ToxCast Chemical and Bioactivity Profiles for in vitro Targets in the Retinoid Signaling System

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Introduction

Retinoid signaling is central to many developmental processes and toxicities. The retinoid pathway starts with dietary retinol (Vitamin A) that is transported into embryonic tissues and converted to retinaldehyde and then retinoic acid (RA), the ligand for the RAR and RXR heterodimer.

Objective: identify the chemicals in ToxCast with effects on the retinoid receptors and relate the activity to developmental phenotypes in the biomedical literature.

ToxCast assays in the retinoid signaling pathway

ToxCast (http://actor.epa.gov/dashboard) includes HTS data on reporter assays for trans-activation of retinoic acid receptors and cis-activation of the DR5 response elements by RAR/RXR. A predictive model for prenatal developmental toxicity using ToxCast Phase I showed the RAR assay set to be the strongest weighting factor (Sipes et al. 2011).

With regards to retinoid metabolism (KEGG pathway hsa00830: Retinol metabolism), ToxCast lacks information on retinal dehydrogenase (EC: 1.2.1.36; RALDH), the enzyme that generates RA from retinol, and on cytochrome-P450 family 26 (EC: 1.14.-.-; CYP26), the enzyme specific to its breakdown. However, the dataset does have results on cytochrome-P450 family 1, subfamily A, polypeptide 1 (EC:1.14.14.1; CYP1A1) also capable of RA breakdown.

Table 1. ToxCast assays in the retinoic acid pathway

ToxCast Assay (iCSS Dashboard)	Gene Symbol	Readout (liver cells, or cell-free)
ATG_RARa_TRANS_up or down	*RARA*	Trans-activation of GAL4-RARa reporter
ATG_RARb_TRANS_up or down	*RARB*	Trans-activation of GAL4-RARb reporter
ATG_RARg_TRANS_up or down	*RARG*	Trans-activation of GAL4-RARg reporter
NVS_NR-hRAR_Antagonist	RAR	Cell-free, time-resolved FRET
ATG_RXRa_TRANS_up or down	*RXRA*	Trans-activation of GAL4-RXRa reporter
ATG_RXRb_TRANS_up or down	*RXRB*	Trans-activation of GAL4-RXRb reporter
ATG_RXRg_TRANS2_up or down	*RXRG*	Trans-activation of GAL4-RXRg reporter
NVS_NR_hRARa_Agonist	*RARA*	Cell-free, time-resolved FRET
ATG_DR5_CIS_up or down	RARA, RARB, RARG	Cis-activation of RARE elements by RAR/RXR
NVS_ADME_hCYP1A1	*CYP1A1*	Enzymatic changes to fluorescent substrate
NVS_ADME_rCYP1A1	*Cyp1a1*	Enzymatic changes to fluorescent substrate

Active Chemicals in ToxCast (of 1858)

589 chemicals had activity (AC50 value) in one or more of the 11 assays interrogating the RA pathway. Because a number of chemicals were active in more than one assay, this represents 879 assay – chemical pairs. Counts for selected assays are listed in Table 2.

Table 2. Active chemicals for RA pathway assays.

Gene Symbol	Active chemicals	Number of chemicals with AC50 at or below 2 uM
RARA	80	9
RARB	19	4
RARG	33	6
RAR	61	3
RXRA	68	9
RXRB	321	23
RXRG	0	0
RARA, RARB, RARG	235	51
CYP1A1	74	18

View of the most potent chemicals: the micromolar AC50 values for some of the top hits are listed below. We observed that trans-retinoic acid and retinol are potent hits at several of the targets, including RARa, where they are the most potent. The organotin compounds have significant activity at both RXRa and RXRb, but not RARa or RARb. Several other compounds displayed similar selective activity. Based on these observations, we chose two sets of chemicals to examine further using text-mining methods. Set 1 includes trans-retinoic acid, retinol, and several polychlorinated compounds, and Set 2 includes organotins and 4-nonylphenol.

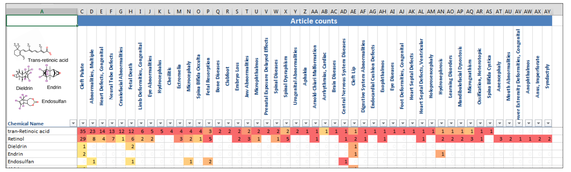
Table 3. Partial list of ToxCast results for RA pathway targeted assays

	ToxCast ssays in the retinoic acid pathway									
Chemicals ranked by potency	ATG_DR5_CIS_up	ATG_RARa_TRANS_up	ATG_RARb_TRANS_up	ATG_RARg_TRANS_up	ATG_RXRa_TRANS_up	ATG_RXRb_TRANS_up	NVS_ADME_hCYP1A1	NVS_ADME_rCYP1A1	NVS_NR_hRAR_Antagonist	NVS_NR_hRARa_Agonist
trans-Retinoic acid	0.0063	0.0004			0.0003	1.0400	1.3200			0.9430
Retinol	0.1470	0.0690		0.2080	1.5400	0.4730				
Tributyltin benzoate	0.0227				0.0053	0.0364				
Tributyltin methacrylate	0.0055				0.1470	0.0250				
Tributyltin chloride	0.0028				0.1760	0.0777				
Tetrabutyltin	0.2790				0.7410	0.0333				
2,4,6-Tris(tert-butyl)phenol	1.8300				0.4770	0.1850				
Pyraclostrobin	0.5420	0.7790					1.6800			
Dieldrin	0.5790	0.7700		1.6800						
Endrin	0.8060		1.6100	1.7000						
SR271425	0.8340					0.7600				
Triflumizole	0.2980	1.4500								
2,6-Di-tert-butyl-4-methoxyphenol		1.0700				0.7000				
Coumaphos	1.6000						0.2740			
CP-532623	0.5030	1.5000								
Imazalil		0.9080					1.4100			
Prochloraz							0.4130	1.9300		
Endosulfan I	1.8300	1.3800								

Comparing developmental effects using text-mining

Text-mining goal: compare reported effects of Set 1 (RAR and RXR actives) and Set 2 (RXR actives only) in development. **Methods:** the EPA literature database of extracted MeSH terms was queried for all articles in which the chemical was a major topic and annotated as toxic and developmental effects were annotated. Articles counts were output to Table 4 (Set 1 chemicals) and Table 5 (Set 2 chemicals). Note: chemicals with few or no articles in PubMed were omitted.

Table 4. Literature reported developmental toxicities for Set 1 chemicals.



Among the many development toxicities associated with this group of chemicals, cleft palate has the most literature and the collection includes a number of malformations.

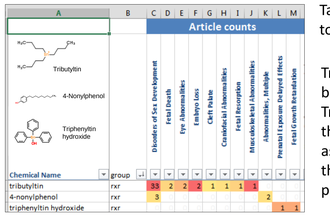


Table 5. Literature reported developmental toxicities for Set 2 chemicals.

Tributyltin, 4-nonylphenol, and triphenyltin are in Set 2 because they had strong activity at RXR but not RARa or RARb. Tributyltin (the MeSH term comprising a number of forms of the compound) is associated in the literature with 33 articles associating it with disorders of sex development. Although there is a report of tributyltin causing cleft palate, the overall profile of adverse effects for Set 2 is very different from Set 1.

Summary

- ToxCast includes 11 assays that test activity in the retinoic acid pathway.
- Approximately 5% of ToxCast chemicals show the potential to disrupt retinoic acid activity at submicromolar concentrations.
- Retinoic acid was the most potent chemical tested in RARa and RXRa trans-activation assays, followed by organotin compounds in the RXR assays.
- Retinoic acid and tributyltin have overall different profiles of activity in the retinoic acid pathways assays: retinoic acid hits both RAR and RXR while tributyltin has no activity at RARa or RARb in ToxCast.
- Retinoic acid and tributyltin also have very different patterns of developmental toxicity.
- Because RAR and RXR heterodimerize with different nuclear receptor families (e.g. RARa with RXRa; RXRa with PPARg, LXRb or FXR), it is possible that chemical activity at these receptors may disrupt multiple signaling pathways.

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