Using *in silico-in vitro* to *in vivo* Extrapolation (IS-IVIVE) to Predict the Oral Dose Required to Activate the Aryl Hydrocarbon Receptor (AhR)

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Introduction

- Activation of AhR can have toxic effects on mammalian hosts
  - Activation of AhR depends on the *in vivo*, steady state, unbound, plasma concentration of the compound and its relationship to the AC50 value and the Emax value in order to exhibit toxicity
- We used IS-IVIVE to predict the oral dose needed to achieve this steady state plasma concentration[1]
- AhR active compounds (AC50 < 100 µM) from a TOX21 assay were downloaded from PubChem[2]
  - 1,063 substances were active
- *In vitro* fraction unbound in plasma (Fu_p) and human hepatocyte intrinsic clearance data was extracted from the httk R-package[3]
  - 1,425 records contained Fu_p values
    - 789 records were from EPA publications[4,5]
    - The remaining data came from various publications
  - 1,020 records contained human hepatocyte CL_{int} values
    - 828 records were from EPA publications[4,5]
- The final data set consisted of **160 compounds** that were in all three data sets
  - The data set contains agrochemicals, pharmaceuticals, and industrial chemicals
- All other input properties, e.g., solubility, logP, pKa, were predicted from machine learning models[1]

Methodology

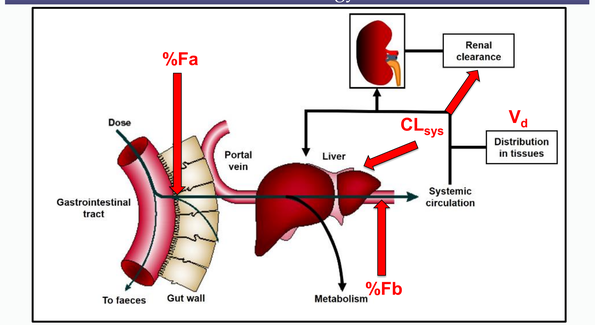


Figure 1 - The HTPK Simulation module[1] was used to predict the dose required to reach the *unbound* AC50 concentration. It uses the ACAT™ model[4] to simulate dissolution and absorption (%Fa) of an immediate release tablet in the gastrointestinal tract.

Results

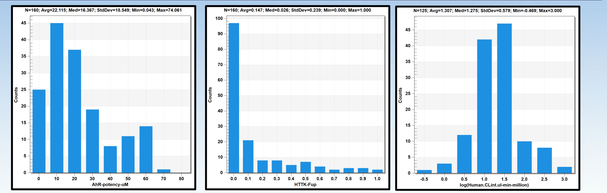


Figure 2– Distribution plots of *in vitro* data. The AhR AC50 values (left) ranged from 0.043 to 74.1 µM. Fu_p values (middle) stretched from 0.0 to 1.0 with 38 values less than or equal to 0.005. Human hepatocyte CL_{int} values (right) varied from 0 to 1,000 µl/min/million cells.

- AC50 values were divided by Fu_p to yield the total plasma concentration needed to achieve the *unbound* AC50 concentration
  - Compounds with Fu_p = 0.005 (highly protein bound) need to reach a total plasma concentration 200 times greater than their AC50 value
- Figure 3 shows a plot of the predicted dose versus the log of the total plasma concentration
  - 18 compounds were able to reach their unbound AC50 value with a daily dose of less than 1,000 mg
    - These compounds had lower unbound AC50 values
      - Average value is 70 µM compared to 1,715 µM
    - 17/18 compounds had %Fa > 90%
    - These compounds had low human hepatocyte CL_{int}
      - Average value is 1.2 µl/min/million cells compared to 51

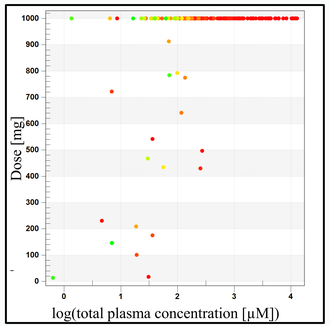
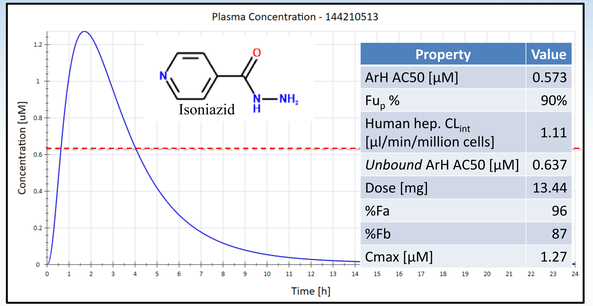


Figure 3 - Plot of the predicted dose versus the log of the plasma concentration (equal to the unbound AC50 value). The points are colored by Fu_p (red – low, yellow – medium, and green high).

Results

- Isoniazid was predicted to have the lowest dose (13.4 mg) needed to reach its unbound AhR AC50 value.



Property	Value
ArH AC50 [µM]	0.573
Fu_p %	90%
Human hep. CL_{int} [µl/min/million cells]	1.11
Unbound ArH AC50 [µM]	0.637
Dose [mg]	13.44
%Fa	96
%Fb	87
Cmax [µM]	1.27

Figure 4 – Total plasma concentration versus time curve for a 13.4 mg dose of isoniazid. The red, dashed line indicates the total plasma concentration needed to achieve the *unbound* AC50 value. The compound is highly permeable and soluble which results in a high %Fa of 96%. The compound is cleared fairly quickly, none of it remains in the blood stream after 24 hours. This combination of parameters means that the compound reaches steady state quickly.

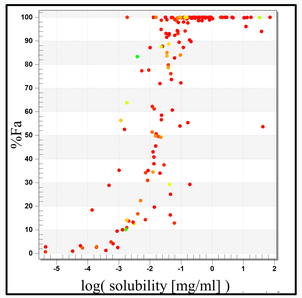
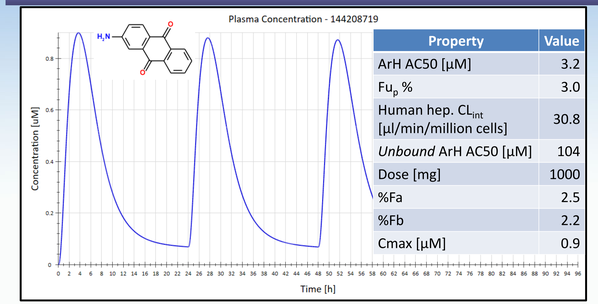


Figure 5 - Plot of fraction absorbed versus log of the aqueous water solubility. Simulations were performed at the dose predicted to achieve their unbound AC50 value. The points are colored by their ArH unbound AC50 values. The compounds in the lower left hand corner have low %Fa values due to low solubility.

References

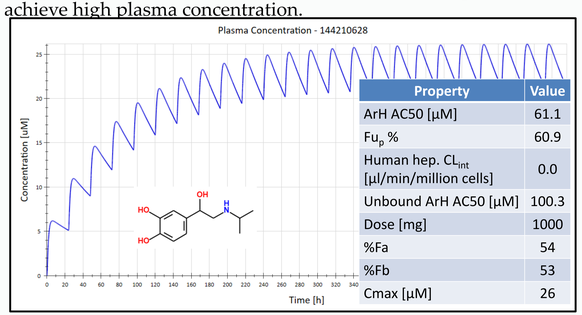
[1]Simulations were performed using the HTPK Simulation module in ADMET Predictor® 9.5 from Simulations Plus, Inc. Lancaster CA
[2]qHTS assay to identify small molecule that activate the aryl hydrocarbon receptor (AhR) signaling pathway. PubChem AID 743122.
[3]Pearce RG, Setzer RW, Strope CL, Sipes NS, and Wambaugh JF. httk: R Package for High-Throughput Toxicokinetics, J Stat. Software, 2017, 79, 4.
[4]Wetmore BA et al. Integration of Dosimetry, Exposure, and High-Throughput Screening Data in Chemical Toxicity Assessment. *Tox. Sci.*, **2012**, 125(1), 157-174.
[5]Wetmore BA, Incorporating High-Throughput Exposure Predictions with Dosimetry-Adjusted *In Vitro* Bioactivity to Inform Chemical Toxicity Testing. *Tox. Sci.* **2015**, 148(1), 121-136.
[6]Advanced Compartmental Absorption and Transit (ACAT™) Model. Simulations Plus, Inc. Lancaster California.

Results



Property	Value
ArH AC50 [µM]	3.2
Fu_p %	3.0
Human hep. CL_{int} [µl/min/million cells]	30.8
Unbound ArH AC50 [µM]	104
Dose [mg]	1000
%Fa	2.5
%Fb	2.2
Cmax [µM]	0.9

Figure 6 – Example of a low predicted solubility (0.92 µg/ml) compound. This results in low %Fa (2.5%) so the compound cannot achieve high plasma concentration.



Property	Value
ArH AC50 [µM]	61.1
Fu_p %	60.9
Human hep. CL_{int} [µl/min/million cells]	0.0
Unbound ArH AC50 [µM]	100.3
Dose [mg]	1000
%Fa	54
%Fb	53
Cmax [µM]	26

Figure 7 – Example of a low predicted human jejunal permeability (0.7 x 10^{-4} cm/s) compound. This results in 54% absorption and Cmax of 26 µM which is far below the concentration needed to activate AhR.

Conclusions

The compounds that were able to reach their unbound AC50 values tended to have low unbound AC50 values, good absorption, and low hepatic clearance. Compounds with low Fu_p required higher total plasma concentration to activate AhR. Low solubility compounds have low %Fa values that prevent them from achieving high enough plasma concentrations to activate AhR.

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