A Systems Approach to Assessing Risk: The Role of Metabolism Research in Describing and Predicting Exposure

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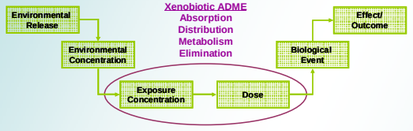
John F. Kenneke[1], Christopher S. Mazur[1], A. Wayne Garrison[1], Candice K. Cunningham[1], Rebecca D. Miller[2],
[1]National Exposure Research Laboratory, U.S. Environmental Protection Agency; [2]Student Services Authority

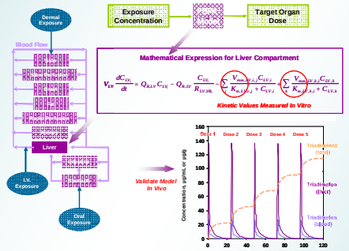
United States Environmental Protection Agency

LINKING EXPOSURE AND DOSE

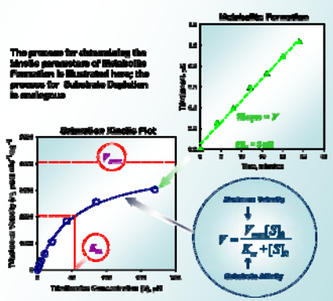
Of the approximately 80,000 chemicals used in U.S. commerce, relatively few have undergone extensive testing to provide a thorough evaluation of risk. In an effort to address this issue, the U. S. Environmental Protection Agency (EPA) is moving from a risk assessment paradigm that requires a "one size fits all" battery of hazard testing, to one that uses a focused, risk-based, hypothesis driven approach to identify the specific information most relevant to the assessment. In support of this philosophy, EPA has proposed using a systems approach for assessing the human health risks of the chemicals it must manage. This approach integrates exposure and toxicity information across the source-to-outcome continuum, with exposure science providing the linkage between environmental concentration and internal dose.



Physiologically-based pharmacokinetic (PBPK) models facilitate the estimation of internal dose metrics (i.e. internal exposure to relevant tissues), which are ultimately used to derive acceptable limits of exposure for regulatory purposes. These estimations are calculated as a function of exposure, which allows extrapolation between dose levels, exposure routes, and species.



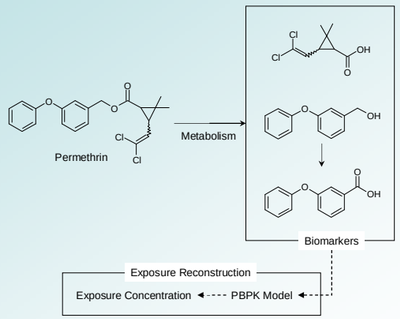
We focus our research on defining exposure, dose and the linkage between the two. We utilize in vitro and in vivo metabolism assays, specific enzyme inhibitors, purified enzymes, stereochemical approaches, and molecular docking to elucidate the kinetics, mechanisms, and pathways of xenobiotic metabolism. From these results we develop PBPK models and tools for prioritizing chemicals for testing and informing human health and ecological risk assessment.



METABOLISM-BASED FACTORS TO CONSIDER WHEN DESCRIBING AND PREDICTING EXPOSURE AND DOSE

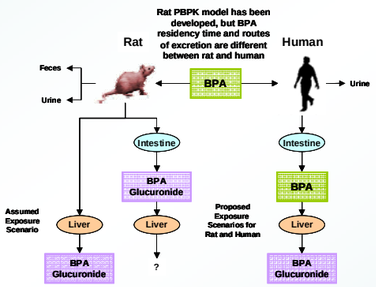
Importance of Metabolite Formation Kinetics for Exposure Reconstruction

Xenobiotic metabolites are often used as biomarkers of exposure for anthropogenic chemicals. Exposure reconstruction (i.e., reverse dosimetry) can utilize biomarker data along with PBPK models to predict potential exposure scenarios leading to the observed biomarker concentrations. In the case of pyrethroids, very little metabolite formation data is available to develop PBPK models. We have studied metabolite formation from cis and trans permethrin in hepatic microsomes and found that the cis isomer is metabolized orders of magnitude slower than the trans isomer. The binding affinity of the cis isomer, however, is greater than trans, which suggests the cis isomer could act as an inhibitor of trans permethrin metabolism. This is an important issue since most applications of permethrin utilize a 40:60 cis:trans mixture..



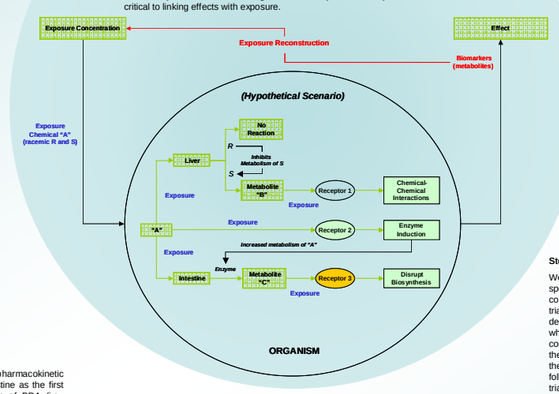
Species and Organ Differences for Bisphenol A

Current modeling efforts for bisphenol A (BPA) metabolism are based on allometric scaling of liver pharmacokinetic parameters for the parent compound from rodents to humans. Our assessment of the small intestine as the first site of BPA exposure via an oral exposure route demonstrates that significant transformation of BPA (i.e., glucuronidation) occurs in rodents but not humans. The end result is that in a rat the liver is primarily exposed to the BPA-conjugate while in human it is exposed to BPA. Thus, this interspecies difference may potentially alter systemic uptake and toxicological endpoints and increase uncertainty and inaccuracy in human health risk assessment. Our results suggest that the one-compartment liver model currently being used may not be sufficient to adequately describe BPA dosimetry..



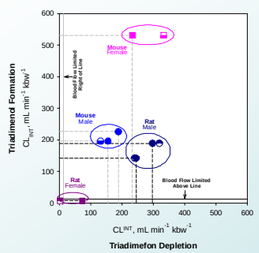
Overview

An important component of assessing risk is defining the exposure of a chemical stressor to a target organism. Often the chemical stressor is assumed to be a single compound even when it is comprised of different stereoisomers (e.g., pyrethroids and 1,2,4-triazole fungicides), which may possess different physical, chemical and toxicological properties. Additional uncertainties in exposure assessment arise when the chemical undergoes transformation inside the organism, especially when the transformation is organ, species, and/or gender specific. Thus, a "simple" exposure scenario of a single chemical may ultimately involve multiple stressors (i.e., stereoisomers and metabolites) resulting in multiple internal exposures. Understanding the relationship between exposure and dose is critical to linking effects with exposure.



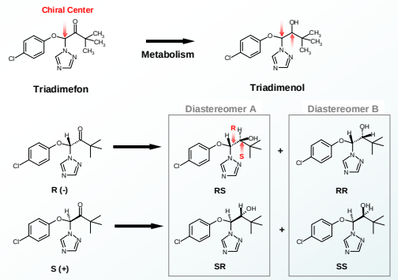
Gender and Species Differences in Metabolism Kinetics

Intrinsic clearance addresses the ability of an organism to metabolize a particular chemical. In "flow limited" systems, the ability of a tissue to metabolize and clear the chemical substantially exceeds the rate at which the blood perfuses that tissue; that is, the transport of the substrate and product to and from the tissue is the rate-limiting step in chemical clearance. Intrinsic clearances derived from kinetic parameters for both triadimefon depletion and triadimenol formation in rat and mouse indicate that triadimefon metabolism is blood-flow limited with the exception of female rat. This suggests that female rat would be sensitive to variation in V_{max}, as can occur with enzyme induction or inhibition. Additionally, since triadimefon is known to be a developmental toxicant as well as a teratogen, slower clearance in the female rat has important implications for pregnant females. Studies are now being conducted with male and female human hepatic microsomes.



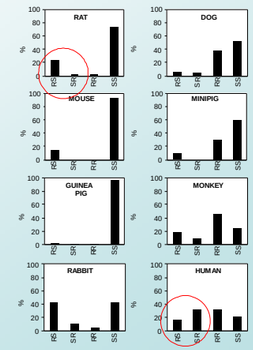
Importance of Stereochemistry

Triadimefon, a 1,2,4-triazole fungicide, has one chiral center and exists as two enantiomers. The metabolic transformation of triadimefon to triadimenol, which is also a commercial fungicide, involves the reduction of a prochiral carbonyl to an alcohol, resulting in formation of a second chiral center. Thus, triadimenol consists of two diastereomers: A (enantiomers RS and SR) and B (enantiomers RR and SS), for a total of four stereoisomers. These stereoisomers have different chemical, physical and toxicological properties, yet they are typically viewed in toxicological studies as only one chemical. It is noteworthy that analytical standards of triadimenol from different manufacturers may not contain the same ratios of diastereomers A and B as the commercial pesticide formulation (80:20). Thus, it is critical to use the commercial pesticide formulation standard in fate and effect experiments.



Stereoselective Metabolite Formation

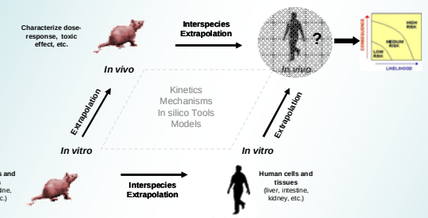
We have shown that the exposure of triadimefon to liver microsomes from fourteen vertebrate and invertebrate species resulted in the formation of triadimenol; however, the exposure-dose scenario is actually much more complex. Triadimefon metabolism occurs via the reduction of a prochiral carbonyl that yields a unique set of four triadimenol stereoisomers (RS, SR, RR, and SS) for each species. The stereoisomers have different toxicities and degrees of binding with endogenous receptors (e.g., enzymes involved in steroidogenesis and nuclear receptors), which could impact the mode-of-action of triadimefon. The implications of this for risk assessment are worth considering since triadimefon exposure to human liver microsomes produced a significantly higher percentage of the more toxic stereoisomers than rat (i.e., RS and SR, which are 10-fold more toxic in rat than RR and SS), and therefore, results extrapolated from rat to human may under predict risk. This concept is further illustrated in the following example: We showed that both black fly larvae and trout exhibit similar LC_{50}s for triadimenol, but triadimefon was significantly more toxic to black fly larvae than to trout. In vitro metabolism studies revealed that black fly larvae produced five-fold more of the toxic triadimenol stereoisomers (RS and SR) than did trout, which could explain the greater toxicity of triadimefon to black fly larvae.



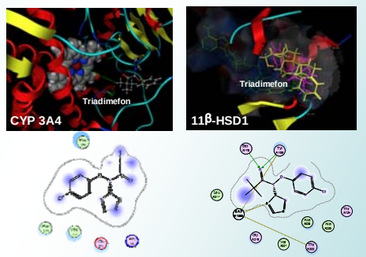
ADVANCING RISK ASSESSMENT

A key component in developing a more consistent and unified approach to risk assessment is having a comprehensive understanding of the key events involved in the development of cancer and non-cancer outcomes. Toward that end, the EPA's Office of Research & Development is working on elucidating key events in toxicity pathways for select chemical classes, from source to response, for the purpose of improving both individual chemical and cumulative risk assessments. As part of this effort, we are helping provide both screening level and sophisticated computational approaches and tools for estimating environmental exposures and for prioritizing chemicals for toxicity testing and risk assessment.

Quantitative risk assessment is needed to prioritize chemical hazards and to determine safety margins. The frequent use of rodent hepatic in vitro assays in toxicological investigations challenges the extrapolation of results to in vivo systems and between species (e.g., rat to human). A parallelogram approach has been proposed as an alternate method to allometric scaling for estimating human values for risk assessment. The parallelogram approach can be used to extrapolate results from in vitro to in vivo and between species in order to estimate toxicity that cannot be assessed directly. This approach also provides a framework for utilizing molecular and cellular approaches to extrapolation for risk assessment and provides a process for systematic, comparative biology. An important component of this parallelogram approach is understanding the kinetics and mechanisms of xenobiotic metabolism. Specifically, we work with other EPA collaborators to develop, evaluate, and apply innovative screening-level PBPK models to link exposures with tissue dosimetry, and integrate PBPK models with comparative in vitro and in vivo data along with computational chemistry techniques to provide risk assessors with an enhanced understanding of how human exposures result in tissue dosimetry.



The ability to quickly prioritize chemicals for hazard testing based on potential human exposure and health risks has been a goal of many regulatory agencies, but has been fraught with failure due to the sheer number of chemicals in use coupled with a lack of chemical specific data. Consequently, in silico techniques such as molecular docking now represent an important component for developing high-throughput screening tools for prioritizing chemicals for testing. We utilize in silico approaches to formulate hypotheses and to target laboratory studies toward generating critical data that addresses the greatest uncertainties in developing tools for prioritizing chemicals for hazard testing and managing chemical risk.



Following a systems based approach to risk assessment, prioritized lists of chemicals from exposure based models serve as input data for effects based models to focus toxicity testing on chemicals having the greatest risk. For example, a chemical that is predicted to be highly toxic but is expected to be cleared or metabolized rapidly would provide only a low tissue dose and therefore may not pose a serious risk. The integration of exposure and toxicity based chemical prioritization models establishes a holistic approach to assessing risk.