In silico Modeling of Aryl Hydrocarbon Receptor (AhR) Activation

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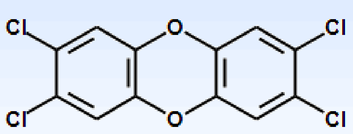
Introduction

Sustained activation of AhR is the molecular initiating event (MIE) in several adverse outcome pathways (AOPs)[1]:

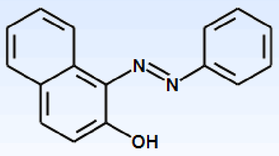
- Lethality to fish and bird embryos
- Causing uroporphyria in birds
- Producing liver tumors in rodents

Results from a quantitative high throughput screening (qHTS) assay, run as part of the U.S. Tox21 program[2] designed to detect AhR activators, were recently made public.[3] Our objective was to use those data to develop an *in silico* classifier to identify AhR activators from their structure.

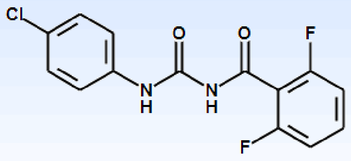
- Compounds with AC_{50} < 100 μM were categorized as activators ("positives").
- A few of the most potent activators are below (SID = PubChem substance ID).
- The data set is highly skewed towards negatives, with only 11% of the compounds tested being categorized as positives.



2,3,7,8-TCDD
(dibenzodioxin)
SID 144209990
AhR AC_{50} = 0.694 nM
AhR Efficacy = 113%



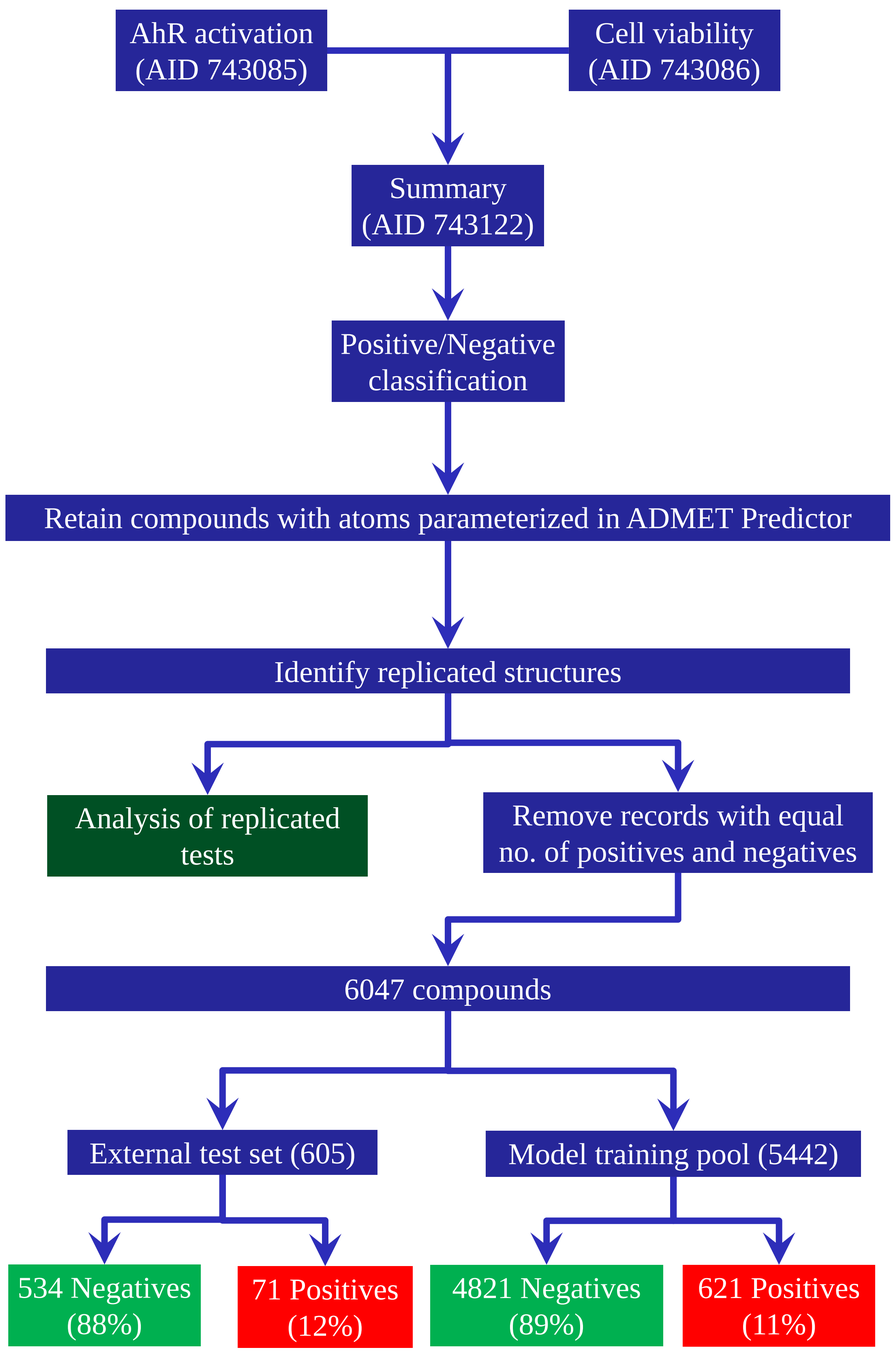
C.I. Solvent Yellow 14
(dye)
SID 144210617
AhR AC_{50} = 137 nM
AhR Efficacy = 82%



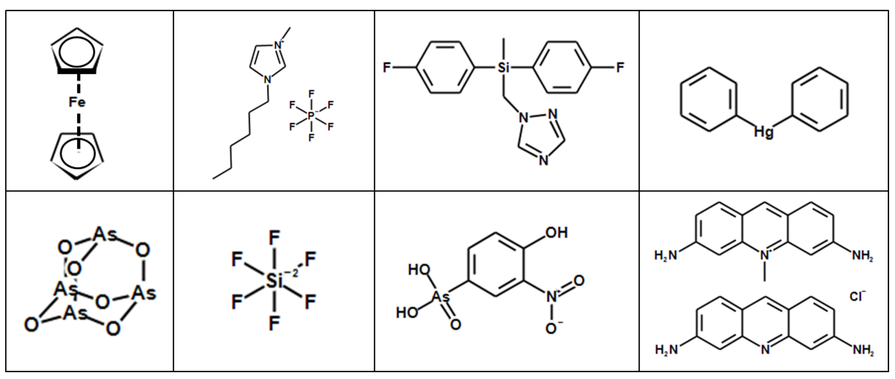
Diflubenzuron
(insecticide)
SID 144213455
AhR AC_{50} = 142 nM
AhR Efficacy = 110%

Preparation of the Modeling Set

AID743122 (AID = PubChem bioassay ID) has two components: a cell-based AhR activation assay (AID743085) run in triplicate at 15 concentrations, and a cell viability counter screen (AID743086). AhR results were reported in binary format: 1 for positives and 0 for negatives. ADMET Predictor™ [4] descriptors are parameterized for H, B, C, N, O, P, S, Cl, Br, I, and F, so compounds containing other elements were removed. Ionic salts were also removed. Salts and other mixtures were resolved based on molecular size. Categorization of molecules tested more than once was by majority vote, with ties being set aside. The data set was then divided into a training set for developing models and a completely external (blind) test set for validating them after models were trained. Both subsets contain a similar balance of positives (about 11%) to negatives (about 89%).



Examples of Compounds Removed

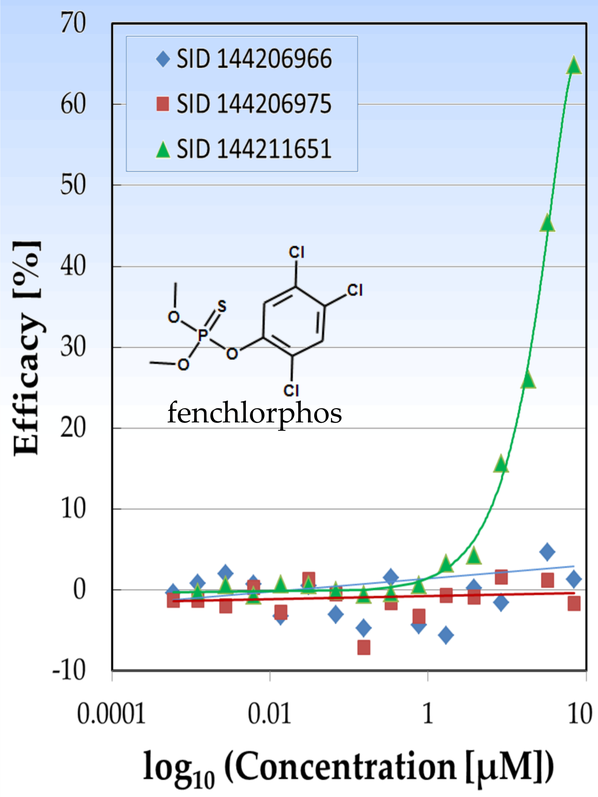


Analysis of Replicated Tests

Each substance tested was identified with the molecular structure of the presumed active ingredient. Some structures (1363) were identified with more than one substance - e.g., as different salt forms or as the same compound obtained from different sources. For 1151 of these, all replicates were categorized as negative; for 153, all were categorized as positive. Results for the remaining 59 structures were ambiguous, i.e., some were categorized as positive and some negative.

In order to confirm how often a positive experimental result, on average, was *reproduced*, we analyzed the 212 structures (153+59) that were categorized positive at least once. There were 469 total replicates resulting from these 212 structures; 400 were positives (85%). Thus, our confidence in any positive result is only 85%.

Fenchlorphos, for example, was tested as the main ingredient in three samples, i.e., the same compound from different suppliers, each with its own SID. Their dose-response curves are shown at right. SID 144211651 activates AhR when assayed at higher concentrations, whereas the other two samples were inactive. Taken together, these results led to the fenchlorphos *molecule* being categorized as inactive. The activity seen for SID 144211651 is quite possibly due to some minor contaminant in the sample.

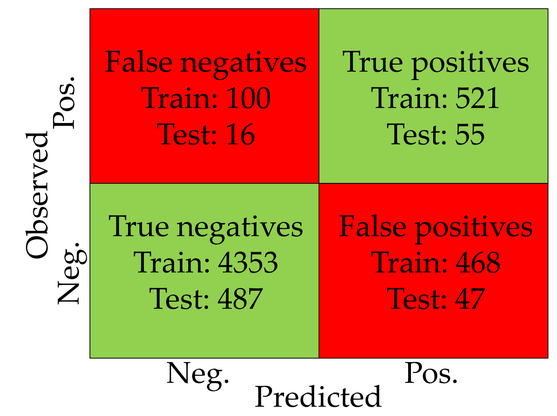


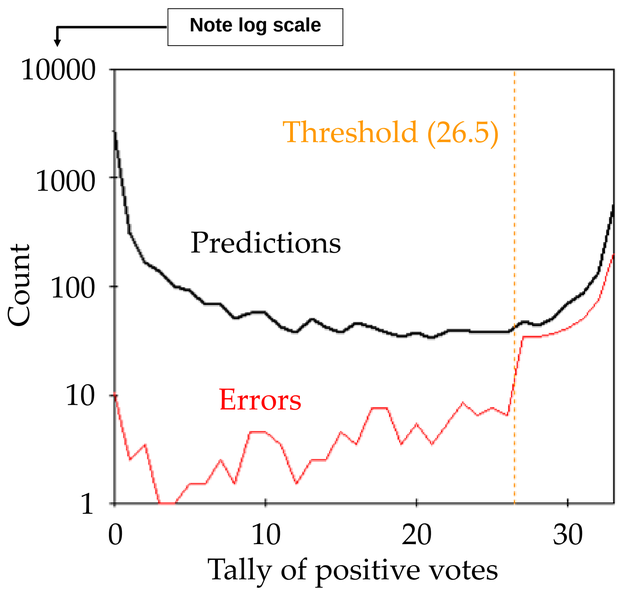
Aryl Hydrocarbon Receptor Model

ADMET Modeler™ [4] was used to create an ensemble classification model comprised of 33 individually trained artificial neural networks, each of which classifies each query molecule as negative or positive. The tabulated performance statistics of the ensemble model and the corresponding truth table are shown below.

	Concordance	Sensitivity	Specificity	PPV	NPV
Train	90%	84%	90%	53%	98%
Test	90%	77%	91%	54%	97%

PPV (positive predictive value) is the number of true positives divided by the number of positive predictions. NPV (negative predictive value) is the number of true negatives divided by the number of negative predictions. Note that the training set sensitivity is very close to the observed positive confirmation rate mentioned above.





The figure at left shows the distributions of predictions and errors for the data set across the tally of positive votes. The black line shows the number of predictions for each degree of consensus and the red line is the number of predictions that were incorrect. The ensemble prediction for a structure is negative unless at least 27 networks classify it as a positive, a threshold of consensus that is derived from the distribution of errors across the data set.[5]

Summary

Data from a qHTS assay that was performed as part of the US Tox21 program were used to create a model to identify AhR activators. AhR activators that were assayed more than once were used to determine that the positive reproducibility of the assay was about 85%. The data set was highly skewed towards negatives. Overall, the classification model obtained when compounds with an AC_{50} less than 100 μM are categorized as positives is highly predictive with a sensitivity of 84% and a concordance of 90%.

References

1 AOP wiki (https://aopkb.org/aopwiki/index.php/Event:18)

2 NTP, (2004) *A National Toxicology Program for the 21st Century: A Roadmap for the Future*. NRC, (2007) *Toxicity Testing in the 21st Century: A Vision and a Strategy*. The National Academies Press.

3 Assay results are available from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=743122)

4 Simulations Plus, Inc. Lancaster CA USA

5 Clark RD et al., *J. Cheminfo.* **2014** ; 6:34.

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