SMALL SCALE FIELD TESTS (PHASE 2) AND LABORATORY TESTS (PHASE 1) WITH OMS–2014 (VETRAZIN),* AN INSECT GROWTH REGULATOR, AGAINST *CULEX QUINQUEFASCIATUS* AND *AEDES AEGYPTI* IN JAKARTA, INDONESIA

M. Soekirno **, St. Aminah**, and H.L. Mathis ***

ABSTRAK

Telah dilakukan uji laboratorium dan uji lapangan dalam skala kecil dengan vetrazin (OMS–2014) terhadap larva *Culex quinquefasciatus* dan larva *Aedes aegypti* di Jakarta. Hasil uji laboratorium menunjukkan bahwa larva *Cx. quinquefasciatus* lebih rentan terhadap OMS–2014 dibanding dengan larva *Ae. aegypti*. LC_{50} untuk larva *Cx. quinquefasciatus* adalah 0,13 mg/l, sedangkan untuk larva *Ae. aegypti* adalah 0,48 mg/l. Uji laboratorium menunjukkan pula bahwa beberapa larva dapat menjadi pupa, tetapi kebanyakan mati dalam bentuk pupa. Di samping itu terdapat pengaruh terhadap terjadinya pupa, sehingga kematian yang terbanyak terjadi pada stadium larva. Hasil uji lapangan menunjukkan bahwa OMS–2014 lebih efektif terhadap larva *Ae. aegypti* daripada larva *Cx. quinquefasciatus*. Hasil uji lapangan dalam skala kecil dengan OMS– 2014 terhadap larva *Ae. aegypti* efektivitasnya sama dengan hasil uji dengan methoprene dan sedikit lebih pendek daripada diflubenzuron. Untuk memberantas larva *Cx. quinquefasciatus* dalam selokan yang airnya tercemar, efektivitasnya sama seperti dengan methoprene dan diflubenzuron.

INTRODUCTION

Environmental contamination is continuing to be an increasing factor in control of vector borne diseases. Except in emergency situations when insecticide has to be used, environmental management is the desired method for vector control. However, more often than not, this is not possible or practical and consequently, insecticides usually become the main tool. Moreover environmentalists admit that insecticides are necessary, but advocate compounds with nil or very short residual effect, and compounds with a narrow killing spectrum. The control specialist uses compounds with a long residual life for more efficient, practical and economical operations. As far as practicable he also uses compounds which affect the target and little else, but few with compounds exist for most tasks.

With the advent of Juvenile Hormones for insect control, a new group of insecticidal compounds came into being. These were synthesized, and the group came to be known as Juvenile Hormone Mimics in the eraly 1970s. Still later, compunds different from the insect's hormones were found to affect the growth physiology of the immatures, and today this broad group is most commonly referred to as Insect

* Vetrazin (CGA–72662), supplied by CIBA–GEIGY

** Biologist, National Institute of Health Research and Development, Jakarta.

*** Entomologist, WHO, Vector Biology and Control Research Unit, Jakarta.

Growth Regulators (IGR).

The insecticidal action of the IGRs meets some of the requirements of the environmentalist as well as being of value to the control specialist. These compounds often have a residual life of several weeks, but they must be ingested to be effective; there is no "contact" effect, thus less environmental effect. Most important to the environmentalist is that IGRs tend to be highly specific and normally have little or no effect on nontarget such as fish and birds. Most important to the control specialist is the effective kill IGRs often give to some insects with high levels of multiple resistance to the more classical insecticides.

The IGR reported here was given the code number of OMS–2014 by the World Health Organization and is one of the newer compounds. Results from laboratory and small scale field tests are reported herein.

LABORATORY SUSCEPTIBILITY TESTS, PHASE 1 OF WHOPES*

Methods. Eggs from colony of *Aedes aegypti* were hatched, and the following day 25 larvae were put into each of several beakers with 250 ml of water. During each series of tests three replicate beakers were used for each dosage rate tested, and three were left untreated.

The 96% technical OMS–2014 was diluted in ethanol to convenient concentrations, and from 0.25 to 1.0 ml of the diluted material was added to the beakers to give desired final dosage rate.

A small pinch of ground dog biscuit was added to each beaker every second day. Daily counts were made and recorded of larval mortality, pupation, pupal mortality, adult emergence, and sex of the successfully emerged adults.

After completing several series of tests with the *Aedes aegypti, Culex quinquefasciatus* eggs were collected from the field, hatched in the laboratory, and tested as previously described.

Results. Beyond six days there was little difference in daily mortalities between the exposed and the untreated immatures. In other words, by the end of six days all the larvae and pupae that were going to die from the treatment had died; mortalities beyond six days were probably from other causes, as indicated by the untreated checks. There was no significant difference of the sex ratio of successfully emerged adults between the control and the test mosquitoes.

Table 1 *(Ae. aegypti)* and table 2 *(Cx. quinquefasciatus)* show the results of these susceptibility tests. *Cx. quinquefasciatus* were more susceptible to OMS–2014 than *Ae. aegypti;* the former consistently showed 100% mortality within three days at 0.4 mg/l. whereas the latter species gave 100% kill in only one of two test series in six days (twice the exposure time) at 0.8 mg/l (twice the dosage rate).

The pupal mortality of *Ae. aegypti* exposed to 0.2 and 0.4 mg/l was about the same – 49.7% and 48.3%, respectively. With this one exception, the proportion of pupal deaths to the number of pupae increased with both species as the dosage rate increased. *Cx. quinquefasciatus* had little pupation and none above 0.1 mg/l; *Ae aegypti* had none above 0.4 mg/l.

* WHO Pesticide Evaluation Scheme

Table 1. Percent Mortality of Immature *Aedes aegypti* During Three and Six Days of Laboratorv Exposure to OMS-2014[a/]

milligrams of active ingredient per litre

	test series	untreated	0.05	0.1	0.2	0.4	0.8	1.6
3 days exposure	I[b/]	6.7	–	0	2.9	22.9	60.0	–
	II	0	1.3	4.0	13.3	57.3	98.7	100 (3 days)
	III	0	1.3	2.7	4.7[c/]	17.3[c/]	–	–
	mean	2.2	1.3	2.2	7.0	32.5	79.3	100
6 days exposure	I[b/]	9.3	–	45.6	11.8	27.9	95.6	–
	II	0	4.0	33.3	98.7	100 (6 days)	100 (6 days)	–
	III	1.3	4.0	5.3	64.7[c/]	95.3[c/]	–	–
	mean	3.6	4.0	28.1	58.4	74.4	97.8	-
	no. dead pupae/ pupations	0/161	1/111	46/168	88/177	42/87	–/0	–/0

a/ 25 late I instars exposed in each of three replicate beakers for each dosage rate in aech series
b/ test mortalities corrected by Abbott's formula
c/ six replicate beakers, each with 25 larvae.

Table 2. Percent Mortality of Immature *Culex quinquefasciatus* During Three and Six Days of Laboratory Exposure to OMS–2014[a/]

milligrams of active ingredient per litre

	series	untreated	0.05	0.1	0.2	0.4	0.8
3 days exposure	I	0	6.7	26.7	88.0	100 (2 days)	100 (2 days)
	II	0	6.7	36.0	92.0	100 (3 days)	100 (3 days)
	III	1.3	6.7	21.3	68.0	100 (3 days)	100 (2 days)
	mean	0.4	6.7.	28.0	82.7	100	100
6 days exposure	I[b/]	6.7	17.1	24.3	94.3	–	–
	II[b/]	8.0	14.5	52.2	100 (5 days)	–	––
	III[b/]	5.3	9.9	31.0	95.8	–	–
	mean	6.7	13.8	35.8	96.7	–	–
	no. dead pupae/ pupations	2/13	5/16	13/17	–/0	- /0	–/0

a/ 25 late I instars exposed in each of three replicate beakers for each dosage rate in each series.
b/ test mortalities corrected by Abbott's formula

SMALL SCALE FIELD TESTS, PHASE 2 OF WHOPES

Aedes aegypti

Method. Each test series consisted of the use of 16 earthenware jars. i.e. four replicate jars for each of three dosage rates and four replicates as untreated checks. Each jar held 30 litres of water to which 10 first instar colony larvae were introduced three times a week. The jars were screened to prevent the introduction of wild eggs. To simulate field usage, one half of the water was replaced with new tap water three times weekly, care being taken that no larvae were removed.

The proper quantities of 2% sand granules of OMS–2014 were weighed for each jar, and the water was agitated immediately after treating. Dosage rates for the first test series were from 0.004 to 0.033 mg/l active ingredient, the second series rates were from 0.033 to 0.3 mg/l, and the third series were from 0.3 to 1.2 mg/l. No test series was started until about 50% or more of the introduced larvae were successfully emerging as adults.

New pupae were removed from the jars daily and their fate recorded in the laboratory. Daily record of the number of pupations, the number of successfully emerged adults, and pupal and emergence mortality were kept. Larval mortality was taken to be the difference between the number of first instars introduced and the number of pupae removed from the jars.

Results. Table 3 shows the weekly mortalities of the immatures before and after treatments. At dosage rates of 0.1 mg/l and higher, the mortalities during the second week were always higher than during the first.

Table 3. Percent of Immature Mortality of *Aedes aegypti* Introduced into Water Jars Treated with OMS–2014[a/]

mg/l (a.i)	weeks after treatment											
	pretreatment[b/]	1	2	3	4	5	6	7	8	9	10	11
0.004	40.0	87.5	59.2	54.2	–	–	–	–	–	–	–	–
0.011	46.7	90.8	76.7	50.0	31.7	–	–	–	–	–	–	–
0.033[c/]	34.5	87.1	82.9	60.0	56.3	–	–	–	–	–	–	–
0.10	42.1	84.2	91.7	86.6	80.8	64.2	54.2	–	–	–	–	–
0.30[c/]	47.7	93.3	97.9	100	98.8	87:5	71.7	65.4	59.6	–	–	–
0.60	53.8	90.8	100	100	100	94.2	87.5	79.2	71.7	65.8	55.0	–
1.2	55.0	92.5	100	100	100	100	100	100	95.0	83.3	65.0	66.7
untreated check	51.7[d/]	43.9[d/]	44.2[d/]	43.0[d/]	48.2[d/]	45.0[c/]	43.8[c/]	47.1 c/	50.8[c/]	45.0	40.0	50.8

a/ total of 120 I instars introduced into four replicate jars weekly for each dosage rate in each test
b/ a two week period.
c/ mean of two test series
d/ mean of three test series

Most of the first week's survival was from the first day after treatment. Pupae could always be found at first day and usually the second day, and most of these produced healthy adults, thus more survival in the first week. At dosage rates lower than 0.1 mg/l this was not true, probably because the material was losing its effectiveness the second week and allowing more survival than those couple of days immediately after treatment.

Table 4 shows the relative duration of effectiveness. At 0.3 mg/l no adults emerged for three weeks, and during the fourth week less than 10% emerged. At 1.2 mg/l there was less than 10% emergence for eight weeks.

Table 4. Number of Weeks of Decreased Emergence of Adult *Aedes aegypti* from I Instar larvae Introduced into Water Jars Treated with OMS–2014

mg/l	rate of successful emergence		
(a.i.)	0%	< 10%	< 30 %
0.004	0	0	1
0.011	0	1	2
0.033	0	0	2
0.10	0	2	4
0.30	3	4	6
0.60	4	5	8
1.2	7	8	9

Most mortality was of larvae, however, the pupae were affected. Of course, as the dosage rate increased the number of pupations decreased, but as the dosage rate increased the pupal mortality rate increased when based on the number of available pupae. This comparison can be seen in Table 5.

Table 5. *Aedes aegypti* Pupal Mortality During Second and Third Weeks after OMS–2014 Treatment of Water Jars

mg/l (a.i.)	no. of test series	no. of pupations	% mortality
0.004	1	114	9
0.011	1	100	12
0.033	2	154	11
0.10	1	41	37
0.30	2	18	72
0.60	1	3	100
1.2	1	0	--
check	3	385	3

Culex quinquefasciatus.

Methods. Concrete drains 50 cm wide and from 30 to 50 m in length were selected on the bases of sluggish or nil water movement and high larval and pupal populations. The water was 9 to 13 cm deep. Relative population indices were taken by the standard dipping technique. The mean of ten dips per drain was taken. Counts were made and recorded in three categories, i.e. I and II instars, III and IV instars, and pupae. No dipping was done within five meters of either end of the drains. At the time of dipping, samples of approximately 100 pupae per drain were brought to the laboratory for observation of mortality or successful adult emergence.

During one test series two replicate drains were treated at a target dosage rate of 0.5 mg/l a.i. of OMS–2014 and two at 1.0 mg/l. This was followed by a second series with two drains treated at 1.0 mg/l and two at 2.0 mg/l a.i. During each test series, two untreated drains were monitored as checks. Water volumes were calculated, and proper amounts of 2% OMS–2014

sand granules were weighed for each drain that was to be treated. The granules were dispersed in the water by hand. For maximum uniformity of coverage, about one third of the granules was dispersed with one walk of the measured drain, thus each drain was covered very close to three times.

Results. Rates of larval and pupal reduction are shown in Table 6. In no case was 100% control shown, and in general, the populations were well on the way to recovery in one or one and a half weeks after treating. In all cases there was a considerable increase in the number of pupae during the first two days after treating indicating that the material initiated some early pupation of the IV instars. The highest levels of kill were at 2.0 mg/l with just above 99% reduction of I and II instars by day 2, and just above 90% reduction of pupae by day 4. During the period of both test series, there was a slight natural increase of all immature stages in the check drains.

Observations of the pupae brought to the laboratory showed no difference between adult emergence rates from pupae taken from treated drains and emergence rates from pupae taken from untreated drains. This shows that if there was some pupacidal effect, the pupae were dying soon after pupation, and collections were being mostly made of older pupae that had already passed their critical stage. The decrease in number of pupae was mostly or entirely caused by the decrease in number of larvae available for pupation.

Table 6. Percent Reduction of *Culex quinquefasciatus* from Pretreatment Population Levels in Drains Treated with OMS -2014 (parenthesis = mean number per dip)

dosage rate of active ingredient in milligrams per litre

days after treatment	untreated[a]			0.5[b]			1.0[a]			2.0[b]		
	larvae			larvae			larvae			larvae		
	I & II instars	all stages	pupae	I & II instars	all stages	pupae	I & II instars	all stages	pupae	I & II instars	all stages	pupae
mean of −5 & −3 days	(176.0)	(325.8)	(42.6)	(153.0)	(243.1)	(61.0)	(170.8)	(318.4)	(45.6)	(218.8)	(410.8)	(68.2)
1	>	>	>	94.6	64.4	>	91.4	67.4	>	93.1	68.0	>
2	>	>	>	97.1	67.5	>	92.1	79.3	>	99.1	89.5	>
4	>	>	>	65.7	51.6	66.6	58.5	61.4	62.1	44.2	56.5	90.8
7	>	>	>	91.1	78.4	60.5	75.0	64.1	33.7	36.0	38.5	47.5
10	>	>	>	37.9	27.9	68.0	16.2	13.1	>	7.1	6.1	1.0
12	>	>	>	72.4	49.2	20.5	17.9	14.3	>	3.0	3.4	>

a/ mean of four drains
b/ mean of two drains

CONCLUSIONS

In the uncontaminated laboratory water, *Culex quinquefasciatus* was more susceptible to OMS–2014 than *Aedes aegypti*. From a log-probit regression line fitted by eye, the three day LC_{50} value for the former species was 0.13 mg/l, while for the latter species it was 0.48 mg/l. These laboratory tests showed that some larvae were able to pupate, but many had ingested lethal quantities of the OMS–2014 and died as pupae. In other words, there was some pupacidal effect, however, most mortality was of larvae.

In contrast to the laboratory susceptibility tests, the phase 2 field testing showed OMS–2014 to be considerably more effective against *Ae. aegypti* than *Cx. quinquefasciatus*. The former species showed 100% control at three weeks after treating with 0.3 mg/l, whereas the latter species never showed 100% control at 2.0 mg/l. Suspended particulates can influence the availability of some material[1]. This drain water is highly polluted and has a considerable amount of suspended particles, thus the effectiveness of OMS–2014 was reduced.

The laboratory tests described here measured dosage rates of OMS–2014 in mg/l over three and six days; similar tests by Herald et al.[2], using triflumuron (Bay Sir 8514) against *Ae. aegypti*, measured dosage rates in ppb over 24 hours. The duration of effect of OMS–2014 against *Ae. aegypti* in water jars simulating field conditions in these tests was similar to methoprene and slightly shorter than from diflubenzuron under the same testing conditions[3]. The poor results shown here of OMS–2014 in the polluted water of these drains against *Cx. quinquefasciatus* were similar to the results shown by Axtell et al.[4] with methoprene and diflubenzuron against the same species in polluted swine waste lagoons.

ACKNOWLEDGEMENTS

We are grateful to the Head, National Institute of Health Research and Development, Prof. DR. A.A. Loedin and the Head of Health Ecology Research Centre, Dr. I.F. Setiady for their support of this study. Thanks are also due to DR. C.P. Pant, Cheif ECV/HQ Geneva, for his criticisms, comments and advice; and to DR. Lim Boo Liat, WHO Consultant, for his assistance in preparing the manuscript. We also express our gratitude to all the staff of HERC for their involvement and assistance in this study without whom the survey would not have been possible.

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