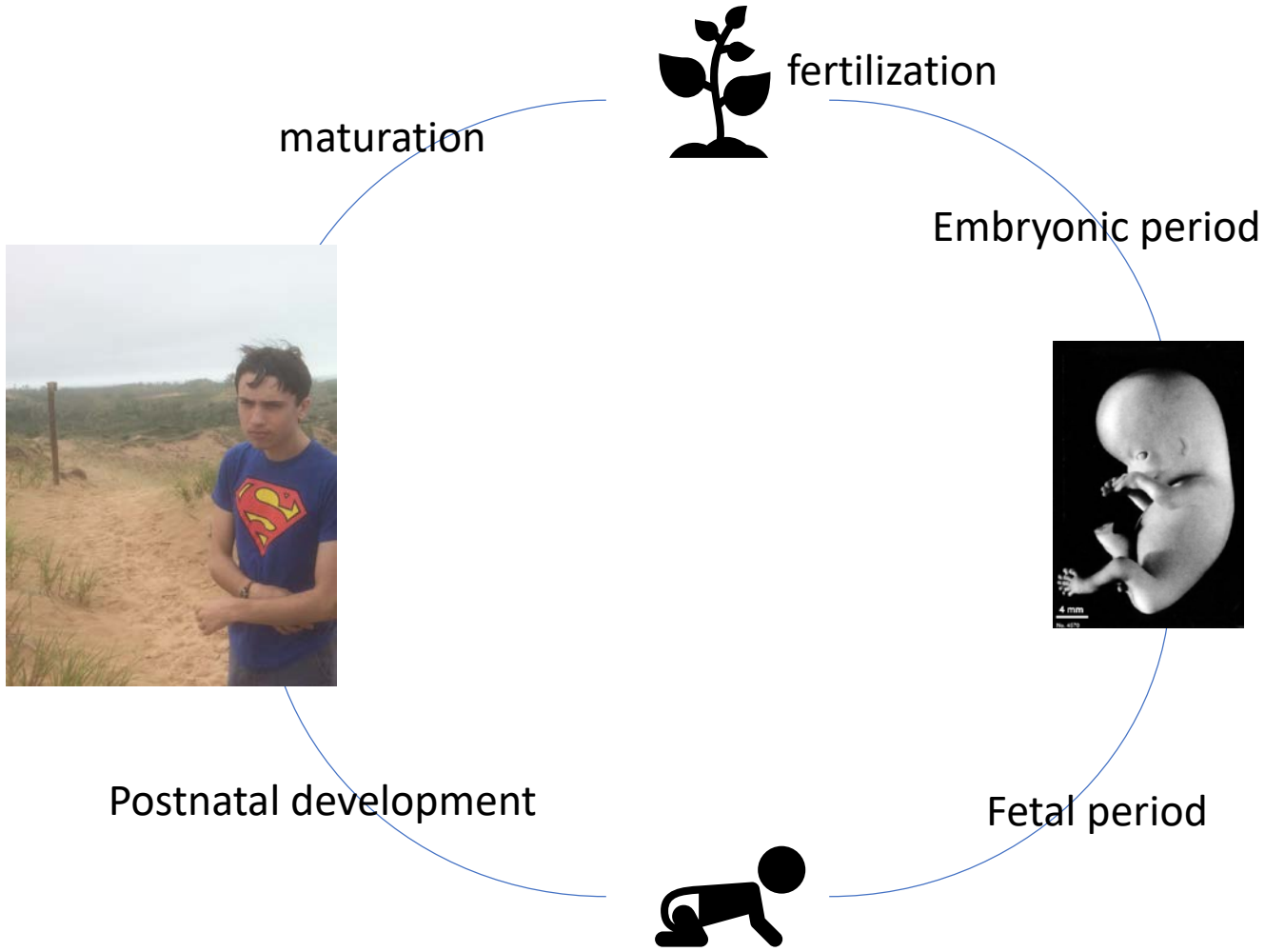
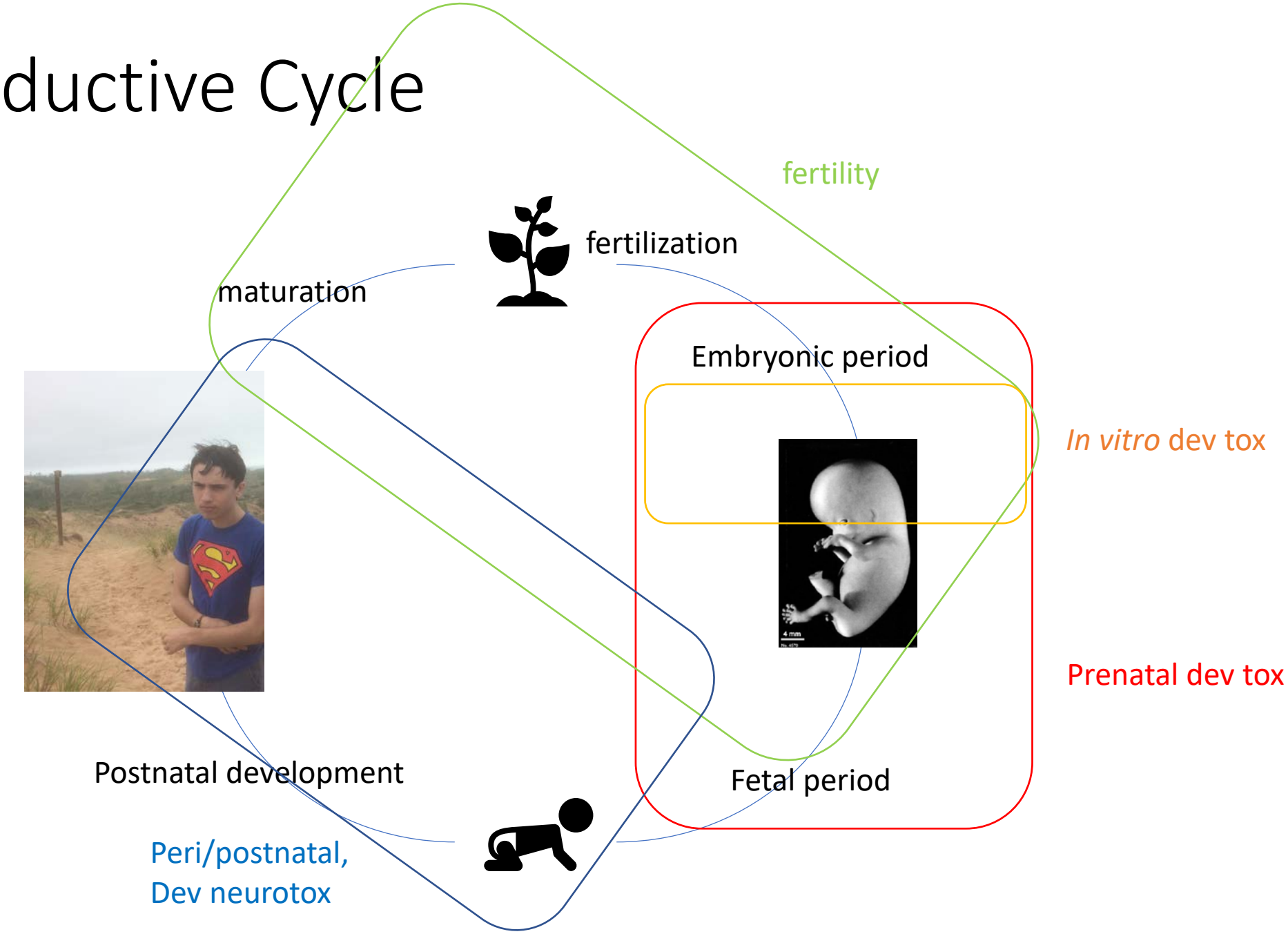
# State of the Science for Predicting Developmental Toxicity Using New Approaches

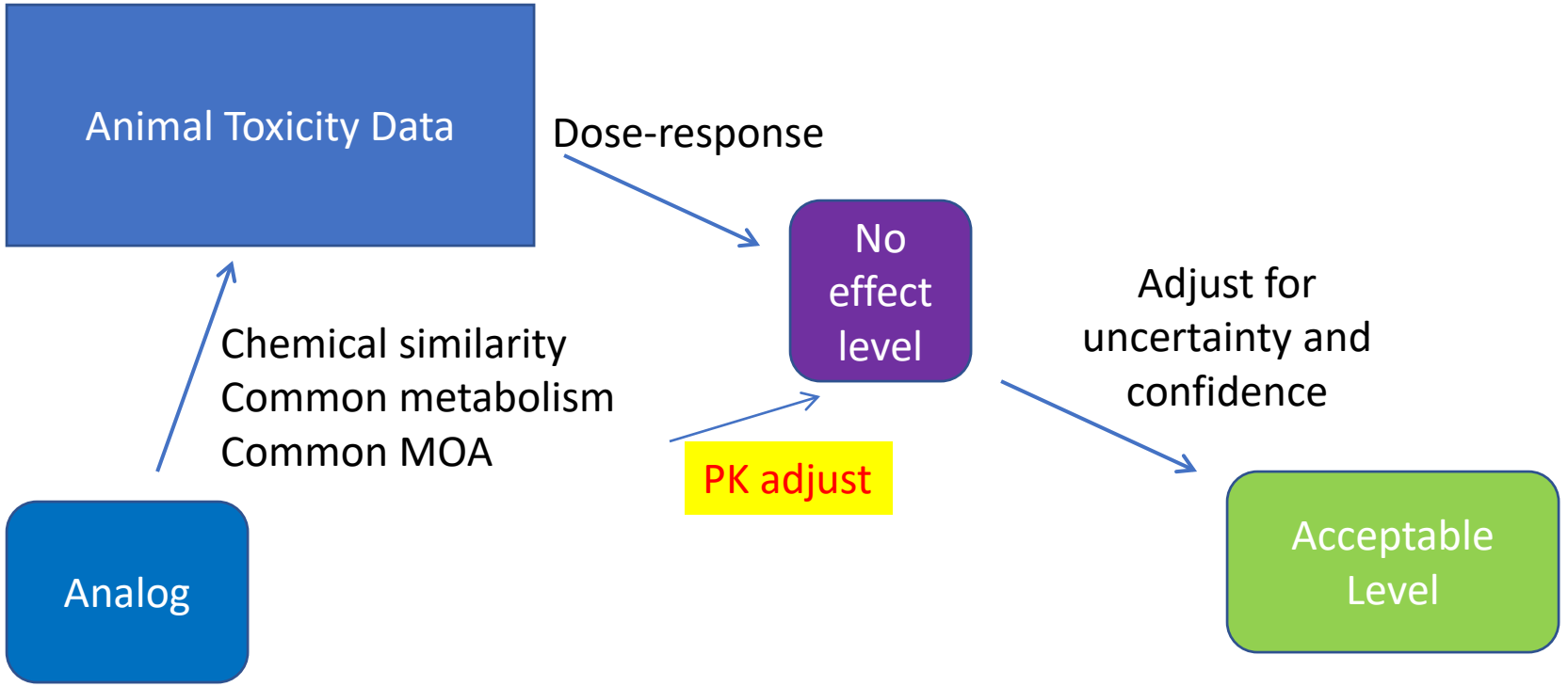
# Reproductive Cycle



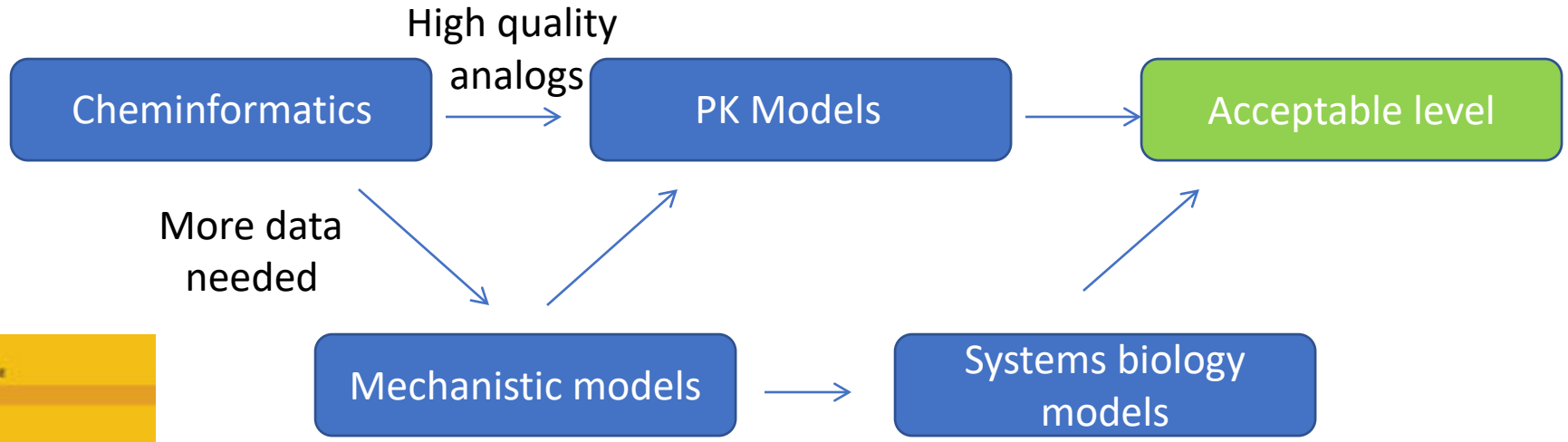
# Reproductive Cycle



# Risk Assessment by Analogy



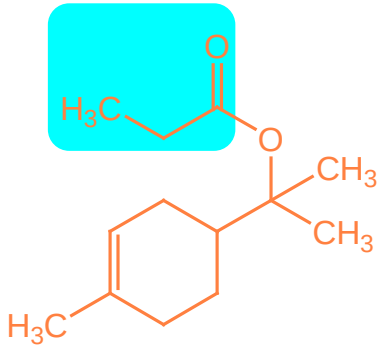
# Predictive Toxicology Workflow



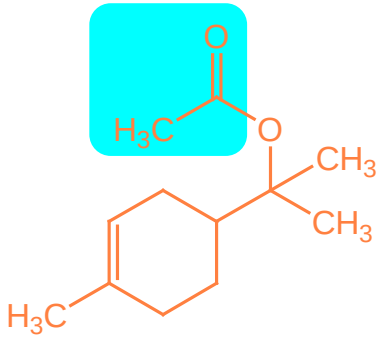

# Chemistry/Toxicity Databases

- EPA CompTox Chemistry Dashboard: 875,000 chemicals
- GRASP: over 800,000 chemicals
- Number of literature references by endpoint in GRASP:
  - 36,000 DART records
  - 21,670 unique chemicals
  - Sources include ECHA, TSCATS, RTECS, NTP, published literature
- Data are searchable by chemical structure

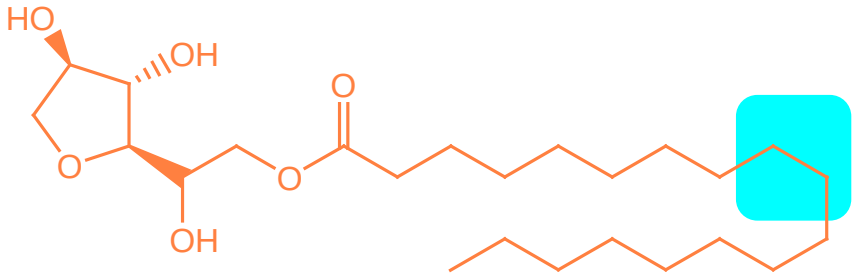
# Suitable Analogs



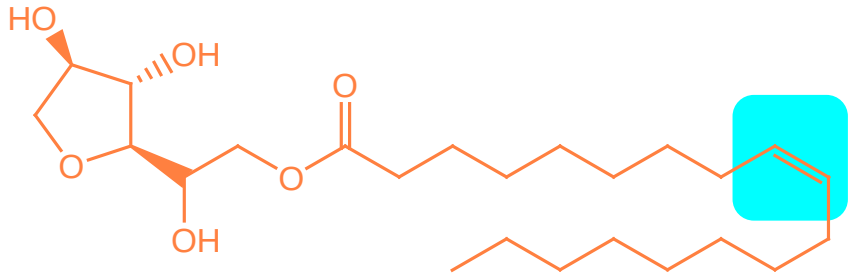

**CAS# 80-27-3**




**CAS# 8007-35-0**

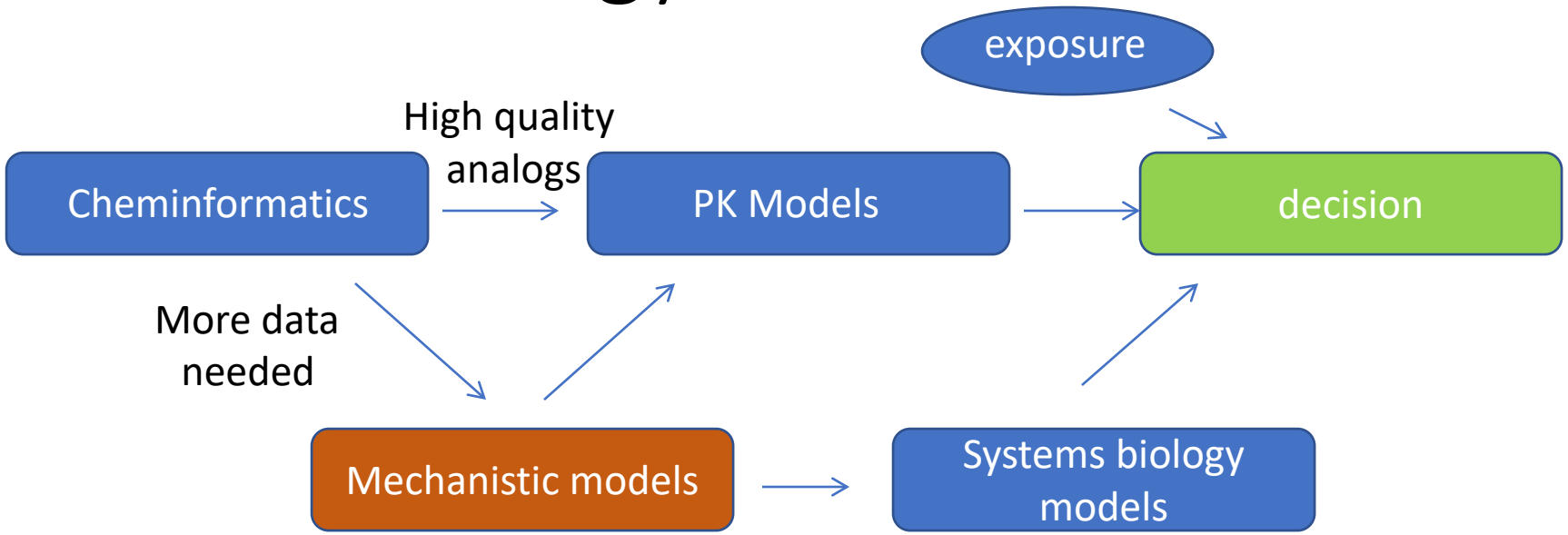



**CAS# 1338-43-8**

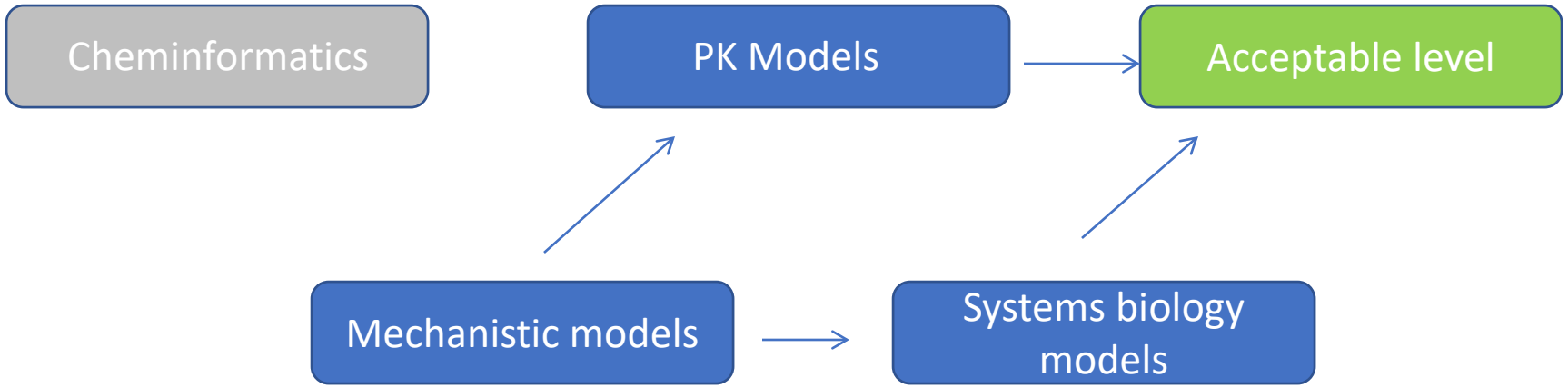



**CAS# 8007-43-0**

# Predictive Toxicology Workflow



# Predictive Toxicology Workflow



# In Vitro Assays for Developmental Toxicants

- Rodent whole embryo culture
  - Morphological development
- Stem cell assays
  - Germ layer formation (gastrulation)
  - Differentiation into specific cell types
  - Metabolomic ratios
- Free-living embryos
  - Zebrafish

# Criteria for Believing in a NAM

- Covers a defined range of modes of developmental toxicity
- Can be combined with other assays that cover the remaining modes of action for universal coverage
- Are responsive to human developmental toxicants
  - Particularly for receptor-mediated toxicity where species differences in receptor-ligand affinity are likely to exist
  - Potency is important: exposure-based validation

# DART Mode of Action Ontology: Categories

- Nuclear hormone receptor ligands
- Retinoic acid synthesis inhibition
- Thyroid hormone synthesis inhibition/ TPO inhibition
- Steroid synthesis inhibitors
- Shh inhibitors and cholesterol synthesis inhibitors
- Tubulin polymerization/ depolymerization inhibitors
- Angiotensin converting enzyme inhibition/ angiotensin receptor antagonism
- Nucleotide derivatives/ nucleotide pool imbalance
- Anti-metabolites
- Anti-angiogenesis
- Anti-coagulants
- HDAC inhibition
- Altered cardiovascular function in embryo
- Acid-base imbalance
- Macromolecule alkylation
- Radicals, oxidizers and oxidative stress
- Inhibition of essential metal function
- Disputed or unknown mechanisms

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# Exposure-Based Validation List (partial)

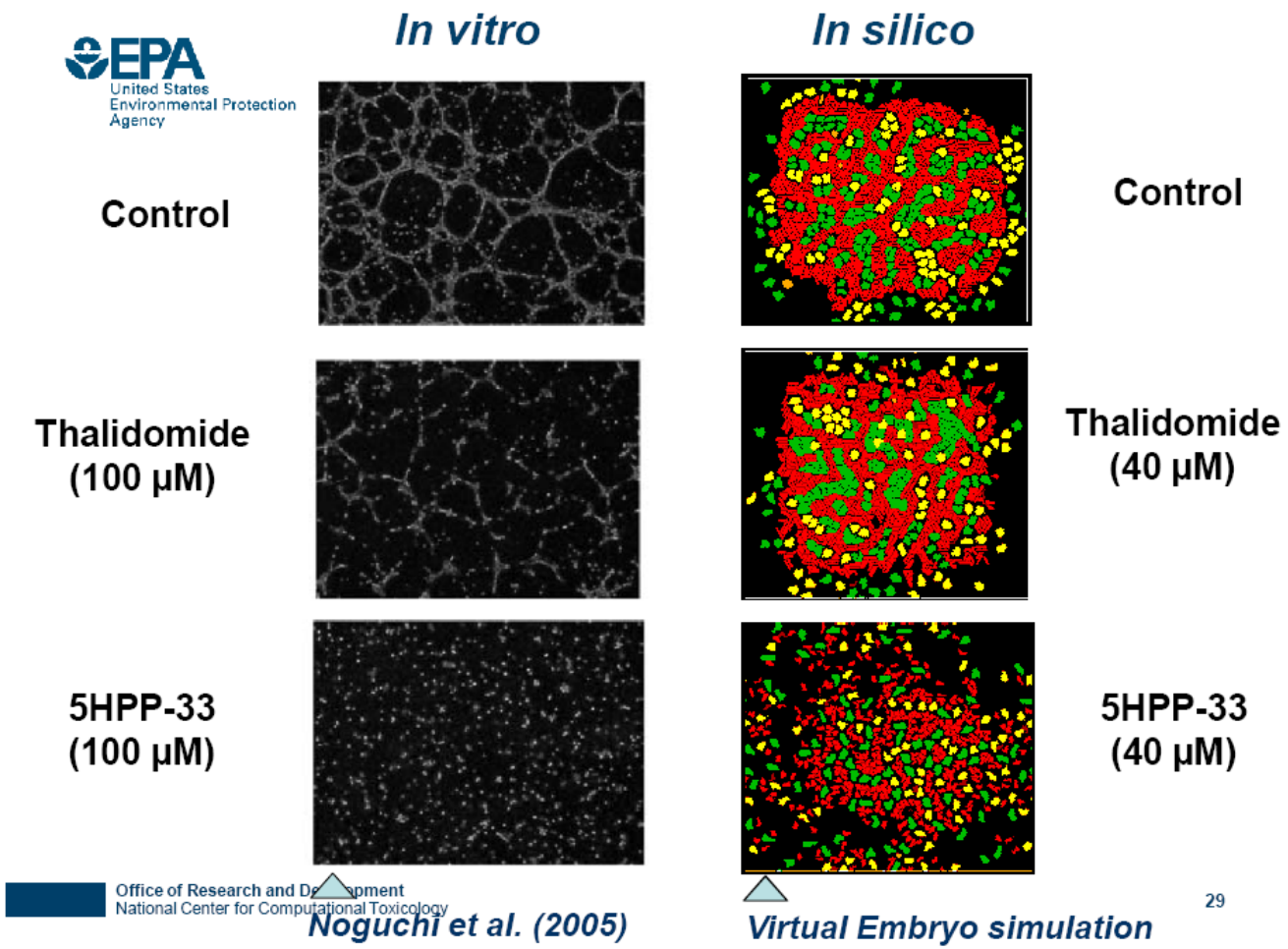
| Compound | Effect Level (uM) | No Effect (uM) |
|---|---|---|
| Abacavir | 80 | 18 |
| All-trans retinoic acid | 0.2 | 0.002 |
| Caffeine | 325 | 7.7 |
| Dabigatran | 7 | 1 |
| Fingolamid | 0.067 | |
| Glycolic acid | 5000 | 275 |
| Methanol | 270,000 | 22 |
| SB-209770 | 500 | 4 |
| zaleplon | | 12 |

# Validation Studies in the Literature

- Warkus and Marikawa, Tox Sci, 2017
  - In vitro gastrulation model using mouse stem cells
  - Positive exposures: 10/17 correctly classified
  - Negative exposures: 14/17 correct
- Marikawa et al., Reprod. Toxicol 2019
  - Human stem cell aggregates
  - Positive exposures: 15/16
  - Negative exposures: 11/12
- Cassar et al., Reprod. Toxicol 2019
  - Zebrafish embryos
  - 75% predictivity

# Computational Models for Development

- Virtual embryo (EPA, Knudsen lab)
  - Cell-agent-based models for specific developmental events and their perturbation by toxicants



# Conclusions

- Read-across is currently the best (and only) method for assessing developmental toxicity on a broad basis
  - HTS and high-content methods can support conclusions about similar biological activity
- Cataloging the universe of developmental toxicity modes of action will be critical in ensuring that non-mammalian methods adequately evaluate toxicity potential