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# REPORT OF THE FOURTH WHOPES WORKING GROUP MEETING

## WHO/HQ, GENEVA
## 4-5 DECEMBER 2000

## REVIEW OF :

## IR3535; KBR3023; (RS)-METHOPRENE 20% EC, PYRIPROXYFEN 0.5% GR; AND LAMBDA-CYHALOTHRIN 2.5% CS

World Health Organization
Geneva

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## 1. INTRODUCTION

The 4th WHOPES Working Group Meeting, the scientific committee to assist the WHO Pesticide Evaluation Scheme (WHOPES) in the review of the reports of testing/evaluation of pesticides in the Scheme, was held in WHO/HQ, Geneva, 4 to 5 December 2000.

The meeting was opened by Dr Lorenzo Savioli, Coordinator, Strategy Development and Monitoring for Parasitic Diseases and Vector Control (PVC). Dr Savioli briefly introduced the structure of the Programme on Communicable Disease Prevention, Eradication and Control (CPE) and that of PVC at the WHO headquarters. He highlighted that prevention through vector control is an integral part of vector-borne disease management and noted the renewed interest in vector control at global level. He informed the participants of the PVC's plans to hold a meeting with WHO Regional Offices in the near future, to revisit the strategic framework for the vector control related activities of the CPE.

Dr Morteza Zaim, Scientist in charge of WHOPES recalled that the first, second and third meetings of WHOPES Working Group have been held in 1997, 98 and 1999, and their reports have been issued as WHO documents and widely distributed. He informed that the present meeting was convened to review the reports of the testing and evaluation of two insect repellents, KBR 3023 (Bayer AG, Germany), IR3535 (Merck, Germany), two insect growth regulators (IGRs) for mosquito larval control, (RS)-methoprene 20% emulsifiable concentrate (EC) (Babolna Bioenvironmental Centre, Hungary), pyriproxyfen 0.5% granule (GR) (Sumitomo Chemical, Japan), as well as lambda-cyhalothrin 2.5% capsule suspension (CS) (Zeneca, UK).

Dr Zaim provided an overview of the Scheme and informed the participants of the role of WHOPES in collection, consolidation and dissemination of information on the use of pesticides for public health. He noted that the recommendations of WHOPES are expected to expedite the registration of pesticides by the Member States. National authorities are encouraged to minimize requirements for local testing of products that have given satisfactory results of trials for similar circumstances. However, Dr Zaim noted that the **WHOPES recommendations are based on the review of the data and information of the products of the above-mentioned manufacturers and do not necessarily apply to nominally similar products of other manufacturer(s), nor to those where the active ingredient is produced by other methods of synthesis.**

Dr Zaim also noted the close collaboration of the Scheme with the International Programme on Chemical Safety (IPCS). Normally WHOPES field studies are carried out only when the human and environment safety of the product has been assessed by IPCS.

Dr Zaim also informed the participants of the meeting of the recent initiative of the Scheme, relating to alternative mechanisms to accelerate the discovery and development of public health pesticides. He briefed the participants on the meeting held in WHO/HQ, Geneva, 18 October 2000, with major manufacturers of public health pesticides and representatives of Malaria Consortium, USNIAID, the Wellcome Trust, the World Bank, in which the great significance of vector-borne diseases and constraints on their control by current methodologies were discussed. The meeting reviewed the future requirements for public health pesticides and unanimously recognized the urgent need to pursue development of alternative pesticide products for vector control, especially as it relates to malaria (treatment of mosquito nets and indoor residual spraying) and dengue/dengue haemorrhagic fever (larviciding and adulticiding). The meeting emphasized

the unique role of WHOPES in coordinating activities related to development of alternative pesticide products for public health and recommended that WHO should visit and discuss with manufacturers of vector control products, under appropriate confidentiality agreements, potential new technologies and compounds for vector control. The meeting requested WHOPES to produce an inventory of potential compounds and technologies available for development and a range of actions for collaborative activities. The report of the meeting is available on WHO homepage on the Internet at >www.who.int/ctd/whopes<.

Once a product is found to meet the requirements of the Scheme, specifications are prepared and published. The specifications include a description of the pesticide concerned and the formulations suitable for use in public health, together with sections concerning their physical and chemical characteristics. If necessary, the maximum contents of impurities are also included in the specifications. Methods for measuring the characteristics of the products are also described. The specifications are part of the International Code of Conduct on the Distribution and Use of Pesticides and are used in international trade and for quality control.

Dr Zaim informed the Group that a memorandum of understanding has been prepared with the Food and Agriculture Organization of the United Nations (FAO) to establish a Joint Meeting on Pesticide Specifications, by which joint FAO-WHO specifications will be developed for technical materials and technical concentrates. Following this new initiative, the WHO specifications for public health pesticides developed do not necessarily apply to nominally similar products of other manufacturers and WHOPES may extend the scope of the specifications to notionally similar products, if it has been satisfied that the additional products are equivalent to those which formed the basis of the evaluation and reference specification.

The meeting was attended by 8 scientists (see list of participants, Annex 2). Professor Arshad Ali, was appointed as Chairman, and Dr Carlo Costantini, as Rapporteur. The meeting was convened in plenary sessions for comprehensive discussion on aspects relating to the public health use of the above-mentioned products and divided into three small working groups to consider the results of the testing and evaluation of different products in detail. The reports of the safety assessments of the International Programme on Chemical Safety (IPCS), WHOPES supervised trials and relevant published literature, as well as the reports submitted by the national disease and vector control programmes (see bibliography, Annex 1) were fully discussed and recommendations on the use of the above-mentioned products were made.

# 2. REVIEW OF INSECT REPELLENT IR3535

## 2.1 Safety assessment[1]

IR3535 (3-(N-acetyl-N-butyl)aminopropionic acid ethyl ester) is of low acute toxicity, $LD_{50}$ being >14,000 mg/kg orally and >10 mg/kg dermally. In a 4-week oral toxicity study, no compound-related effects were observed at the highest dose tested, 2700 mg/kg.

In rabbits the "no observed adverse effect level" (NOAEL) in 2- and 4 week studies was 500 mg/kg; at 6000 mg/kg, a decrease in food consumption, and in body weight development were observed at 600 mg/kg in two weeks. In a 90-day dermal toxicity study in rats, the NOAEL was 3000 mg/kg, the highest dose tested. No mortality was observed in rats in a 4-h inhalation exposure to an air concentration of 5.1 $g/m^3$.

In short term studies in rats, mice and dogs, and in a 90-day study in rats, IR3535 showed a low skin irritation capacity. In several studies, 10, 15, and 20% dilutions of, as well as undiluted IR3535 caused marked conjunctival irritation in rabbits; corneal opacities, which recovered slowly, were also observed.

In single studies, IR3535 did not phototoxic of photoallergic reactions in guinea pigs. In limited studies, IR3535 did not demonstrate skin sensitisation potential

IR3535 did not induce developmental toxicity in rats or rabbits in valid studies. In one study in Himalayan rabbits, marked embryotoxicity was observed at dose levels significantly toxic to the does.

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[1] This assessment is based on the condensed confidential summary of toxicity studies, provided by Merck, Germany, and was performed by IPCS Secretariat.

In a valid 2-generation study in rats, there were no effect of treatment on sperm parameters, oestrous cycles, mating, fertility, duration of pregnancy, numbers of litters or implantations, or growth or development of the pups. In the first generation litters, there was a higher incidence of stillborn pups and pup deaths in the mid and high dose groups; no such finding was observed in the second generation. Because of this inconsistency, the authors considered that this effect was not related to the treatment.

IR3535 was not mutagenic to *S. typhimurium* or *E. coli*, did not induce point mutations (HGPRT) in CHO or V79 cells, or chromosomal aberrations in CHO cells *in vitro* at levels not toxic to the cells. It did not induce micronuclei in bone marrow cells in exposed mice treated intraperitoneally with IR3535 at a dose level 73% of the $LD_{50}$.

IR3535 is readily absorbed through intact skin in rats: within 24 hours 40% were reported to be absorbed. cultured hepatocytes from rat and man metabolised IR3535 effectively to a single metabolite, the carboxylic acid derivative. Similar metabolite pattern was observed in studies *in vivo* in rats and rabbits. Radioactivity from IR3535 disappears rapidly from the plasma, with a half time of 0.5 - 0.7 h in rabbits and rats after an intravenous administration. The excretion takes place mainly via urine.

After single or repeated skin application, no reactions were observed in human volunteers. Preparations containing IR3535 have been on the market for many years in several countries; no adverse effects have been reported. The producer reports that, upon request, 11 companies using this active ingredient in their products have specifically stated that no adverse effects have been observed.

No long-term toxicity and carcinogenicity studies have been reported. However, consistently negative findings in

genotoxicity testing, together with the apparently innocuous chemical structure of the chemical, make it unlikely that IR3535 were carcinogenic to humans. However, long term toxicity and carcinogenecity studies would be advisable.

## 2.2 Efficacy - background/supporting documents

Manufacturer internal research (Marchio, 1996) tested IR3535 against a range of nuisance and vector insects in different experimental conditions. Two ml of a 25% (w/v) ethanol formulation of IR3535 was applied on one leg of 6 subjects, giving an approximate 0.6 mg/cm$^2$ target dose, while the other leg was kept as a control. Tests were carried out outdoors for 6 nights with different subjects and up to 6 hours after treatment in two riverine villages in Liberia between 1900 and 0700 h. The percentage reduction of malaria vectors *Anopheles gambiae* and *An. funestus*, which constituted ~96% of the 1,157 mosquitoes collected landing on the control human baits, was 92% after 6 hours.

The time until a second bite from 500 *Aedes aegypti* freed in a 43-dm$^3$ cage was measured when three increasing target doses (0.1, 0.2, and 0.3 mg/cm$^2$) were applied on the forearm of subjects who exposed them for 5 minutes every hour. The length of action was directly correlated with dose, plateauing off at about 7–8 hours from application with the two higher doses tested. A similar experiment comparing a 30% formulation of IR3535 to a 33% formulation of DEET on 10 test subjects produced mean protection times of 7.6 and 6.3 hours, respectively.

Immediate and long term effectiveness against *Pediculus humanus* was evaluated under laboratory conditions by measuring the escape response of 96 adult lice put in a 1x1 square tissue treated with 20% IR3535. A total of 94% left the treated tissue after 32 minutes, 71% reaching the outer ring of the arena 32 cm from the treated centre, whereas more than 90% of the control batch remained on the central tissue. Soaking the

square tissue with IR3535 48 h before the test, still produced an escape response in 90% of the exposed lice. In Madagascar, three groups of schoolchildren aged 6 to 16 years with similar lice infestations were treated with an insecticidal shampoo and then applied 20% ethanolic formulations of IR3535 or DEET, or otherwise left untreated as a control. On the seventh day after treatment the control group had an infestation about 50% of the pre-treatment value, whereas in the other two groups this was less than 2%.

*Czech Republic* – An ethanol formulation of IR3535 was compared to DEET both in the laboratory and in the field (Rettich, 1999). Cohorts of 50 nulliparous *Ae. aegypti*, 7–14 days old, kept in a 12-$dm^3$ cage were exposed for 1–3 minutes to arms of 4 male and 4 female subjects who treated their left forearm with IR3535 and the right forearm with DEET. Repellents were applied at increasing doses in different experiments: 0.15 $mg/cm^2$ (34 tests), 0.30 $mg/cm^2$ (7 tests), and 0.45 $mg/cm^2$ (14 tests). The number of biting mosquitoes was recorded immediately after application and at 0.5, 1, 1.5, 2, 3, and 4 hours intervals. When the biting activity on an untreated arm exposed before and after each test was lower than 0.5 bites/second, the test was not executed or was discarded.

As expected, the average number of bites increased with time from application at all doses tested and for both repellents. The arm treated with IR3535 received on average 1.6x–20.8x more bites than that treated with DEET. This difference decreased with time and reduced to zero at 4 hours from application at the highest dose tested.

Field tests were carried out in a flood-plain forest in Central Bohemia where biting rates of about 714 landings/person/hour by *Ae. cantans* and *Ae. annulipes* were measured during landing collections on untreated human baits for 1–5 minutes just before repellent application. Five ml of a 15% ethanol solution of IR3535 or DEET were applied on the legs (from hips to ankles) of subjects, giving an application target dose of ~0.125 $mg/cm^2$. The human baits collected biting mosquitoes off their legs for 5

minutes, then moved 5–10 m away and repeated so in 2–3 sites in the forest. The tests lasted until the repellent effect faded away or up to 6 hours from application. During resting periods, the subjects slowly walked outside the forest where biting was negligible. Maximum likelihood re-analysis of data showed significant differences between the two repellents, giving approximate 95% protection times of 4.8 and 9.7 hours for IR3535 and DEET, respectively.

*Thailand* – An ethanol formulation of IR3535 was compared to DEET both in the laboratory and in the field (Thavara *et al.*, unpublished report). In laboratory tests, 0.1 ml of a 20% (w/w) solution of each repellent was applied at the start of a test onto a 30-$cm^2$ marked area on either forearm of three subjects 23-35 years old. This treatment achieved a target dose of 0.55 $mg/cm^2$. The treated skin was exposed for 3 minutes every half an hour to 250 females of disease vectors *Ae. aegypti*, *Culex quinquefasciatus*, *Cx tritaeniorhynchus*, or *An. dirus*, kept in a 27-$dm^3$ cage. Depending on the biting cycle of each species, tests were performed for eight hours at corresponding times during the day or night. Protection time was defined as the time elapsed from the application of repellent and the second bite. IR3535 had the same median protection time as DEET for culicine species, but about half (46%) the median protection time of DEET for *An. dirus*, a significantly lower score compared to either the culicines or DEET.

Field tests at five selected peninsular and continental sites were carried out over 5 months with six pairs of human baits 18–42 years old collecting mosquitoes biting or landing on their legs: 3 ml of the same formulation used in the laboratory tests was applied on one leg of six collectors, whereas the remaining six collectors acted as controls. The other leg of the treated collectors was applied with 3 ml of an identical DEET formulation. This application protocol produced a target dose of approximately 0.3 $mg/cm^2$. Collections alternated 10-min catching bouts with10-min resting bouts carried over for up to

eight hours at day (0900–1700 h) or five hours at night (1900–2400 h). The percentage reduction in mosquitoes landing on the treated collectors as compared to the control collectors was calculated at hourly intervals.

*Aedes albopictus* accounted for 76% of 1,083 mosquitoes caught in two days by the untreated collectors during the daytime tests, giving an average biting rate for this species of 17.1 landings/person/hour. Other predominant species caught by the control collectors were *Armigeres subalbatus* (13%) and *Coquillettidia crassipes* (11%), giving biting rates of 3.1 and 2.0 landing/person/hour, respectively. Only 23 *Ar. subalbatus* could be caught by the treated collectors during these tests, giving a specific percentage reduction of 94% and 90% for IR3535 and DEET, respectively.

During the night-time tests, 1,076 mosquitoes belonging to 12 species were caught in 6 days by the control collectors. Of these, *Cx gelidus* accounted for 32% of the total, *An. hyrcanus* for 16%, *Cx quinquefasciatus* for 14%, and *An. minimus* for 13%. The remaining 25% was composed by *Cx tritaeniorhynchus*, *Mansonia dives*, *Cx sitiens*, *Ma. annulifera*, *Ma. annulata*, *An. maculatus*, *An. sawadwongporni*, and *An. pseudowillmori* in decreasing order of frequency. Another 49 mosquitoes of unspecified species were also collected. The species-specific and overall biting rates varied widely from one site to another, ranging 0.5–23.2 and 3.7–36.3 landings/person/hour, respectively. IR3535 and DEET-treated collectors caught 6 and 7 mosquitoes during the tests, belonging to *An. hyrcanus*, *An. minimus*, and *Cx sitiens*, giving overall percentage reductions of 99.5% and 99.4%, respectively. There was no significant difference at each site in the percentage reduction of IR3535 compared to DEET. When comparing different sites, however, it appeared that IR3535 efficiency was significantly reduced where anophelines were the most abundant species.

In both field experiments there was no clear decrease in the percentage reduction with time, presumably due to the small

sample sizes obtained. No rash, skin irritation, or hot sensation was reported by the subjects treated with IR3535 during and after its application. The main conclusions of this study are: i) under conditions of low biting pressures IR3535 performs equally well than DEET for up to 5 hours in the case of several culicine species belonging to the genera, *Culex* and *Mansonia*, and up to 8 hours against *Ae. albopictus*; ii) as in the case of DEET, IR3535 performs comparatively worse against *Ar. subalbatus* and several other South-East Asian anopheline species.

## 2.3 WHOPES supervised trials

*Burkina Faso* – The protection time of IR3535 against bites from *An. gambiae* complex mosquitoes was compared to that of DEET in field studies carried out for six months throughout one rainy season in a rural village near Ouagadougou (Costantini & Ilboudo-Sanogo, unpublished report). Eight human subjects applied four target doses (0.10, 0.30, 0.60, and 0.80 mg/cm$^2$) of an ethanolic formulation of either repellent on their lower limbs just before the start of the trials. Four groups of two collectors each were allocated to a 4x4 (sites x nights) latin square which was replicated six times for each of the target doses tested. Each group tested one repellent on any one night, whereas one group of collectors acted as a placebo (ethanol only) control; the remaining fourth group tested another repellent (see below). All mosquitoes landing on the exposed legs and feet were aspirated out of doors from 1800 to 2200 h, and indoors from 2400 to 0400 h, with a resting pause of two hours in-between, thereby allowing evaluation of protection efficacy for a period of up to 10 hours.

Almost 30,000 mosquitoes belonging to 15 species (or species complexes) were caught by the control collectors during 96 test nights, giving an average landing rate of 19.2 landings/person/hour. About 93% were *An. gambiae* s.l., followed by *An. nili* (3.8%), *An. funestus* (1.3%), *An. pharoensis*

(0.2%), and several *Aedes* species, among which important vectors of dengue and yellow fever. As expected, median protection times for *An. gambiae* s.l. increased with the application dose, ranging 4.6–22.6 hours for 1R3535, and 5.2–14.7 hours for DEET. At the 90% endpoint, protection times ranged –2.0–6.7 hours for IR3535 (the negative value indicating a reduction <90% immediately after application, as extrapolated from the maximum likelihood linear model relating the percentage reduction of mosquitoes landing on the treated subjects compared to the control subjects) and 2.1–10.9 hours for DEET. Thus, protection times were generally longer for DEET than IR3535.

The linear model relating the log-dose at application with the 90% protection time allowed estimation of the repellents' loss rate, and their $ED_{90}$ (effective dosage). Loss rates of the two repellents were similar, whereas the $ED_{90}$ was more than twice higher for IR3535 than DEET. This was confirmed by laboratory trials on the F1 progeny of wild females using the "separate arms" protocol of Curtis *et al.* (1987), which gave a 0.032 relative potency estimate for IR353 compared to DEET. The proportion of *An. gambiae* s.l. harbouring sporozoites in their salivary glands (estimated by ELISA) was not significantly different across treatments, indicating that a reduction in the number of bites afforded by the repellents reflected a reduction in the number of infective bites as well. Perception of the repellents cosmetic properties by the test subjects revealed a better assessment for IR3535, which was never deemed irritant and its odour was acceptable instead of unpleasant most of the time.

*Malaysia* – An ethanol formulation of IR3535 was compared to DEET both in the laboratory and in the field (Yap 1998 a,b). In laboratory tests, 0.18 ml of a 25% (w/v) solution of each repellent was applied at the start of a test onto a 90-$cm^2$ area on the right forearm of human subjects. This treatment achieved a target dose of 0.50 $mg/cm^2$. The left arm was consistently left untreated as a control. Both arms were contemporarily offered

for 3 minutes to 50 *Ae. albopictus*, or 200 *An. dirus*, aged 3–10 days kept in a 216-$dm^3$ cage, by exposing an area of 25 $cm^2$ of each arm. The number of mosquitoes landing or biting was counted 1, 2, 4, 6, and 8 hours after the application of the test samples. A fresh batch of starved females was introduced at every assessment hour. The same procedure was followed for the three tests comparing an untreated arm *vs.* either a placebo (an arm treated with 75% ethanol), an IR3535-treated arm, or a DEET-treated arm. Three replicates (i.e. test days) were performed for each species. The percentage reduction in mosquitoes landing on the treated arm as compared to the control arm was calculated for every testing bout.

A maximum likelihood re-analysis of the data showed that IR3535 had a median protection time of 5.8 hours and a 95% protection time of 1.9 hours against *Ae. albopictus*. Both estimates were 10% and 26% lower than those of DEET. The median protection time was 5.3 hours for *An. maculatus*, but the 95% endpoint could not be calculated, because even extrapolation of the functional relationship at time of repellent application gave a percentage reduction less than 95% (74%). The median protection time was 32% lower than that afforded by DEET. Thus, the response of the two species was substantially different, albeit in both cases IR3535 produced a lower percentage reduction than DEET. The difference in efficacy between the two repellents, however, was greater in the case of *An. maculatus*. Moreover, this species had longer median protection times but fairly lower percentage reduction estimates than *Ae. albopictus* for both DEET and 1R3535 at the dose tested.

A similar protocol and the same repellent formulations as for the laboratory tests were employed during field tests carried out over three days in two coastal areas of north-western peninsular Malaysia harbouring high frequencies of *Ae. albopictus* (forest area) and *Cx quinquefasciatus* (urban area). A total of 0.75 ml and 1.5 ml of repellent formulation or placebo were applied on the right arm (wrist to elbow) and leg (knee to ankle),

respectively, of human baits instructed to protect all other areas of the body from mosquito bites and to stay at least at 5 m from each other. The application achieved an approximate target dose of 0.20 mg/cm$^2$. In the forest area, 9 human baits were involved (3 treatments x 3 repetitions of each treatment), whereas in the urban area 18 subjects were involved (3 treatments x 3 repetitions of each treatment x 2 groups). The efficacy of the repellents was tested over up to 7.75 hours, with collections performed during 45 minutes 0, 1, 3, 5, and 7 hours after application (from 0900 to 1645 h in the forest area, and from 2100 to 0045 h in the urban area). In the urban area, one group of human baits applied the samples 4 hours before the start of the tests to gather data on the sixth to eighth hours post-treatment.

In contrast with expectations, due to unfavourable climate *Ae. albopictus* comprised only about 49% of the 1,963 mosquitoes collected in the forest area, the remaining 51% accounted mainly by *Cx bitaeniorhynchus*, *Cx gelidus*, *Cx sitiens*, *Cx tritaeniorhynchus*, and to a lesser extent by *Ae. gardneri*. From the data presented it is not possible to extrapolate results for each species, therefore –even if this is not the legitimate procedure– results were considered for the pool of species. Biting rates during the experiment averaged 42.4 landings/person/hour. Only 17 and 7 mosquitoes were caught after 5 hours from repellent application by the IR3535- and DEET-treated collectors, giving a cumulative percentage reduction of 96.3% and 98.2%, respectively. In the urban area, *Cx quinquefasciatus* comprised ~91% of the 2,514 mosquitoes caught during the experiment, giving an average biting rate of 52.0 landings/person/hour. Other species caught were *Cx gelidus* (7.5%), and *Ae. aegypti* / *Ae. albopictus* (1.7%). Only 3 and 1 mosquitoes were caught after 5 hours from application by the IR3535- and DEET-treated collectors, giving a cumulative percentage reduction of >99% for both repellents.

*USA* – Five subjects assessed the protection time of IR3535 compared to DEET in the Everglades National Park, Florida,

against attack from *Ae. taeniorhynchus* (Barnard, unpublished report). On each test, two human baits applied 1 ml of a 25% formulation of either repellent on 650 $cm^2$ of skin surface of one arm, chosen at random. This treatment achieved a target dose of 0.38 $mg/cm^2$. Another two subjects applied different repellents. The last subject acted as a control, treating the arm with an ethanol placebo. All body parts other than the treated arm were protected with appropriate clothing, boots, gloves, and head-nets. At hourly intervals, treated subjects collected mosquitoes landing off their arms for 3 minutes during six hours. Control subjects only collected for one minute, their yield was subsequently adjusted by 3x multiplication. During five tests performed over three days, when all human subjects received each treatment once, a total of 1,462 *Ae. taeniorhynchus* were collected. Two endpoints were assessed from the data, namely percentage reduction in landing mosquitoes on treated collectors compared to control collectors (i.e. percent repellence), and complete protection time defined as the time elapsed between repellent application and the observation period immediately preceding that in which the first mosquito landing on treated skin was observed.

Mean landing rates on control collectors generally decreased across each test, ranging 184–2,532 landings/person/hour. Both repellents provided ≥89% repellence throughout the test, hence median protection times extrapolated from the data-set are highly imprecise. Maximum likelihood re-analysis provided median protection time estimates higher for IR3535 than DEET (9.3 *vs.* 7.1 hours, respectively), but at the 95% end point the reverse was true (4.9 *vs.* 5.4 hours). Mean complete protection time was significantly higher for DEET (5.2 hours) than IR3535 (3.0 hours) using parametric ANOVA, but re-analysis of the data-set using survival analysis procedures showed that median complete protection time of IR3535 (3 hours) was just marginally lower than that of DEET (6 hours) employing Cox's F-test ($P$=0.05011).

## 2.4 Conclusions and recommendations

1. Although IR3535 has been in the market for more than 20 years, there are no reports of adverse effects on human health. Based on current information, chemical structure and mode of action, it is unlikely that IR3535 is hazardous to humans or, when used as an insect repellent, to the environment. Because of the eye-irritating potential, appropriate labelling of IR3535-based products to prevent eye exposure is recommended.

2. Ethanolic preparations of IR3535 showed good repellent properties under temperate and tropical conditions. The manufacturer's recommended target dose of 0.3 mg a.i./$cm^2$ of skin achieves 95% protection for approximately 2-3 hours against several *Aedes* and *Culex* mosquito species. Additional studies are recommended to further assess the repellent properties of IR3535 against anophelines as some of the studies under review showed its lower efficacy as compared to culicines.

3. IR3535 is recommended as a safe and effective insect repellent for human use.

# 3. REVIEW OF INSECT REPELLENT KBR 3023

## 3.1 Safety assessment[2]

KBR 3023 (1-piperidinecarboxylic acid 2-(2-hydroxyethyl)-1-methylpropylester) is of low acute toxicity in rats ($LD_{50}$: 4743 mg/kg body weight) and mice after oral administration and in rats after dermal ($LD_{50}$: >5000 mg/kg body weight) and inhalation exposure ($LD_{50}$: >4364 mg/kg body weight).

The chemical was demonstrated to have negligible dermal and limited ocular irritation capacity in rabbits. In the different skin sensitization tests, KBR 3023 remained negative. Phototoxicity was studied in 50 persons, and no phototoxicity was observed.

Upon repeated administration, the changes observed at the lowest doses, were induction of the hepatic cytochrome P450-dependent reactions, concomitant with an increased relative liver weight. In a 13-week study, low toxicity was observed. Locally skin changes that subsided after the cessation of the treatment, were observed in all treated groups, i.e.- even at the lowest dose of 80 mg/kg/day.

No carcinogenic activity was observed in a long-term dermal study in rats, in which at the highest dose, after two years, cystic degeneration of the liver was observed. In this study dermal effects similar to those seen in the 13-week study, but no other (except those in the liver) systemic effects, were observed.

No neoplastic response, related to the treatment, was observed in a long term study using mice. However, the study has limited power, since no treatment-related effects on any parameter were observed, i.e., it is not clear that the MTD was reached.

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[2] This assessment is based on the condensed confidential summary of toxicity studies, provided by Bayer AG, Germany, and was performed by IPCS Secretariat.

No reproductive toxicity was observed in a 2-generation study, using dermal application, at a daily dose of 200 mg/kg body weight. In an oral embryotoxicity and teratogenicity assay, no teratogenicity, but slight retardation of skeletal development was observed at a dose that was toxic to the dams (500 mg/kg body weight/day). In a dermal teratogenicity study with rats, no signs of embryotoxicity or teratogenicity was observed at a daily dose of 400 mg/kg, which was the highest dose that could be applied dermally; this dose level induced an increase in the relative and absolute liver weight in the dams, but no signs of overt toxicity. In a range-finding teratogenicity study in rabbits, at a dose of 1000 mg/kg body weight/day, that induced mortality in dams, decreased pregnancy rate was observed. In the main study, a dose level of 200 mg/kg body weight/day was used. No treatment-related effect on the pregnancy outcome was observed; the number of dams with soft feces was increased at a dose level of 200 mg/kg body weight/day.

Genotoxicity was studied using *Salmonella typhimurium* point mutation tests with 4 different strains, with and without metabolic activation, Chinese hamster ovarian cells hgprt-assay, and clastogenicity assays (chromosomal aberrations) with CHO cells, and with bone marrow micronucleus cell investigation *in vivo* in mice, as well as using the unscheduled DNA synthesis test on rat primary hepatocytes. No indication of genotoxicity was observed although concentrations that were cytotoxic (or induced mortality in case of the *in vivo* study) were used, with the exception of the chromosomal aberration test which gave a positive response at a cytotoxic concentration.

In acute (2000 mg/kg bw dermally) or subchronic (200 mg/kg bw/d no sing of behavioural or pathological anatomical neurotoxicity was observed.

After intravenous administration of KBR 3023, 14C labeled in the hydroxyethyl moiety, some 80-95% of the radiolable was recovered either in the urine (75-90%) or in faeces 5-16%,

mostly as hydroxylated metabolites, within 48 days. Following dermal application, a dose-dependent absorption was observed in rats, with approx. 60% absorbed at low (20 mg/kg) dose level, and 40-55% at a high (200 mg/kg) dose level.

In dermal absorption studies in healthy male volunteer humans, 15 mg of KBR 3023 were applied on the skin either as the undiluted technical grade material or as a 15% ethanol solution; no occlusion was used. Urine and faeces were collected for six days after the 8-h application. In marked contrast to rats, only 2-4% of the applied radioactivity was recovered in urine. No metabolites were observed in human urine that were not found in rats and that the metabolite patterns were similar in rats and in humans.

The study on the acute toxicity of KBR 3023 on *Daphnia magna* and rainbow trout in a static test were performed according the guidelines of OECD, and conducted in compliance with OECD GLP standards in a certified laboratory. Effective concentrations for *Daphnia magna* and lethal concentrations for *Oncorrhynchus mykiss* were in excess of 100 mg/L, and thus indicate low toxicity.

## 3.2 Efficacy - background/supporting documents

*Czech Republic* – An ethanolic formulation of KBR 3023 was compared to DEET both in the laboratory and in the field (Rettich, 1999). Experimental procedures were described previously in the section concerning IR3535 testing. In 138 laboratory tests, at the lower dose of 0.15 mg/cm$^2$ KBR 3023-treated subjects received 53–88% of the average number of bites received by the DEET-treated subjects. Biting activity of *Ae. aegypti* commenced for both repellents after 1 hour of testing. In 42 tests at the higher dose of 0.30 mg/cm$^2$, however, KBR-treated subjects received 1.7–2.5 more bites than DEET-treated subjects, and biting activity commenced 0.5 hours in advance for KBR 3023 compared to DEET.

During 3–4 field tests in a flood-plain forest in Central Bohemia experiencing high biting rates of *Ae. cantans*, *Ae. annulipes*, and *Ae. sticticus* (1,200–2,400 landings/person/hour), two human baits were treated with 2 or 5 ml of 20% KBR 3023 or 20% DEET, giving approximate target doses of 0.07 and 0.17 mg/cm$^2$. Considering a constant biting rate of 100 landings/person every 5 minutes, maximum likelihood estimates did not evidence significant differences between the two repellents, giving approximate 95% protection times of 2.3 and 5.4 hours for doses of 0.07 and 0.17 mg/cm$^2$, respectively.

*Malaysia* – KBR 3023 protection time against day-biting from *Ae. albopictus* and night-biting from *Cx quinquefasciatus* was compared to DEET outdoors in a forested orchard and indoors in an urban squatter of peninsular Malaysia (Yap *et al.*, 1998). Amounts of 0.5 and 1 ml of 10% and 20% ethanolic formulations of KBR 3023 and DEET were applied on the right arm (wrist to elbow) and leg (knee to ankle) of eight human baits positioned at least 5 m away from each other. Such treatment achieved approximate target doses of 0.07 and 0.15 mg/cm$^2$, respectively. The left limbs were left untreated as controls. During the day-time study, collections of landing/biting mosquitoes were performed for 8 hours from 0900 to 1700 hours, whereas in the night-time study collections lasted four hours (2100–0100 h). Thus, in order to assess the repellents efficacy after 8 hours from application, two groups of 8 collectors each were employed in the latter experiment, and the first group applied the repellents four hours before the start of each trial.

Three replicates of each experiment yielded a total of 5,525 and 6,633 mosquitoes giving average landing rates on the control limbs of 28.8 and 34.5 landings/person/.hour, respectively. *Aedes albopictus* constituted about 89% of the total catch from the orchard, the other species collected belonging to the genera *Armigeres* (~9%), *Aedes* (~1.2%), *Culex* (~0.6%) and *Mansonia*

(0.01%), whereas *Cx quinquefasciatus* represented more than 99% of the total catch from the urban squatter.

A maximum likelihood re-analysis according to the model proposed by Rutledge *et al.*, (1985), assessing the functional relationship of the decrease with time in the percentage reduction of mosquitoes attempting to land/bite the treated collectors, showed consistently longer protection times of KBR3023 than DEET in both experiments. Both repellents showed longer protection times for *Cx quinquefasciatus* than *Ae. albopictus*. Tested formulations did not cause any problem of skin irritation or other adverse effects during and after application; moreover, the human subjects reported to be much more comfortable with the odour of KBR 3023 than that of DEET.

### 3.3 WHOPES supervised trials

*Burkina Faso* – The protection time of KBR 3023 against bites from *An. gambiae* complex mosquitoes was compared to that of DEET in field studies carried out in a rural village near Ouagadougou (Costantini & Ilboudo-Sanogo, unpublished report). Experimental procedures and general results were presented previously in the section concerning IR3535 testing.

As expected, median protection times for *An. gambiae* s.l. increased with the application dose, ranging 4.6–183.7 hours for KBR 3023, and 5.2–14.7 hours for DEET. At the 90% endpoint, protection times ranged 3.2–38.7 hours for and 2.1–10.9 hours for DEET. The protection times of KBR 3023 at the two higher doses tested were highly imprecise, as they were extrapolated from the maximum likelihood linear model relating the percentage reduction of mosquitoes landing on the treated subjects compared to the control subjects, and only a few mosquitoes were caught by the KBR 3023 treated collectors. In

any case, protection times were always longer for KBR 3023 than DEET.

The linear model relating the log-dose at application with the 90% protection time allowed estimation of the repellents' loss rate $\lambda$, and their $ED_{90}$. The loss rate of KBR 3023 was lower than DEET, whereas the $ED_{90}$ of DEET was lower than that of KBR 3023. This ranking was confirmed by laboratory trials on the F1 progeny of wild females using the "separate arms" protocol of Curtis *et al.* (1987), giving a 0.792 relative potency of KBR 3023 compared to DEET. The proportion of *An. gambiae* s.l. harbouring sporozoites in their salivary glands (estimated by ELISA) was not significantly different across treatments, indicating that a reduction in the number of bites afforded by the repellents reflected a reduction in the number of infective bites as well. Most of the time the human subjects deemed the odour of KBR 3023 acceptable instead of unpleasant as in the case of DEET.

*Scotland* – Experiments were carried out in summer 1999 in a peat marsh in Argyllshire where high densities of the Scottish biting midge *Culicoides impunctatus* can substantially impair outdoor activities (Mordue, unpublished report). Five subjects applied on one arm two target doses (0.17 and 0.30 mg/cm$^2$) of a 20% w/v KBR 3023 formulation, leaving the other arm untreated as a control. Experiments were performed during 31 test nights between 1930 and 2230 h, 0, 2, 4, 6, and 8 hours after repellent application. After allowing 10 minutes for build up of the midge population, landing parous female *C. impunctatus* were pooted off the arms of a subject acting as bait by two collectors for 3 minutes. Repeated exposures of the five human baits (4 replicates) allowed comparison of between-subjects variation in response to the repellent, and the decrease of effectiveness with time. A larger sample of ten subjects was used on one replicate to establish a more general response at time zero from application.

Immediately after application, 0.30 mg/cm$^2$ of KBR 3023 afforded a ~79% significant reduction in landing midges as compared to the control arm. There was no significant difference in response between the five subjects tested. The repellent effect at this application dose declined to zero after about 7 hours. The decline with time was substantially slower at the 0.30 mg/cm$^2$ dose. Biting rates could be established only once on the basis of the inflammatory reaction on the skin of both arms: the lower proportion of bites (5/142) compared to landings suggested that not all the midges that landed on the treated skin would have completed the behavioural sequence leading to the taking of a blood meal. Moreover, the extremely high landing rates on control arms (up to 40,000 landings/person/hour) reduced the pootering efficiency. The efficacy of KBR 3023 in preventing bites, therefore, may have been underestimated in this experiment.

*USA* – The protection time of KBR 3023 against attack from *Ae. taeniorhynchus* was compared to that of DEET in the Everglades National Park, Florida (Barnard, unpublished report). Experimental procedures and general results were presented previously in the section concerning IR3535 testing. Both repellents provided ≥89% repellence throughout the test, hence median protection times extrapolated from the data-set are highly imprecise. Maximum likelihood re-analysis provided median protection time estimates higher for KBR 3023 than DEET (8.5 *vs.* 7.1 hours, respectively); this ranking was maintained at the 95% end point (6.1 *vs.* 5.4 hours). Parameter estimates of the functional relationship relating percent repellence with time from application, however, were not significantly different. Median complete protection times were identical for DEET and KBR 3023 (6 hours).

## 3.4 Conclusions and recommendations

1. KBR3023 has a good safety profile and cosmetic properties. Alone or in typical formulations it does not significantly attack common household materials including plastics, coatings, foils, and varnishes.

2. KBR3023 was tested under temperate and tropical conditions against important disease vectors *Ae. albopictus*, *An. gambiae* and *Cx quinquefasciatus* and several pest mosquito demonstrating excellent repellent properties comparable to, and often superior, to those of the standard DEET.

3. At the manufacturer's recommended target dose of 0.3 mg a.i./cm$^2$ of skin, KBR3023 confer more than 95% protection up to 6-7 hours after application. At comparable doses, KBR3023 showed significantly longer protection times than DEET against *An. gambiae* complex malaria vectors; although further studies are needed to assess its efficacy against a broader range of anopheline vector species, KBR3023 can be recommended as the repellent of choice for malaria prevention.

4. Given the promising results shown by KBR3023, efficacy test of this chemical for treatment of mosquito nets, garments, and other materials is recommended.

5. KBR3023 is recommended as a safe and effective insect repellent for human use.

# 4. REVIEW OF INSECT GROWTH REGULATOR (RS)-METHOPRENE EC

## 4.1 Safety assessment

Methoprene (Isopropyl (2E,4E)-11-methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate) is a juvenile hormone analogue racemate. It is an insect growth regulator (IGR), and when ingested by the larvae, it interferes with the normal process of mosquito development, preventing the emergence of adults from pupae. Methprene products are available in the market as (RS)-methoprene or (S)-methoprene. The latter is the biologically active enantiomer (2E, 4E, 7S) of (RS)-methoprene (a racemic mixture of 2E, 4E, 7R and 2E, 4E, 7S). There is no significant difference in toxicity to mammals between (RS)-methoprene and (S)-methoprene.

The toxicity of methoprene has been assesed by the WHO/FAO Joint Meeting on Pesticide Residues (JMPR)(FAO 1984 and 1987) and the following conclusions are noted:

In mammals, a single oral dose of methoprene is eliminated via the urine, the faeces and the expired air. The compound is degraded to primary metabolites and to natural body constituents. Methoprene and its known metabolites have a low order to acute oral toxicity with the $LD_{50}$ values being over 5000 mg a.i./kg body weight for the parent compound in both rats and dogs and for the metabolites in rats.

A three-generation (one litter/generation) reproduction study in rats demonstrated a no-effect level on reproduction of at least 500 ppm. Teratology studies in both mice and rabbits gave no evidence of teratogenicity under the conditions of the experiments. However, pregnant mice and rabbits were treated with methoprene only from days 7 to 13 and from the days 7 to 18 of gestation, respectively; the entire period of organogenesis, therefore, was not covered. The available mutagenicity studies

were negative. A 78-week mouse and two-year rat oncology study were negative.

A minimum no-effect level (NOEL) of 500 ppm for rats was based on a three-generation study, and a no-effect level of 500 ppm for dogs was based on a 90-day feeding study. The acceptable daily intake for man has been established at 0 – 0.1 mg a.i./kg body weight.

WHO has classified methoprene technical grade active ingredient as "unlikely to present acute hazard in normal use".

The following are extracts from the Material Safety Data Sheet (MSDS) of the manufacturer for (RS)-methoprene 20% EC:

| | |
|---|---|
| Acute oral $LD_{50}$ (rat) | >5,100 mg/kg |
| Acute dermal $LD_{50}$ (rabbit) | >2,100 mg/kg |
| Skin irritation (rabbit) | Minimal |
| Eye irritation (rabbit) | Moderate |
| Acute inhalation (rat) | >5.4 mg/l (4 hour study) |

### 4.1.1 Effects of methoprene on aquatic nontarget organisms

Nontarget effects of methoprene on aquatic organisms have been reported in a large number of studies. Miura and Takahashi (1973) using technical grade (RS)-methoprene in the laboratory reported no acute toxicity of methoprene at mosquito control rates to 35 organisms including Protozoa, Platyhelmenthes, Rotatoria, Annelida, Arthropoda (20 species), Mollusca, Chordata and Thalophyta. In field pond studies, most nontarget organisms were unaffected, except that chironomids, ephydrids and psychodids were somewhat depressed. In a subsequent study, Miura and Takahashi (1974) used a liquid slow-release suspension of (RS)-methoprene at 0.11 kg a.i./ha and reported no adverse effects on the backswimmer *Notonecta unifasciata*

and some zooplankton species. Norland and Mulla (1975) using an EC of (RS)-methoprene at 0.1 ppm reported high mortality in young naiads of mayfly *Callibaetis pacificus*. Repeated applications at 5-day intervals at 0.1 ppm (302 g a.i./ha) to field ponds reduced larval chironomids, mayfly naiads and larvae of the predacious beetle *Laccophilus* sp. The ostracod *Cyprinotus* sp. and the predacious odonate naiads (*Tarnetrum corruptum, Erythemis simplicicolis, Enallagma civile, Anax junius*) were not affected. Population recovery of most affected groups occurred within 1-2 weeks after cessation of treatments.

In a field evaluation of the effects of slow-release wettable powder formulation of (RS)-methoprene applied at 21, 42 and 84 g a.i./ha to ponds, no adverse effects were observed on snails, adult and larval Coleoptera (Dytiscidae and Hydrophilidae), nymphal and adult Hemiptera (Notonectidae and Corixidae) and Dryopidae (Creekmur *et al.* 1981). Recently, Lawler *et al.* (2000) reported no adverse effects of (S)-methoprene pellets (4.25% a.i.) applied at 10.4 kg/ha and monitored for 99 days posttreatment for effects on water boatmen *Tricocorixa reticulata* and the brine fly *Ephydra millbrae*. Pinkney *et al.* (2000) treating experimental ponds (multiple treatments) with microencapsulated liquid suspension (S)-methoprene at 11 ppb at 3 week interval between May and July reported no adverse effects on Collembola, mayflies, *Chaoborus* sp. and Odonata.

Chu *et al.* (1997) studied the effects of (S)-methoprene on survival and reproduction of the freshwater cladoceran *Moina macrocopa* in the laboratory and reported 48-hour $LC_{50}$ value of 0.34 ppm. They also studied survival, longevity and fecundity of this cladoceran. At 0.005 and 0.01 ppm, longevity and fecundity increased slightly. They concluded that environmental concentrations of 0.05 ppm will not induce detrimental effects on natural cladoceran populations.

Bircher and Ruber (1988) investigated toxicity of a technical grade (RS)- methoprene to all stages of the salt marsh copepod

*Apocyclops spartinus*. They showed that eggs and early stage nauplii were most sensitive to the IGR, concluding that 0.1 ppm field concentration of methoprene should not induce population declines and only early stages of *Apocyclops* would be affected if methoprene was used at > 0.1 ppm rate which rarely is the case in most mosquito control efforts. Marten *et al.* (1993) used (S)-methoprene against nauplii of *Macrocyclops albidus* and reported 24 hour $LC_{50}$ of 0.67 ppm. This copepod was able to sustain a long-term population at methoprene concentration up to 0.21 ppb with some adverse effects on reproduction.

A few studies have examined the use of methoprene for mosquito control in combination with invertebrate predators, such as planaria, *Dugesia tigrina* (Nelson *et al.* 1994) and *D. dorotocephala* (Levy and Miller 1978), and pre- and postparasitic stages of the nematode *Romanomermis culicivorax* (Levy and Miller 1977, Winner *et al.* 1978) and found no adverse effects on the predators or on different stages of the nematode parasite at mosquito control rates.

Methoprene was reported to be non-toxic to the marine amphipod *Elasmopus bampo (*Reish *et al. 1985)*; the 96-hour $LC_{50}$ was greater than 100 ppm. Gradoni *et al.* (1976) determined laboratory toxicity of a technical grade (RS)-methoprene to field-collected adult female, adult male, and young amphipod *Gammarus aequicauda* and reported 96 hour $LC_{50}$ values of 2.15, 1.95 and 0.32 ppm (24 hour) for the male, female and the young, respectively. Embryos of *G. aequicauda* were not affected at 1 ppm concentration of the IGR.

Batzer and Sjogren (1986) reported no adverse effects on population density or size of fairy shrimp *Eubranchipus bundyi* in a wetland in Minnesota, USA, treated for 3 years with experimental controlled-release (RS)-methoprene briquettes sustaining 1.5 ppb concentration of the active ingredient. McKenney and Matthews (1990) studied the effects of technical grade (RS)-methoprene and (S)-methoprene on developing

larvae of the estuarine grass shrimp *Palaemonetes pugio*. No grass shrimp larvae successfully completed metamorphosis when continuously exposed to 1 ppm methoprene. Completion of metamorphosis was significantly reduced by exposure to 0.1 ppm of the isomeric mixture (RS)-methoprene and not to the single isomer (S)-methoprene. Methoprene exposures did not alter either the duration of the total larval development or the total larval stages prior to metamorphosis. McKenney and Celestial (1996) studied survival, growth and reproduction of the estuarine mysid *Mysidopsis bahia* in the laboratory. They reported that all juvenile exposed for 4 days to 125 ppb of (S)-methoprene died. At sub-lethal concentration of 62 ppb, significant weight loss was recorded and release of the first brood was delayed. At ca. 8 ppb, number of young produced was significantly reduced. They concluded that methoprene may be interfering with an endogenous endocrine system in this crustacean which utilizes juvenile hormone-like compounds.

Juvenile hormone analogs including methoprene have been shown to affect gametogenesis in several species of marine crabs, to alter larval development in marine crabs, lobsters, and shrimp and to affect both development and reproduction in the *Daphnia magna* (Templeton and Laufer 1983). Brown *et al.* (1996) studied acute toxicity of (S)-methoprene to the adult stage of estuarine shrimp *Leander tenuicornis* and reported 96 hour $LC_{50}$ value of 14.32 ppm. In another laboratory and field evaluation, Brown *et al.* (1999) reported 24-hour $LC_{95}$ value of 51 ppm for field-collected late juvenile/adult *L. tenuicornis*. Field applications of microencapsulated liquid suspension of (S)-methoprene at 0.06 kg/ha did not affect survival of the shrimp monitored in cages up to 120 hours pretreatment. Forward and Costlow (1978) using (RS)-methoprene at 0.001, 0.01, 0.05 and 0.1 ppm concentrations reported no adverse effects on swimming rates of the crab *Rhitropanopeus harrisii*. However, the IGR at 1 ppm was acutely toxic to early larval instars ($1^{st}$ zoeal stage) of this crab (Christiansen *et al.* 1977). Horst and Walker (1999) studied the effects of a technical grade

(RS)-methoprene on the blue crab *Callinectes sapidus* and reported that exposure of the crab to 0.6 to 3 ppm environmental concentrations produced morbidity and mortality in the form of an overall reduction in the number of successful hatching and lethargic behavior exhibited by the surviving zoeae. The IGR at 1 ppm was also toxic to megalopa, delaying the molt to the first crab form and resulting in the death of 80% of larvae after 10 days.

Against fish, methoprene seems to be generally safe although at the high end of field use rates for mosquito control, it may be a cause of limited concern. For example, under prolonged constant-exposure laboratory condition, (S)-methoprene concentrations of 84-160 ppb had some adverse effects on the growth of fathead minnow fry *Pimephales promelas* (Ross *et al.* 1994). However, such concentrations of the IGR are unlikely to be sustained in the field when used for mosquito control. Laboratory tests showed that mosquitofish *Gambusia affinis* survived concentrations over 80 ppm of technical (RS)-methoprene (Miura and Takahaski 1973). Also, a 40-day exposure (simulated field test) to this IGR at 0.11 kg a. i./ha yielded no apparent toxic effects in *G. affinis* (Miura and Takahaski 1974). (RS)-methoprene at 36 g a.i./ha applied monthly for 5 months did not have any adverse effects on *G. affinis* in outdoor ponds (Takahaski and Miura 1975). In another study, acute toxicity to 3-5 days old *G. affinis* was not detected when exposed to 30 ppm microencapsulated liquid suspension of (S)-methoprene (Tietze *et al.* 1991). Also, no adverse sub-lethal effects on locomotor activities of *G. affinis* and goldfish *Carassius auratus* monitored for 2 weeks in the presence of 0.2 ppm (RS)-methoprene were noted (Ellgard *et al.* 1979).

Tietze *et al.* (1992) exposed 12-16 day old inland silverside *Menidia beryllina* to microencapsulated liquid suspension of (S)-methoprene in the laboratory and reported 48 hour $LC_{50}$ value of 2.78 ppm. The 96-hour $LC_{50}$ for this IGR against

mummichog *Fundulus heteroclitus* was 124.95 ppm (Lee and Scott 1989). Brown *et al.* (1998) studied the effect of microencapsulated liquid suspension of (S)-methoprene on adult Pacific blue-eye *Pseudomugil signifer* and reported 96-hour $LC_{50}$ value of more than 4 ppm. McKague and Pridmore (1978) had reported 96 hour $LC_{50}$ of 86 ppm (RS)-methoprene to juvenile coho salmon *Oncorhynchus kisutch* and 106 ppm to juvenile rainbow trout.

Miura and Takahashi (1973) reported no adverse effects of (RS)-methoprene at mosquito control rates on western toad tadpole *Bufo boreas halophilus*. However, Ankley *et al.* (1998) reported that (S)-methoprene at 500 ppb caused lethal developmental effects but did not cause limb malformations or any teratogenic effects in northern leopard frog *Rana pipiens*.

To date, the most comprehensive studies on the nontarget effects of (S)-methoprene have been conducted in Minnesota, USA (Hershey *et al.* 1998, Niemi *et al.* 1999). In these studies, some wetlands (treatment and control) were sampled (1988-1990) to examine natural variability in invertebrate populations. Beginning in 1991 and continuing through 1992 and 1993, the wetlands were treated 6 times during spring and summer at 3-week intervals with (S)-methoprene (3-week release granules) at 0.05-0.58 kg a.i./ha. No significant differences in benthic invertebrate density during the first year of treatment were noted. In the 2$^{nd}$ and 3$^{rd}$ years, however, significant decreases in the biomass and density of chironomids, tipulids, ceratopogonids and stratiomyids were recorded. Specifically, insect densities were reduced by 57-83% and biomass 50-83% in the 2$^{nd}$ (1992) and 3$^{rd}$ (1993) years of treatment, respectively, but zooplankton populations were not affected nor was the reproductive success of red-winged blackbird (*Agelaius phoeniceus*) which for food items partially depended upon the treated wetlands (Hanowski *et al.* 1997).

## 4.2 Efficacy - background/supporting documents

### 4.2.1 Laboratory trials

There are numerous laboratory studies conducted on the larvicidal efficacy (adult emergence inhibition) of methoprene against several species of *Aedes, Anopheles, Culex,* and *Psorophora* mosquitoes (Table 1). The $LC_{50}$ or $EI_{50}$ (50% emergence inhibition) values against *Ae. aegypti* ranged from 0.13 ppb (Sawby *et al.* 1992) to 60 ppb (Qureshi *et al.* 1981), but mostly the values were in the range of 0.13 to 3.0 ppb. Methoprene was also highly effective against *Ae. albopictus* ($LC_{50}$ = 2.2 ppb) (Ali *et al.* 1995), *Ae. funereus*, and *Ae. notoscriptus* with $LC_{50}$ values of 0.072 and 0.36 ppb, respectively (Ritchie *et al.* 1997). *Aedes sollicitans* ($LC_{50}$ = 0.01 ppb) (Hseih and Steelman 1974), *Ae. taeniorhynchus* ($LC_{50}$ = 0.23 ppb and 0.45 ppb) (Rathburn and Boike 1975, Dame *et al.* 1998) were highly susceptible to methoprene. The IGR against other species of *Aedes, triseriatus* ($LC_{50}$ = 0.14 ppb) (Wells *et al.* 1975) and *vigilax* ($LC_{50}$ = 0.022 ppb) (Ritchie *et al.* 1997) was also highly effective.

The biological activity of methoprene against species of *Anopheles* was almost in the same range as that of *Aedes* mosquitoes. *Anopheles albimanus, An. dirus* sp. A, *An. dirus* sp. B, *An. farauti, An. gambiae, An. quadrimaculatus* and *An. stephensi* were susceptible to methoprene at the ppb level (Table 1). Among these species, *An. farauti* was the most susceptible ($LC_{50}$ = 0.06 ppb) (Ritchie *et al.* 1997) and *An. stephensi* the least ($LC_{95}$ = 50 ppb) (Jakob 1972).

Among *Culex* mosquitoes, methoprene was effective against *Cx. annulirostris* ($LC_{50}$ = 0.09 ppb), *Cx. pipiens fatigans* ($LC_{50}$ = 10 ppb), *Cx. p. molestus* ($LC_{50}$ = 0.23 – 0.57 ppb), *Cx. p. pallens* ($LC_{50}$ = 20 ppb), *Cx. nigripalpus* ($LC_{90}$ = 0.35 ppb) and *Cx. quinquefasciatus* ($LC_{50}$ = 0.29 – 100 ppb) (Ali *et al.* 1999, Amin and White 1994, Georghiou *et al.* 1975, Robert and Olson

1989). Methoprene showed good activity against *Cx. sitiens* ($LC_{50}$ = 1.12 ppb) (Ritchie *et al.* 1997), *Cx. tarsalis* ($LC_{50}$ = 0.6 ppb) (Hseih and Steelman 1974) and *Cx. tritaeniorhynchus* ($LC_{50}$ = 20 ppb) (Noguchi and Ohtaki 1974).

Methoprene was highly effective against larvae of *Ps. ferox* ($LC_{50}$ = 0.1 ppb), *Ps. varipes* ($LC_{50}$ = 0.2 ppb), also showing good activity against *Ps. confinnis* (Hsieh and Steelman 1974).

In addition to the data given in Table 1, other laboratory studies were those of Schaefer and Wilder (1972) who reported 100% kill of *Cx. p. quinquefasciatus* with methoprene at 100 ppb. Jakob (1972) studied the activity of methoprene against *Ae. aegypti, An. albimanus, An. stephensi* and *Cx. p. quinquefasciatus* and reported good activity (95% larval mortality) at 100 ppb or less; this IGR was more active against the late than against the early 4$^{th}$ instar larvae of these mosquitoes.

Arias and Mulla (1975a) studied the concentration effects (1 ppt to 100 ppb) of methoprene on *Cx. tarsalis*. At almost all concentrations, morphogenetic aberrations were noted in both pupae and adults resulting from treated 4$^{th}$ stage larvae of this mosquito. Besides larval-pupal intermediates and pupal-adult intermediates, abnormal constriction in the cephalothoracic area of the pupae and inability to detach legs and wings from the pupal cast-skin in adults were the common malformations induced by methoprene treatments. Arias and Mulla (1975b) also reported that treating the 4$^{th}$ stage larvae of *Cx. tarsalis* with 0.1 and 1 ppb of methoprene did not result in significant mortality of emerging adults. Adults resulting from 4$^{th}$ stage larvae treated with 0.1 and 1 ppb concentrations of this IGR showed a 5 and 14% reduction in the number of egg rafts, respectively. The higher concentration (1 ppb) of methoprene affected egg viability by 30%. Mosquitoes treated with 0.1 ppb of methoprene showed no appreciable difference in percentage

of egg hatch, whereas the progeny yield of adults was reduced by ca. 36% at the higher treatment rate (1 ppb).

Naqvi *et al.* (1978) studied the stage-activity relationships of methoprene on early (24 hour old) and late (72 hour old) 4th instar larvae of *An. stephensi*. They found that late 4th instar larvae were more susceptible to methoprene treatment than the early 4th instars. Significant reduction in oviposition and egg hatch was found in surviving adults from the early and late 4th instars exposed to 1 and 10 ppb concentration of methoprene, respectively. Sterility in these adults (resulting from early 4th instar treatment) was 78.6% and from late 4th instar, 86%. Based on the data, these authors suggested a concentration of 1 ppb to be most effective against late 4th instar larvae of this species.

It is obvious from the above discussion that methoprene at low concentrations demonstrated high biological activity against a variety of mosquito species under laboratory conditions and at certain concentrations the IGR also caused some reproductive effects.

**Table 1. Laboratory efficacy of methoprene against mosquito larvae[a,b].**

| Species | $LC_{50}$ /$EI_{50}$[c] | $LC_{95}$ /$EI_{95}$[c] | Reference |
|---|---|---|---|
| *Aedes aegypti* | 3.0 | - | Busvine *et al.* 1976 |
| *Ae. aegypti* | 0.2 | - | Pridantseva *et al.* 1978 |
| *Ae. aegypti* | 65.0 | - | Qureshi *et al.* 1981 |
| *Ae. aegypti* | 0.4 | 1.31[d] | Ritchie *et al.* 1997 |
| *Ae. aegypti* | 0.13 | - | Sawby *et al.* 1992 |
| *Ae. albopictus* | 2.2 | 8.1[d] | Ali *et al.* 1995 |
| *Ae. detritus* | 0.9 | 8.5[d] | Majori *et al.* 1977 |
| *Ae. funereus* | 0.072 | 0.69[d] | Ritchie *et al.* 1997 |
| *Ae. notoscriptus* | 0.36 | 1.12[d] | Ritchie *et al.* 1997 |
| *Ae. sollicitans* | 0.01 | 1.3[d] | Hsieh and Steelman 1974 |

| | | | |
|---|---|---|---|
| *Ae. taeniorhynchus* | 0.2 | - | Dame *et al.* 1976 |
| *Ae. taeniorhynchus* | 0.45 | 1-2[d] | Dame *et al.* 1998 |
| *Ae. taeniorhynchus* | 0.23 | 14.5[d] | Rathburn and Boike 1975 |
| *Ae. triseriatus* | 0.14<br>0.09 | 0.97<br>0.36 | Wells *et al.* 1975 |
| *Ae. vigilax* | - | 0.2 | Brown *et al.* 1999 |
| *Ae. vigilax* | 0.022 | 0.17[d] | Ritchie *et al.* 1997 |
| *Anopheles albimanus* | - | 2.5 | Jakob 1972 |
| *An. dirus* sp. A | 0.21 | - | Sithiprasasna *et al.* 1996 |
| *An. dirus* sp. B | 0.17 | - | Sithiprasasna *et al.* 1996 |
| *An. farauti* | 0.06 | 0.99[d] | Ritchie *et al.* 1997 |
| *An. gambiae* | 1.6 | - | Busvine *et al.* 1976 |
| *An. quadrimaculatus* | 1.5 | - | Busvine *et al.* 1976 |
| *An. quadrimaculatus* | 4.0 | 11-15[d] | Dame *et al.* 1976 |
| *An. stephensi* | - | 50 | Jakob 1972 |
| *Culex annulirostris* | 0.09 | 0.34[d] | Ritchie *et al.* 1997 |
| *Cx. pipiens fatigans* | 10.0 | - | Busvine *et al.* 1976 |
| *Cx. p. molestus* | 0.23-0.57 | - | Kono *et al.* 1997 |
| *Cx. p. pallens* | 20.0 | - | Noguchi and Ohtaki 1974 |
| *Cx. nigripalpus* | - | 0.35[d] | Rathburn and Boike 1975 |
| *Cx. quinquefasciatus* | 17.0 | 52.0[d] | Ali *et al.* 1999 |
| *Cx. quinquefasciatus* | 2.3-6.4 | 9.0-27[d] | Amin and White 1994 |
| *Cx. quinquefasciatus* | 0.29 | - | Georghiou *et al.* 1975 |
| *Cx. quinquefasciatus* | 0.1 | 0.5 | Robert and Olson 1989 |
| *Cx. sitiens* | 1.12 | 6.54[d] | Ritchie *et al.* 1997 |
| *Cx. tarsalis* | 0.6 | 32.5 | Hsieh and Steelman 1974 |
| *Cx. tritaeniorhynchus* | 20 | - | Noguchi and |

| | | | |
|---|---|---|---|
| | | | Ohtaki 1974 |
| *Psorophora confinnis* | 7.1 | 100[d] | Hsieh and Steelman 1974 |
| *Ps. ferox* | 0.1 | 0.7[d] | Hsieh and Steelman 1974 |
| *Ps. varipes* | 0.2 | 2.0[d] | Hsieh and Steelman 1974 |

[a]mostly 4[th] instar larvae
[b]all toxicity values are in ppb
[c]lethal concentration to inhibit 50% or 95% adult emergence
[d]$LC_{90}/EI_{90}$ values

### 4.2.2 Field trials

A vast majority of field efficacy assessments or large-scale field treatments of methoprene in a variety of formulations for mosquito control purposes has been conducted in the USA. Mian and Mulla (1982) provided a review of field efficacy of this IGR covering about first 6-8 years of nearly 3 decades of laboratory/semi-field/field assessment and practical field use of methoprene. Because of the rather rapid degradation of methoprene in the aquatic environment, a number of liquid and solid slow- release formulations have become available in the past 2 decades (Ali 1991), for mosquito control purposes.

In simulated field trials in small plots in Florida, USA, a rate of 0.11 kg a.i./ha of (RS)-methoprene on sand or vermiculite provided complete control of the salt marsh mosquito, *Ae. taeniorhynchus* (Rathburn and Boike 1975); this rate of application was, however, much lower than the usual field rate (0.6 to 0.65 kg a.i./ha) against this mosquito (Rogers *et al.* 1976). In California, at a field application rate of 0.11 kg a.i./ha, this compound was found to be effective against *Culiseta inornata, Cx. peus*, and *Cx. tarsalis* breeding in irrigated pastures (Mulla and Darwazeh 1975). In another study, (RS)-methoprene at 10 ppm (high concentration) in the form of round

discs, yielded complete control of *Cx. pipiens* in catch basins for 49 to 64 days (Dunn *et al.* 1975). In North Carolina, satisfactory control of *Ae. taeniorhynchus* in temporary water pools in depressions within dredge soil disposal areas was achieved with a slow-release formulation of (RS)-methoprene applied at 45 g a.i./ha (Axtell *et al.* 1979). However, control of *Cx. p. quinquefasciatus* in anaerobic swine waste lagoons at as high as 0.45 kg a.i./ha of this IGR was not satisfactory (Axtell *et al.* 1980) which was probably due to rapid biodegradation processes existing in these habitats. In another field test by Dame *et al.* (1976), (RS)-methoprene at 0.028 kg a.i./ha was effective against natural populations of *Cx. nigripalpus* and *Cx. salinarius* in salt marsh mangrove habitats. At a rate of 0.028 kg in 38 to 95 liters of aqueous formulation/ha, applied by helicopter, the IGR also provided complete control of *Ae. taeniorhynchus*.

Case and Washino (1978) using a liquid charcoal suspension formulation of (RS)-methoprene applied at 0.11 kg a.i./ha achieved only up to 50% control of *Cx. tarsalis* in ricefields in California. In dairy lagoons in California, (S)-methoprene pellets containing 4% a.i, applied up to 1.12 kg a.i./ha produced unsatisfactory control of *Cx. quinquefasciatus* and *Cx. peus* (Mulla and Darwazeh 1988). However, in freshwater ponds, the same pellets applied at 0.11-0.56 kg a.i./ha induced complete inhibition of *Cx. tarsalis* for 7 days and reduced emergence of *Cx. peus* by 81-100% (2 days posttreatment) and 78-100% (7 days posttreatment) (Mulla *et al.* 1989).

Rathburn *et al.* (1980) experimented with 2 formulations of (RS)-methoprene, sand granules at 0.022 kg a.i./ha and liquid formulation at 0.03 kg a.i./ha in small replicated field plots and reported that 2 applications per week with either formulation were required to achieve satisfactory control of *Cx. nigripalpus* asynchronous broods in Florida. Floore *et al.* (1988) reported that a rate of 11 kg/ha of 4% a.i. (S)-methoprene pellets was required as a pre-flood treatment for *Cx. quinquefasciatus*

control, which lasted for 7-10 days after flooding. McCarry (1996) applied (S)-methoprene 4% a.i. pellets to catch basins at a rate of 11.3 kg/ha (7 g pellets/catch basin) and reported an average of 82% emergence inhibition of adult *Cx. pipiens* and *Cx. restuans* for 15 weeks.

A slow-release briquette formulation containing 1.8% a.i. (S)-methoprene applied at 1 briquette/9.3 $m^2$ to 37.2 $m^2$ rice plots gave 98.2% reduction of *Ps. columbiae* for 58 days (Weathersbee and Meish 1991). (S)-methoprene pellets (4% a.i.) evaluated at 3.4 kg/ha in tidal, saltwater marsh against *Ae. dorsalis* gave >92% control up to 42 days, 86.4% up to 131 days and 67% up to 240 days posttreatment (Kramer *et al.* 1993). Nasci *et al.* (1994) reported 100% control of *Ae. albopictus* for 150 days in Louisiana with. (S)-methoprene pellets (4% a.i.) and sand granules (1.3% a.i.). Kramer (1990) reported up to 90% but short-term control of *Cs. incidens* in used tires (placed in sunny and shady areas) treated with (S)-methoprene at a rate as high as 2.5 ppm. Woodrow *et al.* (1995) tested (S)-methoprene pellets (4% a.i.) at 5-5.6 kg/ha in 4 ha plots as well as on a large-scale in a 260 ha area in a swamp complex for the control of *Cs. melanura* and reported an average of 81% emergence inhibition of this mosquito over a 5 week period. Ranta *et al.* (1994) tested (S)-methoprene 4% a.i. pellets at 5.6 kg/ha and an (S)- methoprene 1.8% a.i. extended residual briquette) (150 day) formulation at 1 briquette/9.3 $m^2$ against *Coquillettidia perturbans* in cattail wetlands in Minneapolis – St. Paul area and achieved 92 and 95% control with pellets and briquettes, respectively for nearly 3 months. Lawler *et al.* (1999) using microencapsulated liquid suspension of (S)-methoprene at a rate of 213 ml/ha in 3.2-27.6 ha sites in mangrove swamps and salt marsh habitats in Florida reported only partial success in reducing *Ae. taeniorhynchus* emergence. The authors discuss the possibility of resistance in *Ae. taeniorhynchus* because of the use of (S)-methoprene 1.8% a.i. extended residual briquettes (150 day) for 6 years in the area (Dame *et al.* 1998).

Outside of the United States, in Canada, Baldwin and Chant (1976) reported complete inhibition of adult emergence in *Ae. communis* breeding in a pond treated with (RS)-methoprene at 28 g a.i./ha; the IGR also effectively controlled several other species of *Aedes* (*Ae. canadensis, Ae. cinereus, Ae. excrucians, Ae. fitchii, Ae. implicutus* and *Ae. vexans*) following treatment of 3rd and 4th instar larvae. Treatment of 1st to 3rd instars, however, resulted in partial control of these species. The formulated methoprene remained active in the study pools for 13 days. In another study in Canada, (RS)-methoprene at 0.028 kg AI/ha effectively controlled several species of spring *Aedes* and early summer *Ae. vexans* for 2 weeks (Rodrigues and Wright 1978). In Indonesia, application of 1 ppm of (RS)-methoprene to larval habitats (1 $km^2$ area) resulted in complete arrest of adult emergence in *Cx. p. quinquefasciatus* for five days (Self *et al.* 1978). In Japan, Itoh (1979) obtained 100% control of adult emergence in *Cx. tritaeniorhynchus* in experimental rice fields treated with 0.1 ppm of (RS)-methoprene (liquid charcoal suspension and briquette formulations). The briquettes at 0.1 ppm were not effective against *Cx. p. molestus* larvae in septic tanks and underground water pools. However, both briquettes and a sand granule formulation of (RS)-methoprene at 0.1 ppm were effective against *Aedes albopictus* larvae; 100% inhibition of adult emergence in this species was noted for about 5 weeks after treatment with (RS)-methoprene at 10 ppm (an extremely high concentration).

In Australia, Ritchie and Broadsmith (1997) reported >90% control of *Ae. aegypti* in tank bromeliads for 6-12 months with (S)-methoprene pellets and granules applied at 2 and 0.5 g, respectively. In Italy, 40 g a.i./ha of a liquid slow-release suspension formulation of (RS)-methoprene yielded complete control of *Ae. detritus* for 4 days posttreatment in salt marsh habitats (Majori *et al.* 1977). In Cypress, Burgess and Chetwyn (1983) reported 80% control of *Ae. detritus* up to 12 days posttreatment when a 200 ha area on the Akrotiri peninsula was

treated with a liquid slow-release suspension of (RS)-methoprene (200 ml/ha) mixed with sand. In Kenya, (S)-methoprene pellets application at 0.22 kg a.i./ha to floodwater *Aedes* habitats gave 88-98% control of *Ae. mcintoshi, Ae. dentatus, Ae. cumminsii*, and *Ae. circumluteolus* for 7-14 days after flooding; *Culex* spp. were controlled up to 91% for 15-31 days (Logan *et al.* 1990). In Malaysia, Sulaiman *et al.* (1994) reported complete emergence inhibition of *Ae. albopictus* for 66-72 days posttreatment from plastic containers placed outdoors and treated with (RS)-methoprene briquettes, 1/container (14 mg a.i./liter).

It is obvious from the field reports that the activity of methoprene against mosquitoes for control purposes depended upon a number of factors, such as the target species, larval stage, type of formulation, rate of application, number of applications, time of application, nature and size of the habitat and water quality. In general, liquid formulations of (S)-methoprene, have been reported to successfully control mosquitoes in different habitats when used at 11 to 45 g a.i./ha. Because of the instability of emulsifiable concentrates of methoprene and the narrow window of susceptibility in the life cycle of mosquitoes to this compound, only short term control (3-7 days) have been reported with conventional EC formulations (Mulla 1995).

### 4.3 WHOPES supervised trials

*USA.* Laboratory (in trays) and semi-field (in fish-tubs placed outdoors) activity of (RS)-methoprene EC was compared to that of microencapsulated liquid suspension (S)-methoprene, against laboratory reared 3$^{rd}$ and 4$^{th}$ instar larvae obtained from laboratory colonized 3 species of Florida mosquitoes, *Aedes aegypti*, *Anopheles quadrimaculatus*, and *Culex quinquefasciatus*. Also, laboratory bioassays with technical grade (RS)-methoprene against the 3 mosquito species were conducted.

In the laboratory studies, *An. quadrimaculatus* was the most susceptible ($LC_{90}$ = 3.2 ppb), followed by *Ae. aegypti* ($LC_{90}$ = 10 ppb), and *Cx. quinquefasciatus* ($LC_{90}$ = 27 ppb). *Culex quinquefasciatus* was nearly 8 times more tolerant than *An. quadrimaculatus*. The $LC_{99}$ values of *An. quadrimaculatus, Ae. aegypti* and *Cx. quinquefasciatus* were 34, 99 and 96 ppb, respectively.

At 50 and 100 ppb concentrations of the two IGR products, 3-5 week posttreatment cumulative mean reductions of *Ae. aegypti* adult emergence from the trays, respectively amounted to 100 and 97.8% [microencapsulated liquid suspension (S)-methoprene and 84.5 and 90.9% (RS-methoprene EC)]. Against *An. quadrimaculatus*, the cumulative mean reductions at 50 and 100 ppb concentrations, respectively amounted to 98.5 and 100% (microencapsulated liquid suspension S-methoprene) and 98.5 and 93.7% [(RS)-methoprene EC].

The 200 and 400 ppb concentrations of the two IGR products against *Cx. quinquefasciatus*, respectively produced cumulative mean (3-5 weeks) adult reductions of 71.7 and 93.5% [microencapsulated liquid suspension (S)-mthoprene] and 40 and 50.6% [(RS)-methoprene EC].

The 50 ppb concentration of each formulation at 1 and 2 week posttreatment respectively produced 100 and 100% [microencapsulated liquid suspension (S)-methoprene] and 100 and 87.4% [(RS)-methoprene EC] adult suppression of *Ae. aegypti* from the out-door, shaded tubs. The 100 ppb rate against *Ae. aegypti* in the 1st, 2nd and 3rd week posttreatment respectively resulted in 100, 100 and 100% [microencapsulated liquid suspension (S)-methoprene], and 100, 100 and 100% [(RS)-methoprene EC] adult reductions.

Using methodology of Ali *et al.* (1994), the adult reductions of *An. quadrimaculatus* at 1, 2 and 3 weeks posttreatment at 50 ppb in out-door tubs, respectively amounted to 100, 98 and 96.8%

[(RS)-methoprene EC] and 100, 100 and 40.1% [microencapsulated liquid suspension (S)-methoprene]. At the higher rate of 100 ppb these reductions were: 100, 100 and 100% [microencapsulated liquid suspension (S)-methoprene] and 100, 87.1 and 94.9% [(RS)-methoprene EC], respectively. At 200 ppb, 100 and 93.4% [microencapsulated liquid suspension (S)-methoprene] and 97.9 and 25.2% [(RS)-methoprene EC] adult reductions occurred at 1 and 2 weeks posttreatment, respectively. The 400 ppb concentration of the formulations induced 100, 82.8 and 32.2% [microencapsulated liquid suspension (S)-methoprene] and 99.1, 23.3 and 3.7% [(RS)-methoprene EC] adult emergence reductions of *Cx. quinquefasciatus* at 1, 2 and 3 weeks posttreatment, respectively.

*Iran.* Efficacy of (RS)-methoprene was studied in laboratory, using technical grade material, against 3$^{rd}$ and 4$^{th}$ instar laboratory-colonized larvae of *An. stephensi* and $EI_{50}$ and $EI_{90}$ values were established at 6.51 and 26.36 ppb, respectively. Complete inhibition ($EI_{100}$) was obtained at 125 ppb.

Field evaluation of (RS)-methoprene EC, at 20 and 40 g a.i./ha, was carried out in artificial ponds (each 1 x 1 m and 35 - 50 cm deep) in the village of Jades, southern Iran, where the soil type was clay loam and the ponds became filled with natural drainage water slightly brackish and clear at first, gradually becoming muddy/organic. Two ponds served as replicates of each treatment while 2 untreated ponds were used as controls. The experiment was repeated 3 times between June and September 1999. In addition to monitoring immature mosquito populations in each pond, 15 x 15 x 15 cm cages made of cubic metal frames and mosquito netting with each cage open at the top end were utilized. Two to three cages appropriately hung were used in each treatment and control pond with the bottom half immersed in the pond water. To the cages 25 - 30 early 4$^{th}$ instar anopheline larvae collected from nearby areas of the ponds were added and exposed for 24-hour period posttreatment. This routine was continued 2$^{nd}$ day (48 hours) and 3$^{rd}$ day (72 hours)

posttreatment, thus exposing 3 different batches of 25 - 30 larvae to pond water. These field-exposed larvae (to treatments and control) were taken to the laboratory to study their survival and emergence as adults. Efficacy and duration of effective control provided by (RS)-methoprene EC is presented in Table 2.

**Table 2. Corrected inhibition (against controls) of adult emergence of anopheline mosquitoes in the laboratory with the early 4th instar larvae held for 24 hour periods at 1, 2, 3, 4 and 7 days posttreatment in exposure cages placed half-immersed under water surface in treatment and control artificial ponds treated with (RS)-methoprene EC at 20 and 40 g a.i./ha, Jades village, southern Iran, July – September 1999.**

| Treatment rate (g a.i./ha) | Percent mean ± SE corrected adult emergence inhibition posttreatment (days) | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 7 |
| 20 | 74.6 ± 4.2 | 63.2 ± 4.2 | 27.5 ± 4.1 | 20.3 ± 5.9 | 16.6 ± 4.6 |
| 40 | 83.7 ± 3.0 | 76.6 ± 3.2 | 32.2 ± 4.5 | 24.3 ± 7.5 | 21.0 ± 4.8 |

The lower rate of 20 g a.i./ha produced a maximum mean inhibition of 74.6% at 1 day posttreatment, declining to 63.2% at day 2 posttreatment and then suddenly to 27.5% at day 3 posttreatment. The higher rate of 40 g a.i./ha was more effective, inducing 83.7% emergence inhibition at day 1 posttreatment, 76.6% at day 2 posttreatment, and sharply declining to 32.2% at day 3 posttreatment. Although the higher rate consistently produced slightly higher magnitudes of emergence inhibitions compared to the lower rate (20 g a.i./ha), the differences were not statistically significant. However, both treatment rates were significantly different ($P < 0.01$) from controls in terms of emergence inhibition. In general, the highest and statistically significant activity by both rates of treatment was exhibited within 2 days of the applications compared to 3, 4 and 7 days posttreatment. In this evaluation the higher rate of 40 g a.i./ha

did not cause complete inhibition of adult emergence of mosquitoes.

Routine laboratory examination of a portion of the preserved larval dip samples for species identification revealed that during the 1st set of treatments, *An. stephensi*, *An. dthali*, *An. fluviatilis*, and *An. algeriensis* representing 33, 33, 21 and 13% of the total anophelines were present in the artificial ponds, respectively, while during 2nd set of treatments *An. stephensi* (53%), *An. fluviatilis* (24%), *An. superpictus* (18%) and *An. dthali* (4%) were present. During the 3rd set of treatments the composition changed to *An. fluviatilis* (69%), *An. dthali* (17%) and *An. stephensi* (14%). Thus, the species make up and the % composition changed during the 3 - 4 months of field experimentation.

Larval dip samples collected pretreatment and at 1, 2, 3, 4 and 5 days posttreatment in all treated ponds with (RS)-methoprene EC and control ponds showed some significant larval declines in the treated ponds at 2 to 4 days posttreatment.

(RS)-methoprene EC was further evaluated in southern Iran, in plots in rice fields at the rate of 40 g a.i./ha. The rice field supported relatively high densities of anopheline larvae. The surface area of plots which received the EC formulation ranged from 24 - 30 $m^2$. Control plots were selected nearly 100 m upcurrent (irrigation water) of the treatment plots. Two replicates were allocated to treatment and two to control. The experiment was repeated 3 times during June – September 1999.

The percent mean (± SE) corrected inhibition (against controls) of adult emergence of anopheline mosquitoes in the laboratory with the early 4th instar larvae held for 24 hour periods at 1, 2 and 3 days posttreatment in exposure cages placed half-immersed under water surface in treatment and control experimental plots in ricefields were 67.8 (± 3.4), 62.2 (± 3.5)

and 35.8 (± 4.2) in 1, 2 and 3 post-treatment days, indicating rather rapid degradation of the IGR under field conditions.

## 4.4 Conclusions and recommendations

1. Methoprene, mainly (S)-methoprene, has been used for mosquito control for over two decades. This IGR has been shown to be safe to humans and the environment at mosquito control application rates. Liquid formulations of (S)-methoprene have been satisfactorily used in a variety of mosquito habitats at rates ranging from 11 to 45 g a.i./ha. (S)-methoprene is the biologically active enantiomer of (RS)-methoprene (a racemic mixture).

2. Under semi-field conditions in Florida, USA, (RS)-methoprene EC yielded results for *Aedes* and *Anopheles* species comparable to liquid formulation of (S)-methoprene at the rates of 50 and 100 ppb, providing emergence inhibition for 3 weeks. However, the efficacy and duration of effectiveness of the former product was markedly less against *Culex quinquefasciatus*.

3. Under field conditions in southern Iran, 20 and 40 g a.i./ha of (RS)-methoprene EC gave 62-84% control of anophelines for 2 days post-treatment. There was no appreciable difference between the high and the low rates of application.

4. (RS)-methoprene EC can be used for mosquito larviciding at the application rates of 20 to 40 g a.i./ha. The higher application rate is recommended for water with high organic content. Expected residual activity of the product is relatively short and weekly applications of the larvicide may be required.

# 5. REVIEW OF INSECT GROWTH REGULATOR PYRIPROXYFEN GR

## 5.1 Safety assessment

Pyriproxyfen (4-phenoxyphenyl (RS)-2-(2-pyridyloxy)propyl ether) is a juvenile hormone mimic which affects the physiology of morphogenesis, reproduction and embryogenesis. These effects on insect growth and development are specific to arthropods.

The toxicity of pyriprofyfen has been assesed by the WHO/FAO Joint Meeting on Pesticide Residues (JMPR)(FAO 1999) and the following conclusions are 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 and after exposure by inhalation, with an $LC_{50}$ value >1.3 mg/l air in mice and rats. Pyriproxyfen is rapidly excreted in animals, primarily in faeces (88-96% within 48hrs).

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

Pyriproxyfen was not genotoxic nor carcinogenic. The acceptable daily intake (ADI) for man has been established at 0 – 0.1 mg a.i./kg body weight on the basis of the NOAEL of 10 mg/kg body weight per day in the one-year study of pyriproxyfen in dogs and a safety factor of 100.

WHO has classified pyriproxyfen technical grade active ingredient as "unlikely to present acute hazard in normal use".

The following are extracts from the Material Safety Data Sheet (MSDS) of the manufacturer for pyriproxyfen 0.5% GR:

| | |
|---|---|
| Acute oral $LD_{50}$ (rat) | >5,000 mg/kg |
| Acute dermal $LD_{50}$ (rat) | >2,000 mg/kg |
| Skin irritation (rabbit) | Mildly irritating |
| Eye irritation (rabbit) | Mildly irritating |

### 5.1.1 Effects of pyriproxyfen on aquatic nontarget organisms

There are a few laboratory bioassay studies and field data assessing the adverse effects and margin of safety of pyriproxyfen to a variety of planktonic, nektonic and benthic aquatic organisms. In a laboratory study on field-collected organisms in Australia, the 48 hour $EC_{50}$ or $LC_{50}$ (median effective or lethal concentration) of the amphipod *Austrochiltonia subtenuis*, the ostracod *Candonocypris novaezelandiae*, the mayfly *Cloeon fluviatile*, the corixid water bug *Micronecta robusta*, and the fish *Pseudogobius olorum* amounted to 0.12, 6.21, 0.17, 1.25 and 3.92 ppm, respectively (Pinder *et al.* 1991a). As is evident from the data, the amphipod was most susceptible to pyriproxyfen followed by the mayfly species, and the ostracod least. In another study in Australia, neonates of *Daphnia carinata* s.l. exposed to pyriproxyfen in acute toxicity trials produced 48 hour $LC_{50}$ of 0.08 ppm. However, continuous exposure to pyriproxyfen at 0.01 ppm during a 3-brood, 14-day life cycle test, suppressed growth of *D. carinata* and reduced reproduction by as much as 80% (Trayler and Davis 1996). Brown *et al.* (1996) tested pyriproxyfen against adult estuarine shrimp *Leander tenuicornis* and reported 96 hour $LC_{50}$ of 0.098 ppm; against pacific blue-eye, *Psuedomugil signifer* fish, 96 hour $LC_{50}$ was 0.854 ppm (Brown *et al.* 1998). In California, USA, laboratory evaluation on field-collected Cladocera (*Simocephalus* sp. and *Alona* sp.) and

Copepoda (*Cyclops vernalis*) revealed no significant adverse effects of pyriproxyfen at 0.01 ppm tested in glass aquaria (Schaefer *et al.* 1988). Miyamoto *et al.* (1993) during a 21-day reproduction study, showed that *Daphnia pulex* and *D. magna* exposed continuously to a 60 ppb concentration of pyriproxyfen in the laboratory were affected in terms of their body lengths shortened and the number of young ones reduced, but their survival and molting rates were not affected. Moreover, the adverse effects on body length and reproduction were quickly reversed in one week when *D. pulex* were transferred to fresh water free of pyriproxyfen. Unpublished laboratory data provided by Sumitomo Chemical Company, Japan on a variety of organisms including species of Cladocera, Copepoda, Mollusca, brine shrimp, freshwater shrimp, dragonfly, juvenile rainbow trout, juvenile and adult carp, and juvenile and adult killifish indicate $EC_{50}$ or $LC_{50}$ values for most of these organisms for various time exposures to be in the ppm range, with Copepoda and dragonfly nymphs being the most susceptible.

In field situations, pyriproxyfen GR applied to experimental ponds at 0.011 to 0.028 kg a.i./ha and monitored 21 days posttreatment, induced no ill effects on mayfly naiads (*Callibaetis pacificus*), dragonfly naiads (*Tarnetrum corruptom* and *Anax junius*), larvae and adults of hydrophilid and dytiscid beetles, and two species of ostracods (*Cyproidopsis* sp. and *Cyprinotus* sp.) (Mulla *et al.* 1986). Pyriproxyfen applied at 0.0056 – 0.11 kg a.i./ha to field plots resulted in minor suppression of reproductive capacities of daphnoid cladocerans and ostracods at the highest rate of 0.11 kg a.i./ha. Also, a low degree of induction of morphogenetic aberrations in Odonata (Anisoptera and Zygoptera) at adult emergence was exhibited 4-10 days after treatment with 0.056-0.11 kg a.i./ha pyriproxyfen. Hydrozoa, Nematoda, Oligochaeta, Turbellaria, Hirudinea, Gastropoda, notonectid bugs, larvae and adult Coleoptera and a few other aquatic invertebrates were not affected by the treatments (Schaefer *et al.* 1988; Schaefer and Miura 1990).

Pyriproxyfen GR application at 0.05 kg a.i./ha to a lake in western Australia induced no adverse effects on populations of invertebrates in 5 x 5 m *in situ* enclosures. These invertebrates included cyclopoid copepod *Mesocyclops* sp., ostracod *Cypricercus salinus*, amphipod *A. subtenuis*, mayfly *Tasmancoenis tillyardi*, certopogonid *Nilobezzia* sp., hydracarinids, water bug notonectids, caddisfly *Ecnomus pansus* and some other invertebrates (Pinder *et al.* 1991b). Thus, the above laboratory and field data clearly indicate 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. In case of some adverse effects on certain organisms, the populations of affected organisms will recover in relatively short time periods.

## 5.2 Efficacy - background/supporting documents

### 5.2.1 Laboratory trials

There are numerous laboratory studies conducted primarily in the USA and Japan on the larvicidal efficacy (adult emergence inhibition) of pyriproxyfen against several species of *Aedes, Anopheles* and *Culex* mosquitoes (Table 3). The $LC_{50}$ or $EI_{50}$ (50% emergence inhibition) against *Aedes* ranged from 0.01 ppb (*Ae. taeniorhynchus*)(Schaefer *et al.* 1988) to 0.33 ppb (*Ae. aegypti*) (Estrada and Mulla 1986). However, in several other studies the $LC_{50}$ of *Ae. aegypti* amounted to 0.0039 ppb (Henrick 1995), 0.023 ppb (Hatakoashi *et al.* 1987), and 0.056 ppb (Itoh *et al.* 1994). Emergence of *Ae. albopictus* was inhibited by 50 and 90% with 0.11 and 0.38 ppb concentrations of pyriproxyfen, respectively (Ali *et al.* 1995). Against species of *Anopheles*, the $LC_{50}$ of pyriproxyfen amounted to 0.0017 ppb (*An. farauti*) and 0.016 ppb (*An. albimanus*) (Kawada *et al.* 1993), 0.025 ppb (*An. gambiae*) (Kawada *et al.* 1993), 0.04 ppb (*An. balabacensis*) (Iwanaga and Kanda 1988), 0.043 ppb (*An. stephensi*) (Hatakoshi *et al.* 1987), and 1.3 ppb (*An. quadrimaculatus*) (Estrada and Mulla 1986). Against species of

*Culex*, pyriproxyfen was most effective against *Cx. pipiens pallens* ($LC_{50}$ = 0.0046 ppb) (Hatakoshi *et al.* 1987), followed by several reports on *Cx. quinquefasciatus* with $LC_{50}$ range of 0.018 to 0.29 ppb (Ali *et al.* 1999; Mulla *et al.* 1986; Schaefer *et al.* 1988). *Culex p. molestus* and two laboratory populations of *Cx. tarsalis* had $LC_{50}$ values of 0.029 ppb (Kawada *et al.* 1994), 0.021 ppb (Schaefer *el al.* 1988) and 0.085 ppb (Estrada and Mulla 1986), respectively. These laboratory studies clearly demonstrate the superior activity of pyriproxyfen against a large number of disease vector mosquito species.

Although at least half of the studies listed in Table 3 give only $LC_{50}$ or $EI_{50}$ values, those which also provide $LC_{90}$ or $LC_{95}$ levels of pyriproxyfen facilitate estimation of higher LC levels by plotting the data on probit paper. For example, data of Schaefer *et al.* (1988) on *Cx. quinquefasciatus* have 0.018 and 0.16 ppb $LC_{50}$ and $LC_{95}$ values, respectively. Plotting these data on probit paper to evaluate dosage response indicates a nearly complete emergence inhibition of this species within 0.3-0.4 ppb active ingredient concentration range of pyriproxyfen. In a field situation, even 10-fold or slightly higher concentration of pyriproxyfen (i. e., 3-4 ppb) would probably give complete control of *Cx. quinquefasciatus* although in this estimation the formulation type to be used as well as prevailing physico-chemical conditions in the study habitat would have a significant influence on the resulting level of control. Nevertheless, the activity of pyriproxyfen at ppb and in many cases at sub-ppb levels against a variety of mosquito species is evident from the laboratory data. Hirano *et al.* (1998) have provided similar information on laboratory activity of pyriproxyfen against mosquitoes.

**Table 3. Laboratory efficacy of pyriproxyfen against mosquito larvae[a,b].**

| Species | $LC_{50}$/ $EI_{50}$[c] | $LC_{95}$/ $EI_{95}$[c] | Reference |
|---|---|---|---|
| *Aedes aegypti* | 0.33 | 2.6 | Estrada and Mulla (1986) |
| *Ae. aegypti* | 0.023 | - | Hatakoshi *et al.* (1987) |
| *Ae. aegypti* | 0.056 | - | Itoh *et al.* (1994) |
| *Ae. aegypti* | 0.0039 | - | Henrick (1995) |
| *Ae. albopictus* | 0.11 | 0.38[d] | Ali *et al.* (1995) |
| *Ae. taeniorhynchus* | 0.01 | 0.052 | Schaefer *et al.* 1988) |
| *Anopheles albimanus* | 0.016 | - | Kawada *et al.* (1993) |
| *An. balabacensis* | 0.04 | - | Iwanaga and Kanda (1988) |
| *An. farauti* | 0.0017 | - | Kawada *et al.* (1993) |
| *An. gambiae* | 0.025 | - | Kawada *et al.* (1993) |
| *An. stephensi* | 0.043 | - | Hatakoshi *et al.* (1987) |
| *An. quadrimaculatus* | 1.3 | 17 | Estrada and Mulla (1986) |
| *Culex pipiens pallens* | 0.0046 | - | Hatakoshi *et al.* (1987) |
| *Cx. pipiens molestus* | 0.029 | - | Kawada *et al.* (1994) |
| *Cx. quinquefasciatus* | 0.04 | 0.3[d] | Mulla *et al.* (1986) |
| *Cx. quinquefasciatus* | 0.018 | 0.16 | Schaefer *et al.* (1988) |
| *Cx. quinquefasciatus* | 0.29 | 1.1[d] | Ali *et al.* (1999) |
| *Cx. tarsalis* | 0.085 | 0.32 | Estrada and Mulla (1986) |
| *Cx. tarsalis* | 0.021 | 0.25 | Schaefer *et al.* (1988) |

[a]mostly 4th instar larvae
[b]all toxicity values are in ppb
[c]lethal concentration to inhibit 50% or 95% adult emergence
[d]$LC_{90}/EI_{90}$ values

### 5.2.2 Field trials

A summary of field evaluations of pyriproxyfen as a mosquito control agent is presented in Table 4. In almost all the studies (except one) either an emulsifiable concentrate (most 10% EC) or 0.5% granule (0.5% GR) were used and a majority of these studies were conducted in the USA, with others reports from New Guinea, Panama, Tanzania, Thailand and Japan. In one study, a 5% microencapsulated formulation of pyriproxyfen against *Cx. tarsalis* was tested in experimental ponds (Mulla *et al.* 1986). It is evident from Table 4 that both EC and GR formulations of pyriproxyfen were highly effective against *Ae. melanimon* and *Ae. nigromaculis* at rates ranging from 0.0028 – 0.011 kg a.i./ha in small plots in irrigated pastures in California, USA, inducing 100% adult emergence inhibition of these species within 3-4 days posttreatment, with a range of emergence inhibition of 20-100% (Mulla *et al.* 1986; Schaefer *et al.* 1988). Control of *Ae. aegypti* (98-100%) with pyriproxyfen lasted for >3 weeks with 25 – 50 ppb of 0.5% GR (Adames and Rovira 1993). Against *Anopheles* spp. (*An. farauti* and *An. punctatus* in New Guinea, and *An. minimus* and *An. maculatus* in Thailand) pyriproxyfen was very highly effective at rates ranging from 5 - 100 ppb in accumulated ground water, temporary pools and slow-moving streams resulting in 70-100% adult emergence inhibition (in most cases 100%) of these species for 20 days to beyond 2 months (Kerdpilbule 1989; Suzuki *et al.* 1989; Okazawa *et al.* 1991), indicating a rather extended control of these species. Pyriproxyfen exhibited superior activity against species of *Culex* (*Cx. peus, Cx. quinquefasciatus, Cx. tarsalis* and *Cx. tritaeniorhynchus*) in a variety of habitats including dairy lagoons, man-made ponds, blocked drains and ditches. In dairy lagoons in California, complete control of *Cx. quinquefasciatus* and other *Culex* mosquitoes was reported in two separate studies for >51 days and up to 2 months at a rate 0.11 kg a.i./ha of 10% EC

formulation of pyriproxyfen (Schaefer *et al.* 1988, 1991), while in man-made ponds the same formulation at lower application rates of 0.0056-0.045 kg a.i./ha resulted in complete control of *Cx. quinquefasciatus* for 2-14 days posttreatment (Schaefer *et al.* 1988). In another study in dairy lagoons, *Cx. quinquefasciatus, Cx. tarsalis*, and *Cx. peus* were controlled 17-100% for 7-68 days with single and multiple applications of 10% EC pyriproxyfen formulation applied at 0.1 kg a.i./ha (Mulligan and Schaefer 1990). Mulla and Darwazeh (1988) reported 26-66% adult emergence inhibition of *Cx. peus* and *Cx. quinquefasciatus* (mixed populations) for 7 days posttreatment in some California dairy lagoons receiving 0.028-0.056 kg a.i./ha of 0.5% GR pyriproxyfen. Mulla *et al.* (1986) reported excellent control of *Cx. tarsalis* (78-100%) for 7 days posttreatment with a microencapsulated formulation applied to man-made ponds at 0.011-0.056 kg a.i./ha, and with GR formulation (85-100% adult emergence inhibition of *Cx. tarsalis* for 7 days) applied at 0.0056-0.028 kg a.i./ha to the ponds. In artificial containers, cesspools and open sewers in Japan, *Cx. pipiens pallens* was controlled (91-100%) for 3-6 weeks with GR formulation of pyriproxyfen applied at 1-100 ppb, while adult emergence of *Cx. tritaeniorhynchus* in some ditches was reduced 43-100% for more than 3 weeks by the same formulation applied at 100 ppb (Kamimura and Arakawa 1991). Pyriproxyfen was also highly effective against *Psorophora columbiae* in irrigate date gardens where GR formulation applied at 0.0056-0.011 kg a.i./ha induced complete inhibition of adult emergence of this species observed for 4 days posttreatment (Mulla *et al.* 1989). It is obvious from the field data that a single, low application rate of pyriproxyfen ranging from 1-100 ppb or 0.0028-0.11 kg a.i./ha generally resulted in good control of a variety of mosquito species in the field. This result is due to the high activity and stability of pyriproxyfen under field conditions. This IGR is perhaps adsorbed onto organic matter in the mosquito larval feeding zone and the active ingredient remains available for ingestion by the larvae for relatively extended time period (Schaefer *et al.* 1988, 1991).

Chemical stability is important especially for juvenile hormone analogs and mimics, since they have to be available at specific susceptible stages in the insect's development, i. e., late 4th larval instar of mosquitoes (Mulla 1995). Controlled release formulations that sustain a minimum level of active ingredient in water sufficient for control is an important factor in the efficacy for JH analogues as insecticides. Granular formulation of pyriproxyfen showed the most stable activity in the field among the tested formulations (Mulla *et al.* 1986).

**Table 4. Field efficacy of pyriproxyfen against mosquitoes in different habitats**

| Species | Formul-ation[a] | Dosage (a.i.)[b] | %IE[c] (range) | Control Duration | Reference |
|---|---|---|---|---|---|
| *Aedes aegypti* | GR | 25-50 ppb | 98-100 | >3 weeks | Adames & Rovira (1993) |
| *Ae. melanimon* | GR | 0.0028-0.011 kg/ha | 20-100 | 4 days | Mulla *et al.* (1986) |
| *Ae. nigromaculis* | GR | 0.0028-0.011 kg/ha | 69-100 | 4 days | Mulla *et al.* (1986) |
| *Ae. nigromaculis & Ae. melanimon* | EC | 0.0028-0.0056 kg/ha | 39-100 | 3 days | Schaefer *et al.* 1988 |
| *Anopheles albimanus* | GR | 25-50 ppb | 95-100 | >3 weeks | Adames & Rovira (1993) |
| *An. farauti* | EC | 0.1 ppm | >70-100 | >2 months | Suzuki *et al.* (1989) |
| *An. punctatus* | GR | 0.02-0.1 ppm | 100 | 20 days->2 months | Okazawa *et al.* (1991) |
| *An. minimus & An. maculatus* | GR | 5 ppb | 70-100 | 4 weeks | Kerdpibule (1989) |
| *Culex* spp. | EC | 0.11 kg/ha | 100 | >51 days | Schaefer *et al.* (1988) |
| *Cx. pipiens pallens* | GR | 1-100 ppb | 91-100 | 3-6 weeks | Kamimura & Arakawa (1991) |
| *Cx. quinquefasciatus* | EC | 0.0056-0.045 | 100 | 2-14 days | Schaefer *et al.* (1988) |

| | | | | | |
|---|---|---|---|---|---|
| | | kg/ha | | | |
| *Cx. quinquefasciatus* | EC | 0.11 kg/ha | 100 | 2 months | Schaefer *et al.* (1991) |
| *Cx. quinquefasciatus* | GR | 25-50 ppb | 100 | >3 weeks | Adames & Rovira (1993) |
| *Cx. quinquefasciatus* | EC & GR | 0.1 ppm | 100 | 4-11 weeks | Chavasse *et al.* (1995) |
| *Cx. quinquefasciatus, Cx. tarsalis, Cx. peus* | EC | 0.1 kg/ha (single and multiple) | 17-100 | 7-68 days | Mulligan & Schaefer (1990) |
| *Cx. peus & Cx. quinquefasciatus* | GR | 0.028-0.056 kg/ha | 26-66 | 7 days | Mulla & Darwazeh (1988) |
| *Cx. tarsalis* | MC & GR | 0.011-0.056 kg/ha, 0.0056-0.028 kg/ha | 78-100<br>85-100 | 7 days<br>7 days | Mulla *et al.* (1986) |
| *Cx. tritaeniorhynchus* | GR | 0.01 ppm | 43-100 | >3 weeks | Kamimura & Arakawa (1991) |
| *Psorophora columbiae* | GR | 0.0056-0.011 kg/ha | 100 | 4 days | Mulla *et al.* (1989) |

[a] GR = granular; EC = emulsifiable concentrate; MC = microencapsulated
[b] a.i. = Active Ingredient
[c] %IE = % inhibition of adult emergence

## 5.3 WHOPES supervised trials

*USA.* Relative efficacy of pyriproxyfen GR and (S)-methoprene GR against laboratory-reared late 3rd and early 4th instars of *Ae. aegypti, Ae. albopictus, Ae. taeniorhynchus, An. quadrimaculatus* and *Cx. nigripalpus* were studied at application rates of 0.02 and 0.05 ppm (active ingredient) in the laboratory (in trays) and under semi-field conditions.

In the laboratory studies, 100 late 3rd and early 4th instar larvae were utilised in each tray with appropriate amount of mosquito larval food added to each tray at the onset of each evaluation and thereafter at predetermined intervals posttreatment. Three replicates of each treatment were used and 3 untreated trays were maintained as controls in each evaluation. The trays were examined daily to score posttreatment larval and pupal mortality or survivorship and adult emergence. The live pupae from each tray were collected and maintained in corresponding cups containing well water (clear medium) and placed in mosquito cages to check for emergence. Each week when all larvae and pupae had either died or survived as adults in the control trays, all live and dead immatures from the treated trays were removed and a new batch of 100 laboratory-reared late 3rd and early 4th instar larvae of the same mosquito species added to each treated and control tray and the routine daily posttreatment mortality/survivorship observations were continued. Six, weekly batches of mosquito larvae of a species were introduced to the trays to determine residual activity of each formulation.

The semi-field evaluations were conducted in 1-m diameter polyethylene fish-tubs lined with polyethylene sheets and containing 100 liters well water. The tubs were maintained outdoors under a canopy to protect from rain and direct sunlight. One hundred 3rd and 4th instar mosquito larvae of a species were exposed to 0.02 and 0.05 ppm active ingredient of each formulation and the same method of daily observations and procedures as for laboratory trays was followed, except that 1 gm of larval food was added to the tubs at the introduction of each mosquito larval batch and on alternate days thereafter; six larval batches were tested. The tubs were covered with fine mesh plastic screen to protect from air-borne debris and wild insects, as well as oviposition by wild mosquitoes.

Table 5 summarises the result of the laboratory and semi-field efficacy trials of the two products.

**Table 5. Laboratory and semi-field efficacy of pyriproxyfen GR and (S)-methoprene GR**

| Species | Formul-ation | Dosage ppm | Range of % inhibition of adult emergence posttreatment (weeks) | |
|---|---|---|---|---|
| | | | Laboratory (trays) | Semi-field (tubs) |
| *Aedes aegypti* | (S)-methoprene GR | 0.02 | 63-87% (2 wk) | 42-84% (2 wk) |
| | | 0.05 | 75-100% (3 wk) | 48-100% (3 wk) |
| | Pyriproxyfen GR | 0.02 | 99-100% (6 wk) | 94-100% (6 wk) |
| | | 0.05 | 100% (6 wk) | 100% (6 wk) |
| *Ae. albopictus* | (S)-methoprene GR | 0.02 | 9-16% (2 wk) | <13% (6 wk) |
| | | 0.05 | 27-44% (2 wk) | 17-32% (6 wk) |
| | Pyriproxyfen GR | 0.02 | 53-100% (6 wk) | 100% (6 wk) |
| | | 0.05 | 93-100% (6 wk) | 100% (6 wk) |
| *Ae. taeniorhynchus* | (S)-methoprene GR | 0.02 | 35-100% (3 wk) | 59-100% (6 wk) |
| | | 0.05 | 62-100% (4 wk) | 92-100% (6 wk) |
| | Pyriproxyfen GR | 0.02 | 81-100% (6 wk) | 99-100% (6 wk) |
| | | 0.05 | 95-100% (6 wk) | 98-100% (6 wk) |
| *Culex nigripalpus* | (S)-methoprene GR | 0.02 | 45-83% (3 wk) | 60-94% (4 wk) |
| | | 0.05 | 47-92% (5 wk) | 67-100% (4 wk) |
| | Pyriproxyfen GR | 0.02 | 82-100% (6 wk) | 100% (6 wk) |
| | | 0.05 | 98-100% (6 wk) | 100% (6 wk) |

| | | | | |
|---|---|---|---|---|
| *Anopheles quadrimaculatus* | (S)-methoprene GR | 0.02 | 39-80% (3 wk) | 34-98% (4 wk) |
| | | 0.05 | 39-79% (4 wk) | 42-99% (5 wk) |
| | Pyriproxyfen GR | 0.02 | 91-100% (6 wk) | 77-100% (6 wk) |
| | | 0.05 | 100% (6 wk) | 97-100% (6 wk) |

In this study the activity profile of (S)-methoprene GR in laboratory trays and in outdoor tubs was very similar. The lower concentration of 0.02 ppm of this formulation generally provided lower levels (ranges) of control of all mosquito species tested although these differences in some species were negligible. The higher concentration of 0.05 ppm of (S)-methoprene GR provided 1-2 weeks extended control of the test species than the lower concentration. Generally, 80% or higher levels of emergence inhibition by (S)-methoprene GR were achieved only for 1-3 weeks, except for *Ae. albopictus* which was relatively tolerant.

By contrast, pyriproxyfen GR at 0.02 ppm and 0.05 ppm of active ingredient caused emergence inhibition in *Ae. aegypti, Ae. albopictus, Ae. taeniorhynchus, An. quadrimaculatus* and *Cx. nigripalpus* invariably of greater magnitude and duration than (S)-methoprene GR at comparable treatment rates of the active ingredients. At both treatment rates, pyriproxyfen provided complete inhibition of adult emergence of all 5 test mosquitoes, with inhibition generally ranging from >80 - 100% (except for *Ae. albopictus* ranging from 53-100%) and >90-100% in laboratory trays at 0.02 ppm and 93-100% and 99-100% in the tubs at the higher concentration of 0.05 ppm. Although the evaluations were terminated at 6 weeks posttreatment, the very high levels of emergence inhibition sustained at 6 weeks posttreatment (except for *Ae. albopictus*) indicate more prolonged activity of pyriproxyfen GR beyond the 6 weeks of

posttreatment monitoring period. The results obtained in the trays and the tubs resulting from this IGR were very similar; the lower concentration of 0.02 ppm was highly effective and produced complete inhibition as did the higher concentration of 0.05 ppm in terms of magnitude and duration.

*India.* Pyriproxyfen 0.5% GR was tested in the field at 0.1, 0.25 and 0.5 kg/ha (or 5, 12.5 and 25 g a.i./ha, respectively) in cesspits, stagnant and slow-moving drains and disused (abandoned) wells. Seven cesspits (ranging from 1-3 m diameter and 1-9 $m^2$ surface area) were treated at each application rate and 7 similar cesspits were left untreated as controls thus utilizing a total of 28 cesspits. In another set of treatments in stagnant and slow-moving drains, 0.1, 0.25 and 0.5 kg/ha rates of 0.5% GR pyriproxyfen were tested separately in stagnant and slow-moving drains. In this trial, 8 drains, 2 for each of the 3 treatment rates of 0.1, 0.25 and 0.5 kg/ha and 2 for control were utilized. The same rates of pyriproxyfen treatments were also utilized in another set of treatments in abandoned wells where a total of 8 wells was utilized, 2 for each treatment and 2 for control. The surface area of these wells ranged from 2.01 to 7.07 $m^2$. In another type of habitat, cement tanks, pyriproxyfen 0.5% GR was tested only at 0.1 and 0.5 kg/ha in replicated tanks.

Dip samples were collected from each treatment and control habitat during each evaluation. These samples were collected twice weekly 1 to 5 weeks prior to the treatments and up to 28 weeks posttreatment in the various trials. In the cesspits and cement tanks, 3 dips were randomly taken; in the drains 3 dip samples from every 10-m length of the drains were collected. In the abandoned wells, the immature mosquitoes were sampled using a 20-cm diameter bucket. Samples of pupae with water from the corresponding sites (treated and control) were taken to the laboratory and observed for inhibition of adult emergence (EI). Posttreatment observations were made 24 hours after a treatment and thereafter twice per week until adult emergence

levels increased and were comparable to the corresponding controls. Data were pooled to obtain weekly means and % larval and pupal reductions were calculated using Mulla's formula (Mulla *et al.* 1971). The duration(s) for which >90% larval and pupal mortality or >90% emergence inhibition occurred was considered as effective control duration. A summary of the dose determination for further field evaluation is given in Table 6.

**Table 6. Efficacy of pyriproxyfen 0.5% GR against *Culex quinquefasciatus* in a variety of habitats in Pondicherry, India.**

| | | Range of % reduction (larvae/pupae) or % inhibition of adult emergence posttreatment | | |
|---|---|---|---|---|
| Habitat | Rate (kg/ha) | Larvae | Pupae | Adult emergence inhibition |
| Cesspits | 0.1 | 0-44% | 0-65% | 97-100% for 6 weeks, thereafter 50-80% for 10 weeks |
| | 0.25 | 0-46% | 22-82% | 80-100% for 9-11 weeks and 60-70% in the 12th week |
| | 0.5 | 0-52% | 0-50% | 95-100% for 9-11 weeks and 60-70% in the 12th week |
| Drains (stagnant) | 0.1 | 6-60% | 12-75% | 50-80% for 1-2 weeks |
| | 0.25 | 0-40% | 5-80% | 78-100% for 8-9 weeks |
| | 0.5 | 0-50% | 0-58% | 90-100% for 10 weeks |
| Drains (slow-moving) | 0.1 | 42-62% | 0 | 35-40% for 3 weeks |
| | 0.25 | 0% | 42-60% | 60% for 1 week |
| | 0.5 | 0-20% | 42-90% | 70-100% for 6 weeks |
| Disused (abandoned) wells | 0.1 | 20-92% | 100% | 60-90% for 18 weeks |
| | 0.25 | 20-90% | 5-80% | 90-100% for 26 weeks |
| | 0.5 | 26-99% | 100% | 90-100% for 26 weeks |

| | | | | |
|---|---|---|---|---|
| Cement tanks | 0.1 | 60-95% | 40-60% | >90% for 4 weeks |
| | 0.5 | 60-95% | 100% | >90% for 5 weeks |

Subsequent to the dose determination trials, medium scale field evaluation of pyriproxyfen 0.5% GR was conducted. This evaluation was conducted in 1.5 $km^2$ area where the study habitats (cesspits, soak pits and drains) were demarcated and enumerated. A total of 13 cesspits and 2 soakpits (42 $m^2$ total surface area) and 5 drains (960 $m^2$ total surface area) were treated at 0.1 kg/ha (cesspits and soak pits) and 0.5 kg/ha (drains) of 0.5% GR pyriproxyfen. A total of 4 cesspits and 2 drains (505 $m^2$ total surface area) were left untreated as controls. Twice weekly pretreatment (for 1-4 weeks) and posttreatment samples of larvae and pupae from the cesspits and soakpits (3 dip samples from each treated and control habitat) and from treated and control drains (5 dip samples from each 20-m long drain segment) were collected. The posttreatment % larval and pupal reductions and % adult emergence inhibition (field-collected pupae) were assessed in the laboratory as already described. The habitats where the % adult emergence inhibition decreased to <50% were retreated during the study. In cesspits and soakpits, pyriproxyfen GR caused >90% emergence inhibition of *Cx. quinquefasciatus* for 8 weeks and 80-90% thereafter for 12 weeks. Larvae and pupae were reduced by >40% to 75% for 6 weeks in these habitats. In the drains, the 0.5 kg/ha rate of treatment induced 80-100% inhibition of adult emergence for 5 weeks; re-treatment at $8^{th}$ week after the first treatment again caused 80-100% adult emergence inhibition for 4 weeks. This study revealed that a re-treatment at 0.5 kg/ha of pyriproxyfen 0.5% GR was required every 6 weeks to significantly suppress *Cx. quinquefasciatus* adult emergence from these drains. This study did not reveal any definite trend of larval reduction in the treated drains although some reductions were noted. However, pupal abundance was reduced

by 40-80% during the 12 week posttreatment period, which included a re-treatment after 8 weeks of the first treatment.

## 5.4 Conclusions and recommendations

1. Pyriproxifen has been used for mosquito control for over five years. This IGR, at mosquito control application rates (ppb or sub ppb), has been shown to be safe to humans.

2. Under semi-field conditions in Florida, USA, pyriproxyfen GR at application rates of 20 and 50 ppb a.i., yielded similar results, inhibiting adult emergence of the following mosquito species for at least 6 weeks: *Ae. aegypti* (94-100%), *Ae. albopictus* (100%), *Ae. taeniorhynchus* (98-100%), *An. quadrimaculatus* (77-100%) *and Cx. nigripalpus* (100%).

3. Against *Cx. quinquefasciatus* in heavily polluted habitats in India, the same formulation, at application rates ranging from 25 to 100 g a.i./ha gave 80-100% inhibition of adult emergence for at least 6 weeks (cess pits), 35-100% control for 1-10 weeks (stagnant and slow moving drains), 60-100% for 18-26 weeks (abandoned wells) and more than 90% for 4-5 weeks (cement tanks).

4. The use of this IGR for mosquito larviciding is recommended at rates of 5 to 10 g a.i./ha. However, higher rates, up to 100g a.i./ha., may be required for control of mosquitoes in heavily polluted waters. Noting the potential adverse effects of pyriproxifen on aquatic non-target organisms in laboratory and small scale field studies, the long term application of this product in permanent water bodies is not recommended until further environmental studies support such uses. Therefore, the use of this product in situations where there is no concern for non-target

organisms, e.g. in highly polluted habitats, in temporary water bodies and in artificial containers is recommended.

5. Recognizing that pyriproxyfen GR is effective in controlling container breeding mosquitoes at extremely low application rates with long residual activity, further investigations are warranted to assess its potential for operational use.

# 6. REVIEW OF LAMBDA-CYHALOTHRIN CS FOR TREATMENT OF MOSQUITO NETS

## 6.1 Safety assessment

Lambda-cyhalothrin (alpha-cyano-3-phenoxybenzyl 3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropane carboxylate, a 1:1 mixture of the (Z)-(1R, 3R), S-ester and the (Z)-(1S, 3S), R-ester) is a synthetic pyrethroid insecticide, consisting of one of the enantiomer pairs of cyhalothrin.

The toxicity of cyhalothrin has been assessed by WHO (1990) and FAO (evaluation report 463/1999[3]) and the following conclusions are noted:

Lambda-cyhalothrin has moderate acute toxicity when administered orally to the rat ($LD_{50}$ 56-79 mg/kg body weight). Clinical signs are consistent with pyrethroid toxicity (e.g. abnormal motor function).

In the rat, lambda-cyhalothrin is less toxic by the dermal route ($LD_{50}$ 632-696 mg/kg body weight) but is highly toxic by inhalation ($LD_{50}$ 0.06 mg/l air). Lambda-cuhalothrin is a mild irritant to the rabbit eye and skin. It is not a skin sensitizer in the guinea pig. Lamda-cyhalothrin gave negative results in a range of *in vivo* and *in vitro* assays designed to detect gene mutations, chromosomal damage, and other genotoxiv effects.

Degradation of lambda-cyhalothrin in water in the dark is rapid by hydrolysis under alkaline conditions, but very slow under neutral and acid conditions. In the light aqueous degradation of lambda-cyhalothrin under acid conditions is rapid.

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3 http://www.fao.org/waicent/faoinfo/agricult/agp/agpp/pesticid/Specs/pdf/lam_cyha.pdf.

Lambda-cyhalothrin is extremely dangerous to fish and *Daphnia.*

WHO has classified lambda-cyhalotrhin technical grade material in Class II "moderately hazardous". The following are extracts from the material safety data sheet (MSDS) of the manufacturer for lambda-cyhalothrin 2.5% CS:

| | |
|---|---|
| Acute oral $LD_{50}$ (rat) | >5,000 mg/kg |
| Acute dermal $LD_{50}$ (rat) | > 4,000 mg/kg |
| Skin irritation | May cause transient (lasting up to 24 hours) tingling or numbness in exposed areas (paraesthesia). The effect may result from splash, aerosol or transfer to the face from contaminated gloves and hands. |
| Eye irritation | May cause mild eye irritation |

## 6.2 Efficacy - background/supporting documents

*Tanzania.* Curtis *et. al* (1996) compared lambdacyhalothrin CS formulation with various formulations of insecticides (a carbamate and four pyrethroids), that were impregnated into bednets and curtains made from cotton, polyester, polyethylene or polypropylene fabric for insecticidal efficacy in the laboratory bioassays and experimental huts, in Tanzania. For bioassays, female *Anopheles gambiae* mosquitoes were exposed for 3 min to the treated nets (3 and 10 mg/m$^2$) used by the local residents.

An initial treatment rate of polyester nets at 10 mg/m$^2$ and one time washing after 8 months and with no re-impregnation, lambdacyhalothrin CS gave 85 to 100 % mortality of *An. gambiae* for 15 months and 50% for 2 years. On polyethylene nets, it lasted for about 5 months (>80%). The effect was better

than deltamethrin SC and much better than permethrin EC and etofenprox EC. The tests yielded low mortality on *Cx. quinquefasiatus.*

Most of the impregnated nets provided significantly better prevention of blood feeding by *Anopheles* than untreated intact nets. There was, however, no difference between the treatments of different pyrethroids on polyester nets. Lambdacyhalothrin was as effective as permethrin at preventing feeding but better at killing.

At 10 mg/$m^2$ or more, on holed nets all pyrethroids gave much better protection than with untreated holed nets. After 15 months, use of holed nets impregnated with lambda-cyhalothrin CS at 10 mg a.i./$m^2$ was still effective. At low rate of 3 mg/$m^2$, it lost effectiveness after washing, which was regained by re-treatment.

*Tanzania.* In an intensely malarious area in north-east Tanzania, lambdacyhalothrin 10% CS was used (1995 -1996) at the dosage of 10 and 20 mg a.i./$m^2$ in four villages for treatment of polyester bednets (Curtis *et al.* 1998). In another four villages, the same insecticide (30 mg a.i./m2) was used for house spraying. Another four villages received neither intervention until the end of the trial but were monitored as controls. Re-spraying was done after 7 to 8 months. The nets were washed after 7 months and retreated. WHO cones were used for bioassay and *An. gambiae* were exposed for 10 min to the sprayed surface and 3 min to the nets.

The mortality rate was 100% after 7 months of spraying. There was a significant effect on the knock down time of nets washed after initial treatment. There was no effect on the nets washed after re-treatment. There was no difference between the doses. Monthly light trapping and ELISA testing showed reduction of the malaria vector populations and the sporozoites rates, leading to a reduction of about 90% in the entomological inoculation

rate as a result of each treatment. Window exit trap and pyrethrum spray catches were carried out in treated rooms. Analysis of variance showed highly significant impact of the intervention (P=<0.001). But no significant different between spray and nets or between the species was observed.

Incidence of re-infection was measured by weekly monitoring of cohorts of 60 children per village, after clearing pre-exiting infection with chlorproguanil-dapsone. There was a reduction in probability of re-infection per child per week by 54-62%, with no significant difference between the two vector control methods. Cross-sectional surveys carried out monthly on 50 samples of children (1to 6 years) for fever, parasitaemia, haemoglobin and weight showed beneficial effects of each intervention in reducing anaemia. However, passive surveillance by resident health assistance showed no evidence for reduced prevalence of fever of parasitaemia. Since net treatment consumed only about one sixth as much insecticides as house spraying and it was concluded that the former intervention would work out cheaper (>2 times) and nets were actively demanded by the villagers, whereas spraying was only passively accepted.

*Philippines.* Quilala *et. al* (1996) (unpublished report) carried out a field trial in selected barangays in Conner, Apayao, Phillippines to determine the effectiveness of lambdacyhalothrin CS treated mosquito nets (10 mg a.i./$m^2$) made of polyethylene fibres on the vector, *An.flavirostris* and malaria transmission. Tests were conducted in randomly assigned group of barangays with households around 500 and population of about 2500 each for treated and control. The mosquito net ownership was <65% in these areas. Dipping method was used to treat the nets initially. Re-impregnation was done after 6 months by soaking the net into the insecticide solution poured into a plastic bag.

Bioassays were carried out (WHO type cones) on the treated nets that were actually in use with mosquitoes exposed for 3 minutes, which gave 100% kill of *An. flavirostris* for 6 months.

The man-landing rate remained the same on the month following treatment but was reduced by 90% on the 2nd month and 54% on 3rd month, compared to densities in control area. Overall, the indoor man-landing rate was significantly lower (1.48 mosquitoes/man/night) in the treated area than control area (5.3 mosquitoes/man/night). Culicine population remained unaffected.

Structured interviews with randomly selected household informants of about 108 in control and 124 in treated areas, within first week, one month and three months after treatment showed that 91.6% and 97.2% perceived reduction in mosquito bites. There were no major complaints of side effects, except for bad odour for a week and cough for two days (this has not been quantified). Inspection of 247 and 224 nets in the treated and control area showed that 78% and 53% were actually used.

The slide positivity rate was assessed by quarterly blood film surveys of 34-42% of non-random samples, from treated and control areas, before and after intervention. Initially, the parasite rates and *P.falciparum* rates were similar in treated (4.63%) and control area (4.26%). The parasite rates were significantly different after the intervention. The investigators, for lack of appropriate analysis, could not make any quantitative observations.

*Malaysia.* Vythilingam *et al* (1999) compared the residual effectiveness of lambdacyhalothrin CS with that of EC formulation. *An. maculatus and Ae. aegypti* were exposed for 2 minutes to treated nets made up of polythene monofilament and polyester multifilament fibres at 15 mg a.i./$m^2$ and after repeated washing (for 3 min) with water and with soap and water.

In the bioassays carried out with WHO insecticide resistance test kits, all the four combinations (2 formulations in 2 types of nets) of unwashed nets gave more than 90% mortality for 9 months (except EC formulation on polyester nets with *An. maculatus).*

When washed with water, the polyester nets treated with CS formulation, provided higher mortality compared to EC formulation (P <0.001). With both the fabrics the CS formulation caused more than 90% mortality of *An.maculatus* upto 4 washes. With *Aedes aegypti*, microencapsulated formulation gave >85% mortality ever after 5 washes with water and >90% after 2 washes with soap and water on both fabrics. The mortality rate was above 80% for EC formulation after 2 washes with soap and water.The CS formulation treated polyethylene nets washed with soap and water performed better than CS treated polyester nets (P<0.005).

*Tanzania.* Miller *et al.* (1999) studied the efficacy of a low dose, frequent wash and re-treatment system of mosquito nets. Initially, lambdacyhalothrin EC, lambdacyhalothrin CS, deltamethrin SC and permethrin EC were compared with a control (untreated hut) at different doses. Bioassay with *An. gambiae* using "wire ball' system (10 cm dia.) exposed for 3 min showed that lambdacyhalothrin CS was the only insecticide showing consistently high mortality (>80%), even at the lowest dosages of 3 mg a.i./$m^2$ and 1 mg a.i./$m^2$ throughout 10 weeks of testing.

Therefore, only lambdacyhalothrin CS treated nets were taken for residue analysis and successive washings. Washing was done by hand with soap for 5 minutes. The gas chromatography and bioassay showed that the deposits after first treatment without washing were close to the target dosages. The mortality rate on the nets that were treated with low dose initially and frequently washed and not retreated declined slowly and after 12 washes the mortality rate was closer to control levels. The insecticide deposit, however declined much more slowly and only about 1/3

of it had been washed out after 12 washes. The amounts of pyrethroids in the nets that were treated with 1 or 3 mg/m2 accumulated rapidly over the first four wash–re-treatment cycles, remained apparently stable over the next 4 cycles, but increased again between 8th and 12th cycle. These nets gave consistently high bioassay mortality throughout.

In the experimental hut trial with low dose treated, frequent wash and re-treatment regime, the numbers caught was too low for statistical analysis. However, the estimated number of mosquitoes that entered huts from untreated ones were not lower in the treated huts suggesting no deterrent effect. The mortality rate and the mosquito that entered the huts were larger and this proportion was showing higher in the huts with treated nets that had been retreated after each wash. The effect on Culicines was low. With a 1 mg a.i./$m^2$ re-treatment after each wash, the amount found by Gas chromatography were at the lower and of range that conventionally recommended; while with a 3 mg a.i./$m^2$ re-treatment cycle, they were in the upper end of this stage and slightly beyond it. The investigators recommended a regular post wash re-treatment of about 3 mg a.i./$m^2$ would be suitable.

*Tanzania.* The strategy of low dose, frequent wash–retreatment regimen was tested in a community wide trial for acceptability and determining users' perceptions (Miller *et al*, 1999). Nets treated with permethrin EC (200 or 500 mg a.i./$m^2$) or lambdacyhalothrin CS (3 or 15 mg a.i./$m^2$) was distributed to the community, without informing them of the type of treatment they received. Users' response was compared from focus group discussions after 2, 8,12 weeks post-treatment. Few participants experienced side effects or expressed fears about the safety of treatment. Low dose gave perceptible lesser protection and therefore an initial high dose followed by frequent lower dosages has been suggested.

## 6.3 WHOPES supervised trials

*Côte d'Ivoire*. The efficacy of lambdacyhalothrin CS was tested in laboratory bioassays and experimental huts (WHOPES Phase II trial), by Darriet *et al* (1999) in M'be'Valley. They considered two dosages, 10 and 15 mg a.i./$m^2$ to treat polyester nets (nylon) and carried out an experiment for 10 months against *An.gambiae & An.funestus.*

The local strain of *An. gambiae* was susceptible, though the $KD_{95}$ was about 2 times longer than for the reference strain (Kisumu strain). However, bioassay was carried out with *An. gambiae,* Kisumu strain (susceptible). Knock down was relatively low after exposure period of 3 minutes (<30%) for first 3 months and < 10% (KD) for 4 to 10 months. The mortality rate was above 80% for 8 months at 10 mg a.i./$m^2$ and 9 months at 15 mg a.i./$m^2$ with unwashed nets.

The entry rate of *An. gambiae* was reduced by 55% in the hut with a net treated at 10 mg a.i./$m^2$ and 68% at 15 mg a.i./$m^2$. For *An. funestus*, the reduction was 57% and 89% respectively indicating considerable deterrent effect of the formulation.

The exit rate of *An. gambiae* was increased by 2 times in the huts with treated nets compared to control, indicating a repellent effect. In control hut, the exit traps veranda collection was 38% of the total number collected, while it was 80 - 83% in huts with nets treated at 10 and 15 mg a.i./$m^2$. In case of *An. funestus*, the exit rate was increased by 2.4 times (71%), and in control it was 29.2%. There was no significant difference between the dosages for *An. funestus* and the exit rate was significantly higher for *An. gambiae,* at 10 mg a.i./$m^{2)}$.

The blood feeding rate of *An. gambiae* was also reduced significantly by 32% in the huts with nets treated at 10 mg

a.i./$m^2$ and 47% at 15 mg a.i./$m^2$. For *An. funestus,* the reduction was 73% at 10 mg a.i./$m^2$ and 81% at 15 mg a.i./$m^2$.

The mortality rate was 30-35% for *An. gambiae* and the same was 41-43% for *An.funestus*. The mortality rate increased with the dose only in case of *An.gambiae.*

Residue analysis was made with treated nets maintained in the laboratory as well as with nets used in the field after 10 months and compared. The results showed that the dosage was almost same for 10 mg a.i./$m^2$ while it was lesser for 15 mg a.i./$m^2$.

*Tanzania.* Maxwell et.al (1999) carried out a more comprehensive trial of mosquito nets (polyester fibre) treated with alphacypermethrin SC and lambdacyhalothrin CS formulations in a village scale in Muheza, Tanzania. Nets were treated with 10, 20 and 40 mg a.i./$m^2$ of alphacypermethrin and 10, 20 mg a.i./$m^2$ of lambdacyhalothrin CS and 6 hamlets were allocated for each treatment and 4 for untreated control.

In the experimental hut, the treated nets were found to cause significantly higher mortality among the anophelines compared to the untreated nets. Alphacypermethrin was significantly better at preventing feeding but lambdacyhalothrin was significantly better at killing anophelines. There were no consistent dose effect and washing (five times) caused no effect on protection from biting but slightly significant effects on mosquito mortality. The persistence of effect after washing of treated nets was the evidence for good wash resistance of lambdacyhalothrin. There were reductions in number of fed mosquitoes exiting from treated rooms by 97% - 95% due to treated nets, the reduction in infective bites was 99%.

Administration of structured questionnaire to assess the users' perception and side effects showed that nasal irritation was more with lambdacyhalothrin (19 out 105 users) than

alphacypermethrin (3 out of 117 users). There were a few other symptoms but there was no difference between the two insecticides. The nets were effective against bedbugs and head lice and other household pests. The beneficial effects were equally perceived.

The two treatments had similar effect on man-vector contact assessed by light trapping (reduced by 80%), the sporozoite infection rates as monitored by ELISA test and entomological inoculation rates (reduced by 95-96%). There was no reduction in the number of *Culex quinquefasciatus.*

Incidence of re-infection of malaria was measured by weekly monitoring of cohorts of children, after clearing pre-exiting infection with chlorproguanil-dapsone. Compared to the incidence in the previous year, about 72% reduction occurred from each treatment. Analysis of variance showed no difference between children with the insecticide treatments and protection was about 81% compared to children with no nets. There was a significant difference between those with treated (80%) and untreated net (33%) but not significant difference between those with untreated and with no nets. The untreated nets gave protection of about 33%.

It was concluded that the two pyrethroids had a similar impact on mosquitoes and malaria incidence. There were relatively more reports of nasal irritation with lambdacyhalothrin and if alphacypermethrin can be obtained cheaply, these could be the pyrethroid of choice for net treatment.

*Côte d'Ivoire.* The effect of lambdacyhalothrin CS treated mosquito nets on malaria transmitted by pyrethroid resistant *An. gambiae* was studied in a large scale in Korhogo area, in West Africa. This was a WHOPES phase III trial, in which the evaluation was done in 8 villages, 4 receiving insecticide treated nets (population: 3500) and 4 matched villages were considered as control to monitor natural change. Previous epidemiological

situation in these villages was available. The nets were treated with lambdacyhalothrin CS at 15 mg a.i./m2. Re-treatment was done at an interval of 6 months, consistent with the period of transmission.

The safety and acceptability of the nets were determined by informal discussions. No complaint was registered in the study with the use of treated nets. The amount of insecticide on nets was of same range as determined by residual analysis during the period of 5-6 months of trial.

Bioassays were carried out with 3min. exposure of susceptibility strain of *An. gambiae* (Kisumu), just after treatment, 3 months, 5 months and after washing on 6$^{th}$ month and re-treatment. The mortality ranged from 60 to 90% for after the treatment indicating irritancy or repelling effect of freshly treated material. Subsequently, the mortality rate remained at 100% during 5 months after treatment. Even after washing (on 6$^{th}$ month after treatment) mortalities were still almost 95 – 100% on nets in use and on those maintained in the laboratory.

Night biting catches showed that there was a significant reduction in the biting rates, survival rate, life expectancy, vectorial capacity and inoculation rates. The inoculation rate was reduced 10 folds. Impact on *An. funestus* was even greater with complete interruption of transmission treated villages.

Data on malaria mortality and incidence were collected by active case detection on randomly selected samples. The parasito-clinical survey were conducted two weeks after the entomological survey. The incidence remained at 2.1 crisis/child/year in villages where no vector control was implemented while it dropped from 3.3 to 1.0 after introduction of ITN and then re-treatment 6 months later.

The parasite rate and gametocyte rats were also reduced. The percentage of carriers of high *P. falciparum* parasitaemia was

40% less among children of villages with treated nets than among children living in no net villages.

*Vietnam.* Tran Duc Hinh *et. al.* (2000) carried out a comparative evaluation of residual effects of bednets impregnated with permethrin EC (200 mg a.i./$m^2$), deltamethrin SC (20 mg a.i./$m^2$), lambdacyhalothrin CS (20 mg a.i./$m^2$), etofenprox EW (200 mg a.i./$m^2$) and alpha-cypermethrin SC (25 mg a.i./$m^2$) concurrently in three sites, North, Centre and South of Vietnam (1998, 1999). Coded double polyester nets allocated randomly to each 366 households in North, 222 in Centre and 207 in the South. Washing of nets was monitored with marks of water soluble marker ink. Bioassays were carried out with *An. dinus* (Susceptible) from the colony on the net pieces selected randomly from a lot of coded net pieces collected from households. Chemical analysis was done to determine target dosages.

The residual effect (>70% mortality) of lambdacyhalothrin CS lasted for 7-11 months, alphacypermethrin SC for 6-10 months followed by permethrin EC (4 months), etofenprox EW (4 months) and deltamethrin SC (3 – 4 months) on unwashed nets in the field. The difference between the insecticides was significant ($P< 0.001$). Analysis of mosquito mortality after the second washing of nets in the field showed that lambdacyhalothrin CS and alphacypermethrin SC killed more mosquitoes (>70%) as compared with others. lambdacyhalothrin CS was found to be the most effective one. Subsidiary tests in the laboratory after two washings showed that lambdacyhalothrin CS still killed >90% of mosquitoes tested whereas all remaining insecticides killed not higher than 70% of tested mosquitoes. Low amount of alphacypermethrin SC, permethrin EC and lambdacyhalothrin CS are required for dipping bednets (1 litre for 75 nets) compared to deltamethrin SC and etofenprox EW.

A questionnaire was administered to 180 randomly selected householders as well as to the dippers. The side effect reported by dippers included headache, skin irritation, dizziness, running nose and nausea in decreasing order. Many complained of strong odour. However, the bednet usage was > 90 % in 3 sites and all the 5 insecticides caused minimal rate of negative side effects (ranging from 0 to 2 %) and were well accepted by community.

It was observed from the study that alphacypermethrin SC and lambdacyhalothrin CS were the two insecticides having the longest residual effect and can be used for bed net impregnation at least once per year. Imperator 50 EC, K-othrine, 1SC and Vectron 10 EW can be used for bed net impregnation at least twice per year. The result on lambdacyhalothrin CS was in agreement with other studies (Curtis *et al.,* 1996; Vythilingam *et al.*, 1999).

## 6.4 Conclusions and recommendations

1. Lambdacyhalothrin CS is safe for the treatment of mosquito nets when the instructions and recommendations are followed. This applies both to persons who treat and who use the treated nets at the recommended dosage.

2. Review of the studies indicates good persistence of insecticidal effect of lambdacyhalothrin CS and its protective efficacy on the various fabrics at the recommended dosages. With unwashed treated nets, this formulation at a target dose of 10-15 mg a.i./ $m^2$ remained effective for up to 11 months.

3. Lambdacyhalothrin CS is relatively more wash resistant than other pyrethroid formulations currently recommended for the treatment of mosquito nets. It remains effective even after 2 washes with water and soap and up to 4-5 washes

with water alone. However, because of improved wash resistance, the risk of insecticide accumulation is greater if nets are treated after each washing, even at low concentration.

4. In addition to killing effect and blood feeding inhibition comparable to other α-cyano pyrethroids, lambdacyhalothrin CS was found to have a significant excito-repellent effects on *An.gambiae*.

5. Lambdacyhalothrin CS formulation is effective against a range of malaria vectors, reducing malaria incidence and morbidity in various epidemiological settings, including in areas with *kdr* pyrethroid resistance in the major vector.

6. Occasional reports of transitory symptoms such as sneezing and cough related to lambdacyhalothrin have also been reported with other α-cyano pyrethroids. However, acceptability of treated nets was not affected.

7. Lambdacyhalotrhin CS is recommended for the treatment of mosquito nets at a dose of 10 to 15 mg a.i./$m^2$. The expected duration of efficacy is up to 11 months if nets are not washed more than twice with water and detergents. In areas where nets are to be washed frequently or mosquitoes are perceived as a nuisance, the use of the upper limit of the dose range (15 mg a.i./$m^2$) is recommended.

## 7. GENERAL RECOMMENDATIONS

1. Due to the large number of variables affecting the outcome of repellents tests, it is recommended that WHOPES initiate standardization of laboratory and field testing procedures. For sound statistical inference and comparative purposes proportional endpoints (e.g. 95% reduction in biting) should be preferred to fixed endpoints (e.g. complete protection time). Experimental protocols should aim at assessing the functional relationship of percent repellency with both dose and time after application. Key target arthropod species should be identified for laboratory and field tests.

2. Evaluation of potential use of repellents for protection against other major vectors, e.g., sand flies, ticks and simuliid black flies, is recommended.

3. In testing pyrethroid efficacy for the treatment of mosquito nets, KDT (Knock Down Time) should not replace mortality recorded 24 H after standard 3 minutes exposure. Although KDT is a sensitive indicator of pyrethroid efficacy, it is difficult in this case to interpret on its own. If measured, it should be in addition to 24 H mortality and KD rate observed 60 minutes after 3 minutes exposure.

4. Although impact of pyrethroid resistance induced by *kdr* on the overall efficacy of nets treated with lambdacyhalothrin CS was limited, it should also be assessed for other pyrethroids and when resistance mechanisms other than *kdr* are involved, e.g. detoxification mechanisms.

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