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Combining NAM Data and IVIVE for Evaluating Potential Inhalation Toxicity

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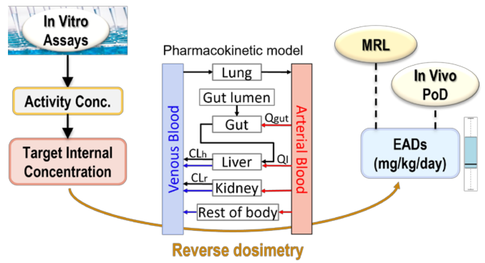
Introduction

- 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).
- The MRL is derived for each route of exposure from the in vivo no- or lowest-observed-adverse-effect level (NOAEL or LOAEL) for the most sensitive adverse effect for that exposure route. Uncertainty factors accounting for species extrapolation, human variability, and data gaps are applied when deriving MRLs.
- Time and resource requirements prohibit conducting in vivo multi-target organ toxicity assessments for the large number of chemicals of potential concern.
- New approach methodologies (NAMs), such as in vitro assays, have the potential provide estimates of safe exposure levels for a chemical when combined with an in vitro to in vivo extrapolation (IVIVE) approach.
- Due to the complex nature of inhalation exposure, the applicability of using NAM data and IVIVE for predicting in vivo LOAELs requires evaluation.

IVIVE: Predicting In Vivo EADs Using In Vitro Activity Concentrations



- IVIVE uses pharmacokinetic models to relate concentrations of substances that induce in vitro responses to a corresponding equivalent in vivo dose.
- It estimates an equivalent administered dose (EAD) that would result in plasma or target tissue (e.g., lung) concentrations equivalent to the in vitro activity concentrations.

In Vitro and In Vivo Data for IVIVE

Chemicals

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

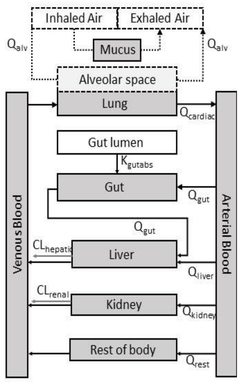
In vitro assay data

- The lowest activity concentrations derived from in vitro assays were obtained from various public resources:
  - Curated high-throughput screening assays data from the Integrated Chemical Environment (ICE, https://ice.ntp.niehs.nih.gov/).
  - Published articles from a limited PubMed search (https://pubmed.ncbi.nlm.nih.gov/)
- In vitro assays covered diverse endpoints (e.g., genotoxicity, cytochrome p450 activation, transcriptome analysis).
- The lowest activity concentration at cut-off (ACC) was used for IVIVE when multiple in vitro values were reported, since they are more comparable to in vivo LOAELs.

In vivo assay data

- MRLs and LOAELs were obtained from the ATSDR Toxic Substance Portal (https://wwwn.cdc.gov/TSP/index.aspx).
- Most data presented here are from acute inhalation studies in rats. Due to data gaps, in some instances, human data were used to replace rat data.
- The sensitive adverse effects included neurotoxicity, respiratory toxicity, hepatotoxicity, immunotoxicity, etc.

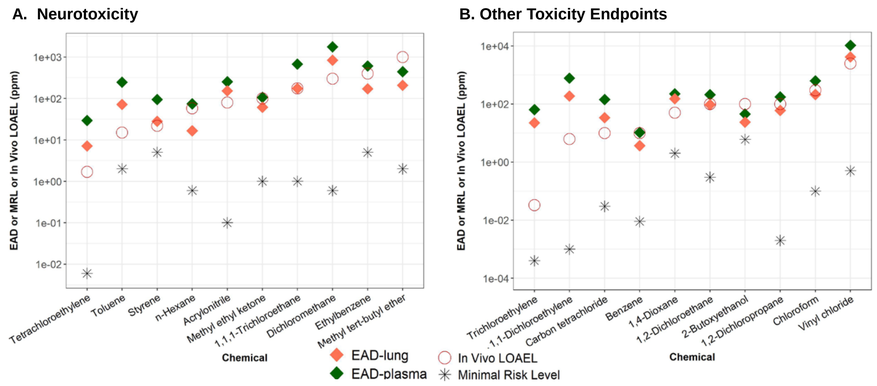
Inhalation route-specific PBPK Model Used in IVIVE



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

- The physiologically based pharmacokinetic (PBPK) model used is provided by 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 also accessible through the ICE PBPK and IVIVE tools.
- The gas exposure units are ppm (one part per million by volume) or μM (micromolar).
- The input physiochemical and pharmacokinetic parameters are obtained from OPERA model predictions (v2.8, Mansouri et al. 2018) or provided by the httk package.
- The plasma and lung maximum chemical concentrations (Cmax) following daily 6-hour gas exposures for 2 weeks are estimated using the rat PBPK model and used for reverse dosimetry.

EADs Compared to MRLs or In Vivo LOAEL of Different Toxicity Endpoints



Figures present EAD estimates compared to in vivo LOAELs and MRLs. EAD-plasma, an EAD estimate that would result in plasma Cmax equal to in vitro ACC (μM). EAD-lung, an EAD estimate that would result in lung Cmax equal to in vitro ACC (μM). The chemicals are grouped by whether their most sensitive in vivo adverse effect is neurotoxicity (A) or not (B). For each figure, the chemicals are ordered from left to right based on their LOAEL values, from low to high.

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

Tables contain 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.

EAD > LOAEL
EAD < LOAEL

Abbreviations:

AChE, acetylcholinesterase; CYP, cytochrome P450; Hemato., hematotoxicity; Immuno., immunotoxicity; LDH, lactate dehydrogenase; miRNA, microRNA; Neuro., neurotoxicity; Resp., respiratory toxicity.

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Results and Discussion

- Previous application of ICE for IVIVE was demonstrated for oral exposures. This work extends the methodology to inhalation exposures to illustrate the utility of NAMs to inform risk for human exposure to inhaled hazardous substances.
- EADs generated using lung Cmax as the target internal concentration (i.e., EAD-lung) were 1.5 to 5-fold lower than those generated using plasma Cmax as the target internal concentration (i.e., 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. There are close agreements between both types of EADs and in vivo LOAELs for the majority of chemicals. EAD-plasma and EAD-lung are within 5-fold of in vivo LOAELs for 15/20 and 18/20 chemicals, respectively.
- Most EADs are at least 10-fold higher than MRLs, suggesting that a "modifying factor" may need to be established to approximate MRLs based on in vitro assay data.
- Physicochemical properties such as volatility impact the quality of the in vitro data, and test systems should be tailored to ensure reliable data for regulatory purposes.

Future Directions

- Evaluate the impact of the mechanistic relevance of an in vitro assay selected for IVIVE to the sensitive adverse effects used for deriving MRLs
- Evaluate the impact of other important factors on IVIVE outcomes:
  - Comparability between in vitro and in vivo exposure regimens (e.g., exposure frequency and duration)
  - Complexity of in vitro assay system (e.g., monolayer versus 3D culture, single cell type versus co-culture)
- Expand the literature search to obtain more information on in vitro activity concentrations

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