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

Fit for Purpose Evaluation of In Vitro Assays for IVIVE

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Abstract ID #15

Introduction

- Compared to animal studies, in vitro assays are quicker, more cost-effective, and provide mechanistic information. However, it can often be difficult to determine how in vitro assay results align with in vivo effects.
- In vitro to in vivo extrapolation (IVIVE; see figure at right) translates in vitro activity concentrations to equivalent in vivo exposure. It estimates the daily equivalent administered doses (EADs) that would result in plasma concentrations equivalent to the selected in vitro activity concentrations.
- Several factors impact IVIVE outcomes, including in vitro assay selection, in vitro concentration metrics, and pharmacokinetic (PK) modeling assumptions (see Hines et al. Poster ID #16 for more details).
- User-friendly tools now exist that facilitate access to data from new approach methodologies (NAMs) and make IVIVE analysis more widely accessible.
  - One of these tools is the Integrated Chemical Environment (ICE: https://ice.ntp.niehs.nih.gov/) (Bell et al. 2020; Abedini et al. 2021).

Objectives

- To evaluate the impacts of in vitro assay selection on IVIVE outcome using in vitro assay data and tools provided in ICE.
- To understand strategies for in vitro assay selection under different fit-for-purpose scenarios.

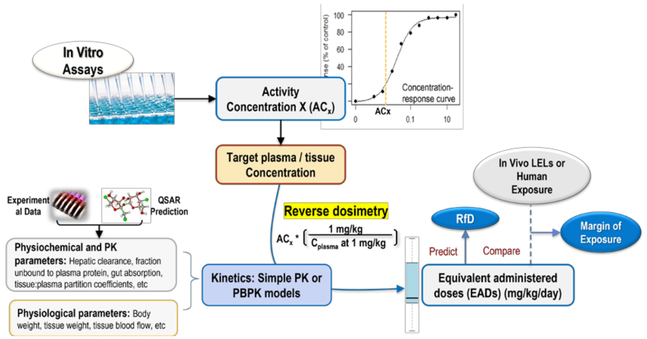
Design and Methods

- We obtained in vitro activity concentrations at cutoff (ACCs) for each selected assay in ICE and used a one-compartment population-based PK model (1C) in the ICE IVIVE tool to predict EADs. The estimated EADs corresponding to the upper 95th percentile of the steady-state plasma concentration were compared to in vivo data for evaluation.
- **Case Study 1 – effects of mechanistic targets:**
  - Chemicals: 19 reference chemicals listed as “estrogen receptor (ER) In vivo agonists” in ICE.
  - In vitro assay groups:
    a) Assays targeted to estrogen metabolic process and ER signaling;
    b) Assays targeted to measure cellular stress responses (e.g., DNA damage, oxidative stress).
  - In vivo data: lowest effect levels (LELs) in "guideline-like" rodent uterotrophic studies as described in Browne et al. 2015.
- **Case Study 2 – effects of toxicological endpoints:**
  - Chemicals: 6 chemicals in the ICE genotoxicity reference list, with known Ames test results and clear genotoxicity calls (Kirkland et al. 2016).
  - In vitro assay groups:
    a) All available cHTS assays; b) Cellular cytotoxicity assays.
  - In vivo data: LD50 values in the acute oral rat toxicity assays.

Acknowledgments and Disclaimer

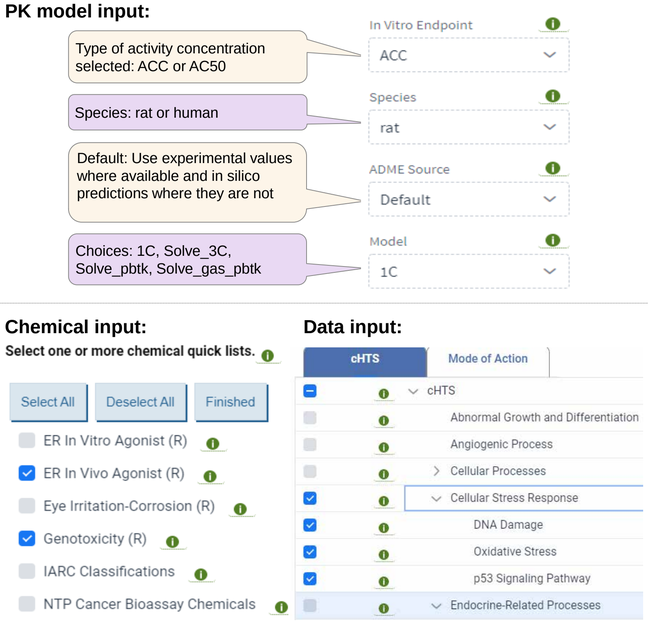
This project was funded by the National Institute of Environmental Health Sciences, National Institutes of Health, under Contract No. HHSN273201500010C. The authors declare that there is no conflict of interest. The views expressed above do not necessarily represent the official positions of any federal agency.

IVIVE Example Workflow



Rfd: reference dose, the U.S. Environmental Protection Agency's maximum acceptable oral dose of a toxic substance; LEL, lowest effect level; AC_x, chemical concentration that produces x% of the maximum activity in the assay.

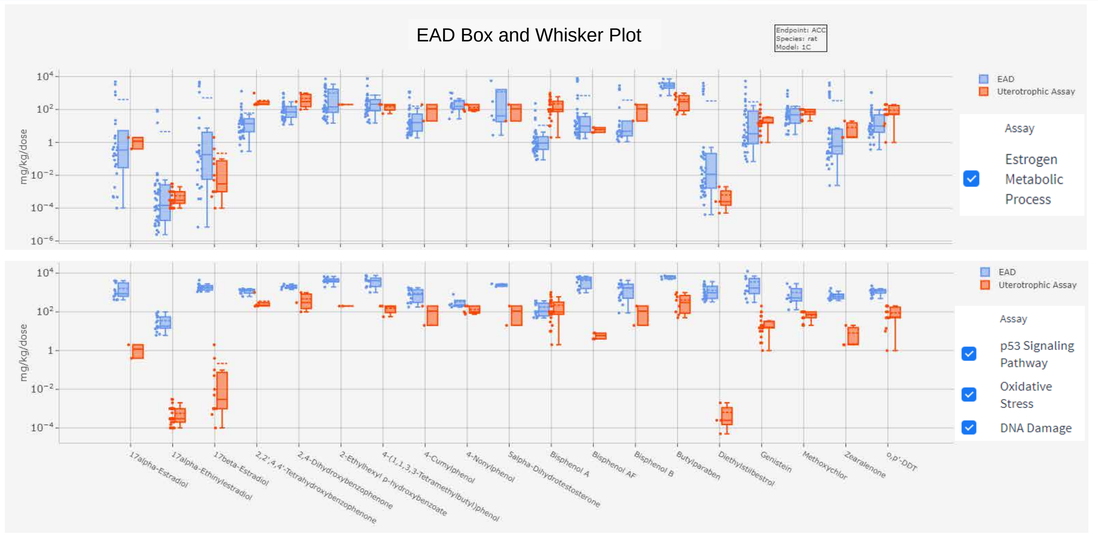
ICE IVIVE Tool User Interface



References

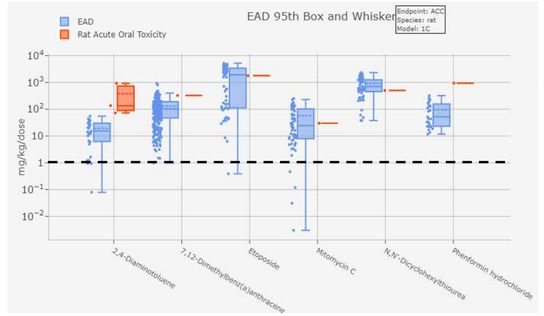
Abedini et al. (2021). Comput Toxicol Vol 20
Bell et al. (2020). Toxicol In Vitro Vol 67
Kirkland et al (2016). Mutat Res / Genet Toxicol Environ Mutagen 795: 7-30
Browne et al (2015). Environ Sci Technol 49(14):8804-14

Case Study 1: EADs Compared to LELs from Rodent Uterotrophic Assays

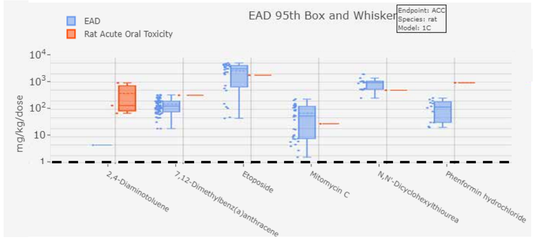


Case Study 2: EADs Compared to LD50 from Rat Acute Oral Toxicity Studies

a) Select **all** cHTS assays in ICE



b) Select in vitro **cytotoxicity** cHTS assays only



In the figures above, blue boxplots represent the range of EADs estimated from in vitro ACC in cHTS assays. EADs corresponding to the upper 95th percentile of the steady-state plasma concentration are shown. Orange boxplot represent lowest effect levels (LELs) from rodent uterotrophic studies (for Case Study 1) or LD50 from acute oral rat toxicity studies (for Case Study 2).

Results

- Using in vitro assays that are more mechanistically relevant to in vivo endpoints of interest (e.g., assays targeted to estrogen metabolic process and signaling) provided more accurate predictions of in vivo toxicity doses (e.g., rat uterotrophic LELs).
- Compared to using all cHTS assays provided in ICE, using only in vitro cytotoxicity assays provided a narrower EAD range but similar median values.
- IVIVE using all ICE cHTS assays provided the lowest EAD estimate, which could be used as a conservative estimate for risk assessment.

Conclusions

- The biological relevance of selected in vitro assay(s) to the in vivo endpoint of interest impacts the ability of IVIVE to provide a reasonable estimation of an exposure corresponding to the in vitro bioactivity.
- ICE provides open access to curated data and user-friendly tools to support improved understanding and appropriate application of NAMs.