New Approach Methods' Model Analysis and Adverse Outcome Pathway Development Validating Retinoid Signaling Associated with Skeletal Dysmorphogenesis

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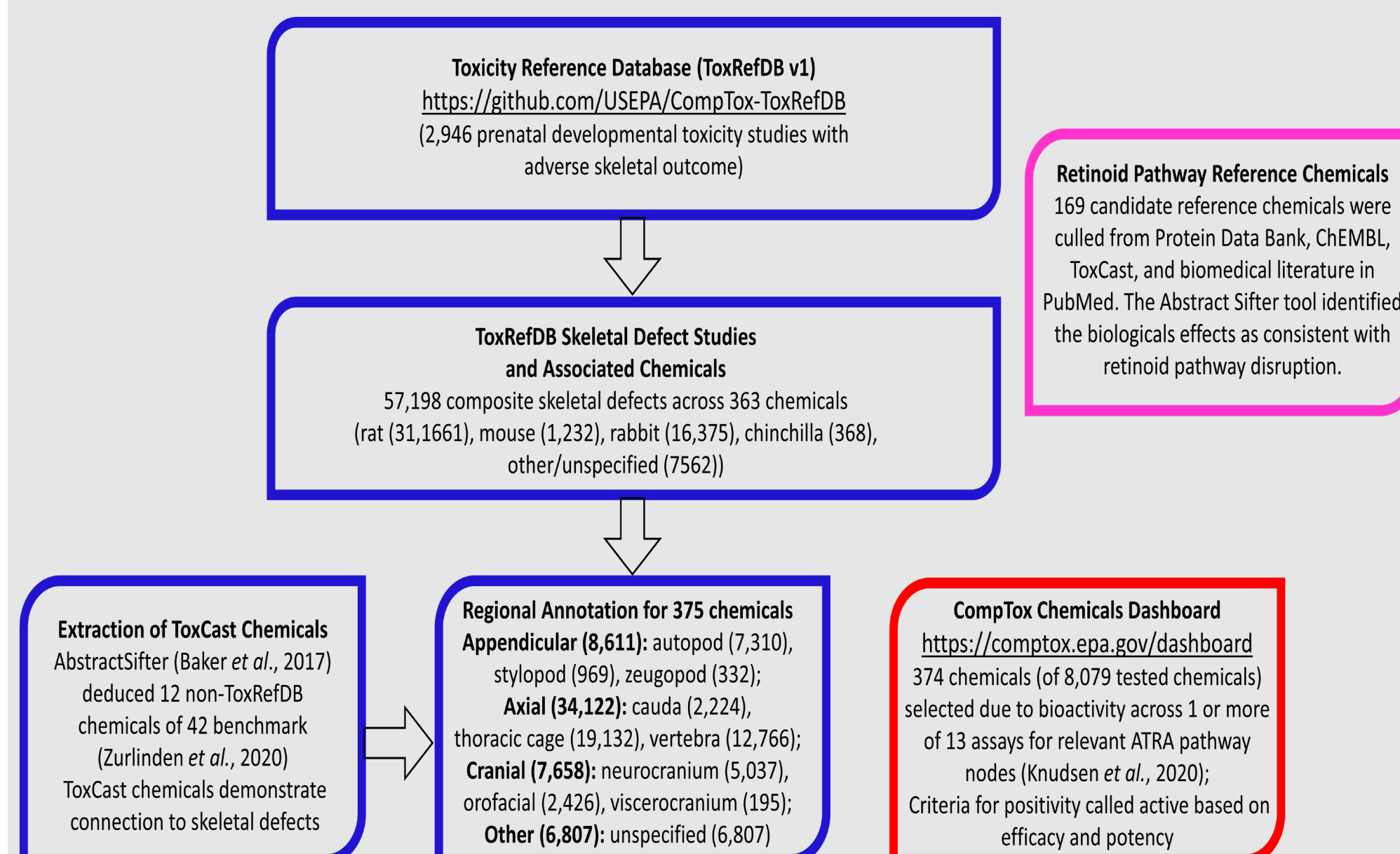
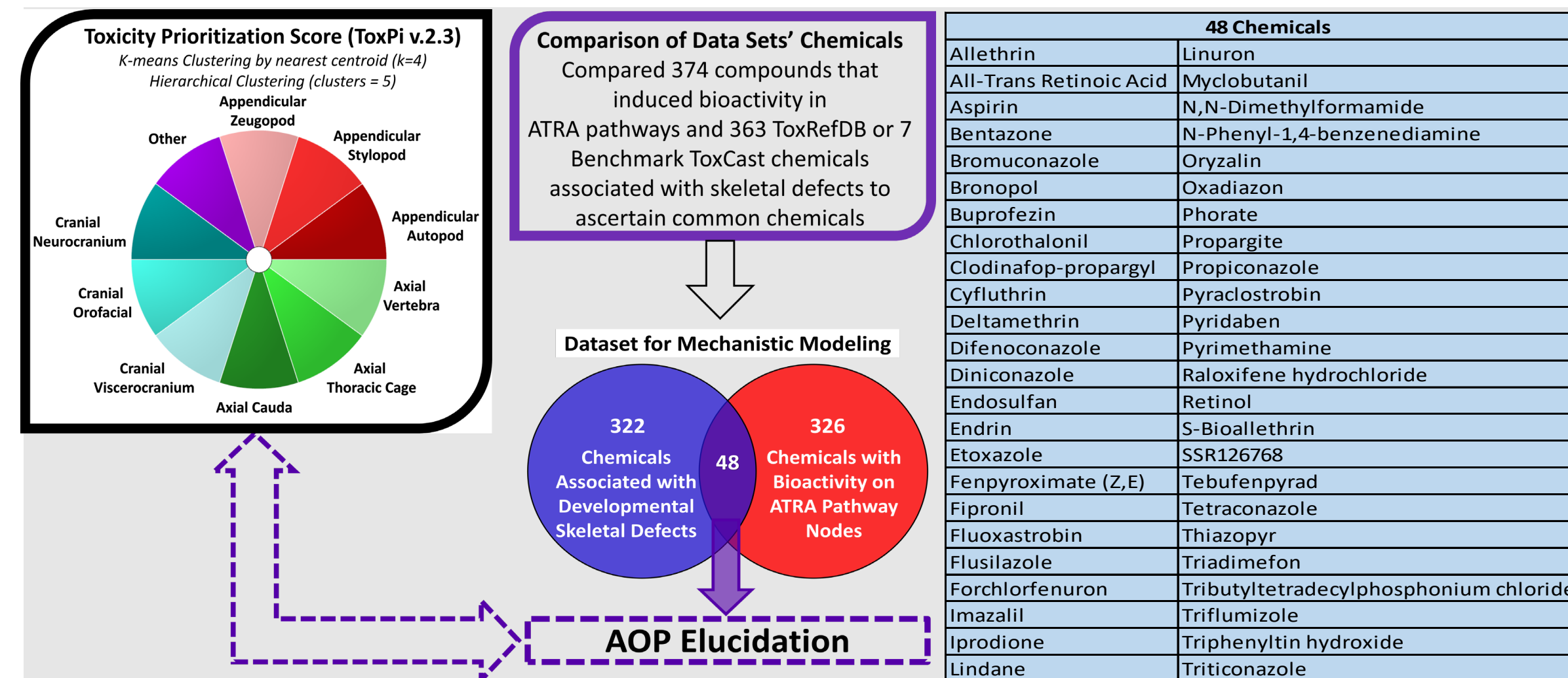pierro.jocylin@epa.gov | 919-541-5184

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 [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]
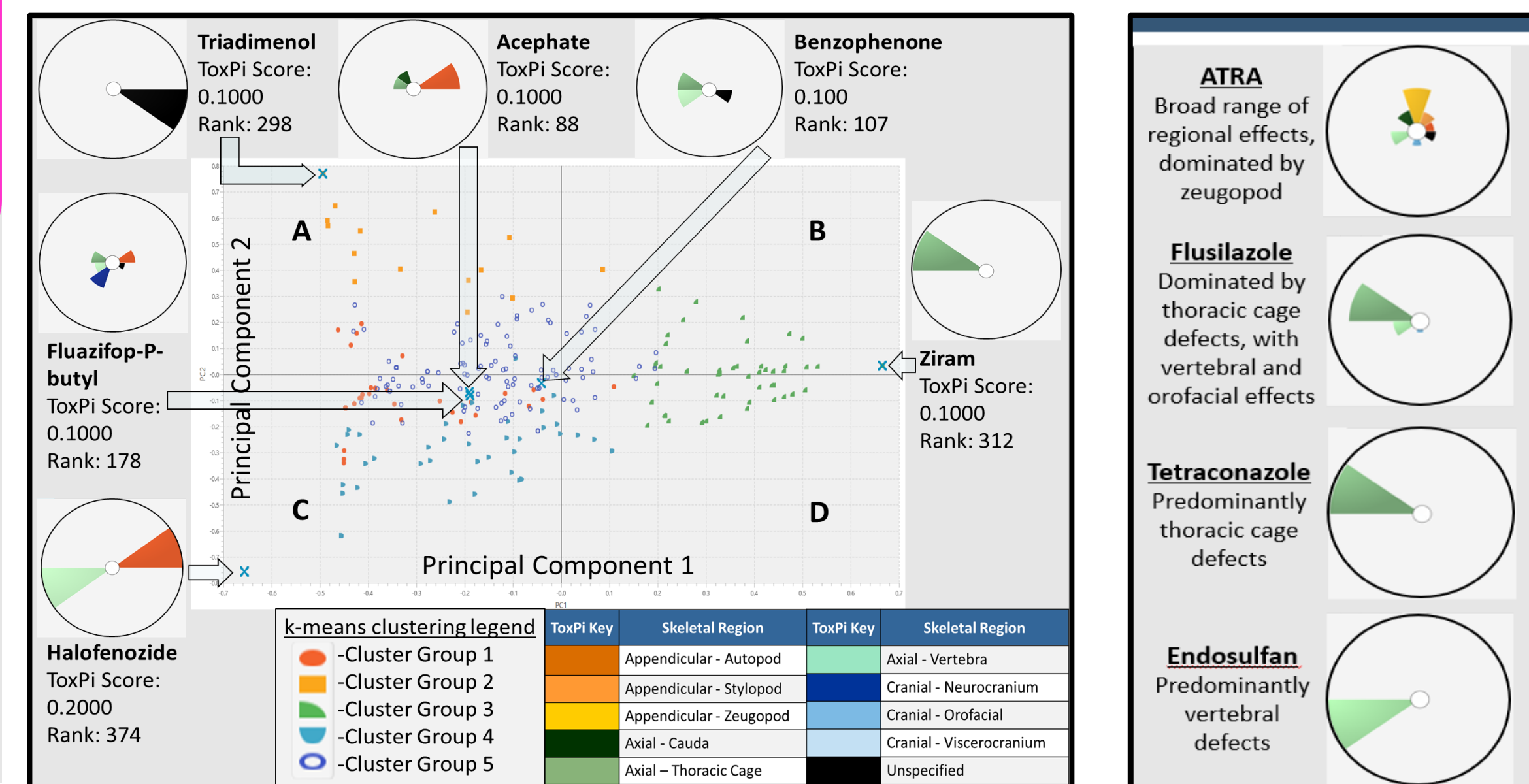
2. Multi-Database Workflow

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))

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.

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

Toxicity Prioritization Score (ToxPi v.2.3)
K-means Clustering by nearest centroid (k=4)
Hierarchical Clustering (clusters = 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

322 Chemicals Associated with Developmental Skeletal Defects | 48 | 326 Chemicals with Bioactivity on ATRA Pathway Nodes

AOP Elucidation

48 Chemicals

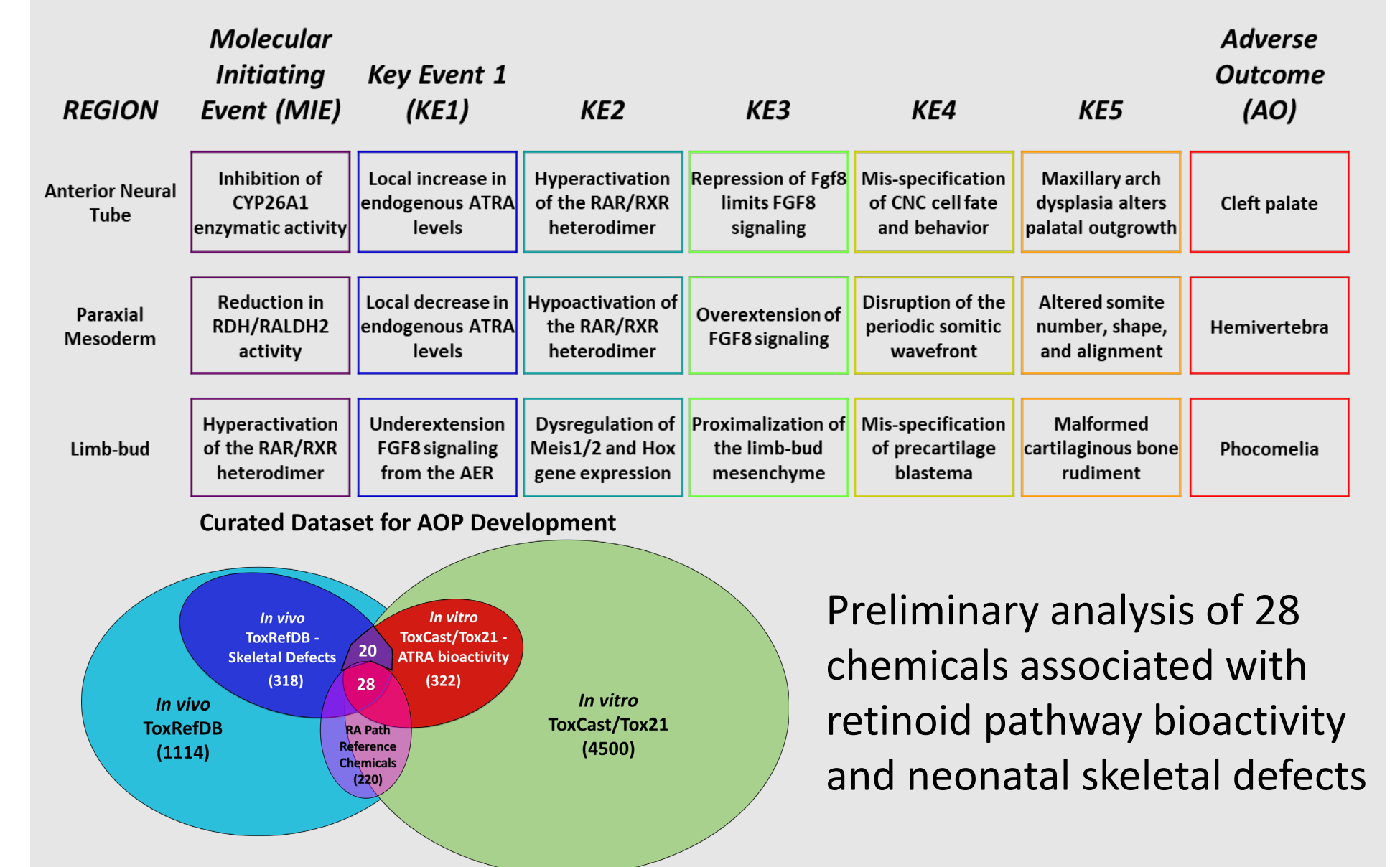|	
Allethrin	Linuron
All-Trans Retinoic Acid	Myclobutanil
Aspirin	N,N-Dimethylformamide
Bentazone	N-Phenyl-1,4-benzenediamine
Bromuconazole	Oryzalin
Bronopol	Oxadiazon
Buprofezin	Phorate
Chlorothalonil	Propargite
Clodinafop-propargyl	Propiconazole
Cyfluthrin	Pyraclostrobin
Deltamethrin	Pyridaben
Difenoconazole	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. 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

Triadimenol ToxPi Score: 0.1000 Rank: 298
Acephate ToxPi Score: 0.1000 Rank: 88
Benzophenone ToxPi Score: 0.100 Rank: 107
Fluazifop-P-butyl ToxPi Score: 0.1000 Rank: 178
Ziram ToxPi Score: 0.1000 Rank: 312
Halofenozide ToxPi Score: 0.2000 Rank: 374

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 vertebral defects

k-means clustering legend:
- Cluster Group 1
- Cluster Group 2
- Cluster Group 3
- Cluster Group 4
- Cluster Group 5

ToxPi Key	Skeletal Region	ToxPi Key	Skeletal Region
	Appendicular - Autopod		Axial - Vertebra
	Appendicular - Stylopod		Cranial - Neurocranium
	Appendicular - Zeugopod		Cranial - Orofacial
	Axial - Cauda		Cranial - Viscerocranium
	Axial – Thoracic Cage		Unspecified

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

Curated Dataset for AOP Development

In vivo ToxRefDB (1114); In vivo ToxRefDB - Skeletal Defects (318); In vitro ToxCast/Tox21 - ATRA bioactivity (322); In vitro ToxCast/Tox21 (4500); 20; 28; RA Path Reference Chemicals (220)

Preliminary analysis of 28 chemicals associated with retinoid pathway bioactivity and neonatal skeletal defects

7. Summary and Conclusions

- Developed curated list of 28 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.