A Review of Treatment Options For Control of Varroa Mite in New Zealand

Report to the Ministry of Agriculture and Forestry

MAF Biosecurity

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Important Notice

It is an offence under the Animal Products Act 1999 to use any drug, substance, or mixture of substances for the prevention or treatment of varroa, unless it has been approved.

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Notes:

Tthe following varroa control products mentioned in this report are registered trade names – Apicure, Apiguard, Apilife VAR, Apistan, Apitol, Apivar, Bayvarol, BeeVar, Check-Mite+, Folbex, Kramer, Nassenheider, Perizin, Vapidifus.

This review focuses on varroa control products commonly in use in countries with a regulatory environment for agricultural chemicals similar to that found in New Zealand.

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

February 2001

The objective of this project was to provide a comprehensive review of the treatment options available and potentially available to New Zealand beekeepers for the control of varroa. The project was carried out under contract to the New Zealand Ministry of Agriculture and Forestry (Contract number RFQ/BER/4/2000).

Resources used to carry out the review included a database search of Commonwealth Agriculture Bureau (CAB) abstracts, an Internet web search, and a search of files and books held by HortResearch on varroa and related subjects. Pesticide safety and LD50 data was obtained from various materials safety and pesticide information profile sources.

For each treatment option, the review covers the following:

a) common trade name(s)
b) active ingredient of the product
c) chemical class
d) method of application
e) effectiveness
f) adverse effects
g) operator safety
h) LD50
i) residues
j) maximum residue limits (MRL's)
k) resistance
l) cost (product and application)
m) impediments that may reduce the likelihood that the product may be approved

Table 1 List of treatments shown to have significant efficacy for varroa control

Product Trade Name	**Active Ingredient**	**Chemical Class**
Apiguard	thymol	essential oil
Apilife VAR	thymol, eucalyptol, menthol, camphor	essential oil
Apistan	fluvalinate	synthetic pyrethroid
Apitol	cymiazole	iminophenyl thiazolidine derivative
Apivar	amitraz	amadine
Bayvarol	flumethrin	synthetic pyrethroid
Check-Mite+, Perizin	coumaphos	organophosphate
Folbex	bromopropylate	chlorinated hydrocarbon
generic	formic acid	organic acid
generic	lactic acid	organic acid
generic	oxalic acid	organic acid

A range of other substances (eg., mineral/vegetable oil, neem oil, wintergreen oil, icing sugar) show some promise as varroa control treatments. However, in the opinion of the authors, insufficient research data currently exists for these substances to be considered as suitable control treatments. Short reviews of several of these substances are included at the end of this paper.

Trade Name(s): Apiguard, generic

Active Ingredient: Thymol (12.5g/tray in Apiguard; 10-15g per treatment for other methods)

Chemical Class: Essential oil

Method of Application: Thymol has been applied to beehives using various methods, including powdered thymol suspended between frames in mesh bags and liquid thymol poured on a sponge on the top bars. Generally 2 to 4 treatments are made at 8g per treatments. A continuous evaporator placed between the brood combs has also been developed[79].

Apiguard is a gel formulation of thymol designed to be easy to apply, while at the same time providing a more controlled release of vapours than other methods. Each Apigard tray contains 50g of gel, with a second tray applied 2 weeks after the first one. The product should be used in the autumn after brood rearing has finished, but when temperatures are above 15^{o}C.

Effectiveness: Pure thymol has been trialled on a number of occasions, showing mite mortality rates of between 54 and 98%[99; 53; 63]. Rates are similar for different application methods[68]. Reproducing varroa in capped cells are protected from thymol, and in areas where year-round brood rearing takes place, thymol has shown a lower mite mortality of between 54 and 85%[53].

A trial in Italy showed Apiguard killed 98% of varroa[30] although subsequent trials in Canada showed mite mortality rates for the product of 68-82%[103] and 76%[101]. Increasing the time period of treatment to 30 days did not improve efficacy. However, in both Canadian trials the product was not applied in the autumn as recommended. The volatility of the product is temperature dependent, and likely to require similar temperatures as for generic thymol (daytime 15-20^{o}C, without falling below 12^{o}C for long periods). For best effect, the hive should also be broodless.

Adverse Effects: Increased adult bee mortality for thymol has been assessed at 9.5 bees/day over 10 days[52], although in another study only young larvae survival was affected (74-87% v. 89-95% for controls), with no differences in sealed brood or adult survival[102].

Operator Safety: Thymol is a skin irritant, although it is generally regarded as safe to use and apply. Safety glasses should be worn when handling the pure compound.

LD50: Rats – 980mg/kg (oral); 100mg/kg (dermal)

Residues: Only taste threshold residues (see MRL).

MRL's: In the EU, thymol is a group II non-toxic veterinary drug that does not require a MRL. Nevertheless, thymol does leave taste residues in honey and wax, although the residues do not persist for long periods of time. The Swiss have set an MRL for thymol of 0.8ppm to ensure honey does not exceed the taste threshold of 1.1ppm[11]. When continuous evaporators are used, there is a chance of thymol residues in honey above this level, although the chance is slight[14]. Thymol is not recommended for application during the honeyflow[68].

Resistance: There are no published reprts of the resistance of varroa to thymol.

Cost: For Apiguard, approximately NZ$8.11 per hive (2 trays) based on bulk UK price. Labour costs are greater than for strip and liquid applications, since 3 visits are required. Bulk price for thymol is NZ$28.40/kg. Total cost for 4 treatments is NZ$1.45. Labour costs for generic thymol application would be similar to Kramer plates (see *Formic Acid*), at 15 minutes per hive.

Impediments to Registration: Apiguard is expected to complete EU registration in the near future. It is likely, therefore, that sufficient data exists to meet registration requirements in New Zealand. Thymol is a generic product, and while sufficient data probably exists for registration, an applicant would need to be found either a) within the chemical supply industry, or b) with a proprietary continuous evaporator device.

Trade Name(s): Apilife VAR

Active Ingredient: Thymol (76%), eucalyptol (16.4%), menthol (3.8%) and camphor (3.8%); 20g of active ingredients/tablet

Chemical Class: Essential oil

Method of Application: Apilife VAR comes in the form of a vermiculite tablet. One tablet is placed on the top bars of the hive at the end of the summer after honey has been removed. The tablet is replaced with a fresh one 3-4 weeks later. Hives should ideally be broodless and daytime temperatures should not fall below 12^{o}C.

Effectiveness: A review of the efficacy of Apilife VAR has shown that in 14 of 22 applications, mite mortality was greater than 90%[68], although some studies have found mortality rates of below 70%[56; 115; 25]. Mite mortality has been shown to correlate with evaporation rate[27]. Levels of active ingredients in Apilife VAR in addition to thymol do not appear to be significant in causing mite mortality[73], and a study comparing generic thymol to Apilife VAR showed similar levels of mite mortality[15].

Adverse Effects: Colony development is not thought to be impaired by use of Apilife VAR[91], although one study reported loss of 50% of bees in treated colonies over winter, and significant reductions in honey crops the next year[112]. Colonies are reported to have problems storing winter feed during Apilife VAR autumn treatment[67].

Operator Safety: As for thymol. No safety issues for operator.

LD50: Thymol: Rats – 980mg/kg (oral); 100mg/kg (dermal)
Camphor: Rats – 2000mg/kg (oral); 3000mg/kg (dermal)
Eucalptol: Rats – 2480mg/kg (oral); 50mg/kg (dermal)
Menthol: Rats – 3300mg/kg (oral); 710mg/kg (dermal)

Residues: An autumn application of Apilife VAR resulted in residues of 0.02-0.48ppm of thymol in honey produced the next spring. Beeswax had low residue levels of both thymol and menthol, but did not increase with the number of treatments. Thymol was not removed during comb melting, but did reduce rapidly when processed wax was exposed to air during storage[9].

MRL's: None of the active compounds in Apilife VAR are toxic at the levels found in the product. The Swiss have set a taste MRL for thymol of 0.8ppm to ensure honey does not exceed the taste threshold of 1.1ppm[11]. Thymol is not recommended for application during the honeyflow[68]. No taste MRL's have been set by the Swiss for any of the other active ingredients.

Resistance: There are no reports of varroa having developed resistance to Apilife VAR, or any of its active ingredients.

Cost: Cost for Apilife VAR is NZ$3.21 per tablet, based on bulk price from European distributor. Total cost for treatment (2 tablets) is therefore NZ$6.43. Labour costs are greater than for strip and liquid applications, since 3 visits are required. Labour would be similar to gel products, at 5 minutes per hive.

Impediments to Registration: Apilife VAR is registered for use in Italy, but not in any other European or North American countries. Nevertheless, it appears likely that there is sufficient efficacy data available to meet registration requirements in New Zealand should the manufacturer or an importer choose to register the product.

Trade Name(s): Apistan

Active Ingredient: Fluvalinate (8.8g/strip)

Chemical Class Synthetic pyrethroid

Method of Application: Apistan consists of a plastic polymer embedded with fluvalinate, a synthetic pyrethroid. The strips should be placed in the hive with one strip used for every 5 frames of bees in each brood chamber. The strip is hung between the frames, with the frames separated slightly so that both sides of the strip come into contact with the bees. The bees rub against the strips as they move through the brood chamber, and then pass the chemical on to other bees as they rub up against each other in the hive. The strips should be removed after 42 days.

Effectiveness: Use of fluvalinate to control varroa was discovered in France and Israel, using Mavrik impregnated wood strips[16; 97]. Development of Apistan resulted in mite mortality in excess of 95%, and often exceeding 99%[60; 17; 46; 42]. However, in mite populations that have developed resistance to Apistan, effectiveness can drop to between 30 and 50%, depending on the concentration of fluvalinate in the product[120].

Adverse Effects: Increased adult bee mortality for fluvalinate has been assessed at 2.7 bees/day over 60 days[52]. Significantly less (86% vrs. 97%) drones emerged from colonies treated with Apistan than control colonies, but survival was greater for both compared to varroa infested colonies (59%). Both varroa and Apistan caused reductions in drone body weight and various glands[125]. In queen cages, exposure to 1% fluvalinate for 3 days caused significant mortality in worker attendants and increased supersedure in queens. Exposure for 7 days caused significant mortality in queens[31].

Operator Safety: Fluvalinate is moderately toxic orally, but practically non-toxic dermally. Because of the strip formulation, so as long as the instructions on the label are followed, the product is of minimal risk to the operator. Fluvalinate is highly toxic to fish and aquatic invertebrates, so it is classified as a Restricted Use Pesticide in the US. Because of the risk to fish, Apistan strips should be disposed of in an approved chemical waste site.

LD50: Rats - 261-281mg/kg (oral); 20,000mg/kg (dermal)

Residues: Fluvalinate is a stable, non-volatile, fat-soluble compound. Fluvalinate accumulates in beeswax over time, and because of its stable nature does not break down naturally in beeswax. In one study, combs with fluvalinate residues continued to kill varroa one year after treatment[111]. Fluvalinate accumulates in beeswax comb as a result of repeated applications of the chemical. Recycling of beeswax into comb foundation does not destroy the fluvalinate residues in the product and fluvalinate in foundation can result in increased residues even in newly established hives[12]. Treatment with ozone or hydrogen peroxide did not produce any reduction in fluvalinate residues in rendered beeswax[136]. Heating of wax to over 100^{o}C at high pressure also did not affect residue levels[139].

There is also the potential for fluvalinate to produce residues in honey, caused either by particles of wax becoming incorporated into the honey at the time of extraction, or by migration of the product directly into the honey. However, because fluvalinate binds so strongly to beeswax, concentrations of over 400ppm in beeswax are required before residues are found in associated honey[139]. In one study, only 1 of 215 honey samples in Belgium tested positive for fluvalinate (0.004ppm), even though 95% of wax samples tested had more than 0.1ppm. Most wax samples had 1-10 ppm and some had more than 50 ppm[32]. Fluvalinate residues are most likely to be a problem for comb honey where the wax has been drawn out from contaminated foundation.

Fluvalinate added at the rate of 100ppm to beeswax foundation was present at similar rates afterwards, and slightly increased mite mortality in the first brood cycle. However, honey bee cocoon acted as a barrier for chemical transfer in subsequent brood cycles. Residues of 10ppm or less in foundation are not likely to affect mite fertility[51].

Fluvalinate residues have also been reported in propolis. In one study in Switzerland, 28 out of 29 samples of propolis contained fluvalinate residues, with an average of 9.8 ppm[13]. Alcohol extracts of propolis also had fluvalinate residues, suggesting the fluvalinate binds with refined propolis, not just the beeswax component of raw propolis[139].

Fluvalinate at 0.2ppm in samples of spiked honey took 28 weeks to degrade below detectable limits of 0.005ppm[5].

MRL's: Official maximum residue levels (MRLs) for fluvalinate in honey range from 0.01ppm in Italy and Germany to 0.05ppm in the Netherlands and the US. The EU does not have an MRL for fluvalinate in honey[140].

The US is the only country that has an official MRL for fluvalinate in beeswax, at 6ppm[140]. It is recommended that residues of fluvalinate in beeswax foundation are kept below 1ppm to ensure MRL's are not exceeding in comb honey[139].

Resistance: Resistance to fluvalinate has been reported in a number of countries[42; 113; 75]. Resistance to Apistan was first reported in Lombardy in Italy in 1991[94], spread throughout Italy due to migratory beekeeping, and was then found later elsewhere in Europe[108]. Varroa resistant to Apistan have now also been reported in various areas in the US[39]. The LC50 (lethal concentration to kill 50% of mites) for resistant mites was 25-50 times greater than for susceptible mites[107]. A field assay has been developed to screen mite populations for resistance to fluvalinate[121]. Varroa resistant to fluvalinate also show a cross-resistance to flumethrin [107]. Apistan should therefore not be used as an alternating product with Bayvarol. Cross-resistance between fluvalinate and amitraz has also been confirmed in the US[38].

Cost: Approximately NZ$7.60 per treatment, based on New Zealand bulk price (assuming 10 frames of brood, and therefore 2 strips per hive). Labour costs are minimal, with two visits per hive 6 weeks apart. One or both visits could be incorporated into normal hive management. Time to apply Apistan has been estimated at 2 minutes per beehive per visit[50].

Impediments to Registration: Apistan has a temporary registration for 2 years until residue data is produced from New Zealand. The effect of this data on final registration cannot be predicted.

Trade Name(s): Apitol

Active Ingredient: Cymiazole (130mg/application)

Chemical Class Iminophenyl thiazolidine derivative

Method of Application: Apitol is a granular product that is mixed with syrup and fed to adult bees. Two applications are made 7 days apart. The best results are achieved when there is little brood in the hive, but with temperatures above 10^{o}C. Apitol should not be used during the honey flow. Cymiazole is a systemic miticide, meaning that it works through the bees' haemolymph[40].

Effectiveness: Apitol has been reported to reduce varroa populations by between 83 and 98% in colonies without brood[100; 143]. However, in comparative tests it has not been as effective as fluvalinate and flumethrin[77].

Adverse Effects: Adult bee deaths have been reported with Apitol[61; 45]. Cymiazole was found to be moderately toxic to honey bees when ingested at a rate of 3500ppm[119]. Cymiazole fed to bees reduced development of hypopharyngeal glands and increased the amount and acidity of rectal contents[118].

Operator Safety: No significant risks identified.

LD50: Rats – 725mg/kg (oral); >3100/kg (dermal)

Residues: Cymiazole is a non-volatile, water soluble chemical, and therefore should easily make its way into honey. However, there do not appear to be any reports of cymiazole residues in either commercial honey or wax. This is possibly because it breaks down in honey provided it is fed in the autumn outside the honey production period. In one study the residues reduced from 2.45 ppm to 0.23ppm after 112 days[45]. While cymiazole is a systemic miticide, the amount of the chemical in bees' haemolymph decreases quickly[24].

MRL's: Official maximum residue levels for cymiazole in honey range from 0.01ppm in Italy and Germany to 1ppm in the EU. The US does not have an MRL for cymiazole in honey[140]. No MRL's have been established for beeswax.

Resistance: There are no reports of varroa having developed resistance to cymiazole.

Cost: Cost for Apitol is NZ$3.51 per treatment, based on bulk price from European distributior. Cost for recommended treatment regime (2 treatments) is therefore NZ$7.02. The use of a liquid application product like Apitol is likely to take more time per beehive than strip products, although both require two visits to the apiary. Total time per hive for liquid products has been estimated at 4 minutes per application, or 8 minutes total[50].

Impediments to Registration: Although there is less information in the literature about cymiazole than for fluvalinate or coumaphos, Apitol is registered for use in Italy, the Netherlands, Germany and Spain. It is therefore likely that sufficient technical data exists to meet the requirements of registration in New Zealand.

Trade Name(s): Apivar

Active Ingredient: Amitraz (500mg/strip)

Chemical Class: Amadine

Method of Application: Apivar consists of a plastic polymer embedded with amitraz, a contact miticide. The strips should be placed in the hive with one strip used for every 5 frames of bees in each brood chamber. The strip is hung between the frames, with the frames separated slightly so that both sides of the strip come into contact with the bees. The bees rub against the strips as they move through the brood chamber, and then pass the chemical on to other bees as they rub up against each other in the hive. The strips should be removed after 6 weeks.

Amitraz has also been used for varroa control in the past applied as a spray (Mitac), and as a fumigant impregnated on potassium nitrate soaked filter paper and ignited on the bottom board (Taktik).

Effectiveness: Amitraz was one of the earliest chemicals tested for varroa control, with studies dating back to 1979. Early studies are reviewed by Merrington[106]. More recently, amitraz in plastic strips has been shown to produce varroa mortality of 97-99%[6]. Amitraz sprayed once and twice killed 90 and 96% of mites respectively[93]. Various concentrations of amitraz killed over 98% of mites, and fumigation strips killed over 99% of mites[60].

Adverse Effects: A preparation of amitraz (Apivarol) was found to increase mortality of 1-3 day old larvae (61% vrs. 83% for control)[4]. A fumigation strip of amitraz caused some bees to leave their hive and form clusters[98]. Fumigation strips also caused high adult bee mortality in package bees[60].

Operator Safety: Amitraz is classified by the EPA in the US as Class III – Slightly Toxic. There are unlikely to be operator safety issues for amitraz in the plastic strip form.

LD50: Rats – 523-800mg/kg (oral); >1600mg/kg (dermal)

Residues: Amitraz is a fat-soluble compound, but unlike other such compounds used as varroacides, it is volatile and unstable in honey, degrading in 3-4 weeks[76]. Amitraz has therefore not been found as a residue in honey[95]. Beeswax appears to accelerate the degradation of amitraz, with the product not being detectable within hours of application[140]. Amitraz was not detectable when added at 100ppm in beeswax foundation, although fluvalinate and coumaphos were present in levels similar to the amount added[51].

MRL's: Official maximum residue levels for amitraz in honey range from 0.01ppm in Italy, Germany and Switzerland to 1ppm in the US. The EU level is 0.2ppm[140]. No MRL has been established for amitraz in beeswax, since the substance has never been found as a residue in beeswax.

Resistance: Amitraz was found to be ineffective in killing mites in the former Yugoslavia, even though the product provided good mite control in the 4 previous years. The mites were believed to be resistant to amitraz[34]. Amitraz resistance was also confirmed in a population of mites in the US that showed resistance to fluvalinate[38]. Amitraz resistance has been shown in laboratory assays[108].

Cost: Between NZ$8.10 and NZ$14.95 per treatment, based on French prices (assuming 10 frames of brood, and therefore 2 strips per hive). According to the manufacturer, the low end price may be influenced by government subsidies.

Labour costs for Apivar are minimal, with two visits per hive 6 weeks apart. One or both visits could be incorporated into normal hive management. Estimated time necessary for application has been determined at 2 minutes per beehive per visit for strip products. Spray application would be 21 minutes per beehive. No figures are given for fumigant products[50].

Impediments to Registration: While there is substantial data available on the use of amitraz as both a spray and fumigant, there appears to be far less on the efficacy of strips. Because of good dosage control, however, the strips are likely to provide better efficacy without side effects on bee behaviour and mortality. Apivar is registered for use in Portugal, Ireland, Italy, Belgium and France. There is therefore likely to be sufficient efficacy data available to meet registration requirements in New Zealand should the manufacturer or an importer choose to register the product.

Trade Name(s): Bayvarol

Active Ingredient: Flumethrin (3.6mg/strip)

Chemical Class Synthetic pyrethroid

Method of Application: Bayvarol consists of a plastic polymer embedded with flumethrin, a synthetic pyrethroid. The strips should be placed in the hive, with two strips used for every 5 frames of bees in each brood chamber. The strip is hung between the frames, with the frames separated slightly so that both sides of the strip come into contact with the bees. The bees rub against the strips as they move through the brood chamber, and then pass the chemical on to other bees as they rub up against each other in the hive. The strips should be removed after 6 weeks.

Effectiveness: Bayvarol used correctly is very effective at controlling varroa. Mite mortality is usually in excess of 95% with kill rates often exceeding 99%[110; 129; 56; 1].

Adverse Effects: No increase in bee mortality was observed in a field study of honey bee tolerance to the chemical, and was the lowest of 6 chemicals tested[52].

Operator Safety: Flumethrin is moderately toxic. However, because of the strip formulation, so long as the instructions on the label are followed, the product is of minimal risk to the operator.

LD50: Rats – 258mg/kg (oral); >150mg/kg (dermal)

Residues: Unlike the closely related chemical fluvalinate, residues are not a major problem for flumethrin, probably because the amount of active ingredient in miticide strips is significantly lower for Bayvarol (3.6mg of flumethrin) than for Apistan (880mg of fluvalinate). A number of studies have not been able to find flumethrin residues in wax, although residues of other miticides were identified[138; 13]. Low residues of flumethrin have been found, however, in brood combs (0.051 ppm), suggesting that an accumulation effect of the chemical in wax over repeated applications is possible[12].

Studies have not found flumethrin residues in samples of commercial honey[134; 59].

Residues of flumethrin have been reported in propolis. In one study in Switzerland, 2 out of 29 samples of propolis contained flumethrin residues, with an average of 2.4 ppm[13]. No MRL’s have been established for beeswax.

MRL’s: Official maximum residue levels (MRLs) for flumethrin in honey range from 0.005ppm in Switzerland to 0.01ppm in Italy and Germany. Neither the US nor the EU have an MRL for flumethrin in honey[140].

Resistance: Populations of varroa showing resistance to fluvalinate have also been shown to have resistance to flumethrin, since the two substances have similar chemical structures. Flumethrin resistance has also been shown in laboratory assays[108]. The LC50 for resistant mites was 1-60 times greater than for susceptible mites[107]. Bayvarol should therefore not be used as an alternating treatment with Apistan.

Cost: Approximately NZ$7.00 per treatment, based on likely New Zealand bulk price (assuming 10 frames of brood, and therefore 4 strips per hive). Labour costs are minimal, with two visits per hive 6 weeks apart. One or both visits could be incorporated into normal hive management. Time to apply Bayvarol has been estimated at 2 minutes per beehive per visit[50].

Impediments to Registration: Bayvarol has a temporary registration for 2 years until residue data is produced from New Zealand. The effect of this data on final registration cannot be predicted.

Trade Name(s): Check-Mite+, Perizin

Active Ingredient: Coumaphos (10%/strip; 32mg/liquid application)

Chemical Class: Organophosphate

Method of Application: Check-Mite+ consists of a plastic polymer embedded with coumaphos. The strips should be placed in the hive with one strip used for every 5 frames of bees in each brood chamber. The strip is hung between the frames, with the frames separated slightly so that both sides of the strip come into contact with the bees. The strips should be removed after 6 weeks.

Perizin is a solution of coumaphos that is trickled over bees. It is best used in the late autumn or winter, ideally in broodless conditions. Two treatments one week apart are recommended[126]. There is some question about the systemic activity of the product, since Perizin was not found to be distributed significantly by food exchange between bees[22], and was present in the haemolymph of bees for only a maximum period of 12 hours after ingestion[21].

Effectiveness: Coumaphos impregnated strips (10%) used correctly are very effective at controlling varroa. Mite mortality rates are between 85 and 97%[109; 6]. Coumaphos solutions are also effective, with rates between 88 and 99% [52; 1].

Adverse Effects: Increased adult bee mortality for Perizin has been assessed at 15.7 bees/day over 7 days[52]. Perizin was assessed as having a low toxicity for bees (LD50 = 14.39µg)[78].

Operator Safety: Coumaphos is highly toxic orally, and has moderate dermal toxicity. However, the low dose formulation of both Check-Mite+ and Perizin greatly reduces the danger to the beekeeper.

LD50: Rats - 13-41mg/kg (oral); 860mg/kg(dermal)

Residues: Coumaphos is a non-volatile, fat soluble substance and can migrate from wax into stored honey. Residues are a major problem for coumaphos, at least in the solution form. A number of studies have found residues in wax[8; 13; 140] and in samples of commercial honey[44; 140]. Coumaphos is the most commonly found miticide in honey in Germany[140]. Coumaphos was found in newly produced wax 6 months after hives were treated with Perizin[23]. Coumaphos added at the rate of 10ppm to beeswax foundation was present at a similar level thereafter, and increased mite mortality dramatically in the first brood cycle. Honey bee cocoons acted as a barrier for chemical transfer in subsequent brood cycles[51].

MRL's: Official maximum residue levels for coumaphos in honey range from 0.01ppm in Switzerland to 0.05ppm in Holland and the Netherlands. The EU does not have an MRL for coumaphos in honey[140]. MRL's have recently been established in the US for coumaphos (0.1ppm for honey, 100ppm in beeswax)[3].

Resistance: Coumaphos resistance has been shown in laboratory assays [108]. In another study, a population of mites showed less than 50% mortality to coumaphos at 20ppm[135].

Cost: Approximately NZ$7.60 per treatment with Check-Mite+, based on US bulk price (assuming 10 frames of brood, and therefore 2 strips per hive). Cost of Perizin is NZ$7.91 for 2 treatments/hive, based on price from a Swiss distributor.

Estimated time necessary for application has been determined at 2 minutes per beehive per visit for strip products, and 4 minutes per hive per visit for liquid products[50].

Impediments to Registration: The frequent and high residues reported in honey from this product and its relatively high mammalian toxicity may have impact in New Zealand registration. However, the product has been given approval by the Environmental Protection Agency (EPA) in the United States, so considerable product safety documentation required for registration is likely to exist.

Trade Name(s): Generic; Apicure, BeeVar (gel); Kramer (plates); Vapidifus, Nassenheider (evaporator)

Active Ingredient: Formic acid (65%)

Chemical Class: Organic acid

Method of Application: Formic acid is a fumigant that kills varroa mite by respiratory inhibition[70]. Methods of application concentrate on ensuring high levels of formic acid vapour are present in the colony for various periods of time. The chemical is generally applied in the late summer and/or spring.

Short term treatments involve placement of a small amount (15ml) of formic acid on absorbent pads, either on the top bars or as a direct application on the bottom board. Release of formic acid is uncontrolled and must be repeated, usually at 1-4 day intervals for a total of 5-6 applications.

Long term treatments reduce labour costs and involve placement of larger amounts of formic acid, either in plastic pouches, gel or evaporators, in order to slow the release of formic acid vapour.

Apicure consists of 30g of formic acid incorporated into a slow-release gel matrix. BeeVar is also a gel, and requires 2 treatments 15 days apart. Both products are placed on the top bars and windows are cut into the plastic container.

The Kramer plate involves putting 250ml of formic acid on a 20 x 24 x 1.5cm piece of soft fibre building board, then sealing it in a freezer bag. 1.5cm holes are punched in the plastic with a round tool. The number of holes depends on the hive type and temperature[82].

The Vapidifus evaporator is a patented device that sits on a hive mat above the top bars. The holding tank takes 130ml of formic acid and controls the release of vapours through a flow regulator. The Nassenheider evaporator is incorporated into a hive frame and has a wick for chemical distribution. It has a 180ml holding tank.

Formic acid plastic pouches containing absorbent paper have been approved for varroa and tracheal mite control by the Canadian federal government. 250ml of formic acid is soaked into the paper. Windows are cut in the bag before placing it on the top bars of the hive. The number of windows depends on the size of the hive. One application is sufficient, although 20 days after placement another 50ml of formic acid can be added[54].

Effectiveness: Formic acid has been used successfully to control varroa since at least 1980[80; 128]. Efficacy rates for short and long term control methods have been comprehensively reviewed[70]. A kill rate of 94% was achieved using short term control on bottom boards for 4 days repeated 4 times[137]. Kramer plates have produced 95% mortality when used in spring and autumn[83]. Nassenheider evaporators showed 94% efficiency[122], while 2 long term treatments with 5 different dispensers showed a total efficiency of between 92 and 98%[28]. Formic acid gel in a paste was 70% effective in one application during spring, compared to 61% for 4 liquid applications. Evaporation rates were above those needed for good control[43].

Significant differences in control efficiency exist depending on weather conditions and time of year, ranging from 92% to 60% for different methods[131]. A spring treatment produced 77% mite mortality[55].

Failures in varroa control have been experienced in the US and elsewhere related to either too low or too high temperatures for proper extended vaporisation[142; 36].

Temperature at time of application is critical for achieving adequate kill rates. In one study, an autumn treatment had average daily release of formic acid below the recommended level, resulting in only 56% mite mortality[26].

Optimum efficiency is obtained when treatment begins in late summer after the honey flow, and when ambient temperature is 18-25^{o}C, with minimum night temperature no lower than 12^{o}C[74].

The efficiency of the treatment can be judged by weighing the application device. If evaporation is 7g or more per day, 95% of mites can be expected to die during a 14 day treatment period, increasing to 97% for 10g or more per day[72].

It is suggested that formic acid is able to kill varroa in capped brood cells as well as on adult bees. Tests where brood combs were fumigated in closed plastic foam boxes at 50ml for 1 hour killed 100% of mites in the brood cells with 90% of brood surviving the treatment[48]. However, vapours may not be sufficient to produce similar results in field conditions.

Adverse Effects: Use of Formic acid gel resulted in removed drone eggs, delayed drone production and reduced adult drone survival (24% survival at 10 days old vrs. 49% for controls). Unlike fluvalinate, formic acid did not reduce drone weight or weight of glands. Surviving drones had higher levels of sperm than controls[33]. Formic acid may also have adverse effects on open brood and hatching bees, depending on ambient temperature and device used, although loss of brood did not have a negative effect on colony overwintering in mid-European conditions[92]. Formic acid produced the highest rate of adult bee mortality (35.3 bees/hive/day) of 6 substances tested[52].

Operator Safety: Formic acid is a corrosive chemical that is potentially hazardous to humans unless proper precautions are taken. Skin and eye contact, as well as vapour inhalation must be avoided. Overalls, chemical goggles and impervious gloves must be worn when using the product, and if inhalation risk exists a vapour respirator must be used. Formic acid is heavier than air and concentrations of 18-57% are combustible if they come into contact with an open flame or spark. Unused chemical and used absorbent pads should be taken to an approved chemical waste disposal facility.

LD50: Rats – 1100mg/kg (oral); 1500mg/m3/15 min. (inhaled).

Residues: Honey contains naturally low levels of formic acid, ranging from 5 to 600ppm, depending on the type of honey. Formic acid used for varroa control in autumn resulted in increased formic acid in syrup stores and honey, although levels dropped to those normally found in honey after a few months[132; 57]. Autumn treatments can be recommended without affecting honey quality, although spring treatments should only be made on an emergency basis because residues may not evaporate in time[11].

MRL's: Even though formic acid is naturally present in honey, use of formic acid as a miticide can leave taste residues in honey. However, taste threshold for formic acid of 150-600ppm (depending on honey aroma and taste) determined by the Swiss does not exceed the worldwide MRL for organic acid in honey of 40 milli equivalent (meq) acid/kg[11].

Resistance: No reports have been made of varroa developing resistance to formic acid. It is suggested that varroa will not develop resistance to organic acids since they are natural parts of the metabolism of all organisms and cannot be rendered harmless through enzymatic effects[50].

Cost: Price for formic acid is NZ$1.83/litre, based on bulk price of 85% product diluted to 65% for use.

Price for Apicure is NZ$7.27 per tray x 2 trays = $14.54/hive, based on US retail price.

Price for the Nassenheider device is NZ$5.61 + 180ml of formic acid (NZ$0.32) = $5.93/hive, based on bulk rate from German distributor.

Price for a Kramer board is NZ$5.02 (includes formic acid), based on price from German distributor. Boards can be reused by adding 250ml formic acid (NZ$0.46).

Cost for short-term application on bottom board is 2.7 cents (15ml) x 6 applications = NZ$0.16.

Cost for plastic pouch application is NZ$0.46 (250ml of formic acid) + NZ$0.10 (pouch) = NZ$0.56.

Labour differs markedly depending on application method. Gel products and the plastic pouch would require 2 visits, with about 5 minutes per hive. Bottom board application has been assessed at 9 minutes per beehive per application (or 36 minutes for 4 applications), while Kramer plates have been assessed at 15 minutes per hive[50].

Impediments to Registration: Formic acid posses potentially unacceptable applicator safety risks, which may affect registration of generic product. However, because the product is also readily available, it is likely to be used by beekeepers despite registration. It is also unlikely that a chemical supply company would progress with registration because of the low cost of the chemical and the inability to recoup registration costs. Nevertheless, generic formic acid has been approved by the Canadian federal authorities, suggesting sufficient data exists to meet the requirements of the New Zealand registration process.

Formic acid in a gel has been approved for use in some US state jurisdictions, which also suggests that sufficient data exists to meet the requirements of the New Zealand registration process should the manufacturer or an importer choose to register the product.

Trade Names: Generic

Active Ingredient: Lactic acid (15%)

Chemical Class: Organic acid

Method of Application: Lactic acid is a contact miticide that must be sprayed directly onto bees. Generally, 5-6ml of 15% lactic acid is sprayed on each comb face using a hand or back-pack sprayer. Two to four treatments per year are recommended[71].

Effectiveness: Lactic acid sprayed onto bees twice in the autumn has been shown to be effective in reducing varroa populations, with mite mortality figures of between 83 and 99%[85; 87; 115], although figures below 80% have also been recorded[66]. In a comparative study, lactic acid killed only 68.5% of mites, compared to formic acid at 91.7%[35]. A spring treatment had an efficacy of 41%[52].

Adverse Effects: Lactic acid sprayed on combs at 8ml/dose caused bees to remove 60% of eggs[86]. In another experiment, bee mortality increased 4-fold for 2 days following treatment, but then returned to normal[84]. However, this may have been related to poor dosage control, since a precise dosage did not produce any adverse effects significantly different than coumaphos treatment, either in the autumn or the next spring[87]. Bee mortality was much lower for lactic acid (1.1 bee/hive/day) compared to formic acid (35.3 bees/hive/day)[55].

Operator Safety: Lactic acid is not considered highly hazardous, although eye or skin contact may cause severe irritation or burns. Safety goggles and chemical resistant gloves should be worn when mixing and applying the product. Unused product should not enter sewers or waterways.

LD50: Rats – 3750mg/kg (oral); 4500mg/kg (dermal)

Residues: Lactic acid residues can rise to 1500ppm in honey stores immediately after application, but decrease to 500ppm within 4 weeks. The average level of naturally occurring lactic acid in honey is 200ppm[133]. Lactic acid is safe to use in the autumn for varroa control, and can be applied in spring until 4 weeks before the beginning of the honey flow[11].

MRL's: Even though lactic acid is naturally present in honey, use of the lactic acid as a miticide can leave taste residues in honey. However, the taste threshold of 800-1600ppm for lactic acid (depending on honey aroma and taste) determined by the Swiss does not exceed the worldwide MRL for organic acid in honey of 40 milli equivalent (meq) acid/kg[11].

Resistance: No reports have been made of varroa developing resistance to lactic acid. It is suggested that varroa will not develop resistance to organic acids since they are natural parts of the metabolism of all organisms and cannot be rendered harmless through enzymatic effects[50].

Cost: Bulk price for lactic acid (99.5%) is NZ$4.15/kg. For spray application, cost per hive (at 15% and 6ml per brood frame side/20 frames) x 2 applications = NZ$0.15. Estimated time necessary for application has been determined at 21 minutes per beehive[50].

Impediments to Registration: Lactic acid does not appear to have been registered as a varroa control product, either in Europe or North America. Nevertheless, it is in widespread use in Europe. Less data exists on lactic acid as a mite control substance than for the other two organic acids. It is also unlikely that a chemical manufacturer would be interested in registering the product because of its low cost as a generic. No proprietary products have been developed using lactic acid.

Trade Names: Generic

Active Ingredient: Oxalic acid (3% - sprayed; 2.1% or 3.2% in sugar syrup)

Chemical Class: Organic acid

Method of Application: Oxalic acid can be sprayed directly on bees at the rate of 3-4ml per comb side. For best results, application should only be made to broodless hives, since the chemical has a direct contact effect on mites on adult bees. Two autumn treatments are generally required.

Oxalic acid can also be mixed in sugar syrup and poured between combs in the autumn, directly on bees. The colony should be broodless, and outside temperature should be above 0^{o}C. The amount of oxalic acid put into the syrup depends on the geographic area (and brood rearing time period), and the amount of syrup depends on the size of colony. In Europe, recommendations suggest 60g of oxalic acid for south Europe (long brood rearing period), 35g for central Europe (medium brood rearing period), and 45g for north Europe (short brood rearing period). The recommendation for feed amount is 25ml for a small colony, 30ml for a normal colony (2 story), and 35 ml for a large colony[49].

Effectiveness: Oxalic acid sprayed on combs has produced mite mortality of 82-99%[69; 114; 47]. Oxalic acid in sugar syrup trickled between combs shows mortality rates of 89-97%[117]. A European study seeking to optimise mite mortality rates and reduce adverse effects from oxalic acid in sugar syrup treatment showed 92.2% efficiency using 3.2% oxalic acid in 60% sugar syrup. 4.2% oxalic acid produced slightly better efficiency, but more adverse colony effects. 2.9ml of syrup/comb was more effective (92% mite kill) than 2.5ml/comb (80%)[116].

Because oxalic acid works through contact, rather than evaporation, ambient temperature is not a critical consideration. Mode of action appears to be the low pH of the oxalic acid solution[116].

Adverse Effects: Long term (4) spray applications of 3% oxalic acid in autumn and spring showed significantly negative effects on brood development and queen survival[62], although other studies did not show such effects[114; 19]. Trials also suggest that some hives show adverse effects from oxalic acid syrup treatment [29].

Operator Safety: Oxalic acid is a corrosive chemical that is potentially hazardous to humans unless proper precautions are taken. Skin and eye contact must be avoided, and eye contamination can cause permanent injury. Overalls, chemical goggles and impervious gloves must be worn when using the product. If a risk of inhalation of the powder exists, a vapour respirator must be used. Unused chemical should be taken to an approved chemical waste disposal facility.

LD50: Rats – 375mg/kg (oral).

Residues: Use of oxalic acid as a mite treatment in autumn did not increase the concentration of oxalic acid in honey the next season[114]. The chemical can therefore be used in the autumn without affecting honey quality[11].

MRL's: Even though oxalic acid is naturally present in honey, use of oxalic acid as a miticide can leave taste residues in honey. However, the taste threshold of 400-900ppm for oxalic acid (depending on honey aroma and taste) determined by the Swiss does not exceed the worldwide MRL for organic acid in honey of 40 milli equivalent (meq) acid/kg[11].

Resistance: No reports have been made of varroa developing resistance to oxalic acid. It is suggested that varroa will not develop resistance to organic acids since they are natural parts of the metabolism of all organisms and cannot be rendered harmless through enzymatic effects[50].

Cost: Bulk price for oxalic acid (99.5%) is NZ$4.13/kg. For spray application, cost per hive (at 3% and 4ml per brood frame side/20 frames) is 10 cents chemical = 10 cents. For syrup application, cost per hive (at 3.2% and 35ml of syrup/hive) is 0.5 cents chemical + 1.5 cents sugar = 2 cents. Estimated time necessary for spray application has been determined at 14 minutes per beehive[50]. It is likely that a feed application would be incorporated in normally wintering-down management, but regardless would take less than 5 minutes per hive.

Impediments to Registration: Oxalic acid does not appear to have been registered as a varroa control product, either in Europe or North America. Nevertheless, it is in widespread use in Europe because of its ease of application, high efficiency and low cost. A substantial amount of data exists on oxalic acid as a mite control substance. However, it is unlikely that a chemical manufacturer would be interested in registering the product because of its low cost as a generic. No proprietary products have been developed using oxalic acid.

Trade Names: Folbex

Active Ingredient: Bromopropylate (chlorbenzilate)

Chemical Class: Chlorinated hydrocarbon

Method of Application: Folbex is a fumigant, and contains bromoprophylate in paper strips. The strips are lit and the resulting smoke distributes particles of the chemical around the beehive. Four applications of one strip at 4 day intervals are recommended. The product should not be used during the honey flow, while surplus honey is on hives, or when the bees are in winter cluster.

Effectiveness: Folbex can be highly effective (>90%) in killing mites[130; 93] although efficacy levels are variable[81]. Folbex was less effective than Apistan (74% vrs. 97%) when brood was present[77]. Folbex killed 90% of mites when the colonies were broodless, but only 55% when brood was present[2].

Adverse Effects: Use of Folbex did not cause adverse effects on queen lifespan, brood area or honey production[7]. Folbex did not affect brood survival compared to controls[4].

Operator Safety: Likely to be low risk, although smoke from the product should probably not be inhaled. Chronic skin exposure to chlorobenzilate may cause inflamed skin, rashes and conjunctivitis.

LD50: Rats – 2784-3880mg/kg (oral); >10,000mg/kg (dermal). Chlorobenzilate is a "Restricted Use Pesticide" in the US, based on its ability to cause tumours in mice and its effects on the testes of rats.

Residues: Bromopropylate is a non-volatile fat soluble compound that accumulates in wax and can transfer to honey. Bromopropylate was present in 22 of 50 samples of German honey in 1987[89]. Although the product was discontinued 8 years previously, 11% of German honey samples tested in 1996 had levels between 0.002 and 0.01ppm. German beekeepers voluntarily discontinued use of the product because of residue concerns[140].

Bromopropylate residues accumulate in brood comb wax and were more than twice as high in colonies treated once a year for 5 years than in colonies treated only once[12]. High levels (56.9ppm) were found in brood comb wax compared to fluvalinate and coumaphos. The chemical was also found in recycled beeswax even after its use was discontinued. The half-life of the product as a residue in wax was calculated at 4 years[10]. Beeswax samples taken 8-9 months after 4 treatments of Folbex showed bromopropylate levels of 25-86ppm. In Denmark, the permitted level of bromopropylate residues in fruit and vegetables is 1-3ppm[58].

MRL's: Official maximum residue levels for bromopropylate in honey range from 0.01ppm in Italy to 1ppm in Germany and Switzerland. No level has been established for the EU or the US[140]. No MRL's have been established in beeswax.

Resistance: Gradual resistance to bromoprophylate was noted in nucleus colonies continuously treated with small doses of the product under laboratory conditions[127].

Cost: According to the European distributor, old stocks of Folbex are sold at a discounted price of NZ$1.43 per full treatment (4 strips), but only in a few selected countries in Asian markets. Labour costs are greater than for strip and liquid applications, since 4 visits are required. Labour required is at least 5 minutes per application, or 20 minutes total.

Impediments to Registration: Because of significant residue problems and possible food safety issues, it may be difficult for bromoprophylate to meet New Zealand pesticide registration requirements as a varroacide. Folbex does not appear to be registered for use in either the EU or the US.

Other Treatment Options Not Considered Due to Lack of Efficacy Data

Vegetable Oil: Vegetable oil has been used as a varroa control. The substance is administered as vegetable shortening in a sugar patty placed on the top bars of the hive. The vegetable oil generally has an emulsifier added.

Vegetable oil patties have been shown to be an effective treatment for tracheal mite, and laboratory tests using vegetable oil on filter paper showed the material was effective against varroa on worker bees. Field trials, however, showed a mite mortality rate of 38% [88]. Vegetable oil with high levels of an emulsifier, either sprayed or administered as patties, killed up to 97% of mites, but also had a side effect of significant die-off of bees (>50%). Lower concentrations of the emulsifier were not effective in killing mites[18]. Canola oil spray (20% solution) killed 65% of varroa[141].

Mineral Oil: Mineral (paraffin) oil with an emulsifier (5% Tween) has been used for varroa control. The material is sprayed at the rate of 6-10ml of oil per frame in autumn, once a week for 3 weeks.

Mineral oil with an emulsifier (Tween at 5%) sprayed at this rate resulted in 97% bee mortality after two applications, and 99.5% mortality after three. However, bee mortality was significantly higher than controls when mineral oil and emulsifier was sprayed on combs[90].

Neem Oil: Neem oil with an emulsifier (2% Tween) has been used for varroa control. The substance is sprayed at the rate of 400ml per hive, with the frames slightly parted and lightly sprayed with oil.

Neem oil applied to honey bees in the laboratory killed 50-90% of varroa mites within 48 hours, with less than 10% bee mortality, but was less effective than Apistan[105]. Neem oil killed 84-96% of varroa mites, three times more than the control group, and more than sprays of thymol or canola oil, or thymol in a vermiculite block. However, neem oil resulted in 50% queen loss and 2/3's loss of bees and 1/3 loss of brood compared to controls[141]. Neem oil was as effective as formic acid, but not as effective as Apistan, and reduced sealed brood in colonies by 50%[104].

Wintergreen Oil: Wintergreen oil has been used for varroa control. The oil is sprayed on frames of broodless hives at the rate of 5ml per hive. Evaporation of 15ml of wintergreen oil killed 95% of varroa mites and caused 7% bee mortality in laboratory experiments. Field trials of wintergreen oil sprayed on frames, together with a 15 minute heat treatment, killed 55-82% of mites, with a second treatment 7 days later killing 90-95% of mites[65; 64]. Another trial using similar treatments resulted in 31% mortality of adult bees after 5 days and 69% reduction in mites[20].

Icing Sugar: Icing sugar has been trialled for use as a varroa control. The sugar is dusted between the combs of hives at a rate of 15g per hive (2 storey). The treatment is repeated 4-6 times. Icing sugar has proved to be an effective means of surveying bees for varroa mites[37]. Various powders, including glucose, wheat flower and stone dust have been trialled, either as a detection or control method [123; 96; 124]. A field trial used 15g of sugar dusted between combs of hives during mid-summer, at varying combinations of days and times. The average mite fall on the treated colonies was between 47 and 56 times greater than the control colonies, although the exact efficiency could not be determined[41].

Table 2 Information summary – varroa control products

Trade Names	**Active Ingredient**	**Control Efficacy**	**Treatment Period**	**Adverse Effects**	**Residue Problems**	**MRL's (ppm)**		**LD50 (oral)**	**Operator Safety**	**Resistance**	**Cost**	
						Honey	Wax	mg/kg			Materials	Visits
Apiguard; generic	Thymol	54-98%	2x2 weeks	Few, if any	Honey taste	0.8	None	980	Skin irritant	None	$8.11	3; 2-4
ApilifeVAR	Thymol Eucalyptol, Menthol, camphor	70-90%	2x3-4 weeks	Over-wintering problems	Honey taste	0.8	None	980 2480 3300 2000	None	None	$6.43	3
Apistan	Fluvalinate	95-99%	42 days	Drones, queens	Honey, wax, propolis	0.01-0.05	6	261-281	Low risk, toxic to fish	Common	$7.20	2
Apitol	Cymiazole	83-98%	2x1 week	Brood food glands	None	0.01-1	None	725	Low risk	None	$7.02	2
Apivar	Amitraz	90-99%	6 weeks	Larvae and adult mortality	None	0.01-1	None	523-800	Low risk	Cross-resistance	$8.10-14.95	2
Bayvarol	Flumethrin	95-99%	6 weeks	None	Propolis	0.005-0.01	None	258	Low risk	Cross-resistance	$7.00	2
Checkmite+; Perizin	Coumaphos	85-99%	6 weeks; 2x1week	Some bee death	Honey, wax	0.01-0.05	100	13-41	Low risk	Reported	$7.60; $7.91	2; 2
Folbex	Bromopropylate	55-90%	4x4 days	None	Honey, wax	0.01-1	None	2784-3880	Skin rashes	In lab	$1.43 (Asia)	4
Generic; various	Formic acid	61-98%	Various	Drones, brood, adults	Honey taste	150-160	None	1100	Corrosive; combustible	None	$0.16-14.54	2-6
Generic	Lactic acid	41-99%	2-4/year	Possible egg loss	Honey taste	800-1600	None	3750	Skin irritation	None	$0.15	2-4
Generic	Oxalic acid	82-99%	At autumn	Reduced brood in spring	Honey taste	400-900	None	375	Corrosive	None	$0.02-0.15	1-2

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