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

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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 [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)

Retinoid Pathway Reference Chemicals
169 candidate reference chemicals were culled from Protein Data Bank, ChEMBL, ToxCast, and biomedical literature in PubMed. The Abstract Sifter tool identified the biologicals effects as consistent with retinoid pathway disruption.

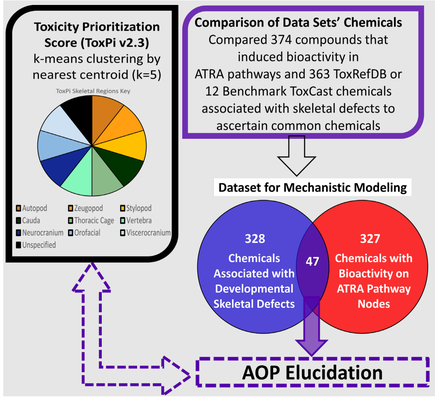
ToxRefDB Skeletal Defect Studies and Associated Chemicals
57,198 composite skeletal defects across 363 chemicals (rat (31,1661), mouse (1,232), rabbit (16,375), chinchilla (368), other/unspecified (7562))

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

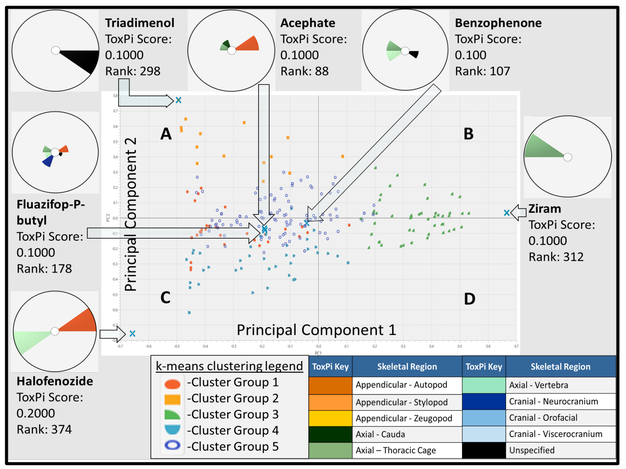


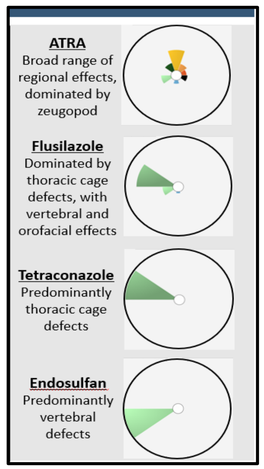
47 Chemicals:
Allethrin, Aspirin, All-trans-retinoic acid (ATRA), Bentazone, Bronopol, Buprofezin, Chlorothalonil, Clodinafop-propargyl, Cyfluthrin, Deltamethrin, Difenoconazole, Diniconazole, Emamectin benzoate, Endosulfan, Endrin, Etoxazole, Fenpyroximate (Z,E), Fipronil, Fluoxastrobin, Flusilazole, 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.

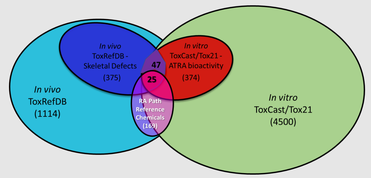
5. Tox-Pi Results





6. 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



Preliminary analysis of 25 chemicals associated with retinoid pathway bioactivity and neonatal skeletal defects

7. Summary and Conclusions

- Developed curated list of 25 chemicals from 4 databases with potential correlation between skeletal defects and retinoid pathway disruption.
- Classification for skeletal phenotypes in 375 chemicals in ToxCast/ToxRefDB (Thoracic cage defects dominant).
- Preliminary findings are consistent with potential for chemical disruption of axial patterning through the retinoid system.
- Will further analyze *in vitro* and *in vivo* experimentation databases to connect ATRA-related MIEs to skeletal AOs.

8. 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). Detailed Review Paper (DRP) of the OECD Test Guidelines Programme (Project 4.97). 2021. *Work in progress.*
- [3] Pierro *et al.* Multi-Database Review of Retinoid Signaling in Skeletal Development for Adverse Outcome Pathways and Computational Toxicology Applications. 2021. *Work in progress.*
- [4] Baker. *et al.* Identifying Candidate Reference Chemicals for *in vitro* Testing of the Retinoid Pathway. 2021. *Work in Progress.*