Special Consideration of Cavity-Nesting Bees for Pesticide Exposure

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An international workshop on pesticide exposure assessment for non-*Apis* bees

- Silvia Hinarejos & Thomas Steeger, Richard Bireley, Jordi Bosch, Natalie Boyle, Wayne Hou, Theresa Pitts-Singer, Rajwinder Singh, & Neal Williams

Routes of pesticide exposure in solitary, cavity-nesting bees

- Andi M. Kopit and Theresa Pitts-Singer
Objectives
- Life history traits relevant in risk assessment.
- Pesticide properties; contribution to risks.
- Exposure routes.
- Bee assay techniques.
Solitary Bees

Most are temperate species. Highest diversity of bees in deserts. Nests composed of multiple cells. Each cell has pollen-nectar provision upon which egg is laid.
**Managed Solitary Bees**

*Osmia cornuta*, Europe

*Osmia bicornis*, Europe

*Nomia melanderi*, western U.S.

*Osmia cornifrons*, Japan, U.S.

*Osmia lignaria*, North America

*Megachile rotundata*, Mediterranean, North America
### Life History Traits of Honey Bees vs Solitary Bees: Implications for Risk Assessment

<table>
<thead>
<tr>
<th>Traits</th>
<th>Honey Bees</th>
<th>Solitary Bees</th>
<th>Expected implications for risk assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecundity</td>
<td>~1,500 eggs/day</td>
<td>Usually ≤ 2 eggs/day (total 10-40)</td>
<td>Social colony resilience makes fitness effects from individual to population level hard to assess in honey bees. Easier in solitary bees.</td>
</tr>
<tr>
<td>Body Size</td>
<td>~100 mg</td>
<td>2 - 400 mg</td>
<td></td>
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<tr>
<td>Nesting substrate, material</td>
<td>Large cavities or hives. Wax and propolis.</td>
<td>Most underground. Others above in cavities.</td>
<td>Ground-nesters exposed via excavation, dwelling in soil, contact of food with soil. Natural cavities less likely routes, but nest materials may be contamination source to solitary bees.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mud, soil, leaves, resin, floral oil, etc.</td>
<td></td>
</tr>
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## Life History Traits of Honey Bees vs Solitary Bees: Implications for Risk Assessment

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<tr>
<td>Nesting period</td>
<td>All or most of the year</td>
<td>Usually 2-3 months in spring or summer.</td>
<td>Honey bees available year-round. Managed adult solitary bees only available for 3-4 months in spring or summer.</td>
</tr>
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# Life History Traits of Honey Bees vs Solitary Bees: Implications for Risk Assessment

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| Larval food             | Royal jelly, bee bread, and honey.       | Pollen mixed with nectar.                  | Honey bees: larval exposure is “filtered” by nurse bees.  
Solitary bees: fresh larval food. |
| Larval food provisioning| Progressive feeding                      | Mass-provisioning                          | Honey bees: food for individual larva collected over extended period; food stored and fed later.  
Solitary bees: larval food collected over short time (1-2 days); feeds within a few days after egg laid. |
Chemical Properties of Pesticides
- Contribute to how / where pesticide exist or persist in environment.

Octanol:Water Coefficient
Lipophilicity, High $K_{ow}$:
a chemical’s affinity for lipids.
- allows permeation of plant and insect cuticular lipid layers.

Hydrophilicity, Low $K_{ow}$:
a chemical’s affinity for water.
- effects environmental accumulation & bioavailability for plant uptake; promotes systemic activity.

http://pubs.acs.org/doi/abs/10.1021/ac300087z
Soil adsorption indicated by the soil partitioning coefficient, $K_{oc} = \text{“stickiness” of compound to organic carbon in soil.}$

High $K_{oc}$ = soil accumulation potential.
Low $K_{oc}$ = movement/leaching via water potential.
<table>
<thead>
<tr>
<th>Family/Class</th>
<th>Mode of Action</th>
<th>Active Ingredient</th>
<th>Log $K_{ow}$</th>
<th>Activity in Environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organo-phosphate</td>
<td>Acetylcholine esterase inhibitors</td>
<td>Dimethoate</td>
<td>0.78</td>
<td>Low persistence (microbial degradation), <strong>low biomagnification</strong>; some soluble in water and runoff.</td>
</tr>
<tr>
<td>Pyrethroid</td>
<td>Prevents sodium channel closure</td>
<td>Bifenthrin</td>
<td>6.00</td>
<td><strong>Quick degradation</strong> by UV, water, $O_2$; <strong>low residuals</strong>; high soil adsorbance; <strong>lipophilic, water insoluble</strong>.</td>
</tr>
<tr>
<td>Neonicotinoid</td>
<td>Affects nicotinic acetylcholine receptor</td>
<td>Imidacloprid</td>
<td>0.57</td>
<td>High water solubility; <strong>systemic</strong>; prone to <strong>groundwater leaching</strong>; moderately persistent; does not biomagnify.</td>
</tr>
<tr>
<td>Benzoylurea</td>
<td>Chitin biosynthesis inhibitor</td>
<td>Novaluron</td>
<td>5.27</td>
<td><strong>Translaminar; lipophilic</strong>; low water solubility; strong soil adsorption; low leaching potential; <strong>persistent</strong>.</td>
</tr>
<tr>
<td>Fungicide</td>
<td>Succinate dehydrogenase inhibitor</td>
<td>Boscalid, Pyroclostrobin</td>
<td>2.96, 3.99</td>
<td><strong>Strong soil adsorption; highly persistent</strong>; possible surface run-off with soil particles.</td>
</tr>
</tbody>
</table>
Chemicals in BOB provisions and soil collected from CA almond orchard, March 2016. Spray application data provided by orchard manager. Analysis by USDA AMS, National Science Laboratories, Gastonia, NC.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Family/Class</th>
<th>Time Since Spray</th>
<th>PPB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bifenthrin</td>
<td>Pyrethroid</td>
<td>8 months</td>
<td>57.4</td>
</tr>
<tr>
<td>Esfenvalerate</td>
<td>Pyrethroid</td>
<td>3 months</td>
<td>35.4</td>
</tr>
<tr>
<td>Methoxyfenozide</td>
<td>Diacylhydrazines</td>
<td>8 months</td>
<td>248</td>
</tr>
<tr>
<td>Oxyfluorfen</td>
<td>Diphenyl ether (herbicide)</td>
<td>2 months</td>
<td>301</td>
</tr>
<tr>
<td>Pendimethalin</td>
<td>Dinitroaniline (herbicide)</td>
<td>2 months</td>
<td>1030</td>
</tr>
</tbody>
</table>

We don’t know if the presence/amount of pesticides is harmful to these bees at any life stage.
Likely exposure risk for honey bees and solitary bees, based on expert opinion. (compare down columns)

<table>
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<tr>
<th>Routes</th>
<th>Adult</th>
<th>Larva</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dust/Spray Particles in Air (contact)</td>
<td>Dust/Spray Particles in Air (contact)</td>
</tr>
<tr>
<td>Honey Bee</td>
<td><img src="%E2%9C%93" alt="Tick" /></td>
<td><img src="%E2%9C%93" alt="Tick" /></td>
</tr>
<tr>
<td><em>Osmia</em> spp.</td>
<td><img src="%E2%9C%93" alt="Tick" /> <img src="%E2%9C%93" alt="Tick" /> <img src="%E2%9C%93" alt="Tick" /></td>
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<tr>
<td><em>Megachile</em> rotundata</td>
<td><img src="%E2%9C%93" alt="Tick" /> <img src="%E2%9C%93" alt="Tick" /> <img src="%E2%9C%93" alt="Tick" /></td>
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</tr>
<tr>
<td><em>Nomia</em> melanderi</td>
<td><img src="%E2%9C%93" alt="Tick" /> <img src="%E2%9C%93" alt="Tick" /> <img src="%E2%9C%93" alt="Tick" /></td>
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</tr>
</tbody>
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Note: 0 indicates no known risk.
Likely exposure risk for honey bees and solitary bees, based on expert opinion. (compare down columns)

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<tr>
<th>Routes (via contact)</th>
<th>Adult</th>
<th>Larva</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mud/Soil</td>
<td>Wax</td>
<td>Water</td>
</tr>
<tr>
<td>Honey Bee</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Osmia spp.</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>Megachile rotundata</td>
<td>✓</td>
<td>✓</td>
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<td>Nomia melanderi</td>
<td>✓</td>
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Routes of Exposure for Solitary Cavity-Nesting Bees

1. Larval Ingestion
2. Adult Ingestion
3. Contact
4. Transovarial
Larval Bee Ingestion Route:
Pollen-nectar mass provision created from natural sources = sole food source.

- detrimental effects on larval development, survival, and/or later adult fecundity.
Larval Bee Ingestion Route:
Nest-building material contain pesticide residues:

Leaching from material into provision:
- nectar is aqueous, thus could attract hydrophilic chemicals
- pollen contains lipids and proteins, thus could attract lipophilic chemicals.
LARVAL INGESTION

- Pollen
- Nectar
- Leaves
- Nest construction
- Larvae
- Provisioning
- Nesting material
- Larvae consumption
- Nest to provision contact

Topical pesticide
Systemic pesticide
Almond tree
Mud / Water
Adult Bee Ingestion Route:
Direct ingestion of contaminated food:
- Nectar for adult energy.
- Pollen for female ovary maturation and egg development.

Incidental Ingestion:
- Grooming of mandibles and tarsi after manipulating / moving contaminated soil and leaf material.
ADULT INGESTION

apple tree

topical pesticide

systemic pesticide

pollen

nectar

leaves

mud / water

direct ingestion

indirect ingestion
Adult Direct Contact Route:
Adult is sprayed or in physical contact with contaminated sources; land on or walk on contaminated surfaces
Transovarial Transmission Route: Results when chemicals are ingested by, or absorbed by, the mother bee. - deleterious effect on offspring. - low or no survival of eggs or reduced egg production
**Transovarial Transmission**

- **Topical Pesticide**
  - alfalfa

- **Systemic Pesticide**
  - ovaries
  - larvae
  - adult ingestion
  - larval ingestion
  - physical contact
Pesticides Used During Bloom in Alfalfa Seed Fields Against Seed Predator, *Lygus*

Insect Growth Regulator = Novaluron (called Rimon)

*Is novaluron killing eggs or larvae? If so, how are bees exposed?*
Is novaluron lethal to ALCB eggs if in provision?

ALCB eggs collected from novaluron-free alfalfa seed fields. Provision doses: 0.5, 1, 2, 10X field rate, water, and blank. Monitor development of immatures; score mortality.

with novaluron: > 90 % mortality, mostly eggs + 1-2 instars

Is novaluron lethal to ALCB brood if mothers are directly exposed through nectar-feeding?

Females fed in laboratory:
10% sugar-water + novaluron
OR
10% sugar-water

Release in cages for nesting; alfalfa never sprayed with novaluron.

Evaluate brood for survival.

ALCB Bee Cages in North Logan, UT

Eggs die if mother is fed novaluron.

Bees drank sugar + novaluron

Bees drank sugar only
Back-to-Back Novaluron Sprays on ALCB

- Cages with novaluron-treated alfalfa
- Brood monitored for 1 wk of nesting after each spray:
  - almost complete failure of egg hatch (“no growth”)
- Chemical analysis of provisions (3 smpls):
  - contained novaluron, 236-656 ppb
1) Mother bee contaminated internally
   - ingestion or direct contact.
2) Provision contaminated
   - contact with leaf pieces
   - transfer from mother bee’s body while making provision.

Labor intensive to get natural provisions from nests, make homogenate, and sometimes sterilize it before using in bioassays.
Bee Acquisition and Designing Protocols

Homogenized provision masses in dishes or well plates.
Well plates used for pesticide dosing of provisions and rearing of bees from egg to adult.
Conclusion:

More work to do, more decisions to make, more protocols to develop, and more topics to address. ★ How do we verify & quantify exposure routes?

Efforts for conceiving conceptual models (mathematical) done at workshop. e.g., residues on leaves for *M. rotundata*