Cheminformatics Analysis of EPA ToxCast Chemical Libraries to Identify Domains of Applicability for Predictive Toxicity Models and Prioritize Compounds for Toxicity Testing

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INTRODUCTION

- EPA ToxCast™ Program aims to predict hazard, characterize toxicity pathways, and prioritize the toxicity testing of environmental chemicals.
- Phase I ToxCast™ has profiled 320 well-characterized chemicals (primarily pesticides) in 524 endpoints, including biochemical assays of protein function, cell-based transcriptional reporter assays, multi-cell interaction assays, transcriptomics on primary cell cultures, and developmental assays in zebra fish embryos. Most of these compounds have been tested also in 76 developmental toxicity, multi-generation studies, and sub-chronic and chronic rodent bioassays.
- As part of ToxCast™ , Toxicity Reference Database (ToxRef DB) includes the following historical toxicity data from 26 *in vivo* chronic/cancer endpoints in rats and mice for 310 food-use pesticides.
  - 16 rat endpoints and 10 mouse endpoints.
  - Each endpoint has both Lowest Effect Level (LEL) (range of -3.99 ~ 1.07 for -log10(LEL)) value and classification index of non-toxic/toxic.
  - On average, there are about 18% toxic compounds for each endpoint.
- Cumulative toxicity indices were also created from some ToxCast™ endpoints, such as "toxic in rats/mouse", "nonspecific tumorigenicity", and "toxic in cell viability".

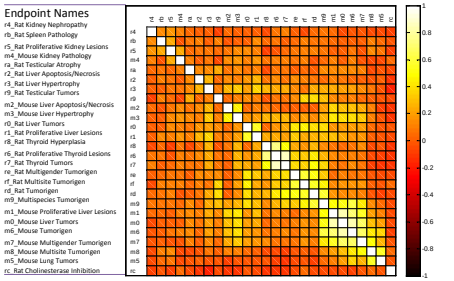


Figure 1 Correlations of 26 endpoints in ToxRef database. Each blot represents the correlation between the toxicity index for all ToxRef compounds of two endpoints.

METHODOLOGY

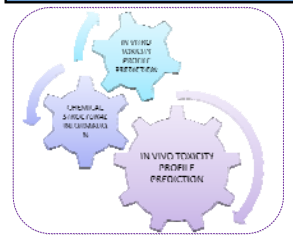


Figure 2 Scheme of the QSAR studies on ToxCast *in vitro* and *in vivo* toxicity data.

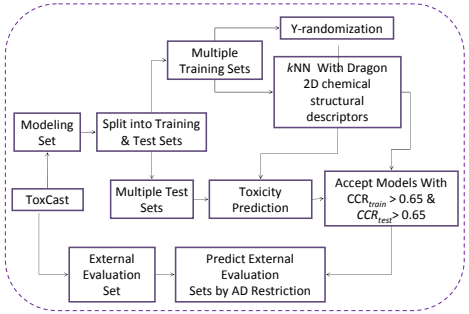


Figure 3 Modeling workflow. Models were developed using kNN QSAR approach, Dragon structural descriptors, and 5-fold external cross–validation. Structural outliers were removed from training sets. Abbreviations: QSAR - Quantitative Structure-Activity Relationships; *k*NN - *K*-Nearest Neighbors; AD - Applicability Domain.

RESULTS

QSAR ON INDIVIDUAL ENDPOINTS

Table 1 QSAR model parameters for some of the 26 ToxRef endpoints.

Endpoint	# of Dragon Descriptors	# of Models	# of Data Sets	CCR_{test}	SE	SP
Rat Liver Hypertrophy	381	11	142	0.70	0.60	0.80
Rat Kidney Nephropathy	381	54	78	0.79	1.00	0.57
Rat Cholinesterase Inhibition	381	1228	94	0.80	0.80	0.80
Mouse Tumorigen	381	240	191	0.71	0.75	0.67
Mouse Multi-gender Tumorigen	381	68	105	0.89	1.00	0.78
Mouse Multisite Tumorigen	381	22	44	0.67	1.00	0.33
Multispecies Tumorigen	381	27	87	0.81	1.00	0.63

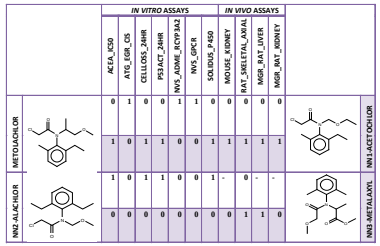
QSAR ON TOXICITY INDEX ENDPOINTS

Table 2 Results of QSAR models on toxicity index endpoints. 5-fold external cross validation was used; mean CCR, SE and SP are shown. Abbreviations: CCR: Correct Classification Rate; SE: sensitivity; SP: specificity .

Category Name	Endpoints Involved#	Non-toxic Comp #	Toxic Comp #	Descriptor#	Model #	Aver. CCR	Aver. SE	Aver. SP
total	26	52	240	705	73	0.58	0.97	0.19
rat	16	86	206	705	80	0.51	0.84	0.18
mouse	9	143	149	705	408	0.6	0.59	0.61
tumor	7	132	160	705	97	0.51	0.56	0.45
rat liver	4	184	108	705	168	0.58	0.38	0.77
mouse liver	4	170	122	705	116	0.63	0.52	0.73
Cell viability	7	123	98	737	104	0.81	0.84	0.81

HARD-TO-PREDICT COMPOUND

Table 3 Example of a hard-to-predict compound and its three nearest neighbors. The *in vitro* and *in vivo* toxicity profiles are compared.



CORRELATION BETWEEN *IN VIVO* AND *IN VITRO* ASSAYS IN TOXCAST

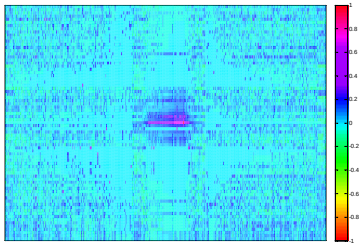


Figure 3 Matthews correlations of *in vivo (y axis)* vs. *in vitro (x axis)* endpoints in ToxCast. In total there are 101 (75+26) *in vivo* and 409 *in vitro* endpoints. The correlations were calculated using data on 320 ToxCast compounds.

DISCUSSIONS

- QSAR models based on chemical structural descriptors are predictive for certain toxicity endpoints (e.g., mouse tumorigen).
- Removing structural outliers and using the applicability domain threshold can effectively increase the prediction power of QSAR models.
- *in vivo* toxicity profiles for some compounds were difficult to predict. A possible reason is that they have fairly different *in vitro* toxicity profiles as compared with their structural nearest neighbors. It appears that chemical structural descriptors alone may be insufficient to enable accurate prediction of *in vivo* toxicity profiles. In order to obtain better predictions for these compounds, their *in vitro* toxicity profiles may need to be incorporated as biological descriptors into modeling process.
- Future studies should concentrate on improving the prediction power of models taking into account the entire chemical structure – *in vitro* – *in vivo* data continuum. We shall consider novel methodologies combining chemical and biological descriptors for building hybrid QSAR models as well as approaches such as multi-task learning.

CONCLUSIONS

- We have developed predictive QSAR models based on chemical structural descriptors for some of the toxicity endpoints.
- *In vitro* data should be used to help improve the prediction power of QSAR models on *in vivo* toxicity endpoints.

REFERENCES:

- Martin et al. *Environmental Health Perspectives* 2009 117(3):392–399
- Dix et al. *Toxicological Sciences* 2007 95(1):5-12

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