2013 TPSA Annual Meeting
Mobile, Alabama

2012 INDIANA BEE KILL INVESTIGATIONS: SAMPLING AND DETECTING PESTICIDE RESIDUES IN BEES AND POLLEN

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Office of Indiana State Chemist

SUMMARY OF BEE MORTALITY CASES

- April to May 2012, seven cases investigated
- All reported large quantities of bees dying/dead (hundreds)
- Complainants (beekeepers) suspected the bee deaths were in response to either pesticide application or corn planting in neighboring fields.
- Dead bees and in some cases, bee pollen and vegetation from the target fields were sampled and tested for pesticide residue.

Sample #	Sample Description	PPB Clothianidin
2012-0093	Dead and dying bees (collected by the beekeeper)	7.0
2012-0094	Dead and dying bees (collected by OISC investigator)	3.4
2012-0106	Dead and dying bees (drunk-like)	3.5
2012-0131	Pollen	>7.4
2012-0132	Pollen	>8.1
2012-0133	Dead bees	>4.8
2012-0134	Dandelions from adjacent farm field	>6
2012-0120	Dead and dying bees (spasms, could not fly) (collected by the OISC investigator)	2.5
2012-0121	Dead and dying bees (collected by the beekeeper)	3.1
2012-0178	Dead and dying bees	3.3
2012-0174	Dead bees from 22 hives	2.5
2012-0199	Dead bees (large bee kill, > 200 hives impacted)	80.0
2012-0197	2x2 swab outside of bee box	20.3 ng/ swab

FIRST AID: If in Eyes: Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. **If on Skin or Clothing:** Take off contaminated clothing. Rinse skin immediately with plenty of water. Use soap if available. **If Swallowed:** Call poison control center or doctor immediately for treatment advice. Have person sip a glass of water if able to swallow. Do not induce vomiting unless told to do so by the poison control center or doctor. Do not give anything by mouth to an unconscious person. **If Inhaled:** Move person to fresh air. If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably by mouth-to-mouth, if possible. Call a poison control center or doctor for further treatment advice. **For emergency medical treatment information, Call Collect Day or Night: (314) 694-4000.**

Hazards to Humans and Animals: Harmful if swallowed, inhaled, or absorbed through skin. Ingestion of treated seed may be hazardous to domestic animals and livestock. Treated seed exposed on soil surface may be hazardous to birds, fish and other wildlife.

Personal Protective Equipment: Minimize skin contact by wearing protective clothing such as long-sleeved shirt, long pants, socks plus shoes, chemical-resistant gloves and protective eyewear when handling treated seed. Handle treated seed in a well ventilated area. Avoid breathing dust. Avoid contact with eyes, skin and clothing. Remove contaminated clothing and wash with detergent and water before reuse. Wash hands and face before eating, drinking or smoking.

Disposal: Cover or collect treated seeds spilled during loading. Dispose of all excess treated seed. Leftover treated seed may be doublesown around headland or buried away from water sources in accordance with local requirements. Do not contaminate water bodies when disposing of planting equipment washwaters. Dispose of treated seed packaging in accordance with local requirements.

WARNING: KEEP OUT OF REACH OF CHILDREN. THIS SEED HAS BEEN TREATED WITH SEED PROTECTANTS CODED BELOW AT THE PRODUCT'S LABELED RATE. DO NOT USE FOR FOOD, FEED OR OIL PROCESSING PURPOSES. DO NOT REUSE EMPTY SACKS. STORE AWAY FROM FEED AND OTHER FOODSTUFFS.

Code	Protectant
A	Metalaxyl
D	Azoxystrobin
H1	Clothianidin* 250
H2	Clothianidin* 500
H3	Clothianidin* 1250
I	Trifloxystrobin
M	Fludioxonil
X	Mefenoxam
W	Ipconazole

Azoxystrobin-treated seed (Dynasty): The active ingredient, Azoxystrobin, in this product can be persistent for several months or longer. Azoxystrobin has degradation products which have properties similar to chemicals which are known to leach through soil to groundwater under certain conditions as a result of agricultural use. Use of this chemical in areas where soils are permeable, particularly where the water table is shallow may result in groundwater contamination. This pesticide is toxic to freshwater and estuarine/marine fish and aquatic invertebrates. Drift and runoff may be hazardous to aquatic organisms in neighboring areas. Do not contaminate water when disposing of equipment washwater or rinsate. **Clothianidin-treated seed (Poncho® 600):** Areas planted with treated field corn or popcorn seed may be replanted immediately with corn, rapeseed and canola. These areas may also be replanted after 30 days with cereal grains, grasses, non-grass animal feeds, soybeans, dried beans, and root and tuber vegetables. These areas may also be replanted after 8 months with sugar cane. Do not plant any other crop in treated area for at least one year after treated seeds are planted. Areas planted with treated sweet corn, canola, rapeseed, or sorghum may be replanted immediately with corn, rapeseed, canola, or sorghum. These areas may also be replanted after 30 days with cereal grains, grasses, non-grass animal feeds, soybeans, dried beans, and root and tuber vegetables. Do not plant any other crop in treated area for at least 4 months after treated seeds are planted.

*Treated with 250 mg, or 500 mg, or 1250 mg per active ingredient per 1000 seed. Poncho® 600 seed treatment contains clothianidin. Poncho is a registered trademark of Bayer CropScience AG.

Trifloxystrobin-treated seed (Trilex): Treated areas may be replanted immediately with any crop for which a tolerance exists for Trifloxystrobin. All crops without Trifloxystrobin tolerances may be planted 30 days after seeding of Trifloxystrobin-treated seed. **Fludioxonil/Mefenoxam-treated seed (Maxim XL):** Corn and sorghum forage may not be grazed until 30 days after planting. **Mefenoxam-treated seed (Apron XL LX):** Mefenoxam is known to leach through soil into groundwater under certain conditions as a result of agricultural use. Use of this chemical in areas where soils are permeable, particularly where the water table is shallow, may result in groundwater contamination.

Call 1-800-8 DEKALB (1-800-833-5252)
with any questions, feedback or additional seed needs.

NOTICE: See bag for limitation of warranties, liability and remedies.

Monsanto Company, 800 N. Lindbergh Blvd., - St. Louis, MO 63167

NET WEIGHT PRINTED ON BAG

4008114-42/53

PONCHO® / VOTiVO®

Net Contents:

2.5 Gallons

A systemic insecticide and biological seed treatment for use on corn, cotton, sorghum, soybean, and sugarbeets for the control of listed insect pests and protection from listed soil plant pathogenic nematodes

ACTIVE INGREDIENTS:

Clothianidin	**40.3%**
Bacillus firmus I-1582	**8.1%**
OTHER INGREDIENTS:	**51.6%**
TOTAL:	**100.0%**

Contains 4.17 pounds clothianidin per U.S. gallon
Contains 0.84 pounds Bacillus firmus per U.S. gallon
(contains a minimum of 2 X10^9 cfu/ml)

EPA Reg. No. 264-1109

KEEP OUT OF REACH OF CHILDREN

CAUTION

Si usted no entiende la etiqueta, busque a alguien para que se la explique a usted en detalle.
(If you do not understand the label, find someone to explain it to you in detail.)

FOR ADDITIONAL PRECAUTIONARY STATEMENTS: See Inside Booklet.

For **PRODUCT USE** Information Call 1-866-99BAYER (1-866-992-2937)

For **MEDICAL** And **TRANSPORTATION** Emergencies **ONLY** Call 24 Hours A Day 1-800-334-7577

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Produced for:
Bayer CropScience LP
P.O. Box 12014, 2 T.W. Alexander Drive
Research Triangle Park, North Carolina 27709

Bayer CropScience

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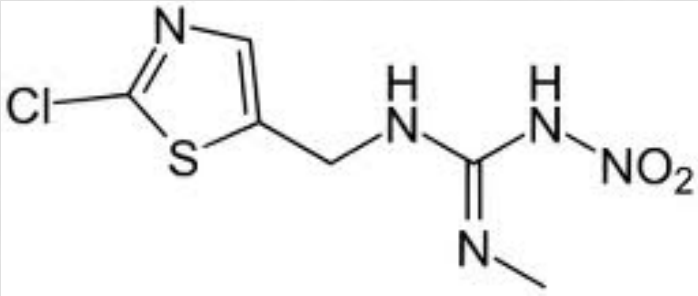
CLOTHIANIDIN

- A neonicotinoid
- Nature, Oct. 2012 Study of Combined pesticide exposure severely affects individual- and colony-level traits in bees: neonicotinoid and pyrethroid
- European Food Safety Authority: EFSA Journal 2013;11(1):3066, Conclusion on the peer review of the pesticide risk assessment for bees for the active substance clothianidin



Case 12-0737

2.5 ppb clothianidin found on dead bees

Case 12-0700 picture 1

3 ppb clothianidin found on dead bees

Case 12-0700 Picture 2

Case 12-0698 Picture 1

Case 12-0698 picture 2

Case 12-0756 Picture 1

Case 12-0756 Picture 2

Case 0658 Picture 1

Case 0658 Picture 2

Case 0693

Case 0733 Picture 1

Case 0733 Picture 2

FIELD SAMPLING

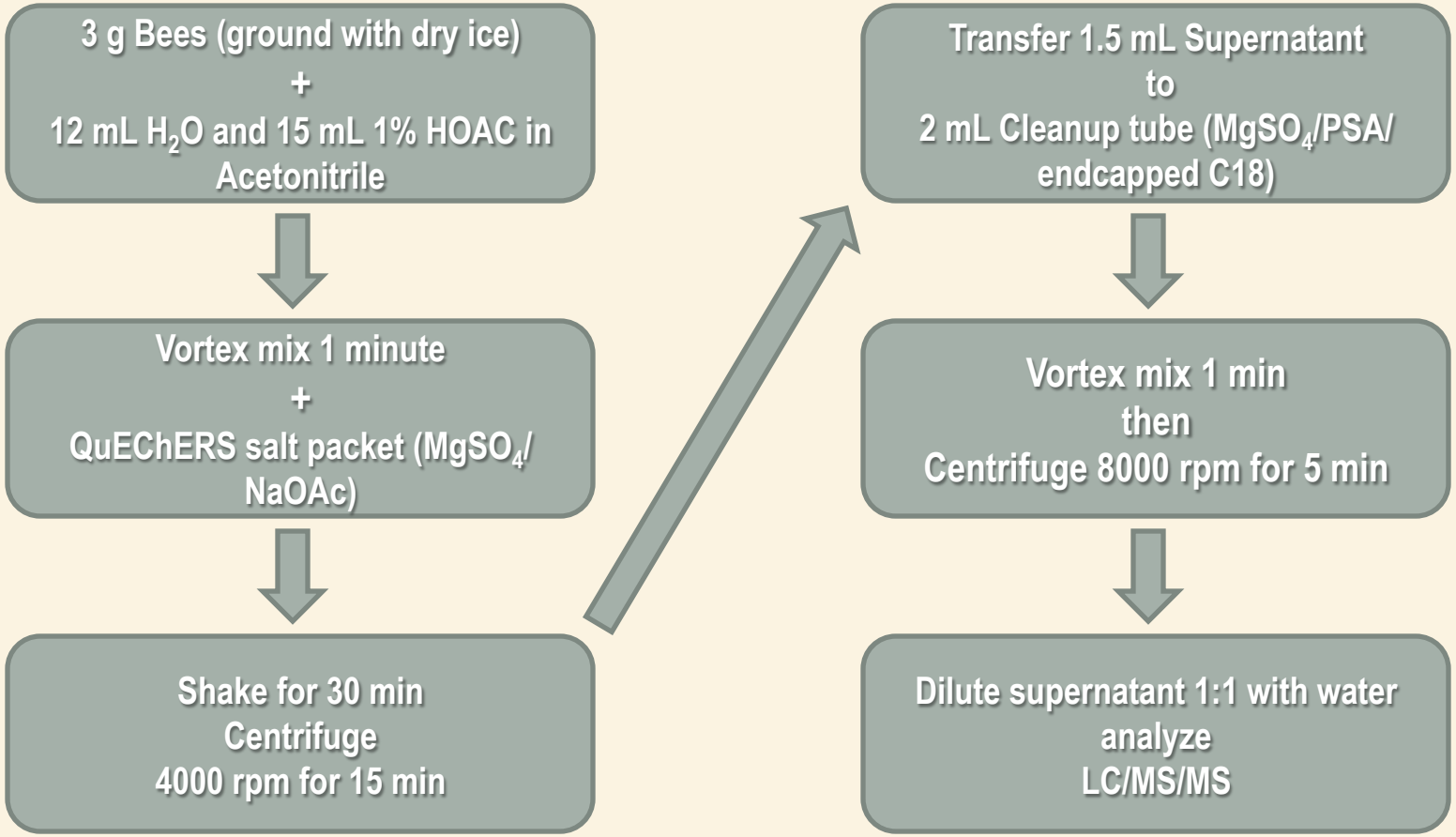
- How many bees? collect (or have bee keeper collect) sufficient amount (1 pint per agency/dept.)
- Mylar bags are good for storing dead bees. Glass jars are preferred for pollen and wax materials.
- Collect foliage/vegetation or soil samples?
- Transfer samples on ice and freeze as soon as samples arrive in lab.
- Information about pesticide applications (i.e. corn planting)
  - What crops are planted
  - What kinds of seed treatment?
  - planting date?
  - planter box cleanout performed?

ANALYTICAL METHOD

- "Refined methodology for the determination of neonicotinoid pesticides and their metabolites in honey bees and bee products by liquid chromatography-tandem mass spectrometry (LC-MS/MS)" J. Ag. Food Chem. 2010, 58(10):5926-31

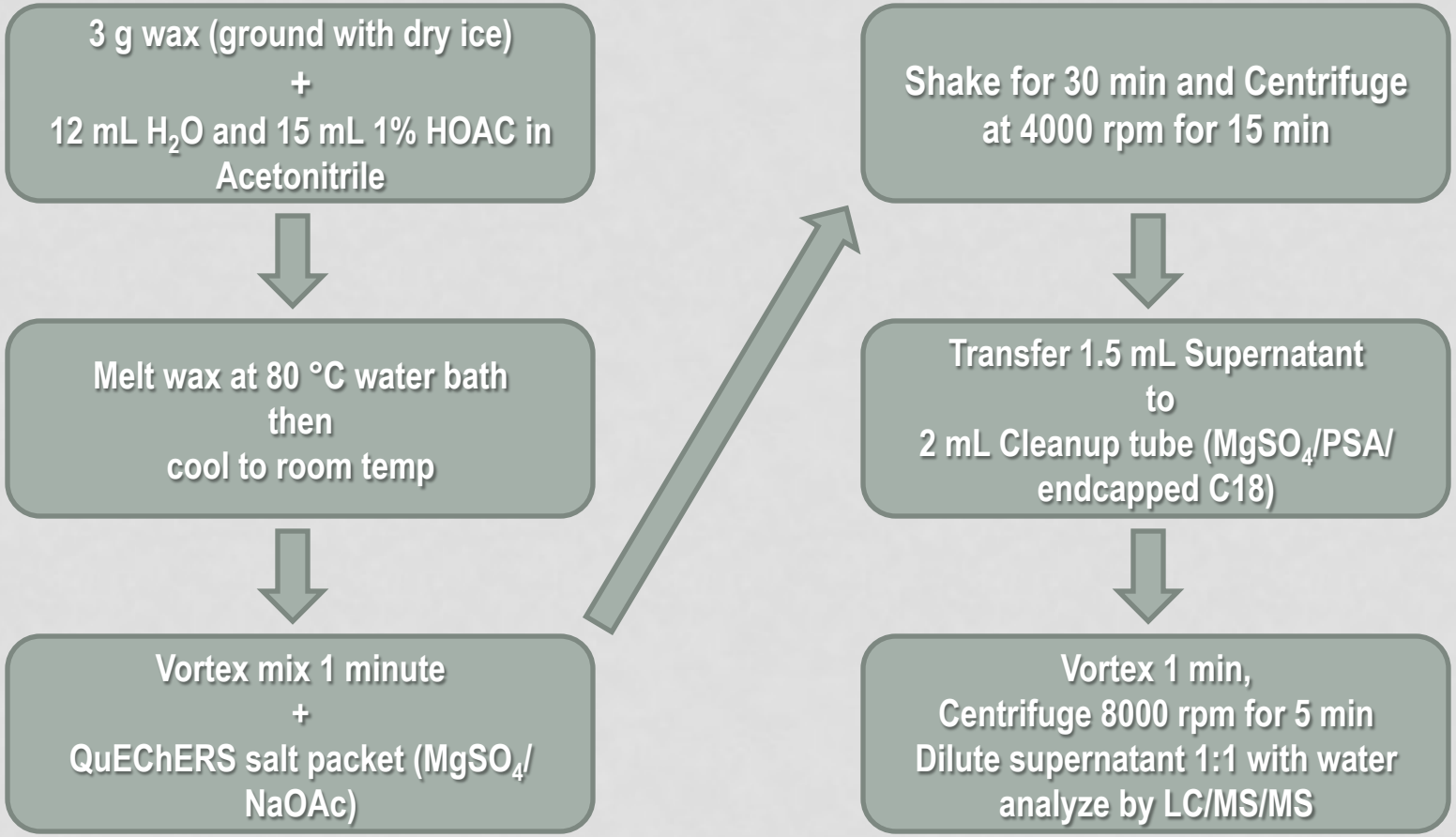
- UCT application note: QuEChERS Analysis of Miticides and Other Agrochemicals in Honey Bees, Wax or Pollen

Bee Extraction by QuEChERS



"Refined methodology for the determination of neonicotinoid pesticides and their metabolites in honey bees and bee products by liquid chromatography-tandem mass spectrometry (LC-MS/MS)" J. Ag. Food Chem. 2010, 58(10):5926-31

Wax Extraction by modified QuEChERS method



"Refined methodology for the determination of neonicotinoid pesticides and their metabolites in honey bees and bee products by liquid chromatography-tandem mass spectrometry (LC-MS/MS)" J. Ag. Food Chem. 2010, 58(10):5926-31

METHOD PERFORMANCE

- Linearity $R^2 > 0.99$
- Limit of Detection = 0.5 ppb
- Limit of Quantitation = 1.5 ppb
- Clothianidin Recovery = 55-75%

CONCLUSIONS

- Clothianidin was detected in bees and pollens.
- No violation of the Indiana Use of Application Law could be documented.