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PYRIPROXYFEN AS A MOSQUITO LARVICIDE

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Abstract Pyriproxyfen is an insect growth regulator that affects the physiology of morphogenesis, reproduction and embryogenesis of insects. It exhibits a high level of activity against mosquito larvae inhibiting adult emergence at very low dose rates. It has low mammalian toxicity and environmental impact. This paper reviews pyriproxyfen efficacy against nuisance mosquitoes and vectors of dengue and malaria. Pyriproxyfen is useful in resistance prevention or management strategies where chemical adulticides are being used since field resistance is unknown. Pyriproxyfen can be transferred by adults to oviposition sites causing effects on egg eclosion and inhibition of emergence.
Key words pyriproxyfen, dengue, malaria, *Aedes, Anopheles, Culex*

INTRODUCTION

Control of urban and semi-urban mosquitoes has been the subject of many new initiatives over the past few years. Initiatives in malaria control have focussed mainly on the use of long lasting insecticidal nets and indoor residual sprays. These interventions can give good levels of control but rarely achieve compete elimination of the vector or the disease agent. There is a need to increase both the level of mosquito control and the percentage of disease reduction by integrating other control methodologies such as mosquito larviciding into control programs. The use of larviciding has decreased over the years in malaria control programs, but is still used as a major tool against *Aedes aegypti* (L.) for the control of dengue. Many countries still prefer the option of space spraying.

The key criteria for an effective mosquito larvicide are low mammalian toxicity, low impact on the environment, broad spectrum of activity against all target species of mosquito and a long duration of effect, thus reducing the frequency of application.

Pyriproxyfen (Sumilarv 0.5G, Sumilarv® is a registered trademark of Sumitomo Chemical Company Ltd) fulfils all of these criteria. Additionally with the potential of resistance to nearly every known class of insecticide currently used as adulticides, there is an urgent need for insecticides such as pyriproxyfen that have a novel mode of action and no recorded field resistance. The effects of pyriproxyfen can go beyond the aquatic stages as the ability of any surviving females to reproduce can also be affected.

Mode of Action and Toxicity

Pyriproxyfen is an insect growth regulator with a unique mode of action affecting the morphogenesis, reproduction and embryogenesis of insects. The morphogenetic effect of pyriproxyfen is primarily seen during larval-pupal transformation. Therefore death occurs at the pupal stage and adult mosquitoes fail to emerge. Pyriproxyfen (technical) mammalian toxicity is as follows: Oral (rat) LD_{50} >5000 mg/kg; Dermal (rat) LD_{50} >2000 mg/kg; Inhalation (rat) LD50 >1000 mg/kg. Pyriproxyfen has a low environmental impact and is suitable for the control of mosquito larvae but like any IGR may have some impact on other arthropods or crustacea. Usually the impact is low and populations rapidly recover but care should be taken to avoid application to natural rivers or lakes. When evaluated through the WHOPES it was concluded: Laboratory and field data clearly indicates that pyriproxyfen will not adversely affect a vast majority of aquatic invertebrates and fish when applied at rates usually <50 ppb in mosquito control programs.'

Pyriproxyfen has been passed by the Joint FAO/WHO Meeting on Pesticide Residues for mosquito larval control in drinking water which is essential if the product is to be used in dengue control. The margin of safety for pyriproxyfen applied to drinking water is shown in Fig 1, where the minimum and maximum recommended dose rates are compared to the maximum dose allowed by WHO (WHO 2003).

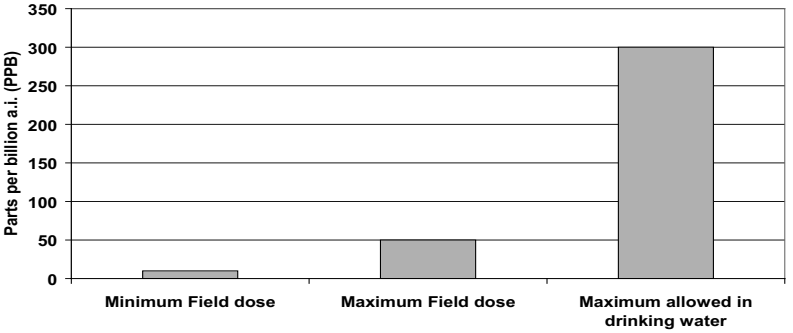

Figure 1. Field dose of pyriproxyfen compared with WHO drinking water limit

Pyriproxyfen can inhibit emergence of *Aedes aegypti* at very low dose rates. The LC_{50} has been shown to be 0.012 ppb (Sichuincha et al., 2005). However in order to achieve complete inhibition of adult emergence and prolong the duration of control the actual field dose rates are higher than this, with label rates of 0.01 - 0.05 ppm of active ingredient (10 - 50 ppb). Using 0.5% pyriproxyfen equates to 2 g -10 g product per m^3 water.

A comparison of the most popular larvicides was conducted in the laboratory but using field collected *Culex quinquefasciatus* larvae. Five to nine different concentrations of each larvicide or IGR were tested at least 3 times. Results for insect growth regulators were measured over 7-10 days due to their different modes of action compared to chemical larvicides. The toxicity of pyriproxyfen to *Culex quinquefasciatus* larvae (Table 1) is greater than the other chemical larvicides or two other IGR`s (Arshad, 1999).

Table 1. (Figure 2) . Toxicity of pyriproxyfen to *Culex quinquefasciatus*.

Larvicide	LC_{90} ppm	Relative Toxicity*	Type
Pyriproxyfen	0.0011	118	IGR
Diflubenzuron	0.0034	39	IGR
Methoprene	0.052	3	IGR
Temephos	0.0096	13	OP
Fenthion	0.130	1	OP
Permethrin	0.017	8	Py

*The more active the insecticide, the higher the number. OP, organophosphate; Py, pyrethroid

Field Trials

***Culex*.** The most common nuisance mosquito and sometimes vector in tropical urban environments is *Culex quinquefasciatus.* It is also invariably the toughest mosquito to control usually requiring much higher levels of insecticide to control both larvae and adults. In addition this species favours highly organic or very polluted environments such as ditches, soakaways and effluent systems. Trials conducted in Dar es Salaam showed that when using pyriproxyfen 0.5G, control of *Cx. quinquefasciatus* was achieved for 4-5 weeks in the rainy season and up to 11 weeks in the dry season, (Fig. 3), (Chavasse et al., 1995). The shorter duration of control in the rainy season was due to heavy rains and continual dilution of the active ingredient in the water. Despite this, duration of control was achieved that exceeds most larvicides in such situations.

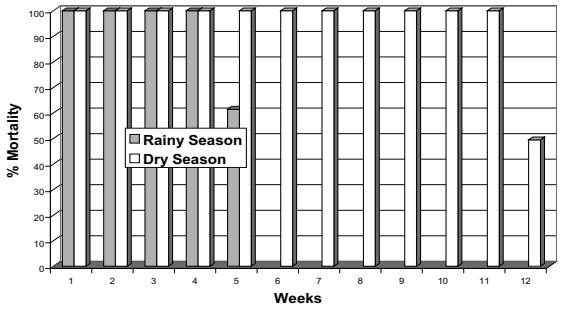

Figure 3. *Cx. quinquefasciatus* larval control with pyriproxyfen 0.5G (Dar es Salaam)

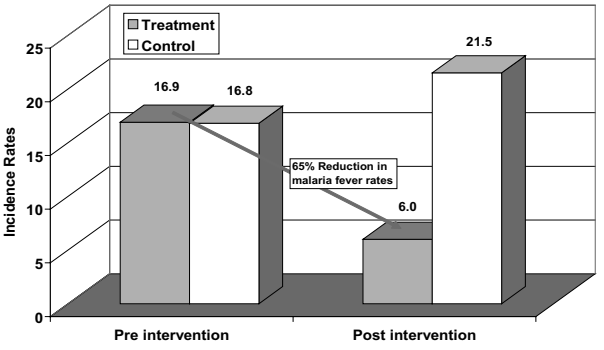

Figure 4. Malaria fever incidence rates, episodes per 1000 person years.

***Anopheles*.** Trials were conducted in Sri Lanka to control *Anopheles* spp. breeding in 12 villages in an irrigated settlement scheme. Treatments were made using pyriproxyfen 0.5G at a dose rate of 0.01 mg a.i./l (0.01 ppm). Entomological bioassays in the treatment sites indicated emergence inhibition of anopheline adult mosquitoes for 190 days in river bed pools. The treatment caused 78% reduction of adult populations of *Anopheles culicifacies* and 72% reduction of *Anopheles subpictus*. The impact of the larviciding caused malaria fever incidence rates to fall by 65% in the treatment area compared to a rise of 22% in the control area. (Fig. 4) (Yapabandara and Curtis, 2004).

***Aedes aegypti*.** Trials were conducted in several countries for dengue control. **Malaysia**. Trials were conducted in Malaysia against *Ae. aegypti* using 60 litre earthenware storage jars. To simulate actual usage 20% of the water was replaced every 2 weeks. Two dose rates of pyriproxyfen 0.5G were used - 0.01 mg/l (0.01 ppm) and 0.02 mg/l (0.02 ppm). Inhibition of emergence of adult mosquitoes was measured. Good control was achieved for 4 months at both dose rates. Additional tests with *Aedes albopictus* (Skuse) showed similar levels of efficacy. Results are shown in Figure 5 (Vythilingam et. al, 2005). **Peru**. A trial was conducted in Iquitos, Peru where 16 water tanks which actively supported breeding *Ae. aegypti* were selected. The volume of these tanks was 200, 300 and 600 litres. They were in constant use as water sources and therefore subject to regular dilution by being topped up with fresh water. They were treated with doses of 50, 67 and 83 ppb pyriproxyfen. Samples of the water were taken back to the laboratory each month for 5 months where batches of 25 laboratory-reared fourth instar *Ae. aegypti* were added. These were compared with control pots containing 1 litre of clean tap water. Larval food was added to all pots and the number of larvae and pupae that had died over a 6 day period was noted and expressed as percentage mortality. Results (Figure 6) demonstrate continued efficacy of pyriproxyfen over the 5 month trial period (Sichuincha et al., 2005). **Cambodia**. Concrete domestic water storage jars are a common larval habitat for *Ae. aegypti* in Cambodia. Those used in these trials had a capacity of 200 litres.

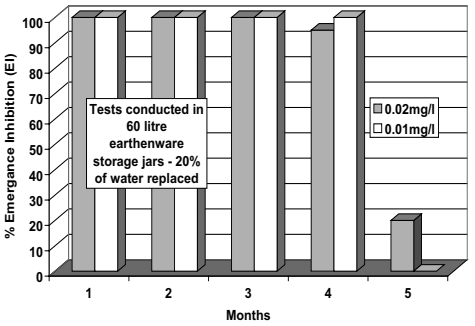

Figure 5. Evaluation of pyriproxyfen 0.5G agains *Ae. aegypti* (Malaysia)

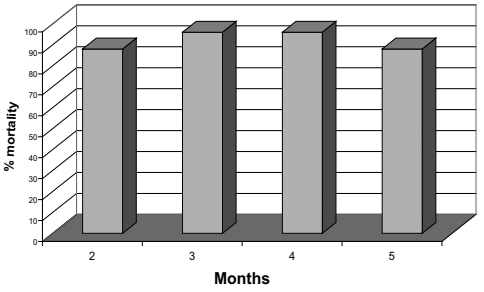

Figure 6. Larval/pupal mortality in water samples from pyriproxyfen-treated water tanks (Peru)

These jars were treated with pyriproxyfen 0.5 granules. Efficacy was good for up to 6 months with the higher dose rates inhibiting emergence of adult mosquitoes by >87% (Fig. 7). At a dosage of 27 ppb pyriproxyfen, monthly removal and replacement of two thirds of the water did not reduce efficacy (Chang Moh Seng et al., 2006).

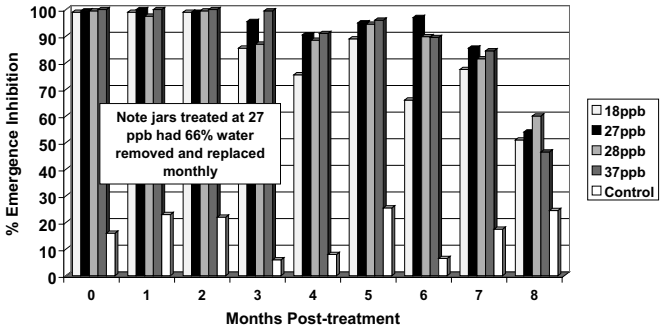

Figure 7. Inhibition of adult emergence of Ae. aegypti using pyriproxyfen 0.5G (Cambodia)

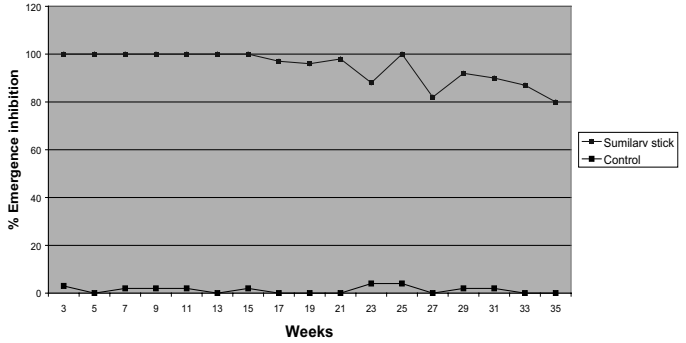

Figure 8. Inhibition of adult emergence of Ae. aegypti from water jars treated with pyriproxyfen sticks at 0.39 ppm (top line) compared with control (bottom line)

Pyriproxyfen Sticks

A trial was conducted using 100 water storage jars in 23 households with capacities of between 300-400 l. The jars were treated with a slow release pyriproxyfen stick formulation at a rate equivalent to 0.039 mg a.i./l (39 ppb). The jars were checked every two weeks and pupae were collected from the surface of the water in each treated and control jar. These were transported with water from the jar back to the laboratory where emergence was monitored. Results, presented in Fig. 8, demonstrate that emergence inhibition remained at 100% for at least 3 months at 39 ppb and above 98% for 22 weeks, remaining above 80% for 35 weeks when the trial ceased (Chang Moh Seng et al., 2007)

Larviciding

Larviciding as a vector control tool has largely fallen out of fashion for malaria control but is still used significantly for control of nuisance mosquitoes in urban environments and of the vectors of dengue. It is generally believed that the control of Anophelines is difficult as the majority of species are rural and their breeding sites are too widespread for practical application. As a consequence the major interventions for malaria control are indoor residual sprays and the use of long lasting insecticidal nets. However these interventions do not provide complete control. The addition of an effective larviciding program would further increase the impact on vectors and their associated disease agents.

Studies conducted in Africa suggest that 80% of Anopheline breeding sites in Africa are man made and close to human habitation. They are therefore relatively easy to find and treat. Studies have shown that many of the so called breeding sites are temporary pools and are not pupal productive. Therefore even if treatment regimes are limited within 100 metres of the confines of a village then the impact of larviciding can still be significant. The most important breeding sites in the village are borrow pits created when soil is removed to make bricks, also standing water around stand pipes and irrigation channels around crop gardens can be also be important.

Resistance

Insecticide resistance has become a major concern in vector control with resistance now present in mosquitoes to all major classes of chemical insecticides. This can lead to product failure and a rise in disease transmission. There is no known field resistance to pyriproxyfen. Several papers have been published supporting this. An organophosphate resistant strain of *Cx. quinquefasciatus* was pressurised with pyriproxyfen for 17 generations to see if resistance developed, and no increased tolerance was observed (Schaefer and Mulligan, 1991). Treatment of *An. subpictus* and *A. nigerrimus* in rice fields in Sri Lanka with two organophosphates showed high resistance resulting in control failure. In contrast pyriproxyfen conferred no selective advantage to larvae with either oxidase or acetylcholinesterase-based resistance mechanisms and continued to provide control for 71 days (Hemingway et al., 1998). Strains of *Culex pipiens molestus* were used to evaluate pyriproxyfen. There were 2 resistant strains, one resistant to organophosphates and the other to both pyrethroids and methoprene. Pyriproxyfen showed potent activity against both strains and even against the methoprene resistant strain, demonstrating a different mode of action and lack of cross resistance, even to another insect growth regulator (Yoshiaki Kono et al., 1997).

Transfer Technology

It has been found by several researchers that if adult mosquitoes are allowed to contact pyriproxyfen treated surfaces they can subsequently transfer it to oviposition sites where it can have a subsequent impact on pupal emergence, causing inhibition of emergence (IE). The first publication demonstrating this effect investigated blood-fed *Ae. aegypti* exposed to 1.0 g/m² of pyriproxyfen for 30 minutes that were then allowed to lay eggs in cups of water containing 4th instar larvae. The IE rate varied according to the numbers of mosquitoes exposed and subsequently allowed to access the oviposition site. With as few as 5 females accessing the oviposition pot there was 100% IE of adults from pupae. The authors also evaluated the effect of treating female *Ae. aegypti* before and after blood meals.

The results showed that mosquitoes exposed to pyriproxyfen 4 days before their meal laid very few eggs but the number of eggs laid increased dramatically if the exposure was 3 days after the blood meal.

This indicates that exposure to pyriproxyfen prior to feeding has an effect on egg development in the female mosquito.

However, pupae developed from the few eggs laid by females exposed 4 days before their blood meal achieved 76.7% adult emergence. In contrast only 5% emergence was recorded from pupae developed from eggs laid by females exposed 3 days after their blood meal. This could be related to the amount of pyriproxyfen remaining on the cuticle of the mosquito and its' resultant transfer to the oviposition site. This was confirmed in another study detecting pyriproxyfen on females from 1-7 days after exposure. At 5 days pyriproxyfen had nearly all disappeared and at 7 days none was detectable on their cuticle. (Itoh et al., 1994)

In another study adults were exposed to pyriproxyfen treated surfaces which resulted in between 43% and 73% IE of *Ae. albopictus*. Rates of inhibition varied depending on the numbers of adults exposed and hence the amount of pyriproxyfen transferred. There appeared to be no impact on fecundity, however egg hatch rate declined by 30% from the 1st to 2nd gonotrophic cycles (Dell Chism and Apperson, 2003). In a more recent study it was shown groups of 5 or 20 blood-fed *Ae. aegypti* exposed to residues of 3 mg pyriproxyfen/m² could transfer enough chemical to new oviposition sites, this preventing approximately 80% of adult emergence from larvae developing in previously uncontaminated water. In addition the authors found that while fecundity was unaffected, the subsequent eclosion of the eggs that these mosquitoes laid was decreased by 70-90% (Sihuincha et al., 2005).

REFERENCES CITED

Arshad. A., Chowdhury, M.A., Hossain, M.I. Ul-Ameen, M., Habiba, D.B. and Aslam, A.F.M. 1999. Laboratory evaluation of selected larvicides and insect growth regulators against field collected *Culex quinquefasciatus* larvae from urban Dhaka, Bangladesh. J. Am. Mosq. Cont. Assoc. 15(1): 43-47.

Chavasse, D.C., Lines, J.D., Ichimori, K., Majala, A.R. Minjas, J.N. and Marijani, J. 1995. Mosquito control in Dar es Salaam. II. Impact of expanded polystyrene beads and pyriproxyfen treatment of breeding sites on *Culex quinquefasciatus* densities. Med. Vet. Entomol. 9, 147-154.

Dell Chism, B. and Apperson, C.S. 2003. Horizontal transfer of the insect growth regulator pyriproxyfen to larval microcosms by gravid *Aedes albopictus* and *Ochlerotatus triseriatus* mosquitoes in the laboratory. Med. Vet. Entomol. 17, 211-220.

Hemingway, J. and Bonning, B.C. 1988. Possible selective advantage of Anopheles spp. (Diptera: Culicidae) with the oxidase- and acetylcholinesterase-based insecticide resistance genes after exposure to organophosphates or an insect growth regulator in Sri Lankan rice fields. Bull. Ent. Res. 78 (3): 471-478.

Itoh, T., Kwada, H., Abe, A., Eshita, Y., Rongsriyam, Y. and Igarashi, A. 1994. Utilization of bloodfed females of *Aedes aegypti* as a vehicle for the transfer of the insect growth regulator pyriproxyfen to larval habitats. J. Am. Mos. Control. Assoc. 20 (3): 344-347.

Kwada, H., Shono, Y., Itoh, T. and Abe, Y. 1993. Laboratory evaluation of insect growth regulators against several species of Anopheline mosquitoes. Jpn. J. Sanit. Zool. 44 (4): 349-353.

Moh Seng, C., Setha, T, Chanta, N., Socheat, D., Guillet, P and Nathan, M.B. 2006. Inhibition of adult emergence of *Aedes aegypti* in simulated domestic water-storage containers by using a controlled release formulation of pyriproxyfen. J. Am. Mosq. Cont. Assoc. 22(1): 152-154.

Moh Seng, C. 2007. Personal communication on field evaluation of controlled 'stick' formulation of 5% pyriproxyfen against *Aedes aegypti* in household concrete water storage containers in Cambodia.

Schaefer, C.H. and Mulligan, F.S. 1991. Potential for resistance to pyriproxyfen: A promising new mosquito larvicide. J. Am. Mosq. Cont. Assoc. 7(3): 409-411.

Sihunincha, M., Zamora-Perea, E., Orellana-Rios, W., Stancil, J.D., Lopez-Sifuentes, V. Vidal-Oré, C. and Devine, G.J. 2005. Potential use of pyriproxyfen for control of *Aedes aegypti* (Diptera: Culicidae) in Iquitos, Peru. J. Med. Entomol. 42 (4): 620-630.

World Health Organization. 2001. Report of the fourth WHOPES working group meeting, WHO/HQ, Geneva 4-5 December 2000. WHO/CDS/WHOPES/2001/2.

World Health Organization. 2003. Pyriproxyfen in drinking water. WHO/SDE/WSH/03.04/113.

Yapabandara, A.M.G.M. and Curtis, C.F. 2004. Control of vectors and incidence of malaria in an irrigated settlement scheme in Sri Lanka by using the insect growth regulator pyriproxyfen. J. Am. Mosq. Cont. Assoc. 20(4): 395-400.

Yoshiaki, K., Omata-Iwabuchi, K. and Takahashi, M. 1997. Changes in susceptibility to pyriproxyfen, a JH mimic during late larval and early pupal stages of *Culex pipiens molestus.* Med. Entomol. Zool. 48 (2): 85-89.

Vythilingam, I., Maria Luz, B., Hanni, R., Siew Beng, T. and Cheong Huat, T. 2005. Laboratory and field evaluation of the insect growth regulator pyriproxyfen (Sumilarv 0.5G) against dengue vectors. J. Am. Mosq. Cont. Assoc. 21(3): 296-300.