
C	Human	CYP2B6		89	days	Prenatal	Male	1	Liver Microsomes	Western Blot	Protein concentration	7.54			pmol/mg protein	

Figure 3. Processes for data extraction into the Enzyme Ontogeny (EO) Database. For each process - A) Digitization and Extraction ; B) Manual Extraction; and C) Direct Import - metadata was collected from the respective source document and added into appropriate fields.

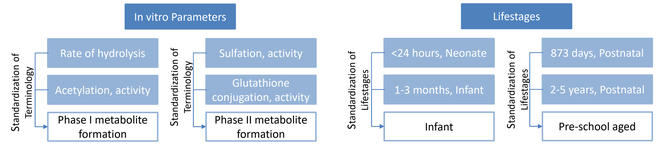
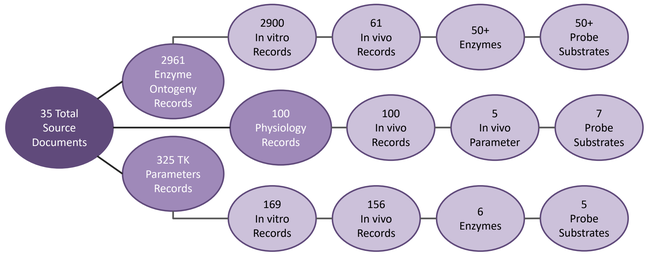


Figure 4 (left). Standardizing lifestage groups and terminology in the Enzyme Ontogeny (EO) Database. Data extraction was initially done verbatim (light blue filled) from source documents. We chose to standardize lifestages and other values (blue outlined) to improve the ability of our database to characterize toxicokinetic lifestage differences.

Results

Figure 5 (right). Metadata on the Enzyme Ontogeny Database. The EO Database contains three separate data tables: Enzyme Ontogeny, Toxicokinetic (TK) Parameters, and Physiology. The EO and TK Parameters data tables contain records in vitro and in vivo records. We include information on the enzyme, probe substrate, age, tissue preparations, and other important metrics when available in the source documents. At present, all records are from human studies.



Results Cont.

Age Bin	Exposure Age Groups[10]	McCarver et al. 2017 Lifestages[6]
Conception to 13 weeks gestation		Prenatal 1st Trimester
>13 to 26 weeks gestation		Prenatal 2nd Trimester
>26 to 40 weeks gestation		Prenatal 3rd Trimester
Birth to <1 month	Infants	Neonate
1 to <3 months	Infants	
3 to <6 months	Infants	
6 to <12 months	Infants	
1 to <2 years	Toddlers	
2 to <3 years	Pre-school aged	
3 to <6 years	Pre-school aged	
6 to <11 years	School aged	
11 to <16 years	Early adolescence	
16 to <21 years	Late adolescence	
21 years and older	Adult	

Table 1. Options for standardization of lifestage categories. Age bins can be grouped to best fit specific assessments. The original reported ages are retained in the database so they may be categorized for different risk assessment needs.

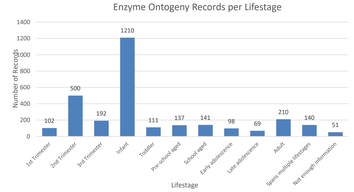


Figure 6. The number of enzyme ontogeny records for each lifestage in the EO Database.

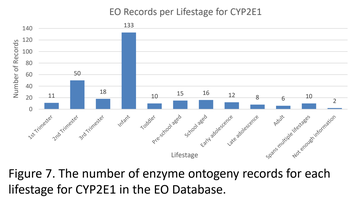


Figure 7. The number of enzyme ontogeny records for each lifestage for CYP2E1 in the EO Database.

Discussion

The Enzyme Ontogeny Database supports comparison of values across sources, addresses the need for more enzyme and TK data across early life stages, and allows for the identification of data gaps to highlight avenues for future research. Additionally, these data can be used as inputs to PBTK (physiologically based toxicokinetic) models designed to estimate internal doses of toxicants in infants and children, two sensitive life-stages. This knowledge is valuable for human health risk assessment.

Continuing with this work, we plan to source additional data via literature searches focused on the activity of xenobiotic metabolizing agents on the tissue level, plasma binding protein and membrane transport protein ontogeny, and life stage related changes in key organ system mass and function. We also hope to expand the focus of the Enzyme Ontogeny Database to include data from animal studies. We would like to emphasize that future animal data is not intended to be used in the same manner as human data for direct comparison.

References

1) Wetmore et al. Incorporating population variability and susceptible subpopulations into dosimetry for high-throughput toxicity testing. Toxicol Sci. 2014 Nov;142(1):210-24. doi: 10.1093/toxsci/kfu169. Epub 2014 Aug 21. PMID: 25145659. 2) Kapraun DF et al. Empirical models for anatomical and physiological changes in a human mother and fetus during pregnancy and gestation. PLoS One. 2019 May 2;14(5):e0215906. doi: 10.1371/journal.pone.0215906. PMID: 31048866; PMCID: PMC6497258. 3) Alcorn, J.; McNamara, P. J.; 2002. 'Ontogeny of hepatic and renal systemic clearance pathways in infants: part I.' Clinical pharmacokinetics. 41(12):959-98. 4) Berthou F, Ratanasavanh D, Alix D, et al. Caffeine and theophylline metabolism in newborn and adult human hepatocytes; comparison with adult rat hepatocytes. Biochem Pharmacol 1988; 37 (19): 3691-700 5) Vulimiri et al. (2013). Ontogeny Database on Enzymes (Phase I and II) of Importance to Chemical Disposition. U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-13/252. 6) McCarver et al. Data from: Developmental expression of drug metabolizing enzymes: Impact on disposition in neonates and young children, 2017. DOI: http://dx.doi.org/10.5061/dryad.71pb6. 7) Hume, R. et al. 1996. 'Differential expression and immunohistochemical localisation of the phenol and hydroxysteroid sulphotransferase enzyme families in the developing lung.' Histochemistry and cell biology. 105(2):147-52. 8) Huen, K. et al. 2009. 'Developmental changes in PON1 enzyme activity in young children and effects of PON1 polymorphisms.' Environmental health perspectives. 117(10):1632-8. 9) Croom EL et al. 2009. 'Human hepatic CYP2B6 developmental expression: The impact of age and genotype.' BIOCHEMICAL PHARMACOLOGY. 78(2):184. 10) US EPA. "Guidance on selecting age groups for monitoring and assessing childhood exposures to environmental contaminants." Washington, DC: Risk Assessment Forum, 2005.

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