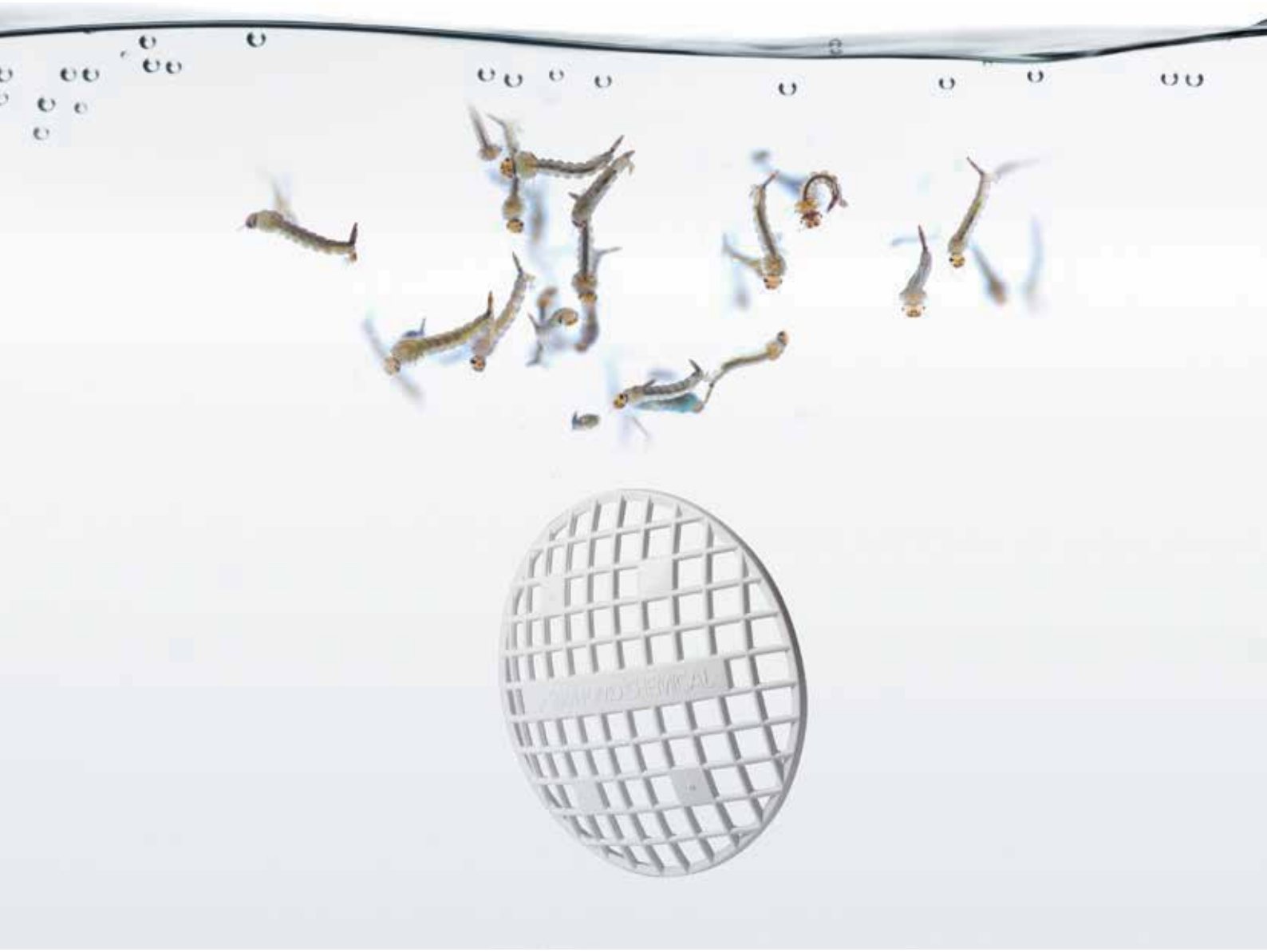
SumiLarv™ 2MR

TECHNICAL BROCHURE

Long lasting control of container breeding mosquitoes



SUMITOMO CHEMICAL

Contents

Introduction and background 3

Product information 4

Mode of Action 5

Impact of pyriproxyfen on egg laying, hatching and larval survival 6

Brazil field simulation - trial 1 8

Brazil field simulation - trial 2 ..... 9 & 10

Thailand field simulation 11

Laos field trial 12

Ethiopia field simulation 13

SumiLarv™ 2MR technical specifications ..... 14 & 15

Introduction and Background

Vector borne diseases are estimated to account for more than 17% of the global burden of communicable diseases and have a major impact on human health and socio-economic development *(World Health Organisation (WHO): Global vector control response 2017-2030. Geneva: WHO; 2017)*.

Aedes mosquitoes control

Amongst vector borne diseases, there are many that are transmitted by *Aedes* mosquitoes, including dengue, chikungunya, Zika and yellow fever.

Dengue fever is a viral infection transmitted by the blood feeding activity of certain species of urban *Aedes* mosquitoes.

Over the past five decades, the global burden of dengue is estimated to have increased massively: recent estimates indicate that there are as many as 390 million dengue infections per year, of which approximately 96 million are considered clinical infections resulting in about 22,000 dengue-related deaths per year *(https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue, March 2020)*.

In recent years other mosquito borne viruses have expanded their distribution resulting in unexpected outbreaks and epidemics.

For example, in 2015 there were 693,000 suspected cases of chikungunya reported to the Pan American Health Organization (PAHO) with Colombia alone contributing 356,000 suspected cases. In neighbouring Brazil, from 2015 to 2017, a total of 464,000 cases were notified *(Emerging Infectious Diseases, vol 24,4, April 2018)*.

In addition, Zika virus which usually presents as a mild flu-like illness in adults, can also result in microcephaly in new-borns when pregnant women become infected.

On February 1st 2016, the WHO declared Zika virus a public health emergency of international concern *(Rachel Lowe et al, International Journal of Environmental Research of Public Health 2018 15(1), 96)*.

In 2017, 120 million people in Brazil were at risk of Zika virus infections, 32 million in Mexico and 29.5 million in Colombia *(www.statista.com, Zika virus - Statistics & Facts, February 2019)*.

In Brazil by the end of 2016 more than 200,000 cases of Zika virus were notified (International Journal of Environmental Research of Public Health 2018 15(1), 96). As of January 2016 there were over 3,174 suspected cases of microcephaly including 38 deaths *(https://www.who.int/csr/don/8-january-2016-brazil-microcephaly/en/, January 2016)*.

Aedes aegypti and *Aedes albopictus* are container breeding mosquitoes occurring in domestic environments that have adapted to colonize artificial containers such as water storage tanks, earthenware jars and cisterns as well as discarded car tyres and items of household rubbish able to hold water.

Ae. aegypti is the primary vector of the viruses that cause dengue, chikungunya, Zika and yellow fever infections in humans, and has caused epidemics of all these diseases throughout many regions of the world.

According to the WHO *(Global Strategy for dengue prevention and control, 2012–2020, WHO)*, in the absence of an effective long-lasting vaccine, prevention of mosquito borne virus transmission depends entirely in controlling the mosquito vectors or the interruption of human–vector contact.

Adult *Ae. aegypti* mosquitoes will rest inside houses most commonly in dark, out of the way places, the females lay eggs indoors and outdoors making use of a wide range of artificial and natural water containers.

In regions where residents use different containers to collect and store water in or around homes, these can become important breeding sites for *Aedes* mosquitoes. As the water is kept for domestic uses the application of larvicides to these containers is an important activity to help reduce populations of *Aedes* and help prevent disease.

While Sumitomo Chemical has already developed SumiLarv™ 0.5G for this application, SumiLarv™ 2MR offers additional benefits of long lasting efficacy allowing one application per season with discs permitting easy removal and replacement from containers as needed.

Anopheles mosquitoes control

Anopheles stephensi is a highly competent Asian urban malaria vector that now poses serious challenges to malaria control and elimination efforts in Africa following its invasion and dramatic continuing expansion across the continent. This species has been associated with an increase in malaria cases in Djibouti- the first country in Africa to be invaded (M. K. Faulde et al., Acta Tropica, 139, 39 -43, 2014), and more recently in Ethiopia (F. Tadesse et al., Preprint, 2023). Modelling suggests malaria cases could surge by 50% if *An. stephensi* becomes established in African cities and no appropriate control strategies are implemented (A. Hamlet et al., BMC Medicine, 20, 135, 2022). The additional risk from *An. stephensi* is because it is an urban mosquito with a preference for container breeding like *Aedes aegypti* and the two species often share the same breeding habitats (M. Balkew et al., Malaria Journal, 20, 331, 2021). SumiLarv 2MR therefore offers a unique opportunity to provide long term control of both vector species.

Product Information

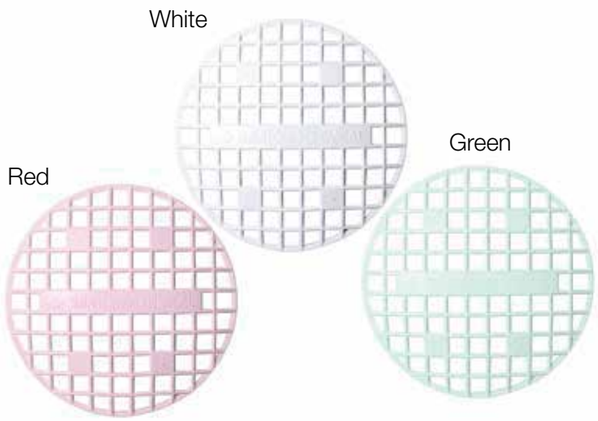
SumiLarv™ 2MR is a polymer matrix release formulation presented as a 5.5 cm diameter plastic disc weighing 2 g containing 2% w/w pyriproxyfen. SumiLarv 2MR prevents mosquito pupae from developing into flying adult mosquitoes which can later bite humans. SumiLarv 2MR has been developed by Sumitomo Chemical Co., Ltd.

How To Use

SumiLarv 2MR should be placed in water containers at a rate of one disc per 40-500 litres of water, depending on local registration. The disc will sink to the bottom of the container and the active ingredient pyriproxyfen will be released slowly from the matrix of the disc to give a long duration of efficacy. As water is used and replaced the disc will release more pyriproxyfen to maintain an effective level to control the mosquitoes. If containers are emptied and cleaned then place the disc in the shade and put back in the container after refilling with water.

Product Profile

- Active ingredient: pyriproxyfen
- Residual efficacy: up to 6 months
- Target vector: *Ae. aegypti, Ae. albopictus, An. stephensi* and other container breeding mosquitoes
- Formulation type: matrix release
- Dosage: 1 disc per 40 - 500 litres water, depending on local registration
- Treatment method: place into water container
- Colours: white, green, red

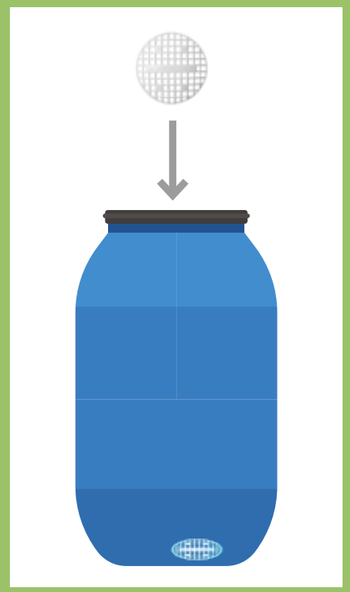


Key Features:

- Effective for up to 6 months – gives an operational cost saving compared to conventional larvicides.
- Easy to use – simply drop into water container.
- Discs are easy to see - helps to identify and track treatments.
- Slow-release technology – ensures prolonged activity of the active ingredient.
- No odour – enhances user acceptance.
- Designed for use in clean water storage containers.

Easy to use:

Simply drop into water container!



SumiLarv™ 2MR (pyriproxyfen): Mode of Action

SumiLarv 2MR is a novel mosquito larval control product based on the insect growth regulator (IGR) pyriproxyfen. Pyriproxyfen was invented and developed by Sumitomo Chemical Co., Ltd. and has a unique mode of action that is insect-specific, stage-specific, and not neurotoxic.

Pyriproxyfen does not directly kill mosquito larvae, but strongly prevents adult emergence.
The late 4th larval stage and pupae just after pupation are the most susceptible to pyriproxyfen (Y.Kono et al., Medical Entomology & Zoology, 48(2), 85-89, 1997).

Since larvae do not die immediately after treatment, users need to adjust to the concept that although larvae can still be seen following an application of SumiLarv 2MR, mortality will normally occur at the pupal/ adult moult thus preventing the emergence of adult mosquitoes. For this reason, evaluation of pyriproxyfen is determined by measuring adult emergence inhibition.

Application of SumiLarv 2MR to the aquatic larval habitat reduces the emergence of adult mosquitoes which in turn reduces biting rates and transmission.

Pyriproxyfen has received U.S. EPA (United States Environmental Protection Agency) status as a reduced risk insecticide and an organophosphate alternative and is recognized by the WHO for treatment of potable water against mosquitoes (Sullivan, J.J. & Goh, K.S., Journal Pesticide Science, 33(4) 339-350, 2008).

Mosquito Lifecycle

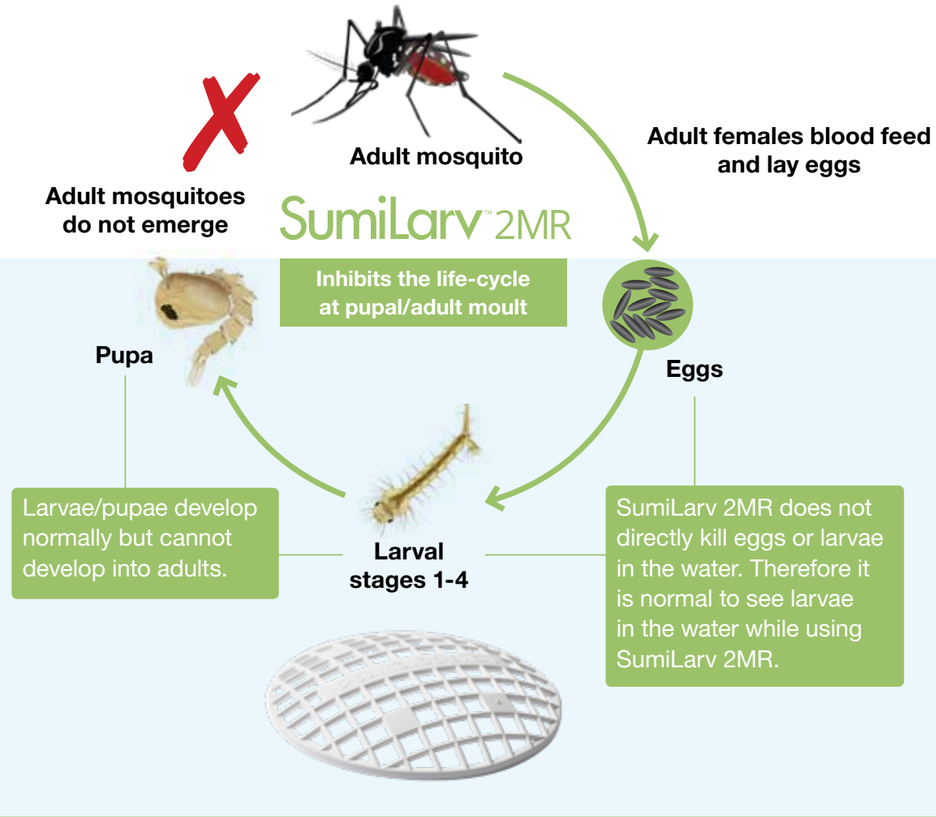


Illustration © Sumitomo Chemical July 2023

Impact of Pyriproxyfen on Egg Laying, Hatching and Larval Survival

The following scientific study demonstrates that treating oviposition sites with pyriproxyfen does not deter egg laying mosquitoes.

In a trial in Iquitos, Peru, it was found that water treated at very 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 affected in the viability, egg laying, egg hatching and larval survival of any hatched eggs. The following paper gives examples of this:

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 and Zoology, 23(2) 186 -193, 1988).



Normal untreated mosquito pupa (the stage vulnerable to SumiLarv™ 2MR)

Treated	Untreated
 Treated *Ae. aegypti* pupa	 Untreated *Ae. aegypti* pupa
 Treated *Ae.aegypti* – failed emergence	 Untreated *Ae.aegypti* – successful emergence
 Treated *Ae.aegypti* – failed emergence	 Untreated *Ae.aegypti* – successful emergence
 Treated *Cx.quinquefasciatus* – abnormal pupa	 Untreated *Cx.quinquefasciatus* – normal pupa
 Treated *An.gambiae* – abnormal pupa	 Untreated *An.gambiae* – normal pupa

SumiLarv™ 2MR - Field Trials



Brazil Field Simulation - Trial 1

In studies conducted by The Oswaldo Cruz Foundation (FIOCRUZ), the effect and persistence of SumiLarv™ 2MR was evaluated in simulated field conditions on the *Ae. aegypti* susceptible strain (Rockerfeller) at four sites: Marília; Recife; Macapá, and Rio de Janeiro.

At the beginning of each test, third instar *Ae. aegypti* were placed in containers floating within the water containers. Soon after, one SumiLarv 2MR disc was placed in the center of the water containers. The first day of treatment was considered as “day zero”. Fortnightly new larvae were introduced in the containers with field and susceptible Rockerfeller strains placed in alternate positions, so that resistant and susceptible populations were exposed to all containers in the experiment.

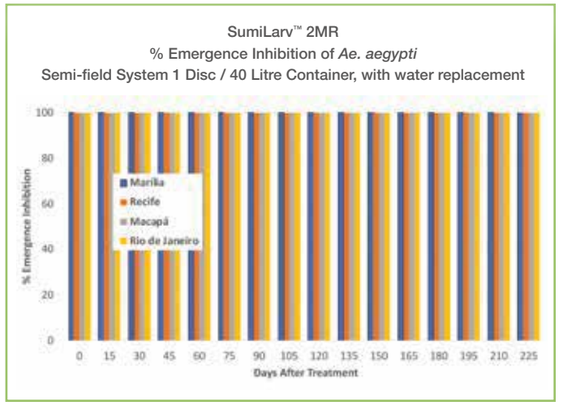


Figure 1



SumiLarv 2MR gave Emergence Inhibition (EI) of *Ae. aegypti* for the duration of the trial (at least 225 days).

Emergence inhibition was determined after the first 72 hours of exposure. Subsequent readings were taken every 48 hours until the emergence or mortality of all the mosquitoes was complete.

The study continued for six months or at the point when two consecutive emergence inhibition readings were less than 80%. Three times a week 1/5th of the volume of water in the tanks was replaced to simulate water replenishment in a residential situation. Results are presented on Figure 1.


FIOCRUZ semi field trial site, Brazil

Brazil Field Simulation - Trial 2

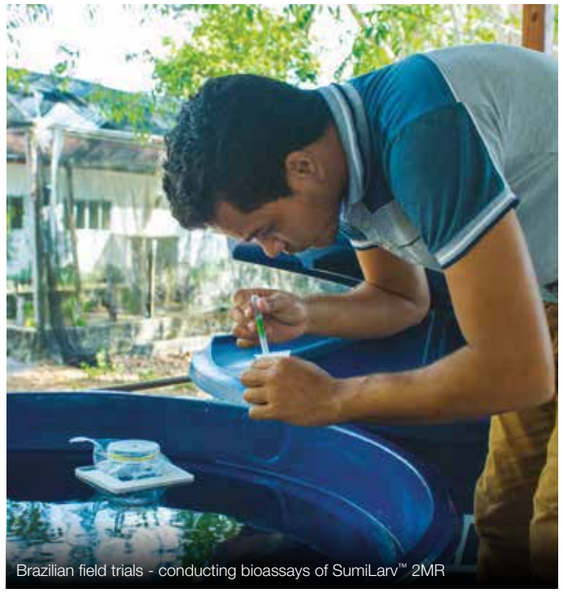
In studies conducted by The Oswaldo Cruz Foundation (FIOCRUZ) in Brazil the effect and persistence of SumiLarv™ 2MR was determined in a field simulation of four naturally occurring populations of *Ae. aegypti*.

The trials were conducted in four regions of the country: North, Northeast and Southeast, the latter being in two distinct regions, Marília and Rio de Janeiro, which represent most of the climatic variations in the country.

At the beginning of each test, 50 third instar *Ae. aegypti* larvae were placed in reservoirs floating in large containers according to WHO methodology (WHO, 2005). Either one or two SumiLarv 2MR discs were added to determine the effect of dose rate on efficacy.



Illustration © Sumitomo Chemical January 2021


Brazilian field trials - conducting bioassays of SumiLarv™ 2MR

In the case of Marília (Figure 2) and Recife (Figure 3) in order to establish the effective dose rate, the disc was divided into pieces: 0.1 disc, 0.2 disc and 0.4 disc. (For the purpose of the test only).These were applied to make three different concentrations of pyriproxyfen. Three 100 litre containers received 0.1 disc, three received 0.2 disc and three 0.4 disc. Larvae were provided with standard laboratory larval food.

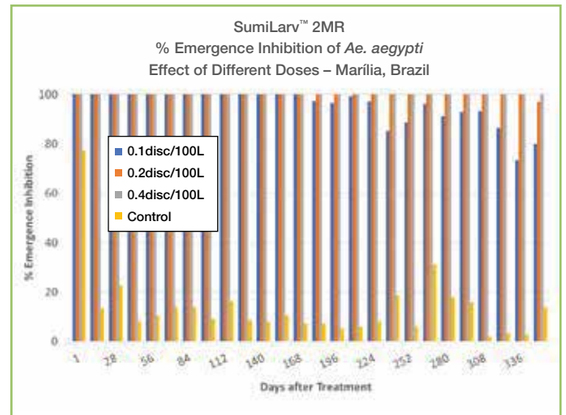


Figure 2

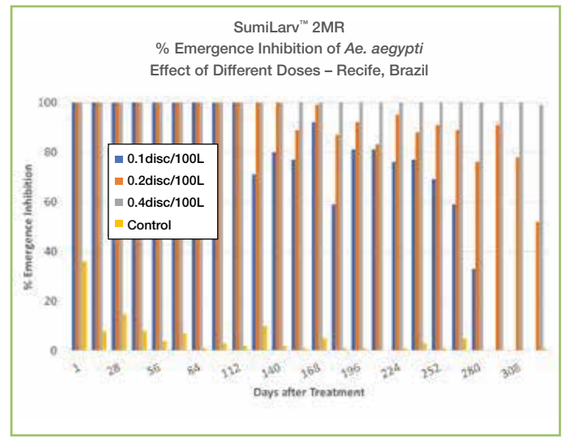


Figure 3

In the case of Rio de Janeiro (Figure 4) and Macapá (Figure 5), SumiLarv™ 2MR treatments were as follows: one disc was applied per 250 litre and 500 litre container. 500 litre containers and 1000 litre containers received two SumiLarv 2MR discs. The remaining containers acted as untreated controls.

Third - fourth instar larvae were introduced to the reservoirs every 2 weeks. Mortality or emergence inhibition was evaluated after 72 hours of exposure, and subsequently every 48 hours until emergence from untreated controls was complete.

Every 2 weeks a new group of larvae were placed in the reservoirs until the six-month period or up to two consecutive readings for which emergence inhibition was less than 80%.

Three times a week one third of the water volume of the tanks was replaced to simulate a residential use situation.

These trials show that SumiLarv 2MR has a residual life of more than ten months, when used at the rate of up to one disc to 500 litres of water, regardless of the location being evaluated.

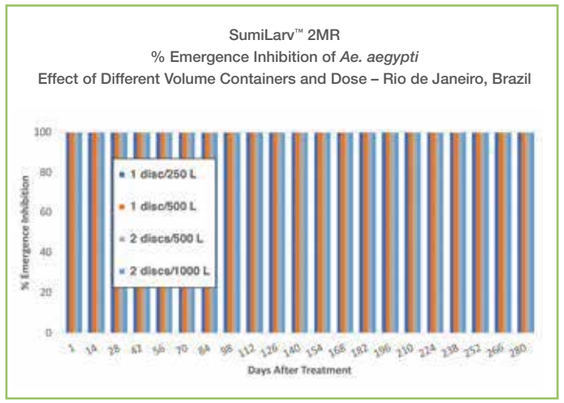


Figure 4

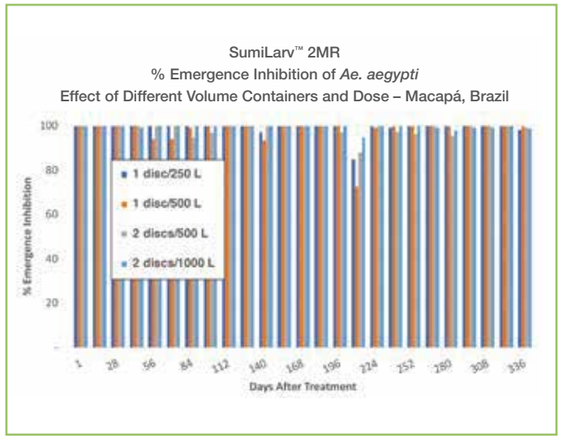
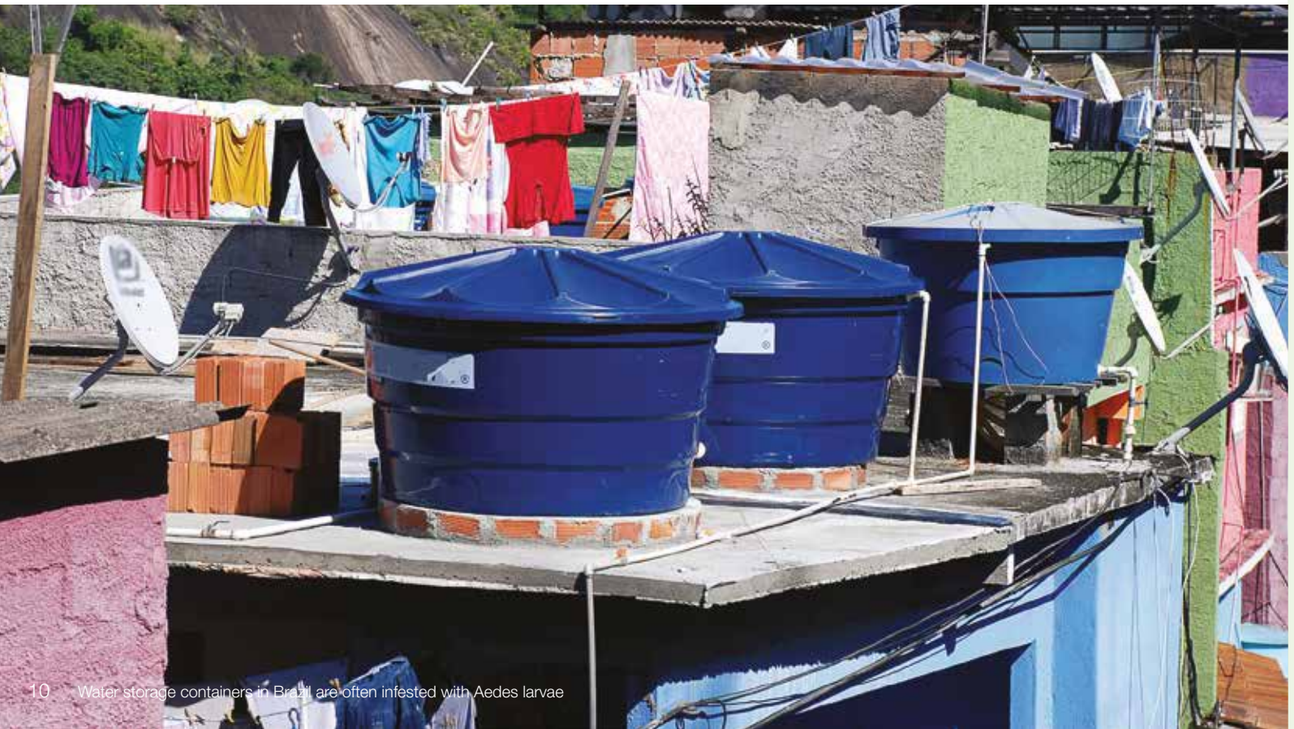


Figure 5



Water storage containers in Brazil are often infested with Aedes larvae

Thailand Field Simulation

A field evaluation of SumiLarv™ 2MR was conducted at a field research station in Nonthaburi Province, Thailand by the National Institute of Health against *Ae. aegypti* larvae in concrete and plastic containers. Multiple dosages of the formulation were used and the treated containers and controls were challenged weekly with larvae for about 6 – 12 months.

Plastic water containers (Figure 6) and concrete containers (Figure 7) were filled with 40L, 80L and 160L of tap water respectively. SumiLarv 2MR discs were placed into each container (1 disc/container). For controls, containers of 40L, 80L and 160L of tap water without SumiLarv 2MR were used. After placing the discs in the containers, each container was covered with a lid in order to minimize evaporation of water and contamination of debris from the air. The water was allowed to settle and remain undisturbed for 1 week.

Treatments were challenged weekly with a fresh cohort of laboratory reared larvae, when 25 third instar larvae of *Ae. aegypti* were added per container. 1 g of ground up mouse food was added to each container as larval food.

Adult emergence was assessed by counting pupal skins 1 week after the larvae had been added. After assessment of efficacy, the water in each container was stirred and 50% of water in each container was removed and replaced with the same amount of fresh tap water. Water replacement was done once every week. Ambient temperatures at the site during the study were between 24°C and 37°C.

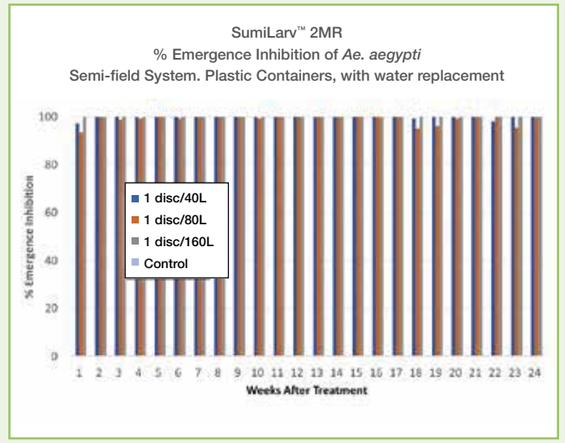


Figure 6

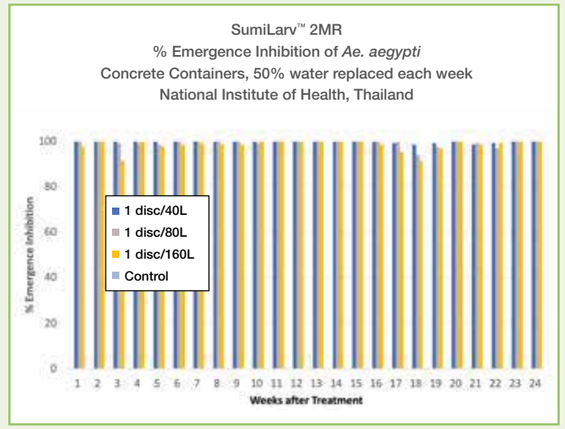


Figure 7

SumiLarv 2MR gave Emergence Inhibition (EI) of *Ae. aegypti* for the duration of the 24 week trial.



Laos Field Trial

A field study to assess the effectiveness of SumiLarv™ 2MR for dengue vector control was conducted in a rural village in a dengue endemic area of Khammouane Province, Laos.

In the treated village consisting of 120 households with a population of 679, SumiLarv 2MR was placed in household water jars and water storage drums at a dosage 1 disc/40L and the discs were replaced every 6 months, with the trial continuing for one and a half years. The containers were inspected for the presence of *Ae.aegypti* larvae and pupae.

The results at the end of the trial are shown in Figure 8. In the village treated with the SumiLarv 2MR, larvae and pupae were not found in water jars, and the positive rate in containers other than water jars was also low.

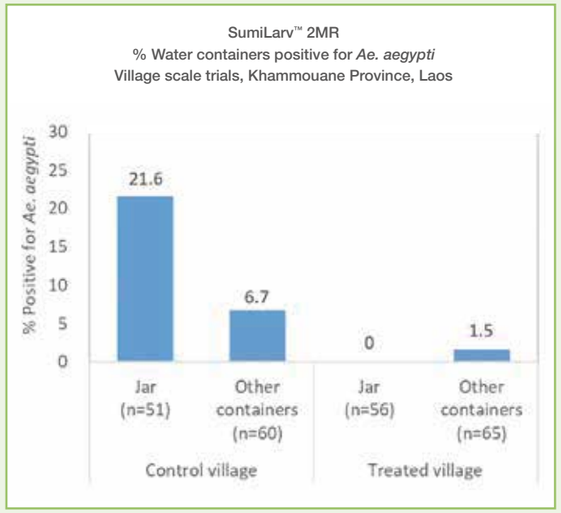


Figure 8

SumiLarv 2MR gave supression of container breeding *Ae. aegypti* in real life village conditions.



Ethiopia Field Simulation - *An. stephensi* Trials

SumiLarv™ 2MR was evaluated by Jimma University against the local pyrethroid resistant population of *An. stephensi* in Awash Sebat Kilo town, Eastern Ethiopia. Three application rates of SumiLarv 2MR namely 1 disc in 100 litres, 1 disc in 250 litres, and 0.5 disc in 250 litres of water equivalent to 1 disc in 500 litres were tested alongside negative controls with no treatment.

Mosquito larvae were collected from the field and reared in an insectary. Batches of 20-25 third to fourth instar larvae from the insectary were introduced to each treated container every 2 weeks. 50% of the tap water was replaced every 7 days to mimic normal water usage.

Pupae were collected daily from containers for 4 days after introduction of larvae. They were counted and transferred to the laboratory for monitoring of adult emergence.

Results are presented in Figure 9.

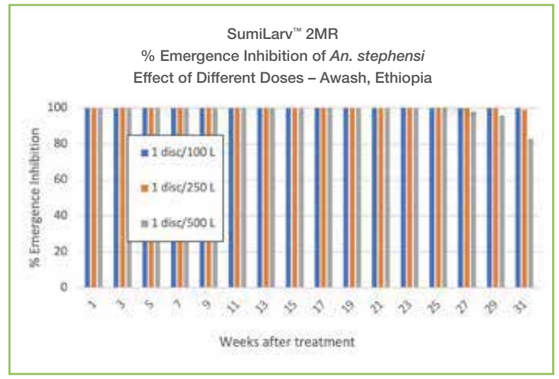


Figure 9

SumiLarv 2MR gave Emergence Inhibition of *An. stephensi* for 31 weeks of the evaluation period.



Jimma University semi-field trial site, Ethiopia

Drinking Water Applications

The risk assessment to humans of applying and using SumiLarv™ 2MR treated water was evaluated by WHO. The FAO/WHO Joint Meeting* on Pesticide Residues assessed the toxicity of pyriproxyfen. An acceptable daily intake (ADI) has been set at 0 - 0.1 mg/kg bw/day.

WHO has recommended pyriproxyfen as a mosquito larvicide for application in drinking-water containers; the recommended dosage of pyriproxyfen (10 µg/L) in drinking water is considered to be without risk to human health.

WHO concluded that SumiLarv 2MR releases pyriproxyfen at a rate that does not generate hazardous concentrations in drinking-water containers, and does not pose a health risk to adults, children, toddlers or new-borns.

* Report of the twentieth Working WHOPES Group Meeting, WHO/HQ, March 2017

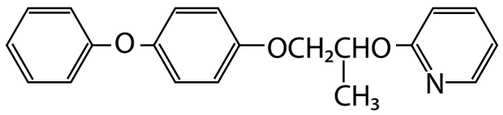
FAO = Food and Agriculture Organization

SumiLarv™ Technical Specifications

ISO common name: pyriproxyfen

Chemical names:
IUPAC 4-phenoxyphenyl *(RS)*-2-(2-pyridyloxy)propyl ether
CA 2-[1-methyl-2-(4-phenoxyphenoxy)ethoxy]pyridine

Structural formula:



Empirical formula: $C_{20}H_{19}NO_3$

Relative molecular mass: 321.37 g/mol

CAS Registry number: 95737-68-1

Analysis

The analytical method for determination of pyriproxyfen as either technical grade material or formulated as SumiLarv™ 2MR 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 2017.

Full analytical methodology is available in CIPAC/ 715/MR/M/3 (CIPAC 2017)

Toxicity
(technical grade pyriproxyfen)

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 LD_{50} values >5000 mg/kg body weight in mice, rats and dogs. The acute dermal toxicity is also low, with LD_{50} >2000 mg/kg body weight in mice and rats. After exposure by inhalation an LC_{50} 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 was 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 bw/day in a one year study of pyriproxyfen in dogs after applying a safety factor of 100. (WHO/CDS/WHOPES/2001.2)

HPLC = High Performance Liquid Chromatography
CIPAC = Collaborative International Pesticides Analytical Council

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
(technical grade pyriproxyfen)

Mammalian Toxicity	
Acute oral LD_{50} (rat)	>5000 mg/kg
Acute dermal LD_{50} (rat)	>2000 mg/kg
Skin irritation (rabbit)	Not irritating
Eye irritation (rabbit)	Mildly irritating

Other:

Not mutagenic. Not carcinogenic in rats and mice.

Not teratogenic in rats and rabbits.

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 some cases however populations of certain organisms, such as crustacea, may experience minor declines when pyriproxyfen 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 *(J. Sullivan, Environmental fate of pyriproxyfen, May 2000).*

Pyriproxyfen 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 *Ae. nigromaculis* larvae) no detectable residues (<0.00005 ppm) were found after 2 days in treated water. Pyriproxyfen 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*, pyriproxyfen 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).

Pyriproxyfen 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 pyriproxyfen showed efficacy against mosquito larvae, a high degree of safety to associated non-target organisms, and chemical persistence that appears to be compatible with the environment. (Schaefer, C.H., et al., Journal of Economic Entomology, 81(6): 1648-55, 1988).

Precautions

SumiLarv™ 2MR 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 SDS for full precautions.

Storage

SumiLarv 2MR should be stored in a secure building that is lockable. The building should be well ventilated and dry.

SumiLarv 2MR should be stored in its original packaging, out of direct sunlight and rain.

Disposal

Dispose of discs appropriately in accordance with local/regional/national/international regulations. Do not dispose of in aquatic habitats, and do not burn.

SumiLarv™ 2MR

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