ADVERSE OUTCOME PATHWAYS
BACKGROUND AND PRINCIPLES

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OUTLINE

• BACKGROUND

• ADVERSE OUTCOME PATHWAYS – DEFINED

• EXAMPLES OF APPLICATIONS

• PRINCIPLES OF AOP DEVELOPMENT
Wide range of diagnostic tests are employed in medicine

Doctors explain to patients, what the results of those tests mean relative to health.
TOXICOLOGY

- HAS GENERALLY FAVORED DIRECT OBSERVATION OF APICAL ADVERSE EFFECTS
  - EXPENSIVE
  - TIME-CONSUMING
  - ETHICAL/SOCIOETAL CONCERNS
• Very limited toxicity characterization for most chemicals in commerce.
“Transform toxicity testing from a system based on whole-animal testing to one founded primarily on in vitro methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin”

“The vision emphasizes the development of suites of predictive, high-throughput assays .....”

“The mix of tests in the vision include tests that assess critical mechanistic endpoints involved in the induction of overt toxic effects rather than the effects themselves.”
Examples

- Enzyme activities
- Gene expression
- Hormone concentrations
- Metabolite abundance
- Histological changes
- DNA damage
- Epigenetic modifications
- Lipid or protein abundance
**ToxCast**

> 600 assays, >2000 chemicals,

**ToxCast HTS Assays**

- **Biochemical Assays**
  - Protein families
    - GPCR
    - NR
    - Kinase
    - Phosphatase
    - Protease
    - Other enzyme
    - Ion channel
    - Transporter

- **Assay formats**
  - Radioligand binding
  - Enzyme activity
  - Co-activator recruitment

- **Cellular Assays**
  - Cell lines
    - HepG2 human hepatoblastoma
    - A549 human lung carcinoma
    - HEK 293 human embryonic kidney
  - Primary cells
    - Human endothelial cells
    - Human monocytes
    - Human keratinocytes
    - Human fibroblasts
    - Human proximal tubule kidney cells
    - Human small airway epithelial cells
    - Rat hepatocytes
    - Mouse embryonic stem cells (Sid Hunter)
  - Biotransformation competent cells
    - Primary rat hepatocytes
    - Primary human hepatocytes

- **Assay formats**
  - Cytotoxicity
  - Reporter gene
  - Gene expression
  - Biomarker production
  - High-content imaging for cellular phenotype

- ~500 Total Endpoints

- **Vision**
  - 1536 well HTS
  - 10,000 chemicals
  - 25 assays per year
21st Century Toxicity Testing is here....

We can rapidly and cost effectively generate pathway-based data
• Activity of 1000s of chemicals in 100s of pathways.

Conceivable that majority of chemicals in commerce could be “tested” within the decade.
1-{2-(2,4-Dichlorophenyl)-2-[(prop-2-en-1-yl)oxy]ethyl}-1H-imidazole
An Adverse Outcome Pathway (AOP) is a conceptual framework that portrays existing knowledge concerning the linkage between a direct molecular initiating event and an adverse outcome, at a level of biological organization relevant to risk assessment.


- Helps us organize what we know
- Utilize mechanistic data to support risk-based decision-making
Potential evidence for more specifically-acting toxicity

There’s an AOP for that...reproductive hazard

AOP 25: Aromatase inhibition leading to reproductive dysfunction
Not only is it biologically plausible – its supported by empirical evidence

Consistent profile of effects have been observed with other cyp19 inhibitors and in other species:

- Prochloraz, fathead minnow: *Toxicol. Sci.* 2005. 86: 300-308
AOP as diagnostic manual: explains what it means for health and why.

- AOPs organize knowledge
- Present it systematically
- Manner that is accessible and usable

Adverse Outcome Pathway
What AOPs are not:

- AOPs are not risk assessments
  - Do not explicitly address exposure

- AOPs are not synonymous with HTT or pathway-based assays
  - Aid interpretation of HTT and pathway-based assay data in the context of apical hazard

- AOPs are not Computational Models
  - Computational models that align with AOPs and can be used to simulate KERs along the AOP and predict state of KEs under various conditions/scenarios termed qAOPs.

- AOPs are not a panacea
  - Don’t solve challenges of in vitro / in vivo extrapolation
  - Don’t account for all known biology or all possible modulating variables
What AOPs can do for us:

• Enhance use of mechanistic data in regulatory decision-making

• Support hypothesis-driven testing - target in vivo testing on endpoints of concern

• Inform appropriate cross-species extrapolation & focus testing on species, life-stages, taxa of concern

• Aid a strategic, knowledge-driven approach to evaluating complex mixtures

• Identify critical knowledge & evidence gaps that impede application
EXAMPLE APPLICATIONS

- Development of Alternative Testing Approaches
- Supporting Tiered Testing Strategies / IATA
- Framework for Organizing and Evaluating Evidence
- Quantitative BMD Estimation
- Bioactivity-Based Environmental Monitoring
ALTERNATIVE TESTING APPROACHES

- **IYD inhibition**: HT assay under development
- **NIS inhibition**: HT assay under development
- **TPO inhibition**: HT assay developed >1000 chemicals screened
- **DIO1 inhibition**: HT assay developed >100 chemicals screened
- **DIO2 inhibition**: HT assay developed >100 chemicals screened
ALTERNATIVE TESTING APPROACHES

Identify assays that could be incorporated and establish why they are relevant

https://aopwiki.org/aops/4

Cyclooxygenase inhibition

Prostaglandin E2 concentrations in eggshell gland mucosa, reduced

Ca\(^{2+}\) and HCO\(_{3}\) transport to shell-gland lumen impaired

Eggshell thickness reduced

Crushed eggs, breeding failure

High Throughput Screening

Avian reproduction study (OPPTS 850.2300; OECD 206)

$>250,000

>30 weeks to perform
Aromatase first appears in common ancestor to amphioxus and vertebrates (Baker 2011).

MIE – likely applicable to most vertebrates

Vitellogenesis-related key events likely applicable to oviparous vertebrates
Using an adverse outcome pathway network to describe the weight of evidence linking nicotinic acetylcholine receptor activation to honey bee colony failure
Model-derived response-response relationships for major KERs along the AOP.

Steady state, after compensation assumed.

APPLICATIONS

Examples are not comprehensive

Some of the most prominent applications to date
An International AOP Development Program

• 2011: US EPA/ORD AOP discovery and development program
• 2012: OECD AOP development programme initiated
• 2013: First guidance document – developing and assessing AOPs
• 2014: Case studies in AOP development and revised guidance
1. AOPs are not chemical-specific

- Not trying to describe what a single chemical does

- Trying to describe what ANY chemical that perturbs the MIE with sufficient potency and duration is likely to do-

- Describing AOP does not require chemical-specific information.

- Applying those motifs in a predictive context requires understanding chemical-specific properties (e.g., potency, ADME) that dictate the magnitude and duration of perturbation at the MIE.

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2. AOPs are Modular

- **Functional unit of observation/verification**
- Observable Δ biological state (measurable)
  - Essential (but not necessarily sufficient)
- Description
  - Methods for observing/measuring
  - Taxonomic applicability
Molecular initiating event (MIE) – A specialized type of KE that represents the initial point of chemical interaction, on the molecular level, within an organism, that results in a perturbation that starts the AOP.

Adverse Outcome (AO) – A specialized type of KE that is generally accepted as being of regulatory significance on the basis of correspondence to an established protection goal or equivalence to an apical endpoint in an accepted regulatory guideline toxicity test.
2. AOPs are Modular

Key Event Relationships (KERs): *Functional unit of inference/extrapolation*

• Define a directed relationship
• Describes the conditions and likelihood $\text{KE}_{\text{up}}$ will trigger $\text{KE}_{\text{down}}$.
• State of $\text{KE}_{\text{up}}$ provides some ability to predict or infer state of $\text{KE}_{\text{down}}$

• Supported by plausibility and evidence
• Quantitative understanding
Increasing level of biological organization

- Essential but not necessarily sufficient
- Defines the conditions under which up-stream domino will cause the next in the sequence to fall
Principles of AOP Development

3. Individual AOPs are a pragmatic functional unit of development and evaluation

- By convention AOP consists of a single sequence of key events connecting an MIE to AO (no branches)
- AOP is a pragmatic simplification of complex biology
Principles of AOP Development

4. For most real-world applications, AOP networks are the functional unit of prediction.

AOPs are not triggered in isolation. They interact.
Principles of AOP Development

4. For most real-world applications, AOP networks are the functional unit of prediction

Key events shared by multiple AOPs

KERs shared by multiple AOPs
Principles of AOP Development

4. For most real-world applications, AOP networks are the functional unit of prediction.

- By building modular AOPs, we gradually describe the complexity of potential interactions.
- AOPs meet systems biology.
An AOP network is an assembly of two or more AOPs that share one or more key events.

AOP 25: Aromatase inhibition leading to reproductive dysfunction (in fish)
Principles of AOP Development

5. AOPs are living documents

• AOPs are a way of organizing existing knowledge

• As methods for observing biology evolve:
  • New possibilities for KEs
  • Ability to measure KEs with greater precision/accuracy

• As new experiments are published:
  • Weight of evidence supporting (or rejecting) KERs grows
  • New AOPs and new branches in AOP networks discovered

• There is no objective “complete AOP”
  • There is only useful or not useful for a given application
AOPs are modular
  • KEs and KERs are shared by multiple AOPs
  • No need to re-write the same descriptions over and over
  • Reusability (best practices)

AOPs are living documents
  • KE and KER descriptions can be expected to evolve over time
  • As descriptions are updated and expanded – all AOP descriptions they link to update automatically

• AOP networks for prediction
  • Entry of structured information in KB allows for de-facto assembly of AOP networks.
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