Adverse Outcome Pathway as an Integrating Framework for Systematic Review
ADVERSE OUTCOME PATHWAY AS AN INTEGRATING FRAMEWORK FOR SYSTEMATIC REVIEW

Katherine von Stackelberg, ScD
Center for Health and the Global Environment
Harvard Center for Risk Analysis
NEK Associates LTD
kvon@hsph.harvard.edu
1.617.998.1037
Synthesizing Evidence from Exposure to Health Outcome

- **Dose**
  - Exposure models
  - PBPK models
  - Exposure → Internal Dose

- **Mode(s) of action**
  - Target tissue dose

- **Disease etiology**
  - Early biological effects
  - Altered structure function

- **Genetic susceptibility/interaction**

**EXPOSURE**
- Biomonitoring
- Modeling
- PBPK

**EFFECT**
- in vivo
- in vitro
- tox assays
- epidemiology
- molecular epidemiology

**DISEASE ETIOLOGY**
- cellular events
- subclinical disease

Intersection and synthesis of effect data, exposures in the environment, and disease etiology
Adverse Outcome Pathway

Exposure links the generalized AOP with fit-for-purpose risk assessment

KE: key event
MIE: molecular initiating event
AO: adverse outcome

Response pathway from cellular to organ to organism

MIE → KE_{up} → KE_{down} → AO

Early cellular key events

Adverse outcomes relevant to risk assessment

Toxicological Data → Adverse Outcome Pathway → Epidemiologic Data
Case Studies as Examples

Review Article
A Systematic Review of Carcinogenic Outcomes and Potential Mechanisms from Exposure to 2,4-D and MCPA in the Environment

Katherine von Stackelberg1,2

1 E Risk Sciences, LLP, 12 Holton Street, Allston, MA 02134, USA
2 Harvard Center for Risk Analysis, 401 Park Drive, Landmark 4046, Boston, MA 02215, USA

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Chlorophenox compounds, particularly 2,4-dichlorophenoxyacetic acid (2,4-D) and 4- (MCPA), are amongst the most widely used herbicides in the United States for both agric and home uses. Epidemiologic studies suggest that exposure to 2,4-D and MCPA may be associated with various neoplastic (NHL), Hodgkin’s disease (HD), leukemia, and soft-tissue sarcoma (STS). Toxicologic studies have also identified possible carcinogenicity in various regulatory agencies worldwide. This systematic review assembles the available data to evaluate epidemiologic exposure, and biomonitoring studies with respect to key cellular events noted in disease. Hypothesized modes of action for these constituents to determine the prevalence of a environmentally relevant concentration of 2,4-D and MCPA and lymphohematopoietic cancer support a genotoxic mode of action. Although plausible hypotheses for other carcinogenic in biomonitoring data to oral equivalent doses calculated from bioassay data shows that envision support a causative relationship. Genetic polymorphisms exist that are known to increase the risk interaction between these polymorphisms and exposures to chlorophenox compounds, but largely unknown.

Perspective
Exposure to Mixtures of Metals and Neurodevelopmental Outcomes: A Multidisciplinary Review Using an Adverse Outcome Pathway Framework

Katherine von Stackelberg,1,2,a Elizabeth Guzy,2 Tian Chu,2 and Birgit Claus Henn1,3

Current risk assessment guidance calls for an individual chemical-by-chemical approach that fails to capture potential interactive effects of exposure to environmental mixtures and genetic variability. We conducted a review of the literature on relationships between prenatal and early life exposure to mixtures of lead (Pb), arsenic (As), cadmium (Cd), and manganese (Mn) with neurodevelopmental outcomes. We then used an adverse outcome pathway (AOP) framework to integrate lines of evidence from multiple disciplines based on evolving guidance developed by the Organization for Economic Cooperation and Development (OECD). Toxicologic evidence suggests a greater than additive effect of combined exposures to As-Pb-Cd and to Mn with any other metal, and several epidemiologic studies also suggest synergistic effects from binary combinations of Pb-As, Pb-Cd, and Pb-Mn. The exposure levels reported in these epidemiologic studies largely fall at the high-end (e.g., 95th percentile) of biomonitoring data from the National Health and Nutrition Examination Survey (NHANES), suggesting a small but significant potential for high-end exposures. This review integrates multiple data sources using an AOP framework and provides an initial application of the OECD guidance in the context of potential neurodevelopmental toxicity of several metals, recognizing the evolving nature of regulatory interpretation and acceptance.

KEY WORDS: Adverse outcome pathway; arsenic; cadmium; developmental; lead; manganese; metal mixtures; systematic review
## Table 1: Summary of Reviews of 2,4-D and/or MCPA.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Evaluation</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>US EPA, SAB [27]</td>
<td>Science Advisory Board consultation on carcinogenicity of 2,4-D</td>
<td>“Data are not sufficient to conclude that there is a cause and effect relationship between exposure to 2,4-D and non-Hodgkin’s lymphoma”</td>
</tr>
<tr>
<td>US EPA [28]</td>
<td>4th carcinogenicity of 2,4-D peer review</td>
<td>Not classifiable as to carcinogenicity</td>
</tr>
<tr>
<td>WHO/IARC [29]</td>
<td>Evaluations of carcinogenic risk</td>
<td>Inadequate and/or limited for 2,4-D specifically and chlorophenoxy compounds generally</td>
</tr>
<tr>
<td>US EPA [5]</td>
<td>Health Effects Division Carcinogenicity Peer Review Committee (2,4-D)</td>
<td>“Evidence is inadequate and cannot be interpreted as showing either the presence or absence of a carcinogenic effect.”</td>
</tr>
<tr>
<td>European Commission [30]</td>
<td>Review report for 2,4-D</td>
<td>Proposed uses have no harmful effects on animal or human health; no evidence of carcinogenicity</td>
</tr>
<tr>
<td>US EPA [3, 4]</td>
<td>Risk assessments and reregistration decision for MCPA</td>
<td>Limited evidence for carcinogenicity</td>
</tr>
<tr>
<td>US EPA [8]</td>
<td>Risk assessments and reregistration decision for 2,4-D</td>
<td>Group D, not classifiable as to carcinogenicity</td>
</tr>
<tr>
<td>Health Canada PMRA [31]</td>
<td>Reregistration decision for 2,4-D</td>
<td>No evidence of carcinogenicity</td>
</tr>
<tr>
<td>Health Canada PMRA [32]</td>
<td>Reregistration decision for MCPA</td>
<td>No evidence of carcinogenicity</td>
</tr>
<tr>
<td>Health Canada [9]</td>
<td>MCPA in drinking water</td>
<td>Not considered a carcinogen</td>
</tr>
<tr>
<td>77FR23135 [33]</td>
<td>Response to NRDC petition to revoke 2,4-D registration</td>
<td>No new evidence that would suggest registration should be revoked</td>
</tr>
</tbody>
</table>
Putative AOP – Generalized Schematic of NHL

Endogenous and exogenous factors
- Obesity
  - Autoimmune conditions
- Viruses
  - Bacteria
- Oxidative stress
  - Chemicals

Chromosomal aberrations / direct DNA interaction

Genetic factors (polymorphisms e.g., DNA repair)
- Activation of response genes
  - Inflammatory factors
  - Anti-apoptotic factors
  - Proliferative factors

Endogenous and exogenous factors
- Chronic inflammation
- Cell survival
- Proliferation

Increased lymphoma risk

FIGURE 5: Schematic of general pathways leading to increased risk of developing lymphoma.
### Genetic Abnormalities in NHL

<table>
<thead>
<tr>
<th>B cells</th>
<th>Somatic mutations</th>
<th>Corresponding lymphoma</th>
<th>Genetic abnormalities</th>
<th>Percent of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem cell</td>
<td>None</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro B cell</td>
<td>None</td>
<td>B-cell lymphoblastic lymphoma (B-LBL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre B cell</td>
<td>None</td>
<td>Mantle cell lymphoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immature B cell</td>
<td>None</td>
<td>Chronic lymphocytic leukemia</td>
<td></td>
<td></td>
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<tr>
<td>Mature naive B cell</td>
<td>None</td>
<td>Burkitt lymphoma (BL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Follicular lymphoma (FL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germinal center</td>
<td>Introduction of somatic mutations</td>
<td>Lymphocyte-predominant Hodgkin lymphoma</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Diffuse large cell B-cell lymphoma (B-LBL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Memory B cell</td>
<td>Somatic mutations</td>
<td>Marginal zone B-cell lymphoma; chronic lymphocytic leukemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma cell</td>
<td>Somatic mutations</td>
<td>Plasmacytoma/myeloma</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anaplastic large T-cell lymphoma</td>
<td></td>
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</tr>
</tbody>
</table>

Bone marrow

Peripheral lymphoid tissue

Terminal differentiation

- Foreign antigen independent
- Foreign antigen dependent

<table>
<thead>
<tr>
<th>Percent of cases</th>
</tr>
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<tbody>
<tr>
<td>&gt;95%</td>
</tr>
<tr>
<td>80%</td>
</tr>
<tr>
<td>5%</td>
</tr>
<tr>
<td>15%</td>
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<tr>
<td>90%</td>
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<tr>
<td>5%</td>
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<td>5%</td>
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<tr>
<td>35%</td>
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<tr>
<td>30%</td>
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<tr>
<td>10%</td>
</tr>
<tr>
<td>5%</td>
</tr>
<tr>
<td>50%</td>
</tr>
<tr>
<td>60% adults; 85% children</td>
</tr>
</tbody>
</table>
Molecular Epidemiology for 2,4-D

- Urinary 2,4-D levels not correlated with chromosome aberration frequency (although significant genomic instability)
- Exposure-related effects observed were reversible and temporary (Source: Garry et al. EHP 2001)
- t(14;18)-positive NHL cases have larger relative risks from agricultural exposures than t(14;18)-negative cases, but show no association with 2,4-D or chlorophenoxyis (Source: Chiu and Blair J Agromed 2009; Schroeder et al. Epi 2001; Agopian et al. J Exp Med 2009)
- t(14;18) occurs frequently in general public (Source: Bende et al. Leukemia 2007; Janz et al. Genes Chrom Canc 2003)
Summary of Toxicological Studies

- 2,4-D and MCPA are capable of interacting with cellular functions
  - Cell proliferation
  - CD38 – MCPA – marker in NHL
  - Mitotic arrest
- Show an impact on immunological parameters in humans exposed *in vivo* but effects are transient and reversible
- Less evidence for direct DNA interaction
  - Equivocal results *in vivo*
  - Some positive *in vitro* but did not come up in ToxCast
- Commercial formulations rather than pure product tend to show positive results
- No evidence for difference in toxicity across salts, acid, ester
Reverse Dosimetry to Obtain Urinary Concentrations Associated with *in vivo/in vitro* Assays to Compare to Biomonitoring Data

**Oral Equivalent Dose**

\[ \text{Oral Equivalent Dose} = AC_{50} \cdot \frac{1 \text{mg/kg} - d}{C_{ss} (\mu M)} \]

*Source: Wetmore et al. Tox Sci 2011*

Predicted concentration in urine at oral equivalent dose in µg/L:

\[ C_{urine} = \frac{OED \cdot BW}{V_{24hr}} \]

*Source: Aylward et al. Reg Tox Pharm 2008*

Compare to biomonitoring data

Use other reference values from bioassays

Evaluate underlying assays with respect to disease etiology
**Figure 7:** Biomonitoring data for 2,4-D in the context of backcalculated urine levels. Reference lines: green = men 575 μg/L (RfD), men 1170 μg/L (ToxCast reverse dosimetry), blue = women 480 μg/L (RfD), women 960 μg/L (ToxCast reverse dosimetry), red = child 630 μg/L (RfD), child 1250 μg/L (ToxCast reverse dosimetry) (see for review [26, 124, 154, 201, 214, 215, 218–227]).
Exposure to Mixtures of Metals

- Superfund Research Program at Harvard
- Mexico city cohort n=493
- Previous study showed U-shaped association between Mn and neurodevelopmental outcomes at 12 mos (Claus-Henn et al. 2010, Epidemiology, 21(4):433-439)
- Exposure to both Pb and Mn associated with greater cognitive deficits than to either constituent individually (Claus-Henn et al. 2012, EHP, 120(1):126-131)
• Evidence for less or greater than additive effect
  – Independent or additive
  – Antagonistic
  – Synergistic

• Multiple exposures leading to a common but non-specific health outcome
  – Reduced performance on a battery of tests including WISC, Bayley’s, etc.

• Frame for the AOP is normal neuronal development and opportunities for perturbations

• May be similar or different mechanisms

• AOPwiki and associated OECD guidance
Strawman for Greater than Additive Effects
## Biological Plausibility and Essentiality

### 1. Support for Biological Plausibility

<table>
<thead>
<tr>
<th>Strong</th>
<th>Moderate</th>
</tr>
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<tbody>
<tr>
<td><strong>MIE: Activation of MEK1/2, ERK in astrocytes</strong>&lt;br&gt;As-Cd-Pt: 63&lt;br&gt;Ax: 121&lt;br&gt;Cd: 122, 123, 124&lt;br&gt;Mn: 125–127&lt;br&gt;Pt: 128</td>
<td></td>
</tr>
<tr>
<td><strong>KE: Increased cellular Ca²⁺</strong>&lt;br&gt;As-Cd-Pt: 60, 63&lt;br&gt;Ax: 135, 136, 137&lt;br&gt;Cd: 92, 123, 124, 138&lt;br&gt;Mn: 125&lt;br&gt;Pt: 96, 97</td>
<td></td>
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<tr>
<td><strong>KE: Reactive organic species and oxidative stress in brain tissue</strong>&lt;br&gt;As-Cd-Pt: 60–63&lt;br&gt;As-Pt: 55&lt;br&gt;Ax: 121, 139, 137, 141&lt;br&gt;Cd: 92, 139, 138, 124&lt;br&gt;Mn: 153, 125, 126, 152&lt;br&gt;Pt: 96, 112, 154</td>
<td></td>
</tr>
<tr>
<td><strong>KE: Apoptosis of astrocytes or neurons</strong>&lt;br&gt;As-Cd-Pt: 60–63&lt;br&gt;As-Pt: 55&lt;br&gt;Ax: 121, 139, 137, 141&lt;br&gt;Cd: 92, 139, 138, 124&lt;br&gt;Mn: 153, 125, 126, 152&lt;br&gt;Pt: 96, 112, 154</td>
<td></td>
</tr>
<tr>
<td><strong>KE: Disruptions in neurotransmitter release</strong>&lt;br&gt;Cd-Mn-Pt: 33&lt;br&gt;Cd-Pt: 38, 40, 47&lt;br&gt;As-Pt: 54&lt;br&gt;Cd-Mn: 59&lt;br&gt;Mn-Pt: 50&lt;br&gt;Ax: 157, 135, 142&lt;br&gt;Cd: 123, 124&lt;br&gt;Mn: 98, 99, 144&lt;br&gt;Pt: 96, 97, 112, 143, 154</td>
<td></td>
</tr>
<tr>
<td><strong>AO: Learning and cognition disorders</strong>&lt;br&gt;As-Pt: 81, 84 reported qualitative interaction, 76–79 reported synergistic&lt;br&gt;Mn–Pt: 14, 87&lt;br&gt;As–Mn: 15</td>
<td></td>
</tr>
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</table>

### 2. Support for Essentiality of KEs

<table>
<thead>
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<th>Strong</th>
<th>Moderate</th>
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<tbody>
<tr>
<td><strong>MIE: Activation of MEK1/2, ERK in astrocytes</strong>&lt;br&gt;117, 119–121</td>
<td></td>
</tr>
<tr>
<td><strong>KE: Increased cellular Ca²⁺</strong>&lt;br&gt;130, 131, 132, 133, 149</td>
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</tr>
<tr>
<td><strong>KE: Reactive organic species and oxidative stress in brain tissue</strong>&lt;br&gt;116, 129, 141</td>
<td></td>
</tr>
<tr>
<td><strong>KE: Apoptosis of astrocytes or neurons</strong>&lt;br&gt;113, 152, 158</td>
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</tr>
<tr>
<td><strong>KE: Disruptions in neurotransmitter release</strong>&lt;br&gt;101, 111, 155, 156, 159</td>
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### 3. Empirical Support for KEs

<table>
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<th>Strong</th>
<th>Moderate</th>
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<tr>
<td><strong>Key event relationships</strong>&lt;br&gt;Empirical evidence/data is insufficient to develop regulatory values for synergistic effects of metal mixtures.</td>
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</table>

Numbers refer to reference number.
Comparison of Levels from Epi Studies to Biomonitoring Data

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<tr>
<th>Cohort</th>
<th>Epi Exposure</th>
<th>Contam Units</th>
<th>Reference</th>
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<tr>
<td>children 6-8</td>
<td>creat-adj urine</td>
<td>µg/g creat</td>
<td>Adams et al. 2009</td>
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<tr>
<td>children 6-9</td>
<td>creat-adj urine</td>
<td>µg/g creat</td>
<td>Calderon et al. 2001</td>
</tr>
<tr>
<td>children 6-9</td>
<td>blood</td>
<td>µg/dL</td>
<td>Calderon et al. 2001</td>
</tr>
<tr>
<td>children 1-2</td>
<td>blood</td>
<td>µg/dL</td>
<td>Claus Penn et al. 2012</td>
</tr>
<tr>
<td>children 1-2</td>
<td>blood</td>
<td>µg/L</td>
<td>Claus Penn et al. 2012</td>
</tr>
<tr>
<td>children 8-11</td>
<td>blood</td>
<td>µg/L</td>
<td>Kim et al. 2009</td>
</tr>
<tr>
<td>women 18-45</td>
<td>blood</td>
<td>µg/dL</td>
<td>Kim et al. 2013</td>
</tr>
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<td>women 18-45</td>
<td>blood</td>
<td>µg/L</td>
<td>Kim et al. 2013</td>
</tr>
<tr>
<td>women 18-45</td>
<td>cord blood</td>
<td>µg/L</td>
<td>Lin et al. 2013</td>
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<tr>
<td>women 18-45</td>
<td>cord blood</td>
<td>µg/L</td>
<td>Lin et al. 2013</td>
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<tr>
<td>children 11-14</td>
<td>blood</td>
<td>µg/dL</td>
<td>Lucchini et al. 2012</td>
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<tr>
<td>children 6-10</td>
<td>blood from hair</td>
<td>µg/dL</td>
<td>Marlowe/Moon et al.</td>
</tr>
<tr>
<td>children 6-10</td>
<td>hair</td>
<td>mg/kg</td>
<td>Marlowe/Moon et al.</td>
</tr>
<tr>
<td>children 6-10</td>
<td>hair</td>
<td>mg/kg</td>
<td>Marlowe/Moon et al.</td>
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<tr>
<td>children 6-6</td>
<td>urine</td>
<td>µg/L</td>
<td>Rosado et al. 2007</td>
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<tr>
<td>children 6-6</td>
<td>blood</td>
<td>µg/dL</td>
<td>Rosado et al. 2007</td>
</tr>
<tr>
<td>children 8-11</td>
<td>creat-adj urine</td>
<td>µg/g creat</td>
<td>Wasserman et al. 2011</td>
</tr>
<tr>
<td>children 8-11</td>
<td>blood</td>
<td>µg/dL</td>
<td>Wasserman et al. 2011</td>
</tr>
<tr>
<td>children 11-14</td>
<td>hair</td>
<td>mg/kg</td>
<td>Wright et al. 2006</td>
</tr>
</tbody>
</table>
Process and mechanistic models – at relevant spatial and temporal scales – can be incorporated at any stage
Putative AOP for Mercury Based on Human Health

**Molecular initiating events/biologic target**
- Blood Plasma
  - Binding to sulfhydryl (SH)
  - Interference with SH/selenium proteins
  - (cysteine group) and transported to sensitive organs
- Thyroid
  - Interference with SH/selenium proteins
- Endocrine System
  - Oxidative Stress
- Brain
  - Interference with SH/selenium proteins
  - Glutathione (GSH) Depletion
- Brain/Mitochondria
  - Oxidative Stress
- Liver
  - ROS increased

**Cellular Effects**
- Endocrine System
  - Tissue and cell damage
  - Apoptosis of steroidogenic cells

**Organ Response**
- Suppressed levels of Sex steroid hormones
  - (testosterone, estradiol, Gonadosomatic index)
- Suppression of gonad development

**Organism Response**
(for use in the Population Model)
- Reproduction
  - Reduced and delayed spawning
  - Reduced Fecundity, small gonads
  - Embryonic development
- Survival
  - Mortality (acute and/or chronic)
  - Young of year survival
- Growth
  - Decreased larval swimming
  - Growth
  - Emaciation

**Additional Effects**
- (considered to be indicators of adverse effects, but the relevance to individuals or population-level effect are not well understood)
  - Changes in gene transcription
  - Biochem processes altered
Initial BayesNet – Relative Risk Model

- Fish Mercury Body Burden
- Mercury AOP(s)
- Survival of reproductive age class
- Population Model (outside of Netica)
- Current population status
- Fish Organic Contaminant Body Burden
- Toxicity
- Fecundity
- Population age structure/
  Population dynamics
- Fish Population Change
- Water Quality
- Habitat
- Ecological Modification
- Food Source
- Recruitment
- Abundance
Conclusions

- AOPs provide a context for data from systematic reviews
- Important that the initial literature review-data extraction follows a structured, transparent approach
  - Toxicological
  - Epidemiologic
  - Exposure and biomonitoring
  - Health outcome etiology
- Institute of Environmental Toxicology at Western Washington University
- Puyallup Research and Extension Service at Washington State University