


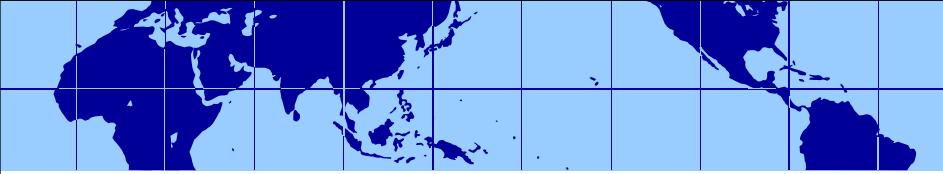

Determination of neonicotinoids and their metabolites in pollen and nectar of field treated pumpkin to assess their potential exposure to honey bees

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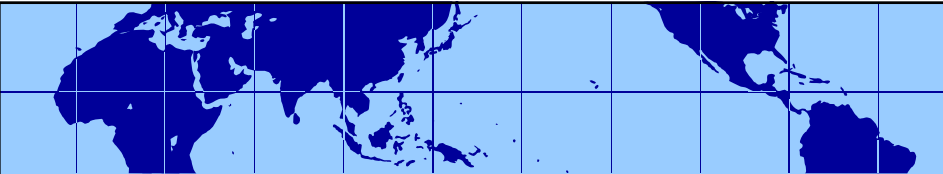

²Department of Entomology, University of Maryland.

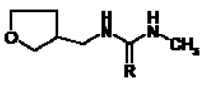


Neonicotinoid insecticides

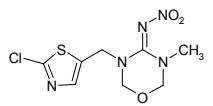
- **Neonicotinoids containing the nitroguanidine moiety such as imidacloprid, dinotefuran, thiamethoxam and clothianidin have very selective toxicity to insects.**
- **They are widely used on cucurbits such as cantaloupe, cucumber, and watermelon crops and other crops pollinated by bees.**
- **Neonicotinoids are systemic insecticides which metabolize into several different metabolites, all of which contain elements of the 6-chloropyridine group.**

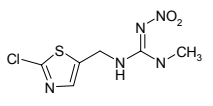


Dinotefuran:
Dinotefuran UF:
Dinotefuran DN phosphate

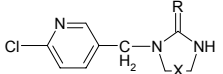
R = N-NO₂
 R = O
 R = NH.H₃PO₄



Thiamethoxam



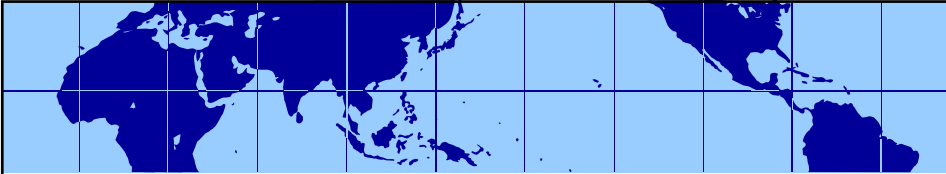

Clothianidin



Imidacloprid:
Imidacloprid olefin:
Imidacloprid, 5 hydroxy
Imidacloprid urea:
Imidacloprid, desnitro olefin:
Imidacloprid, desnitro HCl:

R = N-NO₂
 R = N-NO₂
 R = N-NO₂
 R = O
 R = NH
 R = NH

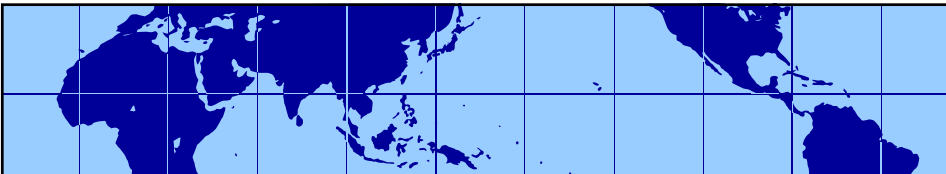

C-X-C = -CH₂-CH₂-
C-X-C = -CH=CH-
C-X-C = OHCH-CH₂-
C-X-C = -CH₂-CH₂-
C-X-C = -CH=CH-
C-X-C = -CH₂-CH₂- , HCl

Toxicity of neonicotinoids & their metabolites to honey bees



- The **nitro-substituted compounds imidacloprid, thiamethoxam, and dinotefuran are the most acutely toxic to the honey bee, with LD₅₀ values as low as 18 ng/bee for imidacloprid, 30 ng/bee for thiamethoxam, and 75 ng/bee for dinotefuran.**
- **Acute LD₅₀ of the metabolites vary, some have LD₅₀>1000.**
- **Metabolites of significant acute toxicity are: the imidacloprid olefin, 5-hydroxy & urea and clothianidin.**
- **In chronic studies, 50% mortality of bees is obtained after 8 days of exposure to imidacloprid or any of its metabolites during which each bee ingested as low as 0.1 ng/bee/day of the toxic substance.**

Suchall et al., *Environ. Toxicol. Chem.* 20, 2001
Iwasa, Tet al., *Crop Prot.*, 23 (5), 371–378, 2004.



Colony Collapse Disorder (CCD)

- **Is the sudden decline in adult honeybee populations without evidence of mortality.**
- **Pesticide use has been identified as a potential contributing factor to these declines among other factors such as new and re-emerging pathogens, habitat loss, pests, and nutritional stress.**
- **Researchers suspect that the declines likely result from a combination of factors representing environmental stressors that compromise the immune system of bees and make them more susceptible to disease.**

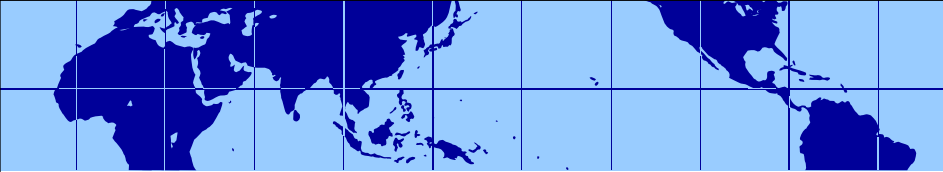

Exposure of honey bees to neonicotinoid insecticides

- **Spraying can poison honey bees through direct contact with the product or through contact with its residue.**
- **Residues of systemic neonicotinoids and metabolites in pollen and nectar, the main food sources of honey bees.**
- **Honey bees visit between 100 and 1500 flowers in order to fill their honey sacs. The honey sac holds almost 70 mg of nectar.**
- **Foraging bees consume their food needs from the nectar of the flowers they visit.**

Objectives



- **Help achieve US EPA Office of Pesticide Programs (OPP)'s goals of the strategic plan by developing more comprehensive testing protocols for evaluating potential sub-lethal and chronic effects of pesticides to brood and adult honey bees under both laboratory and field conditions.**
- **To address the risk management issue involving neonicotinoid insecticides and their potential exposure to honey bees and other pollinators**
- **To assess the levels of residues in pollen and nectar of a cucurbit crop treated with label allowed rates of neonicotinoids using different application methods.**



Experiment design & procedures

Pumpkin (*Cucurbita pepo* L. var 'Howden') has been chosen to represent the cucurbit crop grouping because its large flowers.

- A survey study on cucurbit growers in the Mid-Atlantic region showed that the majority of cucurbit growers treat their transplants with a bedding tray rate of imidacloprid just prior to transplanting or apply the low labeled rate in transplant water.
- Eight treatments consisting of different neonicotinoid insecticide-application method combinations plus an untreated control were evaluated. Each treatment was replicated four times for a total of 36 plots.






- **Imidacloprid (Admire Pro, four treatments)**
 - Bedding tray drench applied to transplants at a rate 6x higher than the label allowed rate for planthouse application but 21x less than the low label rate per acre for field application.
 - Transplant treatments of a low rate of 7 oz/acre
 - Transplant treatment of a high rate of 10.5 oz/acre
 - Split treatments: transplant water treatment followed 4 weeks later by chemigation, which applied one-half the labeled rate through the drip when plants were flowering.

- **Thiamethoxam (two treatments):**
 - **Split applications:** Platinum applied as a half rate (5.5 oz/acre) in the transplant water and the remaining half rate applied 3 weeks later by drip irrigation.
 - **Foliar applications:** Actara 25WDG applied as two foliar sprays, each 5.5 oz/acre, at 3 and 6 weeks after transplanting.

- **Dinotefuran (Venom, two treatments)**
 - **Split treatments:** applied as a half rate (3 oz/acre) in the transplant water and the remaining half rate applied 3 weeks later by drip irrigation.
 - **Foliar sprays:** applied as two foliar sprays, each 3 oz/acre at 3 and 6 weeks after transplanting.

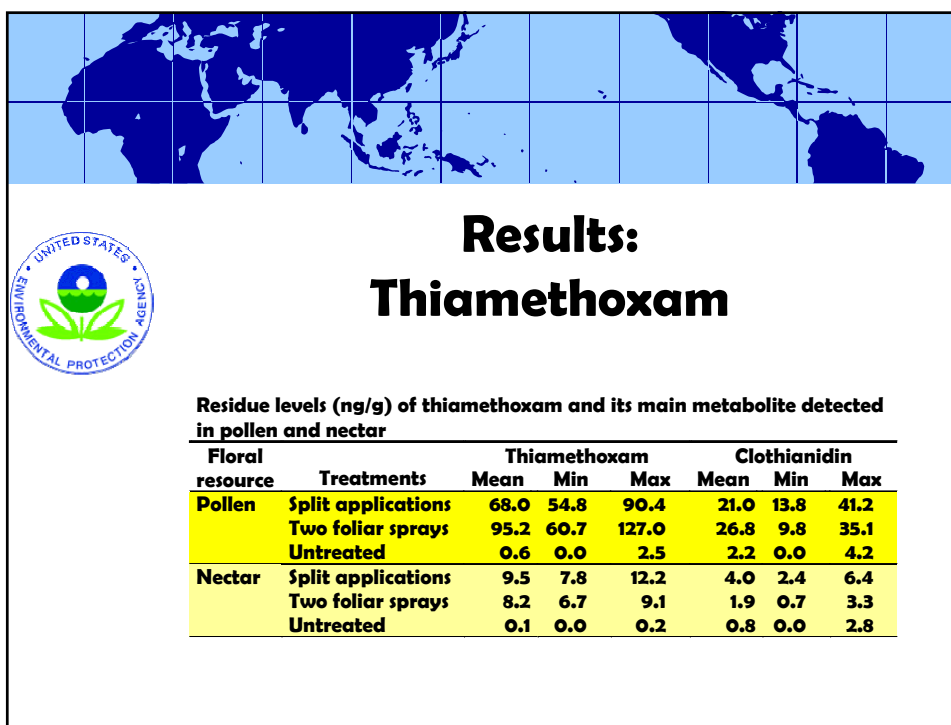
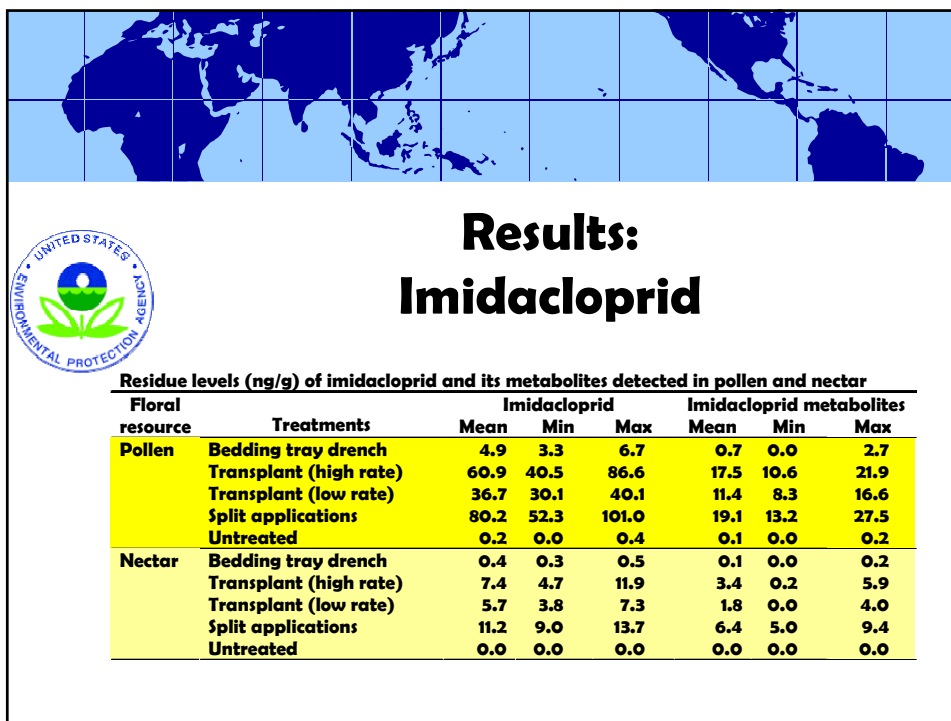
Extraction & cleanup of neonicotinoids & metabolites

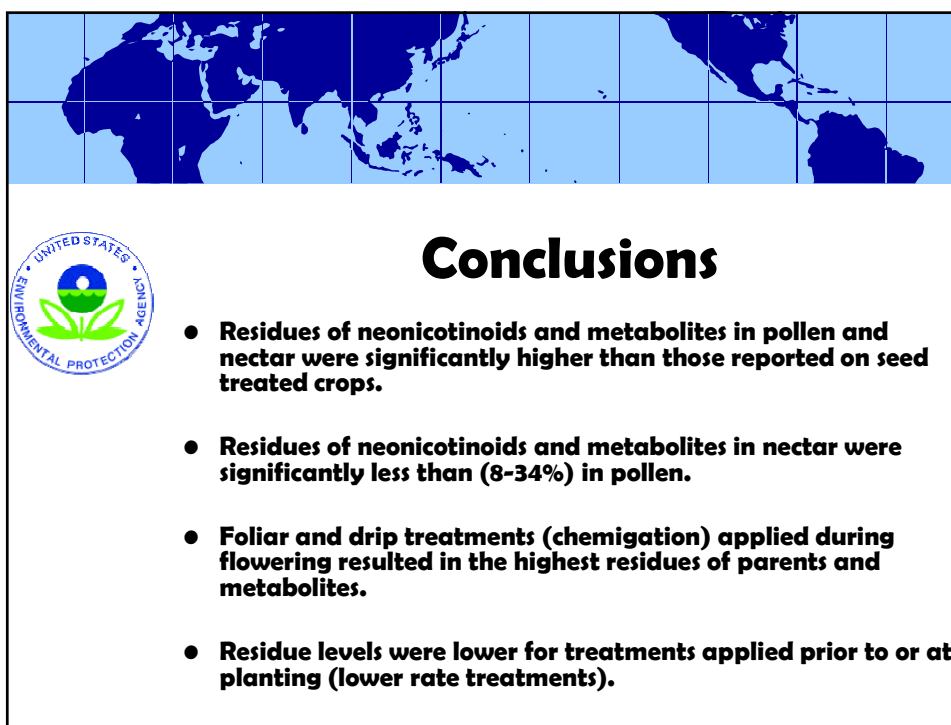
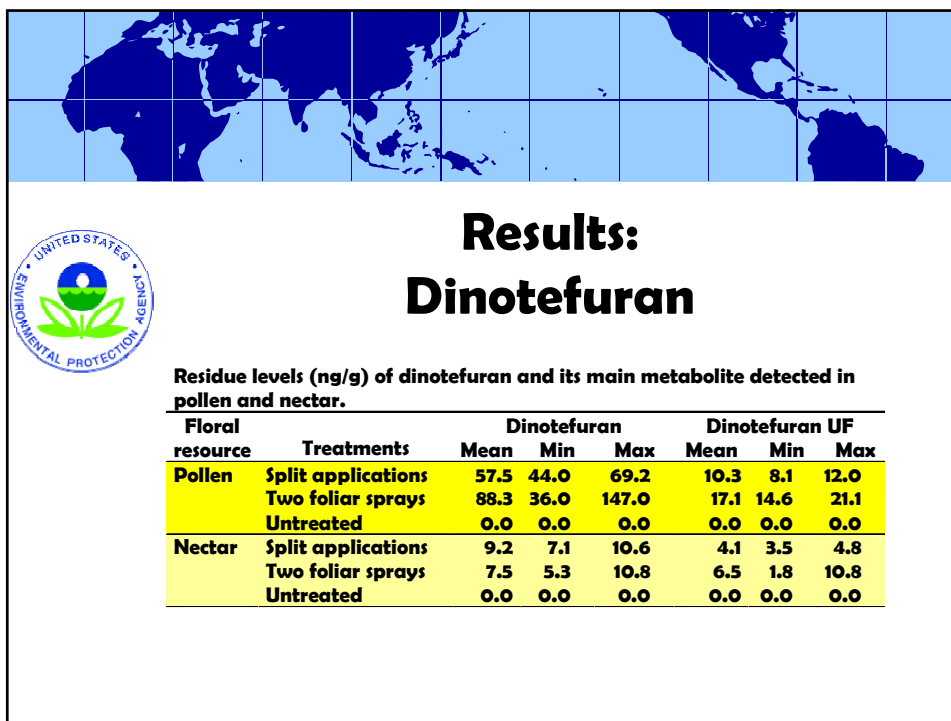
3g pollen or nectar
 ↓ extract with **2% TEA/ACN/H₂O**

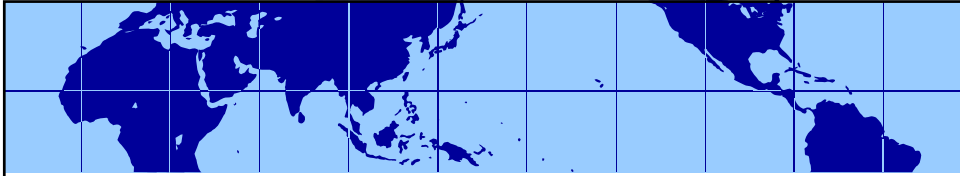
Shaking, salting out (NaOAc, MgSO₄), centrifugation
 ↓ pass through **C-18 SPE**


Concentrate
Waters Xevo LC-MS/MS

Kamel (2010) JAFIC







 **Conclusions**

- **Results show evidence of potential exposure to bees at label rates.**
- **According to this study, residues of neonicotinoids + metabolites may reach up to 100 ng/g in pollen and 15 ng/g in nectar.**
- **Effects depend on how and when pollen and nectar are processed and consumed by worker, queen and brood.**
- **Forager bees transport fine pollen particles to the hive, which are processed into bee bread and consumed/stored as rich protein diet. Each gram of bee bread may contain a significant amount of neonicotinoid residues.**
- **Forager bees consume nectar & deliver the collected nectar to the hive: 70 mg (sac full) of 15 ng/g =1 ng/sac.**



 See you next year at FPRW 2011

