Hemostasis

1 Hemostasis
- Platelet
- Activated platelet
- Fibrin-stabilized platelet plug
- Vascular endothelial damage

2 Hemostasis
- Coagulation cascade
- Fibrin
- Degradation products
- Fibrinolytic cascade

3 Hemostasis
- Blood flow
Anticoagulant Rodenticides

Block Vitamin K cycle

Inhibits formation active clotting Factors II, VII, IX, and X, resulting in hemorrhage
1st Generation Anticoagulant Rodenticides – FGARs

Warfarin (1948)
Diphacinone (1960) intermediate generation
Chlorophacinone (1971) intermediate generation
  Multiple feeding to cause death (short half-life)
Genetic changes in rats (Scotland, Europe, Japan) – “Resistance”

2nd Generation Anticoagulant Rodenticides - SGARs

Brodifacoum (1979), Bromadiolone, Difethialone, Difenacoum
  Single feeding can cause death
More toxic, longer half-life (potentially PBT)
Greater hazard to Non-target Species
Widespread Use
Residential, Urban, Agriculture, Island Restoration
Primary Exposure
Humans (AAPCC: >12,000 calls/yr)
Companion animals (APCC: 8,000 calls/yr)
## Exposure in Predatory Birds and Mammals

High detection rates in liver of wildlife (principally SGARs)

<table>
<thead>
<tr>
<th>Location</th>
<th>Species</th>
<th>Percentage</th>
<th>Number</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canada</td>
<td>Owls</td>
<td>70%</td>
<td>164</td>
<td>Albert et al. 2010</td>
</tr>
<tr>
<td>France</td>
<td>Raptors</td>
<td>73%</td>
<td>30</td>
<td>Lambert et al. 2007</td>
</tr>
<tr>
<td>France</td>
<td>Mustelids</td>
<td>12%</td>
<td>122</td>
<td>Fournier-Chambrillon et al. 2004</td>
</tr>
<tr>
<td>Scotland</td>
<td>Raptors</td>
<td>47%</td>
<td>773</td>
<td>Hughes et al. 2013</td>
</tr>
<tr>
<td>Britain</td>
<td>Raptors</td>
<td>37%</td>
<td>351</td>
<td>Walker et al. 2008</td>
</tr>
<tr>
<td>Britain</td>
<td>Barn owls</td>
<td>26%</td>
<td>717</td>
<td>Newton et al. 1999</td>
</tr>
<tr>
<td>Britain</td>
<td>Polecats</td>
<td>31%</td>
<td>100</td>
<td>Shore et al. 2003</td>
</tr>
<tr>
<td>New Zealand</td>
<td>Various species</td>
<td></td>
<td></td>
<td>Eason et al. 1995, 2002</td>
</tr>
<tr>
<td>United States</td>
<td>Fishers</td>
<td>79%</td>
<td>58</td>
<td>Gabriel et al. 2012</td>
</tr>
<tr>
<td>United States</td>
<td>Bobcat</td>
<td>90%</td>
<td>39</td>
<td>Riley et al. 2007</td>
</tr>
<tr>
<td>United States</td>
<td>Raptors</td>
<td>49%</td>
<td>265</td>
<td>Stone et al. 2003</td>
</tr>
<tr>
<td>United States</td>
<td>Raptors</td>
<td>86%</td>
<td>161</td>
<td>Murray 2011</td>
</tr>
</tbody>
</table>
Adverse Outcome Pathway

**Toxicant**
- Chemical Properties

**Macro-Molecular Interactions**
- Receptor/Ligand Interaction
- DNA Binding
- Protein Oxidation

**Cellular Responses**
- Gene Activation
- Protein Production
- Altered Signaling
- Protein Depletion

**Organ Responses**
- Altered Physiology
- Disrupted Homeostasis
- Altered Tissue Development or Function

**Organism Responses**
- Lethality
- Impaired Development
- Impaired Reproduction
- Cancer

**Population Responses**
- Structure
- Recruitment
- Extinction

Anchor 1 (initiating event)

Anchor 2 (adverse outcome at the organism- or population-level)
Toxicant

Warfarin
hydroxycoumarin
Log Kow 2.6

Diphacinone
indandione
Log Kow 4.27

Brodifacoum
hydroxycoumarin
Log Kow 8.5
Structure Activity Relationship Models

Warfarin

Brodifacoum
Macromolecular Interactions

Vitamin K cycle

Vitamin K hydroquinone

Glutamate residues on clotting factors II, VII, IX, X

γ-Carboxyglutamate residues on activated clotting factors

AR-insensitive

Other?

DT - Diaphorase

VKOR

VKOR

AR-sensitive

AR-sensitive

O

O

OH

OH

γ-Glutamyl vitamin K-dependent carboxylase

Vitamin K

Vitamin K epoxide

AR antidote

Vitamin K₁ (phyllloquinone)
Macromolecular to the Cellular Response
Cell-based Model of Hemostasis

Initiation

| fibroblast |
| Va | TF | IXa |
| Xa | VIIa | IX |
| X | prothrombin |

Propagation

| thrombin |
| prothrombin |
| vWF/VIIa |
| X |

Amplification

| activated platelet |
| Xa | Va | VIIa | Xla |
| XI |

Hoffman and Monroe. 2001. Thrombosis and Haemostasis
Macromolecular to the Cellular Response
Cell-based Model of Hemostasis

Hoffman and Monroe. 2001. Thrombosis and Haemostasis
Lag for Onset of Coagulopathy

Clearance of “functional” clotting factors in humans

<table>
<thead>
<tr>
<th>Clotting Factor</th>
<th>II</th>
<th>VII</th>
<th>IX</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half-Life</td>
<td>48-120 hr</td>
<td>2-6 hr</td>
<td>18-40 hr</td>
<td>30-70 hr</td>
</tr>
</tbody>
</table>

Appearance of “des-carboxy dysfunctional” factors

![Graphs showing prothrombin time and Russell's Viper Venom Time](image-url)
Other Cellular Responses

Vitamin K cycle-related

- reduced bone density
- anti-inflammatory and immune signaling
- inhibits cell proliferation

Uncouple oxidative phosphorylation (mitochondrial toxicity)

Peroxisome proliferator-activated receptor
Multiple Organ Response

Hemorrhage due to coagulopathy:
- Skin
- Musculoskeletal
- Respiratory
- Renal
- Gastrointestinal
- Reproductive
- Central Nervous System

Gabriel et al. 2012. PLOS ONE
Blood Loss and Anemia

Reduced RBC count and hematocrit resulting in pallor

Metabolic acidosis
Increased cardiac output
Hypovolemic shock
Severe hypoperfusion
Localized ischemia, hypoxia
Organ dysfunction
Necrosis

Plausible Linkage
Organism Response

Lethargy (“weakness, fatigue”)
↓ Body condition, reduced fitness
↑ Blood loss from minor trauma
Susceptibility to disease (notoedric mange)
Alter predator-prey dynamics?

Reproductive toxicants

Recovery or Death

Hypothetical Linkage

http://www.urbancarnivores.com/notoedric-mange-a-disease-of/
## Population Response

Incidence of confirmed poisoning of total exposures (~10%)  

<table>
<thead>
<tr>
<th>Country</th>
<th>Species/Number</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canada</td>
<td>6 of 114 owls</td>
<td>Albert et al. 2010</td>
</tr>
<tr>
<td>France</td>
<td>3 of 16 mustelids</td>
<td>Fournier-Chambrillon et al. 2004</td>
</tr>
<tr>
<td>Scotland</td>
<td>15 of 362 raptors</td>
<td>Hughes et al. 2013</td>
</tr>
<tr>
<td>Britain</td>
<td>9 of 187 barn owls</td>
<td>Newton et al. 1999</td>
</tr>
<tr>
<td>USA</td>
<td>4 of 46 fishers</td>
<td>Gabriel et al. 2012</td>
</tr>
<tr>
<td></td>
<td>9 of 139 raptors</td>
<td>Murray 2011</td>
</tr>
</tbody>
</table>

![Graph showing probability vs. log concentration](image-url)
Species of Special Conservation Status

Weka          New Zealand
Red kite      France
San Joaquin kit fox  US
Bald eagle    US
Barn owl      Canada
Adverse Outcome Pathway

**Toxicant**
- First- and second-generation anticoagulant rodenticides

**Macromolecular Interaction**
- Irreversible inhibition of VKOR by binding at Tyr 139
- Limited formation of vitamin K hydroquinone
- Failure to carboxylate clotting factors

**Cellular Response**
- Clotting time (PT, aPTT)
- Acidosis, cell necrosis
- ↓ Bone density
- Anti-inflammatory
- ↓ Cell proliferation

**Multiple Organ Response**
- Hemorrhage
  - Skin, Muscle, Renal, GI, CNS
  - Reproductive, Respiratory
- Acidosis
  - ↑ Cardiac output
  - Hypovolemic shock
  - Organ dysfunction

**Organism Response**
- Anemia
  - Mortality
- ↓ Fitness
  - Body condition
  - ↑ Susceptibility to disease

**Population Response**
- Little consequence for abundant species
- Moderate to serious consequence for species of special conservation status

**Key**
- Established Linkage
- Diagnostic Tool to verify death related to rodenticides
- Hypothetical Linkage
- Plausible Linkage
- Biomarker

Rattner et al. 2014. Environmental Science & Technology
Adverse Outcome Pathway

Toxicant: First- and second-generation anticoagulant rodenticides

Macromolecular Interaction:
- Irreversible inhibition of VKOR by binding at Tyr 139
- Limited formation of vitamin K hydroquinone
- Failure to carboxylate clotting factors

Cellular Response:
- Clearance of carboxylated clotting factors II, VII, IX, X
- ↑ clotting time (PT, RVVT)
- Ischemia, cell necrosis
- ↓ Bone density
- Anti-inflammatory
- ↓ Cell proliferation

Antidote vitamin K

Key:
- Established Linkage
- Plausible Linkage
- Diagnostic Tool to verify death related to rodenticides
- Biomarker
Adverse Outcome Pathway

Multiple Organ Response
- Hemorrhage
- Skin, Muscle, Renal, GI, CNS
- Reproductive, Respiratory

Organism Response
- Anemia
- Mortality

Population Response
- Little consequence for abundant species
- Moderate to serious consequence for species of special conservation status

Key
- Established Linkage
- Plausible Linkage
- Diagnostic Tool to verify death related to rodenticides
- Biomarker

USGS
science for a changing world
Data Gaps and Research Needs

Interspecific differences in sensitivity (raptors > granivores)
  - VKOR activity
  - Are there sensitive sub-populations or conserved across species
  - Metabolism and elimination

Relative potency of ARs for VKOR (additive tox models for mixtures)

Role of vitamin K status

Significance of sublethal effects at individual- and population-level

Quantitative estimates non-target predator mortality
Anticoagulant Rodenticide AOP for “non-target predatory wildlife”

Perkins et al. 2014. Advancing AOPs for Integrated Toxicology and Regulatory Applications
Toxicity Reference Values
Cumulative Probability Survival Curves

Male Hawaiian Hawk

---

Graph: Cumulative Probability vs. grams liver/day to exceed Lowest Lethal Dose

Graph: Prothrombin Times and Russell's Viper Venom Time vs. Chlorphacinone/kg-day ww

BMD 90, BMD 50, BMD 19

---
The train already left the station – major regulatory decisions already made in U.S. EPA, EC and EU in the past 5 years without AOP framework.

AR-AOP could provide biological plausibility for a decision (EIA).

Good communication tool for regulatory agencies and public.

Increase confidence in a risk assessment by using Weight of Evidence Approach in an AOP Framework.
Conclusions

Mechanism of action at molecular level well-known

Relative potency of ARs only partially understood

Limited demographic studies in areas of high AR use

Development of mechanistic dynamic models in silico

Look at other toxicants (Pb) that may have same AO (anemia) through a different MIE
Questions?