The Adverse Outcome Pathway Approach to Ecotoxicology and Its Possible Applicability to Human Health Toxicology

Mixtures and Cumulative Risk Assessment: New Approaches: Using the Latest Science and Thinking about Pathways
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- Rapid, inexpensive *in vivo* testing
- Integrative
- Dose effects
- Metabolism
- Developmental testing
- 24-96 well plate format
- Reproductive testing
- Daphnia 21 d reproductive test
- Fathead Minnow 21-d reproductive test
- Behavioral testing
- Exposure
- Relatively inexpensive

Systems toxicology and molecular analysis  Pathway modeling  Rapid in vivo screening
Application of Research to Levels of Organization Based on Source to Outcome

Source

Environmental Contaminant

Exposure

Key Event

Cellular Effects

Toxicity Pathway

Mode of Action

Adverse Outcome Pathway

Source to Outcome Pathway

Community

Population

Individual

Cellular Effects
Biologic inputs

"Normal" Biological Function

Adverse Outcomes (e.g., mortality, Reproductive Impairment)

Cell injury, Inability to regulate

Early cellular changes

Adverse outcome relevant to risk assessment

Toxicity Pathway

Adverse Outcome Pathway
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.

AOP and alternative animals in human health assessment

<table>
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<th>Toxicant</th>
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Screening for toxicological effects and chemicals
Pathway and network impacts
Mechanistic modeling
Predicted effect
Population impact
Reproductive impairment: **Endocrine Disrupting Chemicals**

**Potent Androgen Receptor Agonists**

**Economic Outcome Pathway**

- 17β-trenbolone (trb-acetate)
- AR binding
- AR-dependent somatic cell proliferation
- Increased muscle mass
- Increased economic yield

**Athletic Outcome Pathway**

- 17β-trenbolone (trb-acetate)
- AR binding
- AR-dependent somatic cell proliferation
- Increased muscle mass
- Improved athletic performance
Example: Potent AR Agonists

Adverse Outcome Pathway: Fathead Minnow

17β-trenbolone (trb-acetate) → AR binding → AR-dependent somatic cell proliferation → Negative feedback, hypothalamic neurons → Tubercle and fatpad formation in females → Reduced steroidogenesis, vitellogenesis → Reduced fecundity

- Testosterone
- Estradiol
- Vitellogenin

Adverse Outcome Pathway: Human

17β-trenbolone (trb-acetate) → AR binding → AR-dependent somatic cell proliferation → Negative feedback, hypothalamic neurons → Increased body hair in females → Testicular atrophy, impaired sperm production → Infertility
Adverse Outcome Pathway

Multiple AOPs converging at common insult/node of impaired vitellogenesis

Ankley et al, 2010
Rat versus fathead minnow *in vivo* tests for endocrine active chemicals

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Chemical</th>
<th>Uterotrophic</th>
<th>Hershberger</th>
<th>Pubertal Female</th>
<th>Pubertal Male</th>
<th>Fathead Minnow</th>
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<tbody>
<tr>
<td>Estrogen Agonist</td>
<td>17α-Ethynylestradiol</td>
<td>1</td>
<td>2</td>
<td>3</td>
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<td></td>
<td>Methoxychlor</td>
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<td>2</td>
<td>3</td>
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<tr>
<td></td>
<td>Bisphenol A</td>
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<td>Androgen Agonist</td>
<td>Methyltestosterone</td>
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<td>5</td>
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<tr>
<td></td>
<td>17β-Trenbolone</td>
<td></td>
<td>5</td>
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<td></td>
<td>11</td>
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<tr>
<td>Androgen Antagonist</td>
<td>Flutamide</td>
<td>2</td>
<td>5</td>
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<td>Vinclozolin</td>
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<tr>
<td></td>
<td><em>p,p'-DDE</em></td>
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<td>Steroidogenesis Inhibitor</td>
<td>Ketoconazole</td>
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<td>Fadrozole</td>
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<td>17</td>
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<td>Fipronil</td>
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<tr>
<td></td>
<td>Prochloraz</td>
<td></td>
<td>19</td>
<td></td>
<td></td>
<td>18</td>
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</table>

*positive*  *negative*  

Fathead minnow effective *in vivo* screening tool
A fish ovary molecular network tool to assess reproductive mode of toxicity of chemicals

Mapping the transcriptional response to Flutamide exposure (an anti-androgen)

genes modulated by flutamide exposure cluster in proximity of module 4 enriched in the GO terms anatomical structure development, cell motility and inflammatory response (FDR<10%).

Mutual information network based on expression and hormone levels

885 microarrays, 9 chemicals, multiple doses, multiple time points
Now over 1700 arrays, more chemicals, more doses

Perkins et al 2011 ET&C
Flutamide impacts androgen and estrogen steroid hormone pathways, possibly at the receptor level.
Identification of potential effects on ovaries caused by Flutamide

Flutamide exposure caused oocyte atresia and greater number of early-staged follicles. It decreased ovary maturation.

Involvement of SDF-1 in oocyte atresia -> Potential non-androgenic mediated effects

Flutamide used as treatment in polycystic ovary syndrome in humans -> reduction in ovarian size and production of androgens

De Leo 1998 JCEM
Flutamide and trenbolone effects on fathead minnow ovaries

Understanding simple mixture effects:

- SDF-1 change in flutamide exposure, but not trenbolone suggests non androgen-dependent mechanism
- Demonstrates interaction of different androgen and anti-androgenic chemicals
Toxicity of nitroaromatics in alternative species

Rat (Rattus norvegicus)

Northern Bobwhite quail (Colinus virginianus)

Fathead minnow (Pimephales promelas)

Daphnia magna
Effect of increasing 2,4 DNT on survival and liver health

![Graph showing survival and liver gene expression with different doses of 2,4 DNT.]

Liver gene expression

Lipids and respiration

![Diagram illustrating lipid and respiration pathways with 2,4 DNT.]

Liver lipid metabolism

![Graph showing liver lipid metabolism with different concentrations of 2,4 DNT.]

Dose and mortality

![Graph showing dose-response for 2,4 DNT with time to mortality.]

Liver gene expression

Genomics: Common pathways impacted by nitrotoluenes -Nrf2, PPAR alpha, fatty acid metabolism

- similar to quail and fathead

Liver lipid metabolism

Lipidomics: 37 individual lipid species affected

![Graph showing lipidomics analysis with 2,4 DNT.]

Wintz et al 2006 Toxicol Sci

Ruwat et al 2010

Deng et al PLoS ONE 2010
## Toxicity of anilines and nitrotoluenes

<table>
<thead>
<tr>
<th>Organ/Tissue</th>
<th>Toxicant → Macro-Molecular Interactions</th>
<th>Cellular Responses</th>
<th>Organ Responses</th>
<th>Organism Responses</th>
<th>Organ/Tissue Effects</th>
<th>Whole Body Effects</th>
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<tr>
<td>Liver</td>
<td>Hepatocyte: NH₂ → NH₉NH₂</td>
<td>Kupffer Cell: Phagocytosis of Damaged Erythrocyte</td>
<td>Fatty liver</td>
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<td>Extramedullary hematopoiesis</td>
<td>Cyanosis/Pale Skin</td>
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<td>Blood</td>
<td>Erythrocyte: NH₉NH₂</td>
<td>Nrf2, PPAR</td>
<td>Hemolysis</td>
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<td>Spleen</td>
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<td>Red pulp: Phagocytosis of Damaged Erythrocyte</td>
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<td>Bone marrow</td>
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<td></td>
<td></td>
<td>Hematopoiesis Increased</td>
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### Pathway

1. Hepatocyte: Monooxygenase
2. Erythrocyte: Peroxidation Lipid Membrane etc.
3. NTs
4. Red pulp: Phagocytosis of Damaged Erythrocyte
5. Hematopoiesis

### Typical Observations in RDT Test

- Met HGB ↑
- Reticulo ↑
- RBC ↑
- HGB ↓
- HTC ↓
- T. bilirubin ↑
- Cyanosis/Pale Skin
- Fatty liver
- Extramedullary Hematopoiesis

### Compensatory Response

- Extramedullary Hematopoiesis
- Congestion
- Organ wt. ↑
- Hematopoiesis Increased
The impact of complex mixtures of nitroaromatics on Daphnia magna

- RDX
- HMX
- Aminodinitrotoluenes
- Dinitrobenzene
- Trinitrobenzene
- Trinitrotoluene
- Dinitrotoluene

LAAP: Louisiana Army Ammunition Plant

Garcia-Reyero et al 2011
Effects of individual and mixtures of chemicals on gene expression

Individual chemical effects

Synthetic mixture interactions

PCR panel

Clear mixture effects
D. magna microarray analysis of mixture effects

Pathway analysis (human orthologs)

Mixtures and Individual chemical pathways

The top (most connected and significant) network for each exposure and their connections

BUT: A number of unique functions were also affected in mixtures -> consistent with non-additivity of chemical effects
Summary: AOP and alternative animals in human health assessment

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