Multi-database Review of Retinoid Signaling in Skeletal Development for Adverse Outcome Pathways and Computational Toxicology Applications

Jocylin D. Pierro[1], Nancy C. Baker[2], and Thomas B. Knudsen[1]
U.S. Environmental Protection Agency, Office of Research and Development
[1]Center for Computational Toxicology and Exposure and [2]Leidos – Research Triangle Park, NC 27711
pierro.jocylin@epa.gov | 919-541-5184

1. Introduction

A Detailed Review Paper of the OECD Test Guidelines Programme (Project 4.97) 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 morphogen 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 (Knudsen et al. *In review*) [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

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

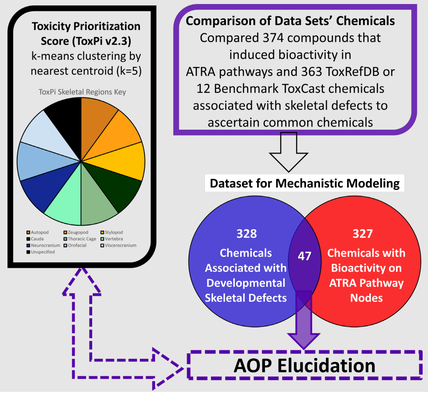
ToxRefDB Skeletal Defect Studies and Associated Chemicals
57,198 composite skeletal defects across 363 chemicals (rat (31,1661), mouse (1,232), rabbit (16,375), chinchilla (368), other/unspecified (7562))

Extraction of ToxCast Chemicals
AbstractSifter (Baker *et al.*, 2017) deduced 12 non-ToxRefDB chemicals of 42 benchmark (Zurlinden *et al.*, 2020) ToxCast chemicals demonstrate connection to skeletal defects

Regional Annotation for 375 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

3. Multi-database & Mapping HTS Data Analyses

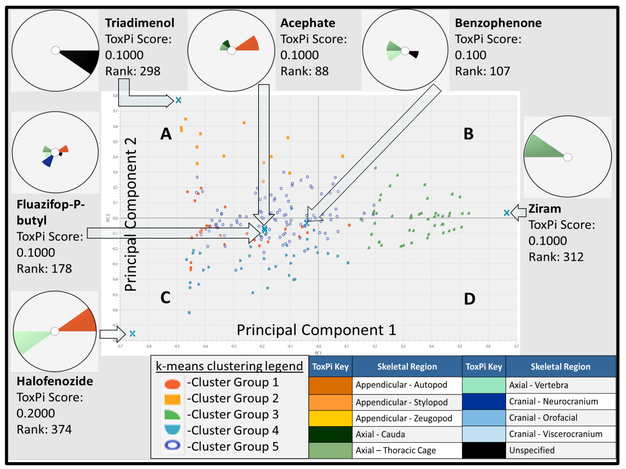


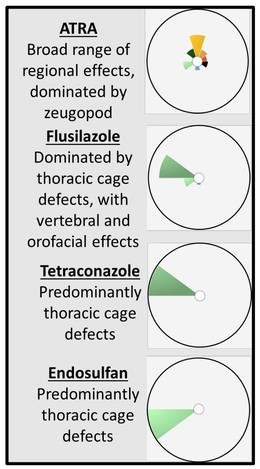
47 Chemicals:
Allethrin, Aspirin, All-trans-retinoic acid (ATRA), Bentazone, Bronopol, Buprofezin, Chlorothalonil, Clodinafop-propargyl, Cyfluthrin, Deltamethrin, Difenoconazole, Diniconazole, Emamectin benzoate, Endosulfan, Endrin, Etoxazole, Fenpyroximate (Z,E), Fipronil, Fluoxastrobin, Flusilazole, Forchlorfenuron, Imazalil, Iprodione, Lindane, Linuron, Myclobutanil, N,N-Dimethylformamide, N-Phenyl-1,4-benzenediamine, Oryzalin, Oxadiazon, Propargite, Propiconazole, Pyraclostrobin, Pyridaben, Pyrimethamine, Retinol, S-Bioallethrin, Tetraconazole, Thiazopyr, Thiram, Triadimefon, Tributyltetradecylphosphonium chloride, Trichlorfon, Triflumizole, Triphenyltin hydroxide, Triticonazole, Zinc pyrithione

4. Annotation of k-clusters

- Group 1 – Primarily driven by autopod defects.
- Group 2 – Primarily unspecified skeletal defects.
- Group 3 – Primarily driven by axial defects.
- Group 4 – Primarily driven by vertebral and thoracic and other.
- Group 5 – Broad regional effects dominated by neurocranial.

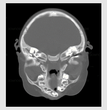
5. Tox-Pi Results

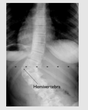


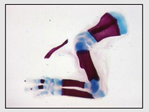


6. Data-Driven AOPs for Skeletal Dysmorphogenesis

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


Cleft Palate [4]


Hemivertebra [5]


Phocomelia [4]

7. Summary and Conclusions

- Data-driven Approach to AOP Elucidation was used
- Analysis of *in vitro* and *in vivo* experimentation databases connect ATRA-related MIEs to skeletal AOs
- Classification for skeletal phenotypes in 375 chemicals in ToxCast/ToxRefDB. (Thoracic cage defects dominant)
- 47 of those chemicals showed disruption of 1 or more ToxCast assay for potential effects on ATRA signaling.
- Preliminary findings are consistent with potential for chemical disruption of axial patterning through the retinoid system.

8. References

- [1] Knudsen et al. Retinoid Signaling in Skeletal Development: Scoping the System for Predictive Toxicology. 2020. *Work in progress.*
- [2] Organisation for Economic Co-operation and Development (OECD). Detailed Review Paper (DRP) of the OECD Test Guidelines Programme (Project 4.97). 2020. *Work in progress.*
- [3] Pierro *et al.* Multi-Database Review of Retinoid Signaling in Skeletal Development for Adverse Outcome Pathways and Computational Toxicology Applications. 2020. *Work in progress.*
- [4] sciencesource.com (2020).
- [5] Texas Scottish Rite Hospital. 2020. Aaos.org
- [6] devtox.org (2020).