Identification of Chemicals Associated with Retinoid Signaling Pathway Disturbance and Skeletal Dysmorphogenesis via New Approach Methods' Model and Adverse Outcome Pathway Development

Jocylin D. Pierro[1], Bhavesh K. Ahir[2], Nancy C. Baker[3], and Thomas B. Knudsen[1]

U.S. Environmental Protection Agency, Office of Research and Development

[1]Center for Computational Toxicology and Exposure, [2]Eurofins, PA, [3]Leidos – Research Triangle Park, NC 27711

pierro.jocylin@epa.gov | 919-541-5184

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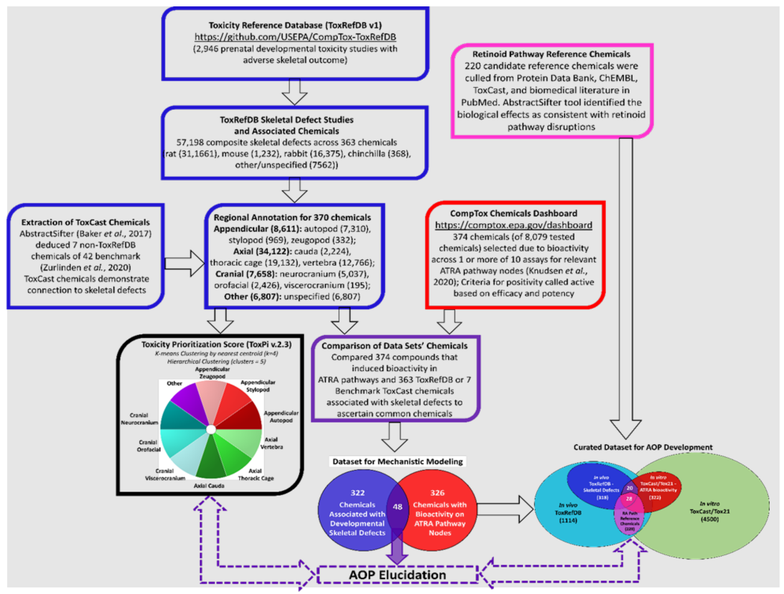
1. Introduction

OECD Testing and Assessment Series No. 343 is supporting recommendations regarding assay development to determine retinoid system toxicants. Here a predictive analysis of the retinoid signaling effects on skeletal development is provided.

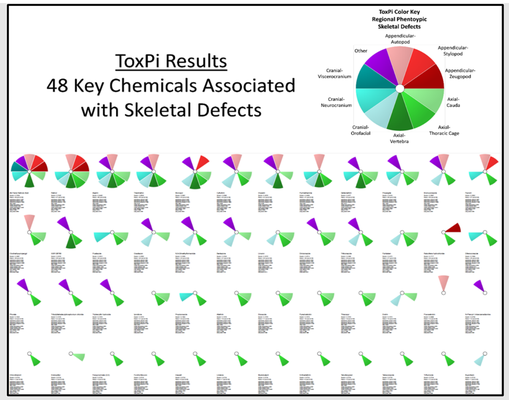
- ATRA (all-trans retinoic acid) signaling is required for patterning the early body plan. Locally-regulated ATRA gradients are important during the initial specification of the body plan (gastrulation) and mesoderm. The retinoid system can be disrupted by genetic or environmental factors, leading to dysmorphogenesis [1, 2, 3]

- An Adverse Outcome Pathway (AOP) framework models how we think chemical disruption of retinoid signaling invokes altered skeletal development. AOPs inform integrated regulatory test method development for predicting developmental defects [1]

2. Multi-Database Workflow

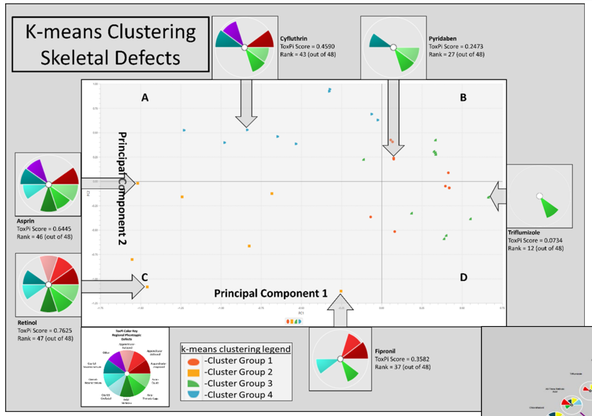


3. Mapping HTS Data Analyses



48 Chemicals	
Allethrin	Linuron
All-Trans Retinoic Acid	Myclobutanil
Aspirin	N,N-Dimethylformamide
Bentazone	N-Phenyl-1,4-benzenediamine
Bromuconazole	Oryzalin
Bronopol	Oxadiazon
Buprofezin	Phorate
Chlorothalonil	Propargite
Clodinafop-propargyl	Propiconazole
Cyfluthrin	Pyraclostrobin
Deltamethrin	Pyridaben
Difenoconazole	Pyrimethamine
Diniconazole	Raloxifene hydrochloride
Endosulfan	Retinol
Endrin	S-Bioallethrin
Etoxazole	SSR126768
Fenpyroximate (Z,E)	Tebufenpyrad
Fipronil	Tetraconazole
Fluoxastrobin	Thiazopyr
Flusilazole	Triadimefon
Forchlorfenuron	Tributyltetradecylphosphonium chloride
Imazalil	Triflumizole
Iprodione	Triphenyltin hydroxide
Lindane	Triticonazole

4. ToxPi Results

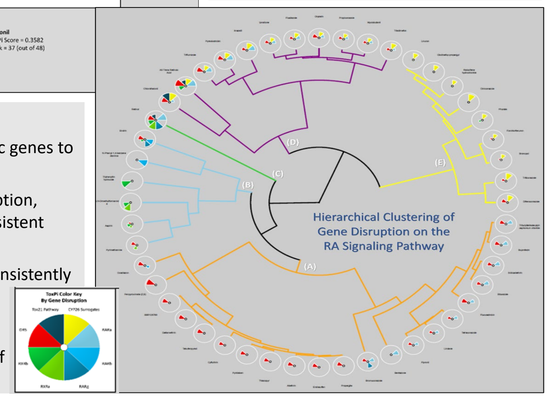


K-means Clustering:

- In examining the sensitivity of phenotypic regions, through the fipronil example it is evident that while limb defects have less specificity than cranial responses, appendicular phenotypes are more sensitive than cranial phenotypes when exposed to chemicals inducing any adverse appendicular outcomes.
- ATRA and retinol are activating through the biological system, with broad effects

Hierarchical Clustering:

- CYP26 surrogates and DR5 are the most specific genes to be activated on the ATRA signaling pathway.
- The overall severity of CYP26 surrogates' disruption, common occurrence of DR5 and RARA are consistent with the k-means clustering.
- Organochlorine pesticides (e.g., endosulfan) consistently activate DR5
- -azole fungicides (e.g., triazoles, imidazoles, pyrazole) have broad association with activation of CYP26 surrogates, RARA, and DR5.



5. Potential AOPs for ATRA-Skeletal Defects

MIE	KE1	KE2	KE3	KE4	AO
Loss of CYP26 activity	Increase in ATRA	Decrease in FGF8 signaling	Modification of axial patterning genes	Alterations in cartilage, rudiments, and ossification	Misshapen, poorly ossified, and missing thoracic cage
Overactivation of RARs	Increased transactivation of RARE	Downregulation of FGF8 expression in the apical ectoderm	Activation of apoptotic pathway	Excessive interdigital cell death (ICD)	Truncation of the autopod
Loss of CYP26 activity	Increase in ATRA	Decrease in FGF8 signaling	Modification of branchial mesenchyme specification	Misrouting of branchial arch mesenchyme	Viscerocranial malformation

6. Summary and Conclusions

- Forty-eight chemicals were found to represent a subset of the chemical landscape having defined *in vitro* (ATRA pathway nodes) and *in vivo* adverse (skeletal) outcomes defined from ToxCast, Tox21, and ToxRefDB (prenatal developmental toxicity).
- Thoracic cage was the first and most frequent skeletal defects in this model, followed by other axial defects (vertebra and cauda), cranial and limb defects.
- DR5 (biomarker of ATRA transactivation) had the greatest occurrence of chemical bioactivity; chemicals disrupting DR5 consistently associated with thoracic cage defects.
- These results have useful applications for building AOP frameworks for ATRA signaling pathway and developmental toxicity (skeletal system and beyond).

7. References

- [1] Knudsen *et al.* Retinoid Signaling in Skeletal Development: Scoping the System for Predictive Toxicology. Reprod. Toxicol. 2021.
- [2] Organisation for Economic Co-operation and Development (OECD). *OECD Testing and Assessment Series No. 343.* 2021.
- [3] Pierro *et al.* Computational model for fetal skeletal defects potentially linked to disruption of retinoic acid signaling. 2022. *Work in progress.*
- [4] Baker. *et al.* Identifying Candidate Reference Chemicals for *in vitro* Testing of the Retinoid Pathway. 2022. *Submitted. [Poster on Thursday of SOT]*