SUMITOMO CHEMICAL

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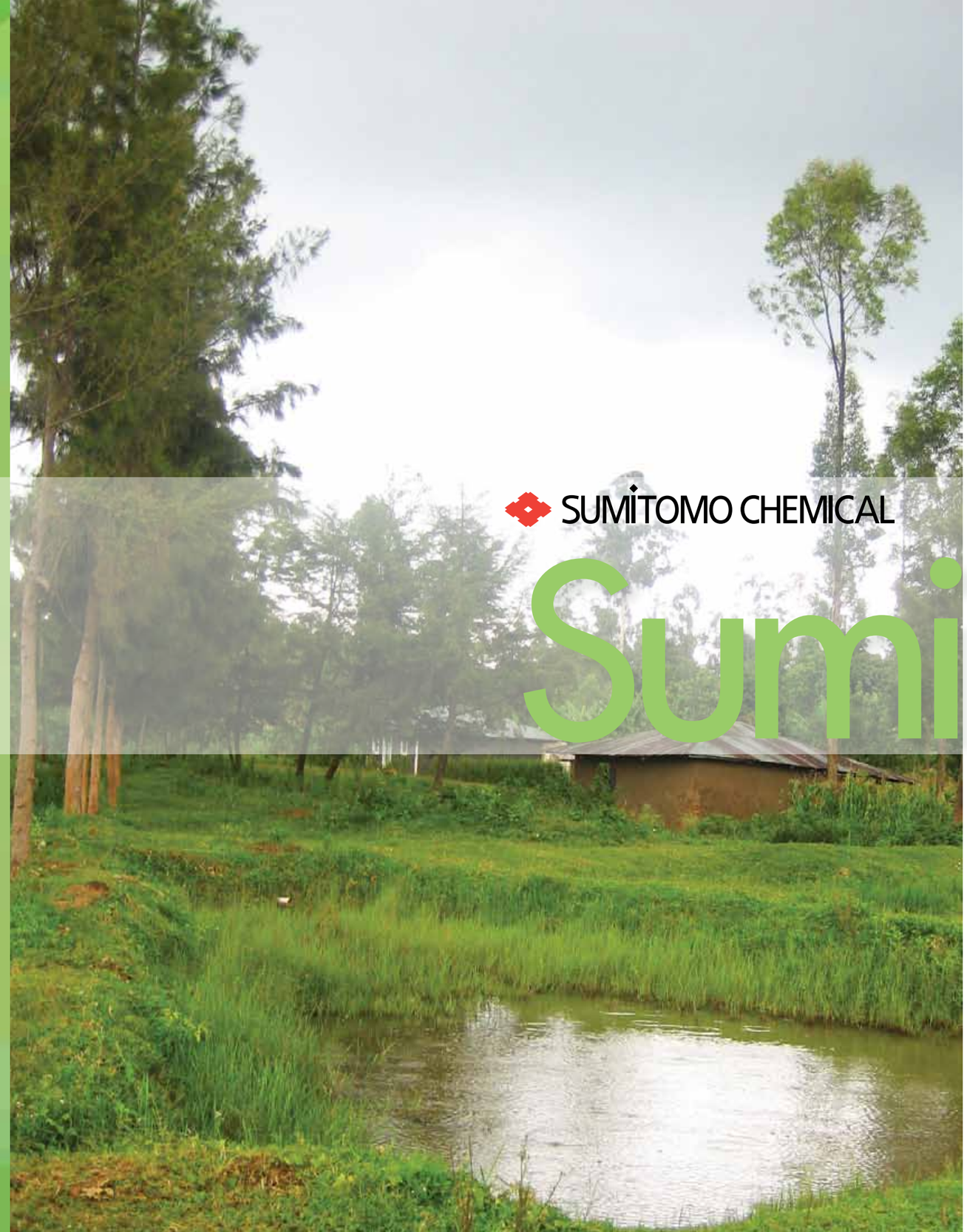


SUMITOMO CHEMICAL

SumiLarv®

Technical Information

SumiLarv is a novel mosquito larval control agent based on the insect growth regulator pyriproxyfen. It was invented and developed by Sumitomo Chemical Co. Ltd.

Contents

Introduction and Background

Control of vector borne disease has become a major priority, with malaria receiving the main focus of attention and funding. However there are many other mosquito borne diseases such as Dengue, Japanese encephalitis, and newly emerging problems such as the Chikungunya virus which has recently spread from Africa into southern European countries.

Currently malaria vector control is very much focussed on the use of Long Lasting Insecticidal Nets (LNs) and Indoor Residual Spraying (IRS). While these interventions can have a significant impact, neither will give total control. For example it has been shown that with LNs 80% coverage is required to achieve a 30% reduction in malaria incidence. Although this is a very significant impact there remains the opportunity to increase it.

Both IRS and LNs only target adult mosquitoes and rely on chemical compounds - largely pyrethroids. Insecticide resistance is increasing in Africa and is a concern for the future effectiveness of these products – the cause of this resistance is unclear but pyrethroid use in agriculture is suspected.

The use of larvicides which are non-chemical such as SumiLarv® will give additional control and target mosquitoes that are resistant to chemical insecticides.

Integrated control has largely been forgotten – it can be used in resistance management and gives additional control to programmes using insecticide treated bednets.

Several scientists have published papers supporting this viewpoint:

1. Trials were conducted in Kenya to demonstrate the additional value of larviciding when integrated with the use of Insecticide Treated nets (ITN`s). The annual rate of 10-12 malaria infectious bites (Entomological Inoculation Rate – EIR) was reduced to 1.68 with the widespread use of ITN`s. Where additional larviciding was carried out this dropped further to 0.39 infectious bites per annum, accounting for an additional 73% reduction in EIR (Fillinger, U. et al., Bulletin World Health Organization; 87:655-665, 2009).
2. Vector control in Africa should target all stages of the mosquito life cycle, yet for the past 50 years it has focussed almost exclusively on adult mosquito control, however, adult based methods are limited in what they can achieve. Larval source management should contribute to greater reductions in transmission than ITN`s alone. Truly integrated and well-managed malaria control efforts have led to major successes in the past, including eradication of *Anopheles gambiae* from Brazil and Egypt, and the eradication of malaria in the United States, Europe and the Middle East, yet these successes have largely been forgotten or dismissed. (Fillinger, U. et al., Bulletin World Health Organization, 87:655-665, 2009).
3. Larval control has long been an option for malaria control, but we suggest that this is an underdeveloped and underused approach, particularly in tropical Africa where it may be of much greater utility than generally appreciated. We suggest that larval control should be integrated with more commonly used approaches such as improved access to screening and treatment, bednets or indoor spraying, (Killeen et al., October 2002, The Lancet 2).
4. Rural communities are more vulnerable to malaria infection thus calling for additional methods to complement personal protection methods for vector control. Targeting the larval stages of malaria vectors is an underutilized malaria prevention measure. (Imbahale et al., Acta Tropica 115, 248 -256, 2010).

Dengue is becoming increasingly important, as it is typically an urban disease and as towns and cities have grown so has Dengue. Latest estimates are that there are between 50 and 100 million dengue infections and 500,000 dengue haemorrhagic fever (DHF) cases per year. While not normally fatal dengue is still responsible for over 20,000 death worldwide a year, which are mostly children. It is estimated that 2.5 billion people live in areas where this disease is present. (http://www.who.int/mediacentre/factsheets/fs117/en/).

Vectors of Dengue such as *Aedes aegypti* are mainly peri-domestic and readily controlled with larvicides. In Africa, it has also been demonstrated that much of the breeding of *anopheline* mosquitoes is within villages, with as many as 80% of the larval breeding sites being man-made and within 100 metres of human habitation. This makes treatment of these larval sites both practical and effective in reducing the number of vectors.

In addition, newly emerging vector borne diseases such as Chikungunya and West Nile Virus, while not usually fatal, can be very debilitating and patients may be unable to work for extended periods.

The mainstay of larviciding for many years has been temephos, however it is an acetylcholinesterase inhibitor, thus prolonged exposure, accidental ingestion or misapplication can be hazardous. Resistance to temephos is now widespread. As a result temephos is being phased out in USA and Europe. (Sihuincha, M. et al., Journal Medical Entomology, 42(4):620-630, 2005).

In summary, there is a renewed case for the use of larviciding in vector control, but the larvicide selected should be effective and safe with ideally no cross resistance to current insecticides.

Product Concept and Mode of Action

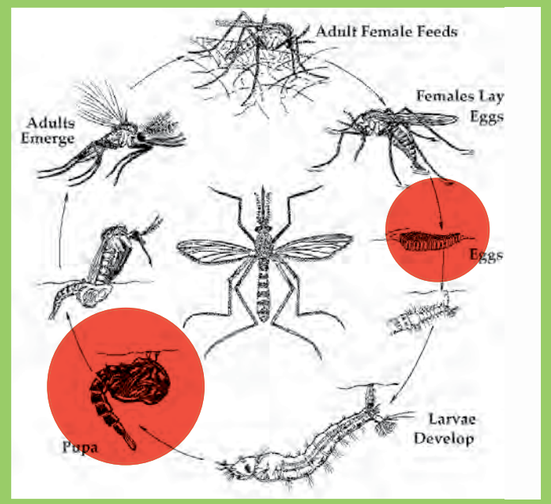
SumiLarv is a novel mosquito larval control agent based on the insect growth regulator (IGR) pyriproxyfen. It was invented and developed by Sumitomo Chemical Co. Ltd.

It does not directly kill mosquito larvae, but disrupts the normal process of insect development at the fourth instar larva to early pupa stage which results in pupal mortality and prevention of adult emergence. The 4th stage larval and pupal stages are the most susceptible to pyriproxyfen (Y.Kono et al., Medical Entomology & Zoology, 48(2), 85-89, 1997).

Since early stages of larvae do not die after treatment, users need to adjust to the concept of the product; although larvae can still be seen after Sumilarv application they will normally die at the pupal/ adult moult stage thus preventing the emergence of adult mosquitoes.

IGR`s are unique in that they are specific for insects and have very low mammalian toxicity. As such pyriproxyfen has received U.S. EPA status as a reduced risk insecticide and an organophosphate alternative and is approved by the World Health Organization for treatment of potable water against mosquitoes. (Sullivan, J.J. & Goh, K.S., Journal Pesticide Science, 33(4) 339-350, 2008).

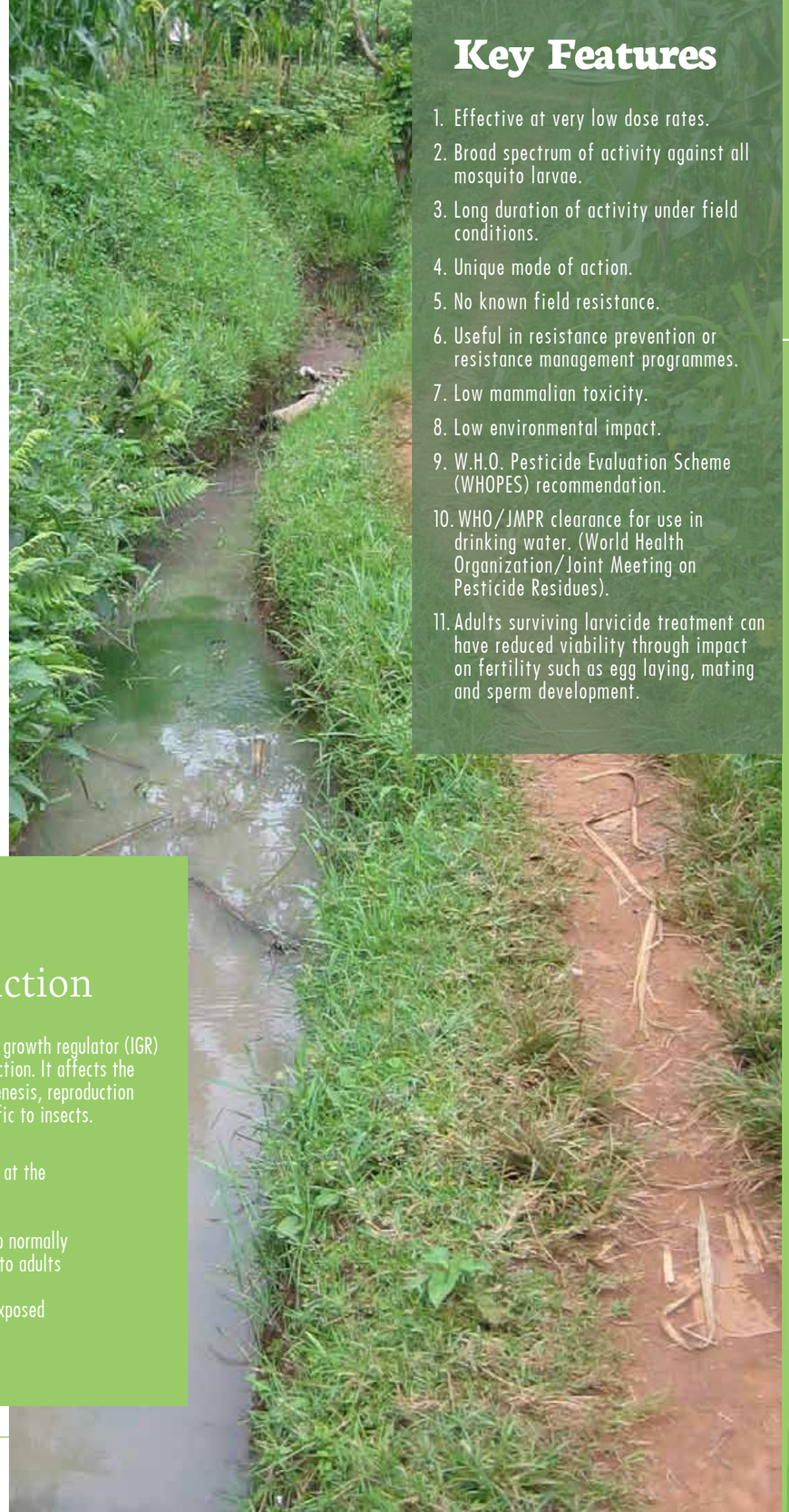
Following application to the aquatic breeding sites of mosquitoes there will be a rapid impact on the adult mosquito population, reducing biting rates and disease transmission.



SumiLarv Mode of Action

Pyriproxyfen is an insect growth regulator (IGR) with a unique mode of action. It affects the physiology and morphogenesis, reproduction and embriogenesis specific to insects.

- Inhibits the life cycle at the pupal/adult moult
- Larvae/pupae develop normally but cannot develop into adults
- Affects viability of exposed female eggs



Key Features

1. Effective at very low dose rates.
2. Broad spectrum of activity against all mosquito larvae.
3. Long duration of activity under field conditions.
4. Unique mode of action.
5. No known field resistance.
6. Useful in resistance prevention or resistance management programmes.
7. Low mammalian toxicity.
8. Low environmental impact.
9. W.H.O. Pesticide Evaluation Scheme (WHOPES) recommendation.
10. WHO/JMPR clearance for use in drinking water. (World Health Organization/Joint Meeting on Pesticide Residues).
11. Adults surviving larvicide treatment can have reduced viability through impact on fertility such as egg laying, mating and sperm development.

SumiLarv Effects On
Culex quinquefasciatus Larvae/Pupae

Impact on Egg Laying

The Impact on Egg Laying in Treated Water, Impact on Survivors, and Potential Transfer Effects of the Active Ingredient

Some larvicides can impact water quality, making them less attractive to adult mosquitoes laying their eggs. This can result in the adults laying eggs in alternative water bodies which are untreated. The following scientific studies demonstrate that pyriproxyfen has no impact on adult oviposition sites.

1. Studies conducted in Thailand analysed the effect of pyriproxyfen on the oviposition response of gravid *Aedes aegypti.* It was found that adults appear to lay more eggs in pyriproxyfen treated containers versus non-treated containers. Additionally results from the larval bioassays provided evidence that gravid adults can horizontally transfer pyriproxyfen from treated water to a non-treated container at levels that can effectively inhibit adult emergence over the long-term. (Ponlawat, A.,et al., Proceedings of the 5th International Congress of Vector Ecology, October, 2009).
2. In a trial in Iquitos it was found that water treated at extremely high pyriproxyfen concentrations of >30,000 ppb (x600 maximum label dose rate) were as likely to be used as oviposition sites as untreated sites (Sihuincha, M. et al. Journal of Medical Entomology Vol. 42, no.4, 620-630, June 2005).

Adult mosquitoes coming in contact with pyriproxyfen or adults surviving pyriproxyfen treatment are effected in the viability, egg laying, egg hatching and larval survival of any hatched eggs. The following papers give examples of this:

1. Some researchers have discovered that pyriproxyfen treated water will have an impact on the hatching of mosquito eggs and the subsequent viability and survival of any larvae that do hatch. Therefore the impact goes beyond direct larviciding (Vasuki, V. Proceedings Indian Academy of Science, 99(6), 477-482, 1990).

Adult mosquitoes which as larvae survived 48 hour immersion in water at 0.005 ppb during their last instar showed considerable reduction in sperm and egg production and also in blood feeding and copulating activity. (Iwanaga, K & Kanda, T. Applied Entomology, 23(2) 186 -193, 1988).


Untreated Larva


Treated Larva


Untreated Pupa


Treated Pupa


Half emerged adult from treated pupa

Application Rates

SumiLarv is commercially available as a 0.5% sand based granular formulation. SumiLarv 0.5G may be applied by means of a measuring spoon (1 teaspoonful = approx. 2 g), or by hand using granule spreaders or knapsack blowers (correctly adapted).

Usage

The choice of dose rate will depend on the level of pollution/organic matter in the water. The lowest dose will give a long duration of efficacy when applied in clean drinking water in vessels stored around houses. The dose rate selected will depend on water quality, e.g., the highest dose rate should be used in septic tanks or ditches where levels of organic pollution are high and the lowest dose in clean drinking water.

Practical Example of Correct Application

For 0.01 ppm a.i., use 2 g (approximately one tea spoonful) of SumiLarv 0.5G per cubic metre of water volume (1000 litres).

For 0.05 ppm a.i. use 10 g (approximately 5 tea spoonfuls) of SumiLarv 0.5G per cubic metre of water volume (1000 litres).

Calculate the approximate volume of water to be treated.

Target water volume (m^3) = Surface area (m^2) x average depth (m).

Frequency of Application

The required frequency of application will depend on three factors: dose rate, degree of pollution of water treated and dilution (caused by rainfall or removal and replacement of water in storage containers).

Generally repeat treatment every 4 – 6 weeks but with drinking water container mosquitoes such as *Aedes aegypti* re-treatment may only be needed every 3 months. A good larval control team should determine the correct dose rate and remove any pupae from time to time and take them back to the laboratory to determine if adult emergence takes place. If pupae emerge then it is time to re-treat.

Note: the presence of actively swimming larvae after a SumiLarv 0.5G treatment is normal and should not be taken as an indication of failure - SumiLarv does not act until the pupal stage.

When treating ditches/pools etc. try to ensure that the granules are distributed as evenly as possible over the area. The treatment of water containers requires only a few granules with the dose applied being based on the volume of the container. It is normal that householders will remove and replenish water in these containers regularly. This is however not a problem since studies have shown that when applied according to label recommendations, SumiLarv 0.5G continues to work even when significant amounts of water are regularly removed and replaced with fresh water (Vythilingham et al., Journal of American Mosquito Control, 21(3)296-300, 2005).

Information for Householders

After application to water storage jars, householders should be instructed to not completely empty and wash out their containers, as the granules will then be lost. In addition householders should be advised that SumiLarv treatments will not taint water, and will have no adverse effects on water quality. The success of such treatments relies on an efficient campaign to identify and treat every container. Householders should also be encouraged to remove unused pots, tires and any other items in which water can collect in the vicinity of their house which could act as mosquito breeding sites.

Field Usage

Small Scale Usage

Water type	Product per 1000 litres	mg/ai/litre	Dose in ppm	Dose in ppb
Clean & drinking water	2 g	0.01	0.01	10
Dirty	4 g	0.02	0.02	20
Polluted	10 g	0.05	0.05	50

Large Scale Usage

Depth of water (cm)	Sumilarv 0.5G/kg/ha*
10	2-10
20	4-20
30	6-30
50	10-50

* Dose selected should be based on water quality, see above.

Comparative Efficacy of SumiLarv to Other Larvicides

SumiLarv is one of the most active larvicides on the market today. This means only small quantities need to be transported in order to treat larval sites. This helps make SumiLarv very cost effective. Some comparisons with other larvicides are shown below. These do vary according to the species, however it is clearly shown that pyriproxyfen is usually the most active.

1. A comparison of the most popular larvicides was conducted in the laboratory using field collected *Culex quinquefasciatus* larvae. Five to nine different concentrations of each larvicide or IGR was tested at least 3 times. Results for IGR`s were measured over 7-10 days after application due to their different modes of action compared to chemical larvicides.

Results are shown in Table 1 and demonstrate the very high relative toxicity of pyriproxyfen, the active ingredient in SumiLarv, to *Culex quinquefasciatus* larvae. For example, pyriproxyfen was shown to be 118 times more active than fenthion, 3 times more active than diflubenzuron and 39 times more active than methoprene. (Arshad, Ali. et al., Journal American Mosquito Control Association 15(1), 43-47,1999).

2. SumiLarv`s activity varies according to species and strain of mosquito. However a study looking at four different anopheline species (a total of 8 strains) of which some were resistant to DDT, dieldrin, malathion, organophosphates and carbamates, showed that pyriproxyfen was more effective than methoprene and diflubenzuron against all strains. Relative to methoprene, pyriproxyfen was shown to be up to four times as active, while pyriproxyfen activity ranged between 19.5X > 470X that of diflubenzuron. See Table 2. (Kawada, H, et al., Japanese Journal of Sanitary Zoology 44 (4) 349-353, 1993).

3. Three species of mosquito were tested in the laboratory against pyriproxyfen (referred to as S-31183) to determine the relative potency for inhibition of emergence (IC50). Pyriproxyfen was the most active compared to the IGR methoprene, a chitin synthesis inhibitor, diflubenzuron and the organophosphate temephos. See Table 3. (Makoto Hatakoshi et al., Japanese Journal of Sanitation & Zoology, 38(4), 271-274, 1987).

Pyriproxyfen vs. Spinosad

These two larvicides were compared in the laboratory and pyriproxyfen was found to be 500 times more active than Spinosad, a bio insecticide.

Pyriproxyfen LC50 0.0001 mg/L and LC95 0.00032 mg/L

Spinosad LC50 0.055 mg/L and LC95 0.20 mg/L

(Darriet, F. and Corbel, V. Journal of Medical Entomology 43(6): 1190 – 1194, 2006).

IGR	Class of insecticide	LC 90	Relative toxicity*	Type
Pyriproxfen (SumiLarv)	IGR	0.0011	118.2	IGR
Diflubenzuron	IGR	0.0034	38.2	IGR
Methoprene	IGR	0.052	2.5	IGR
Temephos	OP	0.0096	13.5	OP
Fenthion	OP	0.130	1	OP
Permethrin	Pyr	0.017	7.6	Py

** The higher the number the greater the activitey of the larvicide*

Table 1 Relative toxicity of pyriproxyfen to *Culex quinquefasciatus* larvae compared to other larvicides

Species	Strain	Pyriproxyfen	Methoprene	Diflubenzuron
An.stephensi	Sus	0.043	0.54 (x 12.6)	0.84 (x 19.5)
	MLT-R	0.025	0.75 (x 30)	1.6 (x 64)
An.gambiae	Sus	0.025	0.067 (x 2.7)	3.7 (x 148)
	DLD-R	0.0098	0.039 (x 3.98)	0.87 (x 88.8)
	DDT-R	0.0040	0.072 (x 18)	1.9 (x 475)
An.albimanus	Sus	0.016	0.16 (x 10)	0.70 (x 43.8)
	OPC-R	0.0042	0.016 (x 38.1)	0.2 (x 476.2)
An. farauti		0.0017		

Table 2. IC 50 values in ppb of IGRs against last instar of anopheline larvae and factors of additional product required vs.pyriproxyfen (in brackets)

Compound	*Cx. p. pallens*	*An. Stephensi*	*Ae. Aegypti*
S-31183 Pyriproxyfen	0.0046	0.043	0.023
Methoprene	0.013 (x2.8)	0.54 (x12.6)	0.77 (x33.5)
Diflubenzuron	0.37 (x80.4)	0.84 (x19.5)	0.6-0.8 (x26)
Temephos	1.7 (x370)	2.7 (x62.8)	4.5 (x196)

Table 3. Inhibition emergence of 4th instar larvae IC50 ppb and factors of additional product required vs. pyriproxyfen (in brackets)

Biological Efficacy

There are many papers on the biological activity of pyriproxyfen. A few have been abstracted below to provide independent evidence of activity. While there are many papers on pyriproxyfen, papers have only been selected where the dose rates used were within or below label recommended application rates.

Laboratory Trials

1. Two granular formulations of IGRs s-methoprene and pyriproxyfen were evaluated against 5 colonized species of mosquitoes – *Aedes aegypti, Aedes albopictus, Aedes taeniorhynchus, Anopheles quadrimaculatus* and *Culex nigripalpus.* Each IGR was applied at 0.02 and 0.05 ppm a.i. While *Culex quinquefasciatus* was exposed at 0.2 ppm and 0.04 ppm a.i. The s-methoprene resulted in variable levels (<39 -100%) of inhibition of adult emergence. Pyriproxyfen at comparable treatment rates to s-methoprene caused very high levels (80 -100%) of initial and residual emergence inhibitions of the tested species. In several species pyriproxyfen induced complete inhibition of adult emergence for several weeks after treatment even at the lower rate of 0.02 ppm. This study clearly demonstraits the superior activity of pyriproxyfen over s-methoprene, on the basis of equal concentrations of the active ingredient against a wide variety of mosquitoes in the laboratory and experimental tubs placed outdoors. (Nayar, J.K. at al., Journal of American Mosquito Control Association,18(3):196-201, 2002).

2. A study was conducted to evaluate the efficacy and persistence of pyriproxyfen at 0.01 and 0.05 ppm on the larvae of *Aedes aegypti, Culex quinquefasciatus and Anopheles albimanus* under laboratory conditions.

   Mortality of all three species was higher among pupae than larvae. Inhibition of adult emergence at 0.05 ppm was 100% for both *Culex quinquefasciatus* and *Anopheles albimanus* throughout the evaluation (16 weeks), while values for *Aedes aegypti* varied from 94.1-100%.

   At 0.01 ppm percentage inhibition of adult emergence in *Culex quinquefasciatus* was 86.6 -100% with corresponding values for *Anopheles albimanus* and *Aedes aegypti* being 82.7 -100% and 76-100% respectively.

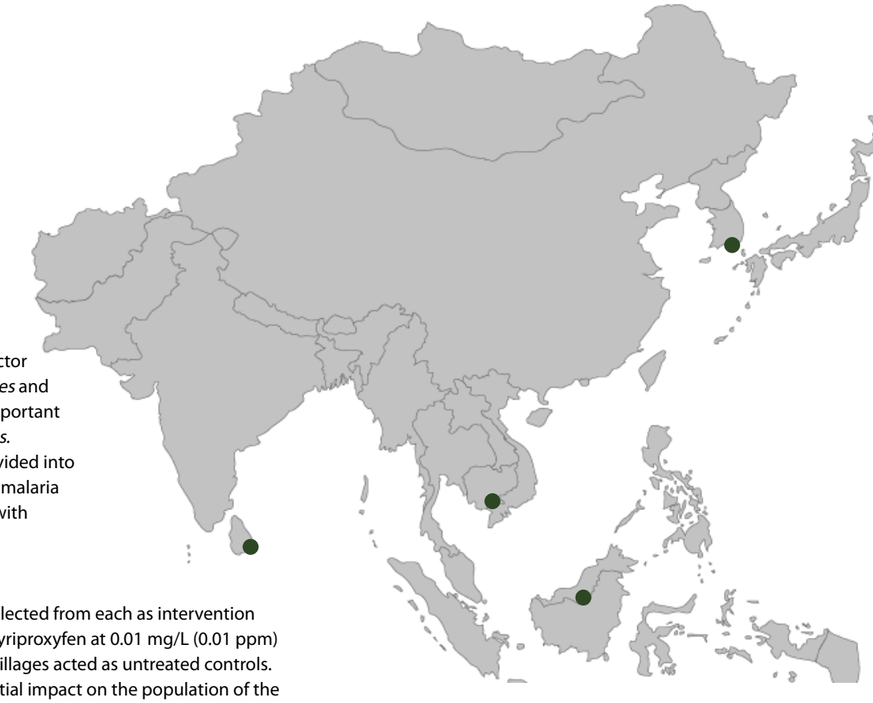
   At 0.05 ppm pyriproxyfen inhibited adult emergence 100% throughout the 16 week evaluation. An additional bioassay carried out on *Anopheles albimanus* at 24 weeks post-treatment showed that total mortality and inhibition of emergence remained at 100%. (Pinzon, MLQ, et al., Final Report, Program de Estudio y Control de Enfermedades Tropicales PECET, Linea de Entomologia, Universidad de Antioquia, Colombia, 2003).

"SumiLarv is one of the most active larvicides on the market today."

Field Trials

Sri Lanka

1. Hand dug gem pits and river bed pools in Sri Lanka are a problem as they create breeding sites for *Anopheles culicifacies* and *Anopheles subpictus*. A small scale field trial was conducted to evaluate SumiLarv 0.5G at a dose rate of 0.1 mg/L (0.01 ppm). This dose rate inhibited adult emergence between 60 – 140 days. It was recommended that pyriproxyfen would only require application twice a year whereas temephos would require 12 applications and hence considerable cost savings could be made. (Yapabandara, A. & Curtis, CF., Acta Tropica, 8,1211-223, 2002).
2. A further study was conducted in eight villages in Sri Lanka where there were many shallow pits dug by gem miners that fill with water. These become breeding places for the main malaria vector *Anopheles culicifacies* and the second most important *Anopheles subpictus.* The villages were divided into 4 villages with high malaria transmission and 4 with lower transmission.



Two villages were selected from each as intervention areas treated with pyriproxyfen at 0.01 mg/L (0.01 ppm) and the remaining villages acted as untreated controls. There was a substantial impact on the population of the two main mosquito vectors compared to the control villages, see **Fig.1**. There was also a reduction in malaria incidence in the population of 85.6% in *P. falciparum* and 57% in *P. vivax,* see **Fig. 2.** (Yapabandara, A. et al., *Acta Tropica* ,80:265-276, 2001).

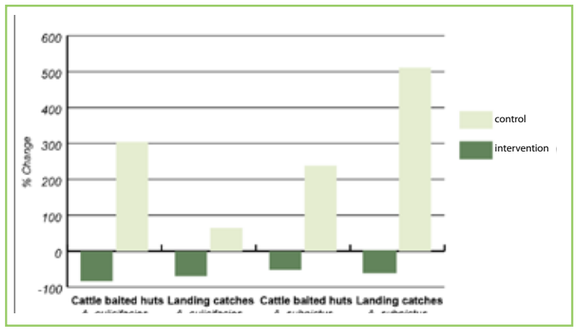


Fig. 1 Impact of pyriproxyfen on mosquito populations using two trapping methods

Malaysia

Trials were conducted in Malaysia against *Aedes aegypti* using 60 litre earthenware storage jars. To simulate actual usage 20% of the water was replaced every 2 weeks. Two dose rates of SumiLarv 0.5G were used - 0.01 ppm a.i. and 0.02 ppm a.i.Inhibition of emergence of adult mosquitoes was achieved for 4 months at both dose rates. See **Fig. 3.** Additional tests with *Aedes albopictus* (Skuse) showed similar levels of efficacy. (Vythilingam, I. et. al, Journal American Mosquito Control 21 (3), 2005).

Cambodia

Concrete domestic water storage jars are a common larval habitat for *Aedes aegypti* in Cambodia. 200L Jars of were treated with an experimental SumiLarv resin chip formulation. Efficacy was good for up to 6 months with the higher dose rates inhibiting emergence of adult mosquitoes by >87%. Monthly removal and replacement of two thirds of the water in the 27 ppb treatment did not reduce efficacy when compared with the 28 ppb treatment rates, with good levels of emergence inhibition maintained for 6 – 7 months following treatment. See **Fig. 4**. (Chang Moh Seng et al., Journal of the American Mosquito Control Association 22(1):152-154, 2006).

South Korea

SumiLarv 0.5G was evaluated for inhibition emergence of *Aedes togoi*, (a vector of malayi filariasis) in brackish water rock pools, at 0.01ppm and 0.05ppm. The lower dose gave >79% emergence inhibition up to 51 days. By 70 days it had fallen to 61%. At the higher dose rate of 0.05 ppm 100% emergence inhibition was achieved up to 70 days (except at after 51 days when it fell to 75%). There was high rainfall throughout the trial causing regular dilution of the pools, however this did not adversely affect the long term efficacy of the product. See **Fig. 5**. The author of this paper concluded that SumiLarv may offer excellent potential for the control of *Aedes togoi* with long term residual activity in mosquito breeding sites, even in brackish water. (Dong-Kyu Lee, Journal of Vector Ecology, June 2001).

In another trial in Korea it was found that SumiLarv provided the greatest initial and residual activities against *Culex pipiens pallens* larvae in marshes and ponds with sewage water. The pyriproxyfen inhibited adult emergence for at least 2 months at a concentration of 0.05 mg/l (0.05 ppm). There is sufficient evidence that pyriproxyfen may offer excellent residual activity in mosquito breeding sites even in sewage water (Lee, Dong-Kyu, Korean Journal of Entomology, 32(1):37-41, 2002).

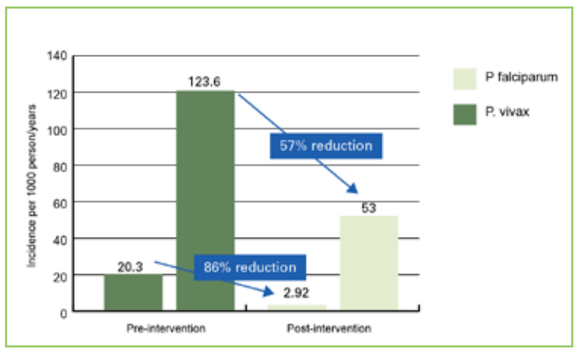


Fig. 2 Comparison of Malaria Incidence Pre and Post Intervention

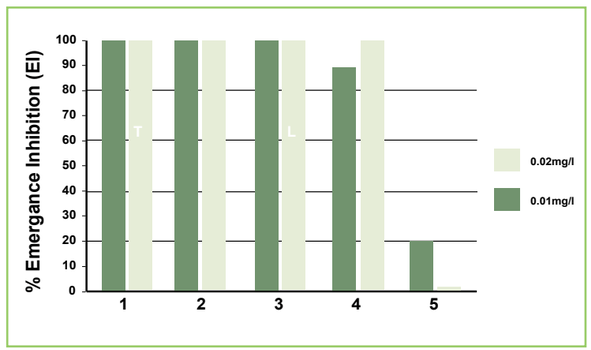


Fig.3 Evaluation of SumiLarv 0.5G against *Aedes aegypti* - Malaysia

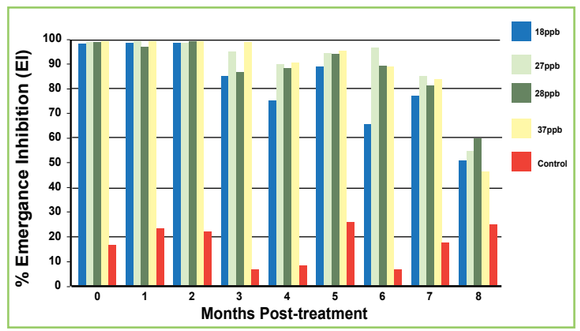


Fig. 4 Inhibition of Adult Emergence of *Aedes aegypti* using SumiLarv 0.5G - Cambodia

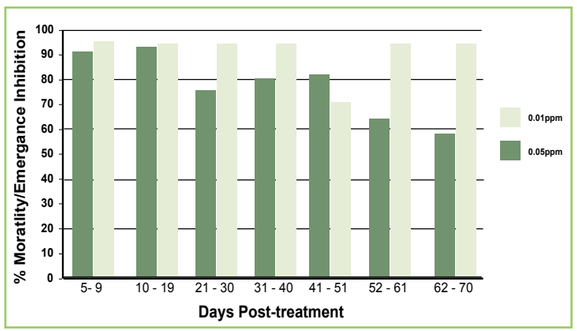


Fig. 5 Average % mortality of *Aedes togoi* in brackish rock pools using pyriproxyfen 0.5% granules

Efficacy Against Chironomids

The increased number of man-made aquatic ecosystems has resulted in a rise in the numbers of aquatic chironomid midges in many regions of the world. Adult midges cause nuisance problems, human allergies, and can have a severe local economic impact.

Laboratory bioassays of SumiLarv 0.5G were conducted against late instar larvae of the nuisance chironomid *Polypedilum nubifer*. Results demonstrated that 0.01 ppm SumiLarv caused a 90% inhibition of emergence of this species. A field trial was then conducted using in situ enclosures at the same dose rate. SumiLarv significantly reduced the emergence of *Polypedilum nubifer* and another chironomid, *Kiefferulus intertinctus* (Skuse), for 24 days. These results show that SumiLarv can provide a satisfactory alternative to the organophosphate larvicides that are currently in use. (Trayler K.M. et al. Journal of the Australian Entomological Society; 33 (2). 127-130. 1994).

Resistance and Resistance Management

The emergence of insecticide resistance in vector mosquitoes presents a significant challenge. Nearly all chemical compounds are now resisted to varying degrees in mosquito species around the world.

"Successful resistance management depends on reducing the selection pressure exerted by a particular mode of action or chemistry on a population" (ref. Prevention and Management of Insecticide Resistance in Vectors and Pest of Public Health Importance. IRAC 2011).

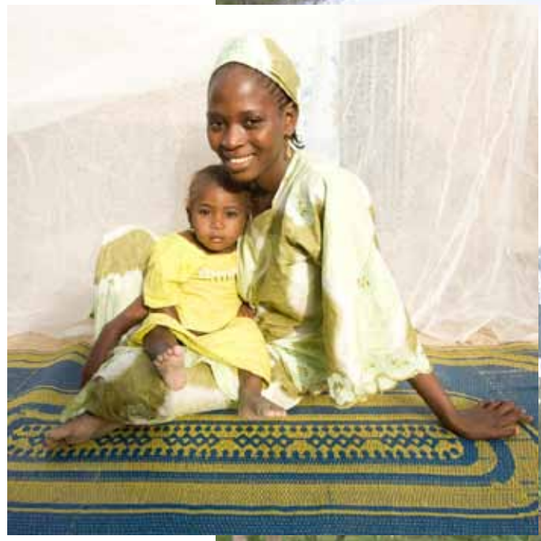
In order to reduce this selection pressure, the use of the same chemical class against both adult and larval insect stages should be avoided.

SumiLarv has a totally different mode of action to chemical adulticides and to all other available larvicides, including other IGR`s and chitin synthesis inhibitors, and is therefore a very valuable tool to use in resistant management programs.

At present there is no known field resistance of mosquitoes to SumiLarv. The use of SumiLarv alone or combined with chemical adulticides will delay or avoid the development of resistance, as survivors of a chemical adulticide treatment will be killed in the aquatic stage by SumiLarv.

Several studies have been conducted to determine if continued use of SumiLarv could select for resistance and also to monitor the impact of SumiLarv treatments against known resistance strains. The potential for resistance to pyriproxyfen has been examined by several researchers:

1. For example, an organophosphate resistant strain of *Culex quinquefasciatus* was pressured with pyriproxyfen (SumiLarv) for 17 generations to determine the impact on resistance to this chemistry. Egg viability began declining in the F7 generation and became lower as the selection process continued and by the F17 generation egg viability was so low that colonies could not be maintained and the experiment had to be terminated. Susceptibility tests on the larvae of the F5, F10, F15 and F17 generations showed no indication of increased tolerance to pyriproxyfen. These results suggest that under normal conditions of field use the development of resistance to SumiLarv by *Culex quinquefasciatus* would be unlikely. (Schaefer, C.H. & Mulligan, F.S. Journal of American Mosquito Control Association, 7(3):409-411, 1991).
2. In other studies, treatment of rice fields in Sri Lanka with monocrotophos at 10 mg/L or pirimiphos-methyl at 0.1 mg/L gave a selective advantage to larvae of *Anopheles subpictus* and *Anopheles nigerrimus* carrying the oxidase- and acetylcholinesterase-based resistance genes. In contrast SumiLarv sprayed at 0.1 ppm conferred no selective advantage to larvae with either resistance mechanism. This difference can be attributed to the greater insecticidal activity of SumiLarv, the lack of any cross resistance with OPs and the slower chemical degradation of pyriproxyfen under field conditions when compared to organophosphates. (Hemingway, J .et al. Bulletin of Entomological Research, 78(3), 471-478, 1988).



3. In laboratory studies five strains of *Culex pipiens molestus* were used to evaluate SumiLarv. The five strains included three different susceptible strains, as well as one resistant to organophosphates and another which showed resistance to pyrethroids and methoprene. Tests were conducted in the laboratory using a range of concentrations. The EC-50 values (Emergence Control) were calculated for pyriproxyfen for all strains and ranged from 0.15 – 0.67 ppb, while methoprene showed a very wide range of ECs, ranging from 0.23 -5.32 ppb. These results demonstrate the potency of pyriproxyfen against all the strains tested and even against the methoprene resistant strain, demonstrating a lack of cross resistance. Results also showed that the mosquito larvae had maximum susceptibility at the late fourth instar to just after pupation. (Yoahiaki Kono et al. Medical Entomology and Zoology, 48 (2) 85-89, 1997).
4. Laboratory studies on the larvae of a resistant strain of *Culex quinquefasciatus* showed that it exhibited very high levels of resistance to permethrin – 2,500 fold. The major resistance mechanism was cytochrome P450 monoxygenases. The strain was tested against six insecticides belonging to organochlorine, organophosphate, carbamate classes and pyriproxyfen. Varying degrees of resistance were found for all insecticide classes but no cross-resistance was found to pyriproxyfen, (Shinji Kasai et al., Archives of Insect Biochemistry and Physiology 37: 47-56,1998).

WHOPES

SumiLarv 0.5G was submitted to the World Health Organisation Pesticide Evaluation Scheme (WHOPES) and has passed all stages (ref. WHO/CDS/WHOPES/2001.S.). SumiLarv 0.5G is therefore fully recommended for use in vector control by WHO.

Drinking (Potable) Water Applications

It is very important that mosquito larvicides can be applied to drinking water. If not, the areas of usage have to be restricted and this limits their value for Dengue control, where the vector species often breed in household water storage containers. In malaria control there are also mosquito breeding sites which are sources of drinking water so larvicides which do not have potable water clearance cannot be used.

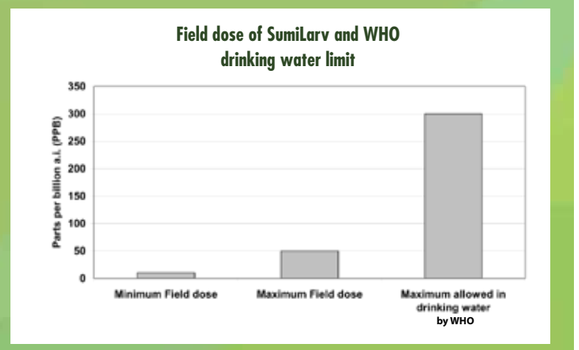
SumiLarv was fully evaluated by the FAO/WHO Joint Meeting on Pesticide Residues or JMPR (WHO/SDE/WSH/03.04/113). The conclusions of this meeting were as follows:

JMPR established an Acceptable Daily Intake (ADI) of. of 0.1 mg a.i./kg of body weight on the basis of the NOAEL of 10 mg a.i./kg of body weight per day, in 1 year studies of toxicity in dogs, and a safety factor of 100.

For water a guideline value of 0.3 mg/litre can be established from the ADI assuming a 60 kg adult consuming 2 litres of drinking water per day and allocating 10% of the ADI to drinking water.

The recommended dose rates of 0.01 – 0.05 ppm a.i. are equivalent to 0.01 – 0.05 mg a.i./litre. Therefore even the top dose rate is 6 times lower than the guideline value advised by JMPR.

The range of application rates from 0.01 – 0.05 mg/L allows the user to be flexible in choice of dose rate depending on container type, quality of water and duration of residual performance against mosquito larvae while retaining dose rates well below the ADI.



Technical Specifications

ISO common name: pyriproxyfen (BSI, draft E-ISO)

Synonyms: none

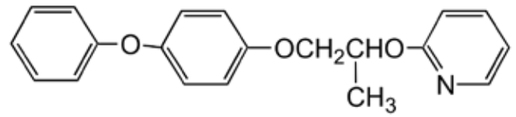
Chemical names:

IUPAC 4-phenoxyphenyl (RS)-2-(2-pyridyloxy)propyl ether

CA 2-[1-methyl-2-(4-phenoxyphenoxy)ethoxy]pyridine

OMS 3019

Structural formula:



Empirical formula: C20H19NO3

Relative molecular mass: 321.37 g/mol

CAS Registry number: 95737-68-1

CIPAC number: 715

Chemical Properties:

Pyriproxyfen is a solid (melting range 48-50ºC) of low volatility and only slightly soluble in water. It has no discernible acidic or basic characteristics and is stable to hydrolysis at pH 4-9 at 25C, but is prone to slow photolysis.

Analysis:

The analytical method for determination of pyriproxyfen as either technical grade material or formulated as SumiLarv 0.5 G is based on reversed-phase HPLC with UV detection at 254 nm and internal standardization with p-benzyldiphenyl. This method has been validated by collaborative studies and was adopted by CIPAC in 2006.

Analytical methods for the determination of impurities use gas chromatography with a flame ionizing detector (GC-FID) using ethyl benzene as an internal standard, for residual solvent, and reversed-phase HPLC with external standardization, for the other impurities.

Physical properties of the formulations are determined by CIPAC methods, as indicated in the specifications.

The proposed specifications are in accordance with the requirementsof the manual (FAO/WHO 2002).

Toxicity

The toxicity of pyriproxyfen was assessed by the WHO/FAO Joint Meeting on Pesticide Residues (FAO 1999) and the following conclusions were noted:

The acute oral toxicity of pyriproxyfen is low with LD50 values >5000 mg/kg weight in mice, rats and dogs. The acute dermal toxicity is also low, with LD50 >2000 mg/kg body weight in mice and rats. After exposure by inhalation an LC50 value >1.3 mg/l air in mice and rats was observed. Pyriproxyfen is rapidly excreted in animals, primarily in faeces, with between 88 to 96% excreted within 48hrs.

Pyriproxyfen is mildly irritating to the eye but not to the skin of rabbits. It did not sensitize the skin of Hartley strain guinea-pigs.

Pyriproxyfen was not genotoxic or carcinogenic. The acceptable daily intake (ADI) for man has been established at 0-0.1 mg a.i./kg per day in a one year study of pyriproxyfen in dogs after applying a safety factor of 100. (WHO/CDS/WHOPES/2001.2)

According to the U.S. EPA while pyriproxyfen is known to produce juvenoid effects on arthropod development, this mechanism of action in target insects and some other arthropods has no relevance to any mammalian endocrine system. Pyriproxyfen is therefore not considered to possess estrogenic or endocrine disrupting properties to mammals. (Sullivan, J.J. & Goh, K.S.,, Journal Pesticide Science, 33(4) 339-350, 2008).

Summary

Acute oral LD50 (rat)	>5000 mg/kg
Acute dermal LD50 (rat)	>2000 mg/kg
Skin irritation (rabbit)	Mildly irritating
Eye irritation (rabbit)	Mildly irritating.

Ecotoxicology

Pyriproxyfen will not adversely affect a vast majority of aquatic invertebrates and fish when applied at rates <50 ppb a.i.(0.05 ppm) in mosquito control programs. In certain cases however populations of certain organisms, such as crustacea, may experience minor declines when SumiLarv is applied at higher label dose rates. Affected populations will recover in relatively short time periods (WHO/CDS/WHOPES/2001.2).

Both crustacea and aquatic insect larvae are sensitive to pyriproxyfen, although adverse effects were found to be reversible. Pyriproxyfen did not exhibit any marked effects on mayfly, dragonfly, ostracods, cladocerans, copepods, or beetles. Planktonic organisms showed no significant adverse effects resulting from 0.01 ppm treatment in experimental aquaria. Pyriproxyfen is not expected to bioconcentrate in fish under environmentally relevant conditions due to the rapid depuration (cleansing of impurities) of the parent compound from fish. (Environmental fate of pyriproxyfen, J. Sullivan, May 2000).

SumiLarv was evaluated against other organisms in mosquito-breeding habitats. When applied at a rate of 0.11 kg a.i/ha to rice plots (20 times greater than required for controlling *Aedes nigromaculis* larvae, no detectable residues (<0.00005 ppm) were found after 2 days in treated water. SumiLarv did not accumulate in soil, there were no residues (<0.005 ppm) after 3 days in fish (*Lepomis macrochirus rafinesque)*, and the residue on rice plants declined to<0.005 ppm after 7 days. Despite slight induction of morphogenetic aberrations in *Odonata* (Dragonflies) at adult emergence and minor suppression of reproductive capacity of *Daphnoid cladocerans* and *ostracods*, SumiLarv was found to be safe to aquatic, non-target organisms, including mosquito predators. (Schaefer, C. H., Miura T. Journal of Economic Entomology 83(5) 1768-1776, 1990).

SumiLarv was highly effective in inhibiting the normal development of mosquito larvae into adults in laboratory and field trials. Late fourth instar larvae were the most sensitive stage. Mortality occurred in the pupal stage and, at lower doses, resulted in the formation of abnormal adults. No long-term bioaccumulation problem was apparent following dynamic or static exposures to fish. Non-target aquatic organisms that co-exist in mosquito breeding habitats were not affected adversely by treatments which were effective against mosquitoes. In summary SumiLarv showed efficacy against mosquito larvae, a high degree of safety to associated non-target organisms, and chemical persistence that appear to be compatible with the environment. (Schaefer, C.H., et al., Journal of Economic Entomology, 81(6):1648-55, 1988)

Precautions

SumiLarv has a very low mammalian toxicity and should not present any problems in normal usage, however as in line with any pesticide good handling practice should be adhered to and protective clothing worn and good personal hygiene practices followed after applying the product. See label and MSDS for full precautions.

Storage

SumiLarv should be stored in a secure building that is lockable. The building should be well ventilated and dry. SumiLarv should be stored in its original packaging, out of direct sunlight and rain.