Pesticides Risks:

Not all bees are alike

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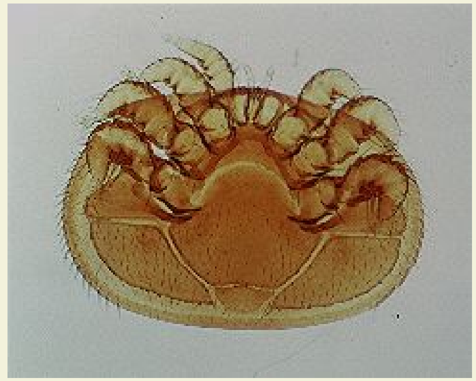
Risk Perception

- Who is asking the question?
  - Who benefits? What are the benefits?
  - Who is at risk? What is the risk?
  - What is known about the risk?

- Is questioner risk averse or a risk taker?

What is a pesticide?

Antibiotics

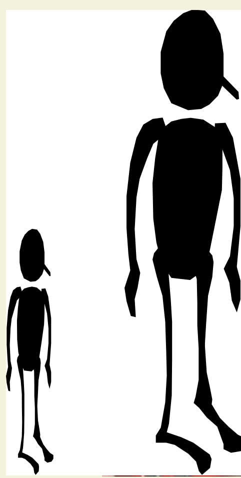


Acaricides

Herbicides

Fungicides.

Nematicides



Rodenticides

Insecticides

Fungicide Target Sites

TARGET SITE	CLASS	EXAMPLES
Nucleic Acid Synthesis: RNA Polymerase I Adenosindeaminase DNA/RNA synthesis **(proposed)** DNA topoisomerase type II	A1: Penylamides A2: Hyroxypyrimidines A3: Heteroaromatics A4: Carboxylic Acids	Benalaxyl, Metalaxyl Dimethirimol Octhilione Oxolinic acid
Mitosis and Cell Division: β-tubulin assembly in mitosis (B1, B2, B3) Cell division (proposed) Delocalization of spectrin-like proteins	B1: Methyl benzimidazole carbamates B2: N-phenyl carbamates B3: Benzamides & thiazole carbamates B4: Phenylureas B5: Benzamides	Benomyl, Thiphanate Diethofencarb Zoxamide Pencycuron Flopicolide
Respiration: Complex I NADH Complex II Complex III – cytochrome bc1 at Qo Complex III – cytochrome bc1 at Qi Uncouplers of oxidative phosphorylation Inhibit oxidative phosphorylation ATP production (proposed) Complex III – cytochrome bc1 at Q **unknown**	C1: Pyrimidinamines C2: Phenyl benzamides C3: Methoxy carbamates C4: Cyano-imidozole C5: Dinitroanilines C6: Organo tin compounds C7: Thiphenecarboxamide C8: Triazolo-pyrimidylame	Diflumetorim Mepronil Azoxystrobin Cyazofamid Fluazinam Fentin acetate Silthiofam Ametoctradin
Amino Acid/Protein Synthesis	D1 – D5: Anilino-primidines	Streptomycin, Oxytetracycline
Signal Transduction	E1 – E3	Vinclozolin
Lipid Synthesis and Membrane Integrity	F1 – F7	Iodocarb, **Bt**
Sterol Biosynthesis in Membranes	G1 – G4	Fenbuconazole, Spiroxamine
Cell Wall Biosynthesis	H3 – H5	Mandipropamid, Polyoxin
Melanin Synthesis in Cell Wall	I1 – I2	Pyroquilon, Fenoxanil
Host Plant Defense Induction	P1 – P5	Probenazole, Laminarin
Multiple Target Sites		Copper, Sulphur, Zineb, Chlorothalonil
Unknown Mode of Action		Cymoxanil, Methasulfocarb

Herbicide Target Sites

TARGET SITE	CLASS	EXAMPLES
Inhibition of Acetyl CoA Carboxylase+	A: Aryloxyphenoxy-propionate, Cyclohexanedione	Alloxydim, Sethoxydim
Inhibition of Acetolactate Synthase*	B: Sulfonylurea, Imidazolinone,	Chlorsulfuron, Cyclosulfamuron
Inhibition of Photosynthesis at Photosystem II*	C1: Triazine, Triazionone, Uracil C2: Urea, Amide C3: Nitrile, Benzothiadiazinone	Simazine, Bromacil Diuron, Fenuron Bromoxynil, Bentazon
Photosystem-1 – Electron Diversion*	D: Bipyridylium	Diquat, Paraquat
Inhibition of Protoporphyrinogen Oxidase+	E: Phenylpyrazole,Pyrimidindione	Bifenox, Butafenacil
Bleaching: Carotenoid biosynthesis inhibition (phytoene desaturase)* Inhibition of 4-hydroxyphenyl-pyruvate-dioxygenase Inhibition of carotenoid biosynthesis (unknown site)*	F1: Pyridazinone F2: Pyrazole F3: Triazole	Norflurazon Benzofenap, Pyrazoxyfen Amitrole
Inhibition of EPSP Synthase*	G: Glycine	Glyphosate
Inhibition of Glutamine Synthetase+	H: Phosphinic acid	Glufosinate-ammonium
Inhibition of Dihydropteroate Synthase*	I: Carbamate	Asulam
Microtubule Assembly Inhibition Inhibition of Mitosis / Microtubule Organization Inhibition of Cell Division	K1: Dinitroaniline, Pyridine K2: Carbamate K3: Chloroacetamide	Pendimethalin, dithiopyr Carbetamide Alachlor, Metolachlor
Inhibition of Cell Wall (cellulose) Synthesis*	L: Nitrile, Benzamide	Dichlobenil, Isoxaben
Uncoupling (Membrane disruption)	M: Dinitrophenol	Dinoseb
Inhibition of Lipid Synthesis	N: Thiocarbamate	Prosulfocarb
Indole Acetic Acid-like Action*	O: Phenoxy-carboxylic-acid	2, 4-D
Inhibition of Auxin Transport*	P: Phthalamate, Semicarbazone	Naptalam

Insecticide/Miticide Target Sites

TARGET SITE	CLASS	EXAMPLES
Nerve Action: Acetylcholine esterase inhibition	1A – Carbamates 1B - Organophosphates	Aldicarb Dimethoate
Nerve Action: GABA-gated chloride channel antagonists	2A – Cyclodienes 2B - Phenylpyrazoles	Chlordane Fipronil
Nerve Action: Sodium channel modulators	3A – Pyrethroids 3B	Permethrin DDT, Methoxychlor
Nerve Action: Nicotinic AChR agonist	4A – Neonicotinoids 4B – Nicotine 4C 4D - Butenolides	Imidacloprid Nicotine Sulfoxaflor Flupyradifurone
Nerve Action: nAChR allosteric activators	5 – Spinosyns	Spinetoram, Spinosad
Nerve & Muscle Action: Chloride channel activators	6 – Avermectins, Milbemycins	Abermectin
Growth Regulation: JH mimics	7A, 7B, 7C	Hydroprene, Fenoxycarb, Pyriproxyfen
Miscellaneous Multi-Target Sites	8A – 8E	Methyl bromide, Borax
Nerve Action: Selective Homopteran Feeding Blockers	9A & 9B	Pymetrozine, Flonicamid
Microbial Disruptor of Insect Gut Membrane	11A & 11B	**Bt**, *Bacillus sphaericus*
Inhibitors of Mitochondrial ATP Synthase	12A – 12D	Azocyclotin, Sulfuramid
Nerve Action: Octopamine receptor agonists	19	Amitraz
Energy Metabolism: Mitochondrial complex I electron transport inhibitors	21 A – METI acaricides & insecticides 21B	Fenproximate Rotenone
Lipid synthesis, growth regulation: **Inhibition of Acetyl CoA Carboxylase**	23 – tetronic & tetramic acid derivatives	Spirodiclofen

Conclusion 1

- Pesticides are classified by target organism.
- Mode of action may be unknown or not fully understood.
- Mode of action for target organisms is identical to mode of action for non-target organisms when target site is the same.
- A pesticide may have multiple target organisms.
- A pesticide may have multiple modes of action.
- It is possible to have same modes of action for different pesticides.

Chemical Characteristics Determine Toxicity

ABSORPTION

Stomach poison
Eaten & absorbed through gut

Contact poison
Absorbed through body wall

Fumigants
Eaten & absorbed tracheae

DISTRIBUTION

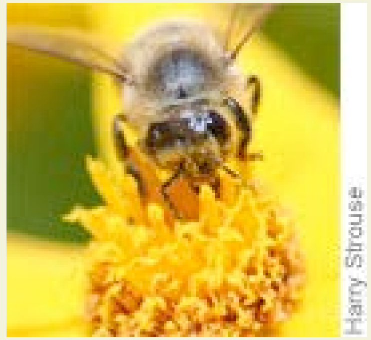
SYSTEMIC
translocations through organism

LOCAL
impacts a localized area

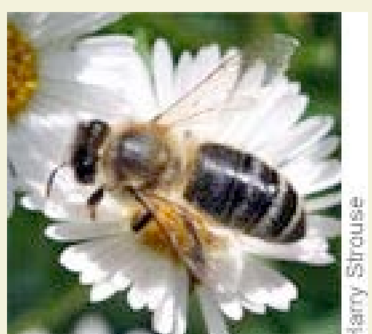
TARGET SITE
(MODE of ACTION)

Exposure and Outcome

Exposure

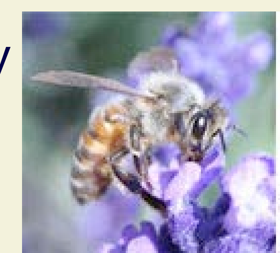


- Acute
  - High dose
  - Short duration
- Subacute/subchronic
  - Moderate dose
  - Moderate duration
- Chronic
  - Low dose
  - Long duration



Outcome

- **Acute**
  - Now/Today
  - Severe (usually)
    - **Death**
- **Subacute/subchronic**
  - Months
  - Sublethal
    - Behavior, immunity
- Chronic
  - Years
  - Carcinogen, mutagen, teratogen, population impacts



Range of Bee Outcomes

- Acute Exposure/Outcome
  - Inhalation?
  - Ingestion (generally assumed that ingestion is more toxic than contact – may not be true)
  - Contact
- Subacute/Subchronic Exposure/Outcome
- Chronic Exposure/Outcome
  - Lethal Toxicity
    - Probability of overwintering, population impacts
  - Sublethal

Range of Bee Outcomes

- Sublethal (subacute/subchronic)
  - Reproduction
    - days to egg hatch or for larval development
  - Mobility
    - Trembling
    - Uncoordinated movement
    - Hyperactivity
  - Behavior
    - Learning/memory (PER)
    - Orientation – maze test
  - Nest development (*B. impatiens, B. terrestris*)
  - Food avoidance (Honey bees & imidacloprid)

Potential for Adverse Outcomes

- Depends upon
  - Systemic/non-systemic
  - Application method
  - Chemical formulation

  - Risk of exposure
    - Prophylactic treatments (seeds, etc.)
  - Dose and duration of exposure

  - How fast chemical decomposes
    - Half-life (Temperature, moisture, sunlight, volatility)
  - Kind of environment "liked" by the chemical
    - pK_a, partitioning (octanol:water) coefficient
  - Soil mobility

Conclusion 2

- Exposure risk depends upon route of entry
- Risk of adverse outcome depends upon route of entry, translocation, detoxification, & target sites
- Toxicity information
  - Target insect/plant/pathogen
    - Most about contact LD_{50}, little about oral LD_{50}, other outcomes
  - Little information on non-target organisms
    - Honey bee adults
      - Little known about eggs, larvae and pupae
- Pesticide formulation and environmental conditions are critical
- Prophylactic treatments are risky.

Comparison of Five Insecticides across Three Species

C.D. Scott-Dupree, et al. (2009) Journal of Economic Entomology 102(1): 177-182

Contact LC_{50}*

Pesticide	*Bombus impatiens*	*Megachile rotundata*	*Osmia lignaria*
Clothianidin	0.39	0.08	0.10
Imidacloprid	**3.22**	0.17	0.07
Deltamethrin	6.90	0.13	8.90
Spinosad	8.95	1.25	4.7
Novaluron	>100	>100	

*LC_{50} – wt:vol (X 10^{-3})

Comparison of Topical LC_{50} in *Apis mellifera* and *Bombus* species

F. Sanchez-Bayo & K. Goka 2014 PLoS ONE 9(4): e94482

Pesticide	***Apis mellifera***	***Bombus***	**Relative Sensitivity $LC_{50\ Am} / LC_{50\ B}$**
Chlorpyrifos	0.07	0.09	0.78
Imidacloprid	**0.06**	**0.02**	3
Deltamethrin	0.02	0.28	0.07
Carbaryl	0.84	41.16	0.02
λ–Cyhalothrin	**0.05**	0.17	0.29
Permethrin	0.6	0.22	2.73
τ-fluvalinate	0.03	0.46	0.06

LC_{50}: μg/bee

If relatively sensitivity is < 1,
honey bees are more susceptible

Comparison of LC_{50} in *Apis melifera* and *Osmia cornifrons*

D.J. Biddinger et al. 2013 PLoS ONE 8(9): e72587

Pesticide µg/bee (formulation)	*Apis mellifera* LC_{50} (LC_{90})	Risk	*Osmia cornifrons* LC_{50} (LC_{90})	Risk	Relative Sensitivity $LC_{50\ Am}$ / $LC_{50\ Oc}$	Rel. Risk
Acetamiprid (Assail)	64.6 (537)	0.12	**5.2** (87.1)	0.06	12.42	2
Dimethoate (Dimethoate)	0.31 (0.46)	0.68	0.09 (1.43)	0.06	3.44	11.33
Phosmet (Imidan)	1.9 (27.5)	0.07	6.1 (14.6)	0.42	0.31	0.22
Imidacloprid (Provado)	**0.15** (1.53)	0.09	**3.82** (57.7)	0.07	0.04	1.28
λ-cyhalothrin (Warrior)	**0.30** (3.4)	0.08	0.91 (5.2)	0.18	0.33	0.24

High Risk if near 1. If Relatively Sensitivity is < 1, honey bees are more susceptible. If Relative Risk is > 1 risk is higher for honey bees.

Single Exposure to Formulated Pesticide and Interactions

D.J. Biddinger et al. 2013 PLoS ONE 8(9): e72587

Pesticide (formulation)	*Apis mellifera* LC_{50}	*Osmia cornifrons* LC_{50}	Relative Sensitivity $LC_{50\ Am} / LC_{50\ Oc}$
Acetamiprid (Assail)	64.6	**4.0**	16.15
Imidacloprid (Provado)	0.2	3.8	0.05
Fenbuconazole (Indar)	(Non-toxic)	(Non-toxic)	
Acetamiprid/Indar (1:1)	14.3	2.1	6.81
Imidacloprid/Indar (2:1)	0.3	6.6	0.04

LC_{50}: μg/bee

If relatively sensitivity is < 1, honey bees are more susceptible

Interaction of Pesticides in *Apis mellifera* and *Bombus* (topical LC_{50})

F. Sanchez-Bayo & K. Goka 2014 PLoS ONE 9(4): e94482

Insecticide	Fungicide	*Apis mellifera*	*Bombus*	Relative Sensitivity $LC_{50\ Am}$ / $LC_{50\ B}$
Cyhalothrin	Propiconazole	0.003	0.01	0.3
	Myclobutanil	0.004	0.02	0.2
	Penconazole	0.011	0.04	0.3
Acetamiprid	Propiconazole	0.076	0.95	0.08
	Fenbuconazole	1.76	22.2	0.08

LC_{50}: μg/bee

If relatively sensitivity is < 1, honey bees are more susceptible

Exposure Comparison: LC_{50}/LD_{95}, Relative Sensitivity (RS), Risk

INGESTED	Pesticide (LC_{50}/LC_{95}) (µg a.i./bee)	*Apis mellifera*	*Melipona quadrifasciata*	RS	Rel. RISK
	Abamectin	0.01/0.02	0.015/0.033	0.67	1.1
	Deltamethrin	0.85/7.00	0.082/0.320	10.36	0.5
	Methamidophos	3.70/5.50	0.066/0.066	5.61	0.7
TOPICAL					
	Abamectin	7.8/13.8	134.6/471.6	0.06	2
	Deltamethrin	112.2/359.6	129.2/460.6	0.87	1.1
	Methamidophos	408.5/1537	296.6/1916	1.38	1.7
CONTACT					
	Abamectin	15.4/83	3.8/14.7	4.05	0.7
	Deltamethrin	6.6/66.6	5.6/29.9	1.18	0.5
	Methamidophos	443/1537	96.1/251.1	4.61	0.7

If Relative Risk is > 1 risk is higher for honey bees

M.C.L. Del Sarto, et al. 2014 Apidologie 45: 626-636

Relative Sensitivity

$LD_{50\ (A.\ mellifera)}/LD_{50\ non\text{-}\mathit{A.\ mellifera}\ subgroup)}$

	Non-*Apis mellifera*	Species	# of cases	Median	Range
Apis mellifera	Andrenidae	1	6	1.47	0.709-3.00
	Apidae (Apinae/Apini)	2	5	1.09	1.04-1.51
	Apidae (Apinae/Bombini)	5	45	0.21	0.001-25.88
	Apidae (Apinae/Meliponini)	7	22	1.29	0.26-2085.7
	Halictidae (Nomminnae)	1	27	0.59	0.012-62.61
	Megachilidae (Megachilinae/Megachilini)	1	29	0.55	0.01-11
	Megachilidae (Megachilinae/Osmiini)	2	16	0.20	0.001-25.88

If relatively sensitivity is < 1, honey bees are more susceptible

M. Arena & F. Sgolastra 2014 Ecotoxicology 23:234:334.

Subchronic Exposure

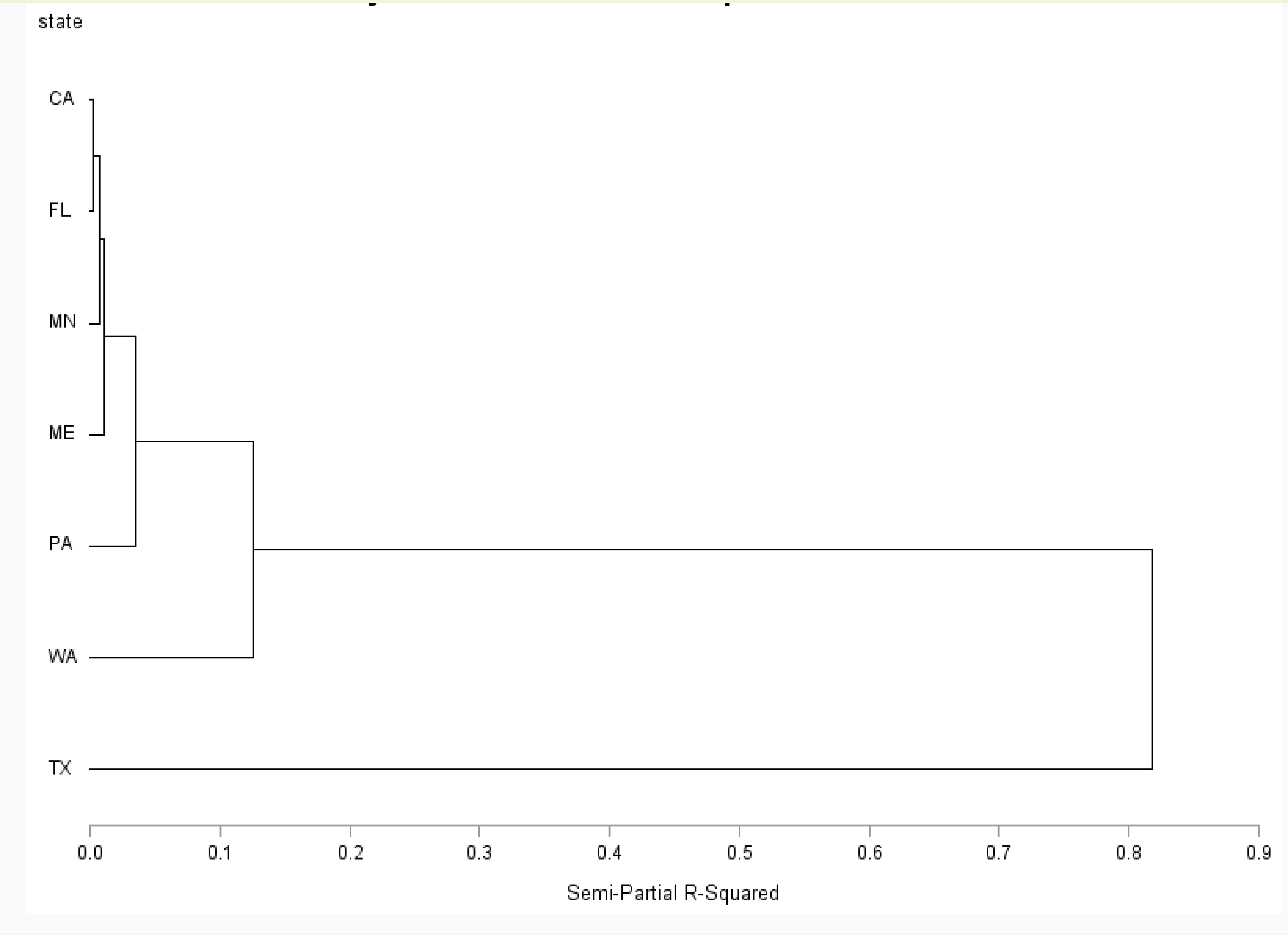
A. Decourtye et al. 2005 Archives of Environmental Contamination and Toxicology 48: 242-250

- Dimethoate and fibronil increase mortality when honey bees exposed for 11 days to subacute concentrations
- No effect on PER observed
- Deltamethrin, prochloraz, endosulfan, fibronil decreased olfactory learning in conditioning trials.

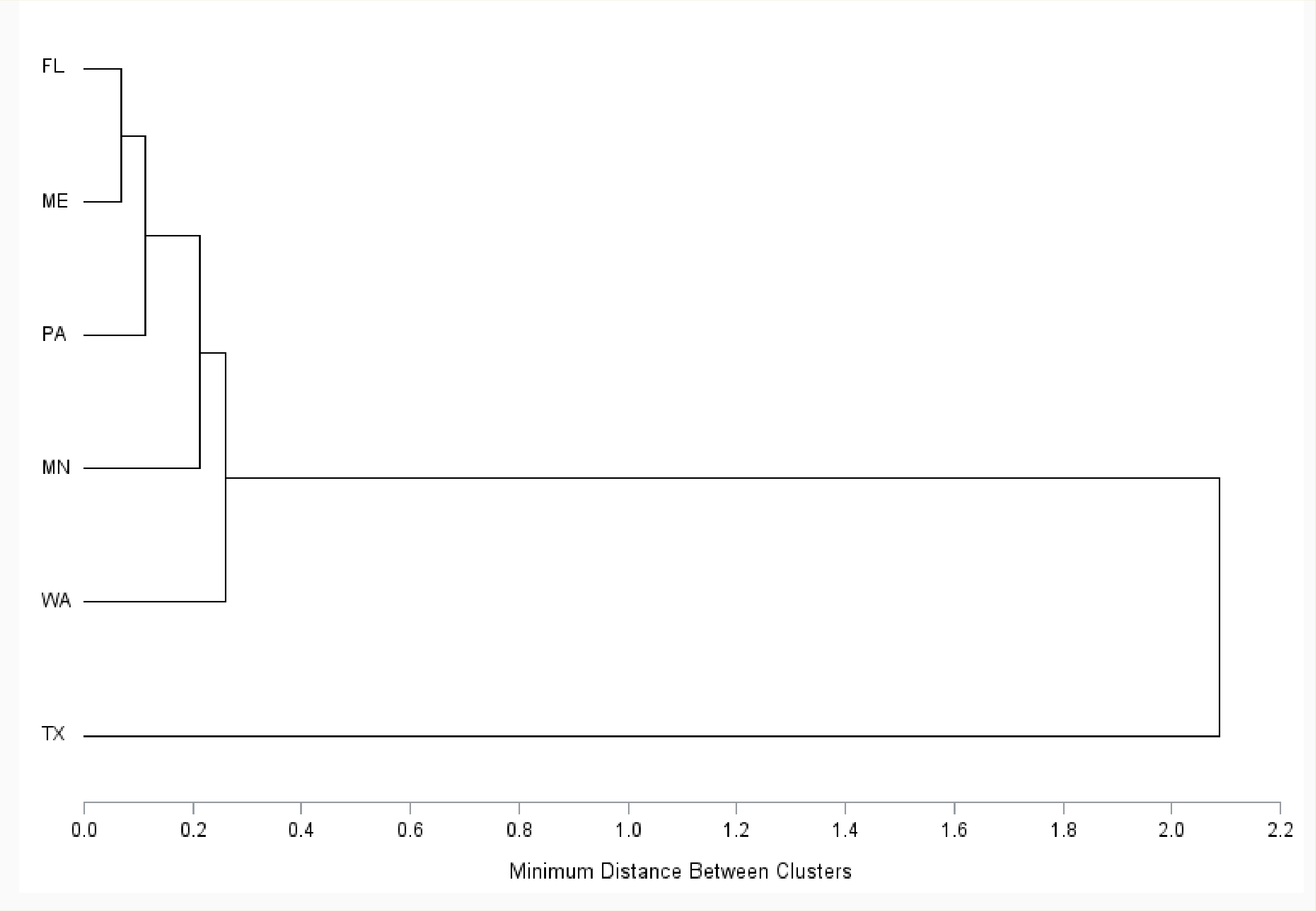
Conclusion 3

- Results among studies difficult to compare
  - Units (μg/bee, μg/kg, μg/l)
  - Conditions
    - Pesticides (a.i.), formulations, solvents
    - Cages, food/water, etc.
  - Species
- Risk (distance between LC_{50} and LC_{90}) varies by species and pesticide
- Exposure routes change LC_{50}, relative sensitivity and relative risk of pesticide
- Interactions change LC_{50}, relative sensitivity and relative risk of pesticide

Pesticides in Pollen:
Quantity Clustered by Mode of Action



Pesticides in Pollen:
Hazard Quotient Clustered by Mode of Action



Neonicotinoids

- Nicotinic Acetylcholine Receptor Agonist
  - Low [ ]: nervous system stimulation
  - High [ ]: blockage of receptor, paralysis, death
- Binds more strongly to insect receptor than mammals
- Four types
  - Neonicotinoids (4A)
    - Acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid, thiamethoxam
  - Nicotine (4B)
  - Sulfoxafor (4C)
  - Butenolides (4D)
    - Flupyradifurone

Imidacloprid – Acute Outcome

- Oral LC_{50} 5 ng/ honey bee
- Given the quantities observed in nectar and pollen, acute risk to honey bee is low
  - Would need to consume nearly 1 g of pollen or 2.6 ml nectar

Application	Nectar	Pollen
Seed dressing	<1 to 8.6 ppb	<1 to 51 ppb
Direct soil	1 to 23 ppb	9 to 66 ppb
Foliar	5 to 11 ppb	36 to 147 ppb

- Chronic risk is higher, thus important to know exposure rate, environmental half-life, rate of metabolism/excretion.
- Little is known about other pollinators.

Neonicotinoids - Sublethal

- Imidacloprid
  - Honey bees
    - Reduce learning, and foraging and homing ability
  - Bumble bees
    - Impaired foraging ability (reduced colony size)
    - Reduced nest growth, reduced queen production
  - Some data (flies, honey bees) indicate food avoidance
- Thiamethoxam (honey bees)
  - Reduce ability to find home

Flupyradifurone

- EPA proposed approval – public comment closed 10/25/14
- Readily translocated through plant
- Degradation
  - DT_{50} 8.3-251 days in soil (field study)
  - DT_{50} 80.6 days in outdoor pond (Germany)
  - DT_{50} photolysis in water – 14 hours
- Mobility in soil
  - Parent compound – low, increases with time (Brazil)
  - Major metabolite (DFA) – high
  - Minor metabolite (6-CNA) - low

Flupyradifurone

	Adult Acute Oral (LD_{50} µg a.i./ honey bee)	Adult Acute Contact (LD_{50} µg a.i./ honey bee)	Adult Acute Contact (LD_{50} µg a.i./ bumble bee)
Flupryadifurone	1.2	122.8	
Sivanto SL200	3.2	15.7	>100
Sivanto FS480	3.4	68.6	

	Adult Honey Bee Chronic Feeding (NOEC µg a.i./ L diet)	Larvae Honey Bee (NOEC µg a.i./kg diet)
Flupryadifurone	≥10000	≥10000

Honey Bee Colonies	Semi Field Studies (up to 200 g a.i./ha during bloom)	Field Studies (up to 205 g a.i./ha during bloom)	Full Colony (600, 2500, 10000 µg a.i./ kg diet)
Flupryadifurone	No adverse effects	No adverse effects	No adverse effects

Conclusions

- Registration data may tell little about impact of pesticide on non-target organisms.
- Little information on, or available to compare, sublethal, subacute/subchronic impacts or quantities needed to produce effect.
- A pesticide’s mode of action in a species tells little about other species.
- Mode of action by class of pesticide tells little about all modes of actions possible for that class of pesticide.
  - Most information on modes of action research focus on target, not non-target organisms.

Conclusions

- Exposure risk depends upon route of entry.
- Risk of adverse outcome depends upon route of entry, translocation, detoxification, & target sites.
- Formulations and adjuvants matter.
- Prophylactic treatments are risky.
- Risk (distance between LC_{50} and LC_{90}) varies by species and pesticide.
- Exposure routes and pesticide interactions change LC_{50}, relative sensitivity and relative risk of pesticide.

THE END

Relative LD_{50} Sensitivity: *Apis mellifera* to non-*Apis* species

	Non-*Apis mellifera*	Cases	Median	Range
Apis mellifera **LD_{50} (A. mellifera)/LD_{50} non-*A. mellifera*)**	*Andrena erythronii*	6	1.47	0.71-3.00
	Apis cerana	3	1.09	1.04-1.51
	Apis florea	2	1.14	1.09-1.18
	Bombus agrorum	3	0.5	0.30-5.00
	B. lucorum	3	0.5	0.30-2.50
	B. terrestris	32	0.20	0.001-25.88
	B. terricola	6	0.05	0.009-0.23
	Melipona beecheii	3	0.92	0.39-1.02
	Nannotrigona perilampoides	6	1.95	1.16-2085.7
	Trigona nigra	3	1.07	0.92-3.23
	T. spinipes	6	1.21	0.26-33.38
	Nomia melanderi	27	0.59	0.01-62.61
	Megachile rotundata	29	0.55	0.01-11.00
	Osmia cornifrons	5	0.33	0.04-12.42
	O. lignaria	11	0.56	0.10-1.72