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Progress and future prospects for assessing the risks posed to pollinators by pesticides – science needs

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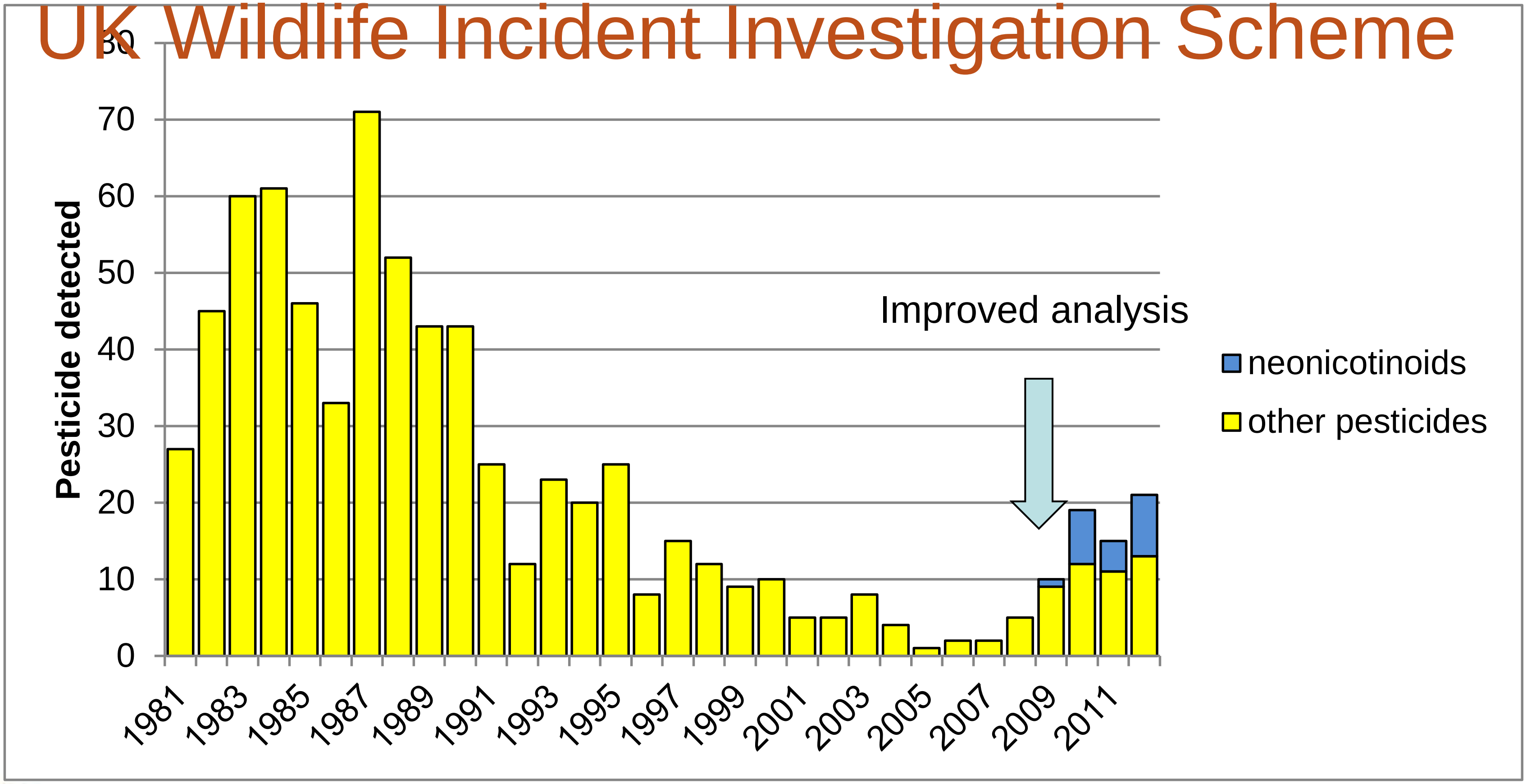
Outline

- Toxicity differences between species
- Exposure differences between species –what is realistic exposure?
- Importance of metabolism
- Interactions between pesticides and between pesticides and veterinary medicines

- Honeybees have been subject of regulatory data requirements at national level within the EU for more than 50 years
- Initial assessment only on toxicity data (hazard) - shown not to be good indicator of effects in the field
- Led to development of Hazard Quotient (HQ= (g ai/ha)/LD50) for sprayed pesticides, i.e. a measure of risk
- Move from laboratory to increasing levels of realism based on HQ (sequential testing) from laboratory to field

UK Wildlife Incident Investigation Scheme

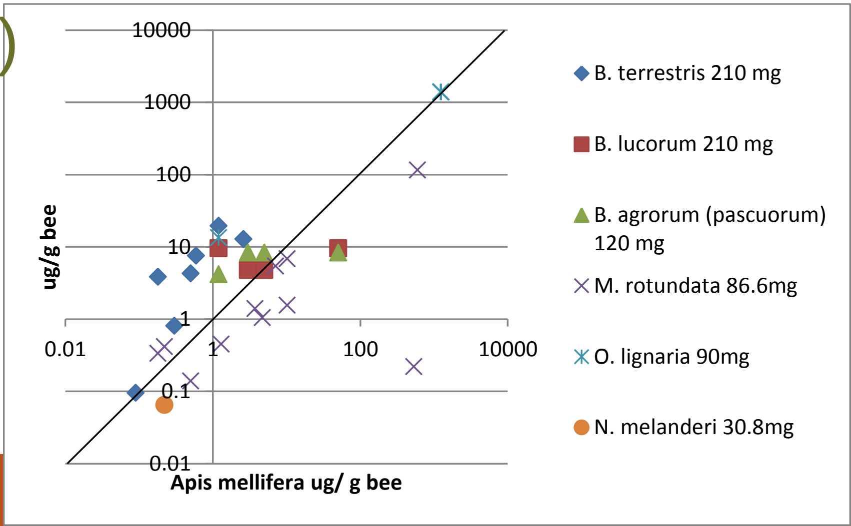


WIIS	Imidacloprid (ng/bee)	Thiacloprid (ng/bee)
2008	-	-
2009	0.1	-
2010	0.05, 0.3	0.008, 0.009, 0.04, 0.07, 0.13
2011	0.047	0.006,0.081, 9.3

Insecticide LD50

- Large dataset for honeybees
- Limited data for non-*Apis* (acute contact toxicity)
- New EFSA guidance requires contact and oral adult and larval oral toxicity
  - Honeybees (acute and chronic) (OECD 213,214, draft OECD larvae)
  - Bumble bee (acute + microcolony)
  - Solitary bee (acute)

Acute contact LD_{50}



Exposure: Nectar

Sprays

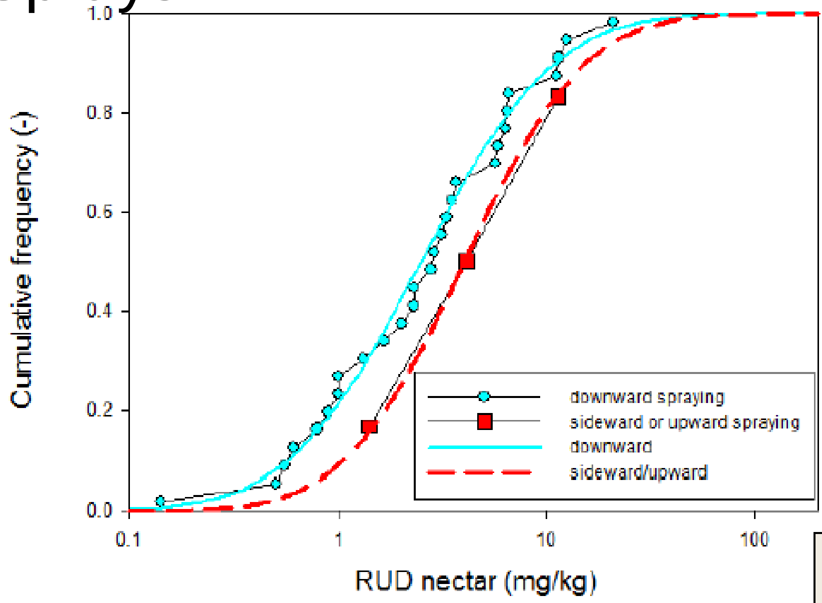


Figure F2: The cumulative frequency distributions of RUD values for nectar for downwards and side/u spraying. Points are measured cumulative frequency distributions and the lines are fitted lognormal distrib

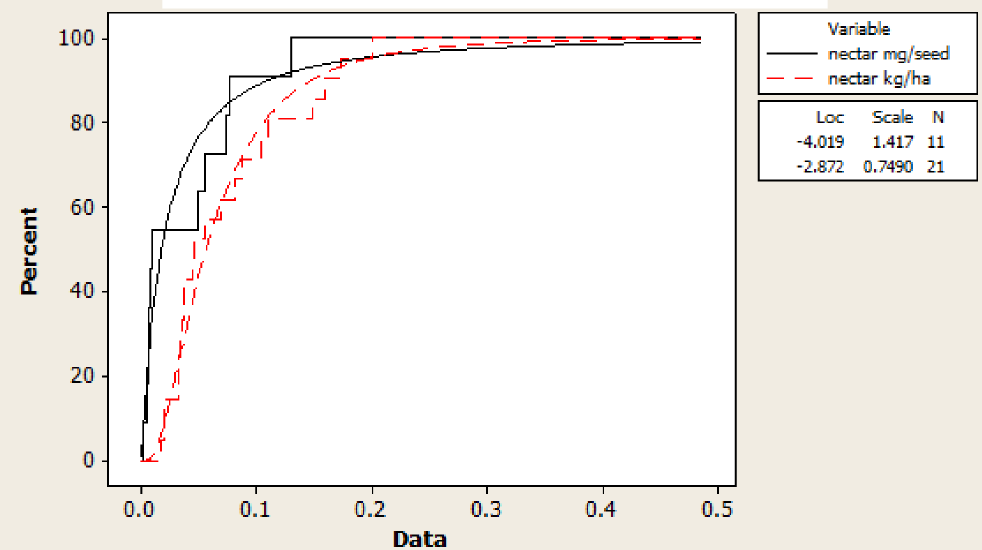
Application	EFSA Median RUD – mg/Kg
1kg/ha sprayed downwards	2.478
1 kg/ha sprayed upwards/sideways	4.018
1kg/ha seed treatment	0.0458
1mg/seed seed treatment	0.0093

Imidacloprid on oilseed rape (canola)

For 0.05 mg / seed = 0.465 µg/Kg nectar

16 g a.i./ha = 0.73 µg/Kg nectar

Seed treatments



Exposure: Pollen

Imidacloprid on oilseed rape (canola)
For 0.05 mg / seed = 0.455 µg/Kg pollen
16 g a.i./ha = 1.3 µg/Kg pollen

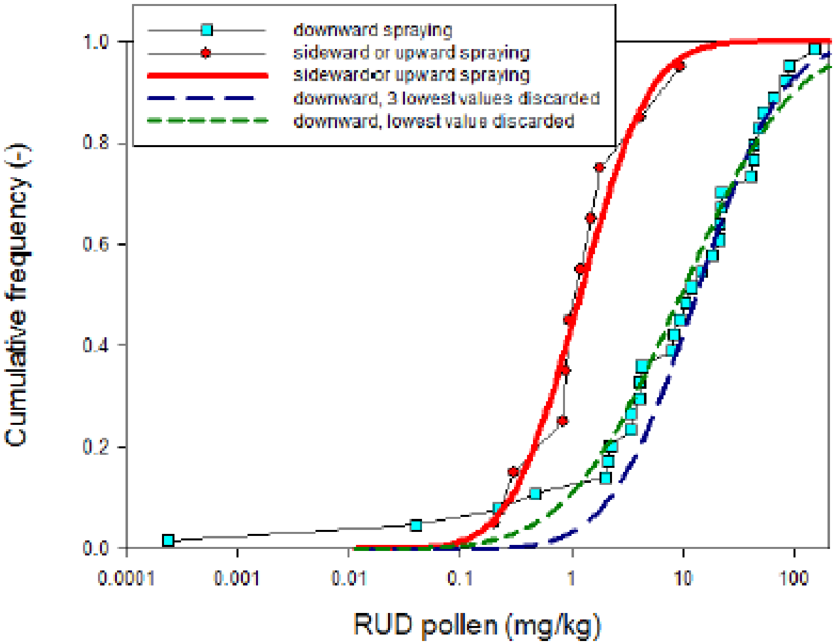
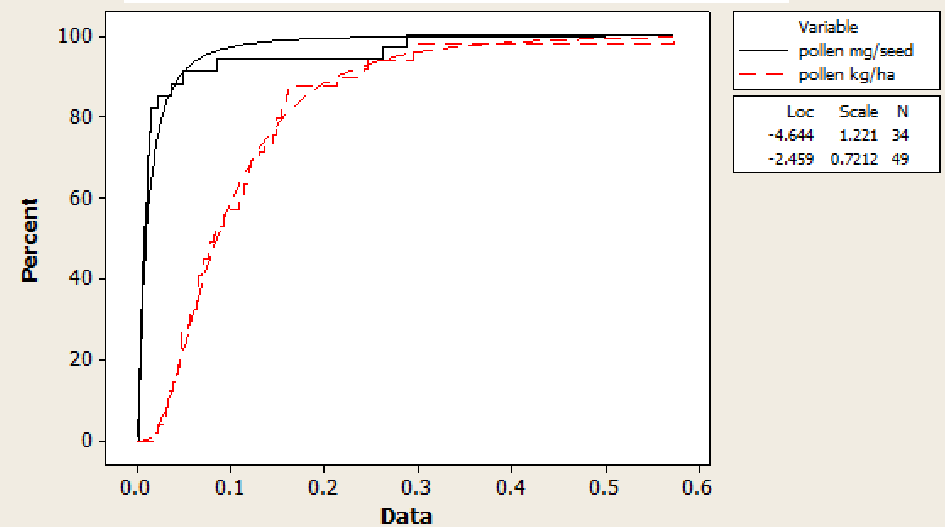


Figure F1: The cumulative frequency distributions of RUD values for pollen for downwards and side/upwards spraying. Points are measured cumulative frequency distributions and the lines are fitted lognormal distributions

Application	EFSA Median RUD – mg/Kg
1kg/ha sprayed downwards	13.02
1 kg/ha sprayed upwards/sideways	1.18
1kg/ha seed treatment	0.0823
1mg/seed seed treatment	0.0091

Seed treatments



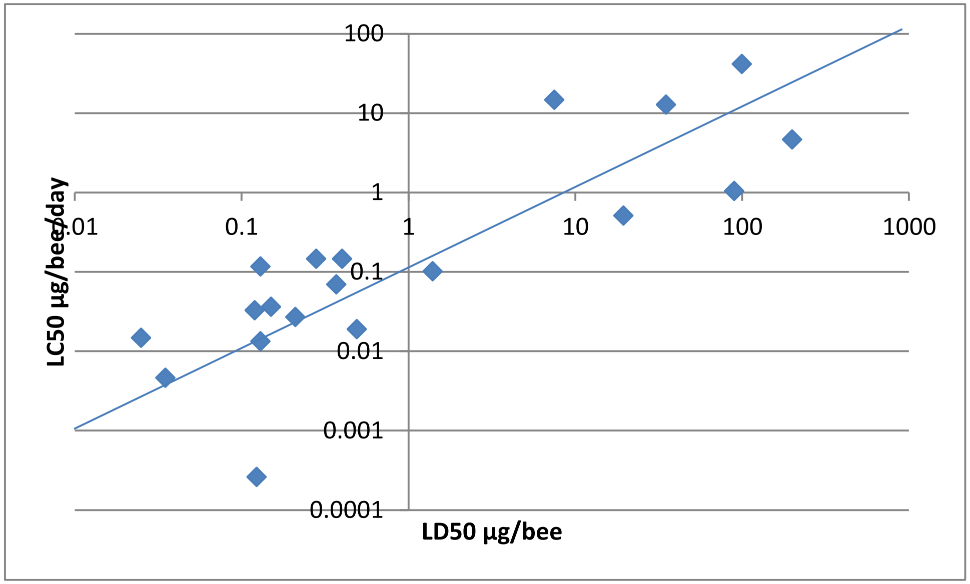
How robust are our intake predictions?

- For honeybees based on worst case - Rortais et al 2005: 128 mg sugar/bee/day = 853 mg nectar/day (15% sugar in canola)

  Bee foraging on oilseed rape requires 8.5 x bodyweight to forage and carry 6 x its bodyweight per day (10 trips carrying 60 µl)

- What are realistic crop contents (sugar content) and exposure profiles for honeybees and for other bee species?

Importance of exposure profile

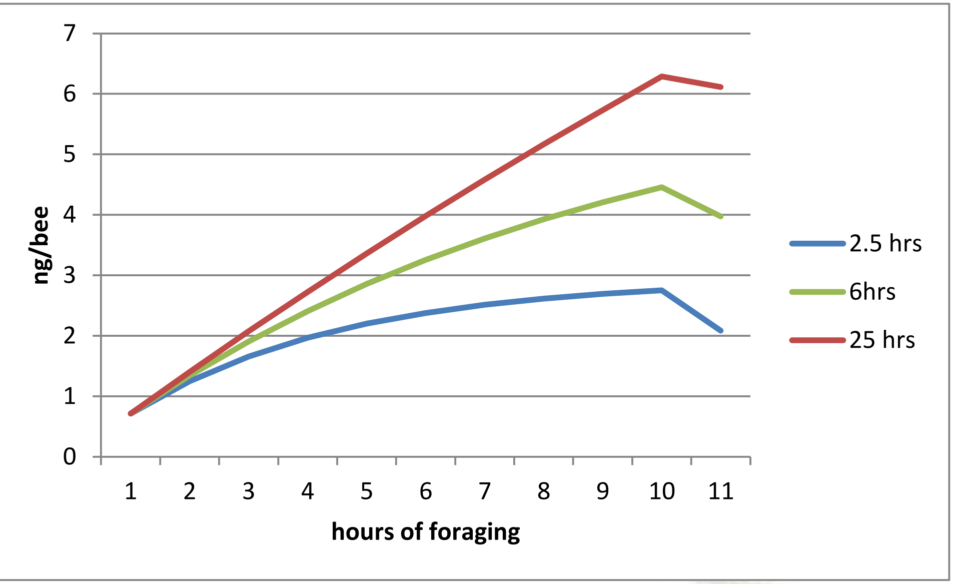


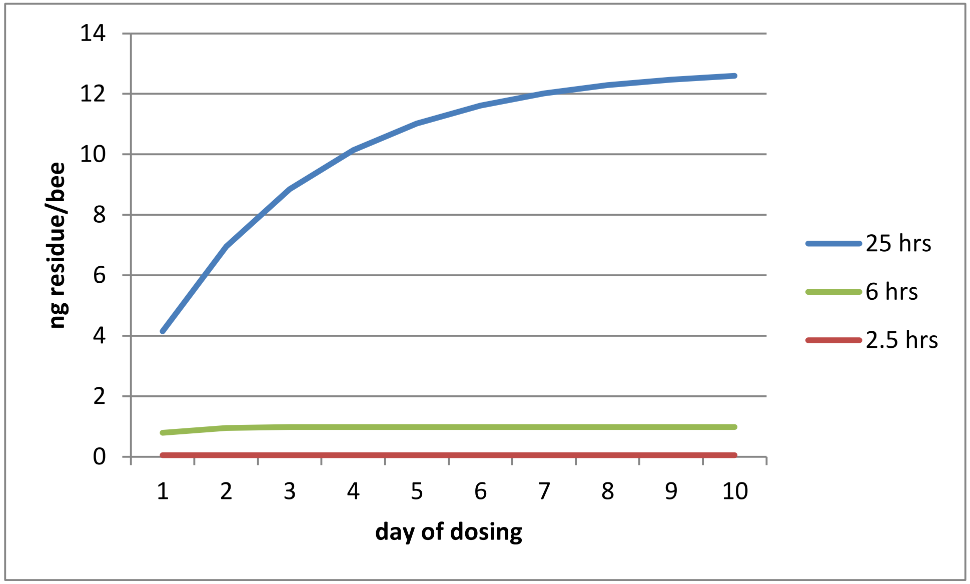
Line oral $LC_{50\ (10\ day\ continuous\ exposure)}$ =acute oral LD_{50}/10 – related to rate of metabolism of pesticide

Metabolism

Residue levels in honeybees based on elimination half life (parent + metabolites) of 2.5, 6 and 25 hrs and 10hrs/day exposure to 5 µg/Kg

Predicted residues





Conclusions

- Need to increase confidence in predicting toxicity between species – lethal and sublethal
- Need to understand and interpret effects of realistic exposure scenarios from field studies:
  - Concentrations in nectar/ pollen and stored honey/bee bread/pollen
  - Exposure profile of foraging bees:
    - Behaviour: relative contribution of crops/ flowering weeds;
    - Exposure time course and metabolism;
    - Responses to contaminated food sources

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Potential interactions between pesticides

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- In EU both active ingredients and products are subject to risk assessments

- Honeybees and other bees may also be exposed to mixtures of pesticide through
  - multiple applications, e.g. tank mixes
  - overspray of residues already present, e.g. systemic pesticides,
  - pollen and nectar collected from a variety of sources
  - use of treatments within hives by beekeepers.

Pesticide tank mixes- UK

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- For arable crops, vegetables and orchards over 50% of the treated area was treated with mixtures.
- For arable crops mixtures contained up to 9 products.
- For vegetables, orchards and soft-fruit mixtures contain up to 7, 8 or 6 products respectively.
- Comparing arable data from 1998 and 2008 means of 2.99 and 3.25 products per mixture

Classes of compounds used in mixtures of 2 to 9 products applied to arable crops

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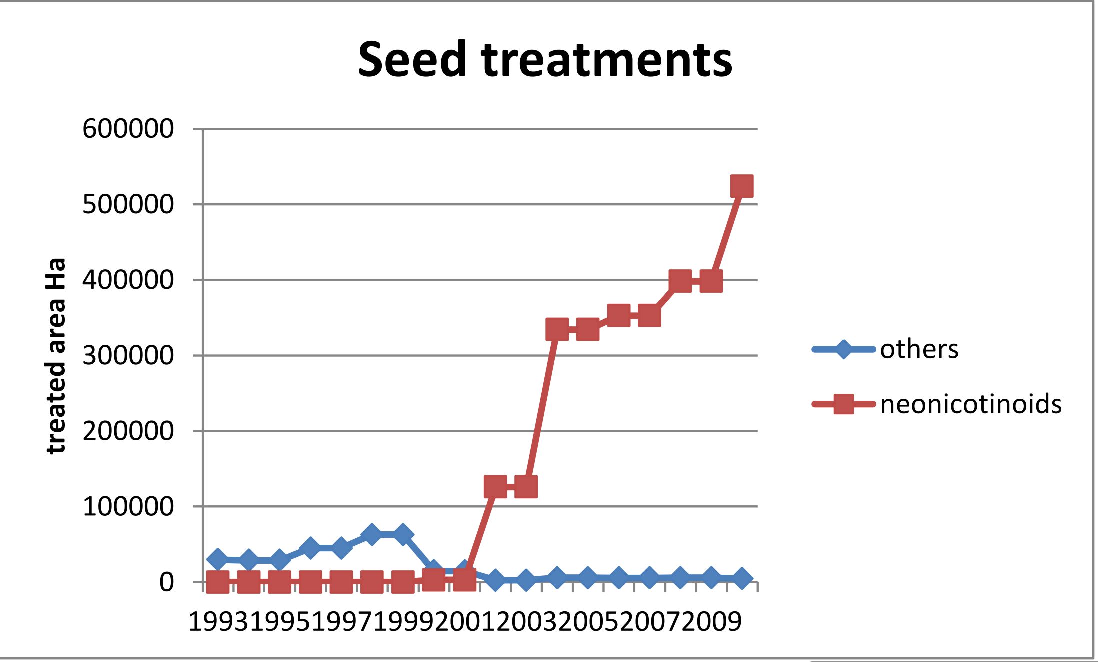
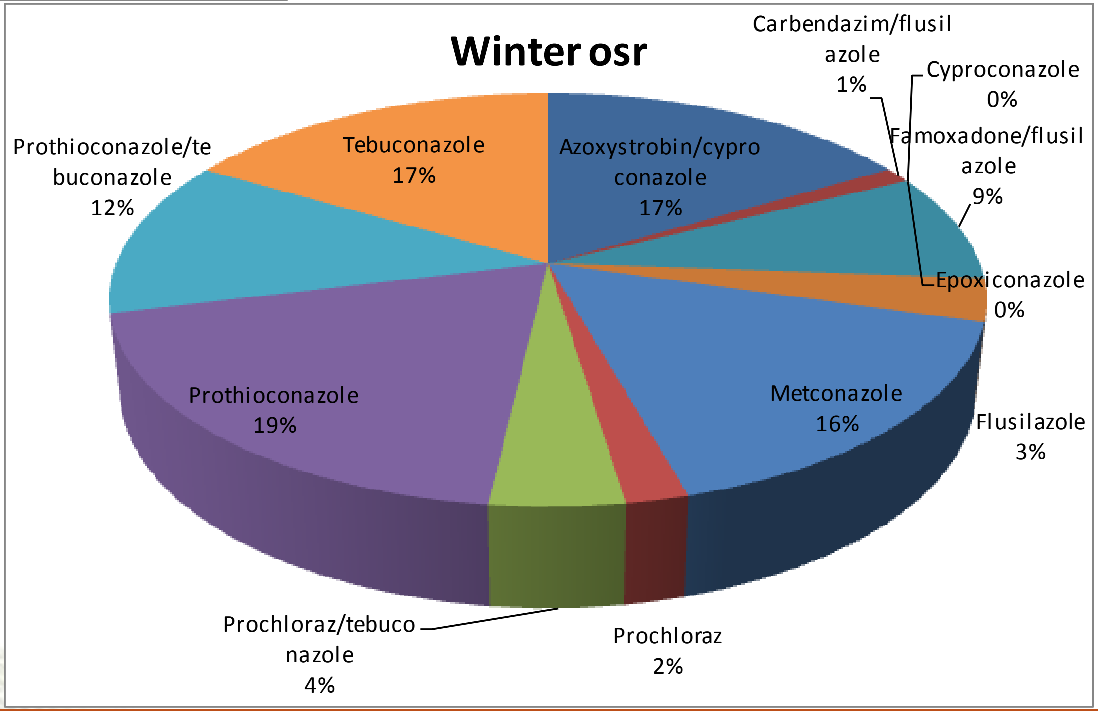
Mixture	N (unique)	Area (ha)	% area
Fungicide(s) + Herbicide(s) + PGR(s)	1117	2585761	19.32
Fungicide(s) + Herbicide(s)	991	1906643	14.24
Fungicides	723	1837802	13.73
Fungicide(s) + PGR(s)	850	1672300	12.49
Herbicide(s) + Insecticide(s)	721	1616471	12.07
Herbicides	618	1488168	11.12
Fungicide(s) + Insecticide(s)	610	1404836	10.49
Fungicide(s) + Herbicide(s) + Insecticide(s)	205	417120	3.12
Herbicide(s) + PGR(s)	78	161796	1.21
Fungicide(s) + Insecticide(s) + PGR(s)	24	121246	0.91
Fungicide(s) + Herbicide(s) + Insecticide(s) + PGR(s)	24	97756	0.73
PGRs	8	27764	0.21
Herbicide(s) + Insecticide(s) + PGR(s)	6	20739	0.15
Molluscicides	4	13559	0.10
Insecticides	10	8670	0.06
Insecticide(s) + PGR(s)	3	6427	0.05

	Pyrethroids									Neonicotinoid
EBI fungicides	Alpha-cypermethrin	Bifenthrin	Cyfluthrin	Cypermethrin	Deltamethrin	Esfenvalerate	Lambda-cyhalothrin	Tau-fluvalinate	Zeta-cypermethrin	Thiacloprid
Cyproconazole	X	X	X	X		X	X	X	X	X
Difenoconazole	X			X	X		X			
Epoxiconazole	X			X		X	X	X	X	X
Fluquinconazole								X		
Flusilazole	X	X	X	X	X		X	X	X	
Prochloraz	X			X		X	X	X		
Propiconazole				X			X	X	X	X
Tebuconazole	X	X	X	X	X	X	X	X	X	
Triadimenol		X	X			X	X	X*	X	
Tetraconazole				X		X				
Metconazole	X	X		X	X	X	X	X	X	X
Prothioconazole	X	X		X	X	X	X	X	X	X
Fenpropidin							X			
Fenpropimorph	X			X		X	X	X*	X	
Spiroxamine				X		X	X	X*	X	



Sprays applied to flowering oilseed rape



Exposure to multiple sources – in hive

- 120 hives from 24 apiaries at 5 sites in France (main types of honey were chestnut, oilseed rape, sunflower, and local mixed flower honey)
- Pollen: 37.8% contained at least two different pesticide residues with 22.2, 12.7, 2.4, and 0.5% containing two, three, four, or five different residues, respectively.
- Honeybees:14.7% contained at least two pesticides with two (11.2%), three (2.3%), four (1.0%), or five (0.2%) active ingredients.

(Chauzat et al., 2011)

In hive treatments

- Varroacides regularly detected
- Live bees - up to 30 µg/Kg bromopropylate, 24840 µg/Kg coumaphos, 326 µg/Kg tau-fluvalinate,
- Bee bread (pollen) - bromopropylate max 20 µg/Kg, chlorfenvinphos max 132 µg/Kg, coumaphos 6.04 ± 25.3 µg/Kg, tau-fluvalinate 221 ±563 µg/Kg
- Wax - up to 7620 µg/Kg chlorfenviphos, 648 µg/Kg coumaphos, 5100 µg/Kg tau-fluvalinate
- Honey - up to 27.5 µg/Kg bromopropylate, 576 µg/Kg coumaphos, 44.7 µg/Kg tau-fluvalinate)

(Chauzat et al., 2011)

Additive toxicity

- Predictable: Toxicity of A+B = [amount of A/toxicity of A] + [amount of B/toxicity of B]
- Can be applied to residues in pollen and nectar to assess the total exposure of adult and larval bees to pesticides.
- Applies to most chemical mixtures

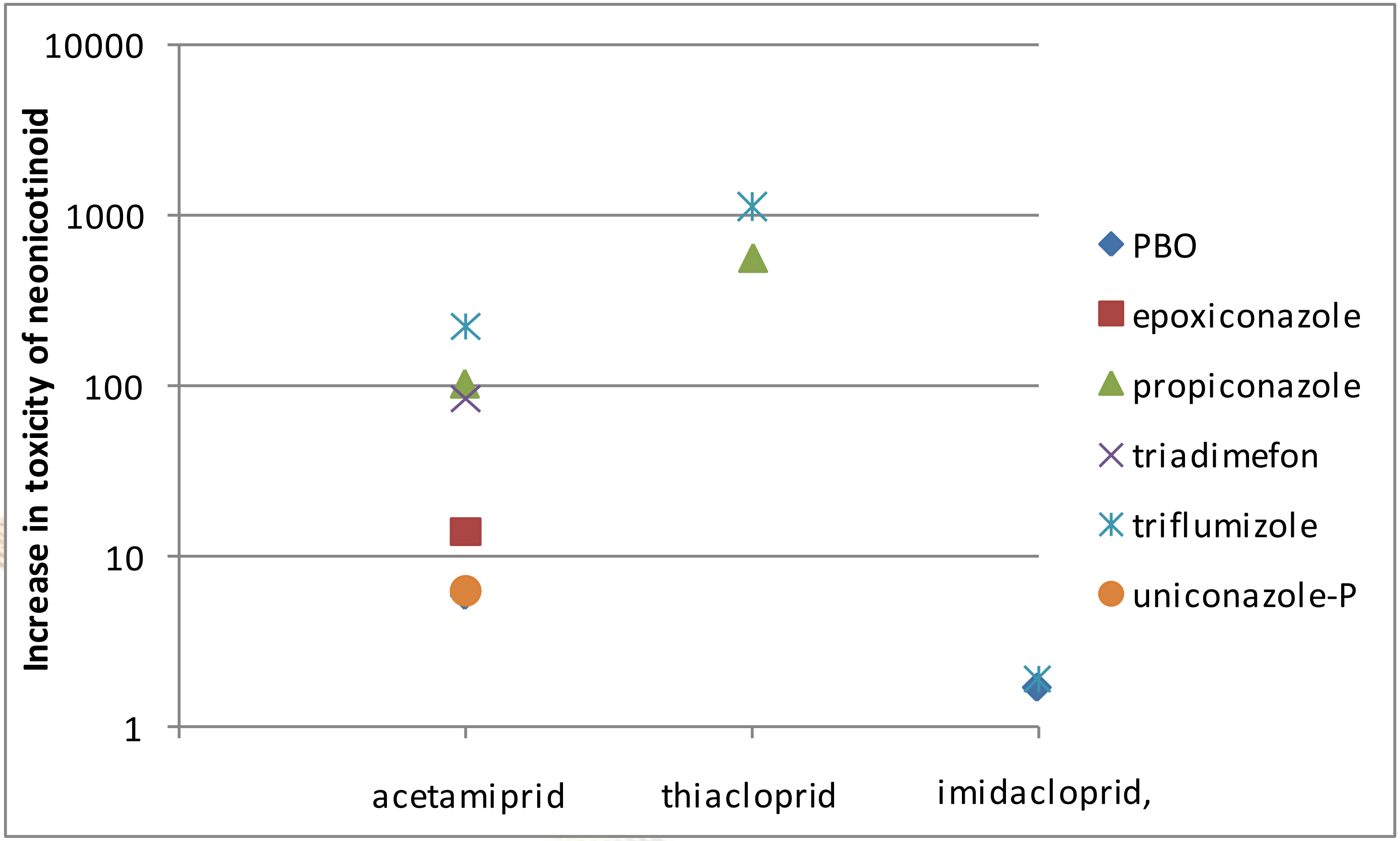
- Applied to bees received through the UK honeybee incident investigation scheme

ead bees	Pesticide	Major contributor to toxic units	% LD50
umble bee	Azoxystrobin; boscalid; cypermethrin	cypermethrin 96%	10.6
oney bee	Bendiocarb; deltamethrin; propiconazole	Bendiocarb 98%	17.2
oney bee	Bendiocarb; *fluvalinate* (varroacide)	Bendiocarb 99%	7.3
oney bee	Bendiocarb; DDE-pp; pirimiphos-methyl	Bendiocarb 86%	6.7
oney bee	*Fluvalinate* (varroacide); tebuconazole	Fluvalinate 75%	0.0037
oney bee	Imidacloprid; tebuconazole	Imidacloprid 96%	0.6
oney bee	Dieldrin; HCH-gamma; permethrin; Propiconazole; thiacloprid	Permethrin 93%	5.1
oney bee	Chlorpyrifos; propiconazole	Chlorpyrifos 99%	0.27
oney bee	*Fluvalinate* (varroacide); propiconazole	Fluvalinate 76%	0.0099
oney bee	DDT-pp; methomyl; propiconazole	Methomyl 96%	7.3
oney bee	DDT-pp; fipronil; propiconazole	Fipronil 99% (veterinary use)	10
oney bee	Chlorpyrifos; dimethoate; *fluvalinate* (varroacide) thiacloprid	Dimethoate 99%	22
oney bee	Chlorpyrifos; cyhalothrin-lambda; difenoconazole; dimethoate; propiconazole; thiacloprid	Dimethoate 94%	28.5
oney bee	Chlorpyrifos; cyhalothrin-lambda; dimethoate; *fluvalinate* (varroacide); thiacloprid	Dimethoate 92%	18.2
oney bee	Bendiocarb; imidacloprid	Bendiocarb 95%	73.3
oney bee	Bendiocarb; permethrin; propiconazole tebuconazole	Bendiocarb 96%	36.4
oney bee	Dieldrin; HCH-gamma; permethrin	Permethrin 39%	1.82
oney bee	Chlorpyrifos; glyphosate; thiacloprid	Chlorpyrifos 97%	1.74

Synergy between pesticides

- Toxicity of the combination is greater than additive - predictable from the mode of action
- Early studies (1980s) identified EBI fungicides increased toxicity of pyrethroids 100 -1000 fold
- More recent studies have shown similar increase in toxicity of mixtures of EBI fungicides and neonicotinoid insecticides
- Inhibition of microsomal monooxygenases (P450s)

Increase in contact toxicity (decrease in LD_{50}) of acetamiprid, thiacloprid and imidacloprid in the presence of 10 µg/bee of a range of P450 inhibitors (data from Iwasa et al., 2004)



Importance of level and route of exposure: Propiconazole + thiamethoxam

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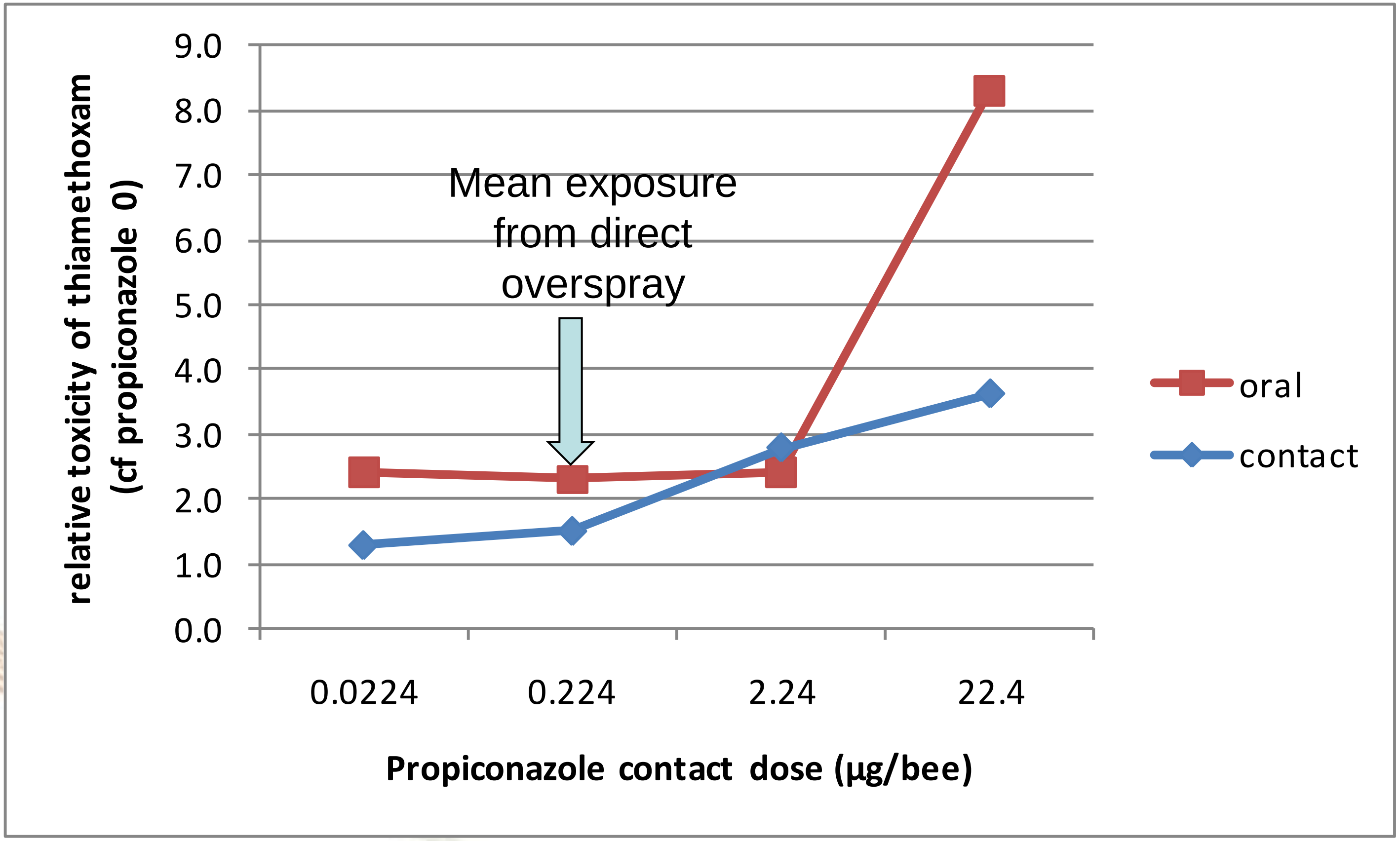
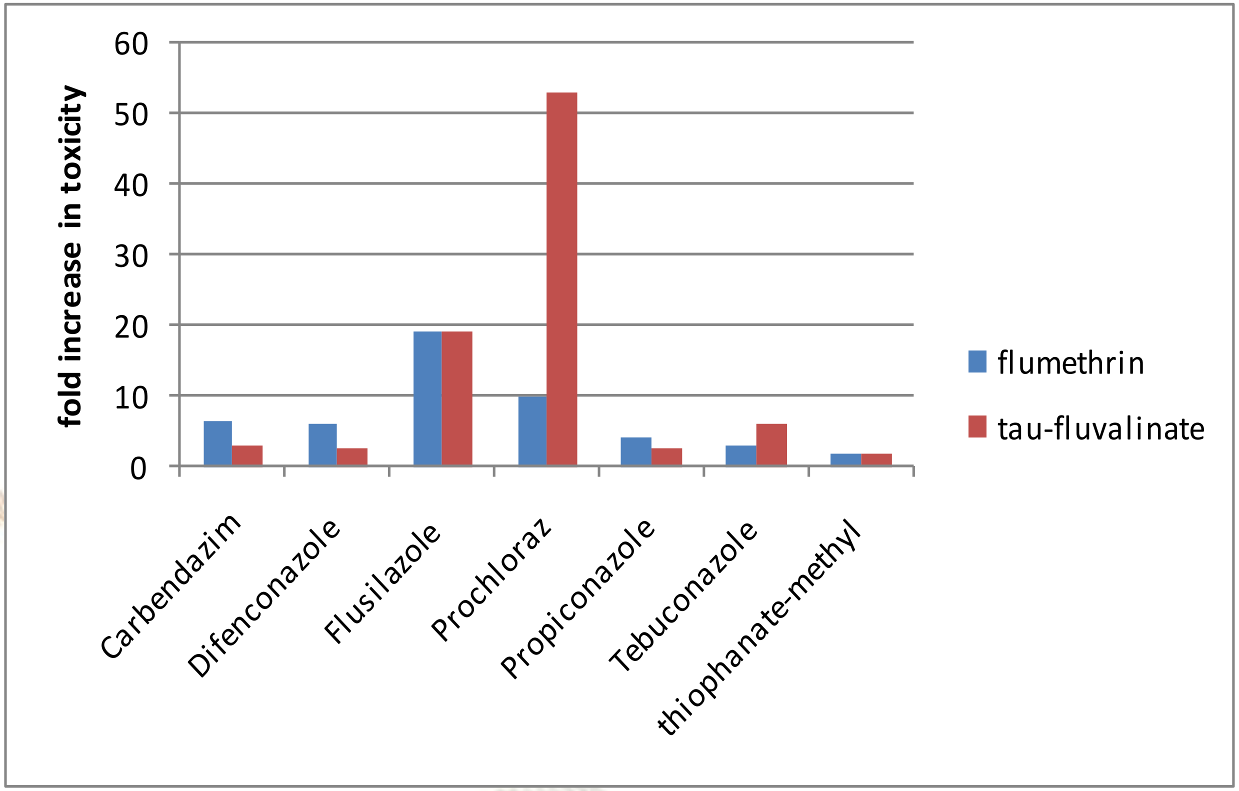


Synergy is dose dependent and many reports use unrealistically high levels/routes of exposure

Interactions with in-hive medicines

- Persistence of residues of in-hive medicines, e.g. varroacides and antibiotics: toxicity of combined effects of these and with agricultural pesticides
- Antibiotics (oxytetracycline) used in hives to control foulbrood diseases increase the toxicity of coumaphos and fluvalinate varroacides (Hawthorne and Dively, 2011)

Effects on the contact toxicity of flumethrin and tau-fluvalinate of co-exposure to fungicides at their maximum field application rate



Conclusions

- The toxicity for most pesticide mixtures is at most additive
- For those that are synergistic (predictable from mode of action) it is important that studies use realistic routes, combinations and levels of exposure to predict effects in the field
- What are realistic levels and combinations of pesticides at the individual and colony level and how do they change over time?

Acknowledgements

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