

# Assessment of residues of fipronil in bees and bee products in Uruguay: a correlation with the hive depopulation

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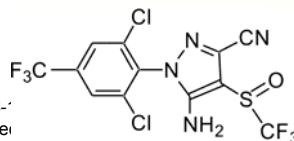
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## Introduction

Monitoring programs of pesticide residues in bee products focus mainly on the determination of residues of synthetic acaricides that are used to control *Varroa sp.* parasites in the hive, but minor attention is paid to environmental contamination due to pesticides used for crop protection that could be introduced into hives by contaminated bees. Recently fipronil was used as an endosulfan substitute to protect crops and prairies from locust attack in Uruguay. Fipronil is a systemic insecticide highly toxic to non-target insects with a very low LD50 to honeybees (6.2 ng/bee) [1]. It induces significant mortality of bees from 0.01 µg/kg, while other sublethal effects in locomotive activity were observed at the µg/kg level. These and other symptoms were observed when 2000 hives were lost in the Flores region of Uruguay, after a fipronil application to protect a soybean plantation from locust attack in 2008. In order to assess fipronil relationship to bee behavior, bee mortality and hive depopulation, nectar and bees from the affected zone were analyzed for pesticide residues.

## Experimental

The pesticides residues were analyzed by a Gas-Chromatograph Shimadzu GC-1 equipped with a flame photometric detector (FPD) and a electron capture detector (ECD). Column: SE54, tickness: 0.25 µm, large: 25 m, d.i: 0.32 mm. Flow 1.7mL/min using He as carrier gas. Injection volume: 1 µL. Split ratio: 1:30 for 30 seconds and later on splitless mode. PTV Injector, Temperature: 60°C (1min), 60-270°C (25 min hold) at 500°C/min. Oven temperature programme: 150°C (2 min), to 270°C (7 min) at 10°C/min.



Death bees in a hive in Flores, Uruguay.



**MS CONFIRMATION:** The pesticides residues were confirmed by a Gas-Chromatograph Shimadzu GC-17A equipped with Q-MS 5050 detector in SIM mode.

### Pesticide extraction for bees by MSPD

- > 1.5g Florisil were blended in a mortar with 0.5g of bees sample.
- > The mixture was placed in a 5mL glass syringe packed with 1.5 g Na<sub>2</sub>SO<sub>4</sub> and 2 g of silica.
- > 15mL of n-hexane were passed through and pesticides were eluted with n-hexane:AcOEt (7:3).
- > Solvent was evaporated until dryness with a mild N<sub>2</sub> stream
- > 1.00mL of bromophos-methyl (internal standard) in AcOEt is added and the solution is GC-ECD and/or GC-MSD directly analyzed

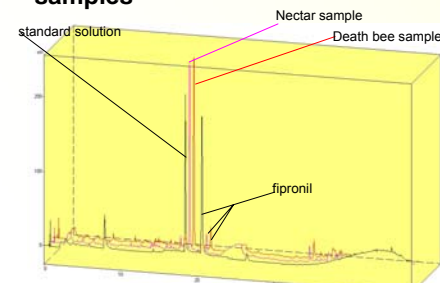
### Pesticide extraction from Nectar by Liquid/Liquid partition.

- > 10 g of raw honey were diluted in 20 mL of water
- > The mixture was partitioned with (3 x 10 mL) AcOEt. The solution is dried and redissolved in n-hexane:AcOEt (1:1) mixture
- > Clean-up was performed with a Florisil cartridge eluting with 2 portions of 10mL of an n-hexane:AcOEt (1:1) mixture.
- > Solvent was evaporated until dryness with a mild N<sub>2</sub> stream.
- > 1.00mL of bromophos-methyl (internal standard) in AcOEt and the solution is GC-ECD and/or GC-MSD directly analyzed

## Fipronil was found in either death or vanishing bees and nectar samples

	Fipronil residues (mg/kg)
Nectar Sample 1	0.04
Nectar sample 2	0.01
Death bees	0.17
Vanishing bees	0.15
<b>DL<sub>50</sub></b>	<b>0.06</b>

Detected fipronil concentrations in analyzed samples



GC-ECD fipronil determination in dead bees and nectar

## Results & Discussion

The clean up methods applied were "fit to purpose"

- > MSPD is a suitable method for the analysis of pesticide residues in insect samples
- > Simple L/L partition of raw honey allowed fipronil extraction with high reproducibility

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- The symptoms observed in vanishing bees correlates with the presence of fipronil
- Hive depopulation in the affected area is probably due to the toxic effects of fipronil.
- Fipronil applications must be avoided in regions where apiculture is performed.

## References

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- [3] Rissato S.R, Galhiane M.S, de Almeida M.V, Gerenutti M, & Apon B.M, *Food Chemistry*, **2007**, 101, 1719-1726