Estimating Likelihood of Fetal *In Vivo* Interactions Using *In Vitro* HTS Data

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Abstract

Tox21/ToxCast efforts provide *in vitro* concentration-response data for thousands of compounds. Predicting whether chemical-biological interactions observed *in vitro* will occur *in vivo* is challenging. We hypothesize that using a modified model from the FDA guidance for drug interaction studies, $\frac{C_{max}}{AC_{50}}$ (i.e., maximal *in vivo* blood concentration over the half-maximal *in vitro* activity concentration), will give a useful approximation for concentrations where *in vivo* interactions are likely. Further, for external doses, where the internal maternal plasma concentration/AC_{50}s>0.1 (i.e., categorized as 'possible') to activate Tox21 targets, where do chemicals accumulate in fetal tissues? *In vitro* to *in vivo* extrapolation was performed using the HTTK R-package from a physiologically-based toxicokinetic model of a human mother and fetus. Here *in silico* parameters of chemical fraction unbound in plasma and intrinsic hepatic clearance were estimated from ADMET Predictor (Simulations-Plus). *In silico* estimated adult C_{max} values predicted *in vivo* human adult C_{max} with median absolute error of 1.03 for 491 chemicals, giving confidence in the R-package and *in silico* estimates. Case examples evaluating $\frac{C_{max}}{AC_{50}}$ values for peroxisome proliferator-activated receptor gamma (PPARγ) and glucocorticoid receptor revealed high rankings for glitazones and corticosteroids, respectively, at pharmacological doses. Doses required to elicit 'likely' interactions across all Tox21/ToxCast assays were compared to estimated daily exposures (Wambaugh et. al., 2014). 87 compounds were estimated to have 'likely' interactions at doses lower than maximum median-estimated daily environmental exposures. The major chemical use-categories included pharmaceuticals, chemical intermediates and natural products. Maximum fetal tissue concentrations (2nd trimester-birth) ranked as follows in decreasing order: rest of body, gut, liver, kidney, lung, and brain, <13x maternal C_{max}. Bisphenols were among the top concentrated across tissues at (due to higher concentrations and accumulating in fetal tissue) sufficient to modulate nuclear receptors. This approach can rapidly prioritize compounds and biological pathways where no experimental pharmacokinetic data exist. Out of domain compounds, passive transport and later stage developmental are issues that require further consideration. Nevertheless, this approach has shown promise toward estimating *in vivo* interaction concentrations for HTS data. *This abstract does not reflect official NTP or EPA views.*

Background

How can we meaningfully relate *in vitro* activity to interactions *in vivo*?

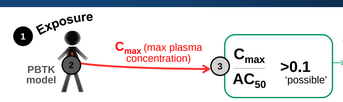
2006 FDA guidance for estimating clinically-impactful effects in humans from *in vitro* assay data[1]:

$\frac{[I]}{K_i} \sim \frac{[I]}{IC_{50}}$ Mean steady-state C_{max} (peak plasma concentration)
Inhibitor concentration required to elicit a 50% reduction of the maximal reaction rate or inhibition effect

As this ratio increases, the likelihood of an interaction increases	X>1 'likely'	1>X>0.1 'possible'	0.1>X 'remote'	& Efficacy > 40%

$\frac{Cmax}{AC_{50}}$ **We *hypothesize* that this approach can quantitatively extrapolate Tox21/ToxCast data to human *in vivo* relevance & estimate impacts to the fetus (via fetal tissue concentrations)**

Methods



1 Exposure Scenarios
Therapeutic – for validating the approach. 495 chemicals with *in vivo* dosing scenarios (Δdose, route, times/day, days) and C_{max}[2].

Environmental – From estimated daily exposure for all ToxCast chemicals (i.e., ExpoCast)[3]. Used maximum median estimated values.

2 PBTK (Physiologically-Based Toxicokinetic Model)
HTTK R-package[4,5] – parameterized using physicochemical properties & measured parameters: fraction of the chemical unbound in plasma (F_{ub}) and intrinsic metabolic clearance (CL_{int}) *in vitro*[6-15], where we estimated and used *in silico* derived F_{ub} & CL_{int} parameters[16].

Assumptions
- Oral absorption at user defined dose
  - Fast absorption rate (1/h)
  - 100% bioavailability
- Chemical's exit
  - Metabolism (Δ into metabolite in liver)
  - Excretion by passive glomerular filtration

Tissues
- Simple & compound
- Explicit & lumped

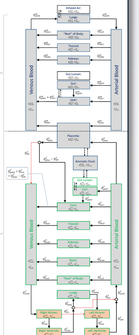


Figure 1. HTTK PBTK model

3 C_{max}/AC_{50} Ratios
AC_{50} and efficacy values from Tox21[17] and ToxCast[18] HTS assays. Data were filtered using inherent wAUC method[17] or curve-fitting flags[18].

4 Fetal Tissue Concs From Environmental Dosing Scenarios
A modified HTTK R-package was used to obtain fetal tissue concentrations at environmental dosing scenarios where the fetal compartment is a PBTK model inside the PBTK model (Kapraun DF, et. al. unpublished).

Novelties of the approach

- **FDA already regulates with this type of approach**
- **Grounded in human clinical effects**
- **Application to entire Tox21 library**
- **Incorporates efficacy & filtered data**
- **Novel *in silico* estimates requiring only chemical structures**
- **Conservative C_{max} vs C_{ss}**

In silico parameters approximate *in vivo* human data at therapeutic doses

In silico parameter evaluation

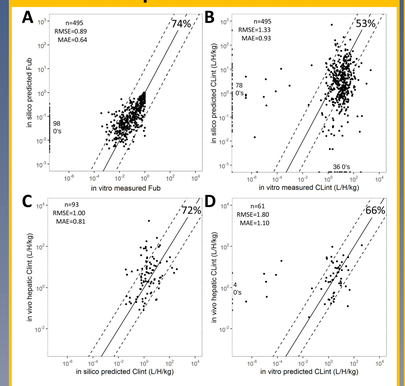
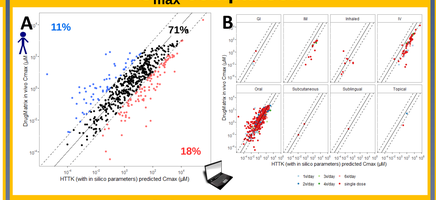
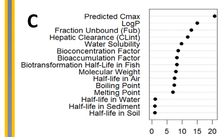


Figure 2. Fraction unbound and hepatic clearance parameter prediction comparisons. F_{ub} and CL_{int} parameters were compared between *in silico* estimated and *in vitro* measured values compiled in the HTTK R package for 495 Tox21 chemicals, respectively (A,B). *In vivo* hepatic CL_{int} values were compared to *in silico* estimated (93 chemicals, C) and *in vitro* measured (61 chemicals, D). Solid line is 1:1, dotted lines are 1 log_{10} difference, with the percentage of data lying within, total number of chemicals (n), root mean squared error (RMSE) and mean absolute error (MAE) noted.

C_{max} comparison



Confidence estimation



D

	10 fold under predicted	within 10 fold	10 fold over predicted
Sensitivity	65%	72%	71%
Specificity	91%	71%	85%
Balanced Accuracy	88%	72%	82%

Figure 3. *In vivo* vs *in silico* C_{max} comparison & assigning confidence. A) HTTK package with *in silico* parameters predicted C_{max} with RMSE = 1.03, MAE = 0.77 for 491 Tox21 chemicals, 613 scenarios at pharmacologically relevant doses. B) Comparison over administration route and colored by dose frequency. GI=gastro-intestinal, IM=intramuscle, IV=intravenous. A&B) Solid line is 1:1, dotted lines are 1 log_{10} difference. C) Fifteen features were used for predicting whether the compound-scenario C_{max} would be over-, under- or within- 10-fold. The features are ordered by importance based on a random forest model, with larger values indicating greater relative importance. D) Statistics were similar for each group, with better specificity for the outside 10-fold ranges leading to higher balanced accuracies.

Case study: glucocorticoid receptor known *in vivo* GR agonists have high $\frac{C_{max}}{AC_{50}}$

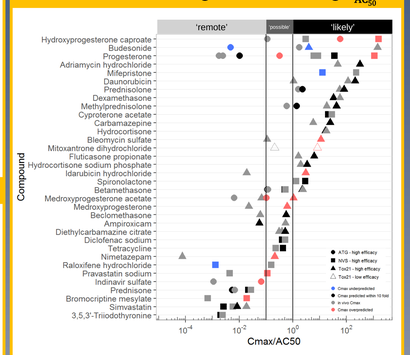


Figure 4. $\frac{C_{max}}{AC_{50}}$ ratios for GR pathway at pharmacological doses. 30 active compounds with therapeutic *in vivo* dosing scenarios and C_{max} values and AC_{50} values in GR Tox21 and ToxCast assays were evaluated for $\frac{C_{max}}{AC_{50}}$ ratios. Colored points indicate the accuracy of the *in silico* predicted C_{max} values (blue, black, red) vs *in vivo* measured C_{max} value (grey). Vertical lines at 0.1 and 1 show regions of 'remote' (<0.1), 'possible' (0.1<X<1) and 'likely' (>1) *in vivo* interaction. Efficacies greater than 40% or 2-fold change were assigned as high efficacy, all others were assigned low efficacy.

Tox21/ToxCast Assays: $\frac{C_{max}}{AC_{50}}$ predicts interactions at estimated environmental doses

57,260 active compound-assay pairs (4083 CASRN, 827 assays)

ExpoCast daily dose 3x's day until plasma conc. @steady state → $\frac{C_{max}}{AC_{50}}$ how many >1, ('likely')? how many>0.1, ('possible')?

A

	'likely'	'possible'
chem-assay pairs	32	202
unique chemicals	18	87
unique assays	20	101

B

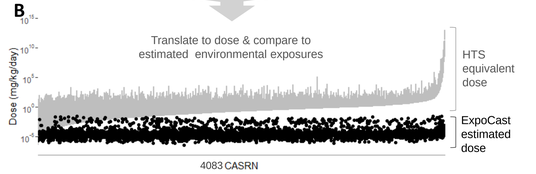


Figure 5. Dose ranges for all active Tox21 compounds eliciting a possible-to-likely human *in vivo* interaction alongside estimated daily exposure. AC_{50}s for active Tox21 compounds with efficacies >40% or 2-fold in the HTS assays were converted to equivalent human *in vivo* doses using exposure scenarios where *in vivo* plasma concentrations were equal to and one tenth of the AC_{50} concentration (likely and possible *in vivo* interactions, respectively) (gray bars). Exposure estimates in terms of dose per day were calculated from NHANES biomonitoring data and physicochemical properties and use categories[3] (black dots).

$\frac{C_{max}}{AC_{50}}$ for 202 'possible' interactions

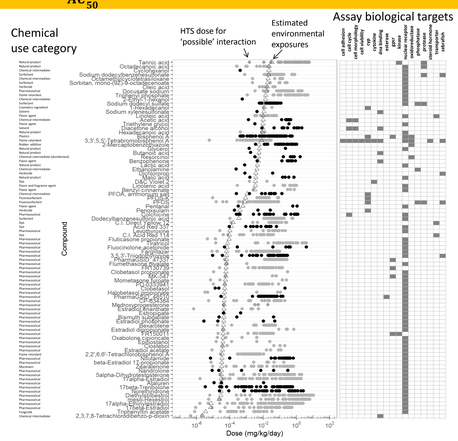


Figure 6. Doses of compounds eliciting a 'possible' human *in vivo* interaction. Assay biological targets are indicated for 'possible' interactions below estimated environmental exposures (triangles). Black (high confidence), gray (lower confidence)

Fetal tissue concentration distribution during 'possible' maternal interactions

Fetal tissue vs. maternal plasma concentrations

Table 1. Summaries of maximal fetal tissue concentrations for all 87 chemicals using 202 dosing scenarios (daily dose, 3x's a day, 30 days) from Figure 6 at 'possible' maternal interactions that are below estimated environmental exposures.

Tissue	Median (μM)	Mean (μM)	Range (μM)
Cplacenta	0.01	11.59	0.0E+00 - 442.90
Cfrest	0.02	2.87	1.9E-05 - 26.67
Cfgut	0.02	2.84	1.4E-05 - 26.53
Cfliver	0.03	2.38	2.4E-05 - 22.22
Cfkidney	0.03	1.87	2.0E-05 - 17.24
Cflung	0.01	1.75	1.4E-05 - 16.80
Cfbrain	0.005	0.82	2.0E-06 - 7.63
Cfplasma	0.02	0.30	4.5E-06 - 2.43
Cplasma	0.02	0.35	4.7E-06 - 3.12

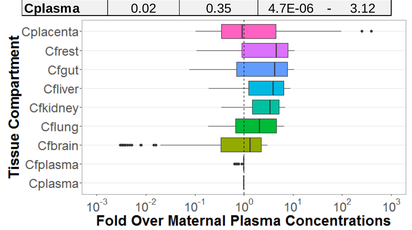
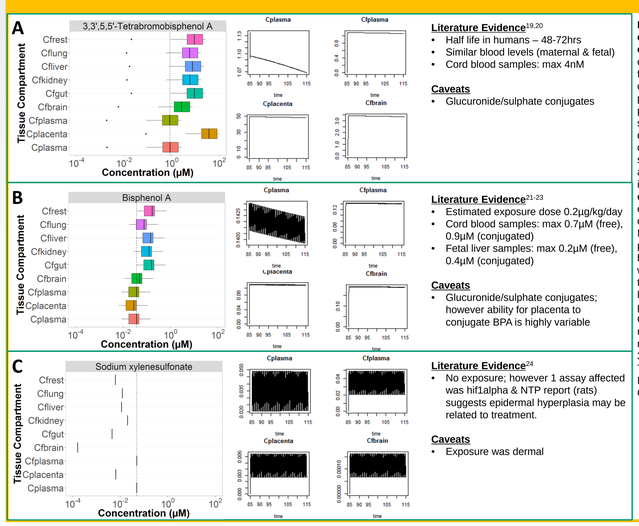


Figure 7. Fetal tissue concentration over maternal peak plasma concentrations. Average concentrations were within 5-fold of maternal plasma concentrations. Placental and fetal brain concentrations varied the most, due mainly to 3,3',5,5'-tetrabromobisphenol A and pharmaceuticals, respectively.

Fetal tissue vs. maternal plasma concentrations for 3 example chemicals



A 3,3',5,5'-Tetrabromobisphenol A

Literature Evidence[19,20]
- Half life in humans – 48-72hrs
- Similar blood levels (maternal & fetal)
- Cord blood samples: max 4nM

Caveats
- Glucuronide/sulphate conjugates

B Bisphenol A

Literature Evidence[21-23]
- Estimated exposure dose 0.2μg/kg/day
- Cord blood samples: max 0.7μM (free), 0.9μM (conjugated)
- Fetal liver samples: max 0.2μM (free), 0.4μM (conjugated)

Caveats
- Glucuronide/sulphate conjugates; however ability for placenta to conjugate BPA is highly variable

C Sodium xylenesulfonate

Literature Evidence[24]
- No exposure; however 1 assay affected was hif1alpha & NTP report (rats) suggests epidermal hyperplasia may be related to treatment.

Caveats
- Exposure was dermal

Figure 8. Fetal tissue concentration for 3 select chemicals. Chemical concentrations were shown for fetal tissue compartments, maternal plasma (Cplasma) & placenta (Cplacenta) for 3 select chemicals showing different patterns of tissue concentrations. Dosing scenarios are from Figure 6 at 'possible' maternal interactions that are below estimated environmental exposures (points to the left of the triangles in Figure 6). Maternal plasma, fetal plasma, placenta and fetal brain tissue concentrations were plotted over gestation time in days. Vertical dotted line is the median maternal plasma concentration. Concentration values to the right are higher than maternal plasma. A) 3,3',5,5'-Tetrabromobisphenol A, B) Bisphenol A, C) Sodium xylenesulfonate

Discussion and Conclusion

Our goal here was to develop a data driven approach to quantitatively relate *in vitro* Tox21 and ToxCast assay data to humans.

A straight forward $\frac{C_{max}}{AC_{50}}$ ratio approach was used with benchmarks of >0.1 (possible) and >1 (likely) in the spirit of methods used to translate *in vitro* human liver enzyme data to clinical relevance.

Predicted C_{max} values for Tox21 chemicals, estimated using *in silico* F_{ub} and CL_{int} parameters, were comparable to *in vivo* plasma concentrations (RMSE = 1.03, MAE = 0.77). IVIVE using this $\frac{C_{max}}{AC_{50}}$ approach for GR agonist assays revealed likely *in vivo* interactions for corticosteroid compounds at therapeutic levels demonstrating the utility of the approach.

Applying this approach to the balance of the Tox21/ToxCast assays revealed 202 chemical-biological interactions as possible/likely *in humans* at everyday exposure concentrations. Evaluating predicted fetal tissue concentrations revealed, on average, similar fetal plasma concentrations vs. maternal plasma concentrations, with the fetal placenta and brain varying most.

This approach provides an intuitive framework to rapidly and quantitatively relate real-world chemical exposures to available *in vitro* bioactivity screening data.

Future Directions

- Explore chemical-target interactions
- Evaluate additional biological targets & assay data
  - Toxicogenomics
- Evaluate alternate models
  - Additional metabolism
  - FDA Drug Guidance document (2012)
  - POD vs AUC vs AC50
  - Css vs Cmax
  - Efficacy limit differences
- Models will continue to improve with the incorporation of more data, specifically publically available data on thousands compounds for
  - *in vitro* HTS/HCS
  - *in vivo* toxicokinetics
  - Estimates for parameters that influence transporters, glucuronidation/sulfonation, fetal F_{ub} & CL_{int} , for example
- This approach can serve as a foundation to develop predictive tools in determining those chemicals likely to accumulate in certain fetal target tissues

References

1. FDA Guidance for Industry: Drug Interaction Studies (2006)
2. DrugMatrix® - https://ntp.niehs.nih.gov/drugmatrix
3. Wambaugh JF et. al., (2014), Environ Sci Technol 48(21):12760-12767
4. HTTK – R package, v1.4 https://cran.r-project.org/web/packages/httk/index.html
5. Wambaugh JF et. al., (2015) Toxicol Sci; 147(1):55-67
6. Ito K and Houston JB (2004) Pharm Res; 21(5):785-92
7. Jones OA, Voulvoulis N, and Lester JN (2002) Water Res; 36(20):5013-22
8. Naritomi Y et. al., (2003) Drug Metab Dispos; 31(5):580-8
9. Obach RS (1999) Drug Metab Dispos; 27(11):1350-9
10. Obach RS, Lombardo F, and Waters NJ (2008) Drug Metab Dispos; 36(7):1385-405
11. Riley RJ, McGinnity DF, and Austin RP (2005) Drug Metab Dispos; 33(9):1304-11
12. Schmitt W (2008) Toxicol In Vitro; 22(2):457-67
13. Tonnelier A, Coecke S, and Zaldivar JM (2012) Arch Toxicol; 86(3):393-403
14. Wetmore BA et. al., (2012) Toxicol Sci; 125(1):157-74
15. Wetmore BA et. al., (2015) Toxicol Sci; 148(1):121-36
16. Ferguson SS et. al., unpublished via ADMET Predictor 7.2, Simulations Plus Inc.
17. Hsieh JH et. al., (2005) J Biomol Screen; 20(7):887-97
18. https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data
19. European Food Safety Authority (2011), EFSA Journal 9(12):2477
20. Cariou R et. al., (2008), Chemosphere 73(7):1036-1041
21. Chou WC et. al., (2011), Envrion Health 10(94)
22. Doerge DR et. al., (2011), Toxicol Appl Pharmacol 255(3):261-70
23. Cao XL et. al., (2012), Chemosphere 89(5):505-511
24. NTP (1998), NTP TR 464, publication no. 98-3380

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