

# In Vitro to In Vivo Extrapolation: Optimizing Parameters for Improved Predictions

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## Introduction

- In vitro high-throughput screening (HTS) assays can accelerate and reduce the overall cost of identifying potentially toxic chemicals. These assays are being developed and evaluated in programs such as the U.S. federal Tox21 consortium (Tice et al. 2013) and the U.S. Environmental Protection Agency's ToxCast project (Kavlock et al. 2012).
- However, before in vitro HTS assays can be used in risk assessment, the ability of in vitro activity to predict in vivo dose–response relationships needs to be evaluated. Central to this evaluation is determining how closely a bioactive chemical concentration in an in vitro assay corresponds to the blood and tissue levels of that chemical that cause adverse effects in vivo.
- Generally, the nominal tested concentration (i.e., the amount of chemical added in the medium divided by volume of the exposure medium) is used to define in vitro concentration–activity relationships. However, the use of free chemical concentration instead of nominal concentration has been suggested to reduce the effect of concentration variability between in vitro assays and between in vitro and in vivo assays (Groothuis et al. 2015).
- We evaluated the performance of in vitro to in vivo extrapolation (IVIVE) of estrogen receptor (ER) pathway activity and impact of critical pharmacokinetic (PK) parameters and dosimetry on IVIVE analysis.

## Data Used in the Analysis

- We selected 10 ER-active chemicals for IVIVE analysis, including two reference estrogens and eight environmental chemicals (Table 1). The chemicals were selected according to availability of data from in vitro assays, data from high-quality in vivo uterotrophic (UT) assay studies, and experimental measurements of pharmacokinetic parameters.
- In vitro data were obtained from 16 ToxCast/Tox21 HTS assays that measure many key events along the ER agonism pathway (e.g. receptor binding, transcription, and cell proliferation) (Judson et al. 2015).
  - For each chemical–assay pair, we calculated the activity concentration at cutoff (ACC) as the lowest effective concentration (LEC) that causes a statistically significant estrogenic effect.
  - We calculated the lowest and median ACC across all 16 assays (Table 1).
- In vivo data were generated in the UT assay, which measures uterine hypertrophy caused by activation of the ER pathway (Kleinstreuer et al. 2015).
  - For each chemical–assay pair, the lowest effective dose level (LEL) that causes an estrogenic effect in UT assay was derived, from which we calculated the lowest and median LELs for each chemical. Data to derive the LEL were available for dosing by subcutaneous or intraperitoneal injection for all 10 chemicals and for oral administration for 7 out of 10 chemicals.

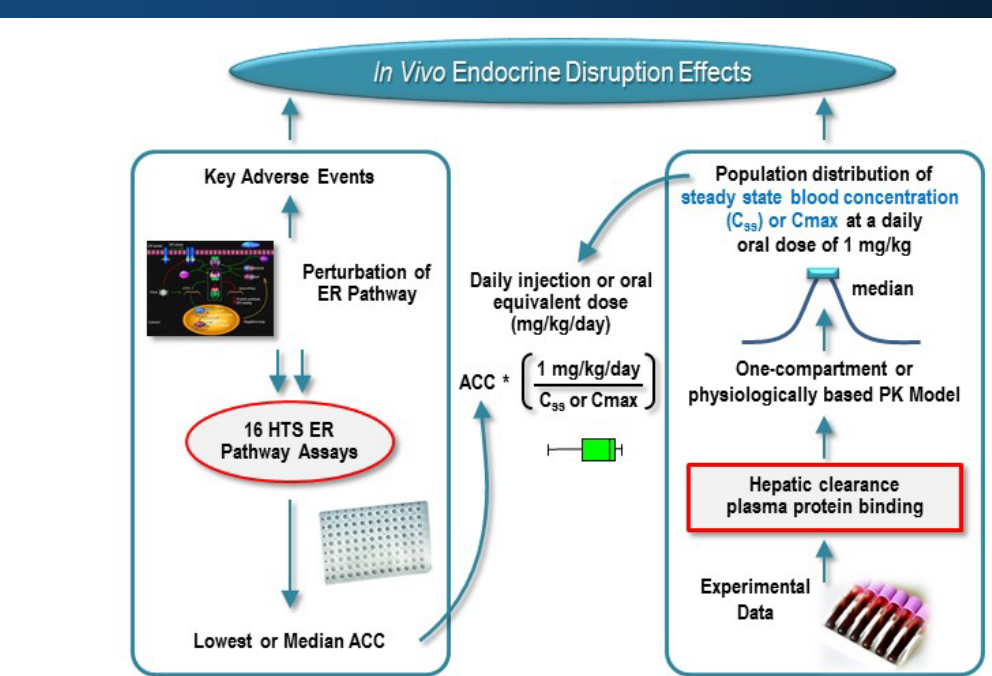
**Table 1. Lowest and Median ACCs for Chemicals Used in the Analysis**

| Chemical Name <sup>a</sup>   | CASRN    | Lowest ACC (μM) | Median ACC (μM) |
|------------------------------|----------|-----------------|-----------------|
| Diethylstilbestrol           | 56-53-1  | 9.020E-07       | 3.404E-04       |
| 17 beta-Estradiol            | 50-28-2  | 8.14E-06        | 1.55E-03        |
| Genistein                    | 446-72-0 | 0.004           | 0.062           |
| o,p'-DDT                     | 789-02-6 | 0.025           | 1.290           |
| Bisphenol B                  | 77-40-7  | 0.028           | 0.079           |
| Bisphenol A                  | 80-05-7  | 0.033           | 0.228           |
| 4-tert-Octylphenol           | 140-66-9 | 0.137           | 0.774           |
| 4-(1,1-Dimethylpropyl)phenol | 80-46-6  | 0.280           | 1.816           |
| Methoxychlor                 | 72-43-5  | 0.674           | 2.525           |
| Butylparaben                 | 94-26-8  | 0.941           | 1.992           |

Abbreviations: ACC = activity concentration at cutoff; CASRN = Chemical Abstracts Service Registry Number.

<sup>a</sup>The table is sorted by lowest ACC in ascending order.

## Figure 1. Use of Pharmacokinetic Modeling for Reverse Dosimetry<sup>a</sup>



Abbreviations: ACC = activity concentration at cutoff; C<sub>max</sub> = maximum blood concentration; C<sub>ss</sub> = steady-state blood concentration; ER = estrogen receptor; HTS = high-throughput screening; PK = pharmacokinetic.

<sup>a</sup>Adapted from Judson et al. 2011.

## Development of a Reverse Toxicokinetic Model for Estrogenic Effects

- Figure 1** depicts a general approach for developing a reverse pharmacokinetic (PK) model for IVIVE.
- The fraction of chemical unbound in the plasma (F<sub>ub</sub>) and intrinsic metabolic clearance rate (CL<sub>intrinsic</sub>) are the two most important parameters for model building.
  - Experimental data on F<sub>ub</sub> and CL<sub>intrinsic</sub> for tested chemicals (Table 2) were obtained from literature (Wetmore et al. 2013).
  - Rat F<sub>ub</sub> or CL<sub>intrinsic</sub> data were preferred. If none were available, human experimental values were used (Wetmore et al. 2012).

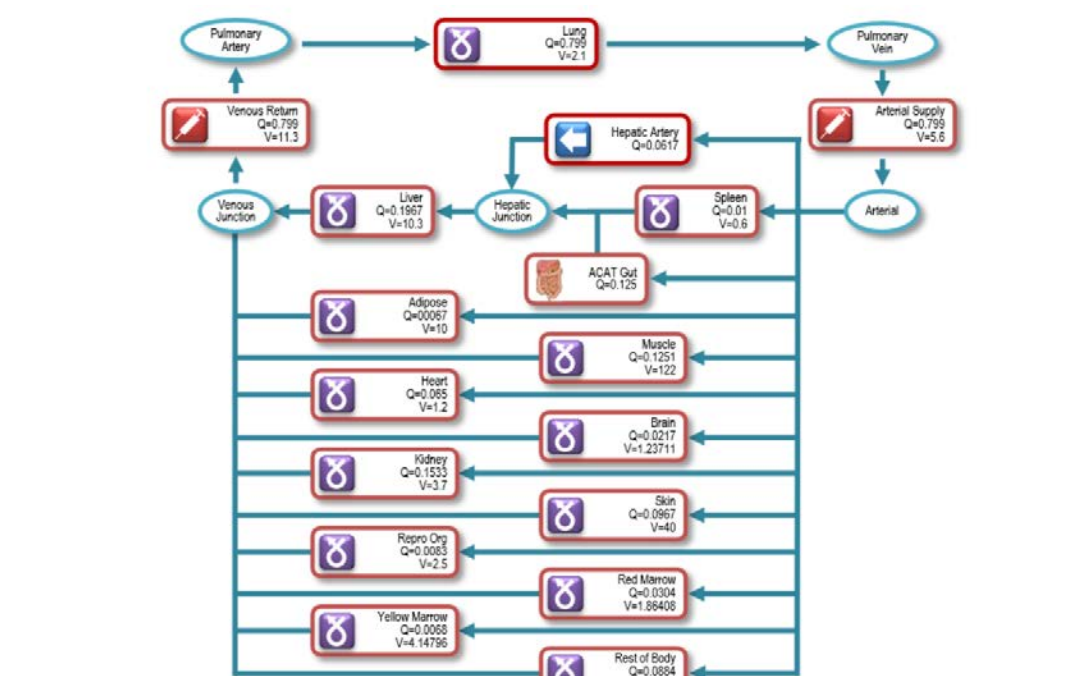
**Table 2. PK Parameters Used in the Models**

| Chemical Name                | Fub   | CL <sub>intrinsic</sub> (L/h) | Hepatic Clearance (L/h) | Renal Clearance (L/h) | Source of PK Parameters (Fub, CL <sub>intrinsic</sub> ) |
|------------------------------|-------|-------------------------------|-------------------------|-----------------------|---|
| Diethylstilbestrol           | 0.005 | 2.753                         | 0.0135                  | 0.0004                | Human, Human  |
| 17 beta-Estradiol            | 0.053 | 1.000                         | 0.0498                  | 0.0042                | Rat, Rat  |
| Genistein                    | 0.300 | 1.246                         | 0.2576                  | 0.0240                | Rat, Human  |
| o,p'-DDT                     | 0.005 | 1.006                         | 0.0050                  | 0.0004                | Human, Human  |
| Bisphenol B                  | 0.018 | 2.378                         | 0.0412                  | 0.0015                | Human, Human  |
| Bisphenol A                  | 0.060 | 0.155                         | 0.0092                  | 0.0048                | Rat, Rat  |
| 4-tert-Octylphenol           | 0.019 | 1.799                         | 0.0329                  | 0.0015                | Human, Human  |
| 4-(1,1-Dimethylpropyl)phenol | 0.005 | 1.817                         | 0.0090                  | 0.0004                | Human, Human  |
| Methoxychlor                 | 0.005 | 1.957                         | 0.0097                  | 0.0004                | Human, Human  |
| Butylparaben                 | 0.042 | 2.621                         | 0.0963                  | 0.0033                | Human, Human  |

Abbreviations: CL<sub>intrinsic</sub> = intrinsic metabolic clearance rate; Fub = fraction of chemical unbound in the plasma; Human = human experimental data reported from literature (Wetmore et al. 2012); PK = pharmacokinetic; Rat = rat experimental data reported from literature (Wetmore et al. 2013).

- We applied either a one-compartment PK or multi-compartment physiologically based PK (PBPK) model to estimate the daily equivalent administered dose (EAD) that would result in a steady-state blood concentration (C<sub>ss</sub>, for one-compartment model) or maximum blood concentration (C<sub>max</sub>, for PBPK models) equivalent to the lowest or median ACC value across assays.
- The daily injection or oral EAD estimates (IED and OED, respectively) were then compared to the lowest and/or median LELs from UT assays with corresponding administration routes.
  - The one-compartment rat population PK (P-PK) model built using the software package R (v. 3.1.2) was used to simulate injection route of administration and estimated C<sub>ss</sub> (Wetmore et al. 2013). The model assumed 100% absorption.
  - Both rat injection PBPK (I-PBPK) and oral PBPK (O-PBPK) models were built using GastroPlus software (Simulations Plus, Inc.), which includes 14 tissue compartments (Figure 2). The I-PBPK model simulated a daily 3-hour intravenous infusion. The O-PBPK model incorporates the Advanced Compartmental Absorption and Transit (ACAT) model to simulate chemical absorption through the gastrointestinal tract. Both PBPK models estimated C<sub>max</sub>.
  - In the case of bisphenol A, we also applied a published PBPK model for oral administration that incorporates glucuronidation (Yang et al. 2013).

**Figure 2. Structure of the GastroPlus Rat PBPK Model**



Abbreviations: ACAT model = advanced compartmental absorption and transit model; PBPK = physiologically based pharmacokinetic; Q = blood flow; V = volume.

- For all models, hepatic clearance (CL<sub>hepatic</sub>) and renal clearance (CL<sub>renal</sub>) were calculated using the following equations:

$$CL_{hepatic} (L/h) = Q_{liver} (L/h) * \frac{Fub * CL_{intrinsic}}{Q_{liver} + Fub * CL_{intrinsic}}$$

$$CL_{renal} (L/h) = GFR (L/h) * Fub$$

- In these equations, GFR is glomerular filtration rate and Q<sub>liver</sub> is liver blood flow rate.
- For both PBPK models, the tissue partition coefficients for each chemical were predicted using ADMET Predictor (Simulations Plus, Inc.).
- The terms IED-free or OED-free refer to the IED or OED, respectively, which could result in free chemical concentrations in the blood equivalent to the lowest and median ACC. To predict IED-free or OED-free across all the in vitro ER assays, we used the following equation: IED-free = IED / Fub; OED-free = OED / Fub.

## Results

- Compared to IED estimates, the IED-free estimates from P-PK model provided a more accurate approximation to injection UT LELs in most cases (Figure 3, Table 3).
  - The lowest IED-free estimates for methoxychlor and o,p'-DDT were only ~1.1-fold less than their corresponding lowest LELs.
  - The lowest IED-free estimates were within ~13-fold of the lowest LELs in injection UT studies for 8 of the 10 chemicals.
  - The median IED-free estimates were within 10-fold of median LELs in injection UT studies for 5 of the 10 chemicals. The largest difference between median IED-free and the median LELs was 86.7-fold for diethylstilbestrol.
- Compared to IED estimates, the IED-free estimates from I-PBPK model also provided a better approximation to injection UT LELs in most cases (Figure 4, Table 4).
  - The lowest IED-free for 17 beta-estradiol was almost equivalent to its lowest LEL.
  - The lowest IED-free estimates were within ~14-fold of the lowest LELs in injection UT studies for 7 of the 10 chemicals.
  - The median IED-free estimates were within 16-fold of median LELs in injection UT studies for 5 of the 10 chemicals. The largest difference between median IED-free and the median LELs was 84.7-fold for bisphenol A.
- Regardless of whether IED or IED-free estimates were used, we did not see a significant difference in performance between the P-PK and I-PBPK models (Tables 3–4, Figures 3–4).
- Compared to OEDs, the range of OED-free estimates from the O-PBPK model provided a much closer approximation to the range of LELs in oral UT studies for all seven chemicals (Figure 5, Table 5).
  - The lowest OED-free estimates were within ~10-fold of the lowest OELs in oral UT studies for 4 of the 7 chemicals.
  - The median OED-free estimates were within 1.4-fold of their respective median LELs in oral UT studies for 17 beta-estradiol and 4-tert-octylphenol.
  - For bisphenol A, the published PBPK model performed better than O-PBPK model in predicting the range of LELs in oral UT studies.
- Using 17 beta-estradiol as an example chemical, we studied the impact of variations in CL<sub>intrinsic</sub> and Fub on IED estimation. As in previous versions of these models (Chang et al. 2015), we observed that Fub and CL<sub>intrinsic</sub> can vary up to 5–10 fold from experimental values without impacting the overall IED estimates (data not shown).

## Table 3. IEDs and IEDs-free Estimated from P-PK Model Compared to Injection LELs in UT Assays

| Chemical Name                | Lowest LEL <sup>a</sup> | Lowest IED <sup>b</sup> | Lowest IED-free | Median LEL | Median IED      | Median IED-free  |
|------------------------------|-------------------------|-------------------------|-----------------|------------|-----------------|------------------|
| 17 beta-Estradiol            | 1.00E-04                | 1.14E-05 (8.8)          | 2.15E-04 (2.1)* | 1.00E-03   | 2.16E-03 (2.2)* | 4.08E-02 (40.8)* |
| Diethylstilbestrol           | 2.50E-04                | 3.20E-07 (780.2)        | 6.41E-05 (3.9)  | 3.25E-04   | 1.21E-04 (2.7)  | 2.42E-02 (74.4)* |
| Genistein                    | 1                       | 0.026 (37.8)            | 0.088 (11.4)    | 15         | 0.443 (33.8)    | 1.478 (10.1)     |
| o,p'-DDT                     | 1                       | 4.60E-03 (217.4)        | 0.920 (1.1)     | 100        | 0.234 (427.9)   | 46.737 (2.1)     |
| Bisphenol A                  | 2                       | 0.010 (197.2)           | 0.169 (11.8)    | 100        | 0.069 (1445.3)  | 1.153 (86.7)     |
| Bisphenol B                  | 20                      | 0.027 (729.0)           | 1.505 (13.3)    | 110        | 0.078 (1413.1)  | 4.270 (25.8)     |
| Methoxychlor                 | 50                      | 0.223 (224.4)           | 44.566 (1.1)    | 100        | 0.835 (119.7)   | 167.018 (1.7)*   |
| Butylparaben                 | 70                      | 1.729 (40.5)            | 41.592 (1.7)    | 300        | 3.661 (81.9)    | 88.075 (3.4)     |
| 4-(1,1-Dimethylpropyl)phenol | 200                     | 0.041 (4849.9)          | 8.248 (24.2)    | 200        | 0.267 (749.1)   | 53.400 (3.7)     |
| 4-tert-Octylphenol           | 200                     | 0.093 (2156.8)          | 4.874 (41.0)    | 200        | 0.522 (382.9)   | 27.456 (7.3)     |

See Table 4 below for abbreviations and table notes.

## Table 4. IEDs and IEDs-free Estimated from I-PBPK Model Compared to Injection LELs in UT Assays

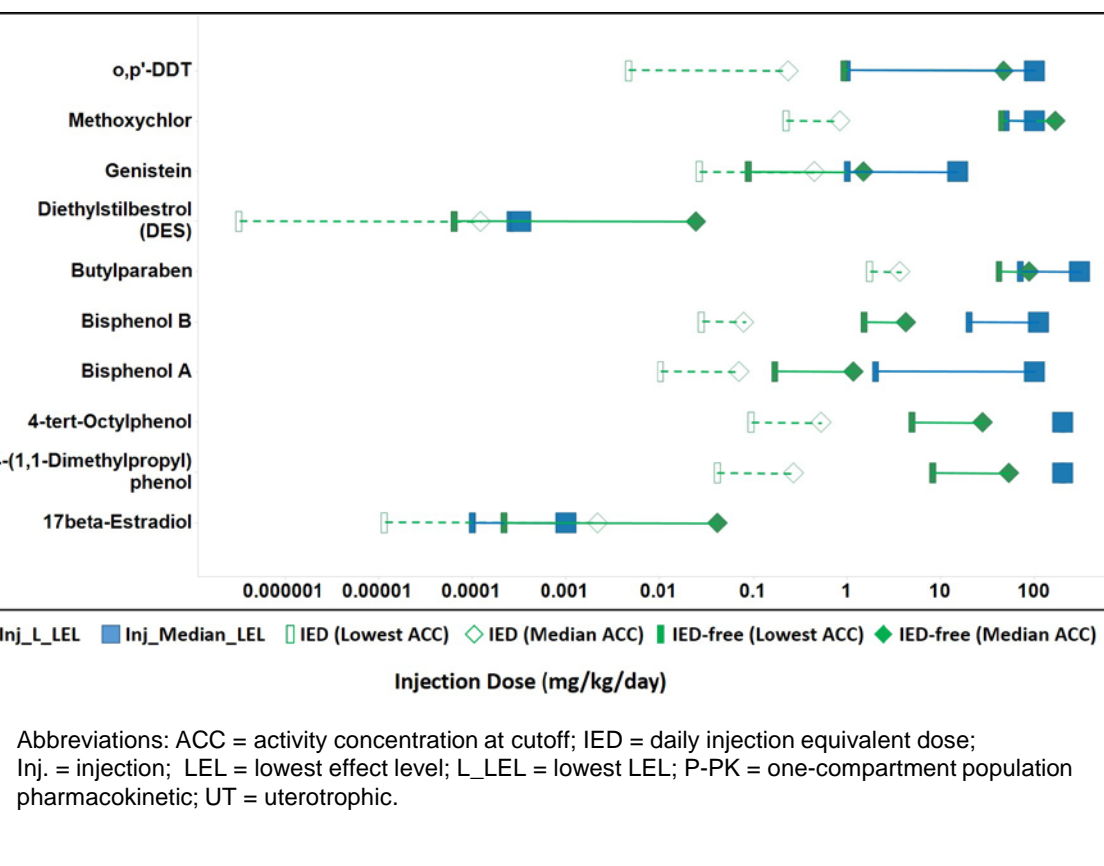
| Chemical Name                | Lowest LEL <sup>a</sup> | Lowest IED <sup>b</sup> | Lowest IED-free | Median LEL | Median IED      | Median IED-free  |
|------------------------------|-------------------------|-------------------------|-----------------|------------|-----------------|------------------|
| 17 beta-Estradiol            | 1.00E-04                | 5.12E-06 (19.5)         | 9.66E-05 (1.03) | 1.00E-03   | 9.74E-04 (1.03) | 1.84E-02 (18.4)* |
| Diethylstilbestrol           | 2.50E-04                | 3.65E-07 (685.8)        | 7.29E-05 (3.4)  | 3.25E-04   | 1.38E-04 (2.4)  | 2.75E-02 (84.7)* |
| Genistein                    | 1                       | 0.0034 (294.8)          | 0.0113 (88.4)   | 15         | 0.057 (263.6)   | 0.190 (79.1)     |
| o,p'-DDT                     | 1                       | 0.021 (46.8)            | 4.273 (4.3)*    | 100        | 1.086 (92.1)    | 217.105 (2.2)*   |
| Bisphenol A                  | 2                       | 0.011 (185.0)           | 0.180 (11.1)    | 100        | 0.074 (1355.6)  | 1.230 (81.3)     |
| Bisphenol B                  | 20                      | 0.013 (1562.0)          | 0.702 (28.5)    | 110        | 0.036 (3027.8)  | 1.993 (55.2)     |
| Methoxychlor                 | 50                      | 0.512 (97.6)            | 102.465 (2.0)*  | 100        | 1.920 (52.1)    | 384.006 (3.8)*   |
| Butylparaben                 | 70                      | 0.368 (190.1)           | 8.857 (7.9)     | 300        | 0.780 (384.8)   | 18.756 (16.0)    |
| 4-(1,1-Dimethylpropyl)phenol | 200                     | 0.024 (8380.0)          | 4.773 (41.9)    | 200        | 0.155 (1294.3)  | 30.905 (6.5)     |
| 4-tert-Octylphenol           | 200                     | 0.264 (757.8)           | 13.872 (14.4)   | 200        | 1.487 (134.5)   | 78.140 (2.6)     |

Abbreviations: IED = daily injection equivalent dose; I-PBPK = intravenous physiologically based pharmacokinetic; P-PK = one-compartment population pharmacokinetic; LEL = lowest effective level; UT = uterotrophic.

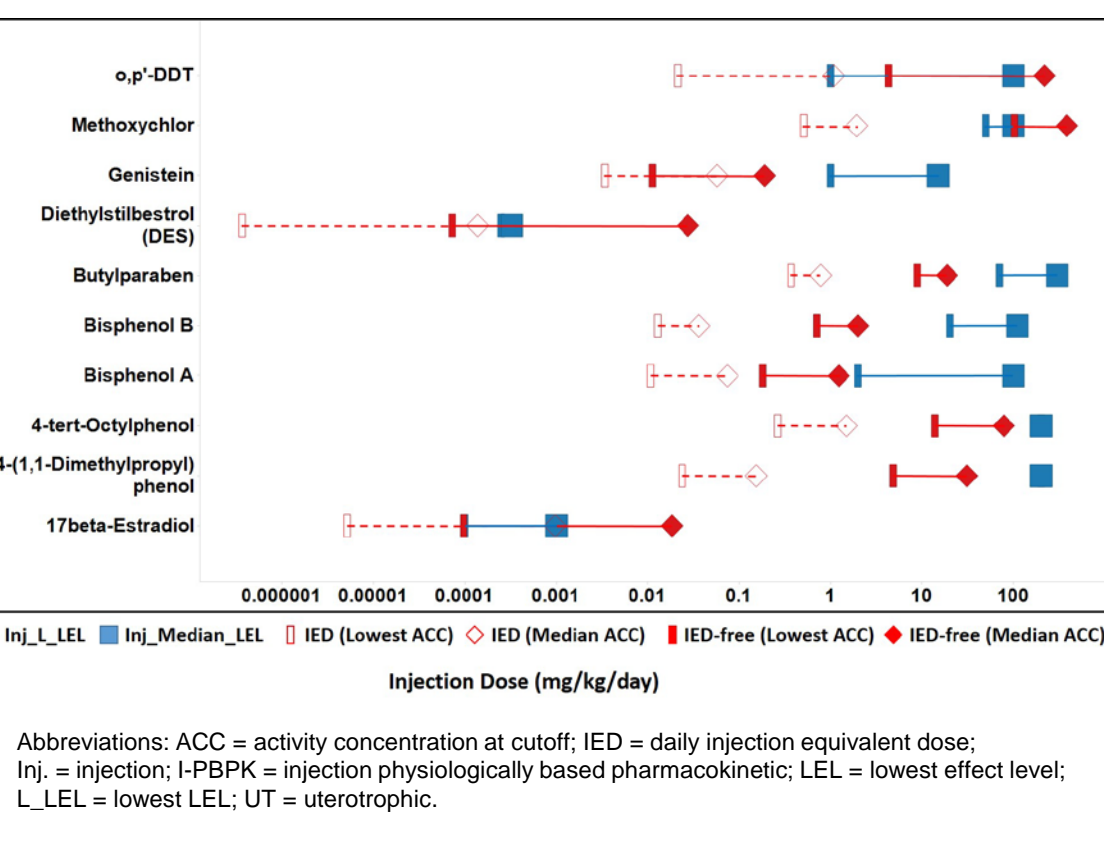
<sup>a</sup>The table is sorted by lowest LEL from injection UT assays in ascending order. IED and LEL are reported in units of mg/kg/day.

<sup>b</sup>The absolute fold differences between lowest IED/IED-free and lowest LEL in injection UT assays or between median IED/IED-free and median LEL in injection UT assays are shown in parentheses. \* indicates overprediction of the LELs.

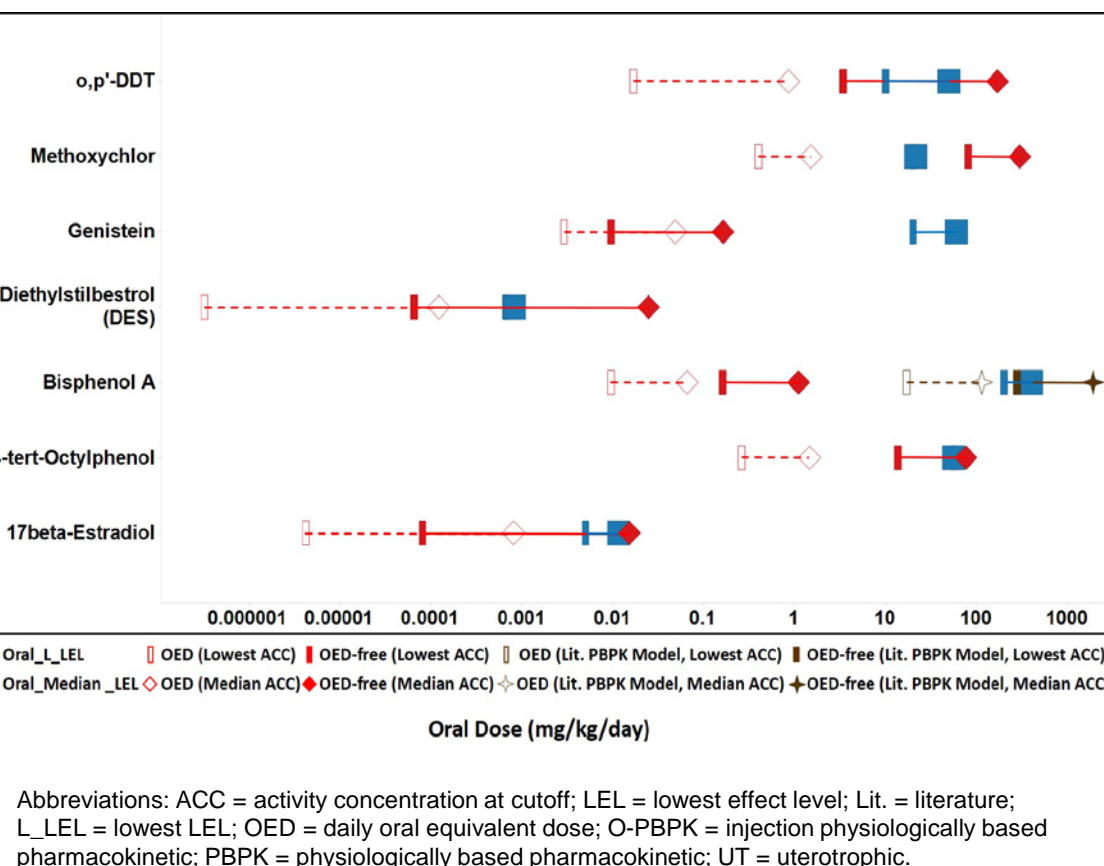
## Figure 3. IEDs and IEDs-free Estimated from P-PK Model Compared to UT Injection LELs



## Figure 4. IEDs and IEDs-free Estimated from I-PBPK Model Compared to UT Injection LELs



## Figure 5. OEDs and OEDs-free Estimated from O-PBPK Model Compared to UT Oral LELs



Abbreviations: ACC = activity concentration at cutoff; LEL = lowest effective level; Lit. = literature; L\_LEL = lowest LEL; OED = daily oral equivalent dose; O-PBPK = injection physiologically based pharmacokinetic; PBPK = physiologically based pharmacokinetic; UT = uterotrophic.

## Table 5. OEDs and OEDs-free Estimated from O-PBPK Model Compared to Oral LELs in UT Assays

| Chemical Name      | Lowest Oral LEL <sup>a</sup> | Lowest OED <sup>b</sup>        | Lowest OED-free                | Median Oral LEL | Median OED                    | Median OED-free               |
|--------------------|------------------------------|--------------------------------|--------------------------------|-----------------|-------------------------------|-------------------------------|
| Diethylstilbestrol | 6.70E-04                     | 3.241E-07 (2067.0)             | 6.483E-05 (10.3)               | 8.30E-04        | 1.224E-04 (6.8)               | 2.447E-02 (29.5)*             |
| 17 beta-Estradiol  | 5.00E-03                     | 4.260E-06 (1173.7)             | 8.038E-05 (62.2)               | 1.17E-02        | 8.104E-04 (14.4)              | 1.529E-02 (1.3)*              |
| o,p'-DDT           | 10                           | 0.017 (586.9)                  | 3.408 (2.9)                    | 50              | 0.854 (58.5)                  | 170.872 (3.4)*                |
| Genistein          | 20                           | 0.0029 (6887.1)                | 0.010 (2066.1)                 | 60              | 0.049 (1233.9)                | 0.162 (370.2)                 |
| Methoxychlor       | 20                           | 0.399 (50.1)                   | 79.792 (4.0)*                  | 21.5            | 1.495 (14.4)                  | 299.098 (13.9)*               |
| 4-tert-Octylphenol | 56                           | 0.258 (217.4)                  | 13.540 (4.1)                   | 56              | 1.462 (38.3)                  | 76.842 (1.4)*                 |
| Bisphenol A        | 200                          | 0.010 (20721.1), 16.79* (11.9) | 0.161 (1243.3), 279.83* (1.4)* | 400             | 0.066 (6071.6), 114.57* (3.5) | 1.098 (364.3), 1909.5* (4.8)* |

Abbreviations: LEL = lowest effective level; O-PBPK = oral physiologically based pharmacokinetic; OED = daily oral equivalent dose; UT = uterotrophic.

<sup>a</sup>The table is sorted by lowest LEL from oral UT assays in ascending order. OED and LEL are reported in units of mg/kg/day.

<sup>b</sup>The absolute fold differences between lowest OED/OED-free and lowest LEL in oral UT assays or between median OED/OED-free and median LEL in oral UT assays are shown in parenthesis. \* indicates overprediction of the LELs.

<sup>c</sup>OED estimates using published bisphenol A PBPK model (Yang et al. 2013).

## Discussion and Conclusion

- Significant differences were not noted in IED predictions between the P-PK and I-PBPK models.
  - The similarity of the results obtained from the two models is potentially due to using the same values for CL<sub>intrinsic</sub> and Fub.
  - The lack of difference highlights the role of chemical bioavailability and metabolic clearance in driving IVIVE analysis, regardless of model structure.
- Good agreement was observed between EAD estimates and in vivo lowest LELs for the majority of chemicals tested. This suggests that combining in vitro assay data and reverse dosimetry could provide an accurate correlation of in vitro and in vivo dosimetry and responses.
- After Fub adjustment, EAD-free better approximated the corresponding LELs for the majority of the tested chemicals, suggesting that free chemical concentration in blood is a closer approximation of in vitro effective concentrations.
- Consideration of metabolic activity could further improve accuracy of our IVIVE approach. For example:
  - All three models overpredicted LELs in UT studies for methoxychlor, which could be due to lack of metabolism in the in vitro assays.
  - The O-PBPK model underpredicted LELs in oral UT studies for bisphenol A, which was improved by incorporating glucuronidation in gut absorption.

## References

- Chang et al. 2015. Applied In Vitro Toxicol 1(1): 33-44.
- Groothuis et al. 2015. Toxicology 332:30-40.
- Judson et al. 2011. Chem Res Toxicol 24:451-62.
- Judson et al. 2015. Toxicol Sci Epub ahead of print
- Kavlock et al. 2012. Chem Res Toxicol, 25:1287-302.
- Kleinstreuer et al. 2015. Environ Health Perspect (forthcoming).
- Tice et al. 2013. Environ Health Perspect 121:756-65.
- Wetmore et al. 2012. Toxicol Sci 125:157-174.
- Wetmore et al. 2013. Toxicol Sci 132:327-346.
- Yang et al. 2013. Toxicol Appl Pharmacol 270:45-59.

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