Review of Retinoid Signaling in Skeletal Development for Adverse Outcome Pathways and Predictive Toxicology Applications

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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 an overview 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.
- 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.

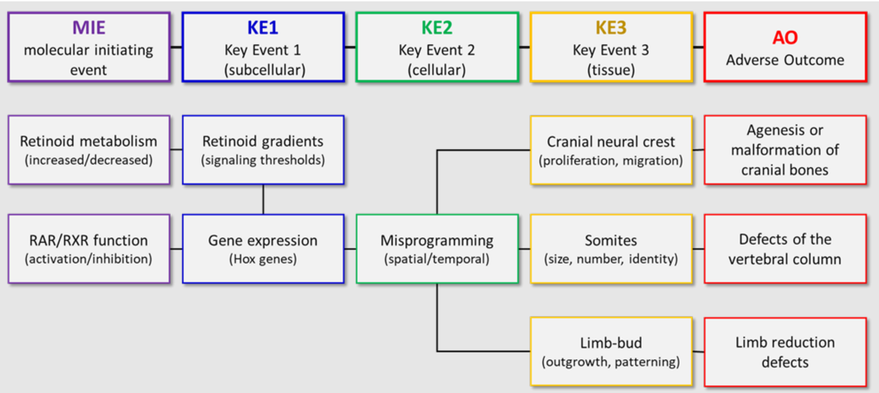
Specific Aims

- Summarize retinoid signaling effects on three skeletal regions: cranial, post-cranial axial, and appendicular.
- Elucidate potential AOPs that can be used to map molecular initiating events (MIEs) linked to disruption of ATRA signaling.
- Use the AOP framework to classify ToxCast chemicals for predictive immoderation of skeletal adverse outcomes (AOs).

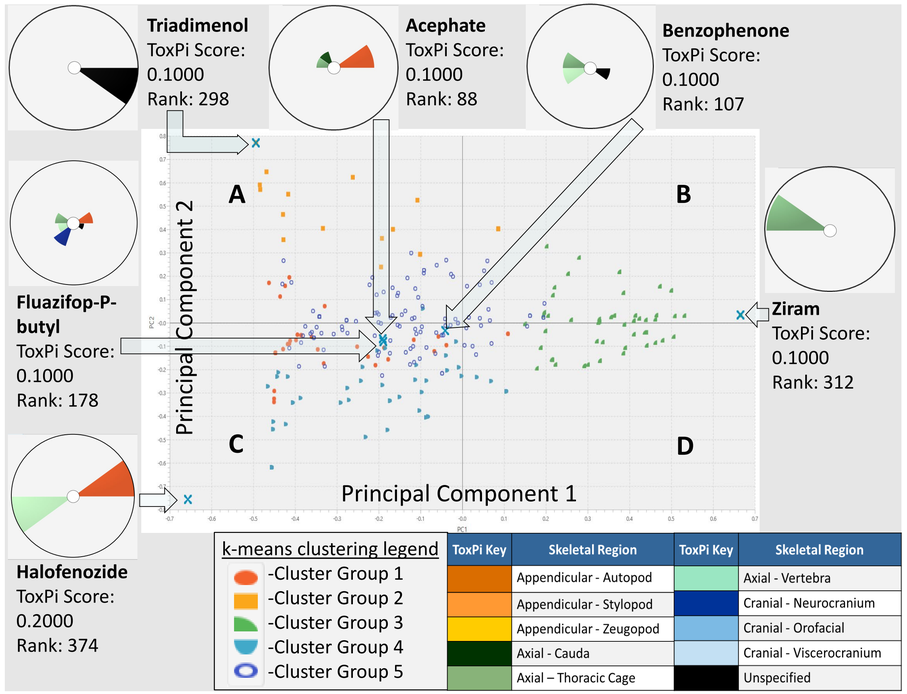
Computational Approach

- AbstractSifter (Baker et al. 2017) tool was used to mine literature on retinoids and skeletal development (approximately 600 articles).
- 375 chemicals available in ToxRefDB and/or ToxCast with observed skeletal phenotypes in prenatal DevTox studies.
- Annotated effects on phenotype regions: cranial (neurocranial, orofacial, viscerocranial); axial (thoracic cage, vertebral, cauda); appendicular (stylopod, zeugopod, autopod); and non-specific other.
- For each chemical, distribution of phenotype(s) was scored as a fraction of 1, based on the lowest effect level (dLEL).

AOP - framework



ToxPi: k-means clustering (375 Chemicals)



Annotation of k-clusters

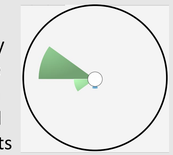
- Group 1 – Primarily drive by autopod defects.
- Group 2 – Primarily unspecified skeletal defects.
- Group 3 – Primarily driven by axial defects.
- Group 4 – Primarily driven by vertebral and thoracic and other.
- Group 5 – Broad regional effects dominated by neurocranial.

Mapping HTS Data from ToxCast/Tox21

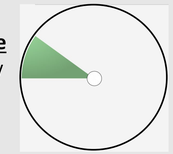
47 Chemicals:
Allethrin, ATRA, Aspirin, Bentazone, Bronopol, Buprofezin, Chlorothalonil, Clodinafop-propargyl, Cyfluthrin, Deltamethrin, Difenoconazole, Diniconazole, Emamectin benzoate, Endosulfan, Endrin, Etoxazole, Fenpyroximate (Z,E), Fipronil, Fluoxastrobin, Flusilazole, Forchlorfenuron, Imazalil,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

ATRA
Broad range of regional effects, dominated by zeugopod

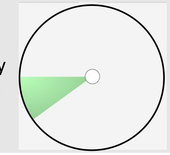
Flusilazole
Dominated by thoracic cage defects, with vertebral and orofacial effects



Tetraconazole
Predominantly thoracic cage defects



Endosulfan
Predominantly thoracic cage defects



Summary and Conclusions

- Classification for skeletal phenotypes observed across 375 chemicals in ToxCast/ToxRefDB. (Thoracic cage defects predominated overall.)
- 47 of those chemicals showed disruption of 1 or more ToxCast assay for potential effects on ATRA signaling.
- These preliminary findings are consistent with potential for chemical disruption of axial patterning through the retinoid system.