# An Open-source, Generalized Workflow for IVIVE Analysis

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## Introduction

- A critical challenge for implementing non-animal approaches for chemical safety testing is linking in vitro assay results to potential in vivo effects.

- In vitro to in vivo extrapolation (IVIVE) predicts the daily in vivo equivalent administered dose that would result in a plasma concentration corresponding to an in vitro activity concentration (**Figure 1**).

- To facilitate IVIVE, we developed an open-source IVIVE tool that incorporates:
  - In vitro assay data
  - Quantitative structure-activity relationship (QSAR) models
  - Reverse dosimetry using either one-compartment pharmacokinetic (PK) or multi-compartment physiologically based PK (PBPK) models

**Figure 1        Predicting In Vivo EAD from In Vitro Activity Concentration**
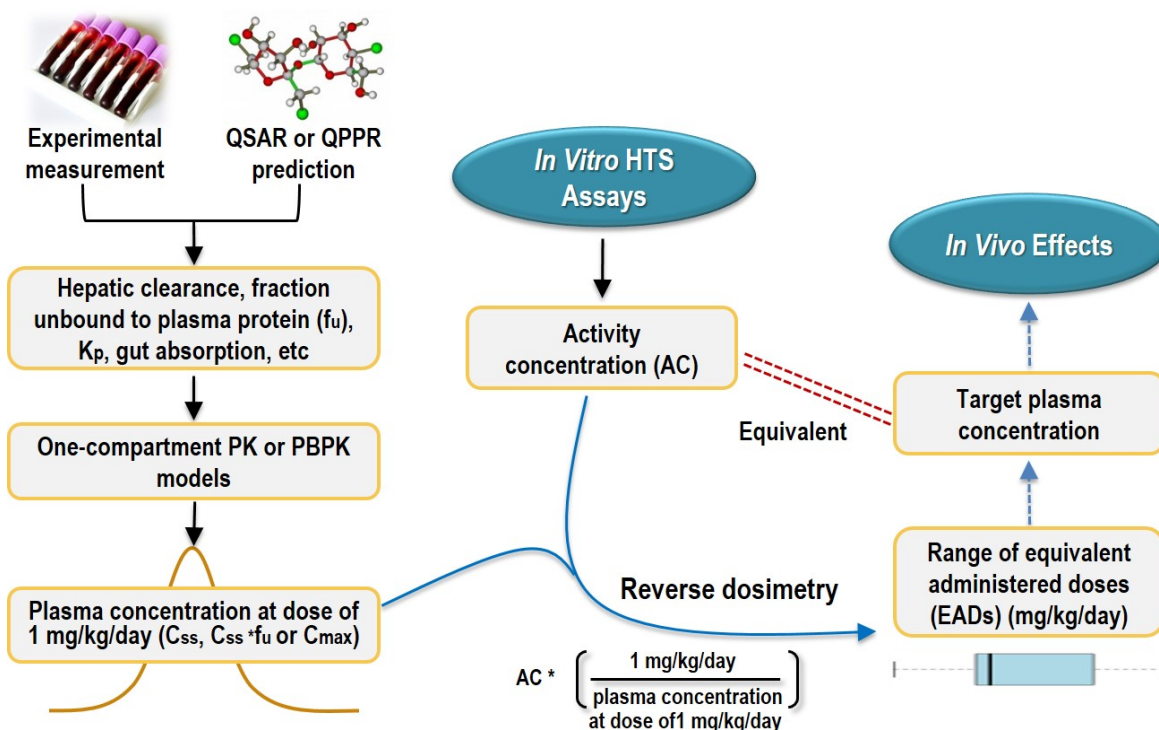
**An Open-source IVIVE Tool**

- The IVIVE tool is publicly accessible through the Integrated Chemical Environment (ICE) web resource (https://ice.ntp.niehs.nih.gov/; Bell et al. 2017). ICE was developed by the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM).

- ICE houses all the information needed to conduct IVIVE, which includes:

  - Curated in vitro and in vivo assay data that cover a range of toxicological endpoints
  - In silico predictions of biophysical and biochemical properties for over 720,000 chemicals
  - Computational tools designed for data exploration

- IVIVE can be conducted using an interactive online tool via the ICE graphical user interface or downloaded as an R notebook to run locally.

- **Figure 2** shows the flowchart of the IVIVE tool. Three different PK models will be used in the IVIVE tool.
  - The one-compartment PK model can be applied to over 7500 Tox21 chemicals with input parameters provided via ICE.
  - The "3compartment" model in the httk R package (httk: high-throughput toxicokinetics; Pearce et al. 2017) can also be applied to Tox21 chemicals with input parameters provided via ICE.
  - The three-compartment (3c) glucuronidation model is applied specifically to chemicals known to undergo gastrointestinal glucuronidation (e.g., bisphenol A).

- Required inputs include:

  - In vitro assay activity provided via ICE and selectable by user; activity is expressed as either:

    - Half-maximal activity concentration ($AC_{50}$)
    - Activity concentration at cutoff (ACC)

  - PK parameters, provided by user or via ICE using experimental data or OPERA model predictions (**Figure 3**) (Kirman et al. 2015; Mansouri et al. 2018):

    - Fraction of chemical unbound to protein
    - Hepatic clearance
    - Renal clearance

  - Additional PK parameters, provided by user or available in ICE, for executing PBPK models:

    - Uptake rate of chemical from the gut

- Vmax and Km for gastrointestinal glucuronidation
  - Vmax, maximum glucuronidation rate achieved at saturating substrate concentration
  - Km, the substrate concentration that is required for the glucuronidation to occur at half of Vmax
- Tissue:plasma partition coefficients (liver, gut, kidney, and rest of the body)

- The workflow output is the daily equivalent administered dose (EAD) that would lead to a plasma concentration equivalent to the activity concentration in an in vitro assay. These EAD values can be compared to in vivo lowest effective levels (LELs) if those data are available.
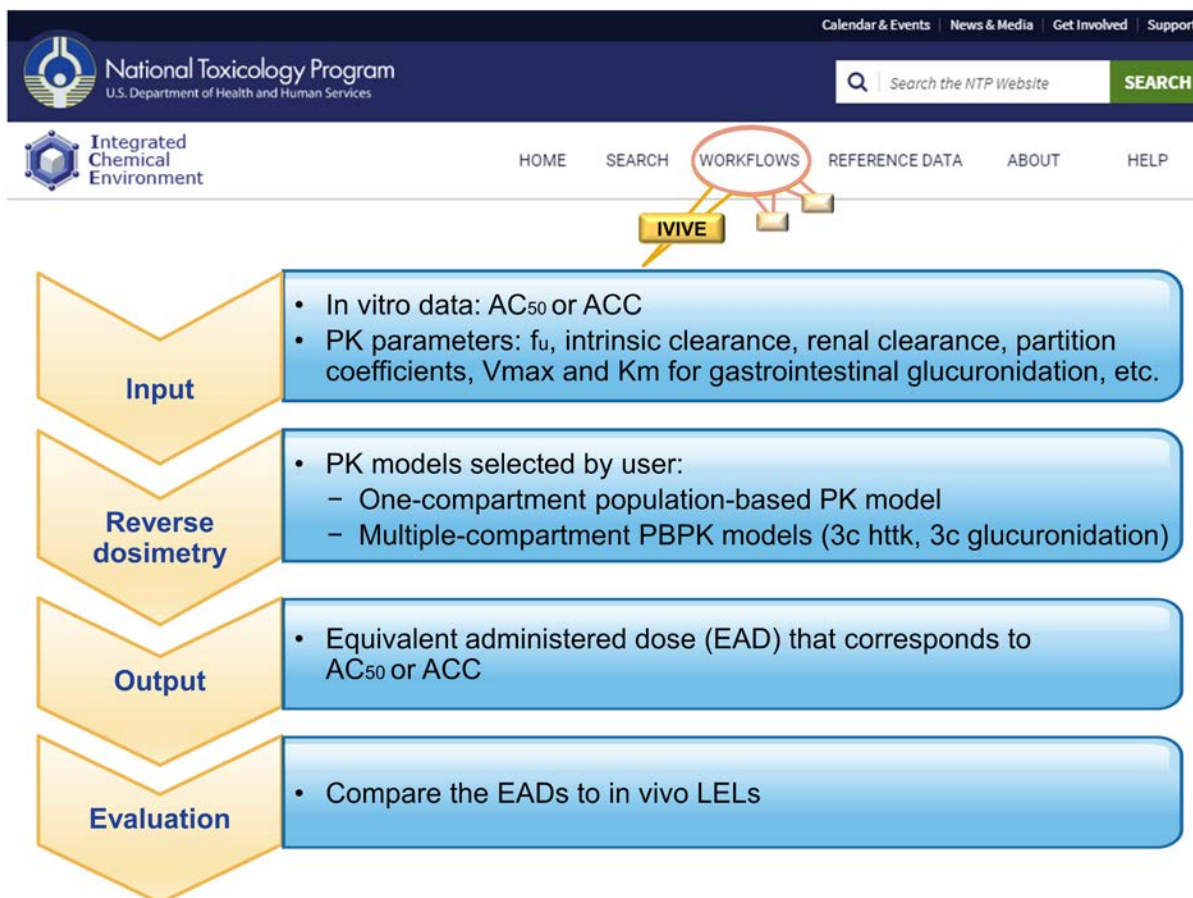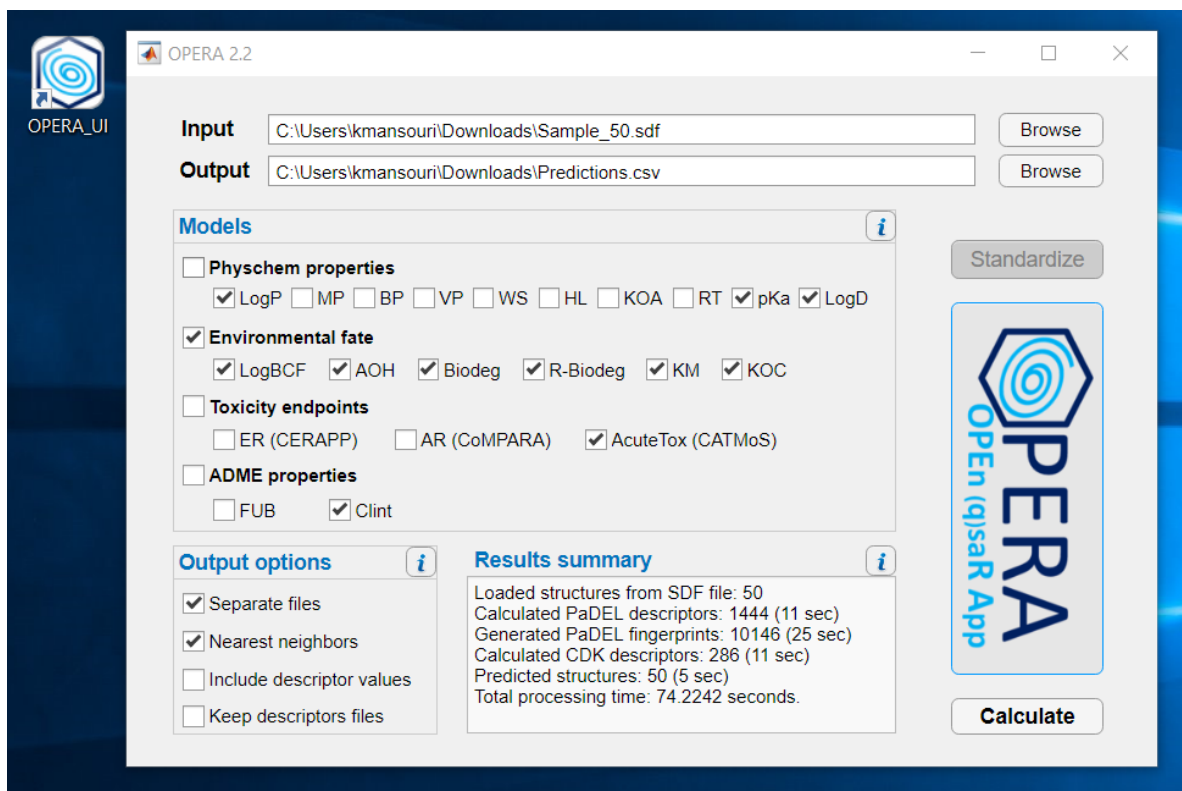
**Figure 2      ICE and IVIVE Tool Overview**

**Figure 3**         **Screenshot of OPERA GUI**



OPERA is a free and open-source suite of QSAR models providing predictions for physicochemical properties, environmental fate, and toxicological endpoints (Mansouri et al. 2018). OPERA is available as downloadable command-line files (https://github.com/NIEHS/OPERA) or a standalone GUI application (shown left). The OPERA predictions are also freely available through the U.S. Environmental Protection Agency's CompTox dashboard (https://comptox.epa.gov/dashboard), and NICEATM's Integrated Chemical Environment (https://ice.ntp.niehs.nih.gov).

**The PK Models Used in IVIVE**

- **Figure 4** shows the structures of the PK and PBPK models used in the IVIVE tool.
- The population-based PK model (**Figure 4A**) is a one-compartment model that
    - Assumes 100% absorption
    - Estimates total clearance as the sum of hepatic and renal clearance
- The model estimates steady-state plasma concentration ($C_{ss}$) following a given dose for a Monte Carlo simulated population that accounts for interindividual variability, covering physical variability across individuals (Wetmore et al. 2012).
- The user has two options for calculating an EAD that would lead to a $C_{ss}$ equivalent to the $AC_{50}$ or ACC from the in vitro assay of interest:
    - EAD corresponding to unbound chemical concentration (default option):
    
    $$EAD_{f_u} = EAD \times \frac{1}{f_u} (mg\,/\,kg\,/\,day)$$
    
    - EAD corresponding to total chemical concentration:
    
    $$EAD = ACC(or\,AC50) \times \frac{1}{C_{ss}} (mg\,/\,kg\,/\,day)$$

- The PBPK model (**Figure 4B**) includes four separate compartments: gastrointestinal tract, liver, kidney, and all remaining tissues (i.e., rest of body).
- The model includes intestinal glucuronidation to simulate specifically chemicals known to undergo gastrointestinal glucuronidation (e.g., bisphenol A). This model can calculate EADs that result in a maximum plasma concentration ($C_{max}$) corresponding to the in vitro activity concentration.

**Figure 4     Structures of Models Used in IVIVE Tool**
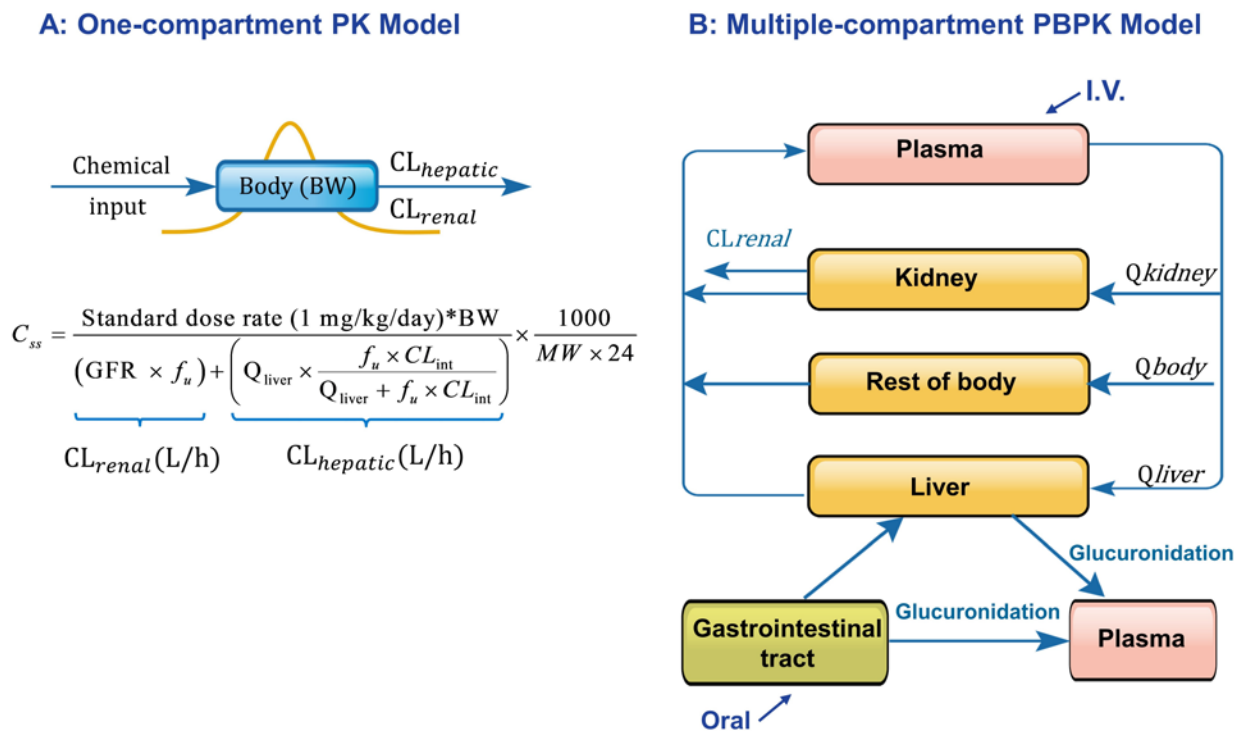


BW, body weight; CL, clearance; GFR, glomerular filtration rate; I.V., intravenous injection; MW, molecular weight; Q, blood flow rate

**IVIVE Tool Application: ER Pathway Assays**

- **Figures 5** and **6** show an example of how the IVIVE tool can be used in ICE.

- We used ACC values from 39 estrogen receptor (ER) reference chemicals tested in 18 ER pathway assays as inputs to the workflow (**Figure 5**).

- **Figure 6** shows the workflow output. EAD values corresponding to unbound chemical concentration (blue boxplots) are displayed along with the ACC values used as inputs (orange boxplots).

- **Table 1** compares the EADs obtained from the ICE tool with LELs from in vivo injection uterotrophic assays (Kleinstreuer et al. 2016; Casey et al. 2018). For majority of chemicals, the $EAD_{fu}$ is within an order of magnitude of the lowest or highest LEL, suggesting the approach is valid in estimating in vivo estrogenic activity from in vitro ER pathway assays.

- **Figure 7** compares the workflow outputs between using the one-compartment PK and published PBPK model (Yang et al 2013) for BPA when the same ER pathway assay ACC values are used as inputs. Improved prediction accuracy was observed with using the PBPK model incorporating glucuronidation, especially for oral exposure.

**Figure 5          ICE IVIVE Tool Input**



The screenshot shows an example of selecting inputs for the IVIVE tool: in vitro metric, group of assays, and chemicals. For this example, we selected the ACC as in vitro metric, the ER pathway assays, and the ER in vivo agonist reference chemical list.

**Figure 6          ICE IVIVE Tool Output**
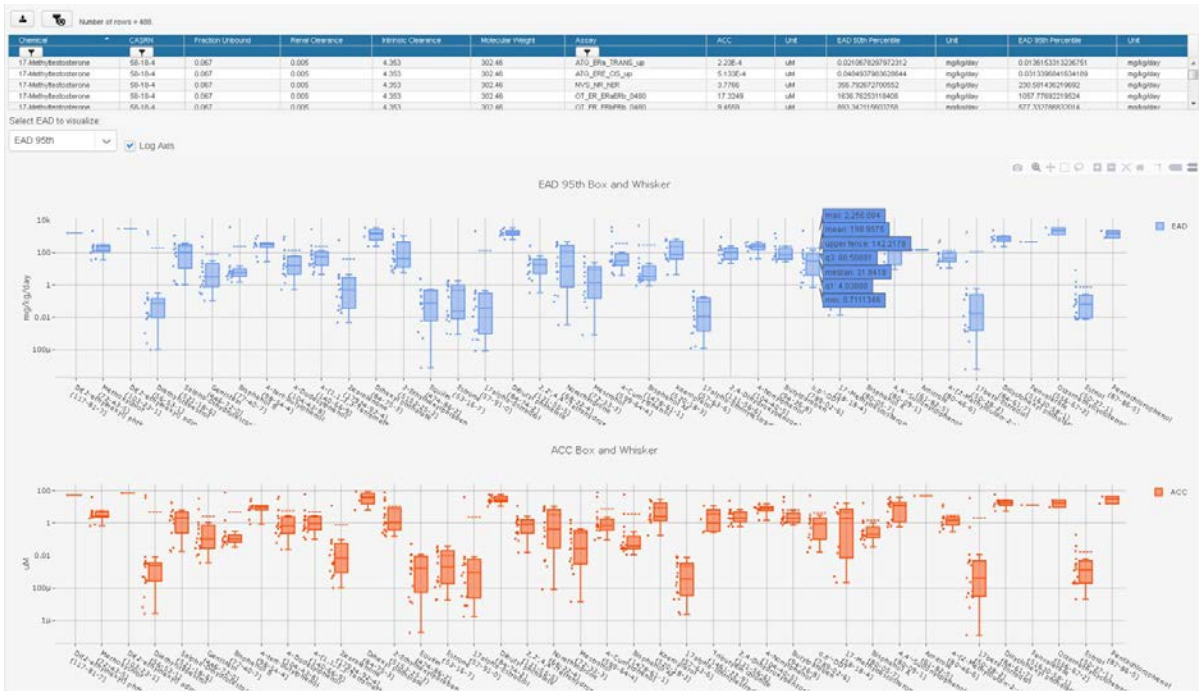


The screenshot shows the EADs (mg/kg/day; blue boxplots) predicted from the ER pathway assay ACC inputs (µM; orange boxplots) using the IVIVE-PK workflow. Data obtained from ICE user interface on Aug. 24, 2018.

**Table 1**      **Comparison of IVIVE-PK Workflow Output with In Vivo Data**

| CASRN | Chemical Name | $EAD_{fu}$ (ICE Output) | Lowest $LEL_{inj}$ | Median $LEL_{inj}$ | Highest $LEL_{inj}$ | Number of studies |
|---|---|---|---|---|---|---|
| 72-43-5 | Methoxychlor | 131.38 | 50 | 100 | 100 | 12 |
| 56-53-1 | Diethylstilbestrol | 0.078 | 5E-05 | 0.00025 | 0.002 | 5 |
| 521-18-6 | 5alpha-Dihydrotestosterone | 102.12 | 4 | 20 | 200 | 3 |
| 446-72-0 | Genistein | 3.22 | 1 | 15 | 35 | 19 |
| 77-40-7 | Bisphenol B | 5.70 | 20 | 110 | 200 | 2 |
| 98-54-4 | 4-tert-Butylphenol | 314.91 | 99.2 | 99.6 | 100 | 2 |
| 104-43-8 | 4-Dodecylphenol | 15.89 | 40 | 40 | 40 | 3 |
| 140-66-9 | 4-(1,1,3,3-Tetramethylbutyl)phenol | 53.60 | 200 | 200 | 200 | 2 |
| 17924-92-4 | Zearalenone | 0.50 | 2 | 2 | 20 | 3 |
| 5153-25-3 | 2-Ethylhexylparaben | 43.77 | 200 | 200 | 200 | 2 |
| 474-86-2 | Equilin | 0.077 | 2 | 2 | 2 | 2 |
| 53-16-7 | Estrone | 0.024 | 0.0018 | 0.102 | 2 | 6 |
| 131-55-5 | 2,2',4,4'-Tetrahydroxybenzophenone | 18.29 | 200 | 200 | 300 | 5 |
| 68-22-4 | Norethindrone | 14.30 | 2 | 11 | 20 | 2 |
| 72-33-3 | Mestranol | 1.44 | 0.0016 | 0.0038 | 0.006 | 2 |
| 599-64-4 | 4-Cumylphenol | 31.84 | 20 | 110 | 200 | 2 |
| 1478-61-1 | Bisphenol AF | 3.39 | 4 | 6 | 8 | 4 |
| 57-63-6 | 17alpha-Ethinylestradiol | 0.012 | 0.0001 | 0.0003 | 0.002 | 35 |
| 131-56-6 | 2,4-Dihydroxybenzophenone | 108.51 | 100 | 200 | 300 | 2 |
| 104-40-5 | 4-Nonylphenol | 235.13 | 100 | 200 | 200 | 3 |
| 94-26-8 | Butylparaben | 69.25 | 50 | 300 | 1000 | 8 |
| 789-02-6 | o,p'-DDT | 31.94 | 1 | 100 | 200 | 9 |
| 58-18-4 | 17-Methyltestosterone | 116.30 | 10 | 10 | 10 | 3 |
| 80-05-7 | Bisphenol A | 12.17 | 2 | 100 | 800 | 29 |
| 80-09-1 | 4,4'-Sulfonyldiphenol | 180.35 | 20 | 20 | 20 | 2 |
| 80-46-6 | 4-(2-Methylbutan-2-yl)phenol | 45.30 | 200 | 200 | 202 | 4 |
| 50-28-2 | 17beta-Estradiol | 0.017 | 0.0001 | 0.001 | 2 | 19 |

$EAD_{fu}$, EAD values corresponding to unbound chemical concentration (ICE output, acceded on Aug. 24, 2018)

**Figure 7        IVIVE Results Using PBPK Model for BPA**
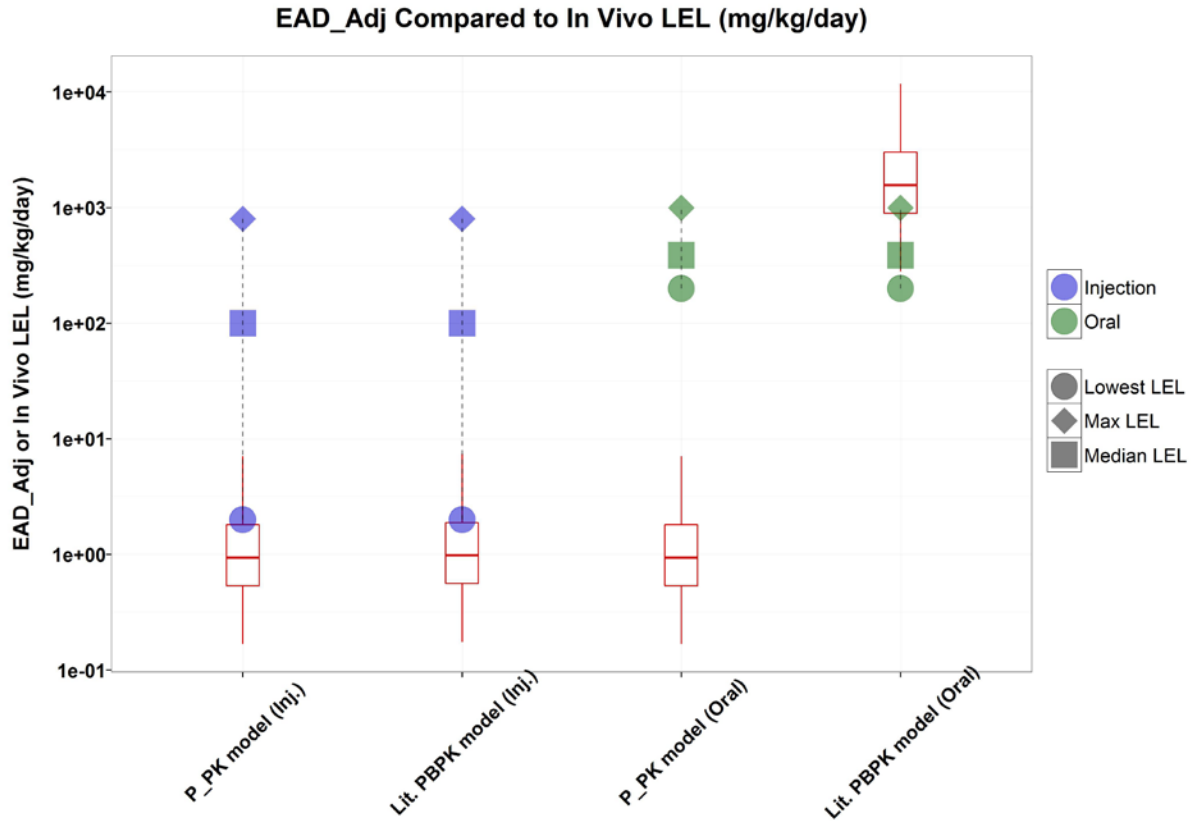


The figure compares EAD values corresponding to unbound chemical concentration (red boxplots) estimated from the one-compartment PK model and PBPK model incorporating glucuronidation to lowest, median, and maximum LELs from injection (blue symbols) or oral (green symbols) uterotrophic studies for BPA. BPA, bisphenol A.

## Discussion and Conclusion

- The ICE tool provides an open-source, easy-to-use tool for IVIVE. The tool can be used to evaluate the correlation between in vitro and in vivo activity for toxicologically relevant endpoints.
- For chemicals lacking in vivo data, the tool can be used to predict relevant toxicity potential, expediting the safety assessment process.
- When evaluating estrogenic activities, the range of EAD estimates produced by the tool correlated well with the range of in vivo uterotrophic LELs for the majority chemicals tested, suggesting the IVIVE approach provides valid estimates of in vivo estrogenic activity from in vitro ER pathway assays.
- The IVIVE workflow using a multiple-compartment PBPK model incorporating glucuronidation improved prediction accuracy for BPA and likely for other BPA-like chemicals.

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