

# STUDY ON FORMIC ACID RESIDUES IN HONEY AFTER TREATMENT OF HIVES WITH MITE AWAY QUICK STRIPS™

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Mite Away Quick Strips (MAQS™) is formic acid in a gel matrix, developed to control varroa mites in honey bee colonies. It is currently being registered as a pharmaceutical veterinary medicine in the UK. In order to measure the amount of formic acid present in honey from the comb immediately after treatment and honey after processing, the Experimental Animal Health Institutes of Latium and Tuscany (IZSLT) and Piedmont, Liguria and Valle d'Aosta (IZSPLV) brought together the beekeeping associations of ; Agripiemonte Honey and ARPAT (Regional Association Produttori Tuscan Bee). Field trials were conducted on 4 different apiaries in Italy (see Figure 1). These clinical trials have been duly authorized by the Ministry of Health as required by DM 11/12/201.

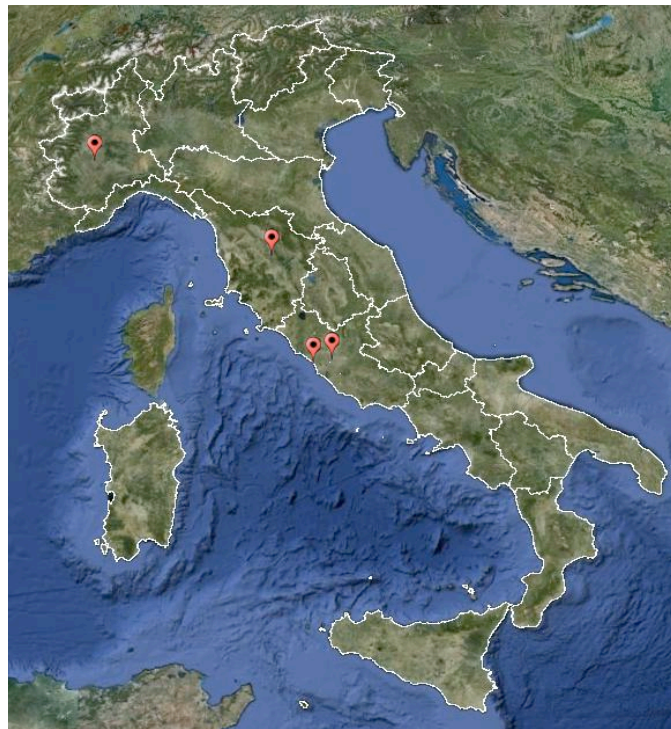


Figure 1: Location of apiaries in which field tests we carried out

## Materials and Methods

In July & August 2011 four tests were conducted: two in Lazio (Bracciano and Morlupo, both in the province of Rome), one in Toscana (Bagno a Ripoli,

province of Florence) and another in Piedmont (Turin). The objective of the tests was to assess the presence of residues of formic acid in honey after the administration of MAQS™ (Box 1 and Figure 2).

**Box 1 – Background Information on MAQS™**

**MAQS:** Formic acid in a gel, wrap in a biodegradable film called Ecoflex® (BASF). It is placed in honey bee colonies to gain control of varroa mites. It is registered and distributed across the USA and Canada. MAQS is currently in the registration process in the UK.

**Active Ingredient:** 136.4 grams of formic acid (2 Strips)

**Dose:** 1.5 strips/hive in hives without honey supers. 2 strips/hive in hives with honey supers.

**Duration of Treatment:** 7 days

**Recommended Temperature:** Between 10° C and 30° C

**Product description and all above information provided by:** NOD Apiary Products Ltd. (Canada)



**Figure 2.** Image 1 shows up close MAQS gel strip with eco-paper wrap, Image 2 illustrates the separating of the strips (2).

**Table 1:** Number of hives treated with MAQS™ for each Apiary involved, if honey supers are on, the start and end dates for treatment and the number of samples analyzed. In total, 78 samples were analysed: 76 samples of honey from the comb, treated as well as not treated, plus 2 samples of the extracted honey bottled for the consumer.

Location of Apiary	Number of hives treated with MAQS™	Honey Supers Present	Day of Treatment	Day Treatment Ended	Number of samples analysed
Lazio (Bracciano)	20	YES	15/07/2011	22/07/2011	Samples analysed: 24 (18T, 6 c)
Lazio (Roma)	8	NO	11/07/2011	18/07/2011	Samples analysed: 14 (6, 8)
Piemonte	9	YES	21/07/2011	28/07/2011	Samples analysed: 34 (15T, 19 c)

Toscana	8	NO	10/08/2011 1	17/08/2011	Samples analysed: 6 (6T)
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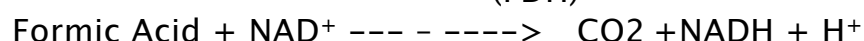
The field test conducted using MAQS was carried out over a 2 month period in 2011 in 4 different apiaries. These samples were taken in terms of a "worst case" scenario; samples of honey from the comb were taken immediately after treatment and with the greatest proximity to the strips. Honey samples were taken before and also immediately after extraction. Samples were taken from a portion of the honey from the comb, 10 cm x 10 cm. The two samples tested after extraction, 5 days after the collection of the honey supers, were taken from the apiary located in the Piemonte region.

The environmental temperatures and relative humidity conditions were recorded in different apiaries since the evaporation of formic acid maybe influenced by climate.

The chemical analyses were carried out by the Regional Centre of IZS PLVA Beekeeping, section of Asti.

The presence and the quantification of formic acid in the samples tested was done by using the enzyme method based on measurement of the variation of the ultraviolet absorption (340 nm) of the Coenzyme NADH (nicotinamide adenine dinucleotide reduced--). In the presence of nicotinamide adenine dinucleotide (NAD) formic acid is oxidised to carbon dioxide by the enzyme formate dehydrogenase (FDH) with the concurrent formation of NADH. The amount of NADH formed is stoichiometric to the amount of formic acid.

(FDH)



The samples submitted have been carefully weighed (10 grams) diluted to final volume of 100 mL with distilled water, filtered and subjected to the tests.

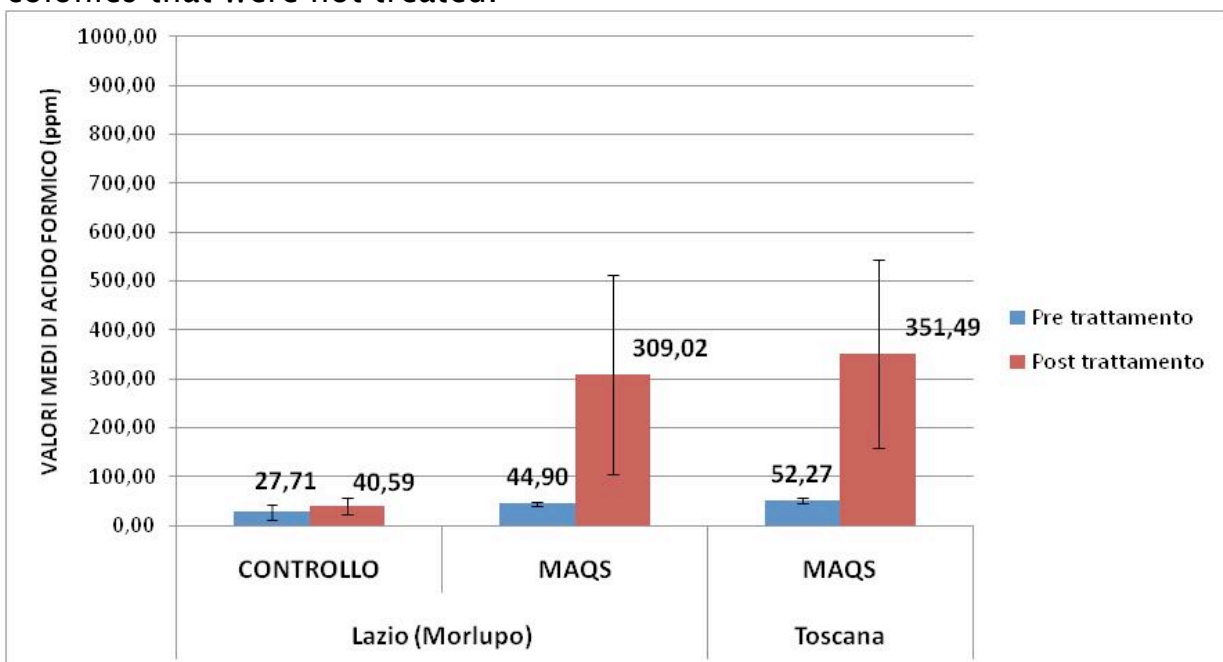
## Results

**Table 2:** Temperature (minimum, maximum and average) and relative humidity registered in each of the different apiaries during the seven days of treatment with MAQS™.

Apiary Location	MAQS Administration Day	Ambient Temperature (C°)	Maximum Temperature (C°)	Minimum Temperature (C°)	Average Relative Humidity (%)
Piemonte	Day 1	30	23	16	52
	Day 2	25	21	18	71
	Day 3	28	21	15	55
	Day 4	26	21	15	46
	Day 5	23	19	15	64
	Day 6	20	18	17	80
	Day 7	29	22	15	67
Toscana	Day 1	28	24	21	54
	Day 2	28	22	16	53
	Day 3	29	24	18	53

Lazio (Bracciano)	Day 4	29	24	19	63
	Day 5	28	24	22	58
	Day 6	26	23	21	53
	Day 7	27	21	16	61
Toscana	Day 1	31	23	14	44
	Day 2	29	22	16	54
	Day 3	30	23	15	61
	Day 4	33	25	17	55
	Day 5	30	25	19	59
	Day 6	35	27	19	59
	Day 7	36	28	20	49
Lazio (Morlupo)	Day 1	27	22	19	71
	Day 2	26	22	18	63
	Day 3	26	20	14	68
	Day 4	26	21	16	71
	Day 5	25	21	18	75
	Day 6	25	22	17	62
	Day 7	25	21	19	59

**Figure 3:** In Lazio and Tuscany the average amount of formic acid found in the samples of honey from the hives without honey supers on that were treated with MAQS™. This data is compared with the amount of formic acid found in colonies that were not treated.



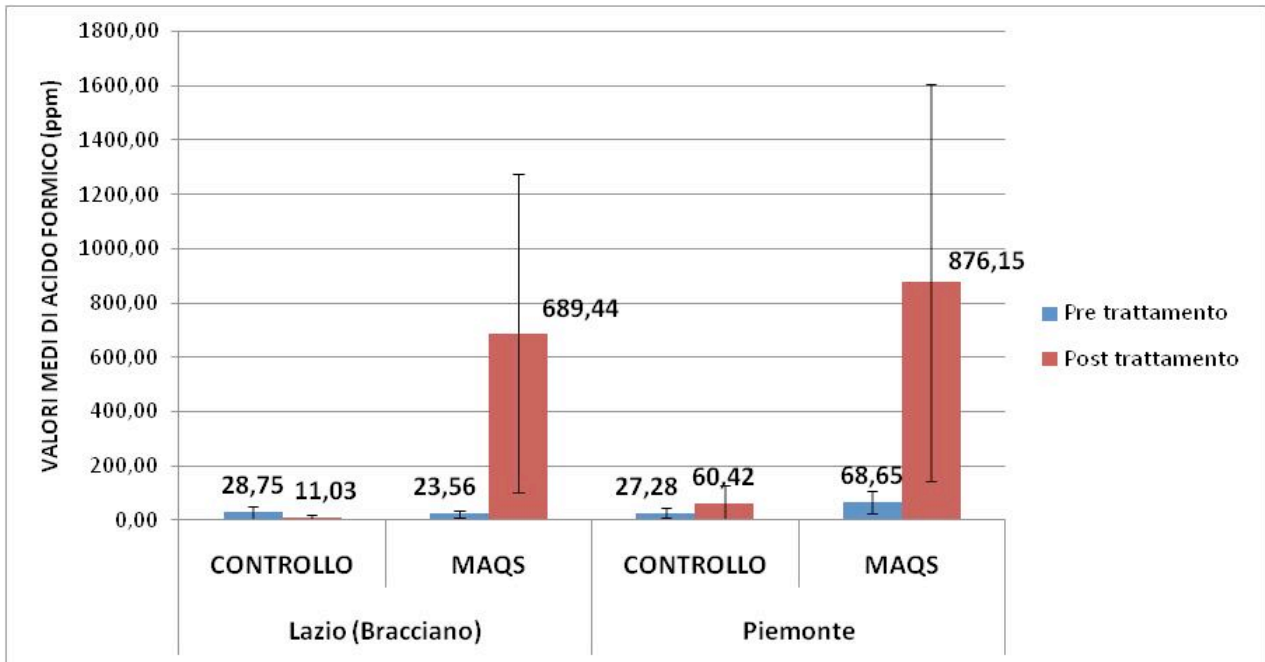
**Figure 3:** Mean values (ppm) of formic acid found in the honeybee colonies that were treated (MAQS) and the untreated colonies (control/controllo)

**Table 3:** Values were obtained by combining the data from the two apiaries.

Table 3: Quantities of the formic acid that were found in the brood area of the hives not treated.				
Formic acid residues in the frames of the brood area	Pre-treatment Media (ppm)	Post-treatment Media (ppm)	Standard deviation pre-treatment	Standard deviation post-treatment

Hives treated in the absence of honey super	48,58	330,26	5,85	179,00
Hives not treated	27,71	40,59	16,53	16,50

**Figure 4:** The average amount of formic acid found in samples taken from the honey comb of the hives treated for varroa mites with MAQS™. The figure was compared with the amount of formic acid found in the honey bee colonies that were not treated.



**Figure 4:** Mean values (ppm) of formic acid found in the honey supers that were treated with formic acid (MAQS) and beehives that were untreated (control).

**Table 4:** The values were obtained by combining the data from the tested hives with honey supers from the two different apiaries.

Formic acid residues from the honeycombs of the honey supers	Pre-treatment Media (ppm)	Post-treatment Media (ppm)	Standard deviation pre-treatment	Standard deviation post-treatment
Hives treated with MAQS	46,10	776,57	37,28	639,19
Hives not treated	27,68	49,02	19,77	63,55

**Table 5:** For the purposes of food contained on average honey samples were taken during the extraction process. Hives treated with MAQS with a honey super had a formic acid level equal to 54.15 ppm. For untreated hives the formic acid level was 65.07 ppm.

Formic acid residues in honey honey-time	Amount of formic acid (ppm)
Hives treated with MAQS™	54,15
Hives not treated	65,07

## Considerations and Conclusions

Formic acid is an active substance listed in Annex I of Regulation (EC) No 37/2010, which includes substances that are not considered dangerous for consumers; therefore no maximum residue limit (MRL) is established. Directive 2001/110/EC, relating to honey, indicates that among the features planned for the composition of honey, a maximum free acid equal to a value of not more than 50 Milli-equivalents acid per 1000 grams of honey EUR 2300 ppm (mg/Kg) is proposed.

The formic acid values obtained during this field test were consistently lower than the maximum values allowed by the Directive 2001/110/EC, in regard to the samples taken directly from the brood area, the honey super and, above all, from the honey prepared for human consumption.

More analytical testing is required to quantify the total acidity of honey samples taken.

## Thanks

Many thanks to Honey Agripiemonte associations, the Associazione Regionale Produttori Tuscany (ARPAT) Bee, Agnese beekeepers, Stefano Masciarelli Grazioli and Masoero Alfredo for the professionalism and cooperation in realization of field trials.

## Bibliography

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