A Data-driven Model Analysis of Retinoid Signaling in Skeletal Dysmorphogenesis and Potential Adverse Outcome Pathways

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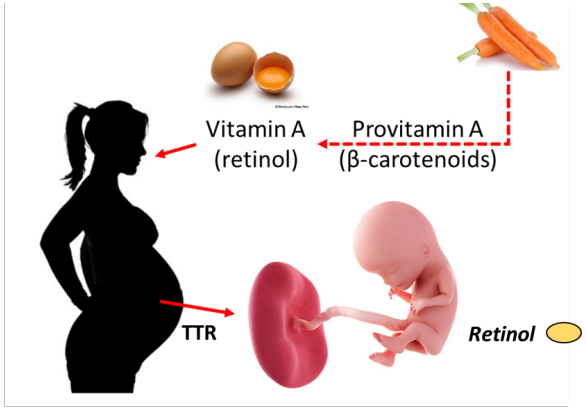
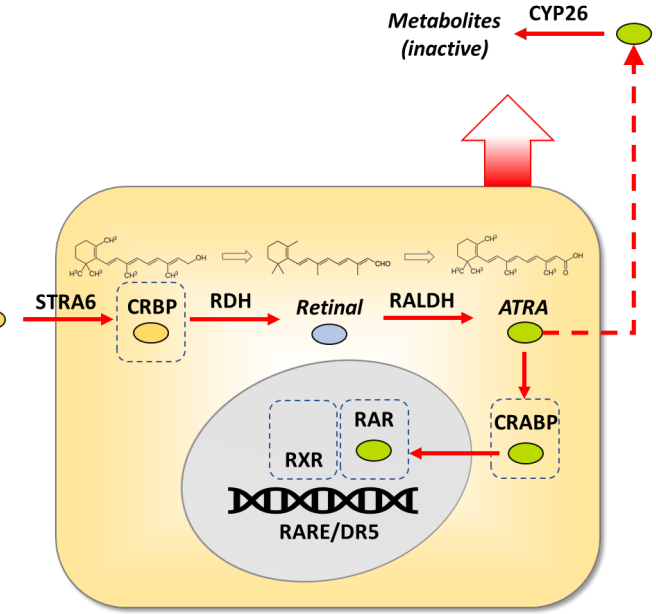
Introduction

- ATRA (all-trans retinoic acid) signaling is required for patterning skeletal development



Adapted from Niederreither and Dolle, 2008



- Retinoid system can be disrupted by genetic or environmental factors, leading to dysmorphogenesis

GOAL:
Develop data-driven models and Adverse Outcome (AOP) frameworks for chemical disruption of retinoid signaling on altered skeletal development

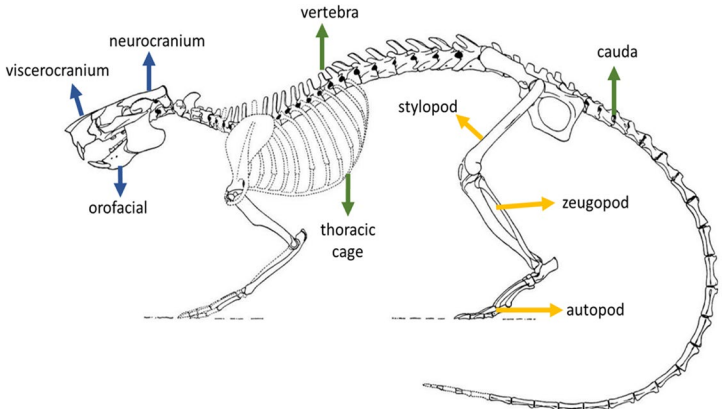
Workflow

Toxicity Reference Database (ToxRefDB v1)
https://github.com/USEPA/CompTox-ToxRefDB
(2,946 prenatal developmental toxicity studies with adverse skeletal outcome)

ToxRefDB Skeletal Defect Studies and Associated Chemicals
57,198 composite skeletal defects across 363 chemicals (rat (31,1661), mouse (1,232), rabbit (16,375), chinchilla (368), other/unspecified (7562))

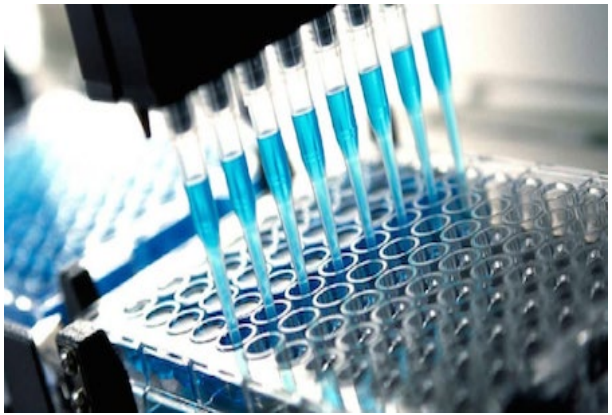
Extraction of ToxCast Chemicals
AbstractSifter (Baker *et al.*, 2017) deduced 7 non-ToxRefDB chemicals of 42 benchmark (Zurlinden *et al.*, 2020) ToxCast chemicals demonstrate connection to skeletal defects

Regional Annotation for 370 chemicals
Appendicular (8,611): autopod (7,310), stylopod (969), zeugopod (332);
Axial (34,122): cauda (2,224), thoracic cage (19,132), vertebra (12,766);
Cranial (7,658): neurocranium (5,037), orofacial (2,426), viscerocranium (195);
Other (6,807): unspecified (6,807)

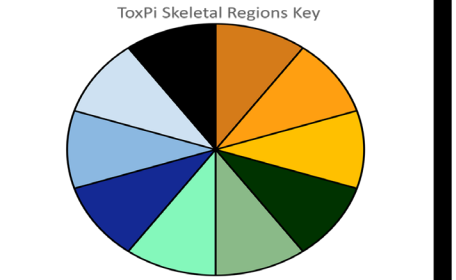


CompTox Chemicals Dashboard
https://comptox.epa.gov/dashboard
374 chemicals (of 8,079 tested chemicals) selected due to bioactivity across 1 or more of 13 assays for relevant ATRA pathway nodes (Knudsen *et al.*, 2020);
Criteria for positivity called active based on efficacy and potency

Mapping HTS Data

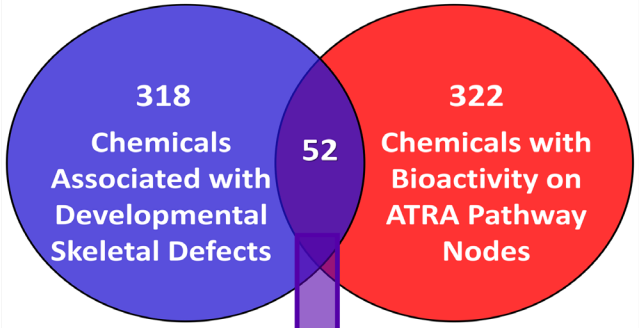


Toxicity Prioritization Score (ToxPi v2.3)
k-means clustering by nearest centroid (k=5)



Comparison of Data Sets' Chemicals
Compared 374 compounds that induced bioactivity in ATRA pathways and 363 ToxRefDB or 7 Benchmark ToxCast chemicals associated with skeletal defects to ascertain common chemicals

Dataset for Mechanistic Modeling



AOP Elucidation

52 Chemicals

Allethrin	Endosulfan	N,N-Dimethylformamide	SAR 150640
all-trans-Retinoic acid	Endrin	N-Ethylperfluorooctane-sulfonamide	S-Bioallethrin
Aspirin	Etoxazole	N-Phenyl-1,4-benzenediamine	SSR126768
Bentazone	Fenpyroximate (Z,E)	Oryzalin	Tebufenpyrad
Bromuconazole	Fipronil	Oxadiazon	Tetraconazole
Bronopol	Fluoxastrobin	Phorate	Thiazopyr
Buprofezin	Flusilazole	Propargite	Thiram
Chlorothalonil	Forchlorfenuron	Propiconazole	Triadimefon
Clodinafop-propargyl	Imazalil	Pyraclostrobin	Tributyltetradecylphos-phonium chloride
Cyfluthrin	Iprodione	Pyridaben	Triflumizole
Deltamethrin	Lindane	Pyrimethamine	Triphenyltin hydroxide
Difenoconazole	Linuron	Raloxifene hydrochloride	Triticonazole
Diniconazole	Myclobutanil	Retinol	Zinc pyrithione

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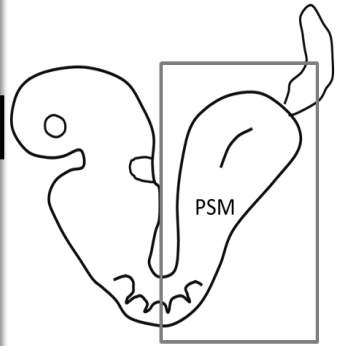
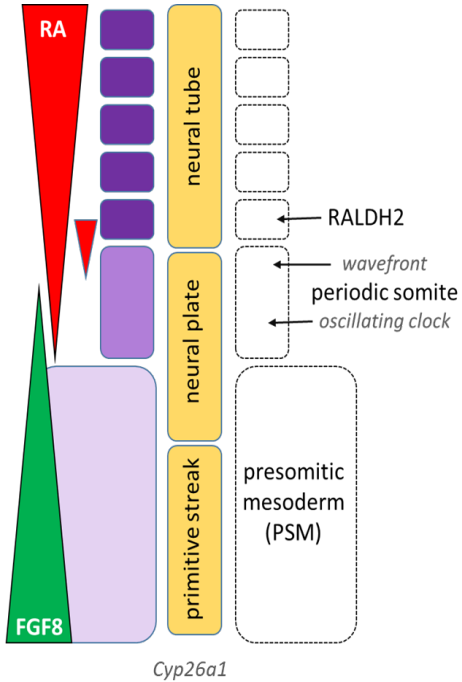
Chemically-associated RA Pathway Disruption and AOs

DSSTOXID	PREFERRED_NAME	CYP1A1 (72)	RARa (65)	RARb (17)	RARg (49)	RXRa (69)	RXRb (299)	RXRg (0)	DR5 (250)
DTXSID7021239	all-trans-Retinoic acid	1.317	NA	NA	NA	NA	1.036	NA	0.006
DTXSID1040619	Bexarotene	NA	7.539	NA	2.655	0.009	0.009	NA	0.014
DTXSID3023556	Retinol	NA	0.076	NA	0.227	2.142	0.464	NA	0.197
DTXSID1020807	2-Mercaptobenzothiazole	0.164	NA	NA	NA	NA	NA	NA	NA
DTXSID2040363	Diniconazole	0.674	NA	NA	NA	NA	NA	NA	NA
DTXSID0032655	Triticonazole	0.793	NA	NA	NA	NA	NA	NA	16.741
DTXSID8024151	Imazalil	1.413	0.908	NA	NA	NA	NA	NA	5.888
DTXSID4032372	Difenoconazole	1.459	NA	NA	NA	NA	NA	NA	2.124
DTXSID3023897	Triademifon	2.085	41.462	NA	NA	NA	NA	NA	11.223
DTXSID7029871	Clotrimazole	2.306	NA	NA	NA	NA	NA	NA	NA
DTXSID3024235	Flusilazole	3.704	8.155	NA	NA	NA	NA	NA	7.718
DTXSID2032500	Triflumizole	4.134	1.453	NA	NA	NA	NA	NA	0.298
DTXSID0021337	Thiabendazole	4.721	NA	NA	NA	NA	NA	NA	NA
DTXSID8024280	Propiconazole	9.010	23.801	NA	NA	NA	NA	NA	6.253
DTXSID9020453	Dieldrin	NA	0.770	NA	1.679	NA	22.531	NA	0.579
DTXSID9037539	Endosulfan I	NA	1.384	NA	NA	NA	NA	NA	1.827
DTXSID6020561	Endrin	NA	NA	1.606	1.698	NA	24.982	NA	0.806
DTXSID1020560	Endosulfan	NA	NA	NA	NA	NA	NA	NA	0.894
DTXSID7020267	Chlordane	NA	NA	NA	6.878	71.470	21.422	NA	1.784
DTXSID7042065	Isodrin	NA	NA	NA	1.077	NA	NA	NA	2.111
DTXSID8020040	Aldrin	NA	NA	NA	0.912	NA	7.167	NA	3.085
DTXSID3042500	Triphenyltin fluoride	NA	NA	NA	NA	0.004	0.001	NA	0.655
DTXSID5034981	Tributyltin benzoate	NA	NA	NA	NA	0.005	0.036	NA	0.023
DTXSID9044796	(Acryloyloxy)(tributyl)stannane	NA	NA	NA	NA	0.015	0.026	NA	0.022
DTXSID2040733	Triphenyltin chloride	NA	NA	NA	NA	0.081	0.037	NA	0.356
DTXSID9035204	Tributyltin methacrylate	NA	NA	NA	NA	0.147	0.025	NA	0.005
DTXSID3027403	Tributyltin chloride	NA	NA	NA	NA	0.176	0.078	NA	0.003
DTXSID4022153	Tetrabutyltin	NA	NA	NA	NA	0.741	0.033	NA	0.279
DTXSID1021409	Triphenyltin hydroxide	NA	NA	NA	NA	NA	0.013	NA	NA
DTXSID9040712	Triethyltin bromide	NA	NA	NA	NA	4.029	0.252	NA	NA

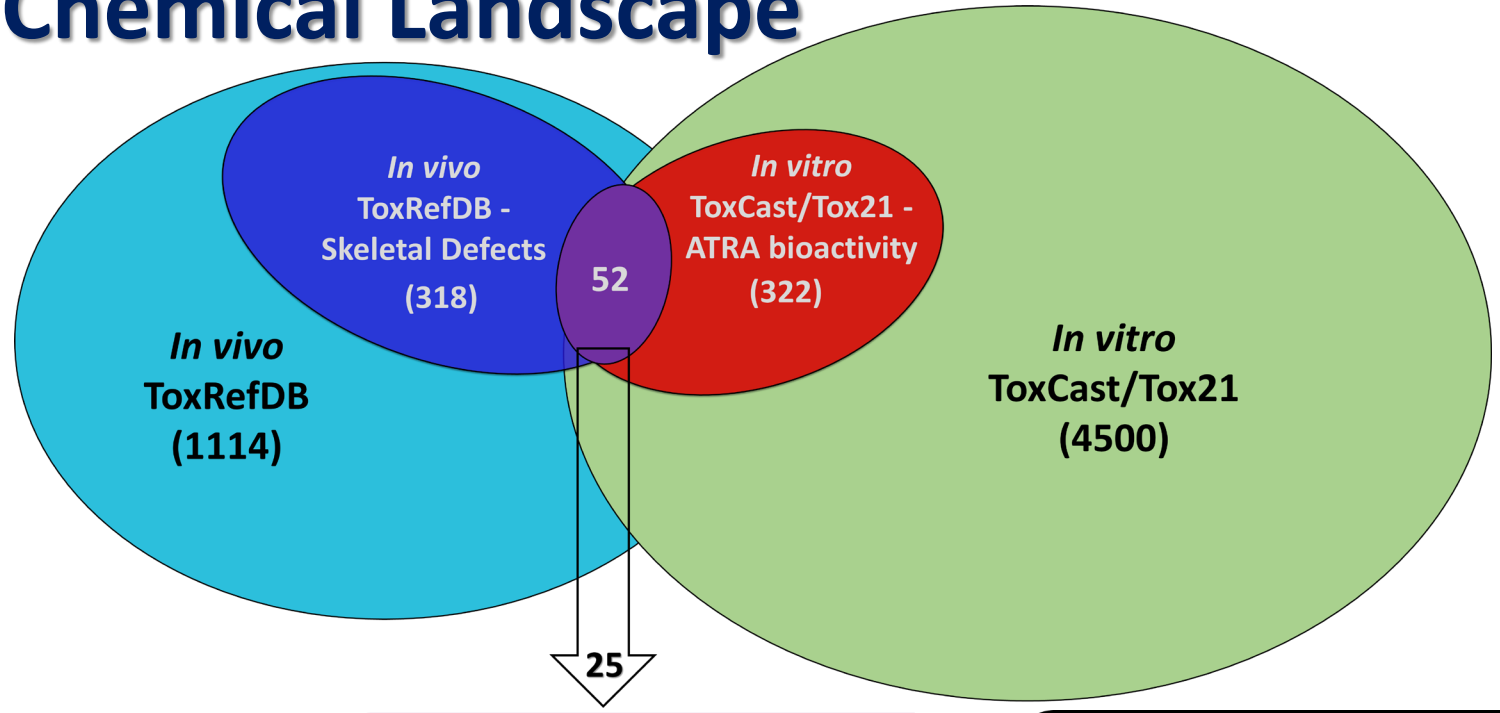




Case examples

ATRA signaling: postcranial axis

Chemical Landscape



25 Retinoid Pathway Reference Chemicals from Protein Data Bank, ChEMBL, ToxCast, and biomedical literature in PubMed were consistent with other databases.

- 1114 Chemicals Tested in vivo recorded in ToxRefDB
- 318 ToxRefDB chemicals associated with *in vivo* skeletal defects
- 4500 Chemicals Tested *in vitro* recorded in ToxCast/Tox21
- 322 ToxCast & ToxRefDB chemicals associated with *in vitro* ATRA pathway bioactivity
- 52 chemicals found in 3 databases establishing association with skeletal defects and ATRA path bioactivity

Phenotype Examined	Percent of Phenotypic Defects Associated with 52 Chemicals
appendicular_autopod	6%
appendicular_stylopod	1%
appendicular_zeugopod	2%
axial_cauda	3%
axial_thoracic cage	48%
axial_vertebra	14%
cranial_neurocranium	11%
cranial_orofacial	4%
cranial_viscerocranium	0%
other	11%

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Potential AOPs for ATRA-Skeletal Defects

REGION	*Molecular Initiating Event (MIE)*	*Key Event 1 (KE1)*	*KE2*	*KE3*	*KE4*	*KE5*	*Adverse Outcome (AO)*
Anterior Neural Tube	Inhibition of CYP26A1 enzymatic activity	Local increase in endogenous ATRA levels	Hyperactivation of the RAR/RXR heterodimer	Repression of Fgf8 limits FGF8 signaling	Mis-specification of CNC cell fate and behavior	Maxillary arch dysplasia alters palatal outgrowth	Cleft palate
Paraxial Mesoderm	Reduction in RDH/RALDH2 activity	Local decrease in endogenous ATRA levels	Hypoactivation of the RAR/RXR heterodimer	Overextension of FGF8 signaling	Disruption of the periodic somitic wavefront	Altered somite number, shape, and alignment	Hemivertebra
Limb-bud	Hyperactivation of the RAR/RXR heterodimer	Underextension FGF8 signaling from the AER	Dysregulation of Meis1/2 and Hox gene expression	Proximalization of the limb-bud mesenchyme	Mis-specification of precartilage blastema	Malformed cartilaginous bone rudiment	Phocomelia

Summary and Conclusions

- NAMs employed to identify, organize, and summarize toxicological and mechanistic data for specific hazard domains
- Established 52 chemicals from 3 databases as reference compounds for developmental skeletal defects and disruption of ATRA signaling
- Apparent chemical disruption of axial patterning through the retinoid system
- Continue to develop ATRA-related MIEs associated with skeletal AOs
- Initiating chemotyping to establish structural similarities between the 52 chemicals with comparable phenotypic effects

References

- [1] Knudsen *et al.* Retinoid Signaling in Skeletal Development: Scoping the System for Predictive Toxicology. Reprod. Toxicol. 2021.
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- [5] Niederreithe *et al.* Retinoic acid in development: towards an integrated view. Nat Rev Genet. 2008 Jul; 9(7):541-53. doi: 10.1038/nrg2340. Epub 2008 Jun 10. PMID: 18542081.

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