Final Study Report

The Tolerance of Honey Bee (Hymenoptera: Apidae) Queens and Colonies to Elevated Levels of Formic Acid in the Brood Chamber Air due to Field Application of Mite Away Quick Strips™ (MAQS) in a Standard Dose or a Double-Dose and the Resulting Levels of Formic Acid in Honey.

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A Study Submitted to the Veterinary Medicines Directorate of the United Kingdom in Support of Application for Marketing Authorizations for Mite Away Quick Strips™ (MAQS)

Study Conducted by
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Conducted under the authority and supervision of the undersigned. We declare that the data generated are valid. The report fully and accurately reflects the procedures used and the raw data generated.

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The Tolerance of Honey Bee (*Hymenoptera: Apidae*) Queens and Colonies to Elevated Levels of Formic Acid in the Brood Chamber Air due to Field Application of Mite Away Quick Strips™ (MAQS) in a Standard Dose or a Double-Dose and the Resulting Levels of Formic Acid in Honey.

**VanderDussen, David\(^1\), Paul Greene\(^2\), Jessika Harding\(^3\), Kathleen Ireland\(^4\).**

**Abstract**

A study was conducted in a bee yard near Stirling, Ontario Canada to determine risk to host species (the honey bee colony) and the levels of formic acid in honey if a Standard Dose (\(n=9\)) or a Double Dose (\(n=10\)) of Mite Away Quick Strips™ (MAQS) is applied mid-August, the time when bees are being raised by the colony for the winter cluster. Formic acid levels within the brood chamber air were monitored. When MAQS was applied the levels of formic acid in the hive air increased to elevated levels within the first hour. With a standard dose, during the period until Day+4 levels peaked then dropped off and held fairly steady at an average of \(\approx 20\) ppm (parts per million). Much higher levels were seen with the double dose. The properly applied standard dose treatment had no apparent negative impact on colony health.

Of the colonies that were going through queen events (supercedure) prior to the application of MAQS, (\(n=5\)) three successfully replaced their queens; two were deemed queenless at Day+44. Even then there was no sign of laying workers or drone-layer queens. The surviving adult bees, along with combs heavy with pollen, were combined with a queen right colony to give the bees a home and in preparation for springtime splits. The results show that queens in the double dose treatment group had an increased (20%) incidence of going queenless due to treatment but superfcedures were successful, giving the colonies healthy young queens going into winter. Supercedure activity post application illustrated that not all eggs and young larva are lost due to treatment, even with a double dose.

Open source feeding was used post treatment as an additional assessment of the colonies’ ability to function. The treated colonies in both groups had excellent bee populations at the time of feeding; they rapidly took up the feed and are deemed to be in very good shape going into winter. Based on this 100% overwintering success is expected from the colonies going into winter that received treatment with MAQS in August.

Samples of honey were taken at the end of treatment and on Day+24. These were analyzed for formic acid levels. With standard dose applications there is a possible slight increase in formic acid

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levels in honey compared to controls at the end of the treatment, however this soon drops off to being within naturally occurring levels.

**Introduction**

Volatilized formic acid has long been used as a method of varroa mite control in honey bee colonies. (Kunzler, K. *et al.* 1979)\(^1\). Charriere, JD, *et al.*, (1992)\(^2\) found that the colonies could withstand the initial concentrations of up to 565 ppm achieved during the first hour of treatment; concentrations then gradually dropped off over the next 5 hours. A variety of methods of applying formic acid were tested. It was concluded that slower, longer release methods needed to be developed. The University of Manitoba, Canada, has used its indoor wintering facility as a controlled environment to test formic acid fumigation. Currie, R.W. (2003)\(^3\) showed significant reductions in the levels of tracheal and varroa mites could be achieved at 40 ppm for 48 hours. Queen loss did not differ from untreated colonies as long as the room was ventilated to keep the temperatures from increasing above 8ºC. Underwood, R.M. and Currie, R.W. (2004)\(^4\) tested 3 concentrations of formic acid (low 11.9 ppm, medium 25.8 ppm, and high 41.2 ppm) for 48 hours. Results showed a reduction in varroa and nosemia spores, but a significant increase in queen loss. Underwood, R.M. and R.W. Currie (2004)\(^5\) followed up on queen performance and determined that formic acid treatment did not have a long-term impact on queen vigorousness or productiveness. Underwood, R.M. and R.W. Currie (2009)\(^6\) continued to follow-up on their work, looking at the impact on tracheal mite and nosemia disease. Tracheal mites were controlled, and there were indications of suppression of *Nosema apis* and *Nosema ceranae* spores.

Ostermann, D.J. and R.W. Currie (2004)\(^7\) documented formic acid levels of up to 222 ppm in hive air in colonies treated with 65% formic acid in water, poured onto paper towels, 3 applications. This pour-on method did not have a negative impact on colony growth or development. However, the results were poor, only showing a reduction in mean varroa abundance of 29.3%. Efficacy in the slow-release methods tested also poor, mite levels were just 33% lower than in untreated colonies. Currie, R.W. and P. Gatien (2006)\(^8\) examined the timing and economic impact of acaricide treatments. The results showed that treatment efficacy of different products can vary with the season. There was no reduction in honey production when formic acid treatments were applied in the spring, however they concluded that formic acid treatments should be applied early in the spring so there is adequate time for the colonies to recover from any temporary suppression of population growth. The importance of monitoring varroa levels to keep them below the economic threshold was emphasized, as well as the importance of not treating until the treatment threshold was reached.

vanEngelsdorp, D. *et al.* (2008)\(^9\) explored a 17-hour application of 50% formic acid. Results showed that >60% of the varroa mites in capped worker brood and on bees could be killed without harming
brood or queens. However, virus levels remained unaffected. In conclusion the researchers stated that short-term treatment with formic acid held promise as a tool for beekeepers, especially when such treatments are necessary during the nectar flow.

**Reproductive Health**

Reproductive health in the wake of using various compounds to control varroa mites has been an evident concern for some time. Wide spread expectations of and experiences with poor queens, high supercedure rates and the inability of colonies to replace queens with the use of the synthetic pyrethroid fluvalinate (Apistan®) or the organophosphate coumaphos (Perizen®, CheckMite+™) led to a number of studies being conducted on the impact of these chemicals on reproductive health.⁹ ¹⁰ There is also evidence that treatment with thymol may contribute to reproductive issues²⁰.

**Materials and Methods**


**MAQS Manufacture Date:** September 13, 2010, Batch #: 10-256-1

**Description of Test Item:** A.I. Formic acid (46.7% w/w) in a saccharide gel matrix wrapped in Ecopaper, forming a strip approximately 10 cm X 20 cm X 0.9 cm. A standard dose is 2 strips, 292 ±3% grams. A double dose is 4 strips, 584 ±3% grams. The doses were weighed at time of application. The strips inside the sachet had become sticky over time but were easy to separate and apply.

**Placebo Treatment:** Standard office printer paper was folded triple thick to match the size of a MAQS strip; two of these placebo strips were used on the control colonies that were having in-hive air monitored (n=2).

**Date of Application (Day+0):** August 22, 2011. Age of test item: 11 months, 9 days.

**Storage Conditions between Manufacture and Application:** Product was stored indoors at the NOD Apiary Products Ltd. warehouse in Frankford, Ontario, Canada. This is not a climate-controlled warehouse. The pail containing the sachets was opened on the day of application, by the lab technician, and a sachet containing one dose was removed from the middle of the pail for analysis. Analysis of the test item showed 47.16% w/w total formic acid, confirming no loss of A.I. over the storage period.
**Test Species:** Honey Bees

**Treatment Groups:** Control $n=3$, Standard Dose $n=9$, Double Dose $n=10$.

**Treatment Period:** 7 days. MAQS residue was removed from the hives when examined on Day+8 or +9 and weighed.

**Trial End Points:** Testing the in-hive air for formic acid levels ended on Day+7 in the control colonies and Day+8 in the treated colonies. Queen/brood status exams were conducted on all colonies at the end of treatment (EoT) period on Days+8 or +9, and on Day+24. Colonies in which no eggs were seen at the EoT period exam were again examined on Days+37 and +44. Honey samples were taken during the EoT exams on Days+8 or +9 and at Day+24. Winter feed uptake was monitored from the time the feed barrels were opened on October 11 (Day+50) until they were almost empty on October 23$^{rd}$. October 23, 2011 (Day+62) marks the end of the study.

**Hive Description:** Standard 10-frame Langstroth hives were used, with full size supers used as the brood chambers and a half-depth super used as a food reserve super on each brood chamber. The food supers are always left in place, even if there is a queen excluder under it. When the queen excluder was above the food super it is noted as a 1.5 brood chamber hive in the raw data. Each hive had a standard full size, just extracted, honey super put on it on Day-4.

Bottom Boards: 2 hives were outfitted with Apinovar style screen bottom boards (SBB). (Figure 1) The collection tray was removed at time of treatment to allow full air circulation. All other hives had solid bottom boards with rims of various heights, from 3/8 inch (0.95 cm) to ¾ inch (1.9 cm) on three sides. The 4th side is left without a rim to provide an entrance for the bees.

**Temperatures and Environmental Conditions:** The hives were situated in a long established bee yard that has South-East exposure, is in the sun from dawn until late afternoon, and is well protected from winds by a mature deciduous tree bush to the West and North and shrubbery to the East and South. A HOBO brand temperature data logger sensor was placed 120 cm above the ground, in the shade, at the North side of bee yard. During the first 3 days of the treatment the highs were 25°C (77°F) on Days 0, +1, and +3, the highest temperature recorded during the treatment period was 26.8°C (80.2°F), on Day+5. See Figure 2. A light honey flow was underway at the start of the study; it ended in early September. By the Day+24 exam (September 15$^{th}$) the bees would immediately engage in robbing if any honey was exposed.
Disease and Treatment History: The bee yard has a brief history of American Foul Brood (AFB), with 3 colonies being diagnosed in the spring of 2010. The bees were destroyed and all hive components burned shortly after diagnosis. No treatments have been used against AFB for over 20 years, burning when found has been the control method. There is no history of European Foul Brood. No treatments have been used for nosema. For varroa control the colonies were treated twice with MAQS in the previous year (2010), in early July and mid-September. No treatments were applied in 2011 prior to the trial.

Colony Health Determination Pre-Application: On Day-4 each colony in the bee yard was visually examined for evidence of deformed wing virus (DWV), American and European foulbrood, chalk brood, and sac brood. The colonies appeared to be healthy and free of these diseases. The colony strength categorization was determined by the crop it had gathered as of August 18th. If the colony had gathered less than 40 pounds (18 kg) in the honey supers it was marked “weak”, 40 to 100 pounds (18 to 45 kg) it was marked “average”, over 100 pounds (>45 kg) it was marked “strong”. Visual exam of the colony during the hive breakdown on Day-4 was used to confirm the colony strength categorization as well as to assess the status of the queen and brood. Weak colonies were seen as very few bees in the honey supers, <4 frames of brood and covering cluster, total number of bees estimated to be less than 9,000. Strong colonies were estimated be at the seasonal peak of 65,000.

Queen Age: Records for the queens of each colony are kept on each hive. The queens were either of the spring of the treatment year or of the prior year. When the status was not clear “unknown” is recorded in the raw data.

Treatment groups: Hives were allocated into treatment groups by dose, queen age, colony strength, bottom board style, placement in the bee yard (shot-gun placement, see “bee yard map”, in photo journal, Appendix 1), and if there was a queen event underway.
**Hive Layout in Bee Yard:** The hives are paired on hive stands, facing south. Treatments were sorted into a shotgun pattern. See “bee yard map” in Photo Journal, Appendix 1.

**Sampling In-Hive Air for Determining the Formic Acid Concentration in the Air in the Brood Rearing Area.**

Air samples were drawn twice a day: early morning while the air was still cool and there was minimal flight activity, and mid-afternoon, to capture the concentration at or near the peak warmth of the day. (Figure 8)

Take an air sample from within the brood rearing area of the colony: on Day-4 a 3/8 inch (0.95 cm) diameter hole was drilled into the back of the lower brood chamber (BC), 14 cm down from the top edge, aligned to the space between the 4th and 5th frame inside the BC. A flap of duct tape was placed over the hole to prevent it from being used as an entrance by the bees. To take an air sample a Dräger Air Sampling tube for either formic acid (calibrated for 1 to 15 ppm) or an acetic acid tube (calibrated to 5 to 80 ppm) was inserted into the hole as far as possible, placing the air sampling point 8 cm inside the hive cavity, approximately centered side-to-side and top to bottom, well away from fresh air coming in from the hive entrance. (Figure 3) The directions for drawing the air sample through the tube were followed. The technicians read the tubes and the results were recorded in the field. (Figure 4)

**Constraints on Air Sampling Technology.** The upper measurement level on the Dräger tubes was 80 ppm. At times the readings were greater than 80 ppm. Samples drawn from treated colonies 1 hour after application in the double dose hives were off the scale, the afternoon of Day+1 again gave readings off the scale in 8 of 10 of the Double Dose colonies. For calculating the results of Days+1 through +8 it was decided that if not all of the remaining reagent above the 80 ppm
marker line had changed colour the results would be designated as 90 ppm. If the entire reagent changed colour the results would be designated as 100 ppm.

**Day+0 Activities:** Starting early afternoon in-hive air samples were taken from each colony using the Dräger tubes for formic acid. Then a wood-chip fueled smoker was lit. A wad of green grass was placed inside the lid to cool the smoke and prevent sparks from leaving the smoker. Each hive was gently smoked; the honey super, half super, and queen excluder were removed, and the MAQS strips or a placebo of folded paper were applied by laying two strips (a single dose) (Figure 5) or 4 strips (a double dose) (Figure 6) across the top bars of the frames of the full depth brood chamber, staggering them so they lay flat and across the full width of the hive body. Then the hive was reassembled and the time noted. One hour later another air sample was taken, using the Dräger acetic tubes for the MAQS treated colonies and the Dräger formic acid tubes for the placebo treated colonies.

**Colony Exams at End of Treatment (EoT):** The treatment period is 7 days. The hives were not opened from the time of application until the end of treatment examination on the afternoons of Days +8 or Day+9. Then the colonies were examined to check for the presence of queens, queen cells, eggs in worker brood, open worker brood and capped worker larva. From one colony in each treatment group drone brood was opened using a cappings fork and the contents were examined in the field for the presence of live and dead varroa.

**Colony Exams for Determination of Queen Status Post-treatment:** The centre three to five frames of the brood chambers were examined on Day+24 (September 15th) to determine if the queens were present or were in the process of becoming mated, whether of not they had become drone-layers, and if they would be suitable for heading-up a colony for going into winter. If there was capped worker brood, eggs and larva present (brood all stages) it was confirmed that the queen had survived the treatment and the colony was deemed to be in good shape for going into winter. No additional exam was required. If the colony did not have “brood all stages”, two follow-up exams were conducted, one on Day+37 and one on Day+44. If a colony did not have “brood all stages” by Day+44 it was deemed hopelessly queenless. See attached Photo Journal of trial.
**Honey Sampling:** To have samples of honey that was gathered during the treatment period, on Day-4 (August 18\(^{th}\)) all honey supers were removed and a newly extracted honey super was put on each hive. A bee-escape was put on the super, and all the honey supers that had been on the hive were put on top. On Day-2 the honey supers above the bee-escapes and the bee-escapes were removed.

A light honey flow was underway at the start of the study and continued through the treatment period. During the end of treatment (EoT) exams on Day+8 or Day+9 a sample of honey was taken from the honey super of each colony that had gathered enough honey to be able to take samples from 3 areas of the honey super. Numbering the 9 frames across the honey supers as 1 to 9, the samples were a mixture of the honey from frames 3, 5, and 7. Areas of open cells with partially to fully cured honey was crushed and scraped with a clean spoon to obtain the samples. The honey samples were sealed into laboratory sample vials and placed in a freezer (-15\(^{\circ}\)C).

The honey flow ended early September. By the Day+24 exam (September 15\(^{th}\)) the bees would immediately engage in robbing if any honey was exposed. It became difficult to take a sample without crushing the bees that would quickly gather on the comb. Under these conditions it was decided to take triplicate samples from each dose group, and only from the centre frame (frame #5), taking care that no bees were included in the samples. The honey samples were sealed into laboratory sample vials and placed in a freezer (-15\(^{\circ}\)C).

**Honey Sample Storage:** The sample vials were kept closed and stored in a freezer at -15\(^{\circ}\)C until shipped to GCL & Chemisar Laboratories Inc. for analysis. Samples were shipped by courier on October 14\(^{th}\), 2011; results were received October 26\(^{th}\).

**Honey Harvest:** The honey supers were set over bee escapes after the Day+44 (October 5\(^{th}\)) examination. They were removed from the hives 2 days later, taken to the honey house of River Valley Apiaries, Stirling, Ontario. They were quarantined until the lab analysis determining formic acid levels was completed.

**Control colonies Post Day+44.** As the honey supers and bee-escapes were being removed it was evident that the control colonies had significantly smaller bee populations. Examination on Day+46 (October 7\(^{th}\)) found visual Parasitic Mite Syndrome (PMS) symptoms, including Deformed Wing Virus (DWV) bees, stressed behavior and shotgun brood patterns. MAQS was applied at the end of the colony exam. On October 11\(^{th}\) the bees appeared to be no longer stressed, the queens were laying in even patterns and foragers were gathering some late pollen.
Feeding for Wintering: Two barrels of 208 litres each of 67.6% liquid sucrose were set out on the North side of the bee yard on October 11th. Mats of straw 3 to 4 inches (15 to 20 cm) thick were put on the syrup to provide a floating platform for the bees. The lid was then replaced but wedged up about 2 inches (5 cm) on one side to provide access for the bees. (Figure 7)

Results and Discussion

In-Hive Air

In our study formic acid was found to be naturally present in brood chamber air at 1 to 3 ppm at all times in the control hives and pre-treatment in the treatment groups. Treatment with MAQS elevated the concentration of formic acid in the air. Many air samples taken from the hives with the double dose went above 80 ppm. See Figure 8 for the air concentrations at 1-hour post application through Day+4, and Figure 9 showing concentrations for the treatment period.

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<th>Pre-trtmt ppm</th>
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Impact on Queens

Queen Event: A queen event is occurring when circumstances are such that the colony takes steps to raise a queen. This is identifiable by the presence of queen cells in the colony that have larva being nursed in them, capped queen cells, hatched queen cell(s), or the presence of virgin queen(s). Queen events are triggered by the swarming impulse, queen killed by beekeeper activities (movement of hives, manipulations, application of miticides, the beekeeper removing the queen) or the bees themselves deciding that it is time to replace the current queen in the normal course of managing their affairs.

A queen event is a serious concern as the honey bee colony relies on its queen as the sole source of the next generation. Most swarming takes place in the spring, so when queen cells are observed mid-August it is assumed that supercedure is occurring: the bees are taking steps to replace the queen before winter.

At the start of this trial (Day-4) five colonies were identified as having queen events underway. Such colonies are often not included in comparable studies, thus not providing a true reflection of the reality the beekeeper must deal with. It was decided to leave them in this study, placing them in different groups. (Figure 15)

Queen Event Results (Figure 15)

By Day+24, 3 of the 5 colonies that had queen supercedure under way at the start of the trial had successfully completed supercedure. Of the 2 that did not 1 was a weak colony in the double dose group and 1 was in the standard dose group. This hive (#20) was on a bottom board that only had a 3/8 inch (0.95 cm) high entrance (bottom board rim). This bee-height entrance may have led to the colony having difficulties in controlling the formic acid vapour concentration in the brood chamber since each bee in the entrance opening would have a considerable impact on restricting air flow.
<table>
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<tr>
<th>MAQS Double Dose vs. Standard Dose vs. Control Trial</th>
<th>Post-treatment Exam, Afternoons of Day +8 and Day +9</th>
<th>Follow-up Exam Day +37** Only colonies with queen status in question checked</th>
<th>Follow-up Exam Day +44(Oct.5). Only colonies with queen status in question checked</th>
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<tbody>
<tr>
<td><strong>Product</strong> Applied</td>
<td><strong>Hive</strong> Status</td>
<td><strong>Bread Status</strong></td>
<td><strong>Colony Status</strong></td>
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<td><strong>Queen Health Status</strong></td>
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<td>Pre-treatment Exam Day -4</td>
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<td><strong>MAQS Beehives (non-GMO)</strong></td>
<td>10-796-1</td>
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<tr>
<td><strong>Controls</strong></td>
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<td>queenless, queen right present &amp; capped queen cells</td>
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The data shows that it is an outlier compared to the other bottom board styles. The use of bottom boards with less than a $\frac{1}{2}$ inch (1.27 cm) high opening the full width of the hive should not be recommended as the only source of fresh air into the brood rearing area. See “Bottom Board Design Effect”.

During treatment 3 additional colonies initiated queen cell building. This was identified at Day+8 when capped queen cells were seen. One still had a laying queen, in 2 hives no eggs or young brood were seen, the queens were missing in action (MIA). The timing indicates that the 2 queens MIA were susceptible to the elevated levels of formic acid vapours in the brood area caused by the application of treatment. Both hives were in the double dose group; they both successfully completed the supercedure, one by Day+24 and one by Day+34.

It is evident that not all the eggs or young larva from which queens can be raised are killed during treatment, even with the elevated levels of formic acid concentrations documented during this study. The age of the queen did not appear to be a factor in the triggering of supercedure. The response of the colonies after treatment with MAQS demonstrated that they are able to adapt to and function effectively within an environment that has significantly elevated levels of formic acid.

**Bottom Board Design Effect:**

Bottom board design appeared to be a significant factor. (Figure 10)

*Screen Bottom Boards:* The concentrations of in-hive formic acid dropped off very quickly when open screen bottoms were used, regardless of the dose received. (Figures 8 &10) It is unlikely that the levels of formic acid will be sufficient to achieve additional varroa mite death after the first 24 hours post application.

*Bottom Board Rim Height:* The entrance to the hive is via the rimless side of the bottom board, which is on the width side of the Langstroth hive. The height of the rim on the other three sides gives the height of the entrance. The standard full size entrance is $\frac{3}{4}$ to $\frac{7}{8}$ inch high, but some beekeepers use a reduced height of $\frac{3}{8}$ inch (0.95 cm) opening. The “bee space”, $1/4$ to $3/8$ inch rim (0.635 to 0.953 cm) is the height of the honey bee, and is utilized in hive designs to minimize the bees’ proclivity to building comb or sealing with propolis any space larger or smaller. One hive in the bee yard had a $3/8$ inch rim bottom board. Rather than switch it to a bottom board with a deeper rim to bring it into consistency with other solid floor bottom boards in the bee yard (0.5 to 0.75 inch rims) it was decided to treat it with a standard dose application. It proved to be an outlier, with the exception that the Day+24 formic acid concentration in honey was within naturally occurring levels. See Figure 10 ($3/8" = 0.95$ cm rim), Figure 15, and “Queen Event Results”.
Feed Uptake: The presence of the open source syrup mimics a honey flow: the bees work the barrels as if they are giant flowers. It does not stimulate robbing between colonies. Open source feeding has been successfully used by the beekeeper as a method for determining the viability of the colony going into winter since 1983. Colonies that have their affairs in order will rapidly recognize the food source and utilize it, putting up food reserves in the brood chamber for the winter, increasing the hive weight. All the colonies treated with MAQS in August rapidly picked up weight and were very heavy by the end of the trial. The control colonies that were treated late in the study (October 5th) had much smaller clusters and were considerably lighter.

Expectations for overwintering: Previous studies have shown that treatment with MAQS will provide good control of mites resulting in the colonies being relieved of the stresses of varroatosis. With the excellent feed uptake and adequate pollen reserves it is projected that the treated colonies will winter well. (Figure 11) Splits will be required to prevent swarming; replacing the colonies lost over the prior year will not be a problem. Expansion or having nucleus colonies for sale will be an option.

Formic Acid Concentration in Honey

Honey contains a number of acids, which includes amino acids (0.05-0.1%) and organic acids (0.57%, range: 0.17-1.17%). The average pH of honey is 3.9 (with a typical range of 3.4 to 6.1)\textsuperscript{xv}. Formic acid is a volatile organic acid naturally occurring in nectar-based honey\textsuperscript{xvi}. 

Figure 11. This is the hive where the queenless colonies were united with a queen right colony. It fed heavily and will likely need splitting in early spring.
The concern is that the formic acid levels would increase due to treatment to a point that it would impact human consumption by affecting taste. Bogdanov, et al (1999)\textsuperscript{xvii} ran a series of tasting trials and concluded that an increase of 150 to 600 ppm would be required to affect taste. A taste effect when formic levels decreased below documented naturally occurring levels in the source honey post treatment was not addressed. In a follow-up field trial, Bogdanov et al. (2002)\textsuperscript{xviii}, after 3 years of field studies concluded that there are no accumulative residues. Honey tested after formic acid applied in the fall remained below the taste threshold. Emergency treatments in the spring could lead to brief increase in formic levels that would take some hives' honey above the taste threshold, but would not cause a statistically significant increase in free acidity. These results were supported by Donders, J., and Cornelissen, B., (2005)\textsuperscript{ix}

The current study was designed to determine the maximum amount of formic acid that would be found in honey to be harvested when using MAQS technology, under a worst case scenario: dilution by honey volume was minimized by a) removing previously gathered honey pre-treatment and b) minimal end-of-season honey flow occurred during treatment. The impact of doubling the dose provides comparison. One colony, Hive #83, was too weak to put up any surplus honey, so it could not be included. The end of treatment samples were taken from open cells within moments of the combs being taken from the hive, they were immediately sealed and stored in a freezer. This honey was not subject to the warming, extracting and processing that would normally occur post-harvest. The Day+24 samples were a mix of sealed and open-cell honey, secured and handled in the same way as the previous samples.

The formic acid concentration in honey was affected by the treatment, increasing dramatically in 2 hives in the double dose groups and slightly in the standard dose group at the end-of-treatment mark. (Figure 12). Levels rapidly dropped off to being within naturally occurring levels in the single dose treatment group. In the double dose group the concentrations also dropped off but, on average, remained just above naturally occurring levels by Day+24. (Figure 13). The range in concentration was lowest in the standard dose treatment group. (Figure 14). This drop-off in elevated levels was also seen in the trial conducted in Sutton, U.K, (Wilkens, S., 2011)\textsuperscript{xx} when feed-source “honey” from hives was tested for formic acid levels.
**Taste Detection Conclusion:** The average increased levels of formic in the honey in the standard dose treatment at the end of treatment was at the lower range of taste detection at 188 ppm over the control high, but by the next sample taking time all standard dose treated colonies were within naturally occurring levels. If MAQS is applied at amounts double the standard dose there is a moderate risk of taste detection 24 days after application. This is for honey taken right from the comb in the field: typical warming and extraction from the comb would likely see these levels reduce prior to the honey reaching the marketplace, due to the volatility of formic acid.

**Efficacy Note:** Determination of efficacy was not part of this study. However, in Day+8 field checks of drone brood, checking approximately 4 square inches (26 square cm) of drone brood (80 to 100 capped cells) in one hive in each group showed 100% varroa kill under the cap in both MAQS treatment groups; live varroa families in the control group. Initial air concentrations and the extended concentrations achieved with MAQS technology indicate that efficacy should be excellent.

**Summary and Conclusion**
There is minimal risk to the colonies when MAQS is applied correctly under the conditions tested. The bees can manage their affairs despite the elevated levels of formic acid in the brood chamber air. MAQS technology allows the bees to maintain an effective level of formic acid for the 3-day period post application, when the hives have solid floor bottom boards which have a full hive-width entrance, height a minimum of ½ inch (1.27 cm). Under the conditions experienced there was minimal to no evidence of damage to the brood, and the queens, regardless of age, handled the treatment well. If the dose is doubled queens may be lost and supercedure triggered, however some eggs and/or young larva will survive the treatment and the colonies are likely to successfully replace the queens, giving the colonies young queens going into winter. MAQS has the potential to provide a solution to the queen health issues experienced with other products.

If supercedure was already underway before MAQS was applied it was an indicator that the colony was already struggling with queen issues, however supercedure was likely to be successful even with the treatment. If it was not successful the colonies did not become drone layers and were easily combined with queen-right colonies, ready to split in the spring.

In the hives that received the double dose of MAQS the formic acid levels in honey went from well above naturally occurring levels at the end of treatment to below naturally occurring levels at the Day+24 mark. On average, at the Day+24 mark levels were just above the levels that were naturally occurring. The maximum level of formic acid in honey was 0.69% in the double dose group, well within the level of organic acids naturally occurring in honey.
Formic acid levels in honey in the standard dose treated hives did increase to just above naturally occurring levels during treatment in this study, but stayed within a tight range, indicating that the bees had control of the environment; after the treatment period the levels soon dropped off to within naturally occurring levels. The formic acid levels in honey are not a concern when MAQS is applied as directed.

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iii Currie, R.W. (2003) Use of Formic Acid to Control Varroa and Tracheal Mites in Indoor Wintering Facilities. ARDI Project #00-413, University of Manitoba, Winnipeg, MB, Canada.


xiii Dräger Safety AG & Co. KGaA, Revalstrasse 1, D-23560 Lubeck, Germany. www.draeger.com

xiv GCL & Chemisar Laboratories Inc., 24 Corporate Court, Guelph, Ontario Canada. N1G 5G5 www.chemisar.com


xvi ibid


xviii Bogdanov, S., J.D. Charriere, Anton Imdorf, V. Kilchenmann, P. Fluri (2002) Determination of residues in honey after treatments with formic and oxalic acid under field conditions. Apidologie 33: 399-409


xx Wilkens, S., Director. Food and Environment Research Agency (FERA). (2011) Assessment of the effect of two application methods of NOD Mite Away Quick Strips (MAQS) on adult bee mortality and colony development, when applied to control varroa mites (Varroa destructor Anderson & Trueman) in naturally infested honey bee (Apis mellifera L.) colonies and a comparison of efficacies and comparative formic acid levels in honey, after treatment, under field conditions in the UK. GLP Study Sponsored by BASF.