Determination of residues in honey, as a consequence of the use of a new antibiotic varroacide product in *Apis Mellifera* Beehives

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Introduction

Due to the severe sanitary and economic damage that varroa causes to beekeeping production and to the producers' growing need of having more efficient, economic and secure tools; trying to improve the global situation of Argentinean honeys in regard to medicine residues, we have developed a product able to fill these needs. With this test, we try to show the benefits our product can provide, in order to get the official permission of the corresponding authority.

Properties of the product

The product is a varroacide based on coumaphos and presented in small cakes of 70 g, this last fact makes it completely novel and gives the product outstanding characteristics. The treatment is carried out in a single application over the combs of the brood chamber in the beehives. Once the product is placed, the bees, in their desire to clean the beehive, start crumbling the cake, leaving exposed its different layers with drug; so that the drug is distributed among the population, eliminating the mature varroa mites that are on the bees. The crumbling process lasts around 35 days.

In this way, the treatment covers at least two varroa reproductive cycles and ensures the elimination of the varroa mites that at the moment of applying were inside the sealed cells (protected from the chemicals action) so, a higher level of efficiency is got.
Regarding this brief explanation, the following benefits can be noticed:

- As it is a small cake, it is very easy and safe to use and reduces the risk of exceeding or lacking the dosage.
- The treatment is carried out in a single application, reducing highly the cost on trips and manpower.
- The product disappears from the beehive 35 days after the application, avoiding the contact of the mites with the varroacide, reducing the risk of generating resistance to the active ingredient.

Composition

Coumaphos	400 mg
Excipients	70 g

Dosage
The dose used was a pill for each beehive (70 gm) for the beehives with 4 brood combs or more. For those beehives with les than 4 brood combs it is used ½ pill.

Objectives
- Determine the level of coumaphos residues in honey and the time of removal to assure levels of residues lower than 10 ppb of coumaphos.

Materials and Methods
4 equal standard beehives were used for the test (each one had 5 brood combs), there was activity inside the brood nests of all the beehives at the beginning of the test.
The beehives were indicated with the letters: F, G, H, I.
Honey samples to determine the existence of active ingredient couldn't be taken before the treatment. All the beehives were treated with slow release cakes. The first residue sampling was carried out fifteen days before the end of the treatment (sampling "-15"). Once the treatment finished, honey samples were taken according to the following chronogram:

Immediately after the treatment (0 days)
10 days after the treatment
30 days after the treatment
60 days after the treatment
90 days after the treatment

The honey samples were taken extracting all the honey from the super, individually, with centrifugal extractor. From this honey, it was taken a sample of around 1 kg per beehive from the different parts of the extractor (in two flasks of ½ kg). Honey extractions were made with washable tools so as to prevent cross-contamination among the different samplings, the washing was carried out with hot water and detergents, then the tools were dried with disposable paper towels before the next extraction. The samples were preserved in the freezer until sending them to the lab. The remaining honey was put inside a drum, properly identified and discarded for human consumption.

The coumaphos residues level was determined, in each sample from all the beehives, by gas chromatography and mass spectrometry.
The substance investigated was coumaphos, since according to ***The European Agency for the evaluation of Medicinal Products Veterinary Medicines and Inspections, EMEA,***
the levels of chlorferron, coroxon, and potasan metabolites in the honey samples tested were below the detection limits of the analytic method used.

Quantification limit: 5 ppb.

For further information consult the analysis protocol from the CIC attached to this report.

Additional information

Safeguard: Coumaphos is highly toxic via oral, and it has a moderated toxicity via dermal.

LD_{50}: Rats - 13-41 mg/kg (oral); 860 mg/kg (dermal)

MRL: The official Maximum Residues Levels for coumaphos are from 0.01 ppm in Switzerland to 0.05 ppm in the Neatherlands. The European Union doesn't have a MRL for coumaphos in honey. The MRL was established in 0.1 ppm for honey and 100 ppm for bee wax in USA.

1. Wallner, K (1999). Varroacides and their residues in bee products. Apidologie 30(2-3): 235- 248.
2. Anon (2000). EPA sets tolerance levels for coumaphos. American Bee Journal 140(10): 778.

Results

The results obtained in the lab for coumaphos are shown in ppb in the following table

	Sampling	-15	0	10	30	60	90
Treatment	Beehive						
AB Var C Cake	A	<LC	ND	ND	ND	ND	ND
	B	11	ND	ND	ND	ND	ND
	C	7	ND	<LC	ND	ND	ND
	D	ND	ND	ND	ND	ND	ND

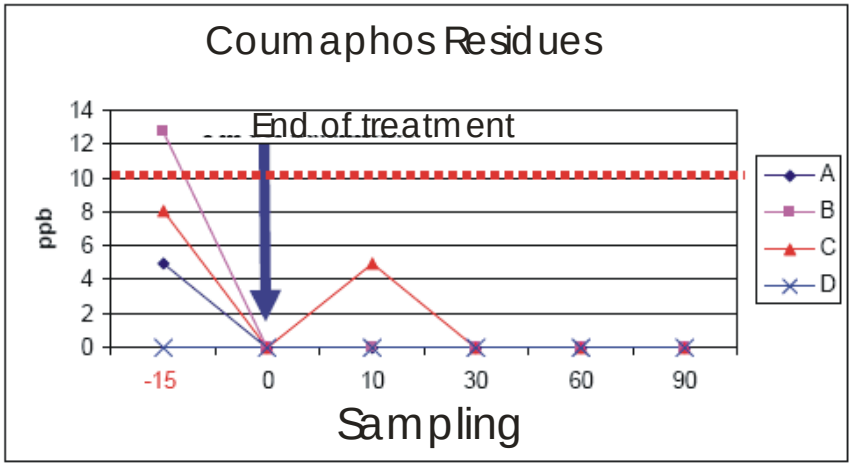
Notes:
<LC, existence of the product below the quantification limit (5 ppb)
ND, non detectable

The percentage of recovery was 84%, so the values were adjusted.
For further information consult the laboratory report attached.

The results corrected are shown in the following table:

	Sampling	-15	0	10	30	60	90
Treatment	Beehive						
AB Var C Cake	A	4,99	0	0	0	0	0
	B	12,76	0	0	0	0	0
	C	8,12	0	4,99	0	0	0
	D	0	0	0	0	0	0

The results of the table are shown in the following graph:



Conclusion
ALL the samplings carried out since the last day of treatment (samplings 0, 10, 30, 60, 90) were below the MRL (10 ppb).

The statistic analysis carried out by the statistic chair of the FCV from UB is in the attached report

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APVMA (Australian Pesticides & Veterinary Medicines Authority) RESIDUE GUIDELINE N° 28 – RESIDUES IN HONEY; June 2001.

A Review of Treatment Options for Control of Varroa Mite in New Zealand
MAF