International Regulatory Approaches and Bee Safety Testing

Standard regulatory study cascade and laboratory to field extrapolations

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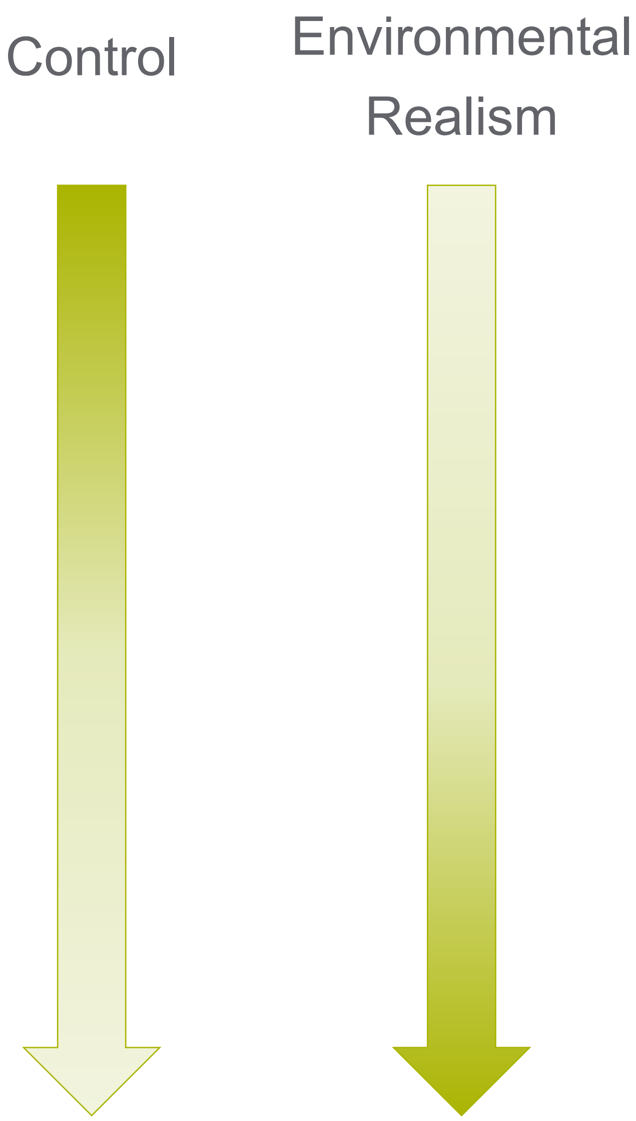
Outline

- Tiered risk assessment from laboratory to field
  - Effects
  - Exposure
  - Use in risk assessment
- Lab to field extrapolation –
  - Hazard vs risk assessment
  - Sublethal effects
- Multi-year field studies

Tiered Risk Assessment Approach for Bees

- Tier I - Laboratory
  - Modelled exposure values
  - Laboratory acute and chronic toxicity data
    - Adults
    - Larvae
- Tier II – Semi-field
  - Measured residues in nectar and pollen
  - Semi-field (tunnel) effects studies
    - Small hives
    - Surrogate crops
- Tier III - Field
  - Full-field studies
    - Multiple hives
    - Target crops



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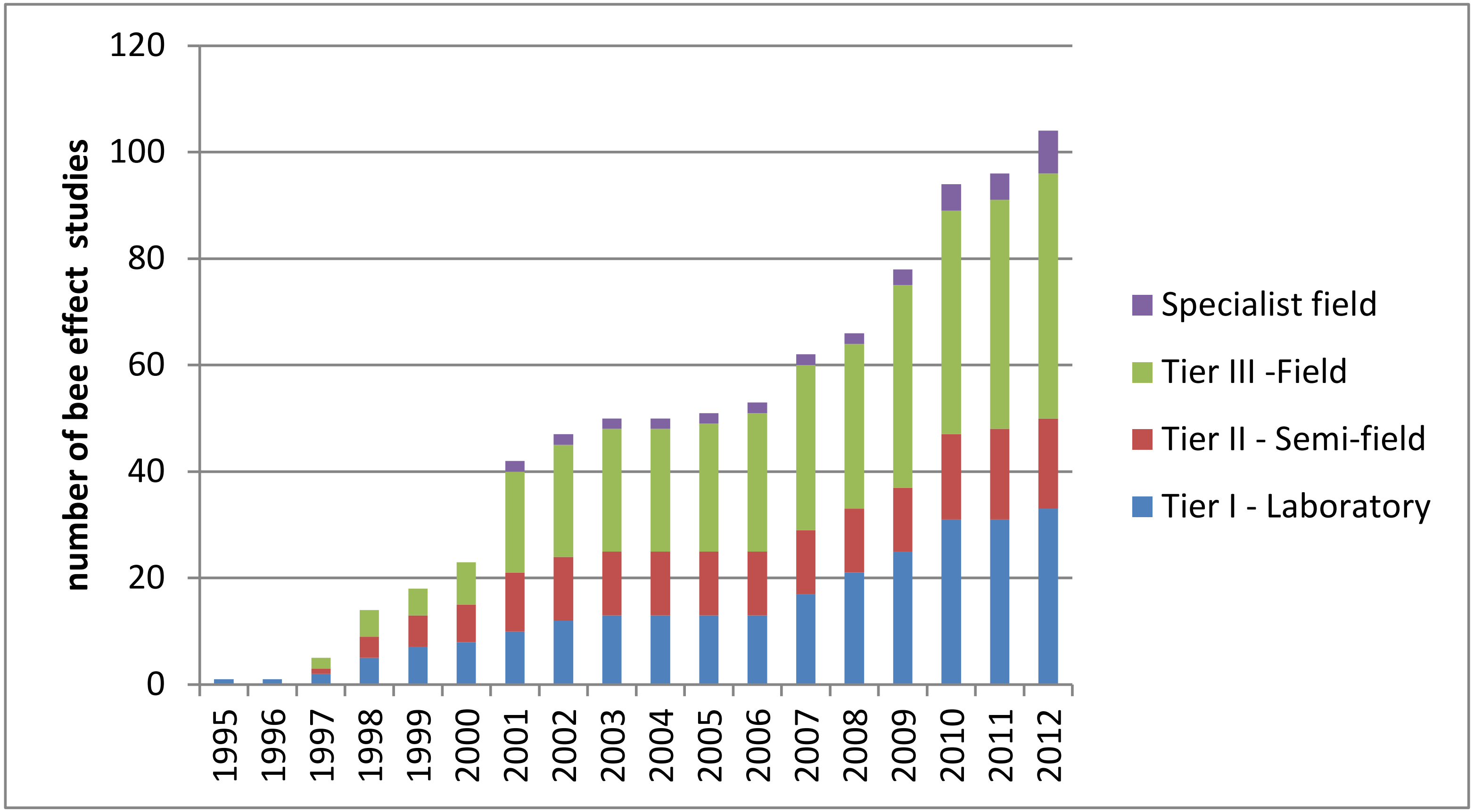
Thiamethoxam – Cumulative number of regulatory studies conducted



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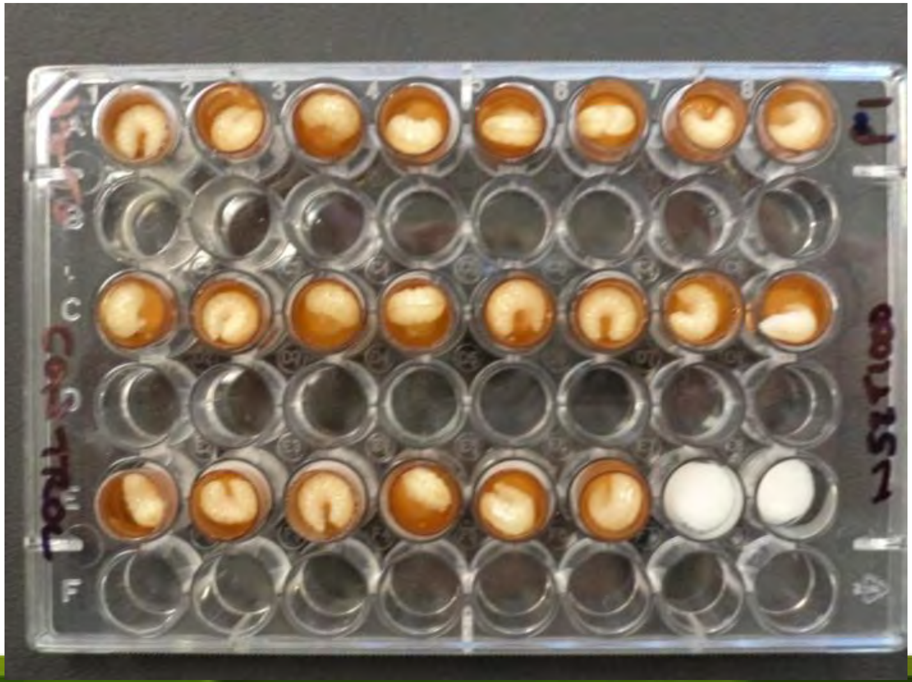
Tier I – Use of Standard Laboratory Studies

- Honeybee acute contact and oral toxicity data required for both active ingredients and formulations for over 20 years in EU
- Sprays
  - Contact and oral honeybee LD_{50} (OECD213; 214)
  - HQ (hazard quotient) = proposed application rate/lowest LD_{50}
  - If HQ > 50 – semi-field and/or field study required
  - If IGR – detailed brood effects study required
- Systemic
  - Comparison of toxicity (adult and larval LC50) with exposure based on residues in nectar with trigger values

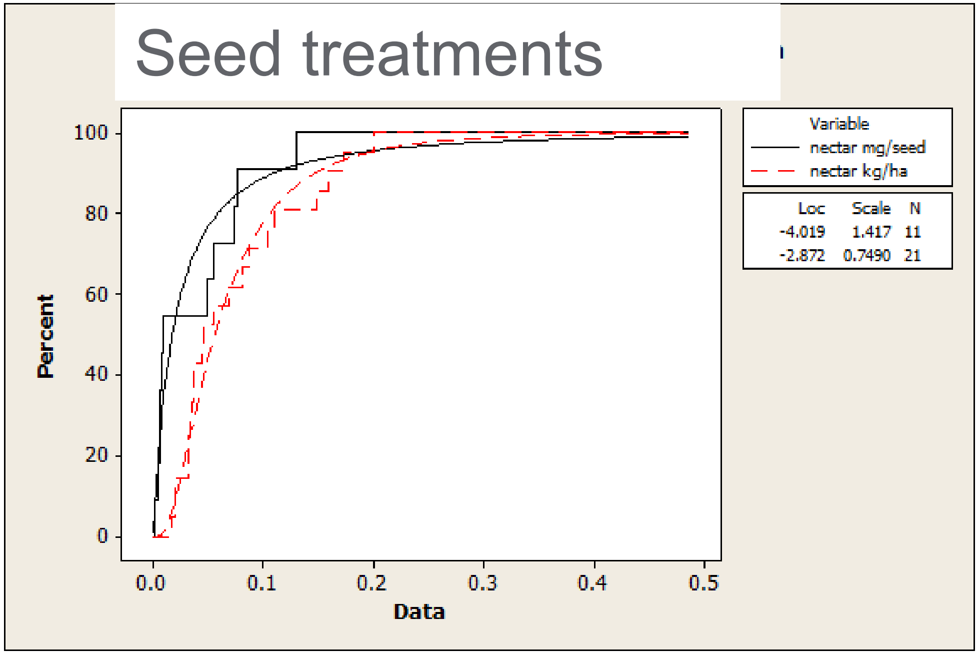
- If concerns raised more detailed studies at higher tier and more realistic exposure

Standard Laboratory Studies

- Adult workers:
  - OECD guidelines (213 and 214) acute contact and oral toxicity
  - 10 day chronic (no current guidelines)
- Larval testing – acute (OECD 237) (chronic/repeated exposure under development)



Exposure - Systemic pesticide residues in nectar can be estimated by the Residue per Unit Dose approach (EFSA)



For seed treatments residues estimated by multiplying the mg ai/seed by 0.0093 to give mg ai/Kg nectar

Imidacloprid on oilseed rape (canola)
For 0.05 mg / seed = 0.465 µg/Kg nectar

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Example: EFSA Tier I risk assessment for seed treatments

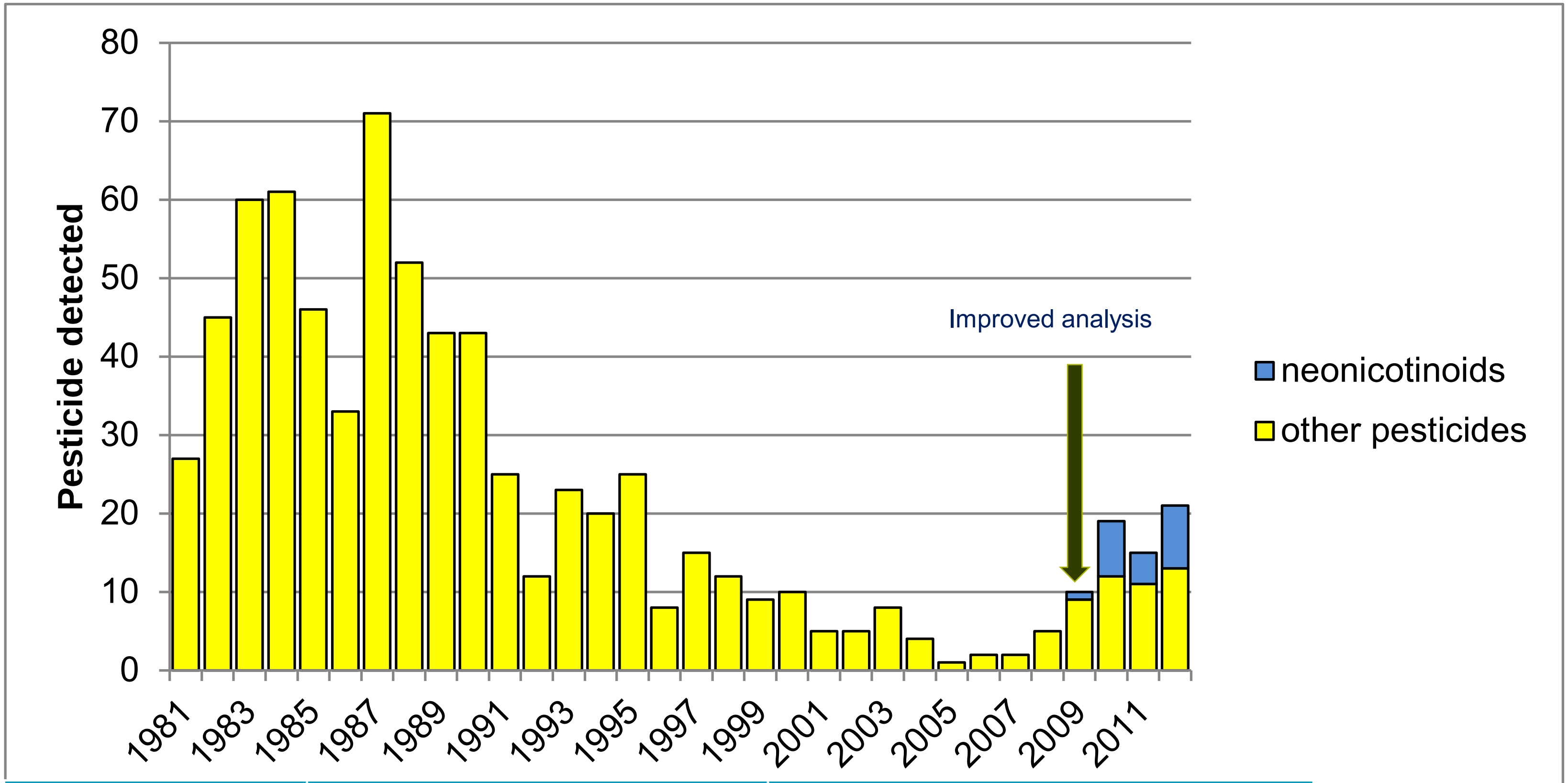
- For chronic adult risk
  = Application Rate x Shortcut Value/LC_{50} (10 day LC50)

- Suggested safety factor 33
  - i.e. trigger for further assessment >0.03

  - For a pesticide with LC50 0.37 ng/bee/day and 50 g ai/ha applied
    0.05 x 0.78/0.37 = 0.11

- [1] Shortcut Value = 0.78
  - estimated exposure per kg/ha applied based on:
    - Residue in nectar per kg ai applied
    - Daily nectar intake by individual bee

Data for validation of risk assessments: UK Bee Incident Scheme



WIIS	Imidacloprid (ng/bee) Oral LD50 4ng/bee	Thiacloprid (ng/bee) Oral LD50 17300 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

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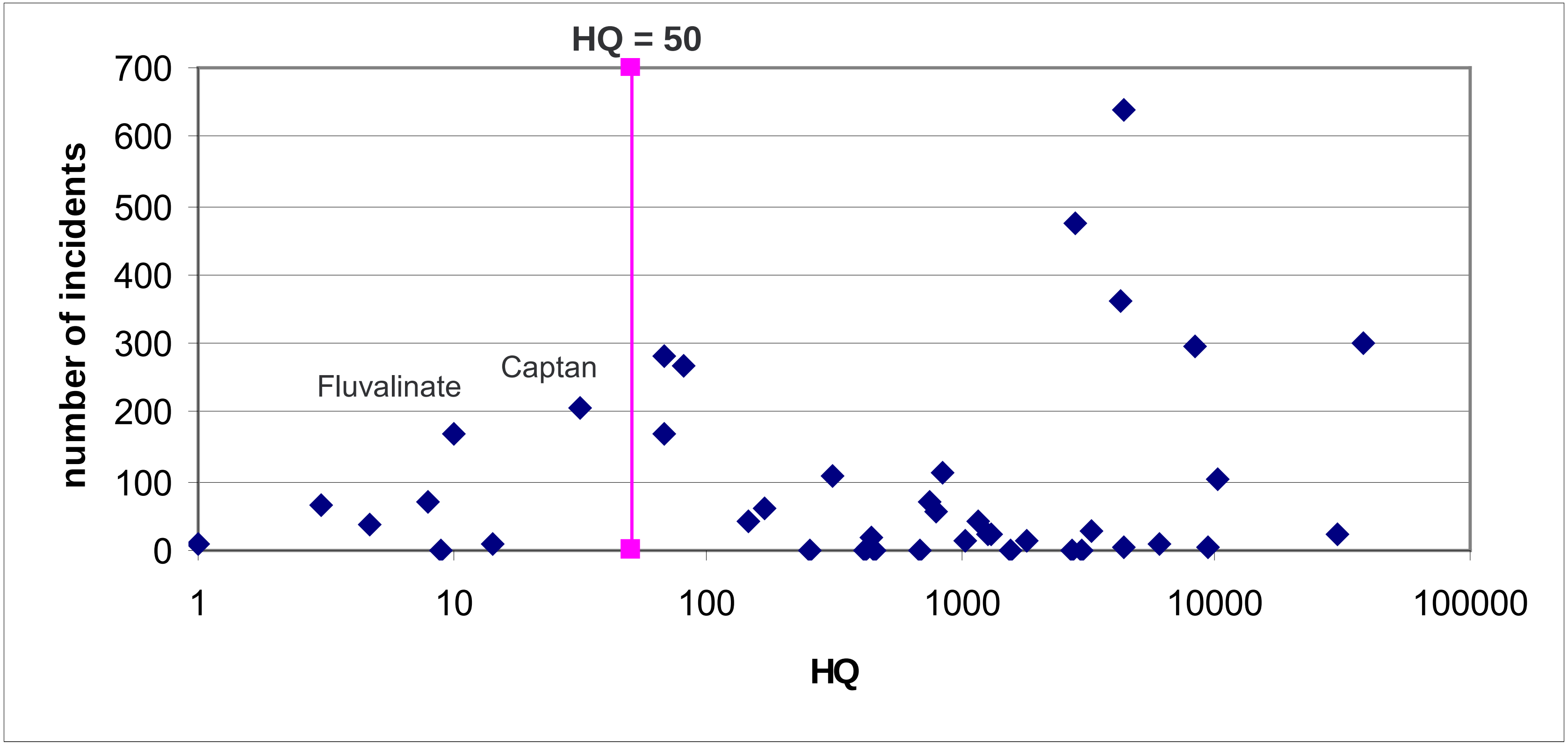
Validation sprayed pesticides HQ vs real-use incidents in UK, NL, DE (1980-2007)



For spray applications the HQ threshold of 50 is appropriate to identify plant protection products with low risk of effects in the field

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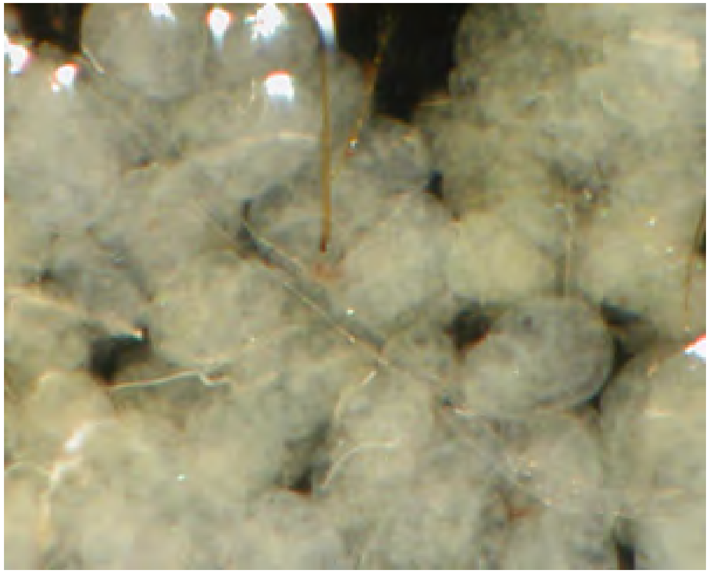
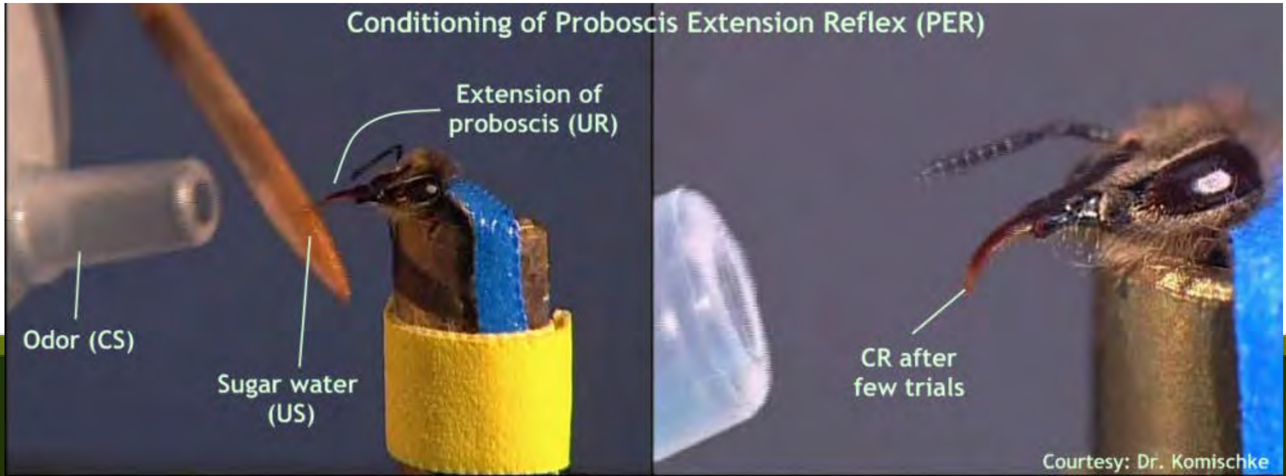
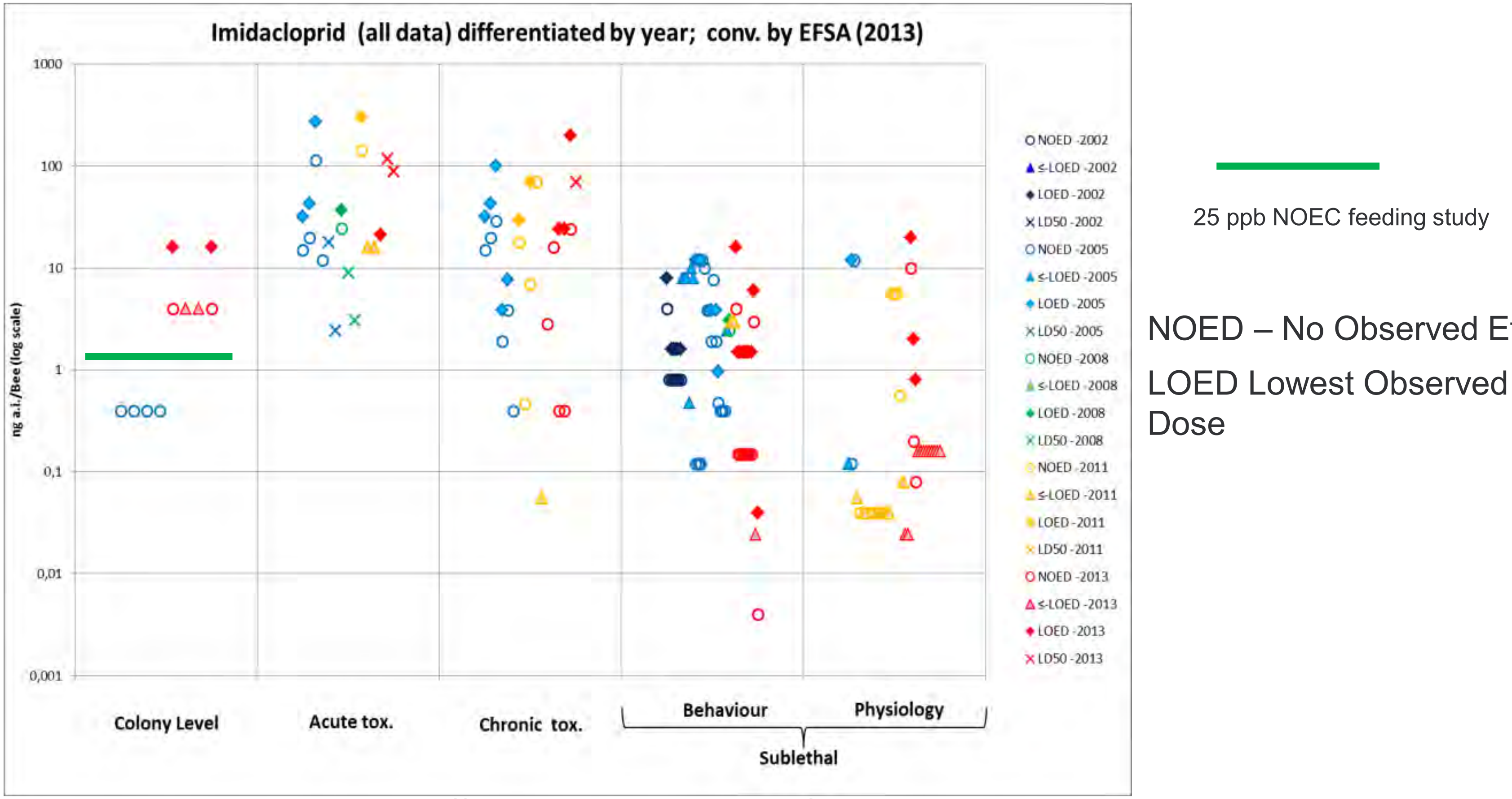
Sublethal effects measured in the laboratory – how do they predict effects at the colony level?

- Hypopharyngeal gland development
- Probocis extension reflex
- Locomotor activity
- Food consumption
- Longevity
- Immunity –individual and social
- Visual T or complex maze
- Morphological/histochemical changes





Sensitivity of lab sublethal effects data vs effects in the field



NOED – No Observed Effect Dose

LOED Lowest Observed Effect Dose

No observable adverse effects at colony level for controlled chronic exposure to

10x 90%ile field residue level (systemic exposure via pollen and nectar)

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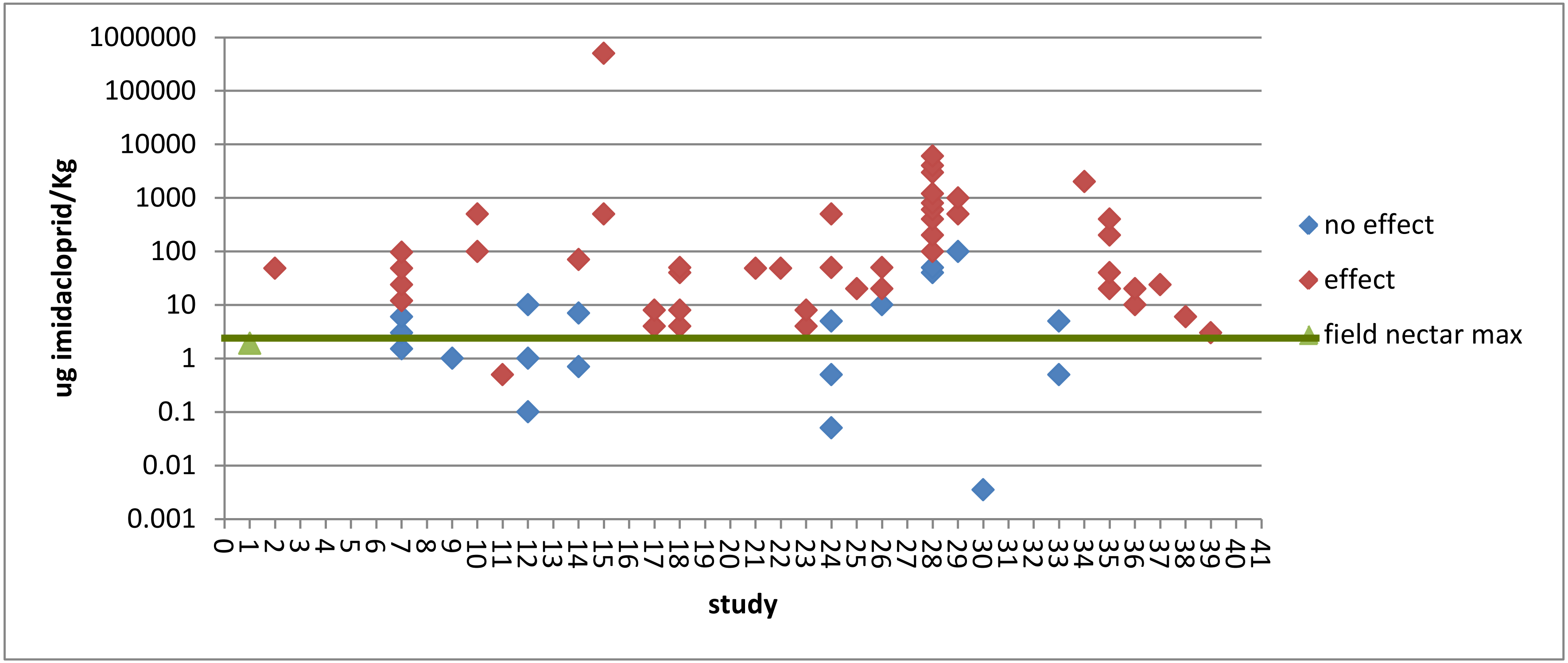
Dose levels used in sublethal studies (residues in nectar from systemic seed treatment)



Most sublethal studies conducted at unrealistic exposure levels and show no effects at field nectar maximum

Field nectar **maximum** residue = worst case

Laboratory studies = potential mode of action **not risk**

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HIGHER TIER RISK ASSESSMENT

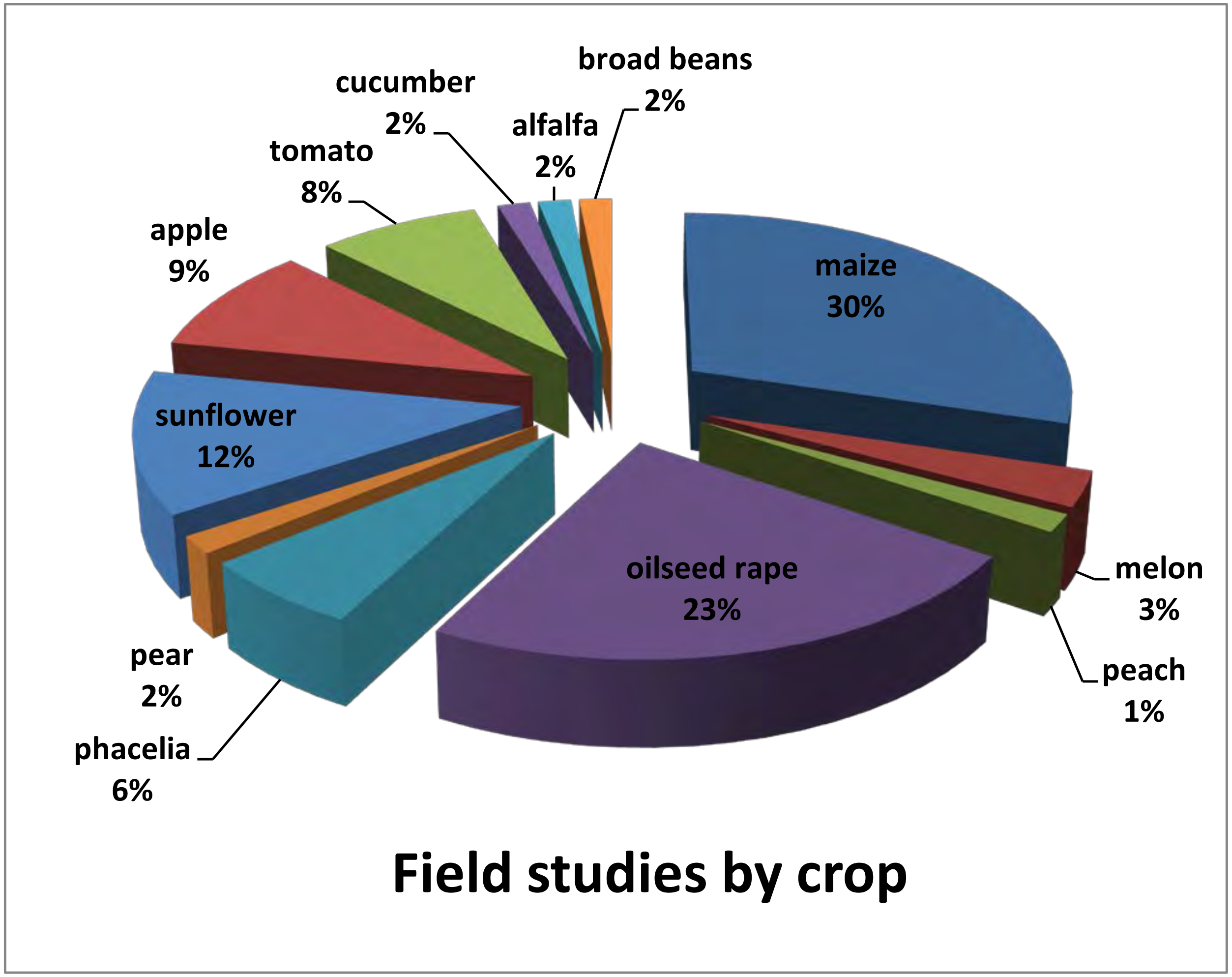
Tier II - Semi-field studies

- Colony confined to treated crop
- Replicated treated and control tunnels
- Adult and brood assessments (OECD75), foraging activity, mortality
- Toxic reference and control
- Limited confinement period

Tier III - Field studies

- Isolated plots (min 1Ha)
- 4-6 colonies per plot
- Control and treated plots
- Assessments from pre-application through minimum10 day post-application (throughout flowering period and overwintering success for systemics)
- Mortality, colony assessments, bee foraging behaviour residues
- Assessment based on experience and expert judgement

Thiamethoxam



Clothianidin seed treatment in Canola – Canada 2012

- 5 treated, 5 control
- 2Ha fields
- 4 colonies per field
- Isolated sites
- Exposure up to 1.9 ppb clothianidin in pollen
- No effect on any parameter including:
  - colony development
  - adult mortality
  - colony weight
  - honey yield
  - overwintering survival

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Multi-year Field Studies in France: Background

- 2002 – thiamethoxam data were submitted to French regulatory authorities to support registration

- French authorities requested more data for honey bees related to uses on corn and oilseed rape
  - Questions about year on year use
  - Concerns about overwintering losses

- A multi-year honey bee field study was proposed
  - Study design was mutually agreed upon with refinements from year to year
  - 2 oilseed rape and 3 maize sites
  - Each site paired treated and control 1 ha plots separated by >1.8Km

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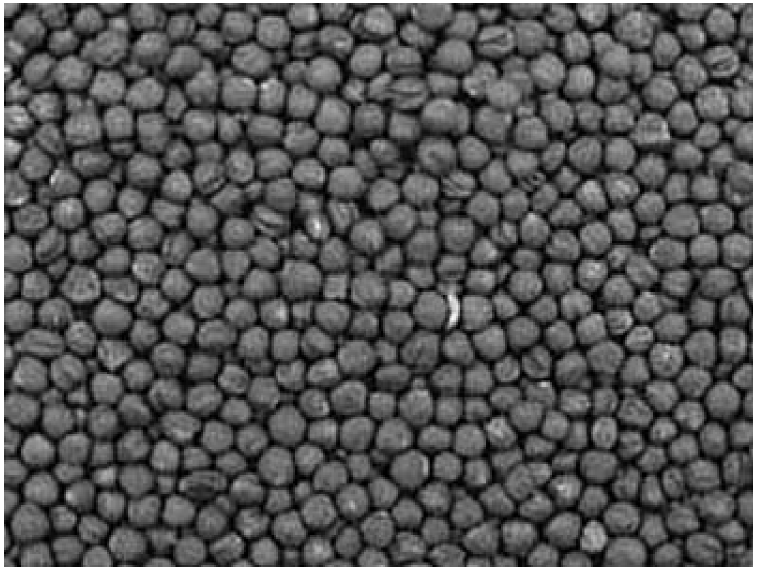
Seed Treatment

- Roxet Winter Oilseed Rape (WOSR) seed
- Test item
  - 282 g/L thiamethoxam
  - 33.4 g/L metalaxyl-m
  - 8.0 g/L fludioxonil
- Control (fungicide only)
  - 2005: thiram
  - 2006-2008: fludioxonil + metalaxyl-m
- Target rate range
  - 0.02 – 0.022 mg ai/seed
- Rate of thiamethoxam applied
  - 12.69 – 21.15 g ai/ha



Honey bees

- 6 Hives per plot
  - Queen-right
  - 10,000 – 20,000 bees/hive
  - 2 brood chambers
  - Additional brood chambers added as colony developed

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Assessment endpoints

Mortality

Flight intensity

Parasites and Disease

Behavior

Brood assessment

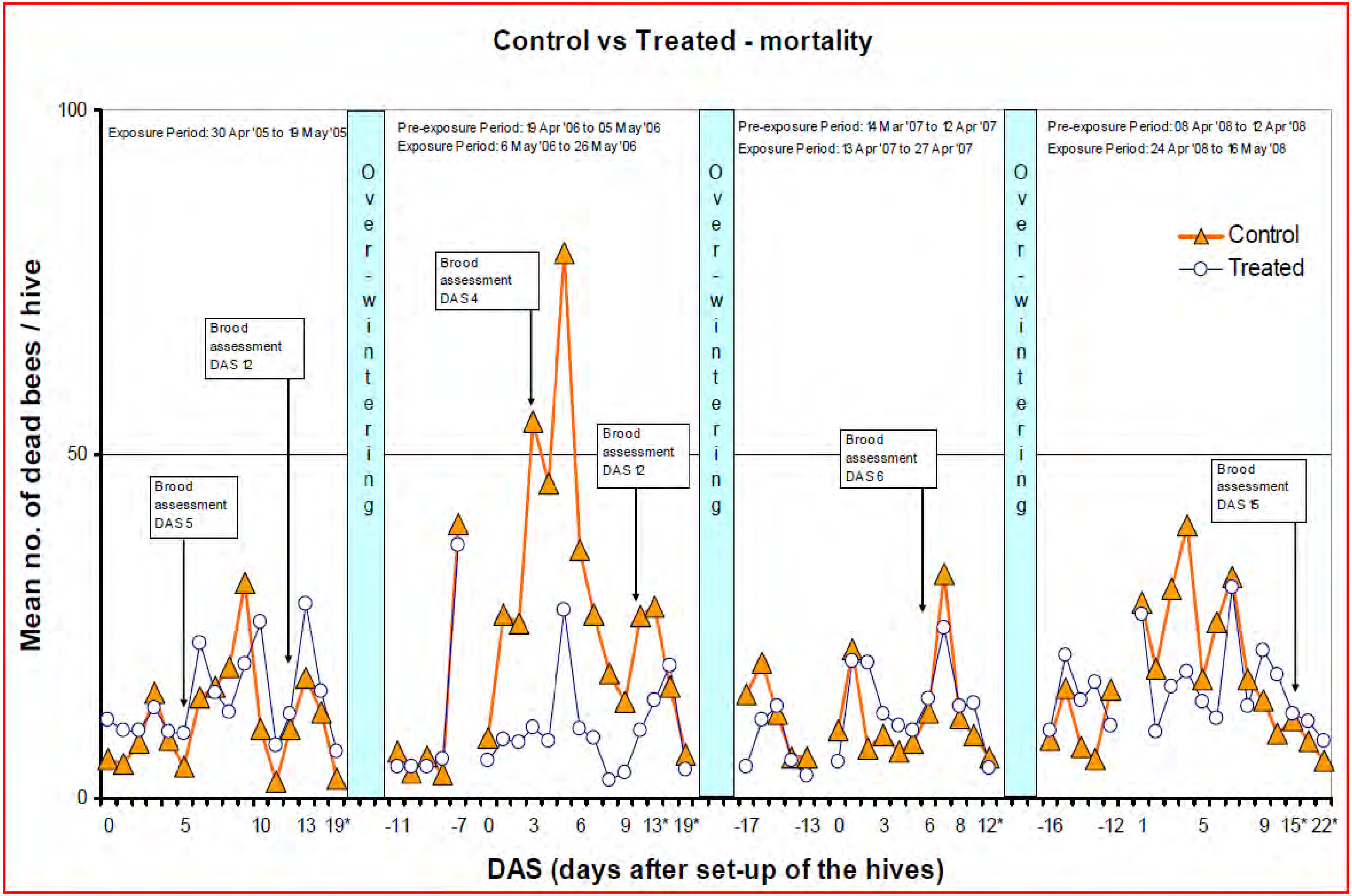
Weight & Strength

Sampling Plants and Bees for Residue Analysis

Results

Example: One site oilseed rape in Alsace (highest residues)

Adult Mortality – no effect



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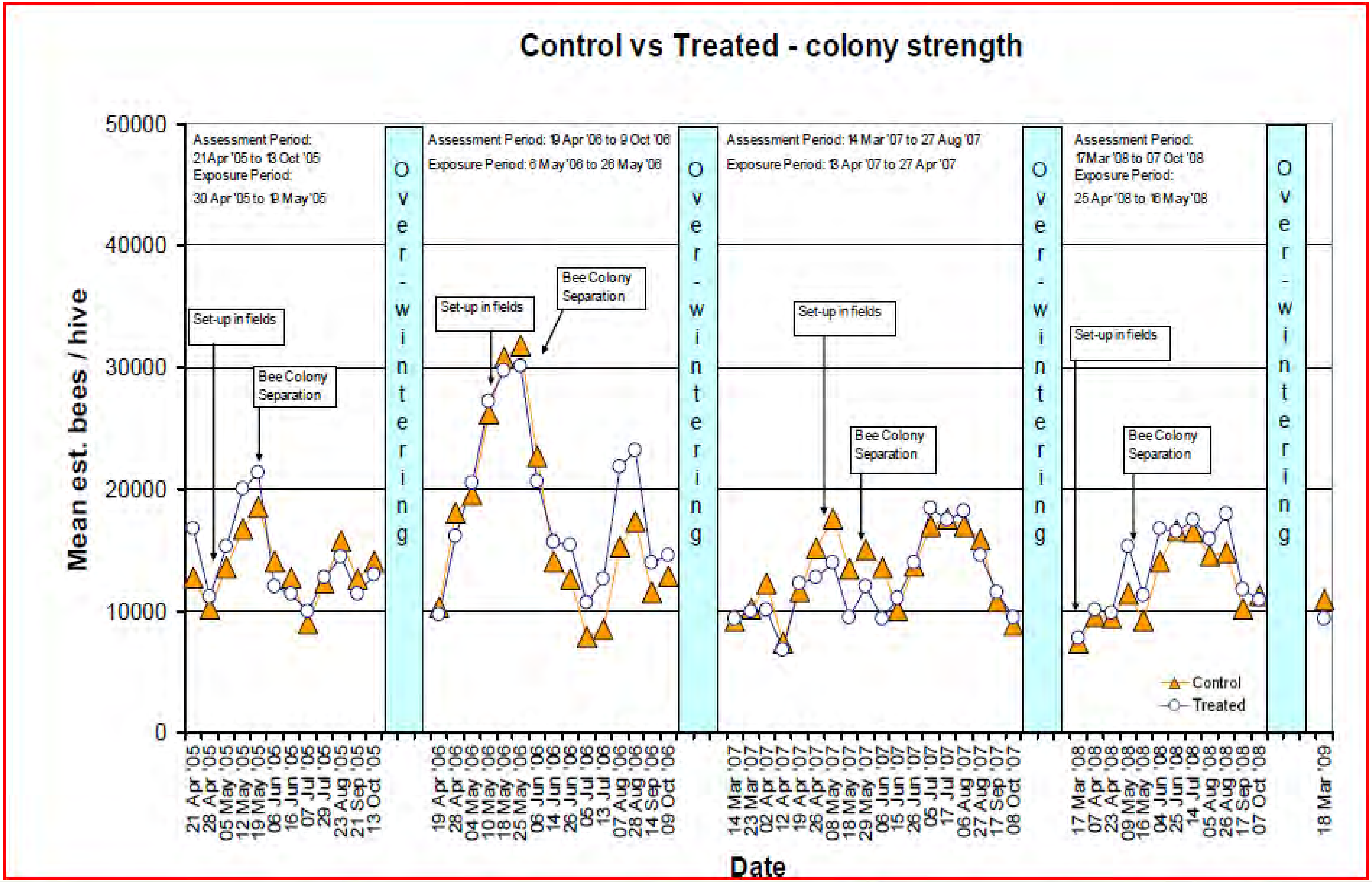
Colony Strength – no effect



Behaviour

- No behavioural differences of the bees in the test item treatment group were observed during the entire exposure period to the crop compared to the bees in the control group in the four years of observations

Summary – All Crops and Sites

- Residues of thiamethoxam and CGA322704 in nectar and pollen were low

Crop	Matrix	TMX (µg/kg)	CGA322704 (µg/kg)
Corn	pollen	<1.0 to 2.0	<1.0 to 2.0
Oilseed rape	pollen	<1.0 to 1.0	<1.0
	nectar	<0.5 to 3.0	<1.0

- Overall, no differences in colony development, adult mortality, flight intensity, behavior and colony health were observed between treated and control colonies foraging on oilseed rape or corn

- Similar multi-year studies have been conducted for clothiandin and imidacloprid

Conclusions

- Laboratory studies provide hazard data and indication of potential risk
- Laboratory sublethal effects studies provide information on modes of action, e.g. neurotoxins cause behavioural effects
- “Well conducted higher-tier studies can be useful in determining, under actual use conditions and typical foraging activity of bees, whether a compound represents a significant risk to bees” (EPA,PMRA,CDPR 2012).
- Field studies provide real-world exposure scenarios and allow “bees to be bees”
- Good beekeeping practices are essential to properly manage confounding factors and maintain optimal bee health
  - Disease, nutrition, parasites

Bringing plant potential to life

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