Improving systematic review and usability of NexGen/high throughput data in studies of chemical toxicity using AOPs

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Overview

• What we consider systematic review
• Study Quality
  – Microarray study quality: SOAR
  – HTS assays: update on developments
• Data Integration, Analysis and Graphical Synthesis
  – Data Integration
    • Orthogonal assays (HTS)
    • Bayesian data integration (all data types)
    • Evidence Maps (graphical synthesis)
  – Adverse Outcome Pathway-based Data Integration
    • AOPXplorer

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Define: Systematic Review

Define the Question

Database

Systematic Database Search

Multiple Studies

Systematic Process/Protocol Synthesis

Systematic Review

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Systematic Review

Predefined Protocol
Predefined Search Terms, Databases, Filtering Criteria

Predefined Protocol
Pre-defined study quality criteria, data integration methods, statistical analysis methods, and exit criteria

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SOAR for Microarray Study Quality

• Specific to toxicology microarray studies
• Structured for in vitro and in vivo studies
• Asks questions about study design
• Most questions come from
  – ToxR Tool
  – MIAME standard (microarray data quality standard)
• Scoring trained and evaluated on datasets of known quality
• McConnell, et al 2014 PLOS ONE;; DOI: 10.1371/journal.pone.0110379
• Developed by EPA/ORD/NCEA in collaboration with US Army Engineer Research and Development Center
HTS Study Quality Considerations
(Work in progress)

- If cell assay
  - Is the cell of the correct type/lineage?
    - BG1-Luc-4E2 may not actually be of ovarian lineage
      (http://web.expasy.org/cellosaurus/CVCL_6571)
    - If different lineage, will this impact interpretation?
    - Metabolically competent?
- If protein assay
  - Evidence that protein operates same as in cell?
  - Evidence that protein has same post-translational modifications as expected if in the cell?
- Transcriptional assays
  - Is a realistic or artificial promoter used? This will impact confidence in the result
- Dose range appropriate for question being studied?
- Are species relevant for question being studied?
DATA INTEGRATION
Challenge

Decisions

Data-to-Decisions Chasm

AOPs

Test Data

Modeling

Toxicogenomics

TOX21/ToxCast

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Rules of AOP Development

AOPs are not chemical-specific
AOPs are modular

• Key Events – functional unit of observation – nodes
• Key Event Relationships – dose/response-response – edges

![Diagram of AOP model with MIE, KEs, and AO]

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AOP List

AOP List

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1 AOPs Ready for Commenting
   1.1 Currently Under OECD EAGMST Review
   1.2 Open for General Comments
2 AOPs Under Development

AOPs Ready for Commenting

Currently Under OECD EAGMST Review

- AFB1: Mutagenic Mode-of-Action leading to Hepatocellular Carcinoma (HCC)
- Alkylation of DNA in male pre-meiotic germ cells leading to heritable mutations
- Androgen receptor agonism leading to reproductive dysfunction
- Aromatase inhibition leading to reproductive dysfunction (in fish)
- Binding of agonists to N-methyl-D-aspartate receptor (NMDAR) in adult brain causes excitotoxicity that mediates neuronal cell death, contributing to cognitive and memory loss
- Binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment of learning and memory abilities
- Estrogen receptor agonism leading to reproductive dysfunction
- Binding to the chloride channel of the ionotropic GABA receptor leading to epileptic seizures
- PPARα activation leading to impaired fertility upon uterus exposure in males
- PPARα antagonism leading to reduced physical endurance
- PPARγ activation leading to impaired fertility in adult female
- Protein Alkylation leading to Liver Fibrosis
- Skin Sensitisation Initiated by Covalent Binding to Proteins
- Xenobiotic Induced Inhibition of Tyrophosphodiase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals

Open for General Comments
AOP as a framework to integration and weight of evidence

A. Receptor
   MIE  →  KE 1  →  KE 2  →  KE 3  →  KE 4  →  AO
   KER1  →  KER2  →  KER3  →  KER4  →  KER5

Indirect KER

B. MIE  →  KE 1  →  KE 2  →  KE 3  →  KE 4  →  AO
   3  →  2  →  1  →  3  →  2

AOP with weighted KER

C. MIE  →  KE 1  →  KE 2  →  KE 3  →  KE 4  →  AO
   2  →  3  →  1  →  2  →  2

Evidence for chemical X causing or initiating AOP

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**Diagram Recap**

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### Key Nodes and Pathways

- **Adipose PPARγ**
- **Adipose adioponectin**
- **FXR**
- **NFE2L2/Nrf2**
- **SHP**
- **LXR**
- **Hepatic PPARγ**
- **Lipogenesis**
- **Steatosis**
- **DHB4/HSD17B4**
- **AMPK**
- **Fatty Acid Beta Oxidation**

### Pathway Descriptions

1. **↓ Adipose PPARγ**
   - **ligand**
   - **p17**

2. **↓ Adipose adioponectin**
   - **ligand**
   - **p18**

3. **↓ FXR**
   - **p11**

4. **↓ NFE2L2/Nrf2**
   - **p6**

5. **↓ SHP**
   - **p7**

6. **↑ Lipogenesis**
   - **ligand**
   - **p1**

7. **↑ Hepatic PPARγ**
   - **ligand**
   - **p16**

8. **↓ free FA uptake**
   - **p27**

9. **↓ Adipose FA storage**
   - **p28**

10. **↑ free FA**
    - **p29**

11. **↑ Lipogenesis**
    - **p30**

12. **↓ Fatty Acid Beta Oxidation**
    - **p24**

13. **↓ AMPK**
    - **p5**

### Regulatory Adjustments

- **MIE?**
- **p12**
- **p13**
- **p14**
- **p15**
- **p19**
- **p20**
- **p21**
- **p22**
- **p23**
- **p25**
- **p26**
- **p27**
- **p28**
- **p29**
- **p30**

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**Note:** The diagram includes various nodes and pathways that represent interactions within cellular processes, such as lipid metabolism and regulation. The labels and connections are indicative of regulatory adjustments and pathways involved in metabolic processes.
Modified Bradford hill criteria as mentioned by John

**Lambda**

**A**

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**B**

Liver

**C**

\[ \text{fecundity} = -0.042 + 0.95V_{tg} \]  
\( r^2 = 0.88 \)

**D**

\[ n_{t+1} = \exp(-rP_t/K) M^*n_t \]

population matrix model

**Forecast of fathead minnow population levels**

**KE1**

**KE2**

**KE3**

**KE4**

**KE5**

**AO**

**AOP**

**MIE**

Aromatase Enzyme Inhibition

Granulosa Cell Reduced E2 synthesis

**Estrogen Receptor**

Agonism

**Hepatocyte**

Reduced Vtg production

**Impair oocyte development**

**Female**

Impair ovulation & spawning

Population Declining trajectory
Application to Developing Screening Level Risk Assessments

— Identify all available data for a chemical or mixture
— Use AOPs to identify potential adverse outcomes (hazard ID)
— Use concentration-response or dose-response data to calculate a POD for an AOP
  • Use sufficient key event – key event sufficient to infer adversity based on network theory
— Reverse dosimetry on POD (if in vitro data) to estimate adult POD
— Determine a safe margin from the POD (divide by 100 if a 100x safe margin is desired)
Orthogonal Assays

Estrogen Receptor alpha

Transactivation Assays
Luciferase or beta-lactamase

Cell Proliferation Assays
MCF-7 proliferation

Cell-Free Assays
Binding/Competition

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Bayesian Data Integration

• **Question**: Is tumor rate in controls from study 1 different from tumor rate in controls from study 2?

• **Statistical testing using ROPEs**
  – ROPE: region of practical equivalence
  – Region where we say it’s all equivalent to the null hypothesis
  – So, for a Bayesian “t-test” situation, the null hypothesis is centered at 0, and we’d put a ROPE up that flanks it by maybe 0.5 on either side
95% Highest Density Interval (HDI): From Orange line (5% frequency) and above on the curve

ROPE: Area between the two black lines (+/- 5%)
ROPE and HDI Decision Rules

- If the 95% HDI is completely within the ROPE, then accept null hypothesis.
- If the 95% HDI contains zero, then zero difference is a credible value, then accept null hypothesis.
- If the 95% HDI does not contain zero and the 95% HDI is within the ROPE, cannot accept or reject null hypothesis. More data is required.
- If the 95% HDI is completely outside the ROPE, reject null hypothesis.
Approach

- **Question:** Is tumor rate in controls from study 1 different from tumor rate in controls from study 2?
- *Markov Chain Monte Carlo (MCMC using Stan)*
  - Model 1: tumor rates in controls study 1
  - Model 2: tumor rates in controls study 2
- **Model set-up (for both studies)**
  - Flat uninformative prior for both (Beta(1,1))
  - Likelihood modeled as Bernoulli
  - Posterior: Beta distribution using information from the likelihood
- **Calculate the difference in tumor rates from MCMC**
  - Null hypothesis: difference in tumor rates is within a ROPE centered on 0 +/- 5% (this is a rather generous ROPE)
95% Highest Density Interval (HDI): From Orange line (5% frequency) and above on the curve
ROPE: Area between the two black lines (+/- 5%)

**Conclusion:** The tumor rates are substantially the same from both studies, and likely represent values that are on either side of the mean due to random sampling
Larger Bayesian Analysis Context

• The tumor rates in controls are likely from the same overall distribution
• We have confidence that we can combine the data from both studies to create a more credible model for statistical analysis
• **New Question**: does chemical X change the tumor rates in exposed mice?
• **Answer**: coming soon, stay tuned!
Is Oxybenzone an EDC?

• **Approach**
  – *Orthogonal HTS Data:*
    • PubChem AID 743075 (part of Tox21)
      – ER alpha agonist assay: ER-alpha-UAS-bla GripTite™
    • PubChem AID 743079 (part of Tox21)
      – ER alpha agonist assay: BG1-Luc-4E2
  – *Bootstrap metaregression (R aop package)*
    • Data from the 2 orthogonal assays were combined and bootstrap together (instead of bootstrapping each assay independently)
  – *Point of Departure determination (R aop package)*
Is Oxybenzone an EDC?

Spaghetti plot

95% Confidence Envelope + Median

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Is Oxybenzone an EDC?

Point of Departure:
5.3uM

Oxybenzone is likely an EDC.

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Risk Screening Level for Oxybenzone Estrogenic Activity

Oxybenzone skin absorption data is modeled adequately by a Poisson (3.9) distribution.

Risk Screening Level for Oxybenzone Estrogenic Activity

- Oxybenzone MW: 228.24
- Assume: 5L of blood in human
- Given:
  - POD: 5.3uM
- Human POD by skin absorption (protect 1:10,000 people): 432g
- Margin of Exposure (MOE): 100x
- Human Risk Screening Level: 4.32g

Real-world application of oxybenzone sunscreen
- 28g of sunscreen applied; 4% oxybenzone = 1.12g of oxybenzone applied
- 3 applications gets us within the 100x MOE (Human Risk Screening Level of 4.32g)
Oxybenzone

Pro-Estrogenicity Arguments
- Evidence in 2 estrogen receptor agonist assays
- Assays are different tissues of origin
- One assay: full-length natively expressed ER
- One assay: ER ligand-binding fusion protein
- Both assays from human tissues

Attenuating Statements
- Not a complete system -- may lack paracrine factors
- Lack of pharmacokinetic model
- Do have skin absorption and urine elimination rates

Contra-Estrogenicity Arguments
- Both assays are cells in monoculture -- not organs or organoids
- Cells may not be able to metabolize oxybenzone

Conclusion: Oxybenzone is an EDC
Confidence: Level 7 (Scale 1-10)
Risk Screening: 4.32g

Confidence Level: We are creating a protocol that describes our levels of confidence on a 1-10 scale (10 being highest confidence)

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AOPXplorer

• Tool being developed at US Army ERDC to facilitate analysis of NexGen toxicology data
• Predict adverse outcomes using toxicogenomic and HTS data
• Overlays data onto adverse outcome pathways (AOPs)
• Using Machine Intelligence and causal network theory to make predictions of adverse outcomes using AOPs and your data
• Facilitate Screening Risk Assessment development and publication
• Ongoing development

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Sneak Peak of AOPXplorerer

Steatosis AOP Network

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Incorporation of AOPs from AOPwiki

Fish fecundity AOPs

AOP Decreased Fecundity via Estrogen Receptor-alpha Antagonism AOP

AOP Decreased Fecundity via Aromatase Inhibition AOP

AOP Decreased Fecundity via PPAR-gamma Activation AOP

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Fish Fecundity AOP Network

Decreased Fecundity AOP Network

- PPAR-gamma
- Aromatase
- Estradiol
- Vitellogenin
- Estrogen_receptor_alpha
- Fecundity
AOPXplorer

• So far, all of the analysis code, ontologies, etc are all up on github
  – All of this is a work in progress, and improvements are constantly being made

• AOPXplorer web interface is still under development
• Coordinating with AOP-KB/wiki
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