National Institute of Environmental Health Sciences
Division of the National Toxicology Program

Application of In Vitro to In Vivo Extrapolation (IVIVE) to Inform In Vivo Point of Departure

M Mumtaz[1], X Chang[2], D Allen[2], N Kleinstreuer[3]

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

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Poster P146

Background

- As part of their chemical risk assessment process, the Agency for Toxic Substances and Disease Registry 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 likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure (ATSDR 2022).
- The MRL for a chemical is based on the in vivo point of departure (PoD) of the most sensitive adverse effect by a specific route of exposure. Applicable uncertainty factors are then applied to the in vivo PoD to derive the MRL.
- Time and resource requirements prohibit conducting in vivo multi-target organ toxicity assessments for the large number of chemicals of potential concern.
- In vitro high-throughput screening (HTS) assays and other new approach methodology (NAMs) data could be used to address this problem by using reverse dosimetry to contextualize HTS concentration responses to an in vivo system, enabling derivation of a safety risk level without animal testing.
- In this study, in vitro activity concentrations from curated HTS assays for 35 chemicals were used as inputs for in vitro to in vivo extrapolation (IVIVE) analyses. The IVIVE analyses estimate the daily equivalent administered doses (EADs) that would result in plasma concentrations equivalent to these in vitro activity concentrations. We then compared the EADs to the in vivo PoDs and MRLs for neurotoxicity, hepatotoxicity, or developmental toxicity.
- IVIVE analyses were conducted using the IVIVE tool in the National Toxicology Program's (NTP's) Integrated Chemical Environment (ICE, https://ice.ntp.niehs.nih.gov/).

In Vivo PoD and MRL

In vivo point of departure (PoD)

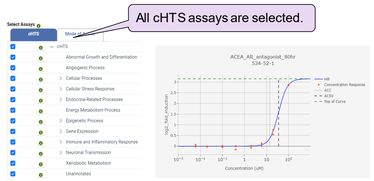
- Lowest observed adverse effect levels (LOAELs), no observed adverse effect level (NOAELs), and benchmark doses (BMDs) were obtained from the published literature.

Uncertainty factors (UFs) for calculating MRL

- Traditionally, UFs account for uncertainties and variability for the following extrapolations:
  - Interspecies (animal to human)
  - Intra-human (normal to sensitive populations)
  - LOAEL to NOAEL data

In Vitro High-throughput Screening (HTS) Assay Data

- ICE provides user-friendly access to high-confidence data curated from published literature, databases, and validation studies. We obtained in vitro activity concentrations from curated HTS (cHTS) assays from ICE. To maximize the number of chemicals for IVIVE analyses, we used all available cHTS assays in this study.
- For chemicals that were active in at least one cHTS assay, the in vitro activity concentrations at cutoff (ACCs) derived from concentration–response curves (Filer et al. 2017) were used as the input concentration for IVIVE.
- For chemicals that were inactive in all cHTS assays, the input concentration for IVIVE was set at 100 µM, which is the typical maximum testing concentration for most cHTS assays (Tice et al. 2013). This approach is expected to provide an EAD value better approximating the NOAEL.



Screenshot of cHTS assay selection and an example concentration–response curve for cHTS data in ICE. ACC, activity concentration at cutoff; AC50, half-maximal activity concentration.

IVIVE Overview and ICE IVIVE Tool User Interface

IVIVE overview. IVIVE was performed to estimate the daily EAD that would result in plasma or tissue concentrations equivalent to the in vitro activity concentrations of selected assays.

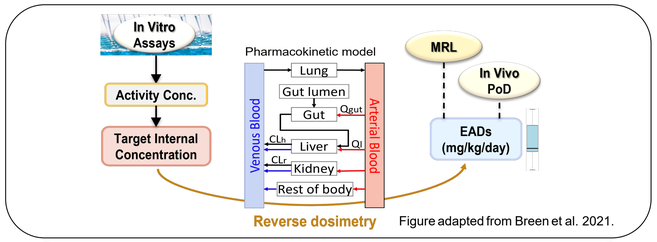


Figure adapted from Breen et al. 2021.

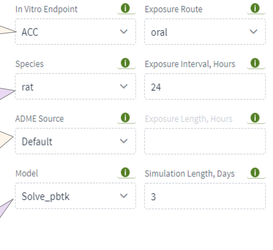
ICE IVIVE Tool User Input

Type of activity concentration selected.

Species: rat or human. Rat model is chosen as majority of the in vivo POD values are obtained from rat studies.

Default option uses experimental values for absorption, distribution, metabolism, and excretion (ADME) where available and provides in silico predictions where they are not.

Solve_pbtk model from the httk R package (Pearce et al. 2017) is selected in this study.

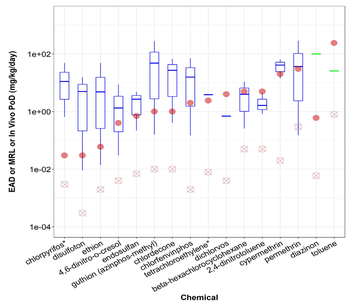


Summary and Future Directions

- For each toxicity endpoint, ICE provided at least one cHTS assay activity concentration for more than half of our chemicals of interest.
- Depending on the endpoints evaluated, our results showed that for most chemicals, the ranges of EAD estimates are within 10-fold of in vivo PoDs.
- The minimum EAD is lower than in vivo PoDs for most of the chemicals, suggesting that IVIVE using the most sensitive in vitro assay provides a conservative estimate for risk assessment.
- The ranges of EAD estimates are higher than most MRLs, suggesting that an "uncertainty factor" may need to be established for in vitro derivation of MRLs.
- This study provides a good example of IVIVE application using ICE and demonstrates the usefulness and limitations of using cHTS data and the ICE IVIVE tool.
- Specific in vitro assays providing the most accurate predictions for in vivo PoDs need further evaluation.

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

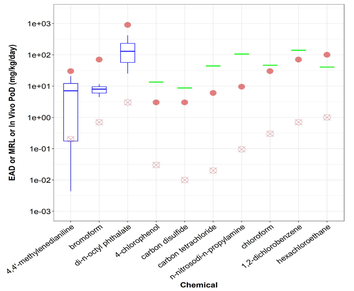
Neurotoxicity



Chemical	MRL	In Vivo PoD	EAD Range
chlorpyrifos*	0.003	0.03	0.7 - 48.7
disulfoton	0.0003	0.03	0.009 - 15.7
ethion	0.002	0.06	0.014 - 48.8
4,6-dinitro-o-cresol	0.004	0.4	0.030 - 8.8
endosulfan	0.007	0.7	0.2 - 4.8
guthion (azinphos-methyl)	0.01	1	0.2 - 277.5
chlordecone	0.01	1	0.4 - 67.2
chlorfenvinphos	0.002	2	0.1 - 70.3
tetrachloroethylene*	0.008	2.4	3.9
dichlorvos	0.004	4	0.7
beta-hexachlorocyclohexane	0.05	5	0.3 - 10.8
2,4-dinitrotoluene	0.05	5	0.8 - 4.5
cypermethrin	0.02	20	14.3 - 64.1
permethrin	0.3	30	0.2 - 290.2
diazinon	0.006	0.6	100
toluene	0.8	240	25.6

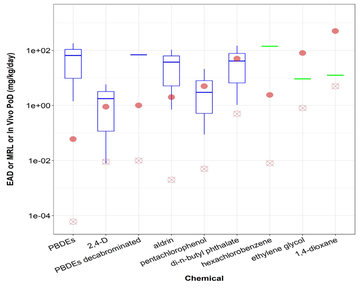
EAD_ACC
EAD_MaxTestConc.
In Vivo PoD
Minimal Risk Level

Hepatotoxicity



Chemical	MRL	In Vivo PoD	EAD Range
4,4'-methylenedianiline	0.2	30	0.0044 - 20.9
bromoform	0.7	70	4.4 - 11.5
di-n-octyl phthalate	3	900	25.0 - 416.3
4-chlorophenol	0.03	3	13.4
carbon disulfide	0.01	3	8.7
carbon tetrachloride	0.02	6	44.0
n-nitrosodi-n-propylamine	0.095	9.5	106.0
chloroform	0.3	30	46.5
1,2-dichlorobenzene	0.7	70	140.1
hexachloroethane	1	100	40.5

Developmental Toxicity



Chemical	MRL	In Vivo PoD	EAD Range
PBDEs	0.00006	0.06	1.4 - 180.2
2,4-D	0.009	0.9	0.008 - 5.8
PBDEs decabrominated	0.01	1	68.9
aldrin	0.002	2	0.7 - 106.0
pentachlorophenol	0.005	5	0.1 - 21.1
di-n-butyl phthalate	0.5	50	1.1 - 148.2
hexachlorobenzene	0.008	2.4	141.1
ethylene glycol	0.8	80	9.2
1,4-dioxane	5	500	12.6

In the figures above, boxplots represent the range of EADs estimated from in vitro ACC in cHTS assays. Red solid circles represent in vivo PoDs from in vivo toxicity studies. Red open circles represents MRLs derived from these in vivo PoDs after applying uncertainty factors. Green bars show the EADs predicted from 100 µM, which is a typical maximum testing concentration for cHTS assays. 2,4-D, 2,4-dichlorophenoxyacetic acid; PBDE, polybrominated diphenyl ethers.

Acknowledgements

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More Information On ICE

Learn more about NICEATM and ICE tools at SOT:

- Rooney et al. Abstract 4000 / Poster P698
- Abedini et al. Abstract 4004 / Poster P702
- Karmaus et al. Abstract 4005 / Poster P703

Visit ICE: https://ice.ntp.niehs.nih.gov/

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