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Division of Translational Toxicology

ATSDR AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY

Using In Vitro Data and PBPK Models to Predict Inhalation Toxicity

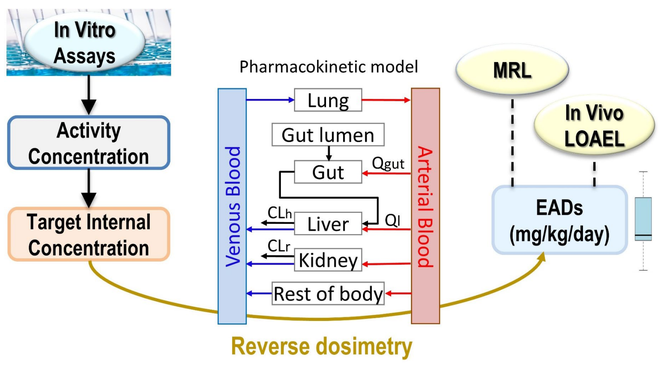
X. Chang[1], E. Reinke[1], A. Daniel[1], D.G. Allen[1], N. Kleinstreuer[2], M. Mumtaz[3]

[1]Inotiv, RTP, NC, USA; [2]NIH/NIEHS/DTT/NICEATM, RTP, NC, USA;
[3]Agency for Toxic Substances and Disease Registry, CDC, Atlanta, GA, USA

Introduction

- As part of its chemical risk assessment process, the Agency for Toxic Substances and Disease Registry (ATSDR) establishes a minimal risk level (MRL) for chemicals of concern.
- The MRL is an estimate of daily human exposure to a hazardous substance that is unlikely to cause adverse noncancer health effects (ATSDR 2022). It is derived for each route of exposure from the no- or lowest-observed-adverse-effect level (NOAEL or LOAEL) for the most sensitive adverse effect after applying uncertainty factors.
- Time and resource requirements prohibit conducting in vivo multi-target organ toxicity assessments on the large number of chemicals of potential concern.
- New approach methodologies (NAMs), such as in vitro assays, have the potential to provide estimates of safe exposure levels for a chemical when combined with physiologically-based pharmacokinetic (PBPK) modeling and in vitro to in vivo extrapolation (IVIVE) approach.
- Previously we evaluated and presented an application of combining in vitro data and IVIVE to inform oral hazard assessment and obtained promising results (Mumtaz et al. 2022).
- The complex nature of inhalation exposure poses challenges to using NAM data to predict in vivo LOAELs and MRLs. We conducted a proof-of-concept study to evaluate application of in vitro data and IVIVE to inform inhalation hazard assessment.

IVIVE: Predicting In Vivo EAD Using In Vitro Activity Concentration



- IVIVE uses pharmacokinetic models to relate in vitro activity concentrations of substances to corresponding equivalent in vivo doses.
- To evaluate IVIVE results, the estimated equivalent administered doses (EADs) can be compared to in vivo LOAELs or MRLs.

Chemical Set, In Vitro and In Vivo Data

Chemicals

- Twenty volatile organic compounds with relatively abundant pharmacokinetic data and published MRLs covering multiple target organs via inhalation exposure were selected for evaluation.

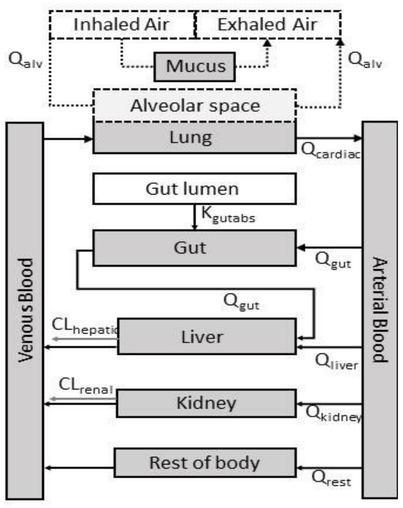
In vitro assay data

- The activity concentrations at cutoff (ACC, μM) derived from in vitro assays were obtained from public resources:
  - Curated high-throughput screening assay data from the Integrated Chemical Environment (ICE, https://ice.ntp.niehs.nih.gov/).
  - Published articles from a limited search of PubMed (https://pubmed.ncbi.nlm.nih.gov/).
- In vitro assays covered diverse endpoints (e.g., genotoxicity, cytochrome p450 activation, transcriptome analysis).
- When multiple in vitro values were reported for one chemical, the lowest ACC was used for subsequent analysis to provide the most conservative prediction.

In vivo data

- MRLs and LOAELs were obtained from the ATSDR Toxic Substance Portal (https://wwwn.cdc.gov/TSP/index.aspx). Most data were from rat acute inhalation studies. In instances of data gaps, human data were used.
- The adverse effects considered included neurotoxicity, respiratory toxicity, hepatotoxicity, and immunotoxicity.

Inhalation Route-specific PBPK Model Used in IVIVE



CL, clearance; K_{gutabs}, gut absorption rate constant; Q, blood flow rate

- The Physiologically Based Pharmacokinetic (PBPK) model used was from the U.S. Environmental Protection Agency's httk (high-throughput toxicokinetics) R package (v2.2.2, Pearce et al. 2017, Linakis et al. 2020). The model is accessible through the ICE tools.
- The gas exposure units used were ppm (one part per million by volume) or μM (micromolar).
- The input physicochemical and pharmacokinetic parameters were predictions obtained from the Open (Quantitative) Structure-activity/property Relationship App (OPERA; v2.8, Mansouri et al. 2018) or provided by httk.
- The plasma and lung maximum chemical concentrations (Cmax) following daily 6-hour gas exposure for 2 weeks were estimated using the rat PBPK model and used for reverse dosimetry to estimate EADs.

Acknowledgments

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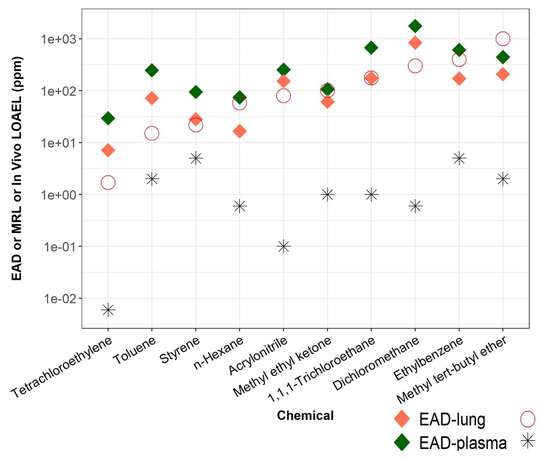
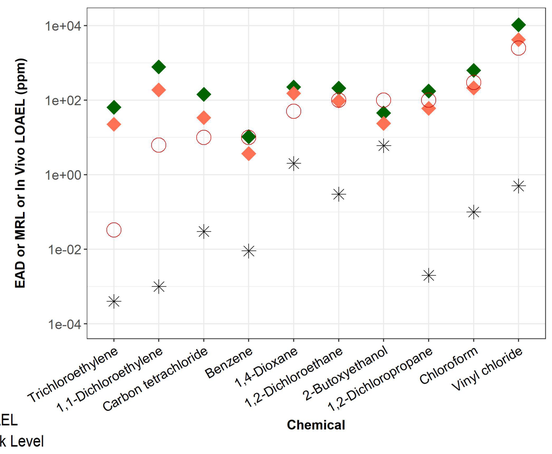
EADs Compared to In Vivo LOAELs and MRLs

A. Neurotoxicity



B. Other Toxicity Endpoints



Figures present EAD estimates compared to in vivo data. EAD-plasma: EAD estimate that would result in plasma Cmax equal to in vitro ACC values. EAD-lung: EAD estimate that would result in lung Cmax equal to in vitro ACC values. Figure A shows chemicals for which the most sensitive endpoint is neurotoxicity. Figure B shows chemicals that induce other endpoints such as respiratory toxicity, hepatotoxicity, and immunotoxicity. For each figure, the chemicals are arranged from left to right by increasing LOAEL values.

Chemical	Lowest ACC (μM)	In Vitro Assay Target	Ratio: EAD-plasma vs. LOAEL	Ratio: EAD-lung vs. LOAEL	In Vivo Toxicity Endpoint	In Vitro Mechanistic Relevance
Tetrachloroethylene	29	Regulation of gene expression	17.4	4.2	Neuro.	High
Toluene	114	Cell viability	16.4	4.8	Neuro.	Low
Styrene	50	Genotoxicity	4.3	1.3	Neuro.	Low
n-Hexane	10	AChE activity	1.3	0.3	Neuro.	High
Acrylonitrile	117	Cell transformation	3.2	1.9	Neuro.	Low
Methyl ethyl ketone	40	LDH leakage	1.1	0.6	Neuro.	Low
1,1,1-Trichloroethane	152	Cell transformation	3.9	1.0	Neuro.	Low
Dichloromethane	535	Oxidative stress	5.9	2.8	Neuro.	Low
Ethylbenzene	500	Oxidative stress	1.5	0.4	Neuro.	Low
Methyl tert-butyl ether	340	Brain endothelial cell tube formation	0.4	0.2	Neuro.	High
Trichloroethylene	28	Cell proliferation	1959	686	Immuno.	Low
1,1-Dichloroethylene	48	Stress protein expression	125.0	30.2	Resp.	High
Carbon tetrachloride	34	LDH leakage	14.4	3.4	Hepatic	Low
Benzene	5	Regulation of miRNA expression	1.1	0.4	Immuno.	High
1,4-Dioxane	300	Genotoxicity	4.5	3.1	Ocular	Low
1,2-Dichloroethane	100	Apoptosis	2.1	1.0	Resp.	Low
2-Butoxyethanol	58	CYP1A1 expression	0.5	0.2	Hemato.	High
1,2-Dichloropropane	50	Regulation of gene expression	1.8	0.6	Resp.	High
Chloroform	170	Inhibition of uptake of bile acid	2.1	0.7	Hepatic	High
Vinyl chloride	568	Cell transformation	4.2	1.7	Develop.	Low

Table contains in vitro ACC values used for IVIVE analysis and ratios between EADs and LOAELs for each chemical. Cells are highlighted when fold difference between EADs and LOAELs is less than 5-fold. AChE, acetylcholinesterase; CYP, cytochrome P450; Develop., developmental toxicity; Hemato., hematotoxicity; Immuno., immunotoxicity; LDH, lactate dehydrogenase; miRNA, microRNA; Neuro., neurotoxicity; Resp., respiratory toxicity.

Within 5-fold difference, EAD > LOAEL
Within 5-fold difference, EAD < LOAEL

Results and Discussion

- This work illustrates the utility of NAMs to inform human risk assessment to inhaled hazardous substances.
- EADs generated using lung Cmax as the target internal concentration (EAD-lung) were 1.5 to 5-fold lower than those generated using plasma Cmax as the target internal concentration (EAD-plasma). This suggests that EAD-lung provides a more conservative estimate of in vivo LOAELs than EAD-plasma.
- In vivo LOAELs of the most sensitive adverse effect by gas exposure were used to evaluate IVIVE outcomes. We observed close agreements between both types of EADs and in vivo LOAELs for most chemicals. EAD-plasma and EAD-lung values were within 5-fold of in vivo LOAELs for 15/20 and 18/20 chemicals, respectively.
- Most EADs were at least 10-fold higher than MRLs, suggesting that, similar to the uncertainty factors applied to derive MRLs from LOAELs, a modifying factor would likely be needed to approximate MRLs based on in vitro data.
- This project demonstrates a promising approach for predicting inhalation toxicity using non-animal approaches.
- **Future work will include:**
  - Further evaluating the impact of mechanistic relevance of an in vitro assay selected for IVIVE to the sensitive adverse effects used for deriving MRLs.
  - Evaluating the impact of other factors on IVIVE outcomes:
    - Comparability of in vitro and in vivo exposure regimens
    - Complexity of in vitro assay systems (e.g., monolayer versus 3D culture)
    - Physicochemical properties such as volatility
    - Standardization of test systems to ensure reliable data for regulatory purposes
  - Expanding literature search to obtain more information on in vitro activity concentrations.

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