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Summary Geraniol 2 Test Reports - Insecticide

Repello Barrier Spray/Equestri Guard/Tick Safe vs Common Pesticides in Killing Insects

STUDY: Laboratory test on spontaneous mortality and long-time effect of Repello Barrier Spray/Equestri Guard/Tick Safe vs. Common Pesticides on flying insects and their stages of metamorphosis, Dr. Alois Muelhofer, LHS Laboratories, Department of Entomology, Wiener Neustadt, Austria July-August 2002

Test products: Repello Barrier Spray/Equestri Guard/Tick Safe:

REF A = 2 x 500 ml DURSBAN

with 5.0 g/l chlorpyrifos 3.7 g/l neopynamine and 19.5 g/l methoxychlor

REF B = 2 x 500 ml INSEX SPRAY with 23.8 g/l permethrin 5.0 g/l bioallethrin and synergist piperonyl butoxide.

MD 1 for laboratory-bred, resistance-free House Fly and Moths imagoes, larvae, pupae (puparia)

MD 2 for *Musca domestica* from a stable, presumably resistance-free, imagoes, larvae, puparia

MD 3 for *Musca domestica* from a stable, fully resistant to organophosphorus compounds and pyrethroids, test specimens as above, and

MD 4 for *Musca domestica* from a stable with unspecified resistance to REF A and REF B, test specimens as above.

No distinction was made between male and female.

Results: Repello Barrier Spray/Equestri Guard/Tick Safe was 100% effective in spontaneously killing flies and moths, also reliably killing insecticide-resistant strains of *Musca domestica*. The long-time effect of Repello Barrier Spray/Equestri Guard/Tick Safe is minor to that of the two reference products on non-resistant flies but far superior with regard to resistant flies.

Laboratory-bred moths did not display unexpected resistance to any of the products.

Larvae Mortality illustrates that all three products have a similar effect on resistance-free *Musca domestica* larvae. The same picture emerged for resistances to REF A and REF B as had with the adults in Table 1. What was striking about the effect of Repello Barrier Spray/Equestri Guard/Tick Safe on all larvae was that it immediately caused them to cease eating and moving shortly after the treatment and to yellow and shrivel up after just 8 hours.

Repello Barrier Spray/Equestri Guard/Tick Safe completely inhibited metamorphosis of *Musca domestica* pupae in the puparia whereas about 80% of the puparia treated with reference products actually ended up emerging.

All pupae cut open with a scalpel after 21 days were immobile. The pupae treated with the reference products which had failed to emerge indicated signs of decomposition; those treated with Repello Barrier Spray/Equestri Guard/Tick Safe were dried out. It can be concluded that the active ingredient had penetrated through the walls of the puparium of the pupae treated with Repello Barrier Spray/Equestri Guard/Tick Safe and killed the pupae; with the reference products, the pupae which failed to emerge were clearly killed as a result of fungus infestation, in a manner commonly found in nature, and not from the effect of REF A and REF B.

Ants

STUDY: Laboratory study on spontaneous mortality and long-time effect of the product Repello Barrier Spray/Equestri Guard/Tick Safe on ants, Family Formicidae, with account taken of the product's repelling effect. Dr. Alois Muelhofer, LHS Laboratories, Department of Entomology, Wiener Neustadt, Austria March 2003

Test Product: Repello Barrier Spray/Equestri Guard/Tick Safe

REF A = 2 x 500 ml DURSBAN

with 5.0 g/l chlorpyrifos 3.7 g/l neopynamine and 19.5 g/l methoxychlor

REF B = 2 x 500 ml INSEX SPRAY with 23.8 g/l permethrin 5.0 g/l bioallethrin and synergist piperonyl butoxide .

Ant species: *Lasius niger* (black field ants), *Pheidole megacephala*, *Formica rufa* (red forest ants) -worker ants found in the wild.

Results: substantiates the 100% spontaneous effectiveness of all three products one has come to expect. The mortality rate among all three species of workers tested was absolute. The three test products had the very low long-term effect over a period of four weeks on adult workers belonging to *Lasius niger*. It should be noted, however, that all three test products proved to have such a strong repellent effect on ants that the tests could only be conducted by stunning the ants with a cold spray before placing them on the slabs treated with the three products. Whereas 80% of the eggs treated with REF A and REF B developed first-stage pupae, the eggs treated with Repello Barrier Spray/Equestri Guard/Tick Safe became yellow and completely dried out.

Clothes Moths

STUDY: Efficacy of Repello Barrier Spray/Equestri Guard/Tick Safe in Controlling Fur and Clothes Moths, Dr. Alois Muelhofer, LHS Laboratories, Department of Entomology, Wiener Neustadt, Austria, May-December 2005

Test Product: Test product: 15g timber chips prepared in vacuum with 4,5g Geraniol.

Results: The repellent effect of wood chips impregnated with Geraniol was 100%.

The life span of the adult moths in the cage was short-lived. After 3 hours at the latest, all the moths of all the placements in the outside cages were dead. This explains why in none of the tests in wardrobes 1 and 2 eggs or larvae were observed. Of the eggs that were applied monthly on the sheep's wool pullovers and fur jackets, no further metamorphosis occurred. After 7 months the test was halted at the instigation of the sponsor. Until this time, the repellent effects and effect on mortality against adult moths and the prevention of egg laying were still fully active.

Flying and Crawling Insects

STUDY: Laboratory tests to determine the effectiveness of product on flying and crawling insects and their eggs and stages of metamorphosis, conducted in accordance with OECD and GLP regulations, Dr. Alois Muelhofer, LHS Laboratories, Department of Entomology, Wiener Neustadt, Austria, February 1, 2002 to May 31, 2002

Test products: Repello Barrier Spray/Equestri Guard/Tick Safe:

REF A= 2 x 500 ml DURSBAN

with 5.0 g/l chlorpyrifos 3.7 g/l neopynamine and 19.5 g/l methoxychlor

REF B = 2 x 500 ml INSEX SPRAY with 23.8 g/l permethrin 5.0 g/l bioallethrin and synergist piperonyl butoxide.

Insect Test Populations

I. *Musca domestica* - The test specimens, designated as

MD 1 for laboratory-bred, resistance-free insects,

MD 2 for insects from a stable where no systematic pest control had been carried out,

MD 3 for insects from a stable where pyrethroids and azamethiphos were used for several years, and

MD 4 for insects from a stable where a product was used which contained 20 g/l chlorpyrifos - methyl, 20 g/l cypermethrin, and synergist.

The resistance trends are clearly indicated and evident in the results from 12/20020515 since all repetitions yielded results comparable to those in the first test.

II. *Tineola bisselliella* - The specimens used were exclusively laboratory-bred since practice has shown that insects caught in the wild are damaged by the time they arrive in the laboratory.

III. Blattodea

III. I *Blattella germanica* - The test animals, designated as BG 1 for those that are resistance-free laboratory-bred specimens

BG 2 for those from a large-scale industrial bakery where alternating types of insecticides have been used to control pests for years, as shown by the resistances from 13/20020515, and

BG 3 for those from a restaurant very recently infested by the insects so they were still free of resistance, as indicated in the comparative test in 13/20020515.

III. II *Blatta orientalis* - The test animals, designated as

BO 1 for those that are resistance-free laboratory-bred specimens

BO 2 for those from a heavily infested restaurant in which pest control up to that point had been carried out exclusively with household aerosol products containing pyrethrum and pyrethroid (Paral, Raid, and the like).

III. III *Periplaneta americana* - This species is seldom found in the wild in Europe and the specimens for this study were obtained from a zoo where they are bred as food for reptiles and guaranteed to be resistance-free.

Field tests with Repello Barrier Spray/Equestri Guard/Tick Safe were conducted in the zoo's administrative offices where escaped insects had established small populations.

IV. *Lasius niger*, *Pheidole megacephala*, and *Formica rufa* - These specimens were all caught in the wild. Different sites were selected to ensure that the population of the *F. rufa*, in particular, was not endangered. This species is protected by law.

V. *Vespula austriaca* in the annex - The specimens for 15/20020515 were laboratory bred; those for 16/20020515 were caught in the wild.

VI. *Xenopsylla cheopis* and *Ornithodoros moubata* for 17/20020515 were laboratory bred

a) Rat Flea - *Xenopsylla cheopis*

b) Soft Ticks - *Ornithodoros moubata*

Results: No differences to results *M. Domestica* were observed in the results for either the test product or the two reference products in terms of spontaneous mortality and long-term effect with respect to the Blattodea specimens, which had been divided into males, females and specimens in the process of metamorphosis.

The presumed resistances were evident throughout all stages of the population.

The application of Repello Barrier Spray/Equestri Guard/Tick Safe resulted in a 100% mortality rate without any incident of reanimation. The period from the time the insects were treated to the time they were in a dorsal position and immobile was less than two minutes in each case. When the reference products were applied to resistance-free cockroaches, this period lasted up to 4 hours, especially with female BG1s bearing oothcae.

Fleas and Ticks

All 50 fleas were completely motionless within 10 seconds and did not recover. When the fleas were examined under a microscope 24 hours later, they showed signs of relatively advanced decomposition.

20 hours after spraying, all ticks showed signs of marked discoloration and no longer drew in their legs on contact. Immediately after the treatment, one tick was taken out of each test container and placed on the shaved abdomen of a hamster sedated with Vetanarcol. These 5 ticks were completely immobile and displayed no signs of boring into the skin and sucking blood at any time during the 24 hour observation period, but rather fell off eventually from their host

The other 20 ticks placed on sedated hamsters 20 hours after the treatment, fell off the host animal immediately with their legs drawn in and were dead. No further changes were observed during the additional three day observation period.

Mode of Killing Insects- Destruction of Chitin

Microscopic examination of insects' chitin layer

To evaluate the manufacturer's claim that Geraniol acts to destroy chitin, 5 adult *Musca domestica*, 5 larvae, and 5 pupae (puparia) as well as 5 adult male and female *Blattella germanica*, 5 oothecae, and 5 nymphs were killed or treated with 0.1 ml of reference product A and 0,1 ml Repello Barrier Spray/Equestri Guard/Tick Safe. After being stored 24 hours at room temperature, the dead adult specimens were examined under a microscope to evaluate their chitinous outer shell in comparison to the images of the insects which had been stunned shortly before with cold spray. The condition of the chitin in the living specimens and in those treated with reference product "A" were completely the same in all instances.

When the adults treated with Repello Barrier Spray/Equestri Guard/Tick Safe were examined under the microscope, they showed definite signs of decomposition, such as broken-off antennas, legs, and stingers and wings in the flies, raw, crater-like constitution of the top of the wings in cockroaches, with no difference detected between imagoes and nymphs. The white larvae of the flies were brown and totally deformed.

The treated puparia and oothecae were incubated in a bedding of sawdust in closed containers and their emergence/hatching rate compared with that of a reference group of 5 untreated specimens. In the untreated control puparia and oothecae and in those treated with reference product A, all flies or nymphs emerged; in those treated with Repello Barrier Spray/Equestri Guard/Tick Safe, not a single emergence was observed. The incubation of puparia and oothecae was repeated a total of six times with specimens from different populations and sites of origin and yielded the same results each time (see Study nos.: 12/20020515 and 13/20020515)

Conclusion: These tests proved conclusively that Repello Barrier Spray/Equestri Guard/Tick Safe has a physical, dehydrating effect on the test specimens as well as a biological effect which destroys chitin. There is no indications that the nervous system is affected.

The condition of the fly larvae and the absence of emergence presumably indicate that there is an inhibitory effect on the enzyme chitinase or a reaction with the disaccharide chitobiose.

Wasps

STUDY: Follow-up study on the effectiveness of the product Repello Barrier Spray/Equestri Guard/Tick Safe on nests of Wasps (*Vespula austriaca*) in a Field Test, Dr. Alois Muelhofer, LHS Laboratories, Department of Entomology, Wiener Neustadt, Austria, June 2001

Results: No specimens capable of flying emerged from any of the treated nests after

Repello Barrier Spray/Equestri Guard/Tick Safe was sprayed into them. Insects incapable of flying were seen crawling out or falling out, squirming in panic, from all of the nests in varying numbers. These insects were all able to be collected by the recorder.

During the observation period, wasps returning from without were seen time and again flying up to the nest, circling the entry hole, and flying off again. From this, it can be concluded that nests treated with Repello Barrier Spray/Equestri Guard/Tick Safe are not able to

be re-inhabited. On cutting apart the nests, the recorder found only dead insects or insects in the throes of death. Not a single one of these insects was able to fly. The evaluation of the reanimation tests in the terrarium showed that not a single insect recovered.

In summary, this field test confirms the laboratory findings, showing that Repello Barrier Spray/Equestri Guard/Tick Safe also causes 100% mortality in *Vespula austriaca* in the field when applied in accordance with package instructions.

Ticks on Cattle

STUDY: Activity of 1% Repello "Insect Killer" in the prevention of tick infestation on Cattle.

Dr. Khalid Khallaayoune, Institut Agronomique et Vétérinaire Hassan II, Département de Parasitologie, Rabat-Instituts, Maroc

Dr. Gérard DUVALLET Centre d'Écologie fonctionnelle et évolutive, Université de Montpellier, France July 2008

Results: 1% Repello Barrier Spray/Equestri Guard/Tick Safe is very well tolerated by cattle, which show no adverse effects from the product. It shows an activity of 99.20% at D1 for Hyalomma sp. ticks. The comparison of Group A (Repello Barrier Spray/Equestri Guard/Tick Safe) with Group B (placebo) indicates a significant reduction in the number of ticks in the group sprayed with 1% Repello Barrier Spray/Equestri Guard/Tick Safe ($p < 0,0001$). In addition, application of the product made it possible to maintain an infestation level for three weeks which was considerably inferior to that of the group which was not treated (1,9 vs 21,9).

Conclusion: Repello Barrier Spray/Equestri Guard/Tick Safe at 1% Geraniol has a highly effective activity rate on ticks, and is perfectly suited to control tick infestation of cattle.

orn Flies on Cattle

STUDY: Efficiency of Geraniol in Repelling Horn Flies on Dairy Cattle Dr. Wes Watson, Dept. Entomology, North Carolina State University, Raleigh Durham, July-August 2008

Product Tested: Repello Products formulation 1% Geraniol in Soybean Oil

Dosing: 120 ml per cow pour on treatment

Results: 1% Repello Geraniol formulation produced a Horn Fly reduction of over 95% for a two week period. The Control Horn Fly count averaged 500/head with treated cows averaging 22 Horn Flies/head.

Conclusion: Repello Products 1% Geraniol formulation is a viable pour on product for dairy and beef cattle and is especially well suited for organic cattle farming as an organic 25b Exempt product.

Poultry Red Mites

STUDY: Investigation into the Effectiveness of Repello Insect Killer Spray Solution for Counteracting Poultry Red Mites (*Dermanyssus gallinae*) in vitro, Prof. Dr. A Liebisch, ZECKLAB, Labor für Klinische Diagnostik und Prüfung, Burgwedel, February 10, 2004

Results: The present investigations proved that SRV is sufficient in controlling *Dermanyssus gallinae* (poultry red mite) in vitro. Dilution A (1:29) was 100% effective against adult mites. 88% effectiveness with Dilution B (1:59) is still very good and sufficient for use in practice. An effectiveness $>75\%$ is required and dilution C (1:79) is not effective enough (67%) against adult mites.