Probabilistic Environmental Hazard Assessments of ToxCast Phase I and II In Vitro Datasets

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INTRODUCTION

- 700+ commercial chemicals are introduced to the US market each year. Over 85% are approved for manufacturing despite the lack of experimental health and safety data.
- EPA ToxCast initiative, launched in 2007, uses high-throughput screening assays to expose living cells to chemicals to evaluate potential toxic and adverse health effects.
  - Phase I & II included 1859 chemicals
- There is a need for computational approaches to utilize existing toxicological data to then predict thresholds of untested chemicals and identify assays for assessing toxicity: estrogen agonist activation and responses to oxidative stress such as:
  - Chemical toxicity distributions (CTDs) utilized to perform probabilistic hazard assessments (PHAs)

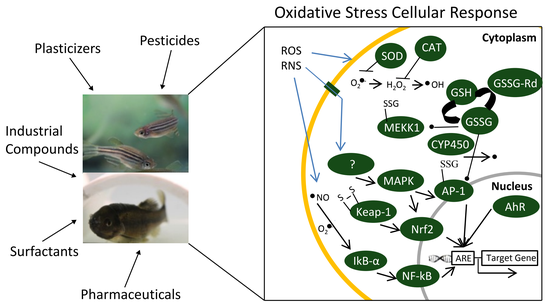

Figure 1: General oxidative stress mechanism. Developed from J. Limón-Pacheco and M.E. Gonsebatt *Mutation Research* 2009; 674: 137-147. Photo credit: Samuel P. Haddad.

STUDY OBJECTIVES

- Using CTDs, develop PHAs for chemicals tested by ToxCast and the Endocrine Disruptor Screening Program (EDSP)
- Identify compounds with the highest potency to activate the estrogen receptor and elicit oxidative stress
- Examine the comparative sensitivity of *in vitro* assays for activity representing a diverse group of chemicals

MATERIALS AND METHODS

- Data were compiled from the U.S. EPA's ToxCast database for *in vitro* estrogen agonist and oxidative stress related assays
- Data from three estrogen agonist and five oxidative stress response *in vitro* promoter assays are presented; descriptions are available in Table 2.
  - Utilized human liver and kidney cells to measure responses
- Percent Rank was assigned to each compound using a Weibull formula:

$$j = (i * 100) / (n + 1)$$

- CTDs for each assay were constructed by plotting EC_{50} values on a logarithmic scale versus a probability scale following methods previously described [1,2]

RESULTS

Estrogen Agonist Assay Comparison

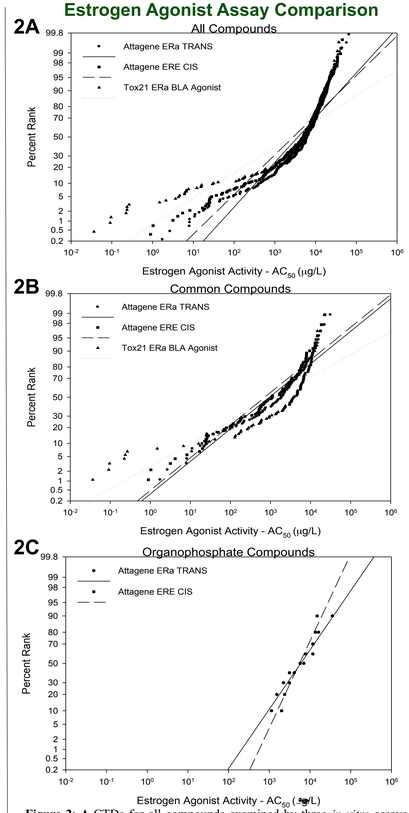

Figure 2: **A** CTDs for all compounds examined by three *in vitro* assays of estrogenicity. **B** CTDs for common compounds examined by the three *in vitro* assays. **C** CTDs for common organophosphate compounds examined by two Attagene *in vitro* assays of estrogenicity.

Exposure Duration Comparison

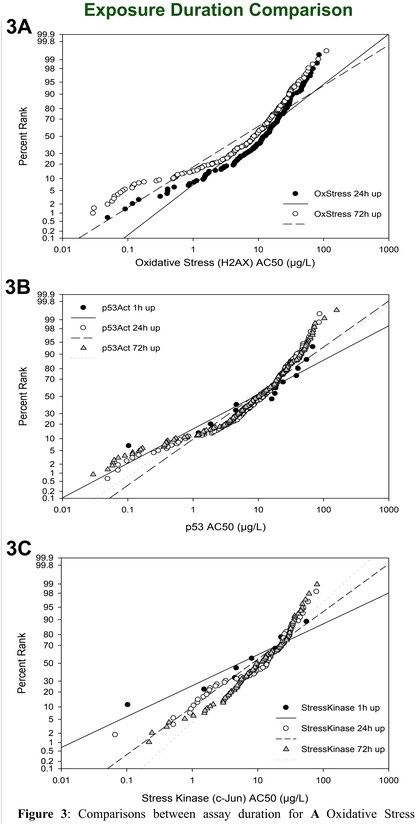

Figure 3: Comparisons between assay duration for **A** Oxidative Stress (H2AX), **B** p53, and **C** Stress Kinase (c-Jun) assays.

Oxidative Stress Assay Comparison

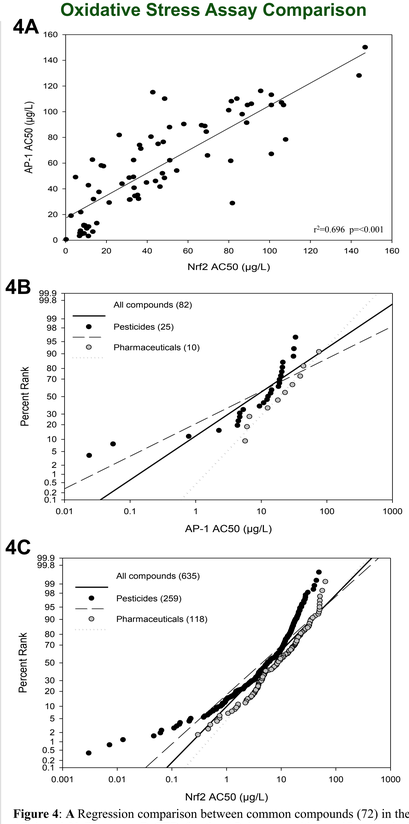

Figure 4: **A** Regression comparison between common compounds (72) in the AP-1 and Nrf2 assays. **B** CTD for the AP-1 assay with specific chemical compound class regressions. **C** CTD for the Nrf2 assay with specific chemical compound class regression comparisons.

Table 1. Equations for regression lines and values corresponding to various centiles for CTDs of: **[2A]** All ToxCast Phase I&II data for all compounds; **[2B]** for common chemicals; **[2C]** and common Organophosphate Compounds. **[3A-C]** Equations for regression lines and CTD centiles for **a**ll ToxCast Phase I and II data for three oxidative stress related assays for three time points. **[4A-C]** Equations for regression lines and CTD centiles for all ToxCast Phase I&II AP1 and NRF2 assays for both all compounds and selected compound classes.

	Assay	n	r^2	a (slope)	b (intercept)	Centile value **(µg/L)** 1%	5%
[2A]	Attagene_Era_TRANS	437	0.859	1.238	-4.417	48.8	173
	Attagene_ERE_CIS	282	0.858	1.097	-3.789	21.5	90.0
	Tox21 ERa BLA Agonist	227	0.724	0.710	-2.400	1.27	11.6
[2B]	Attagene_Era_TRANS	90	0.924	0.903	-2.682	2.48	14.1
	Attagene_ERE_CIS	90	0.939	0.906	-2.587	1.94	11.0
	Tox21 ERa BLA Agonist	90	0.734	0.621	-1.911	0.214	2.69
[2C]	Attagene_Era_TRANS	9	0.975	1.602	-6.045	210	558
	Attagene_ERE_CIS	9	0.941	2.349	-8.797	568	1110
	Assay	n	r^2	a (slope)	b (intercept)	Centile value **(µg/L)** 1%	5%
[3A]	Oxidative Stress (H2AX) 24hr	147	0.897	1.525	-1.472	0.275	0.77
	Oxidative Stress (H2AX) 72hr	205	0.883	1.234	-0.930	0.074	0.263
[3B]	p53 activity 1hr	14	0.890	1.044	-0.987	0.052	0.234
	p53 activity 24hr	160	0.895	1.398	-1.299	0.184	0.565
	P53 activity 72hr	223	0.901	1.294	-1.129	0.119	0.399
[3C]	Stress Kinase (c-Jun) 1hr	8	0.900	0.907	-0.659	0.014	0.082
	Stress Kinase (c-Jun) 24hr	55	0.916	1.397	-1.282	0.179	0.550
	Stress Kinase (c-Jun) 72hr	96	0.945	1.759	-1.717	0.450	1.099
[4A]	Nrf2 vs AP-1	73	0.696	0.876	17.193	--	--
[4B]	**AP-1**	**82**	**0.852**	**1.315**	**-1.179**	**0.134**	**0.442**
	Pesticides	25	0.716	0.972	-0.814	0.028	0.140
	Pharmaceuticals	10	0.962	2.064	-2.622	1.391	2.975
[4C]	**Nrf2**	**635**	**0.899**	**1.648**	**-1.287**	**0.234**	**0.607**
	Pesticides	259	0.887	1.413	-1.145	0.146	0.443
	Pharmaceuticals	118	0.952	2.126	-1.670	0.491	1.028

Table 2: Estrogen agonist and oxidative stress assay descriptions.

Assay Provider	Assay Name	Description
Endocrine Disruption Assays		
Attagene	ATG_Era_TRANS	Estrogen receptor 1 measured using an inducible reporter gene assay to determine agonist/antagonist effects (fluorescent induction of mRNA ESR1)
Attagene	ATG_ERE_CIS	Estrogen receptor 1 measured using an inducible reporter gene (fluorescent induction of mRNA ESR1)
NIH Chemical Genomics Center	Tox21 ERa BLA Agonist	Estrogen receptor 1 measured using a GAL4 BLAM reporter gene assay
Oxidative Stress Assays		
Cellumen/Apredica/Cytoprex	APR_Oxidative Stress	H2AX phosphorylation
Cellumen/Apredica/Cytoprex	APR_p53Act up	p53 activity
Cellumen/Apredica/Cytoprex	APR_Stress Kinase up	c-Jun phosphorylation
Attagene	ATG_Nrf2_ARE_CIS	Nuclear factor erythroid 2–related factor 2 (Nrf2) TF inducible reporter gene
Attagene	ATG_AP_1_CIS	Activator protein 1 TF inducible reporter gene

DISCUSSION

- Estrogen agonist *in vitro* assays from ToxCast vary in their relative sensitivities; the most sensitive assay varied when all data, common compounds or organophosphate compounds were considered. NCGC Agonist assay was most sensitive when all compounds and common chemicals were considered, but the Attagene TRANS assay was most sensitive when organophosphates were compared, with 5th centile values of 11.6, 2.69, and 558 µg/L, respectively. (Table 1; Figure 2A-C)
- Exposure duration altered assay sensitivity; for the oxidative stress (H2AX) assay sensitivity increased with duration (Figure 3A), while for both the p53 and Stress Kinase (c-Jun) assays sensitivity decreased with duration (Figure 3B-C).
- High correlation was seen between the AP-1 and Nrf2 assays (r^2=0.696) (Table 1; Figure 4A). Both assays were more sensitive to pesticides (0.253, 0.443) than pharmaceuticals (2.012, 1.028) as can be seen by their 5th centile values, respectively (Table 1; Figure 4B-C).
- Our findings suggest assay selection for estrogenicity and oxidative stress should consider false negatives from ToxCast, chemical class and experimental assumptions.

NEXT STEPS

- Identify physico-chemical properties of compounds with a wide range and degree of affecting oxidative stress-regulated genes
- Develop a computational model that identifies attributes of less hazardous chemicals and elucidates sustainable molecular design guidelines to minimize health effects of oxidative stress
- Conduct *in vitro* and *in vivo* exposures to evaluate activation of oxidative stress-regulated genes and thus strengthen, expand, and confirm computational modeling

REFERENCES

1. Berninger JP, Brooks BW. Leveraging mammalian pharmaceutical toxicology and pharmacology data to predict chronic fish responses to pharmaceuticals. *Toxicology Letters* 2010;193(1):69-78.

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