Survey of ecotoxicologically-relevant reproductive endpoint coverage within the ECOTOX database across ToxCast ER agonists

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Background & Objectives

The U.S. EPA's Endocrine Disruptor Screening Program (EDSP) has been charged with screening thousands of chemicals for their potential to affect the endocrine systems of humans and wildlife. In vitro high throughput screening (HTS) assays have been proposed as a way to prioritize chemicals for EDSP Tier 1 screening. It is unclear whether the in vitro HTS assays, generated mostly in mammalian cell-lines and mammalian receptors, correlates with in vivo effects in environmentally relevant species.

The objectives of this current study are:

- Identify relevant reproductive toxicity data from the EPA's ECOTOX database.
- Collapse endpoints into effect category "groupings".
- Examine the predictivity of the ToxCast ER Model, and individual ToxCast HTS assays.

ECOTOX Database

ECOTOX knowledgebase is a comprehensive, publically available database that contains single chemical toxicity information for aquatic and terrestrial life.

- Data is added on a chemical by chemical basis.
- Curated predominantly from primary literature. Both standardized and non-standardized assays.
- Results are grouped into 25 effect categories. (Fig 1)
  - Further subdivided into 3,329 measurement categories.
- New additions added quarterly.

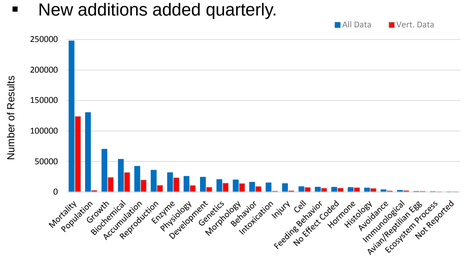


Fig 1. Summary of data availability in the ECOTOX database. Number of results for each effect category are depicted.

Table 1. Summary of ECOTOX database.

	All Data	Vertebrate Data
# Studies	614,909	246,324
# Results	788,626	323,466
Chemicals	10,924	7,428
Species	11,669	1,636

Database Coverage: Reproductive Endpoints

For the purposes of this study, only data from vertebrate species were considered.

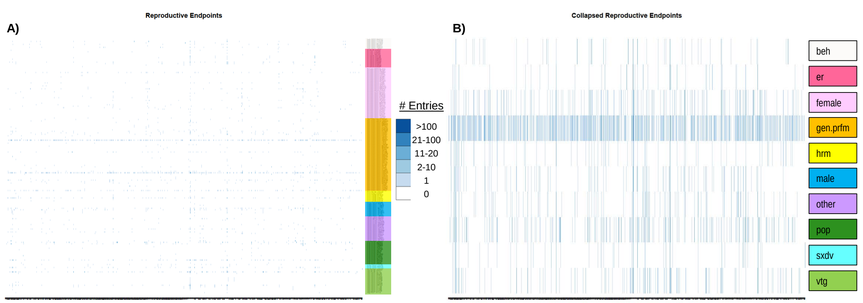


Fig 2. Chemical coverage of vertebrate reproductive effect endpoints. A) There are 172 reproduction-related endpoints in the ECOTOX database, creating a sparse data matrix. B) Endpoints have been collapsed into 10 groupings using manual curation. The number of entries for each chemical/endpoint combination are shaded in blue.

Summary of Reproductive Groupings

Reproductive Endpoints: (Fig 2A)
Approximately 70% of the effect endpoints have results from <25 unique casrns.

- Endpoints with the greatest number of unique casrns include: General reproduction (546), Progeny counts/numbers (495), Viability (252), Sex ratio (180), Vitellogenin (173).

Collapsed Reproductive Endpoints: (Fig 2B)
10 groupings were created through manual curation of ECOTOX endpoints (Table 2).

- Groupings have increased chemical coverage, with an average coverage of ~260 unique casrns.
- Most chemicals have multiple experiments conducted within each group.

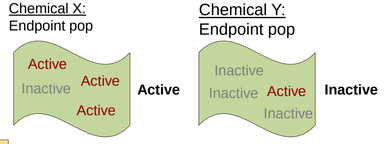
Table 2. Reproductive endpoint groups were created through manual curation. Each grouping contained 3-48 effects endpoints, and tens to hundreds of unique species and casrns.

Groups	Grps	Combined Effects	Casrn	Species	Total Entries	Entries Range	Avg # entries per cas	Med # entries per cas
Behavior	beh	7	71	47	504	1-67	7.10	3
Estrogen Receptor	er	13	81	30	694	1-200	8.57	2
Female Effects	female	34	342	52	1433	1-54	4.19	2
General Repro. Performance	gen.prfm	48	962	186	6816	1-187	7.09	4
Hormone	hrm	8	146	61	658	1-52	4.51	2
Vitellogenin	vtg	16	191	68	2444	1-588	12.80	3
Population	pop	16	355	342	2270	1-118	6.39	2
Sexual Develop.	sxdv	3	117	40	499	1-58	4.26	2
Male Effects	male	9	184	55	910	1-63	4.95	2
Other	other	17	184	86	1209	1-113	6.57	2

Consensus Activity Calling

1. Determine if the test identified a concentration were the chemical was "active".

2. For each chemical/endpoint combination, there are often multiple studies conducted. If 50% or greater of the studies identified an "active" concentration, the chemical was marked as "active" for that endpoint.



Predictivity calculations:

		Condition (as determined by "gold standard")	
		Condition positive	Condition negative
Test outcome	Test outcome positive	True positive	False positive (Type I error)
	Test outcome negative	False negative (Type II error)	True negative

Positive predictive value = Σ True positive/ Σ Test outcome positive	
Negative predictive value = Σ True negative/ Σ Test outcome negative	
Sensitivity = Σ True positive/ Σ Condition positive	Specificity = Σ True negative/ Σ Condition negative

Balanced accuracy (BA) = (sensitivity + specificity) / 2

Predictivity of ToxCast ER Model

ToxCast ER Model: (Judson et al. 2015. Tox Sci. doi: 10.1093/toxsci/kfv168)

- Constructed from 18 ToxCast assays that measure chemical bioactivity at different sites along the estrogen receptor pathway.
- Chemicals with an AUC of >0.1 were considered true positives.

Table 3. Predictivity of the ToxCast ER Model. For each calculation, the ToxCast model and endpoint groups were subset to include only shared chemicals. Groupings with balanced accuracy (BA) <0.5 are poorly predicted by the ToxCast ER model.

Grps	True Positive	False Positive	False Negative	True Negative	Sensitivity	Specificity	BA	PPV
vtg	15	22	15	35	0.5	0.61	0.56	0.41
pop	9	19	15	118	0.38	0.86	0.62	0.32
gen.prfm	9	241	18	157	0.33	0.39	0.36	0.04
hrm	6	24	12	31	0.33	0.56	0.45	0.2
male	6	48	7	23	0.46	0.32	0.39	0.11
other	5	22	19	34	0.21	0.61	0.41	0.19
er	5	18	8	13	0.38	0.42	0.4	0.22
sxdv	3	10	16	31	0.16	0.76	0.46	0.23
beh	3	6	6	13	0.33	0.68	0.51	0.33
female	2	83	8	70	0.2	0.46	0.33	0.02

ToxCast ER Model had limited association with the ECOTOX groupings. The best modeled groupings were vitellogenin (vtg) and population (pop).

- ER Model had greater specificity, ability to identify true negatives, than sensitivity.

Individual ToxCast, Zf Assays:
A single assay within the ER Model may be a stronger predictor of a grouping than the overall model. Both groupings also contain a significant proportion of fish data (vtg: 100%, pop: 51%). Here, we examined the predictivity of the 18 ER pathway assays and all zebrafish (Zf) assays within ToxCast.

Table 4. Predictivity of the vitellogenin (vtg) and population (pop) groupings. Assays with the strongest associations, as determined by balanced accuracy (BA), are highlighted below.

vtg					pop				
Assays	Sens.	Spec.	BA	PPV	Assays	Sens.	Spec.	BA	PPV
NVS_NR_bER	0.50	0.76	0.63	0.61	NVS_NR_mERa	0.56	0.79	0.67	0.48
NVS_NR_hER	0.55	0.68	0.61	0.55	OT_ER_ERbERb_1440	0.46	0.88	0.67	0.45
NVS_NR_mERa	0.56	0.65	0.60	0.56	OT_ER_ERaERb_1440	0.46	0.86	0.66	0.42
ACEA_T47D_80hr_Positive	0.57	0.62	0.59	0.53	OT_ER_ERbERb_0480	0.46	0.83	0.65	0.36
Tox21_ERa_BLA_Agonist_ratio	0.41	0.72	0.56	0.52	Tanguay_ZF_120hpf_MORT_up	0.32	0.93	0.62	0.47
NHEERL_ZF_144hpf_TERATOSCORE_up	0.92	0.19	0.56	0.44	Tox21_ERa_BLA_Agonist_ratio	0.36	0.89	0.62	0.40
					NVS_NR_hER	0.43	0.81	0.62	0.37

- Estrogen receptors had the highest associations for both the vitellogenin and population groupings.
- Interestingly, both groupings also associated with in vivo HTS zebrafish assays.
- Vtg and pop groupings were also compared to all ~1800 assays within ToxCast (data not shown). Assays with a minimum of 10 true positives and a balanced accuracy >0.5 were examined. 3 of the top 15 assays measured the estrogen receptor. Other highly associated assays included measurements of cortisol, progesterone and androgen receptor antagonism.

Conclusions & Future Directions

- ECOTOX database is a very large but sparse data matrix that requires extensive data curation before it can be used for predictive modeling.
- Limited associations exist between ToxCast in vitro assays and ECOTOX reproductive groupings.
- Groupings contain considerable amount of experimental and biological variability. On-going work is focusing on refining the groupings in order to minimize this variability.
- Comparisons between ECOTOX groupings and ToxRefDb repro. endpoints are currently underway.