

REPORT OF THE EIGHTH
WHOPES
WORKING GROUP MEETING

WHO/HQ, GENEVA
1-3 DECEMBER 2004

Review of:
NOVALURON 10% EC



WORLD HEALTH ORGANIZATION

COMMUNICABLE DISEASE CONTROL,
PREVENTION AND ERADICATION
WHO PESTICIDE EVALUATION SCHEME

WHO/CDS/WHOPES/2005.10

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

**WHO/HQ, GENEVA
1-3 DECEMBER 2004**

**Review of:
NOVALURON 10% EC**



WORLD HEALTH ORGANIZATION

**COMMUNICABLE DISEASE
CONTROL, PREVENTION AND ERADICATION
WHO PESTICIDE EVALUATION SCHEME (WHOPES)**

© World Health Organization 2005

All rights reserved.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

All reasonable precautions have been taken by WHO to verify the information contained in this publication. However, the published material is being distributed without warranty of any kind, either express or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall the World Health Organization be liable for damages arising from its use.

CONTENTS

	Page
1. Introduction	1
2. Review of novaluron 10% EC	3
2.1 Safety assessment	3
2.2 Efficacy – WHOPES supervised trials	4
2.2.1 Laboratory studies	4
2.2.2 Field studies	10
3. Conclusions and recommendations	31
Annex 1: References	33
Annex 2: List of participants	35

1. INTRODUCTION

The eighth meeting of the WHOPES Working Group, an advisory group to the WHO Pesticide Evaluation Scheme (WHOPES), was convened at WHO headquarters in Geneva, Switzerland, on 1–3 December 2004. The objectives of the meeting were: (i) to review the reports of the testing and evaluation of novaluron 10% EC (emulsifiable concentrate) by Makhteshim Chemical Works Ltd., Israel, and to make recommendations to WHOPES on its use for mosquito larviciding; and (ii) to review and revise WHO guidelines on laboratory and field testing of chemical and bacterial larvicides and insect growth regulators (IGRs) against mosquito larvae.

The meeting was opened by Dr Lorenzo Savioli, Coordinator, Strategy Development and Monitoring for Parasitic Diseases and Vector Control (PVC). In his opening remarks he referred to the recent development of a *Global strategic framework for integrated vector management*¹ by WHO, with the objective to strengthen vector control activities at the global and national levels, optimizing the use of vector control methods and resources and promoting inter- and intrasectoral collaboration and community participation.

Dr Morteza Zaim, Scientist in charge of WHOPES, presented the objectives of the meeting as well as an overview of the Scheme to the participants. Dr Zaim noted that the recommendations of WHOPES are intended to expedite registration of public health pesticides by Member States. He also noted the close collaboration of the Scheme with the WHO Programme on Chemical Safety (PCS) and emphasized that no public health pesticide is considered by the Scheme for field testing until its

¹ *Global strategic framework for integrated vector management*. Geneva, World Health Organization (WHO/CDS/CPE/PVC/2004.10; available at: http://whqlibdoc.who.int/hq/2004/WHO_CDS_CPE_PVC_2004_10.pdf, accessed 23 December 2004).

safety has been assessed by PCS. He also noted that the reports of the WHOPES Working Group Meetings are a consolidation of the available information on pesticides evaluated by the Scheme and other published/unpublished reports and that these are excellent resource for pesticide registration authorities and national control programmes. He emphasized that every effort is made to ensure that the reports are useful and widely available.

The meeting was convened in plenary sessions and working groups at which the reports of the WHOPES supervised trials and relevant published literature (see Annex 1: References) were reviewed and discussed. The meeting was attended by 11 scientists (see Annex 2: List of participants). Dr Mir S. Mulla was appointed as Chairman and Dr Purushothaman Jambulingam as Rapporteur. Recommendations on the use of novaluron 10% EC were made. The Group also reviewed and revised the draft guidelines on laboratory and field testing of chemical and bacterial larvicides and insect growth regulators against mosquito larvae, to be published as a separate document by WHOPES.

2. REVIEW OF NOVALURON 10% EC

2.1 Safety assessment

Novaluron is an IGR of the benzoyl urea family, acting as a chitin synthesis inhibitor. It has a low vapour pressure and is of low water solubility, which is independent of pH. Novaluron is stable to hydrolysis at pH 5 and 7 but is slowly hydrolysed at pH 9 at 25 °C. It undergoes only slow photolysis (pH 5).

Novaluron is of generally low-acute, subacute and chronic toxicity. Positive assessments were obtained in skin and eye irritation and skin sensitization tests of the EC formulation but did not occur with the technical material, indicating that novaluron itself is neither an irritant nor a sensitizer. Tests on carcinogenicity, mutagenicity and teratogenicity were negative. Novaluron does not show signs of reproductive or developmental toxicity.

Novaluron is of low toxicity to birds, fish, earthworms and aquatic plants but is highly toxic to some crustacea. A WHO assessment of the human and eco-toxicity of novaluron is available on the WHOPES home page on the Internet² as part of the development of specifications for the technical material and the EC formulation of novaluron.

The following are the extracts from the material safety data sheet of the manufacturer, Makhteshim, for novaluron 10% EC.

Acute toxicity – oral	LD ₅₀ > 5000 mg/kg (rat)
Acute toxicity – dermal	LD ₅₀ > 2000 mg/kg (rat)
Acute toxicity – inhalation	LC ₅₀ > 5.15 mg/litre (novaluron technical), 4 hours (rat)

² <http://www.who.int/whopes/quality/newspecif/en/>

Skin irritation	Slightly irritating (rabbit)
Eye irritation	Irritating (rabbit)
Sensitization	Guinea-pig maximization test: sensitizer. However, the field-use dilution (0.1% in distilled water) does not cause skin sensitization

2.2 Efficacy – WHOPES supervised trials

2.2.1 Laboratory studies

California, USA

Laboratory bioassays were carried out against second- and fourth-instar larvae of *Culex quinquefasciatus* and *Aedes aegypti* using technical material (Su et al., 2003; Mulla et al., 2003). The methods and procedures used were those of Estrada & Mulla (1986) and Mulla et al. (1986, 1989). In brief, the procedures were as follows: the technical material was dissolved in acetone, diluted further in acetone, and small volumes of proper dilutions added to disposable paper cups containing 100 ml distilled water and 20 larvae of a given instar. Food was given to the larvae on every inspection and water loss replenished. The larvae were exposed at 26–27 °C and 16:8 light/dark period. Assessment of larval mortality was made every other day, counting and removing dead larvae, pupae and adults (partially ecdosed). Several concentrations in the activity range and control were used, each treatment replicated three times and the test run on two or three occasions. Adult emergence was evaluated by counting and removing pupal skins from the cups. Overall inhibition of emergence and stage-related mortality were recorded and subjected to probit regression analysis using POLO-PC software,³ from which inhibition of emergence (IE%) values and their 95% confidence intervals were determined.

³ LeOra Software (1987). POLO-PC: a user's guide to probit or logit analysis. Berkeley, CA.

The activity of technical material against *Cx. quinquefasciatus* and *Ae. aegypti*, expressed as IE₅₀ and IE₉₀, is presented in Table 1. Novaluron showed a high level of activity against *Cx. quinquefasciatus*: the IE₅₀ values were 0.159 µg and 0.118 µg/litre active ingredient for the second- and fourth-instar larvae; the IE₉₀ values were 0.604 µg and 0.595 µg/litre active ingredient, respectively. Both second and fourth instars of *Cx. quinquefasciatus* had somewhat similar levels of susceptibility, at the IE₉₀, to novaluron. Novaluron caused mortality in larvae on ecdysis. Delayed mortality also occurred in pupae and at the adult stage. The higher range of concentrations caused almost 100% of mortality in the larval stage of *Cx. quinquefasciatus*, while in lower sublethal concentrations larval mortality was not complete; the surviving individuals suffered mortality in the pupal and adult stages.

Novaluron also proved highly active against both second- and fourth-instar larvae of *Ae. aegypti*. The IE₅₀ and IE₉₀ values were 0.026 µg and 0.144 µg/litre active ingredient for the second instar, and 0.045 µg and 0.160 µg/litre active ingredient for the fourth instar. *Ae. aegypti* was 3–6 times more susceptible than *Cx. quinquefasciatus*. As in *Cx. quinquefasciatus*, most mortality in *Ae. aegypti* occurred in the larval stage. However, at sublethal doses where there was some survival of larvae the survivors suffered delayed mortality in the pupal and adult stage.

Matale, Sri Lanka

Serial dilutions of novaluron 10% EC were made in acetone⁴ and tested against the larvae of *Anopheles culicifacies* and *An. subpictus* (Yapabandra, 2004). The test units were beakers containing 240 ml distilled water and 20 fourth-instar larvae (reared from wild-caught females). Five concentrations were tested and each treatment and control replicated four times. During the test, larvae were fed Farex[®] baby food on alternate days. Mortality of larvae, pupae and adults was assessed daily

⁴ WHO procedure requires serial dilution of formulated product in water only.

until all individuals died or emerged as adults. The tests were run at room temperature (26 °C). Stage-related mortality at various concentrations was calculated, and the percentage mortality values obtained at each concentration were subjected to probit regression analysis. The IE₅₀ values for *An. culicifaci* and *An. subpictus* were reported at 0.25 mg and 0.39 mg/litre active ingredient, while the IE₉₀ values were 0.54 mg and 1 mg/litre active ingredient, respectively (Table 2). Mortalities in the control were from 5% to 10%.

Tapachula, Mexico

The susceptibility of wild-caught laboratory-reared larvae and pupae of *An. albimanus*, *An. pseudopunctipennis*, *Ae. aegypti*, *An. albopictus* and *Cx. quinquefasciatus* was determined, following standard procedures of Mulla et al. (1974) and WHO (1981). Novaluron 10% EC was serially diluted with ethyl alcohol⁵ and appropriate aliquots were added to 250 ml of water in flat trays for *Anopheles* or 250 ml of water in plastic cups for *Aedes* and *Culex* mosquitoes. In each container, 20 larvae (first or third instars) or pupae were placed. The units were treated with five dosages (in mg/litre of formulation). Each treatment and control was replicated five times and each bioassay repeated five times. The larvae were fed every other day. The bioassays were carried out in an insectary at 26 ± 2 °C, 80 ± 5% relative humidity and photoperiod 12:12 light and dark period.

Assessment was made every 2 days where live and dead larvae, pupae and adults were counted until all test materials died or became adults. Dead specimens were removed after counting. Adult emergence was determined by counting the exuviae; adults partially ecdoded were counted as dead. Mortality readings were corrected by Abbott formula and log-probit analysis carried out (Finney, 1996) for determining LC₅₀ (IE₅₀) and LC₉₉ (IE₉₉). The percentage inhibition of emergence (IE%) was based on the initial numbers of larvae used.

⁵ WHO procedure requires serial dilution of formulated product in water only.

It was noted that the first-instar larvae were more susceptible than the third instar (Table 1). The pupae of *Cx. quinquefasciatus*, *Ae. Aegypti* and *Ae. Albopictus* were slightly more susceptible than the larvae (Table 1), but the pupae of *An. albimanus* and *An. pseudopunctipennis* were much more susceptible than their larvae (Table 2).

Table 1. Activity of novaluron against *Culex* and *Aedes* mosquito larvae in the laboratory

Species	Location	Formulation	Larval instar	Lethal concentration ($\mu\text{g}/\text{litre a.i.}^{\text{a}}$)	
				IE ₅₀	IE ₉₀ or IE ₉₉
<i>Cx. quinquefasciatus</i>	California, USA	Technical	2	0.159	0.604 (IE ₉₀)
			4	0.118	0.595 (IE ₉₀)
<i>Ae. aegypti</i>	California, USA	Technical	2	0.026	0.144 (IE ₉₀)
			4	0.045	0.160 (IE ₉₀)
<i>Cx. quinquefasciatus</i>	Chiapas, Mexico ^b	10% EC	1	9.45	14.5 (IE ₉₉)
			3	1.31	16.1 (IE ₉₉)
			P ^c	9.56	15.9 (IE ₉₉)
<i>Ae. aegypti</i>	Chiapas, Mexico ^b	10% EC	1	15.36	69.5 (IE ₉₉)
			3	25.35	70.8 (IE ₉₉)
			P ^c	8.91	67.2 (IE ₉₉)
<i>Ae. albopictus</i>	Chiapas, Mexico ^b	10% EC	1	19.17	68.4 (IE ₉₉)
			3	34.99	94.4 (IE ₉₉)
			P ^c	2.05	50.0 (IE ₉₉)

^a a.i. = active ingredient.

^b The values for inhibition of emergence (IE) were derived from 10% EC diluted with ethyl alcohol instead of water.

^c P = pupae.

Table 2. Activity of novaluron 10% EC against *Anopheles* mosquitoes in the laboratory

Species	Location	Larval instar	Lethal concentration ($\mu\text{g/litre a.i.}^{\text{a}}$)	
			IE ₅₀	IE ₉₀ or IE ₉₉
<i>An. culicifacies</i>	Sri Lanka ^b	4	25.0	54 (IE ₉₀)
<i>An. subpictus</i>	Sri Lanka ^b	4	39.0	100 (IE ₉₀)
<i>An. albimanus</i>	Mexico ^b	1	18.9	68.6 (IE ₉₉)
		3	31.0	88.8 (IE ₉₉)
		P ^c	2.9	8.6 (IE ₉₉)
<i>An. pseudopunctipennis</i>	Mexico ^b	1	16.5	73.7 (IE ₉₉)
		3	32.6	99.6 (IE ₉₉)
		P ^c	6.6	15.1 (IE ₉₉)

^a a.i. = active ingredient.

^b The values for inhibition of emergence (IE) were derived from 10% EC diluted with acetone or ethyl alcohol instead of water.

^c P = pupae.

2.2.2 Field studies

California, USA

Novaluron 10% EC was tested for its efficacy against natural populations of *Culex* mosquitoes breeding in outdoor microcosms (Su et al., 2003). A total of 16 fibreglass tubs, each with 1 m² surface area and containing 240 litres of reservoir water, were used. Water depth was 30 cm and water level was kept constant by float valves on the water-lines feeding each tub. These tubs were located in an open, sunlit area at the Aquatic and Vector Control Research Facility of the University of California, Riverside, where natural populations of mosquito larvae were developing. In order to have sustainable mosquito production, the tubs were enriched with rabbit-food pellets before flooding (50 g/tub).

Because of the environmental factors such as ultraviolet light and organic pollution, which would degrade novaluron, approximately 2.5, 5 and 10 times the observed laboratory IE₉₀ (equalling 1.25, 2.5 and 5 µg/litre active ingredient, respectively), were used as dosages in this field test.

Novaluron 10% EC was diluted with distilled water to 0.1% active ingredient; appropriate amounts of this stock suspension (0.3, 0.6 and 1.2 ml) were added to each of the assigned tubs, yielding the 1.25, 2.5 and 5 µg/litre active ingredient, respectively. Three treatments and untreated controls were assigned randomly, with four replicates each. Treatments were made 7 days after flooding when late-instars third and fourth larvae were present in large numbers.

Larvae (early and late instars), pupae and exuviae (pupal skins) were sampled by the dipping technique before and 4, 9, 14 and 21 days after treatment to assess the initial and persistent efficacy. In each tub, four dips were taken, one from each of the four corners. During sampling, one dip was taken from each corner of all replicates and the remaining three dips taken

sequentially from the remaining corners. This sampling procedure minimized sampling error because larvae and pupae diving after physical disturbance resurfaced before the next sampling round. Average densities of larvae, pupae and exuviae in various treatments and controls were calculated and compared by one-factor ANOVA with a repeated-measures design.⁶ Other invertebrates were observed qualitatively during sampling to assess the margin of safety of novaluron for non-targets.

Species composition in larval populations was determined by identification of fourth-stage larvae collected from untreated control tubs on each sampling day. Water temperature was determined by submerging a minimum–maximum thermometer in a tub of water that was at the centre of the microcosm arrangement.

It was noted that the average number of early-instar larvae per dip did not provide as good an indication of level of control as the number of late-instar larvae and pupae, as some newly hatched larvae had not been exposed to the material for a long enough period of time. Nevertheless, the population trend of early-instar larvae did decline after the treatments, especially at the higher dosages. On day 4 after treatment, all treated tubs at the three different dosages had significantly lower counts than the control. A dosage-related difference was noted on this sampling day, as the highest dosage (5 ppb) significantly lowered the average counts of early larvae; no difference was indicated between the two lower dosages (1.25 and 2.5 µg/litre active ingredient). On day 9 after treatment, the lowest dosage (1.25 µg/litre active ingredient) lost its efficacy, while the two higher dosages (2.5 and 5 µg/litre active ingredient) still exhibited comparable activity against early larvae.

Compared with the population trends of early larvae, later larvae provided a better indication of control, which clearly showed

⁶ Abacus Concepts, Inc. (1987). *StatView + graphics*. Berkeley, CA.

that novaluron significantly reduced populations at the lowest dosage for up to 9 days and at the two higher dosages for up to 14 days. On day 14, the lowest dosage was no longer effective, but efficacy was still fairly good for the two higher dosages. On days 21 and 27 after treatment, however, no reduction was shown; the population densities of late-stage larvae in the lowest and the middle dosages even surged above the level in untreated controls (Table 3).

Population trends of pupae and collection of pupal exuviae provide accurate assessment of the impact of IGR treatments on immature mosquitoes. Reduction in pupae and exuviae is reflected in reducing or preventing adult emergence, which is the primary goal of larvicidal treatments. On days 4 and 9 after treatment, all treatments were equally effective in inhibiting pupation, while high numbers of pupae were collected from untreated control tubs. On day 14 after treatment, the number of pupae was still high in control tubs. Statistically, the same numbers of pupae were noted in the tubs treated by the lowest dosage and the control tubs. The two higher dosages were still effective in preventing pupation on day 14. On days 21 and 27 after treatment, low numbers of pupae were present in all treatments and controls; no significant differences were noted among various treatment regimens and controls.

No adult emergence was noted on day 0 (before treatment) and day 4 after treatment, as no exuviae were present in dip samples because of the short time of development. On days 9 and 14 after treatment, large numbers of adults emerged from control tubs, while emergence was almost completely inhibited in treated tubs, except that a very few exuviae were noted on day 14 in the tubs treated with the lowest dosage (Table 3). On days 21 and 27, as in pupal counts, low numbers of exuviae were collected in all tubs and there were no significant differences among various treatment regimens and the controls.

As for species composition during the test period, from day 0 to day 9, *Cx. stigmatosoma* and *Cx. quinquefasciatus* were the predominant species; these two species declined from day 14 and remained at low levels to day 27 when the test was concluded.

The efficacy of novaluron 10% EC was further studied against natural populations of *Culex* mosquitoes breeding in mesocosms (Su et al., 2003). A total of 12 bare-ground dirt ponds, each with 27 m² surface area and containing approximately 8100 litres of reservoir water, were used. Water depth was 30 cm and water levels were kept constant by float valves on the water-lines feeding each pond. These ponds were also located in an open, sunlit area at the Aquatic and Vector Control Research Facility of the University of California, Riverside. In order to have sustainable mosquito production, the ponds were enriched with rabbit-food pellets before flooding (2 kg/pond).

In the pond test, in addition to the factors in tubs such as organic pollution and ultraviolet irradiation, edaphic factors were involved. Therefore, somewhat higher dosages of 1, 5 and 10 mg/m² active ingredient (equalling 3.3, 16.5 and 33.3 µg/litre active ingredient respectively), were evaluated.

Novaluron 10% EC was diluted with tap water to 1% active ingredient; appropriate amounts of this stock suspension (2.7, 13.4 and 26.7 ml) were transferred to a 150 ml squeeze plastic bottle and further diluted using tap water to the final volume of 120 ml and applied to the water surface of the ponds, which yielded 3.3, 16.5 and 33.3 µg/litre active ingredient, respectively. Three treatments and an untreated control were assigned randomly, with two replicates each. Treatments were made 8 days after flooding when late instars (third and fourth) were present in large numbers.

As in the tub test, larvae (early and late instars) pupae and exuviae were sampled by the dipping technique before and 3, 7,

13 and 20 days after treatment to assess efficacy. In each pond, one dip was taken from each of four corners. Average densities of larvae, pupae and exuviae in various treatments and control were calculated and compared by one-factor ANOVA with a repeated measures design (see footnote 4). Other invertebrates were observed qualitatively during sampling to assess the safety margin of novaluron for non-targets.

Species composition in larval populations was determined by identification of fourth-stage larvae collected from untreated control ponds on each sampling day.

As in the tub test, the average number of early-instar larvae per dip provided a less sensitive indication of control efficacy. Nevertheless, the population trend of early-instar larvae did show some decline as a result of treatments, especially at the higher dosages. The initial population densities in all assigned treatments and control ponds before treatment were essentially the same. On day 3 after treatment, the treated ponds at two higher dosages (5 and 10 mg/m² active ingredient) had significantly lower counts than the control. No significant reduction in early larvae was noted in the lowest dosage (1 mg/m² active ingredient). On day 7 after treatment, all three dosages (1, 5 and 10 µg/litre active ingredient) exhibited good activity against early larvae, with the highest dosage being more effective than the two lower dosages. On days 13 and 20 after treatment, some differences in densities of early larvae were noted among the three treatments. However, mosquito populations in untreated controls declined significantly as a result of predation.

Compared with the population trends of early-instar larvae, later-stage larvae provided a better indication of control, which clearly showed that novaluron significantly reduced populations at the lowest dosage for up to 3 days and at the two higher dosages for up to 7 days. The three dosages were equally effective on day 3 after treatment. Some dosage-dependent differences were noted on day 7 after treatment, when the lowest

dosage was no longer effective; the two higher dosages yielded equivalent control. On days 13 and 20, populations of late-instar larvae were present at comparable levels among the three treatments. Population reduction in an untreated control was believed to be caused by predation by tadpole shrimp as well as dragonfly nymphs by days 13 and 20 after treatment in the control ponds (Table 3).

Population trends of pupae and pupal exuviae are considered to be sensitive parameters to assess the impact of IGR treatments on immature mosquitoes. Emergence prevention is the primary goal of IGR larvicidal treatments. On days 3 and 7 after treatment, all treatments were equally effective in preventing pupation, while higher numbers of pupae were sampled from untreated control ponds at the same time. On days 13 and 20 after treatment, as in early- and late-stage larvae, numbers of pupae declined in untreated control ponds as a result of assumed predation by tadpole shrimp and dragonfly nymphs. Pupal densities on day 13 in treatments using the two lower dosages had increased to higher levels than that in the control, while in the highest dosage, pupal counts remained low. By day 20 after treatment, however, all treatments had lost their efficacy based on pupal counts.

Exuvial counts were attempted in order to evaluate the effect of this material on adult emergence. No adult emergence was noted on day 0 (before treatment), as no exuviae were present in dip samples because of the short time of development. On days 3 and 7 after treatment, some adults emerged from the control ponds, while emergence was almost completely suppressed or prevented in treated ponds. On day 13, exuviae counts in untreated ponds were low because of assumed predation on late-stage larvae and pupae by tadpole shrimp as well as dragonfly nymphs. Pupal exuviae were present in fairly high numbers in the ponds treated at the lowest dosage, while exuvial counts were still low in middle dosage; no exuviae were sampled at the highest dosage. On day 20 after treatment, the situation was

similar to that on day 13. Pupal exuviae counts in all treatments, however, had surged to high levels.

As to species composition during the test period, from day 0 to day 7, *Cx. stigmatosoma* and *Cx. quinquefasciatus* were the predominant species; these two species declined from day 13 and remained at low levels to day 20 when the test was concluded. At the dosages 1, 5 and 10 mg/m² active ingredient (equalling 3.3, 16.5 and 33.3 µg/litre active ingredient, respectively), novaluron provided excellent control of immature mosquitoes (*Culex* spp.) in mesocosms for up to 14 days in clear to moderately polluted water, as indicated by the observed average numbers of late-instar larvae, pupae and pupal exuviae. The level of control by novaluron at various dosages was comparable with that of the most active IGR, pyriproxyfen (S-31183) and was better than that by methoprene (Mulla et al., 1986, 1989).

Table 3. Efficacy and persistence of novaluron 10% EC against immature *Culex* and *Aedes* mosquitoes as determined in small- and/or medium- to large-scale field studies in various mosquito developmental sites

Country	Test sites	Mosquito species	Dosage active ingredient	Efficacy days (> 90% IE)	
USA	Microcosm (240 litre)	<i>Cx. quinquefasciatus</i>	1.25–5 µg/litre	14	
	Mesocosms (27 m ²)	<i>Cx. quinquefasciatus</i>	1–5 mg/m ² 10 mg/m ²	7 13	
Thailand	Water storage jars (200 litre)	<i>Ae. aegypti</i>	0.05–0.1 mg/litre	175	
			0.50–1 mg/litre	190	
		<i>Ae. aegypti</i>	1–5 µg/litre	40	
			10 µg/litre	68	
Mexico	Plastic drums (75 litre)	<i>Ae. aegypti</i>	1 µg/litre	26	
			5–10 µg/litre	54	
			20 µg/litre	68	
			0.0166–0.0498 mg/litre	112	
India	Pools (300 litre)	<i>Cx. coronator</i>	0.055–0.165 mg/litre	112	
			0.055–0.165 mg/litre	98	
			<i>Ae. albopictus</i>	1–10 mg/m ²	11–13 (>80% IE)
				1–10 mg/m ²	8–17 (>80% IE)
Cesspit (1–15 m ²)	Drains (4–6 m ²)	<i>Cx. quinquefasciatus</i>	1–10 mg/m ²	33–69 (>80% IE)	
			1–10 mg/m ²		
Wells (1.3–4 m ²)		<i>Cx. quinquefasciatus</i>	1–10 mg/m ²		

It was concluded that novaluron at the low dosage of 2.5 and 5 µg/litre of active ingredient provided excellent control of *Culex* larvae for up to 14 days post-treatment in microcosms (Table 3). Almost complete inhibition of adult emergence was achieved at the 1.25, 2.5 and 5 µg/litre of active ingredient, based on exuviae count. It appears that a concentration of 5–25 µg/litre of active ingredient will be needed for *Culex* control, with higher dosages necessary in actual field situations.

In field mesocosms with dirt bottom and sunlit, a dosage of 1, 5 and 10 mg/m² active ingredient (3.3, 16.5 and 33.3 µg/litre active ingredient, respectively) provided excellent control of *Culex* species for 7 days; only the highest dosage (10 mg/m² active ingredient) yielded control for 13 days (Table 3). In order to achieve somewhat long-lasting control, it will be necessary to increase novaluron dosage to 20–50 mg/m² (66.6–166.5 µg/litre active ingredient). However, longevity of control is prolonged at best with tailor-made formulations for specific purposes.

Nonthaburi, Thailand

Initial as well as long-term efficacy of novaluron 10% EC was studied in two experiments in water-storage containers against *Ae. aegypti* larvae for a period of 6 months (Mulla et al., 2003).

The experiments were carried out in glazed (200-litre) earthen water-storage jars and (75-litre) plastic pails, the most commonly used water-storage containers. The evaluation procedures were those used by Mulla et al. (2004). The units were arranged in a block design on a concrete slab covered with a roof. The units were filled with tap water from a domestic water supply. Larval food consisting of ground mouse-food was added to the water in the amount of 1 g/200-litre jar and 0.5 g/75-litre pail. The treatments were challenged with successive cohorts of 25 third-instar larvae/unit before treatment and then on a weekly basis for the duration of the experiment. Larval food was added once a month and water loss was replenished monthly. Each treatment was replicated four times.

After the addition of the first cohort, the jars and pails were treated and covered. Assessment of larval survivorship was made 48 hours after the addition of third-instar larvae by visually counting all live larvae (no pupation at this time) and then assessing adult emergence by counting pupal skins one week later according to the procedures developed in previous studies (Mulla et al., 2004). One week after the addition of larvae, all surviving larvae had pupated and turned into adults or had died as pupae. The resulting pupal skins (reflecting successful emergence) floating on the surface at the meniscus were visible and easily counted by removing them with a syringe and adding them to water in white enamel pans. All the pupal skins were removed by a syringe or a fish net before the next cohort of larvae was added. Pupal skins persisted for about a week (Mulla et al., 2004), reflecting a precise level of adult emergence.

The first experiment was carried out in earthen water-storage jars painted white on the inside for visual counting of live larvae, pupae and pupal skins. Novaluron 10% EC was diluted with distilled water; aliquots of appropriate dilutions were added to obtain the desired concentrations. Each concentration and control was replicated four times.

One day after they were filled with water, each jar was stocked with 25 second- or third-instar larvae of *Ae. aegypti* from a laboratory colony and larval food was added. The jars were then treated with either 0.05, 0.10, 0.5 or 1 mg/litre of the active ingredient (ppm), or an untreated control. The highest concentration (1 mg/litre) was equal to that of temephos used as sand granules in the present *Ae. aegypti* control programme in Thailand. After the treatment, the jars were covered with 5-mm-thick sheets of celocrete.

A total of 26 cohorts of *Ae. aegypti* larvae were added to the treated 200-litre earthen water jars at weekly intervals over a period of 6 months (25 February–26 August 2002). Larval

survivorship was assessed 48 hours post-addition of larvae, while pupal skins were counted 7 days post-addition of larvae, by which time most larvae had pupated and emerged as adults.

In the first 9 cohorts, second-instar larvae were used, while in the remaining cohorts third-instar larvae (easier to handle and count) were used. Little or no difference in the efficacy of novaluron against these two instars was noted. The first cohort showed a high level of larval mortality and IE, but all the concentrations caused a high level of larval mortality (89–99%) and 100% IE in the second cohort.

Larval mortality in cohorts 6, 7, 8 and 9 was high, the final IE in all these cohorts being 100%. Larval mortality and IE in cohorts 11–14 were 100% in all concentrations; similar results were also noted in cohorts 15, 16 and 17. This trend of complete inhibition of emergence was also noted in cohorts 18, 19 and 20, which covered the period up to 137 days post-treatment, as well as in cohort 21 (144 days) where IE was almost 100% in all treatment regimens.

Beyond the 21st cohort, 5 more larval cohorts were added, the last 26th added 183 days post-treatment. In these cohorts, larval mortality declined, indicating a decline in the concentration of novaluron in the water in the jars. However, delayed mortality occurred in all the concentrations, and the final IE was close to 100% in all the concentrations in cohorts 22, 23 (168 days post-treatment) and 24. However, in cohort 25 (175 days post-treatment), it was noted that there was 13–14% emergence at the two low concentrations (0.05 and 0.1 mg/litre), but the inhibition of emergence was still very high (93% and 96%) at the two high concentrations. The IE declined in all concentrations, in cohort 26, 190 days post-treatment. At this time, further challenging of the treatments with new cohorts of larvae was discontinued as it was evident that the two low concentrations had declined in their efficacy both 175 and 190 days after

treatment and that the two highest concentrations also showed a decline at 190 days post-treatment.

From the data, it is evident that novaluron (10% EC) has considerable residual activity at one twentieth to one tenth of the concentration of temephos (Table 3). At the equivalent concentration of 1 mg/litre, it is likely that temephos sand, zeolite granules (Mulla et al., 2004) and novaluron will have similar residual activity for more than 6 months.

After recognizing the long residual activity of the lowest concentration (0.05 mg/litre active ingredient) in the previous experiment in jars, it was realized that in order to find the minimum effective dosage it was necessary to carry out a second experiment using a low range of novaluron concentrations in two types of water-storage containers: 200-litre earthen jars and 75-litre plastic pails. This experiment was started a little more than 2 months after the start of the previous experiment and terminated 90 days post-treatment when the level of the control became lower than 80%.

In the second experiment, both the 200-litre earthen jars and 75-litre plastic pails were employed. Preparation of the units and procedure for testing was the same as in the previous experiment. The concentrations used in the clay jars were 1, 5 and 10 µg/litre active ingredient (ppb) and in the plastic pails 1, 5, 10 and 20 µg/litre active ingredient (ppb). After the addition of third-instar larvae, the units were supplied with larval food as in the previous experiment and covered. Assessment of efficacy was carried out as described above, with larval evaluation 48 hours and emergence or pupal skins 7 days after addition of larvae. As before, pupal skins were counted visually and removed by syringe or fish net before adding a new cohort of larvae. Pupal skins prevailed for one week (Mulla, 2004) and provided a precise count of adult emergence. Additional larval food was given and water loss was replaced monthly.

The data from cohorts 1 and 4 showed complete inhibition of emergence in the three concentrations used in earthen jars. In the plastic pails there was also complete inhibition of emergence for cohort 1 and IE 97–100% in cohort 4 in all four concentrations used. In cohort 5, the inhibition of emergence was 45% at the 1 µg/litre active ingredient in the jars, but the remaining two concentrations for this cohort as well as all three concentrations in jars for cohort 7 yielded 98–100% IE. The lowest concentration in the jars produced 99% inhibition of emergence in cohort 7 (40 days post-treatment). From the profile of larval mortality, it is clear that larval mortality was dose dependent, higher mortality usually occurring at the high dosages as noted in other benzoyl urea IGRs. It also appears that efficacy of the lowest dosage declined faster in the plastic pails with partly screened lids than in the jars with solid covers.

Cohorts 8, 9 and 10, added 47, 54 and 61 days post-treatment respectively, showed that some of the concentrations in both the jars and pails were losing strength. The two high concentrations of 5 and 10 µg/litre in jars yielded 90–100% IE in cohorts 8 (47 days post-treatment) and 9, but the IE declined to 84–89% in cohort 10 (61 days) in the jars. In the pails, the three high concentrations yielded 100% IE in cohorts 8 and 9, but the IE declined precipitously at 5 and 10 µg/litre in cohort 10, while still realizing 100% IE at the highest concentration (20 µg/litre). This clearly showed that efficacy at all dosages except the 20 µg/litre had declined in both containers after 60 days. It is also evident that, as in the previous experiment, larval mortality was dose dependent, high larval mortality occurring at the highest concentration even in cohort 10. As the efficacy at the three lower concentrations declined markedly in cohort 10, three additional cohorts were used to confirm this decline in efficacy. From these studies it was concluded that 10–20 µg/litre active ingredient can provide excellent control of *Ae. aegypti* for at least 2 months.

In the experiment using low concentrations (1–20 µg/litre active ingredient), almost 100% IE was obtained at all dosages up to 40 days post-treatment. However, the lowest dosage (1 µg/litre active ingredient) failed to yield satisfactory control in the jars 47 days post-treatment and in the plastic drums 40 days post-treatment. The dosages 5 and 10 µg/litre active ingredient in the earthen jars provided more than 90% IE for 54 days; declining control reached 84% and 89% 61 days post-treatment (Table 3). In the plastic drums, the duration of efficacy (IE 100%) at 5, 10 and 20 µg/litre active ingredient was for 54 days (Table 3), the two lower dosages declining to 59% and 82% on day 61. The highest dosage (20 µg/litre active ingredient) still produced IE 100% for 68 days (Table 3).

It was concluded that in simulated field studies, *Ae. aegypti* was completely controlled at 10–20 µg/litre active ingredient for about 2 months. Higher concentrations of 50, 100, 500 and 1000 µg/litre active ingredient in earthen jars yield 100% IE for 168 days post-treatment, with the two higher concentration giving a high level of control (approximately 90%) for 190 days.

Galewela and Elahera, Sri Lanka

Novaluron 10% EC was tested for efficacy and residual activity in riverine pools in Galewela and gem pits in Elahera, Sri Lanka, common habitats of *An. culicifacies* and *An. subpictus* (Yapabandra, 2004).

Four experiments were carried out in 36 riverine pools. Each treatment and control was replicated four times. The pools selected were of about similar size and depths. The diameter was 1 m and the depth about 30 cm. In the first three experiments, various dosages were applied (0.005–10 mg/litre active ingredient) to determine the range of activity of novaluron under the test conditions. The treatments were applied using Hudson hand compression sprayer. The effectiveness of the treatments was evaluated by introducing 10 third- and fourth-instar larvae (laboratory-reared from wild-caught

individuals) of *An. culicifacies* into each of 5-litre sentinel buckets floating in the pool. The bucket covered with net had several screened (100-mesh) port holes on the sides 2 cm from the bottom. These screened holes permitted transfer of water and food but kept the larvae in the bucket. The bucket was held in a hole cut out in styrofoam. The bucket handle was tethered to a pole at the side of the river to prevent the buckets from being carried away with flooding water. The larvae were fed with fish-food daily. Emerged adults in the buckets as well as dead larvae and pupae were counted daily until all individuals had died or became adults. In the absence of *An. culicifacies* larvae, *An. subpictus* larvae were used. In addition to the field bioassay, wild anopheline larvae were sampled by dipping five dips per pool every 8 days.

In the fourth experiment, novaluron was applied to riverine pools at the rate of 0.01 mg/litre active ingredient and evaluated by the sentinel bucket method and assessment of wild populations of larvae by dipping technique. This rate of application provided >90% IE for 63 days, declining to 82% IE 89 days after treatment. The dipping method assessing the wild populations of larvae yielded 100% control for 89 days, declining markedly after 103 days (Table 4).

In gem pits, 24 pits, each with an approximate diameter of 3 m and depth 2 m of water, were selected. Novaluron was applied at seven rates (0.001, 0.01, 0.1, 0.25, 1, 2.5 and 5 mg/litre active ingredient) and temephos 50% EC at 1 ppm (active ingredient) using four gem pits per each treatment and control. Assessment of efficacy was made by sentinel buckets, as above, in riverine pools every 8 days up to 156 days. A total of 10 *Anopheles* larvae were introduced into each bucket and observations made until all larvae/pupae had died or became adults. Since the lowest dosage (0.25 mg/litre active ingredient) in this experiment showed long-lasting residual activity, another experiment using 0.001, 0.1 and 1 mg/litre active ingredient novaluron was started using nine anopheline-infested pits. Anopheline larvae

were sampled in the pits by well net (25 cm diameter), where five sweeps were taken per pit.

The gem pits treated with 0.1–5 mg/litre active ingredient showed 100% mortality of sentinel larvae up to 124–132 days (Table 4), while the 0.01 mg/litre active ingredient gave 100% mortality for 116 days. Even the very low rate (0.001 mg/litre active ingredient) gave satisfactory control (90%+) for 56 days (Table 4). Temephos at 1 mg/litre active ingredient provided excellent control for 32–56 days.

In addition, the impact of novaluron on nontarget fauna was studied in 24 riverine pools. Novaluron was applied at 0.01, 0.1, 1 and 2.5 mg/litre active ingredient and temephos at 1 mg/litre active ingredient. The treatments were applied with a Hudson hand compression sprayer. Four pools were used per treatment and control.

Table 4. Efficacy ($\geq 90\%$ emergence inhibition) and persistence of novaluron 10% EC against immature stages of *Anopheles* mosquitoes as determined in small- and/or medium- to large-scale studies in various mosquito developmental sites

Country	Test sites	Mosquito species	Dosage mg/litre a.i. ^a (ppm)	Efficacy days
Sri Lanka	Riverine pools	<i>An. culicifacies</i> and <i>An. subpictus</i>	0.01	63 ^b
	Gem pits		0.001 0.01–1 2.5–5	56 124 132
Mexico	Artificial pools	<i>An. albimanus</i> and <i>An. pseudopunctipennis</i>	0.0166–0.0498	112

^a a.i. = active ingredient.

^b Assessment by the dipping technique, measuring larval abundance, showed 100% control for 89 days, declining markedly after 103 days.

Two organisms were used: guppies *Poecilia reticulata* and a native fish *Rasbora daniconis*. Aluminium cylinders divided into two equal parts with an aluminium partition were used. Several holes (20 x 20 cm) were made in the sides above the bottom and covered with 100-mesh nylon strainer cloth. The drums were inserted into treated and control pools and five fish of each species were introduced separately. The cylinders were covered with netting. Fish were fed daily. Each treatment was replicated four times. Aquatic beetles were studied in sentinel buckets, introducing five individuals into each bucket. Population of the fish and beetles were monitored every 7 days up to 35 days. Both fish species and the aquatic beetles survived for the duration of the experiment for 35 days at all four concentrations (0.01, 0.1, 1 and 2.5 mg/litre active ingredient) without any noticeable ill effects.

It was concluded that novaluron exhibited a high level of activity against *Anopheles* mosquitoes. The dosage of 0.01 mg/litre active ingredient provided control for more than 2 months of *Anopheles* larvae in riverine pools. In gem pits, novaluron was even more effective: at the low rates (0.01 and 0.1 mg/litre active ingredient) it yielded almost 100% control of sentinel *Anopheles* larvae for more than 4 months.

Tapachula, Southern Mexico

The efficacy of novaluron 10% EC against local species of *Anopheles*, *Aedes* and *Culex* mosquitoes was determined in simulated field studies, 12 km south of Tapachula, Chiapas, Southern Mexico (Arredondo-Jiménez, Valdéz-Delgado, 2004).

Three dosages of novaluron (0.166, 0.332 and 0.498 ppm active ingredient), corresponding to 0.5, 1 and 1.5 ml of the 10% EC formulation respectively, were applied to a set of 18 sun-exposed experimental plots, measuring 1 m² and 40 cm deep, where water depth was 25 cm (capacity about 300 l). For comparison, 5% temephos granules (Abate[®], Clark Co., Roselle, IL) were applied at 1 mg/litre active ingredient (6 g of granules per pool).

The pools were filled with water and left for natural colonization by wild mosquitoes for a month. Water level was maintained by adding water when necessary. Larval abundance was measured by dipping, taking 10 dips per pool. In addition to this assessment, two floating sentinel cages each with 25, field-collected, third-instar larvae of *An. albimanus* and *An. pseudo-punctipennis* were placed in each pool. Mortality readings were taken 1, 3 and 7 days later. Cages were replaced when all adults emerged. Efficacy and the residual activity were determined by monitoring natural populations or the mortality of mosquitoes in sentinel cages.

The mosquitoes breeding in the pools were *An. albimanus* and *Cx. coronator*. All three dosages of novaluron (0.166, 0.332 and 0.498 mg/litre of 10% EC) effectively controlled third and fourth instars and pupae for 16 weeks. The duration of effectiveness of novaluron was one month longer than that of temephos. Similarly, larvae of *An. albimanus* and *An. pseudo-punctipennis* in sentinel cages were completely controlled; inhibition of emergence was 100% for 16 weeks, a month longer than that of temephos (Table 4).

In addition to the pools, a set of 25 20-litre plastic buckets was placed in shaded areas under trees and filled with 18 litres of water (40 cm deep). Water level was kept constant by adding water manually. After several weeks, the buckets were colonized by *Aedes* mosquitoes. Three dosages of novaluron 10% EC (0.55, 1.1 and 1.65 mg/litre), corresponding to 0.055, 0.11 and 0.165 mg/litre active ingredient, were applied to the buckets. Temephos 5% granules applied at 1 mg/litre active ingredient or 0.4 g of granules per bucket, were used for comparison. Efficacy and the residual activity were determined by monitoring natural populations or the mortality of mosquitoes in sentinel cages.

In experimental buckets that were naturally colonized by *Ae. albopictus* and *Cx. quinquefasciatus*, all three dosages of novaluron (0.55, 1.1 and 1.65 mg/litre of 10% EC) were

effective for 14 weeks against *Ae. albopictus* and for 10 weeks against *Cx. quinquefasciatus*. There was no significant difference between novaluron and temephos in efficacy and residual activity. High mortality (>85%) was noted in *Ae. aegypti* and *Ae. albopictus* confined in sentinel cages placed in the buckets, in all three rates of novaluron, for 14 weeks. Surviving larvae failed to develop to the adult stage.

The studies in Mexico indicated that novaluron is highly effective against local *Anopheles* in artificial pools at the rate of 0.166 mg/ litre of 10% EC. The duration of efficacy of this dosage and the two higher dosages was 16 weeks. In experimental buckets, all the dosages (0.55, 1.1 and 1.65 mg/litre of 10% EC) yielded control of wild populations of *Ae. albopictus* for 14 weeks and of *Cx. quinquefasciatus* for 10 weeks. Mortality of *Ae. aegypti* and *Ae. albopictus* ranged above 85% in sentinel cages for 14 weeks.

Pondicherry, India

Novaluron 10% EC was tested against *Cx. quinquefasciatus* in three types of larval habitats, namely cesspits, drains and disused wells, at three application rates (1, 5 and 10 mg/m² active ingredient) in an urban area, Cuddalore, 22 km from Pondicherry, India (Jambulingam et al., 2004).

Adult emergence and larval and pupal densities were monitored using emergence traps and dipper sampling, every 2–3 days before treatment for 1–2 weeks, and after treatment until the abundance in the treated habitats reached pre-treatment level. The habitats with comparable densities were assigned to control and treatments. The percentage reduction of larval, pupal densities and inhibition of adult emergence on each day of sampling was calculated to compare the effect of the dosages.

The number of days during which the emergence inhibition or the reduction of densities of pupae/larvae was more than 80% was considered as the duration of effectiveness. The inhibition

of emergence and the reduction of the density of larvae and pupae were below 50% in all the three types of habitats selected and at all the dosages of novaluron tested (1, 5 and 10 mg/m² active ingredient) on the first day post-treatment. More than 80% emergence inhibition or reduction of larval/pupal density could be observed from day 3 post-treatment onwards; the effect lasted for 13 days in cesspits, 17 days in drains and 69 days in disused wells at the high dosage tested (10 mg/m² active ingredient). The overall effect of novaluron on emergence and pupal density was dosage dependent in the three habitats. The duration of effectiveness was relatively longer and more pronounced, as determined by emergence inhibition, due to the cumulative effect, when compared with the density of pupae and larvae. The residual effect of novaluron was longer in disused wells than in drains and cesspits at all the application rates.

Field dosage for each type of habitat was selected based on the observed level of ≥80% adult emergence inhibition or pupal density reduction during the post-treatment period. The effective duration at 5 and 10 mg/m² active ingredient was 1.2 times higher than that at 1 mg/m² and not significantly different ($P < 0.05$); 1 mg/m² was selected as the field application dosage for cesspits. For the stagnant U-shaped drains, 10 mg/m² active ingredient was chosen as the field application dosage, as its effective duration was 2.1 times and 1.7 times greater than at 1 and 5 mg/m² active ingredient. In disused wells, >80% IE was observed for about a month at 1 mg/m². At 5 and 10 mg/m², the effective duration was twice as long as that at 1 mg/m², with no significant difference between the two higher dosages ($P > 0.05$). Therefore, the medium dosage (5 mg/m² active ingredient) was selected as the optimum field application dosage for disused wells. The medium-scale trial results were in agreement with those of small-scale trials.

3. CONCLUSIONS AND RECOMMENDATIONS

Novaluron is an IGR of the benzoyl urea family. Unlike juvenoids, which act only at later stages of development, novaluron acts across early and late developmental stages. It inhibits the synthesis of chitin and hence interferes with moulting. Given its considerable activity against all developmental stages of mosquitoes, novaluron offers the flexibility of timing of application against asynchronous broods of mosquitoes and also facilitates ease of monitoring and surveillance.

Novaluron has low-acute, subacute and chronic toxicity to mammals, with no indication of carcinogenicity, mutagenicity or teratogenicity. It is unlikely to present acute hazard in normal use. Novaluron is also of low toxicity to birds, fish and aquatic plants but is highly toxic to some crustacea and other aquatic invertebrates.

Laboratory studies have indicated that novaluron is highly effective against mosquito larvae and pupae at low dosages (1–5 µg/litre active ingredient). *Ae. aegypti* was found to be more susceptible to novaluron than *Cx. quinquefasciatus*. *Anopheles* species were less susceptible than the other two genera. In one study, high pupicidal effects were noted in *Anopheles* species, a feature that requires further validation.

Field studies in artificial and natural habitats showed that novaluron 10% EC was effective against populations of *Ae. aegypti* (Mexico and Thailand), *Anopheles* species (Mexico and Sri Lanka) and *Culex* species (India, Mexico and USA) at application rates of 10–50 ppb (µg/litre active ingredient). The higher dosages were needed for polluted water and sunlit and open habitats. Studies on the efficacy of this formulation in water-storage containers against *Ae. aegypti* provided long-lasting residual activity for 5–6 months. Similarly, in confined

sources of larvae such as gem pits and disused wells, novaluron at practical dosages provided control for up to 4 months. However, further field studies characterizing the spectrum of activity of novaluron against a range of species under operational use and in a wider variety of habitats are warranted.

Noting the above, the Meeting recommended:

1. The use of novaluron as a mosquito larvicide for application in temporary mosquito habitats, polluted waters and non-drinking water-storage containers at the target dose of 10–50 µg/litre active ingredient or 10–100 g/ha active ingredient. Within this range, the higher dosages are needed in polluted and vegetated habitats and for obtaining longer residual effects.
2. Short- and long-term ecological assessment of field use of novaluron in semi-permanent and permanent bodies of water should be conducted before its use in such habitats can be recommended.
3. Although the 10% EC formulation of novaluron has been found to show a high level of activity against larvae of various groups of mosquitoes, there is a critical need for the development and evaluation of other formulations (e.g. granules and tablets) operationally suitable for use in specific larval habitats such as containers and other confined sources of larvae.
4. WHO should conduct an assessment of the safety of novaluron for use in drinking-water as a mosquito larvicide.

ANNEX 1: REFERENCES

Arredondo-Jimenez JI, Valdéz-Delgado KM (2004). *Effect of novaluron (Rimon® 10 EC) on various mosquito species (Anopheles albimanus, An. pseudopunctipennis, Aedes aegypti, Ae. albopictus and Culex quinquefasciatus) from Chiapas, México* (unpublished report to the WHO Pesticide Evaluation Scheme).

Estrada JG, Mulla MS (1986). Evaluation of two new insect growth regulators against mosquitoes in the laboratory. *Journal of the American Mosquito Control Association*, 2:57–60.

Jambulingam P, Sadanandane C, Nithyanandam N (2004). *Small and medium scale field evaluation of an IGR, novaluron 10% EC, against Culex quinquefasciatus in India* (unpublished report to the WHO Pesticide Evaluation Scheme).

Mulla MS et al. (1986). Evaluation of new insect growth regulators against mosquitoes with notes on nontarget organisms. *Journal of the American Mosquito Control Association*, 2:314–320.

Mulla MS, Darwazeh HA, Schreiber E (1989). Impact of new insect growth regulators and their formulation on mosquito larvae development in impoundment and floodwater habitats. *Journal of the American Mosquito Control Association*, 5:15–20.

Mulla MS, Darwazeh HA, Norland RL (1974). Insect growth regulators: evaluation procedures and activity against mosquitoes. *Mosquito News*, 10:329–332.

Mulla MS et al. (2003). Laboratory and field evaluation of novaluron, a new acylurea insect growth regulator against *Aedes aegypti* (Diptera: Culicidae). *Bulletin of the Society of Vector Ecology*, 28:241–254.

Su TY et al. (2003). Laboratory and field evaluation of novaluron, a new insect growth regulator (IGR) against *Culex* mosquitoes. *Journal of the American Mosquito Control Association*, 19:408–418.

WHO (1981). *Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate and carbamate insecticides: establishment of the baseline*. Geneva, World Health Organization (WHO/VBC/81.805).

Yapabandra M (2004). *Determination of optimum dosage, efficacy and persistence of novaluron EC in different mosquito breeding sites* (unpublished report to the WHO Pesticide Evaluation Scheme).

ANNEX 2: LIST OF PARTICIPANTS

Dr M.K. Cham, Roll Back Malaria Department, World Health Organization, Geneva, Switzerland.

Dr P. Guillet, WHO Regional Office for Africa, Harare, Zimbabwe.

Dr P. Jambulingam, Vector Control Research Centre, Pondicherry, India.

Dr H. Ladonni, Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran.

Dr C. Lagneau, Entente Interdépartementale pour la Démoustication du littoral Méditerranée, Montpellier, France.

Dr L. Manga, WHO Regional Office for Africa, Harare, Zimbabwe.

Dr M.S. Mulla, Department of Entomology, University of California, Riverside, CA, USA.

Dr M. Nathan, Strategy Development and Monitoring for Parasitic Diseases and Vector Control, Communicable Disease Control, Prevention and Eradication, World Health Organization, Geneva, Switzerland.

Dr L. Savioli, Strategy Development and Monitoring for Parasitic Diseases and Vector Control, Communicable Disease Control, Prevention and Eradication, World Health Organization, Geneva, Switzerland.

Dr E. Walker, Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI, USA.

Dr M. Zaim, WHO Pesticide Evaluation Scheme (WHOPES),
Strategy Development and Monitoring for Parasitic Diseases
and Vector Control, Communicable Disease Control, Prevention
and Eradication, World Health Organization, Geneva,
Switzerland.



WORLD HEALTH ORGANIZATION

COMMUNICABLE DISEASE CONTROL,
PREVENTION AND ERADICATION
WHO PESTICIDE EVALUATION SCHEME