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**REPORT OF THE SIXTH
WHOPES WORKING GROUP MEETING**

**WHO/HQ, GENEVA
6–7 NOVEMBER 2002**

**Review of:
DELTAMETHRIN 25% WG & WP
and
AGNIQUE MMF**

**WORLD HEALTH ORGANIZATION
COMMUNICABLE DISEASE CONTROL,
PREVENTION AND ERADICATION
WHO PESTICIDE EVALUATION SCHEME**

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1. INTRODUCTION

The 6th WHOPES Working Group, 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, from 6 to 7 November 2002.

The meeting was opened by Dr V. Kumar, Acting Director of Communicable Disease Control, Prevention and Eradication (CDS/CPE), who welcomed the participants and highlighted the role of vector control as an integral part of vector-borne disease management. He noted the need for safe and cost-effective insecticides for vector control and stressed the important role of WHOPES in supporting the Member States in this regard.

Dr L. Savioli, Coordinator, Strategy Development and Monitoring for Parasitic Diseases and Vector Control (CPE/PVC), also welcomed the participants and noted the increasing role of WHOPES, in recent years, in promoting the safe and effective application of pesticides in public health. He also noted the close collaboration of WHOPES with industry and other agencies in promoting appropriate pesticide management in public health.

Dr M. Zaim, Scientist in charge of WHOPES, reviewed the objectives of the meeting and informed the participants of the two products under evaluation – deltamethrin 25% WG (Bayer Environmental Science, Lyon, France – formerly Aventis Environmental Science, Germany) for indoor residual spraying against malaria vectors, and Agnique[®] MMF mosquito larvicide and pupicide (Cognis Corporation, Cincinnati, Ohio). He also noted that earlier recommendations of WHOPES relating to use of deltamethrin WP for indoor residual spraying is for review, in light of the new information which has become available.

Dr Zaim also informed the participants that this is the final activity in the phase III of WHOPES, as it relates to evaluation of deltamethrin WG and Agnique. The final stage, WHOPES phase IV, relates to development of specifications for quality control and international trade. He briefed participants on the joint activity with the Food and Agriculture Organization of the United Nations (FAO) on development of specifications and informed them of the development of the first edition of the Manual

on development and use of FAO and WHO Specifications for Pesticides. He noted that this document supersedes all previous FAO and WHO publications on development and use of specifications. It details the standard process, unified requirements and procedures, harmonized definitions, nomenclature, technical guidelines and standards applicable to pesticides for use in agriculture and public health. FAO/WHO specifications for pesticides are now developed through the FAO/WHO Joint Meeting on Pesticide Specifications (JMPS) and are based on the procedures provided in the Manual.

The meeting was attended by 9 scientists (list of participants, Annex 2). Dr H.L. Lee was appointed as Chairman, and Dr P. Jambulingam, as Rapporteur. The meeting was convened in plenary sessions for comprehensive discussion on aspects relating to public health use of the above-mentioned products and divided in two small working groups to consider the results of the testing and evaluation of each product in detail. The reports of the safety assessments of the International Programme on Chemical Safety, WHOPES supervised trials and relevant published literature, as well as the reports submitted by the national disease and vector control programmes (bibliography, Annex 1) were fully discussed and recommendations on the use of the above-mentioned products were made.

2. REVIEW OF DELTAMETHRIN 25% WG AND WP

2.1 Safety assessment

Deltamethrin [(S)-alpha-cyano-3-phenoxybenzyl (1*R*, 3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclo-propanecarboxylate] is a synthetic pyrethroid insecticide. Deltamethrin is not mobile in the environment. With the current usage pattern and under normal conditions of use, environmental exposure is expected to be low.

Deltamethrin has a high to moderate acute oral toxicity and the International Programme on Chemical Safety (IPCS) has classified it as 'moderately hazardous' (WHO, 2001a). It is a type II pyrethroid; clinical signs of poisoning include tremor, salivation, and convulsion. The human and environmental safety of deltamethrin has been reviewed by WHO (1990). In addition, the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) (WHO, 2001b) assessed toxicity of deltamethrin and the following conclusions were drawn:

When deltamethrin was orally administered to rats, the compound was absorbed and was almost completely eliminated from the body within 2–4 days with very little tissue retention except in fat. Dermal absorption is low. Deltamethrin did not produce any irritant effect on the intact and abraded skin of the rabbit. Transient irritating effects however were produced in the eye of the rabbit, with or without rinsing. Deltamethrin is not a skin sensitizer in the guinea pig.

Deltamethrin is highly toxic to fish and other aquatic invertebrates. However, its toxicity to birds is very low. Deltamethrin is not mutagenic in a variety of *in vivo* and *in vitro* test systems. In a study on mouse, there were no teratogenic or reproductive effects, except for a dose-related decrease in fetal weight. No teratogenic effects were observed in rabbits. In rats deltamethrin did not induce neuropathological changes. Tests with adequate ranges of assays, both *in vitro* and *in vivo* gave no evidence of genotoxicity. Long-term experiments in rats and mice showed no carcinogenic effect and exposure to deltamethrin is unlikely to be a carcinogenic hazard to humans. Deltamethrin may cause transient itching and /or burning sensation in exposed human skin. In non-fatal cases of poisoning, numbness, itching, tingling and burning of the skin and

vertigo are symptoms that are frequently reported. Most of these symptoms are transient and disappear within 5–7 days. No long-term adverse effects were reported.

In a 2-year study in dogs, using a 100-fold safety factor, and on the basis of the NOAEL of 1.0 mg/kg body weight/day, the acceptable daily intake (ADI) for man was established at 0-0.01 mg/kg body weight.

On the basis of the NOAEL of 5 mg/kg body weight in the study of acute neurotoxicity in rats and a safety factor of 100, an acute reference dose has been established at 0.05 mg/kg body weight.

The following are the extracts from the Material Safety Data Sheet (MSDS) of the manufacturer, Bayer (formerly Aventis) for deltamethrin WG 250:

Acute oral LD ₅₀ (rat)	> 3465 mg/kg
Acute dermal LD ₅₀ (rat)	> 2090 mg/kg
Inhalation	Not relevant, large particle size prevents uptake into the lungs
Skin irritation (rabbit)	Slightly and reversible irritating
Eye irritation (rabbit)	Slightly irritating

2.2 Efficacy of deltamethrin WG - WHOPES supervised trials

M'be, Côte d'Ivoire – The efficacy of residual spraying of deltamethrin 25% WG was compared to that of 2.5% WP formulation in experimental huts at the dosage of 20 mg a.i./m² for 6 months against *An. gambiae* & *An. funestus* (Darriet *et al.* 2001).

Before the trial, susceptibility of the local strain of *An. gambiae* to deltamethrin was determined in comparison to a reference susceptible strain (Kisumu). Three to five days old non blood fed females were exposed to impregnated papers for one hour at the diagnostic concentration of 0.05% of deltamethrin using the standard WHO adult bioassay test kits and mortality after 24 h was recorded. During the exposure period, the number of knocked down mosquitoes was checked periodically at 5, 10, 15, 20, 30, 40, 50 and 60 minutes. Times for 95% knock down (TKD₉₅) for susceptible strain and M'be were established by means of log-probit analysis. Twenty mosquitoes were exposed to each test

and 5 replicates were taken. The mortality rate of the two strains was 100%. The TKD₉₅ for *susceptible* strain was 17.3 minutes and for the local population it was 36.7 minutes. Thus, the TKD₉₅ was about 2 times longer for local strain than for the reference strain.

The experimental huts (2.55 x 1.75 x 2.0 m) had single rooms with four entry traps and one veranda trap on the backside. Of the 5 huts, two were allocated randomly for each formulation. One was kept as the control. The formulations were sprayed using hand-operated compression sprayer and let to dry one week before evaluation. Mosquitoes including the dead ones, were collected three times a week from the room and the veranda trap. Their feeding status was recorded and the live mosquitoes were observed for mortality after 24 h.

Bioassay was carried out monthly with susceptible strain of *An. gambiae*, using WHO cones. For each test, and in each hut, four replicates of 15 non-blood fed females, 3–5 days old, were exposed to the surface cones for 30 minutes. The number of mosquitoes that were knocked down at the end of the exposure time and dead after 24 h were recorded.

In the huts sprayed with WP, knock down after 30 minutes was 75% for 5 months following the spray, which decreased to 30% in the 6th month. The mortality rate was above 80% for 5 months, which dropped to 36% in 6th month. A low level of KD (5%) was recorded one week after the treatment, probably due to irritant effect of the insecticide. In the huts sprayed with WG, knock down after 30 minutes was about 80% for 4 months following the spray, which decreased to 60% in 5th month and less than 10% in 6th month. A knock down rate of 47% was recorded one week after the treatment, suggesting lesser irritant effect of the formulation compared to WP, although mortalities caused by the two formulations were comparable. The mortality rate was above 80% for 4 months, which dropped to 70% in 5th month and to 34% in 6th month.

Six mosquito collections made in the huts before spray were comparable ($P = 0.87$). During the six-month study, *An. gambiae* constituted 86% of the total catches (8483) and *An. funestus* 5.5%. The number of *An. gambiae* collected per hut was 2375 in control, 1334 in WG and 1129 in WP sprayed huts. The entry rate of

An. gambiae was reduced by 43.8% in the huts treated with WG and 52.5% with WP. In case of *An. funestus*, the reduction was 60% and 64.9% respectively, indicating a deterrent effect of the formulations. The WP formulation was significantly more deterrent than WG ($X^2 = 36.1$; $P = 0.001$), particularly during the first three months after the treatment, after which the difference was less marked or with no difference.

The induced exophily of *An. gambiae* was increased by 1.6–1.8 times in the treated huts compared to the control, indicating a repellent effect. In the control hut, the exit trap's veranda collection was 31% of the total number collected, while it was 49% and 55% in huts treated with WP and WG. In case of *An. funestus*, the induced exophily was increased by 1.7 times in the huts treated with WP (62%) and WG (63%), compared to the control (36%). Treatment with the two formulations significantly increased the exophilic behaviour of the two species. The induced exophily of *An. gambiae* in the huts treated with WG formulation was greater than that in the huts treated with WP formulation ($X^2 = 19.10$; $P < 0.001$). No such difference was found with *An. funestus* ($P = 0.9$). While the induced exophily of *An. gambiae* was almost unchanged in untreated huts and huts treated with WP formulation throughout the six-month period of observation, the exit rate showed a decline over the six months in the huts treated with the WG formulation.

The blood feeding rate of *An. gambiae* and *An. funestus* was 70-75% in the untreated as well as in the treated huts with the two formulations, indicating that the treatment did not inhibit blood feeding. The mortality rates of *An. gambiae* and *An. funestus* caught in untreated hut were 3% and 2% respectively. The overall mortality rates of *An. gambiae* (11%) and *An. funestus* (23%), caused by the WG formulation treatment were significantly greater than that caused by WP formulation (8% and 13%). ($X^2 = 17.94$; $P = 0.05$; $X^2 = 4.52$; $P = 0.03$). The mortality rate of *An. gambiae* was significantly greater during the first two months, compared to subsequent months. Immediate mortalities did not differ between the formulations.

The two formulations performed similarly, although mortality rates in huts treated with both products were surprisingly low.

WG induced significantly greater exophily and higher mortality rates than WP.

Orissa State, India – Deltamethrin 25% WG was tested against the malaria vectors in the area, *An. fluviatilis* (sibling species S) and *An. culicifacies* (B & C), in comparison with deltamethrin 2.5% WP for the residual effect on different surfaces and to select the field dosage (Jambulingam *et al.* 2001). At the dosage selected, the impact of residual spraying of the formulations on vector behaviours, such as house entry, induced exophily and blood feeding success were studied in modified traditional huts.

The study was conducted in the Malkangiri District of Orissa State, India, endemic for *falciparum* malaria. *Anopheles fluviatilis* is the main vector, abundant from September to February, predominantly endophilic and anthropophilic, and resistant only to HCH. *Anopheles culicifacies*, the secondary vector, is predominantly endophilic, abundant from June to August and resistant to DDT and HCH.

Contact bioassays were carried out on simulated surfaces, such as cement, mud, thatch roof and tiles, mounted on wooden frames using modified bioassay kits developed by the Vector Control Research Centre, Pondicherry. Each formulation was tested at the dosages of 12.5, 25 and 50 mg a.i./m² against wild caught fully fed *An. fluviatilis* and *An. culicifacies*. Three replicates of 10 to 15 females were exposed to the surfaces for one hour (instead of the 30 minutes recommended by WHO) and observed for knock down 1 h post exposure and mortality after 24 h. Controls were exposed to untreated surfaces. Tests were carried out with *An. culicifacies* at weekly intervals until the mortality rate dropped to less than 20% and with *An. fluviatilis*, at monthly intervals for 5 months, beyond which the tests could not be continued due to scarcity of mosquito samples.

The two formulations were effective against *An. fluviatilis* causing $\geq 90\%$ mortality for 1.5–2 months at 12.5 mg a.i./m² and for more than 4 months at 25 mg a.i./m² on the surfaces. At 50 mg a.i./m², the two formulations caused 100% mortality for 4–4.5 months. There was no significant ($P > 0.05$) difference in the effect between the formulations and, surprisingly, between the surfaces (Probit

analysis: 95% confidence intervals for effective duration causing > 90% mortality).

More than 90% mortality of *An. culicifacies* was observed for 4 to 6 weeks, 5 to 8 weeks and 7 to 9 weeks on different surfaces treated at 12.5, 25 and 50 mg a.i./m² respectively. The residual effect did not differ between the dosages ($P > 0.05$). No significant difference was observed between the two formulations or between the surfaces ($P > 0.05$).

Since more than 90% mortality of *An. fluviatilis* was observed for at least 4 months at 25 mg a.i./m² of each formulation (which would be adequate to cover the peak transmission season in the area) 25 mg a.i./m² was selected for the hut scale trial. There was no significant ($P > 0.05$) difference between 25 and 50 mg a.i./m² in their efficacy against *An. culicifacies*.

Nine modified form of traditional huts were built and three huts were randomly allocated for treatment with each formulation and three for control. The study was conducted during October–March 2001, when *An. fluviatilis* was abundant. The huts resembling those of local tribes were made of mud walls and thatched roof with an open veranda in front and eaves permitting mosquitoes to enter into the huts. The huts were fitted with two exit traps and one veranda trap. Deltamethrin formulations were sprayed at 25 mg a.i./m² in the huts, using hand compression sprayer. The sprayable surface area in each hut was about 150 m².

Mosquitoes were collected twice a week in each hut, 8 times prior to treatment and 33 post-treatment. Each collection included alive and dead mosquitoes, in rooms, exit and veranda traps. Mosquitoes collected were identified, classified by abdominal condition and the live ones were held for 24 h to record any delayed mortality.

An. fluviatilis was the predominant species forming about 71% of the total collection (13467). *An. culicifacies* constituted only 1% of the collections, due to the cold season, unfavourable to the species. The numbers of *An. fluviatilis* collected in untreated huts were 2427 and 2215 before and after the treatment respectively. In the huts treated with 25% WG and 2.5% WP, the respective numbers were 1481 and 951 and 1549 and 992. The results

showed that the entry of *An. fluviatilis* was reduced by 30% in huts treated with WP as well as with WG. The reduction in the entry rates was not statistically different between the huts treated with WP and WG ($t = 1.05$; $df = 8$; $P = 0.32$). The induced exophily in WG huts was only 10%. In huts treated with WP no induced exophily was observed. The exit rate was considerably high even in untreated huts, as the volunteers slept near a fire to keep the house warm in the cold season. It is not known, however, what the potential impact the use of fire may have had on the outcome. Overall, the reduction in the proportion of fed females was significantly lower (Odds ratio interaction test, $P < 0.05$) in the treated huts. The reduction in the feeding success was 25.1% and 43.5% in the huts treated with WG and WP respectively, indicating relatively a higher deterrent effect of WP formulation.

The immediate and delayed mortalities were 67.5% and 24.8% in huts treated with WG and 63.7% and 27.4% in huts treated with WP, as compared to 0 and 3.0% in untreated huts respectively. There was no significant difference between the two formulations in their effect on immediate mortality ($X^2 = 3.10$; $P = 0.08$), delayed mortality ($X^2 = 1.7$; $P = 0.19$) or the total mortality ($X^2 = 0.92$; $P = 0.34$). Contact bioassays on the sprayed walls of the huts showed 100% mortality throughout the study period, i.e., for 5 months.

Thus, the WG formulation was comparable with WP in its residual efficacy and killing effect. While WG exhibited a higher repellent effect than WP, blood-feeding reduction was higher with WP.

Spray man had no side effect except for slight headache, that lasted for about 15 minutes. There was no complaint of any side effect from the volunteers who slept in the experimental huts. They observed that deltamethrin formulations had no odour or stain on the sprayed surfaces and they could perceive the benefit of reduction in mosquito nuisance.

Danane, Côte d'Ivoire – The effect of deltamethrin 25% WG on *An. gambiae* was studied in a large-scale study in Danane' forested area in West Africa (Dossou-yovo *et al.* 2002). Malaria transmission in the area was perennial and high (> 300 infective bites /man/year). This was a phase III trial in which evaluation was done in 6 villages, 3 receiving insecticide spray (population: 2570)

and 3 matched villages considered as control to monitor natural change. Allocation for treatment and control was done randomly. Baseline information on vectors was collected during March 1998 to March 1999. Forty-eight man landing catches were made, one every 6 weeks in 3 randomly selected houses of each village. The collections were identified, dissected for parity and infection in salivary glands. Cytogenetic analysis was done from semi-gravid females to identify the *An. gambiae* forms.

Spraying of deltamethrin WG was done at 20 mg a.i./m² using a hand compression sprayer. The first round of spray was done in May 2001 and the second in November 2001, six months after the first.

Man landing collections (72 catches before and 72 after the spray) showed that there was a significant reduction (2 times) in the biting rates, survival rate, life expectancy, vectorial capacity and inoculation rates in the sprayed villages. The inoculation rate was reduced 16 folds. In control 6697 (31 bites /man/night) and 2666 (12.3 bites /man/night) females of *An. gambiae* were collected before and after spraying respectively. In treated villages, 4147 (19.2 bites /man/night) and 878 (4.1 bites /man/night) females of *An. gambiae* were collected before and after spraying respectively. The relative reduction of the average biting rate in the treated villages was 46.3%. The impact observed was mainly after the second round of spraying rather than the first round. The second round of spray enhanced the reduction in biting density.

The parous rate significantly decreased in the sprayed villages from 70.0% to 58.8%, while it significantly increased from 73.5% to 82.2% in untreated villages. The sporozoite rate also decreased from 3.8% to 1.1% in the sprayed villages while it increased from 2.6% to 3.7% in the control villages. The relative reduction was about 80%. The vectorial capacity decreased from 9.67 to 0.60 in the sprayed villages, while it increased from 22.98 to 24.5 in control villages. The relative reduction was about 95%. The entomological inoculation rate was reduced by 88% in the sprayed villages.

Similarly, the average biting rate of *An. funestus* was reduced by 43%. The survival rate showed an increase in treated as well as untreated villages, but the increase was lesser in treated villages. The sporozoite rate decreased (46.5%) in treated villages, while it

remained unchanged in control villages. The vectorial capacity and inoculation rates were 8 and 4 times lower in treated villages compared to untreated villages, respectively.

Standard WHO susceptibility tests with *An. gambiae* (Danane') showed that the mortality rate was 96% with DDT, 91% for permethrin and 95% for deltamethrin. After 6 months of spraying, no change was observed in vector susceptibility status. Analysis of KDT also showed a similar result. Cytogenetic analysis indicated that the *An. gambiae* population in the study area was composed of the molecular form "M" (66.4%) and molecular form "S" (33.6%) and the *Kdr* was observed only in S form. The *Kdr* frequencies were in the range of 0.01–0.11. Analysis of *Kdr* allelic frequencies in control and treated villages, before and after spraying, indicated that the allelic frequency of *Kdr* had not changed 3 months after spraying.

Acceptability of the spraying was assessed by conducting a survey in 2 clusters of 14 families per village. The survey showed that people appreciated the spraying as a method of reducing mosquito biting nuisance and not for reduction of malaria.

In summary, two rounds of spraying with deltamethrin WG reduced the average biting rates of both *An. gambiae* and *An. funestus* by 2 times, inoculation rate by 7 times and vectorial capacity by 6 times in one year.

Kheda, India – Large-scale testing/evaluation of deltamethrin 25% WG was carried out in the Kheda district, Central Gujarat, India, against the major malaria vector, *An. culicifacies* (Yadav *et al.*, 2002). The vector is predominantly endophilic and zoophilic and resistant to DDT, HCH and malathion. The vector breeds in riverbeds and irrigation canals and rice fields. The period of malaria transmission extends from July to November. Infections due to *P. falciparum* and *P. vivax* are prevalent, the latter being the predominant. Though DDT and HCH were used in the past, currently malathion is used selectively in high-risk villages.

The objective of the study was to compare the persistence of deltamethrin 25% WG on common sprayable surfaces and to assess the impact of the spray on resting and biting densities, blood feeding preference, survival rate, sporozoite rate and vector

behaviour (entry rate, induced exophilly, immediate and delayed mortality and engorging rate in the houses). The perceived side-effects of spray men and residents in the sprayed huts were also determined.

Among the high-risk villages in the district (Slide Positivity Rate > 3% and *P. falciparum* > 30% during the last three years) twelve were selected for the trial. The villages were made into matching pairs, based on the baseline data collected during June 2000 – June 2001, and six villages representing each pair were allocated randomly to treatment and control. Most houses in the villages were made of mud wall, or brick-walls with mud or cement plastering. Roofs were made of earthen tiles or straw thatching, or cement concrete houses with thatched or tiled roofs, generally with 30 cm wide eaves. Sibling species A, B and C being sympatric, the vectors A and C formed 44% of the total in control villages and 13% in villages to be sprayed. Susceptibility test of the local population of *An. culicifacies sensu lato* to deltamethrin (0.05%) was done using WHO diagnostic test papers at one-hour exposure and 24 h holding.

Deltamethrin 25% WG was sprayed at 20 mg a.i./m² in July 2001 in the villages allocated for treatment and followed by a second round 3 months later. Scrapings collected from five locations of mud wall and wooden surfaces randomly selected in five houses sprayed and samples of Whatman filter paper exposed to spraying were subjected to residue analysis.

Following standard WHO procedures to determine the residual effect of the insecticide spray, contact bioassays were carried out on mud-wall and wood surfaces on day 1, 15, 30 and then every month till 11 months after spraying. Five replicates of 20 females of *An. culicifacies* sibling species C, 48–72 h old and sugar fed were exposed for 30 minutes and mortality was recorded immediately after exposure, as well as after 24 h.

Of the 12 villages, six were selected for entomological monitoring (3 sprayed and 3 unsprayed). In each of the villages four index rooms were selected and another four were selected randomly. In a dwelling room in each house eaves were covered and two exit traps were fitted. Hand and pyrethrum spray catches, exit trap, light trap and human landing collections in two villages from each group

were made at fortnightly intervals. The mosquitoes were processed for identification of sibling species, trophic status and source of blood meal and mortality post 24 h. Presence of sporozoites in mosquitoes was detected using ELISA.

Susceptibility tests showed 100% knock-down/mortality indicating the species was fully susceptible to the insecticide. About 97% and 95% of the dwelling rooms were sprayed in the two rounds. The residue analysis was carried out on samples collected in 1 day and 90 days post-spray. The dosages determined by residue analysis matched with the target dosage on mud walls and on the first day after spraying. On the wooden surfaces and on day 90 post-spray, variation was noticed.

The results of bioassay indicate that on mud walls, 30 min knock-down remained > 80% for three months and after the second round of spraying it remained > 80% until eight months on mud surface and nine months on wood surface, since the first round of spraying. 24 h kill was 100% through out the 11-month observation.

During the baseline period, the geometric mean densities (indoor resting) were comparable between control and treatment villages. After spraying, the densities significantly declined in villages treated with deltamethrin as compared with control ($H = 4.08$; $df = 1$, $P < 0.05$). The density of other mosquito species also showed similar pattern. The geometric mean of light trap density during the pre-spray period was 0.36 and 0.47 per trap / night in treated and control villages respectively. After spraying, it declined to 0.18/trap/night as compared to no change in control. The survival through one day taking a 2-day gonotrophic cycle was 0.65 during the pre-spray period, which decreased to 0.53 after spraying. In control villages the survival rate was 0.59 and 0.62 during the pre- and post spray periods respectively.

The human blood index declined from 3.7% to 0.84% in the treated villages while there was a slight increase from 11.4% to 12.5% in the control villages ($n = 465-786$). The mean landing rate (88 collections) declined from 0.7/man/night to 0.1/man/night in treated villages while there was little change (4.34 and 4.1/man/night) in control villages. The sporozoite rate estimated from landing catches, light trap and indoor collections was 0.16% in

intervention villages, which became zero after spraying. In control villages, the sporozoite rate was 0.41% during the baseline period and 0.14% during the intervention period. The entomological inoculation rate was 0.104 during the baseline period, which became zero after spraying. In control villages, the inoculation rates were 1.77 and 0.6 during the corresponding periods.

The exit rate of *An. culicifacies* (proportion of mosquitoes collected in exit traps to total entry) in deltamethrin sprayed villages was 5.2% (out of 1435), as compared with 3.4% (out of 3951) in control villages. The difference was statistically significant ($P < 0.02$). The proportion of fed to total entry of *An. culicifacies* in rooms sprayed with deltamethrin was 0.47, compared with 0.39 in control rooms. The difference was statistically significant ($P > 0.01$). Delayed mortality among *An. culicifacies* caught from insecticide sprayed rooms was about 23% in treated as well as control villages.

Interview using a semi-structured questionnaire with 40 householders a month after spray showed 10 of them reported of itching or burning sensation on face for a day or two after spraying. None reported about stains on the sprayed surfaces. Medical examinations, which included clinical, haematological, urological, biochemical, nerve conduction and lung function tests, of five spray men were carried out before and three and five days after spraying. Overall, no side effects were found.

Deltamethrin spray significantly reduced the vector density, survival, sporozoite rate and inoculation rate. However, the effect of spraying lasted for three months. Considering the 6-month period of transmission in the area, two rounds of spraying at an interval of three months was recommended. In villages with perennial transmission, additional focal spraying was recommended.

2.3 Review of deltamethrin WP

Based on the results of earlier trials, deltamethrin WP has been recommended by WHO for indoor residual spraying at the dosage 10–25 mg a.i./m² with an expected residual effect of 2–3 months (Chavasse and Yap, 1997).

Much work has been done subsequently on the residual efficacy of WP formulation in different ecological situations. In view of the

additional/recent information available on the efficacy of WP formulation and its relative efficacy with WG formulation, it is felt necessary that the previous recommendations by WHO for WP formulation be reviewed again and, if needed, appropriate modifications are made.

Therefore, the available literature and reports on the residual efficacy of deltamethrin WP formulations have been reviewed and the results are summarized in Table 1. Deltamethrin WP has been tested for the residual efficacy at the dosage of 12.5, 20, 25, 50, 100 and 125 mg a.i./m² against different vector species and on various commonly available spray surfaces. In one trial, the formulation was evaluated at 10 mg a.i./m² only on plant surfaces.

The results indicated that on the common sprayable surfaces, the effect of WP formulation ranged from 2–9 months at different dosages. The efficacy varied with the type of surface sprayed. The formulation was more effective on bamboo, wood and thatch surfaces than mud and cement plastered walls. The second or subsequent round application of deltamethrin WP to the surfaces, 3–6 months after the initial treatment, substantially increased the period of residual effect.

Comparing the dosages, the effective duration of the formulation was longer at 20 mg a.i./m² (12–36 weeks) and 25 mg a.i./m² (8–21 weeks) than at the lower dosage, 12.5 mg a.i./m². There was, nevertheless, no marked difference between 20 mg a.i./m² and 25 mg a.i./m², except in one trial at 25 mg a.i./m² where > 70% mortality was observed in *Ae. aegypti* up to 8 weeks. Higher dosages, 50, 100 and 125 mg a.i./m² did not show significant difference or sometimes were even less effective.

Table 1. Persistence of residual efficacy (in weeks) of deltamethrin WP on different surfaces

Species (% mortality)	Surface	Deltamethrin WP (in mg a.i./m ²)							Country	Reference
		12.5	20	25	50	100	125			
<i>Ae. aegypti</i> * (>70%)(L)	Mud wall (Mossi type)							8	Bobo Dioulasso, Burkina Faso	Cooseman & Sales 1978
	Mud wall (Bobo type)							9		
	Wood ceiling (Bobo)							21		
	Thatch ceiling (Mossi)							21		
<i>Ae. aegypti</i> * (100%)(L)	Mud wall (Mossi)							9	Bobo Dioulasso, Burkina Faso	After 1 st round
	Mud wall (Bobo)							21		
	Wood ceiling (Bobo)							29		
	Thatch ceiling (Mossi)							29		

Table 1 continued. Persistence of residual efficacy (in weeks) of deltamethrin WP on different surfaces

Species (% mortality)	Surface	Deltamethrin WP (in mg a.i./m ²)						Country	Reference
		12.5	20	25	50	100	125		
<i>Ae. aegypti</i> * (100%)(L)	Mud wall (Mossi)			29				Bobo Dioulasso, Burkina Faso	Cooseman & Sales 1978
	Mud wall (Bobo)			22					
	Wood ceiling (Bobo)			29				After 2 nd round	
	Thatch ceiling (Mossi)			22					
	Mud wall (Mossi)			19	28	10		Bobo Dioulasso, Burkina Faso	
<i>Ae. aegypti</i> * & <i>An. gambiae</i> (90–100%)	Mud wall (Bobo)			12	28	12		Bobo Dioulasso, Burkina Faso	
	Wood ceiling (Bobo)			28	28	28		30 min 3 rd round	
	Thatch ceiling (Mossi)			28	28	28			
				28	28	28			

Table 1 continued. Persistence of residual efficacy (in weeks) of deltamethrin WP on different surfaces

Species (% mortality)	Surface	Deltamethrin WP (in mg a.i./m ²)						Country	Reference
		12.5	20	25	50	100	125		
<i>An. gambiae</i> (>70%) –(F)	Mud wall – 1 st round				12			Kaduna, Nigeria	Rishikesh et al., 1978
	Mud wall – 2 nd round				24				
	(90–100%)								
	Thatch roof- 1 st round		-		12				
<i>An. gambiae</i> (>70%) –(F)	Thatch roof – 2 nd round (90– 100%)				24				
	Mud - 1 st & 2 nd round						12	Kaduna, Nigeria	
	Thatch –1 st & 2 nd round						12		
	Mud - 1 st round				12			Guinea Savanna, Kaduna, Nigeria	Rishikesh et al., 1978
<i>An. gambiae</i> (>70%) –(F)	Mud - 2 nd round				20				
	Thatch – 1 st round				12				
	Thatch – 2 nd round				20				

Table 1 continued. Persistence of residual efficacy (in weeks) of deltamethrin WP on different surfaces

Species (% mortality)	Surface	Deltamethrin WP (in mg a.i./m ²)							Country	Reference
		12.5	20	25	50	100	125			
<i>An. flavirostris</i> (100%)	Bamboo	24						Philippines	1979**	
	Wood	24								
	Coconut leaves	24								
<i>An. albimanus</i> (resistant to pyrethrin)	Cotton	24						Guatemala	1981**	
	Wood	24								
	Cement blocks	20								
<i>An. gambiae</i> (90%)	Mud	16						Congo	1981**	
	Cement	16								
<i>An. stephensi</i> (100%) (L)	Mud				0	5		Pondicherry, India	Das & Kalyanasundaram <i>et al.</i> 1984	
	Cement				0	0				
	Thatch				20	17				
	Mud				3	10				
	Cement				14	19				
<i>Ae. aegypti</i> (100%) (L)	Thatch				20	17				
	Mud				3	18		Pondicherry, India	Das & Kalyanasundaram <i>et al.</i> 1984	
	Cement				3	19				
<i>Cx. quinquefasciatus</i> (100%) (L)	Thatch				20	17				
	Mud				3	18				
<i>An. culicifacies</i> (>80%)	Cement				3	19				
	Mud			10	20	17		Ghaziabad dt. UP, India	Kuldip Singh 1989	
	Cement			11						

Table 1 continued. Persistence of residual efficacy (in weeks) of deltamethrin WP on different surfaces

Species (% mortality)	Surface	Deltamethrin WP (in mg a.i./m ²)							Country	Reference
		12.5	20	25	50	100	125			
<i>An. culicifacies</i> (80–100%) (F)	Mud	10	12	12	12				Ghaziabad dt. UP, India	Ansari <i>et al.</i> , 1997
	Cement	8	12	12	12					
	Brick	8	12	12	12					
	Thatch	12	12	12	12				Jagdalpur, MP., India	Gill <i>et al.</i> 1997
<i>An. culicifacies</i> (>80%)	Mud			15	15				South Mexico	Arredondo <i>et al.</i> 2000
	Stone surface			14	14					
<i>An. albimanus</i> (F) >75%	Cement			18	18					
	Wood			20	20					
	Bamboo			19	19					
	Palm thatch			20	20					
<i>An. culicifacies</i> (100%) (F)	Wooden walls		36						Karnatak, India	Ravi Kumar <i>et al.</i> 2001
	Cement wall		35						Ingwavuma dt., KwaZulu-Natal	Mnzava <i>et al.</i> 2001
<i>An. arabiensis</i> (90–100%) (F)	Mud wall			24					Mpumala-linga Province, South Africa	Govere <i>et al.</i> 2001
<i>An. arabiensis</i> (90–100%)	Mud	24								
	Cement	24								

* *Ae. aegypti* was used in place of *An. gambiae*;

** Report: Deltamethrin in vector control by Agrovet Division of Roussel Pharmaceuticals (India) Ltd. Bombay;
(L) Laboratory strain, (F) Field strain.

2.4 Conclusions and recommendations

1. Deltamethrin, a type II pyrethroid, has a high to moderate acute oral toxicity and has been classified as 'moderately hazardous'. It is biodegradable and not mobile in the environment. It is an irritant to the skin or eye of a rabbit. With guinea pigs no sensitization reaction has been noted. In rats, no mutagenic, teratogenic or reproductive effects have been reported. There is no evidence of genotoxicity. Exposure to deltamethrin is unlikely to be a carcinogenic hazard to humans. Deltamethrin may cause transient itching and/or burning sensations in exposed human skin. No long-term adverse effects have been reported.
2. In India, deltamethrin 25% WG was found effective against *An. fluviatilis*, causing > 90% mortality at least for 4 months on common sprayable surfaces at an application rate of 25 mg a.i./m². The spray in the modified traditional huts reduced the entry rate and feeding success of the vector species. The WG formulation was comparable with WP in its residual efficacy and killing effect.
3. In Côte d'Ivoire, spraying of experimental huts with WG formulation showed a relatively high deterrent effect and induced exophily among *An. gambiae* and *An. funestus*, but with low mortality rates of entering mosquitoes. WG formulation was found better than WP in terms of causing more induced exophily and mortality rates of the two species. Contact bioassays provided more than 95% mortality for 4 months.
4. In large-scale evaluations against *An. culicifacies* in India and *An. gambiae* in Côte d'Ivoire, deltamethrin WG sprayed at 20 mg a.i./m² at intervals of 3 and 6 months respectively, significantly reduced man biting rates, vectorial capacity and sporozoite inoculation rates.
5. In experimental hut studies, no major side-effects were observed among volunteers who slept in deltamethrin-sprayed huts. The village-scale studies have shown that deltamethrin 25% WG spray was acceptable to the community and caused no major side effect on spraymen. Considering its efficacy and reduced operator exposure, as well as the reduced bulk thus

reducing storage and transport, deltamethrin 25% WG is recommended for use for indoor residual spray in malaria vector control programmes. Review of existing information on efficacy of deltamethrin WP and the reports of comparative testing of the two formulations available to the Meeting have shown similar efficacy. Therefore, the two formulations are recommended for indoor residual spray at the dosages of 20 to 25 mg a.i./m², with an expected residual effect of 3–6 months.

3. REVIEW OF AGNIQUE[®] MMF MOSQUITO LARVICIDE AND PUPICIDE

Agnique[®] MMF – POE (2) Isostearyl Alcohol [Poly (oxy-1, 2-ethanediyl), alpha-isoctadecyl-omega-hydroxyl] (Cognis Corporation, Cincinnati, OH, USA) and Arosurf[®] MSF [Poly (oxy-1, 2-ethanediyl), alpha-isoctadecyl-omega-hydroxyl] (Sherex Chemical Company, Inc., Cincinnati, OH, USA.) are chemically identical and are made from renewable plant oils. Technical Arosurf MSF is a clear, viscous, light straw-colored liquid, when diluted with water becomes jellified and clogs spray equipment, making it difficult to treat large areas in short time (Mulla *et al.* 1983). Technical Arosurf MSF, however, was evaluated and used as a mosquito larvicide and pupicide from 1977 to 1994, after that Sherex Chemical Company stopped its production. On investigation, Cognis Corporation found that Arosurf MSF formulation contained significant amounts of polyethylene glycols (PEGs), which resulted in these poor effects when the technical Arosurf MSF was mixed with water. Cognis investigated new manufacturing approaches to remove these PEGs and this resulted in a new patented process, which involved changing the type of catalyst. The refining process gave a more usable end-product, Agnique MMF. Thus, the main difference between Agnique MMF and Arosurf MSF is that Agnique MMF is chemically purer than Arosurf MSF because it has a narrower ethoxylation distribution range of 0 moles to 7 moles of EO (isostearyl alcohol with 7 moles of ethoxylation), as compared to the latter product that has a range from 0 moles to 12 moles of EO (within reasonable detection limits). **Studies on Arosurf MSF have been included in this review and it is believed that the results obtained, using Arosurf MSF against mosquito larvae, would be identical to those obtained using Agnique MMF against mosquito larvae. Both products are chemically identical and both products have identical Material Safety Data Sheets (MSDSs, Arosurf MSF, Sherex Chemical Company, 1984 and Agnique MMF, Cognis Corporation, 2000).** Agnique MMF was evaluated by several Mosquito Control Districts in 1998 in the USA, but scientific data from these evaluations has not been published and therefore it cannot be reviewed here.

3.1 Safety assessment

Arosurf MSF (ISA-2OE) belongs to a group of non-ionic surfactants that have been routinely used in detergents and cosmetics for over 20 years. It is a biodegradable, non-ionic, surface control material that spontaneously and rapidly spreads over the surface of the water to form an ultra-thin film (monomolecular layer) that is about one molecule in thickness. Its mode of action is considered to be physical rather than chemical in which it reduces the water surface tension and thus interferes and disrupts behavior and normal development of mosquitoes. Mosquito larvae and pupae normally use the water surface tension to suspend for long periods when breathing and/or resting, and the presence of the monomolecular layer subsequently kills mosquito larvae and pupae by inhibiting proper orientation at the “on-water” surface and/or by wetting tracheal structures and causing anoxia. The safety, properties, application rates, and mosquito-controlling efficacy of Arosurf MSF (ISA-2OE) are assumed to be comparable with that of Agnique MMF. Both of these products are registered larvicide and pupicide with a currently active EPA (Environmental Protection Agency, USA) label.

Safety of Agnique MMF has been assessed by WHO's Programme on Chemical Safety (PCS) on behalf of WHOPES. It was concluded that Agnique is of low acute toxicity and have some irritation capacity, which is not marked on human skin. Agnique did not exhibit mutagenicity in *Salmonella*, and several non-ionic surfactants with rather closely related alcohol ethoxylate structure have been demonstrated to be devoid of mutagenic activity in a wider range of testing, including mammalian cells *in vivo*. It was noted that no long-term carcinogenicity or reproductive toxicity studies have been performed on Agnique. Studies with closely related alcohol ethoxylates, however, have not demonstrated carcinogenicity or reproductive toxicity. With an exception of *Daphnia magna*, Agnique has been shown to be of low environmental toxicity. The assessment concluded that Agnique is safe for its intended application.

Technical Agnique MMF mosquito larvicide and pupicide is a clear, viscous, light straw-colored liquid that is insoluble in water. It may cause minor eye irritation on prolonged or repeated contact. When used according to label directions for larval and pupal control, it

does not pose a risk to human health, wildlife or the environment. The following information is extracted from the Material Safety Data Sheet (MSDS, 2000) of the manufacturer (Cognis Corporation, Ohio, USA) for Agnique MMF:

Oral LD ₅₀ (rat)	> 20 g/kg
Inhalation LC ₅₀ (rabbit)	> 29 mg/L
Dermal LD ₅₀ (rabbit)	> 2 g/kg
Oral LD ₅₀ (Avian acute, Mallard Duck)	> 2,000 mg/kg
Oral LD ₅₀ (Avian dietary, Bobwhite Quail)	> 5,000 mg/kg
LC ₅₀ (Fish acute, Bluegill)	290 mg/kg
LC ₅₀ (Rainbow trout)	98 mg/kg
LC ₅₀ , Aquatic invertebrates (Daphnia)	1.9 mg/L

Monomolecular films have been used in the United States in floodwaters, brackish waters, and ponds. They have been used along with other mosquito control measures in an integrated pest management (IPM) programme. In addition to low toxicity, human exposure to the product is limited by direct application to mosquito breeding sites such as ditches, ponds, marshes, or flooded areas that are not drinking-water sources.

Several studies have been conducted both in the laboratory and in the field assessing the effects of Arosurf MSF (Arosurf 66-E2, ISA-2OE) on several aquatic non-target organisms. Levy *et al.* (1982c) studied the effect of Arosurf 66-E2 on mosquito fish (*Gambusia affinis*) and aquatic snail (*Gyraulus* sp.) in outdoor concrete tanks at the surface dosage of 0.5 and 1.0 ml/m² that was repeated every 7-10 days to assure continuous film presence. They showed no observable adverse effect on fish and snails exposed continuously to monomolecular films for 105 days. Webber and Cochran (1984) observed no detrimental effects on the non-target species they tested with Arosurf 66-E2 (ISA-2OE) in laboratory aquariums and fish tanks at the rate of 0.68 ml/m². The non-target species were a fresh water tree frog (*Hyla cinerea*), the two fresh-water fishes (*Hypostomus plecostomus* and *Gambusia affinis*), and four salt-water fish species. Hester *et al.* (1991) conducted 96 h acute toxicity tests in the laboratory to determine the effects of a 47 ml/m² (a 100 fold excess of a monomolecular film) application of Arosurf MSF on longnose killifish (*Fundulus similis*), grass shrimp (*Palaemonetes pugio*), freshwater shrimp (*Palaemonetes paludosus*), fiddler crab (*Uca* spp.), crayfish

(*Procambarus* spp.), freshwater amphipod (*Gammarus* spp.), freshwater isopod (*Asellus* spp.), fairy shrimp (*Streptocephalus seali*), snail (*Physa* spp.), polychaete (*Laeonereis culveri*) and an unidentified amphipod. The results from this test showed no acute effect on any life stage of the tested organisms exposed to this concentration of Arosurf MSF up to 96 h post-treatment. None of the organisms tested are dependent on the air-water interface during any stages of their life cycle.

In field situations, Mulla *et al.* (1983) tested Arosurf 66-E2 at the rate of 4.67-7.0 L/ha with no apparent effect on non-target organisms such as mayfly naiads (*Callibaetis pacificus*), diving beetle adults (*Berosus metalliceus*), ostracods and copepods. When Takahashi *et al.* (1984) tested ISA-2OE in field plots at rates of 0.25, 0.5, and 1.0 ml/m², however, they showed acute lethal effects on corixids (*Corisella* spp.), notonectids (*Notonecta unifasciata*), clam shrimp (*Eulimnadia* sp.), and *Tropisternus lateralis* beetle adults. Non-targets that did not exhibit mortality were mayfly naiads (*Callibaetis* spp.), chironomid larvae and copepods. These authors indicated that, with the exception of clam shrimp, the organisms affected by Arosurf were insects that have a ventral plastron that may be affected by the reduction in surface tension. Kenny and Ruber (1993) studied effectiveness of Arosurf MSF at the rate of 4.67 L/ha on microcrustacean populations in the field. No toxic effects were detected in microcrustacean populations belonging to the Copepoda and Cladocera groups. The Copepoda were *Acanthocyclops vernalis* (a mosquito predator and a host to at least 2 mosquito parasites), *Ectocyclops polyspinosus*, *Eucyclops serrulatus*, and 2 species of *Macrocyclus* (*M. albidus*, a known mosquito predator, and *M. fuscus*) and *Orthocyclops modestus*; the Cladocera were *Simocephalus expinosus*, *Scapholeberis* sp., *Ceriodaphnia* sp., and *Bosmina* sp. Karanja *et al.* (1994) tested Arosurf MSF at the rate of 4.0 L/ha applied every 14 days in small field plots against 15 species of non-target organisms with no effect. The most commonly encountered non-target organisms belonged to the families Dytiscidae, Hydrophilidae, Planorbidae, Ampullaridae, Corixidae (*Micronecta* spp.), Notonectidae (*Anisops* spp.), Nepidae, Belostomatidae and Glossiphonidae Ranidae (Hyperoliidae). Hester *et al.* (1989) field evaluated phytotoxic effects of Arosurf MSF on five species of aquatic vegetation--black mangrove (*Avicenna germinan*), saltwort (*Batis maritima*), cordgrass (*Spartina alterniflora*), arrowhead

(*Sagittaria* sp.), and rice (*Oryza sativa*)-- at the rate of 0.94 ml AI/m² in their natural aquatic habitats. Their results showed no significant deleterious effect on these species of aquatic vegetation. Thus, the above laboratory and field data clearly show that fish, invertebrates and aquatic vegetation when exposed to different concentrations of Arosurf MSF are not adversely affected. The fish and invertebrates studied are never in direct contact with surface monomolecular films.

3.2 Efficacy-background/supporting documents

3.2.1 Laboratory trials with Arosurf MSF

There are numerous laboratory studies conducted primarily in the USA on the efficacy of Arosurf 66-E2 (ISA-2OE) against several species of *Aedes*, *Culex* and *Anopheles* mosquitoes (Table 2). Among *Aedes* species, Arosurf 66-E2 was tested against 1st to 4th instar larvae and pupae of *Aedes aegypti* at a surface dosage of 0.25 and/or 0.50 ml/m² (Levy *et al.* 1982c). Larval development was delayed for the 1st and 2nd instar larvae lasting 8–35 days before 100% mortality was observed; 3rd and 4th instar larvae were more sensitive taking 2–14 days for 100% mortality, and pupae were the most sensitive, with 100% mortality occurring within one day post-treatment. Later, Das *et al.* (1986) also bioassayed Arosurf MSF (ISA 2OE) at varying doses (0.11 to 0.56 ml/m²) against 1st–4th instar larvae and pupae of *Ae. aegypti* in small trays with similar results. White and Garrett (1977) bioassayed ISA-2OE against 4th instar larvae of *Aedes taeniorhynchus* at the rate of 0.04 ml/m² and found it to be ineffective with only 5.0 and 25.4% mortality in 24 and 72 h post-treatment, respectively. Later, Levy *et al.* (1981) bioassayed ISA-2OE against 1st–4th instar larvae and pupae of *Ae. taeniorhynchus* at a surface dosage of 0.25 ml/m² and did not obtain a clearly defined relationship between percentage mortality of larvae according to instar at various post-treatment intervals. They observed however 100% mortality of pupae 24 h post-treatment. Levy *et al.* (1986b) compared technical Arosurf MSF and water-base Arosurf MSF at the rate of 0.25 ml/m² against the 1st to 4th instar larvae of *Ae. taeniorhynchus* and showed 47 to 97% mortality of the 4th instar larvae within 24 h post-treatment with water-base formulation compared to 0 to 63% mortality with technical Arosurf MSF. At 2 days post-treatment with technical Arosurf MSF, mortality of the 1st–2nd and 4th instar larval was

delayed as compared with water-base formulation. However, they did not find differences in larval sensitivity between these two formulations against the 3rd instar larvae. Levy and Miller (1987) also tested the effect of water quality (12.5 to 75% artificial sea water) with Arosurf MSF water-base formulations made with well, reverse osmosis filtered, tap, and distilled waters at the rate of 0.25 ml/m² against the 3rd or 4th instar larvae of *Ae. taeniorhynchus*. Their results showed no significant correlation between the water quality of the habitat or formulation.

Among the *Culex* species, ISA-2OE was bioassayed against the 1st–4th instar larvae, eggs and adults of *Culex quinquefasciatus* at the surface dosage of 0.25 ml/m². Even though it was not effective in killing 100% of larvae of this species up to 96 h post-treatment, it killed 100% of the pupae 48 h post-treatment (Levy *et al.* 1982a). No detrimental effect was observed on eclosion when the egg rafts were treated topically with 0.0012 ml ISA-2OE or when the oviposition water was treated with 0.25 ml/m² of ISA-2OE. Significantly higher mortality was observed among gravid females maintained in cages when provided with ovipositing dishes treated with ISA-2OE (Levy *et al.* 1982a). Mulla *et al.* (1983) also tested different concentrations of Arosurf 66-E2 against the 4th instar and pupae of *Cx. quinquefasciatus* and showed that pupae (0.0008 µl/cm²) had greater susceptibility (60–fold) to this material than the 4th instar larvae (0.05 µl/cm²) at the LC₉₀ level. Das *et al.* (1986) bioassayed Arosurf MSF (ISA 2OE) at varying doses (0.11 to 0.56 ml/m²) against the 1st–4th instar larvae and pupae of *Cx. quinquefasciatus* with similar results.

Table 2. Laboratory efficacy of Arosurf MSF (ISA-2OE, Arosurf 66-E2) against mosquito larvae

Species	Dosage	Stage tested ^{1,2}	Reference
<i>Aedes aegypti</i>	0.25 – 0.5 ml/m ²	1 st –4 th L ^b & P ^c	Levy <i>et al.</i> (1982c)
<i>Ae. aegypti</i>	0.11 – 0.56 ml/m ²	1 st –4 th L ^b & P ^c	Das <i>et al.</i> (1986)
<i>Ae. taeniorhynchus</i>	0.04 ml/m ²	4 th L ^a	White & Garrett (1977)
<i>Ae. taeniorhynchus</i>	0.25 ml/m ²	1 st –4 th L ^b & P ^c	Levy <i>et al.</i> (1981)
<i>Ae. taeniorhynchus</i>	0.25 ml/m ²	1st–4 th L ^{b,c}	Levy <i>et al.</i> (1986b)
<i>Ae. taeniorhynchus</i>	0.25 ml/m ²	4 th L ^c	Levy & Miller (1987)
<i>Cx. quinquefasciatus</i>	0.25 ml/m ²	1 st –4 th L ^b , P ^c , ER ^a & A ^b	Levy <i>et al.</i> (1982a)
<i>Cx. quinquefasciatus</i>	0.0008 µl/cm ²	P ^c	Mulla <i>et al.</i> (1983)
	0.05 µl/cm ²	4 th L ^b	
<i>Cx. quinquefasciatus</i>	0.11 – 0.56 ml/m ²	4 th L & P ^c	Das <i>et al.</i> (1986)
<i>Anopheles albimanus</i>	0.25 ml/m ²	1 st –4 th L ^b & P ^c , E ^b	Perich <i>et al.</i> (1987)
<i>An. quadrimaculatus</i>	0.04 ml/m ²	4 th L ^c	White and Garrett (1977)
<i>An. quadrimaculatus</i>	0.25 ml/m ²	1 st –4 th L ^c	Levy <i>et al.</i> (1982b)
<i>An. stephensi</i>	0.11 – 0.56 ml/m ²	4 th L ^b & P ^c	Das <i>et al.</i> (1986)
<i>Ae. aegypti</i>	0.35 – 0.40 g/matrix	1 st –4 th L ^c	Levy <i>et al.</i> (1985)
<i>Cx. quinquefasciatus</i>	0.35 – 0.40 g/matrix	1 st –3 rd L ^c	Levy <i>et al.</i> (1985)
<i>Ae. taeniorhynchus</i>	0.35 – 0.40 g/matrix	1 st –4 th L ^c	Levy <i>et al.</i> (1985)

¹ L = Larval instar (s), P = Pupae; E = Egg eclosion; ER = Egg-rafts hatch; A = Adults.

² Mortality: a = none; b = less than 100% in 72–96 h post-treatment; c = 100% in 24–48 h post-treatment.

Among the *Anopheles* species, Arosurf MSF was bioassayed against *Anopheles albimanus* 1st–4th instar larvae and pupae at the rate of 0.25 ml/m² and proved to be effective against the 4th larval instar (83.3%) and pupal (100%) stages within 72 h post-treatment (Perich *et al.* 1987). They also found that egg eclosion was reduced to approximately 25% with Arosurf MSF when placed in a beaker. Earlier, White and Garrett (1977) bioassayed ISA-2OE against the 4th instar larvae of *An. quadrimaculatus* at the rate of 0.04 ml/m² and showed 99.3% to 100% mortality of larvae 24–72 h post-treatment. Subsequently, Levy *et al.* (1982b) bioassayed the 1st to 4th instar larvae of *An. quadrimaculatus* at the rate of 0.25 ml/m² of ISA-2OE and showed that the 4th instar larvae immediately dropped from their normal horizontal position at the surface to the bottom of the beaker, became moribund and died. Whereas, some 1st to 3rd instar larvae, even after dropping to the bottom, could reattach to the surface and prolonged their survival. Das *et al.* (1986) also bioassayed Arosurf MSF (ISA 2OE) at varying doses (0.11 to 0.56 ml/m²) against the 1st–4th instar larvae and pupae of *An. stephensi* and showed that it was effective against the 4th instar larvae and pupae, the latter were more susceptible than the 4th instar larvae.

Levy *et al.* (1985) used solid slow-release matrices (Sherex 0.5 g multiporous biodegradable matrix; 0.35–0.40 g Arosurf/matrix) that were impregnated with Arosurf MSF to control larvae, pupae, and emerging adults of *Ae. aegypti*, *Cx. quinquefasciatus* and *Ae. taeniorhynchus* in the laboratory. They showed that 100% mortality could be achieved for ca. 4.5 months from a single application.

These bioassay studies show that Arosurf MSF, at different concentrations, has little detrimental effect against the 1st to 3rd instar larvae of most Culicine (*Aedes*, and *Culex*) species and of some Anopheline (*Anopheles*) species, as compared to the 4th instar larvae. The main reason for this response is that the earlier instars of mosquito species can withstand prolonged submersions, making use of dissolved oxygen through the skin (Clements 1992). The 4th instar larvae of most Culicine species are killed within 48 to 96 h but some of Anopheline species are killed within 24 to 48 h. Both Culicine and Anopheline pupae are usually killed within 24 h post-treatment with Arosurf MSF.

3.2.2 Field trials

3.2.2.1 Field trials with Arosurf MSF

Field evaluations of Arosurf MSF are presented in Table 3. Most of the field studies were conducted with technical material but in a few cases a water-based formulation was used. Among the *Aedes* species, Arosurf MSF (ISA 2OE) was evaluated against *Ae. aegypti* in wells at the rate of 11.2 L/ha either as technical or water-based formulation (Das *et al.* 1986). This concentration of Arosurf prevented emergence completely, as well as the breeding of *Ae. aegypti* for nearly 2 weeks. Levy *et al.* (1981) evaluated efficacy of ISA-2OE at varying dosages (0.3–0.45 ml/m²) against natural populations of *Ae. taeniorhynchus* larvae and pupae in several salt-marsh habitats. Results of tests with hand-spray applications showed that high mortality (98-100%) of immature stages could be achieved in certain salt-marsh habitats at dosage of 0.45 ml/m² of surface water. Helicopter application of ISA-2OE at a surface dosage of 0.37 ml/m² also resulted in a 100% mortality of larvae and pupae at 24 h post-treatment. In one hand-spray test, 15–20% of the larvae were identified as *Ae. infirmatus* and they also showed high mortality. Takahashi *et al.* (1984) tested ISA-2OE for controlling mosquitoes at application rates of 0.25, 0.5 and 1.0 ml/m² surface area of experimental plots and found it be effective in reducing the 3rd and 4th instar larvae of *Ae. nigromaculatus* and *Ae. melanimon* populations with an average mortality reduction of 88% and 96% for the three-day test period, respectively. It was also effective in reducing pupae of the above species at all three rates.

Table 3. Field evaluations on efficacy of Arosurf MSF (ISA-20E, Arosurf 66-E2) against mosquito larvae

Species	Dosage	Stage tested ^{1,2}	Reference
<i>Aedes aegypti</i>	1.2 L/ha	Mixed L ^c	Das <i>et al.</i> (1986)
<i>Ae. infirmatus</i>	0.3–0.45 ml/m ²	Mixed L ^c & P ^c	Levy <i>et al.</i> (1981)
<i>Ae. melanimon</i>	0.25–1.0 ml/m ²	Mixed L ^c & P ^c	Takahashi <i>et al.</i> (1984)
<i>Ae. nigromaculatus</i>	0.25–1.0 ml/m ²	Mixed L ^c & P ^c	Takahashi <i>et al.</i> (1984)
<i>Ae. taeniorhynchus</i>	0.37–0.45 ml/m ²	Mixed L ^c & P ^c	Levy <i>et al.</i> (1981)
<i>Culex erraticus</i>	0.3–0.4 ml/m ²	Mixed L ^b & P ^b	Levy <i>et al.</i> (1982b)
<i>Cx. nigripalpus</i>	0.33–0.56 ml/m ²	Mixed L ^c & P ^c	Levy <i>et al.</i> (1980)
<i>Cx. nigripalpus</i>	0.20–0.45 ml/m ²	Mixed L ^c & P ^c	Levy <i>et al.</i> (1982a)
<i>Cx. nigripalpus</i>	0.47–1.87 L/ha	Mixed L ^b	Levy <i>et al.</i> (1985)
<i>Cx. quinquefasciatus</i>	0.33–0.56 ml/m ²	Mixed L ^c & P ^c	Levy <i>et al.</i> (1980)
<i>Cx. quinquefasciatus</i>	0.20–0.45 ml/m ²	Mixed L ^c & P ^c	Levy <i>et al.</i> (1982a)
<i>Cx. quinquefasciatus</i>	0.47–1.87 L/ha	Mixed L ^b	Levy <i>et al.</i> (1985)
<i>Cx. quinquefasciatus</i>	11.2 L/ha	Mixed L ^c	Das <i>et al.</i> (1986)
<i>Cx. quinquefasciatus</i>	0.25–1.0 ml/m ²	Mixed L ^b	Takahashi <i>et al.</i> (1984)
<i>Cx. tarsalis</i>	0.25–1.0 ml/m ²	Mixed L ^b	Takahashi <i>et al.</i> (1984)
<i>Cx. tarsalis</i>	0.46–0.93 ml/m ²	Mixed L ^{b,c}	Mulla <i>et al.</i> (1983)
<i>Cx. peus</i>	0.93 ml/m ²	Mixed L ^c	Mulla <i>et al.</i> (1983)
<i>Psorophora columbiae</i>	0.20–0.45 ml/m ²	Mixed L ^c & P ^c	Levy <i>et al.</i> (1982a)
<i>Ps. ciliata</i>	0.20–0.45 ml/m ²	Mixed L ^c & P ^c	Levy <i>et al.</i> (1982a)

Table 3 continued. Field evaluations on efficacy of Arosurf MSF (ISA-2OE, Arosurf 66-E2) against mosquito larvae

Species	Dosage	Stage tested ^{1,2}	Reference
<i>Coquillettidia perturbans</i>	4.67 L/ha	Mixed L ^{a, b}	Kenny & Ruber (1992)
<i>Anopheles albimanus</i>	4.67 L/ha	Mixed L ^c	Perich <i>et al.</i> (1990)
<i>An. arabiensis</i>	4.0 L/ha/14 days	4 th L ^c & P ^c	Karanja <i>et al.</i> (1994)
<i>An. crucians</i>	0.3–0.4 ml/m ²	Mixed L ^c & P ^c	Levy <i>et al.</i> (1982b)
<i>An. crucians</i>	0.47–1.87 L/ha	Mixed L ^b	Levy <i>et al.</i> (1985)
<i>An. quadrimaculatus</i>	0.04 ml/m ²	4 th L ^c	White & Garrett (1977)
<i>An. quadrimaculatus</i>	0.3–0.4 ml/m ²	Mixed ^c & P ^c	Levy <i>et al.</i> (1982b)
<i>An. quadrimaculatus</i>	0.47–1.87 L/ha	Mixed L ^b	Levy <i>et al.</i> (1985)
<i>An. stephensi</i>	11.2 L/ha	Mixed L ^c	Das <i>et al.</i> (1986)
<i>Ae. taeniorhynchus</i>	5.6–11.2 kg/ha/ pellets	Mixed L ^b	Levy <i>et al.</i> (1985)
<i>Cx. quinquefasciatus</i>	5.6–11.2 kg/ha/ pellets	Mixed L ^b	Levy <i>et al.</i> (1985)
<i>Ps. columbiae</i>	5.6–11.2 kg/ha/ pellets	Mixed L ^b	Levy <i>et al.</i> (1985)
<i>Ps. ciliata</i>	5.6–11.2 kg/ha/ pellets	Mixed L ^b	Levy <i>et al.</i> (1985)

¹ L = Larval instar (s); P = Pupae

² Mortality: a = none; b = less than 100% in 72–96 h post-treatment; c = 100% in 24–48 h post-treatment.

Among the *Culex* species, Levy *et al.* (1980) tested ISA-2OE against mixed populations of *Cx. nigripalpus* and *Cx. quinquefasciatus* larvae in sewage settling, polishing and evapo-percolation ponds at 0.33 to 0.56 ml/m² that resulted in 88.6–98.2% and 96.5–99.6% mortality of both *Culex* spp. larvae and pupae at 24 and 48 h post-treatment, respectively, in all water conditions. Later, Levy *et al.* (1982a) evaluated the efficacy of ISA-2OE for controlling *Cx. nigripalpus*, *Cx. quinquefasciatus*, *Psorophora columbiae* and *P. ciliata* under a wide range of environmental conditions at surface dosage of 0.20–0.45 ml/m² and showed an effective control of larvae and pupae (> 90%) of the *Culex* and *Psorophora* spp. can be achieved by 72 h post-treatment. Levy *et al.* (1982b) observed that *Cx. erraticus* in ponds with *An. quadrimaculatus* larvae were significantly more tolerant to the surface film than larvae of *Anopheles* spp. Mulla *et al.* (1983) tested Arosurf 66-E2 at the rate of 0.46, 0.69 and 0.93 ml/m² against *Cx. tarsalis* larvae in experimental ponds and found 80% to 99% reduction of larvae at 2 and 7 days post-treatment, and slightly less mortality (61% to 93%) at 14 days post-treatment. They also showed that in dairy lagoons a rate of 0.93 ml/m² produced an excellent control (100% mortality) of *Cx. peus* for up to 21 days post-treatment. Takahashi *et al.* (1984) evaluated ISA-2OE for controlling mosquitoes at application rates of 0.25, 0.5 and 1.0 ml/m² and found it to be effective in reducing the 3rd and 4th instar larvae of *Cx. tarsalis* and *Cx. quinquefasciatus* populations with mortalities ranging from 49% to 100% for the 3-day test period at the three rates used. Das *et al.* (1986) evaluated Arosurf MSF (ISA-2OE) against *Cx. quinquefasciatus* in simulated field and natural field conditions at the rate of 11.2 L/ha, either as technical or water-based formulation, and showed that the concentration of Arosurf used prevented emergence completely as well as breeding of the vector mosquitoes in various treated habitats for nearly 2 weeks.

Among the *Anopheles* species, White and Garrett (1977) tested ISA-2OE and other monomolecular films in small experimental pools (4 m² pools) in a woodland environment and in large swimming-pools (24–180 m²) outdoors against the 4th instar larvae of *An. quadrimaculatus* at a rate of 0.04 ml/m². ISA-2OE provided 100% mortality of larvae at 24 h post-treatment, both in small and large pools. Levy *et al.* (1982b) evaluated efficacy of ISA-2OE for controlling mixed populations of *An. quadrimaculatus* and

An. crucians in marshy or swampy ponds with a hand-spray applicator and showed 90–100% mortality of larvae and pupae 48–72 h post-treatment at a surface dosage of 0.3–0.4 ml/m². Das *et al.* (1986) evaluated Arosurf[®] MSF–ISA 2OE against *An. stephensi* in wells at the rate of 11.2 L/ha, either as technical or water-based formulation, and found this concentration prevented emergence completely and breeding of the vector mosquitoes in various treated habitats for nearly 2 weeks. Perich *et al.* (1990) tested Arosurf MSF in the field at 4.67 L/ha against different larval instars of *An. albimanus* and showed that it did not provide 100% reduction of the 1st and 2nd larval instar populations at 24 h post-treatment, although 100% mortality was obtained at 48 h post-treatment.

Karanja *et al.* (1994) conducted a field trial to test the insecticidal action of the monomolecular surface film, Arosurf MSF, applied at the rate 4.0 L/ha/14 days by knapsack sprayers, against larvae and pupae of *An. arabiensis* in a rice irrigation scheme in Western Kenya. Larval and pupal densities and the number of emerging adults were determined by dipping and emergence cages respectively. The proportion of daily mortalities among the 1st to 3rd instar larvae for the treated and control were not significantly different. There were high daily mortalities of the 4th instar larvae, with no adult emergence from treated plots compared to lower daily 4th instar mortalities and continuous adult emergence from untreated control plots, indicating the potential of the monomolecular surface films for control of *An. arabiensis* mosquitoes in rice fields.

Levy *et al.* (1985) used an automatic drip-dispensing system to maintain the concentration of 0.47–1.87 L/ha/24 h Arosurf MSF in ponds to control mixed populations of *An. quadrimaculatus* and *An. crucians*, *Cx. nigripalpus* and *Cx. quinquefasciatus* larvae. They obtained 30–100% control of the 1st–4th instar larvae and pupae over the 30-day test period. Levy *et al.* (1985) also conducted field trials in semi-permanent water habitats against larvae and pupae of *Ae. taeniorhynchus*, *Ps. columbiae*, *Ps. ciliata* or *Cx. nigripalpus* and *Cx. quinquefasciatus* at 5.6–11.2 kg/ha of the 1 g Arosurf MSF impregnated pellets. The trial showed that these floating matrices could initially provide effective surface film coverage on the habitat and partial larvicidal and pupicidal control. These formulations however, were extremely attractive to

non-target organisms, especially fishes and birds, and were rapidly destroyed or removed.

Kenny and Ruber (1992) applied Arosurf MSF to a Massachusetts cattail marsh at 4.67 L/ha to prevent emergence of *Coquillettidia perturbans*. They made one application by helicopter and later in the season three , by fixed-wing aircraft. The material appeared to prevent adult emergence for about a week after the helicopter application, but due to large inter-trap variances in untreated controls the results were not statistically significant. Control was spotty with the fixed-wing application. One problem was obtaining good coverage at a site with difficult aerial access; the second was the interference to delivery of the pupicide as the emergent plant canopy developed later in the season.

These studies show that Arosurf[®] MSF is effective in controlling larvae and pupae of *Aedes*, *Culex*, *Psorophora* and *Anopheles* mosquitoes. In mosquito species whose larvae have little or no surface contact for breathing (*Mansonia* spp., *Coquillettidia* spp., *Culex pilosis*, and *Cx. erraticus*) however, there is a need for properly timed applications at sensitive, surface-contacting stages (pupae to emerging adult) for maximum impact.

Arosurf MSF can be used safely in a wide variety of habitats, such as, fresh and salt-water marshes; pastures; ditches; dairy waste ponds, tree holes, sewage treatment vats and storm sewers. These results also suggest that Arosurf MSF can be used for mosquito larval control in areas where the surface film of this material can be maintained for sufficient time to interfere with adult emergence. The main disadvantages of using Arosurf MSF are: (a) that it is nearly invisible on the water surface and requires frequent testing for its presence by placing a few drops of an indicator oil (Adol[®], Oleyl alcohol indicator, Sherex Chemical Company, OH, USA) on the water surface, and checking for a reaction, a time-consuming process, (b) that it is drawn towards vegetation and floating debris, and (c) that sustained winds tend to make the film pile up in localized areas.

3.2.2.2 Field trials with Agnique MMF against mosquitoes and chironomids

Adugna *et al.* (2000) field evaluated Agnique MMF at the rate of 4.7 L/ha in semi-permanent habitats against larvae/pupae of *Culex pipiens* group, *Cx. tigripes*, *Anopheles arabiensis*, and *An. coustani* and showed that mean mortality of larvae/pupae of *Cx. pipiens* and *Cx. tigripes* at 24 and 48–72 h post-treatment was 60.6 and 72.4–98.2%, respectively. The mortality of larvae/pupae of *An. arabiensis*, *An. coustani*, and *Cx. pipiens* group at 24 and 48–72 h post-treatment was 85.7 and 90.8–100%. In general, 100% mortality of the 3rd and 4th instar larvae of *Anopheles spp.* occurred within 48 h post-treatment and was faster than 75% mortality of the 4th instar larvae of *Culex spp.* within 48 h post-treatment. They also tested Agnique MMF in a pond that had *Cx. pipiens* and *Cx. tigripes* populations and showed that although a mean percent reduction difference between pre- and post-treatment count was statistically significant after 24 h ($p < 0.05$), poor mosquito control in pond tests was recorded both at 24 h post-treatment (49.5%) and 48–72 h post-treatment (38.7–67.7%). Numerous larvae, pupae and adults were observed dead, submerged and floating on the water surface in the treated pond, 72 h post-treatment. A re-application of Agnique MMF after 144 h reduced larval/pupal population with more than 90%. In 7.5 m² enamel trays placed in the field however all instar larvae and pupae of *Anopheles* and *Culex spp.* appeared moribund at 24 h post-treatment and 100% were dead within 48 h post-exposure to 0.18 ml/m² of Agnique MMF (Adugna *et al.* 2000).

Ali (2000) evaluated efficacy of Agnique MMF in suppressing emergence of adult Chironomids in man-made earthen ponds at the rate of 0.23, 0.47 and 0.94 ml/m². Midge adults were collected by using two types of traps: (a) in floating traps: adults were not significantly reduced by the 0.23 ml/m² treatment rate, but at 0.47 and 0.94 ml/m² rate they were significantly reduced (73–93%) for 1–2 weeks post-treatment, (b) in metal-cone traps: the lowest rate significantly reduced adult emergence for only 1-day post-treatment, where at the two higher rates emergence was reduced by 78.6–97% for one-week post-treatment. Using either trap, the highest rate of Agnique MMF did not suppress adult emergence of midges more than the middle rate. These results suggest that Agnique MMF can be used for chironomid control in

areas where the surface film of this material can be maintained for sufficient time to interfere with adult emergence.

3.2.2.3 Arosurf MSF in combination with other mosquito larvicides

Arosurf MSF (ISA-2OE, Arosurf 66-2E) kills mosquito larvae, pupae and emerging adults by physical modification of the water interface of the mosquito habitat (White and Garrett, 1977). It can also be used to entrap and drown ovipositing and resting females, as well as sink and/or inhibit eclosion of floating eggs and egg rafts of certain species (Levy *et al.* 1982a). Pupal mortality above 90% occurred at label rates of 0.187–4.67 L/ha within 24 h post-treatment; however, larvicidal action is usually delayed by 24-72 h post-treatment, and therefore its overall effectiveness is dependent upon the persistence of a monomolecular surface film on the habitat for that length of time. Because control of the 1st to 4th instar larvae is usually slow, environmental and climatological factors such as persistent unidirectional winds of moderate to high velocity, run-off, heavy rain, overflow or drainage can disrupt surface film integrity over most or all of a habitat before effective larvicidal action has been obtained. For these reasons the Arosurf MSF monitoring system employing the use of Adol indicator oil for determining the presence or absence of the surface film on the water surface is necessary. Due to these reasons, Levy *et al.* (1984a) suggested that Arosurf MSF could be blended with other commercial mosquito larvicides to produce more rapid control of mixed stages of immature mosquitoes by either of the formulation components. They suggested that formulation of two products might produce joint action properties and have fewer limiting factors than each component alone, and therefore kill larvae and pupae quickly and persist in the mosquito habitat for a longer period than either component alone. They also suggested that combination formulations could be effective against organophosphorus and organochlorine resistant mosquitoes, while producing no adverse impact upon the environment.

Laboratory trials with Arosurf MSF in combination with other larvicides - Bioassays with water-base blends of Arosurf MSF + Teknar[®], Arosurf MSF + Vectobac[®] and Arosurf + Bactimos[®] against mixed larvae and pupae of *Cx. quinquefasciatus* showed that combination products at application rates, which were at and

below label recommended rates for each product, contributed to significantly better control of mixed developmental stages of mosquitoes than either of the formulation components alone (Levy *et al.* 1984b). In another study Levy *et al.* (1984c) bioassayed Arosurf MSF and diesel or a diesel-isopropyl alcohol formulation and produced 100% kill of *Cx. quinquefasciatus* 4th instar larvae at a lower dosage than recommended for either Arosurf MSF or diesel compounds. They also showed that a water base formulation of Abate 4-E and Arosurf MSF at below label recommendation of each compound produced identical efficacy against the 4th instar larvae and pupae of *Cx. quinquefasciatus*, when compared to formulation components alone. These authors suggested that there would be a significant benefit in formulating Diesel oil or Abate[®] 4-E with Arosurf MSF for control of mixed stages of immature mosquitoes. Arosurf MSF is compatible with the 3 *Bacillus thuringiensis* var. *israelensis* (*Bti*) formulations and Abate 4-E as a water-base suspension, if vigorous agitation is applied when the components are being mixed. Levy *et al.* (1986a) also conducted a laboratory evaluation of Arosurf MSF (0.25 ml/m²) in combination with various concentrations of *Bacillus sphaericus* (BSP-1, Strain 2362) against larvae and pupae of *Cx. quinquefasciatus* and indicated that extremely low application rates of commercial grade *B. sphaericus* (i.e., 0.03–0.26 pt/acre) could be blended with Arosurf MSF (2.43 L/ha) to provide a water-base formulation resulting in 100% mortality of larvae, pupae and/or emerging adults 24 to 48 h post-treatment. Higher rates of *B. sphaericus* in combination with Arosurf MSF were not as effective. Perich *et al.* (1987) determined the efficacy of Arosurf MSF (0.59 L/ha) in combination with three preparations of *B.t.i.* (Teknar, Bactimos and ABG-6193 at three different concentrations) against *An. albimanus* larvae, pupae and eggs. All Arosurf MSF and *B.t.i.* combined formulations produced over 90% mortality of all larvae and pupae, at 48 h post-treatment. Egg eclosion was reduced to approximately 25% with all formulations containing Arosurf MSF.

Field trials with Arosurf MSF in combination with other larvicides - Mulla *et al.* (1983) evaluated Arosurf 66-2E + nonanoic acid (1:1) against mixed larval stages of *Cx. tarsalis* in experimental ponds at rates of 0.23 and 0.46 ml/m² and showed 94% control 2–days post-treatment. They also evaluated Arosurf 66-2E + oleic acid (1:1) against all larval stages of *Cx. peus* in a lagoon at the rate of 4.67 L/ha and obtained 89% to 99% reduction of larvae up to

28 days. Levy *et al.* (1984b) field tested water-base blends of Arosurf MSF + Teknar, Arosurf MSF + Vectobac and Arosurf MSF + Bactimos against mixed larval stages and pupae of *Culex*, *Psorophora* and *Aedes* spp. to determine the spectrum of compatibility and efficacy of Arosurf+ *B.t.i.* blends. Their test results of the combinations at application rates being at or below label recommendation for each product indicated that mixed formulations produced significantly better control of mixed developmental stages of mosquitoes than either of the formulation components alone. Perich *et al.* (1990) tested Arosurf MSF combined with Teknar against naturally occurring populations of *An. albimanus* at the rate of 5.84 L/ha and showed that it provided a 100% reduction of all larval instars at 24 h post-treatment and gave significant ($P < 0.05$) control, when compared with similar untreated areas for at least 10 days post-treatment.

3.3 WHOPES supervised trials using Agnique MMF

Pondicherry, India - Agnique MMF was tested against *Cx. quinquefasciatus* first in two types of larval habitats, namely cesspits/pools (0.25 to 12.0 sq. m.) and open drains (10 x 0.5 m) and then in disused wells (1.3 to 2.63 sq. m) in an urban area (Jambulingam *et al.* 2002). Larval and pupal abundance was monitored prior to treatment in the selected habitats using dipper sampling every two–three days for about two weeks to compare with densities in test areas' post-treatment. Post-treatment immature sampling was carried out at 24 h after treatment and subsequently every 2–3 days in the treated and in untreated habitats. The criteria for the selection of the field dosage of Agnique MMF was based on > 80% reduction in the density of larvae, pupae or the emerging adults at 7 days post-treatment.

In the first series of tests, Agnique MMF was tested at three selected application rates, namely, i.e. 3.5, 7.0 and 10.0 L/ha, using a hand compression sprayer, in cesspits/pools and in drains. The mean percentage reductions in cesspits/pools in the density of larvae, pupae and the adult emergence (using emergence traps) were < 50% for larvae and pupae and 50–100% for adult emergence at 24 h post-treatment. The test showed that for seven days these values varied from 5.2–56.4%, 0–52.5% and 0–100%, respectively (Table 4). Similar results were obtained in drains, where the mean reduction in the density of larvae, pupae and the

adult emergence was < 50%, 12.6–56.8% and 57.2–94.1%, respectively, at 24 h post-treatment; the test lasted for seven days with values varying from 0–63%, 0–67.5% and 0–57%, respectively (Table 4). These values were significantly below the 80% reduction desired at 7 day post-treatment for field application. Therefore, in the second series of tests Agnique MMF was tested in the same two habitats at higher application rates, ranging from 15–100 L/ha to determine the optimum field application rate. The mean percentage reduction in cesspits/pools in the density of larvae, pupae and the adult emergence was 43.8–81.3% for larvae, 35.0–72.3% for pupae and 90.9–100% for adult emergence at 24 h post-treatment; and the test lasted for seven days with values varying from 12.6–78.1%, 0–72.3% and 14.8–100%, respectively (Table 4). Similar results were obtained in drains, where the mean reduction in the density of larvae, pupae and the adult emergence was 0–92.4%, 31.8–84.0% and 67.1–100%, respectively, at 24 h post-treatment; the test continued for seven days with values varied from 0–63.6%, 11.5–84.5% and 27.0–93.7%, respectively. These results indicated that at 30 L/ha and above Agnique MMF was effective in reducing the density of larvae and pupa by about 50–80% and adult emergence by > 80% up to 3–5 days. The difference in the percent reduction for 7 days between the dosages was not statistically significant ($P > 0.05$). Larval, pupal and adult densities returned to pre-treatment levels between 5–12 days.

Agnique MMF was also tested in disused wells at application rates ranging from 10–30 L/ha and the reduction was 23–82% in the larval density, 79%–88% in pupal density and 65% to 100% in the adult emergence at 24 h post-treatment; and for five days these values varied from 0–44%, 0.4–84% and 67–86%, respectively. The difference in the percent reduction for 7 days post-treatment between the dosages, however, was not statistically significant ($P > 0.05$).

In conclusion, the reduction of larval and pupal density and adult emergence was not up to the desired level at different dosages tested in the three types of habitats, which limited the scope of fixing the optimum field dosage.

Table 4. Evaluation of a monomolecular surface film, Agnique MMF, against *Culex quinquefasciatus* in different larval habitats, Pondicherry, India

Habitat	Mosquito stage & dosage used	% reduction 24 h post-treatment	% reduction 7 days post-treatment
Cesspits/pools			
Larvae	3.5–10.0 L/ha	<50	5.2–56.4
	15–100 L/ha	43.8–81.3	12.6–78.1
Pupae	3.5–10 L/ha	<50	0–52.5
	15–100 L/ha	35.0–72.3	0–72.3
Adults	3.5–10 L/ha	50–100	0–100
	15–100 L/ha	90.9–100	14.8–100
Drains			
Larvae	3.5–10 L/ha	<50	0–63
	15–100 L/ha	0–92.4	0–63.6
Pupae	3.5–10 L/ha	12.6–56.8	0–67.5
	15–100 L/ha	31.8–84	11.5–84.5
Adults	3.5–10 L/ha	57.2–94.1	0–57
	15–100 L/ha	67.1–100	27.0–93.7
Disused wells			
Larvae	10–30 L/ha	23–82	0–44*
Pupae	10–30 L/ha	79.2– 88.2	0.4–84*
Adults	10–30 L/ha	65.4–100	67–86*

* % reduction 5 days post-treatment in disused well.

Delhi, India - Agnique MMF was evaluated in small-scale, outdoor cement tanks (1 m³) against laboratory colonized *An. stephensi* (Batra *et al.* 2002). The water level in these tanks was maintained at 1 meter depth. The tanks were covered with emergence traps to record daily emergence. Two thousand newly hatched 1st instar larvae were released once a week in each cement tank to be treated with Agnique MMF as well as in control tanks. Agnique MMF was applied once at six selected dosages (0.05, 0.1, 0.2, 0.4, 0.6 and 1.0 ml/m²) to the tanks when previously released larvae reached 3rd instar. The results indicated (Table 5) that about 9% of the larvae released in control sites emerged as adults in the first two weeks, as compared to 0.6, 0.2 and 0.1% in sites treated with 0.4, 0.6 and 1.0 ml/m² of Agnique MMF, respectively. In the four week period, however, the emergence of adults in control sites was 7.1% as compared to 1.5, 1.2 and 0.6% in the above-mentioned treated sites, respectively.

Table 5. Emergence of adult *An. stephensi* from cement tanks treated with various dosages of Agnique MMF and placed outdoor for 4 weeks post-treatment, Delhi, India

Weeks	Control	No. of emerged adults at various dosages (ml/m ²)					
		0.05	0.1	0.2	0.4	0.6	1.0
1	62	32	16	7	0	0	0
2	299	80	54	23	24	8	5
3	168	84	52	19	34	45	2
4	41	69	53	57	65	46	45
Total emerged	570	265	175	106	123	99	52

Agnique MMF was also evaluated in a medium-scale study, in disused cemented water storage tanks (average 4.65 m², varying from 2.25 to 18 m²) and disused wells (average 1.5 m²) that supported breeding of *An. stephensi*. Four replicates at doses of 0.25, 0.5, 1.0 and 2.0 ml/m² and untreated controls were used and an additional one dose of MLO (Mosquito Larvicidal Oil, Indian Oil Corporation - a larvicide used for malaria control) at 20 ml/m² was used for comparison. Agnique MMF was applied manually with a sprayer in selected dosages in the water storage tanks and wells. Observations on larval/pupal density at different dosages were recorded daily, with 1–4 days post-treatment, and thereafter weekly. Efficacy and residual activity of Agnique MMF was determined from the post-treatment counts of larvae and pupae in treated and control sites as compared to the pre-treatment counts. In tanks, in addition to *An. stephensi*, *An. subpictus* populations (a ratio of 65:35) were also found. The results showed that in tanks treated with 0.25 ml/m², the reduction of the late instar was maximum (77.9%) at 4 days post-treatment. The percent pupal reduction was 88.9% at one-week post-treatment, but 100% at 4 weeks post-treatment. In wells with the same dose however a 100% reduction in pupae was achieved at 24 h post-treatment and remained the same up to one-week post-treatment. Thereafter, no

reduction was noticed up to three weeks post-treatment. In tanks treated with the dosage of 0.5, 1.0 and 2.0 ml/m², the percent reduction of the late 3rd/4th instar larvae was above 75% on 4 days post-treatment, but pupal reduction was 100% with these dosages and that lasted for one or two-weeks post-treatment. In wells treated at the rate of 0.5 ml/m², the percent reduction of late instar larvae started after 24 h and reached 100% within one-week post-treatment, no reduction was observed thereafter. In wells treated with a dosage of 1.0 ml/m², 100% reduction of late instar larvae lasted up to 2 weeks post-treatment, whereas in wells treated with a dose of 2.0 ml/m², the reduction of late instar larvae increased to 100% in 2 weeks post-treatment and remained so up to 3-weeks post-treatment. Reduction in pupae was 100% after 2 days post-treatment with all doses in wells.

In tanks where MLO was applied at 20 ml/m², the reduction in later instar larvae was noticed after 1 day post-treatment and reached 100% on 4 days post-treatment, but declined after 2 weeks post-treatment. Pupal reduction of 100% remained up to 2 weeks post-treatment. In wells with the same dose, however, the effect was noticed up to one week post-treatment, but pupal reduction was 100% up to 5 weeks post-treatment.

Kuala Lumpur, Malaysia – Laboratory studies were conducted in 5-liter and 15-liters earthen jars with 5 liters of water using 30 3rd/4th instar larvae of *Aedes albopictus* at three different dosages (equivalent to 0.47, 2.35 and 4.7 ml/m²) of Agnique MMF (Lee, 2002). The dosages of Agnique MMF were carefully pipetted onto the water surface. Larval/pupal mortality was observed and recorded at intervals of 3 days without disturbing the monolayer film on the water surface. Thirty fresh larvae were added after 5 days post-treatment and no larval food was provided in order not to disturb the water surface.

The results showed that the percent reduction at all three dosages in larval/pupal densities varied in the 5-liter earthen jars from 45.3 to 56.0% at 3 days post-treatment and from 86.0 to 92.7% at 11 days post-treatment (Table 6). A similar trend was observed in the 15-liter earthen jars where the percent reduction at all three dosages varied from 70.7–72.00% at 3 days post-treatment and from 85.0–95.0% at 10 days post-treatment (Table 6). The lowest dosage (0.47 ml/m²) was as effective in reducing larval/pupal

densities as were higher dosages (4.7 ml/m²). Considerably higher dosages (equivalent to 7.05–1441.7 ml/m²) were without significant reduction in larval/pupal densities. It was concluded that under laboratory conditions Agnique MMF, applied at the recommended dosages (0.47 ml/m²), suppressed the larval/pupal densities for 3 days post-application and higher dosages, even though there was increased mortality did not justify the cost.

Table 6. Effects of various dosages of Agnique MMF on *Aedes albopictus* in 5 L and 15 liter ceramic earthen jars post-treatment, Kuala Lumpur, Malaysia. Fresh larvae (30 each jar) were added 5 days after first treatment

Dosage *	% reduction 3 days post-treatment \pm SD	% reduction post-treatment \pm SD **
In 5 liter jars		
4.7 L/ha	56.0 \pm 3.7	86.0 \pm 3.2
23.5 L/ha	45.3 \pm 5.2	88.3 \pm 1.5
47.0 L/ha	52.7 \pm 5.4	92.7 \pm 1.1
Control	8.9 \pm 8.4	32.2 \pm 14.2
In 15 L jars		
4.7 L/ha	70.7 \pm 1.6	85.0 \pm 5.7
23.5 L/ha	72.0 \pm 9.6	95.0 \pm 3.1
47.0 L/ha	70.7 \pm 10.9	92.7 \pm 4.8
Control	5.6 \pm 9.6	23.3 \pm 2.9

* Volumes of equivalent concentrations were added to jars.

** % reduction was 11 days post-treatment in 5 L jars and 10 days post-treatment in 15 L jars.

Field tests were also conducted close to human habitation with high *Ae. aegypti* and *Ae. albopictus* populations using four types of containers (ceramic jars, car tires, drink cans and coconut shells) preferred by *Aedes* mosquitoes. Each container was half-filled with rain-water and sets of 10 containers of each type were placed both under shelter and in open spaces until natural *Aedes* larval populations were established. After that a low (equivalent to 1.8 L/ha) or a high (equivalent to 4.7 or higher) dose of Agnique MMF was applied separately to the test containers. The larval/pupal populations in all containers were monitored regularly with minimal disturbance to the water surface until they returned to the original pre-control levels or in 3 months time. Sampling was conducted by using a long glass pipette. Five dips per container were taken and the total number of larvae/pupae was recorded.

Dead adults were also recorded. Larvae/pupae were sampled randomly from positive control containers placed under shelter and in open places which allowed to emerge for identification. *Ae. albopictus* was the predominant species in open places, and was 7 times more abundant in open places than under shelter. Rainfall was also monitored in open places.

The results showed that when Agnique MMF was applied at a lower dose of 1.8 L/ha to sets of containers, larval/pupal densities were not suppressed in ceramic jars (15 L) and in tires placed under shelter or in tires, drink cans and coconut shells placed in open places. Larval/pupal densities were suppressed by 70.8% in ceramic jars (5 L) and by 50% in drink cans placed under shelter for 3 and 6 days, respectively (Table 7). When Agnique MMF was applied at a higher dose of 4.7 L/ha to sets of containers, however, larval/pupal densities were not suppressed in ceramic jars (5 L and 15 L) and in tires placed under shelter, or in tires, drink cans and coconut shells placed in open places (Table 7). The larval/pupal densities in drink cans placed under shelter showed 70% suppression that lasted for 17 days. In ceramic jars (5 L and 15 L) placed in the open, larval/pupal densities showed 36.7–60% suppression that lasted for only 3 days. When a second application of a high dose of Agnique MMF (a total of 9.4 L/ha) was applied to containers placed in open areas, no suppression of larval densities was observed in ceramic jars (15 L), tires, drink cans and coconut shells; but in ceramic jars (5 L) placed in open areas much higher suppression (50.0–85.7%) of larvae/pupal densities was observed for 14 days. In all treated containers where Agnique MMF showed reduction of larval/pupal densities, however, *Aedes* larvae/pupae were not totally eliminated, as they were always present in these containers.

Table 7. Effects of Agnique MMF on Aedes larval/pupal densities in different types of containers maintained in open and sheltered conditions, Kuala Lumpur, Malaysia

Location & Types of Container	Low dose (1.8 L/ha)			High dose (4.7 L/ha)			Second addition of high dose		
	% reduction	duration days	duration days	% reduction	duration days	duration days	% reduction	duration days	duration days
Under shelter									
Ceramic jar (5 L)	70.8	3	0	0.0	0	0	ND	ND	ND
Ceramic jar (15 L)	0.0	0	0	0.0	0	0	ND	ND	ND
Tire	0.0	0	0	0.0	0	0	ND	ND	ND
Drink can	50.0	6	17	70.0	17	17	ND	ND	ND
In open places									
Ceramic jar (5 L)	63.3	3	3	36.7	3	3	50.0 – 85.7	14	14
Ceramic jar (15 L)	50	3	3	60.0	3	3	0.0	0	0
Tire	0.0	0	0	0.0	0	0	0.0	0	0
Drink can	0.0	0	0	0.0	0	0	0.0	0	0
Coconut shell	0.0	0	0	0.0	0	0	0.0	0	0

3.4 Conclusions and recommendations

1. Agnique MMF is a monomolecular surface film that is chemically similar to Arosurf MSF. The latter product was evaluated and used for mosquito control in the USA for about 20 years. Agnique MMF is purified Arosurf MSF. It is a biodegradable, non-ionic, surface control material that spontaneously spreads over the surface of the water to form an ultra-thin film (monomolecular layer) which is about one molecule in thickness. Its mode of action is physical rather than chemical in that it reduces the water surface tension and subsequently suffocates mosquito larvae and pupae interfering with emergence of the adults. It has been shown to be safe to non-targets including humans.
2. Monolayers in general have less detrimental effect against younger larval stages of most Culicine and some Anopheline mosquitoes. The later larval stages, as well as pupae, are more sensitive to the action of such products.
3. In an urban habitat in India, Agnique MMF was tested against *Culex quinquefasciatus* in cesspits/pools and drains at selected dosages of 3.5 to 10.0 L/ha. At these dosages, the reduction in the density of larvae/pupae was below 50%, 24 h post-treatment. Subsequently, higher dosages from 15-100 L/ha were tested and 30.0 L/ha and above was found to be effective in suppressing the density of larvae/pupae by about 50–80% and adult emergence by > 80% up to 3–5 days. In disused wells, rates of 10–30 L/ha produced similar results as those reported for cesspits/pools and drains. The reduction of larval/pupal density and adult emergence did not reach the desired level of > 80% at different dosages tested in the three types of habitats.
4. Agnique MMF was evaluated in cemented tanks and wells against *Anopheles stephensi* in urban areas in Delhi, India. In tanks, the dosages of 5–20 L/ha were effective in reducing late instar larvae of *An. stephensi* and *An. subpictus* by more than 75% by 4 days post-treatment and 100% of pupae for at least one week post-treatment. In wells at the same dosages, control was slightly better than in tanks.

5. In a study in Malaysia, Agnique MMF was evaluated against container-breeding *Aedes* mosquitoes at rates of 1.8 L/ha and 4.7 L/ha and above in both the laboratory and the field. Under laboratory conditions, 4.7 L/ha and above was able to suppress (45.3 to 56.0%) the larval populations at 3 days post-treatment and the residual effect lasted for up to 10–11 days with 86.0 to 92.7% reduction. In the field it was not effective against *Aedes* larvae in the majority of containers being used.
6. Agnique MMF at rates of 5 to 10 L/ha (0.5 to 1.0 ml/m²) in fresh and brackish water habitats can suppress larvae, pupae and emerging mosquito adults, **where the surface films of this material can be maintained for sufficient time to interfere with their development.** Inconsistent efficacy has been observed in different breeding sites, even at high application dosages. Factors affecting the integrity of the surface film, such as wind, emerging vegetation and debris has detrimental effect on the efficacy of the product. Monitoring of application is only possible by use of an indicator oil.
7. Further improvement in the cost-effectiveness of Agnique MMF and research into combination with other mosquito larvicides, especially where resistance to conventional products are of concern, is recommended.

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