

**Susceptibility of *Aedes aegypti*
and *Culex quinquefasciatus*
to insecticides in Paramaribo,
Suriname, 1979-1981,
and experimental selection
for resistance**

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Abstract

Laboratory-reared mosquitoes were tested by WHO standard methods. Larvae and adults of both species were resistant to DDT and dieldrin. Adults of both species were resistant to propoxur but susceptible to malathion and fenitrothion. *C. quinquefasciatus* larvae were susceptible to chlorpyrifos, temephos and fenitrothion. *A. aegypti* larvae had a normal susceptibility to chlorpyrifos (LC_{95} 0.005 mg/l) but an abnormally low susceptibility to fenitrothion (LC_{95} 0.077 mg/l) and temephos (LC_{95} 0.035 mg/l). In September 1981, 2.8 % of 2067 wild-caught *A. aegypti* larvae survived the WHO diagnostic dosage of temephos (0.02 mg/l), 16, 1% of 2098 larvae survived the diagnostic dosage of fenitrothion (0.06 mg/l), and selection of offspring from the fenitrothion survivors with 0.06 or 0.1 mg/l of fenitrothion in each generation increased the survival rate to 88.3 % of 557 larvae of the F_5 generation, dosed with 0.06 mg/l. It is concluded that the *A. aegypti* population has the potential for rapid development of fenitrothion resistance. Larvae of both species were susceptible to a commercial formulation of *Bacillus thuringiensis* H 14 (potency 6 000 ITU/mg), with LC_{50} values of 0.018 mg/l to *A. aegypti* and 0.049 mg/l to *C. quinquefasciatus*.

Key words : Culicidae — Insecticide — Fenitrothion — Susceptibility — Resistance — *Aedes aegypti* — *Culex quinquefasciatus* — Suriname.

Résumé

SENSIBILITÉ D'*Aedes aegypti* ET *Culex quinquefasciatus* AUX INSECTICIDES A PARAMARIBO, SURINAM, DE 1979 A 1981 ET SÉLECTION EXPÉRIMENTALE DE LA RÉSISTANCE. Des moustiques élevés au laboratoire ont été testés par les méthodes normalisées OMS. Les larves et les adultes des deux espèces étaient résistants au DDT et à la dieldrine. Les adultes des deux espèces étaient résistants au propoxur, mais sensibles au malathion et au fenitrothion. Les larves de *C. quinquefasciatus* étaient sensibles au chlorpyrifos, au fenitrothion et au téméphos. Les larves d'*A. aegypti* avaient une sensibilité normale au chlorpyrifos (CL_{95} 0,005 mg/l) mais une sensibilité, anormalement basse au téméphos (CL_{95} 0,035 mg/l) et au fenitrothion (CL 0,077 mg/l). En septembre 1981, 2,8 % de 2067 larves d'*A. aegypti* capturées dans la nature ont survécu au dosage diagnostique OMS de téméphos (0,02 mg/l) ; 16,1 % de 2198 larves de la même espèce ont survécu au dosage diagnostique de fenitrothion (0,06 mg/l) et les sélections successives des survivants à l'exposition au fenitrothion à 0,06 ou 0,1 mg/l ont élevé

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le taux de survie à 88,3 % des 557 larves de génération F5 exposées à 0,06 mg/l. On en conclut que la population d'*A. aegypti* de Paramaribo a la potentialité de développer rapidement la résistance au fénitrothion. Les larves des deux espèces ont été sensibles à une formulation commerciale de *Bacillus thuringiensis* H 14 (à 6000 UI/mg), les CL_{50} étant de 0,018 mg/l pour *Aedes aegypti* et de 0,049 mg/l pour *C. quinquefasciatus*.

Mots-clés : Culicidae — Insecticides — Fénitrothion — Sensibilité — Résistance — *Aedes aegypti* — *Culex quinquefasciatus* — Surinam.

Introduction

The Suriname Ministry of Health has used a variety of organochlorine and organophosphorus insecticides for mosquito control in Paramaribo during the past 35 years. DDT was used against *Aedes aegypti* larvae from 1948-55 and as a residual spray in houses from 1949-55. HCH was used as a residual spray in privies, stables and other out-buildings around 1953 (Bruyning, 1953), and as a perifocal spray against *A. aegypti* from 1955-62, then it was replaced by dieldrin (1963-66) and fenthion from 1966 (Van der Kuyp, 1967). According to unpublished reports of the Anti-Gelekoorts Campagne (AGC) and various technical advisers from the Panamerican Health Organization (PAHO), perifocal treatment with fenthion and focal treatment with temephos was carried out once every 8 weeks over the whole coast region of Suriname from 1968-72, and these two insecticides were used three times per year in Paramaribo alone from 1973-76. Since 1976, perifocal treatment with fenthion and fenitrothion and focal treatment with temephos has continued in Paramaribo, but only around the port and hospitals and in two small test areas, which all together cover only a small part of the city (about 4 % of the total area). Fenitrothion ultralow volume (ULV) spray was used in one of the areas test from January to May 1978, and the entire city was sprayed twice with malathion from truck mounted ULV sprayers from May to June 1982. The public have used household aerosols containing propoxur since 1977 or earlier.

DDT resistance in Paramaribo *A. aegypti* was detected in 1953 and dieldrin resistance in 1963 (Van der Kuyp, 1967). *Culex quinquefasciatus* in Paramaribo were already resistant to DDT by 1953 (Bruyning, 1953). Mouchet and Quiroga (1976) reported that *A. aegypti* larvae from Suriname were resistant to malathion, fenthion, temephos and bromophos. According to Dr J. Mouchet (personal communication, 1980), these conclusions were based on unpublished data in the files of the World Health Organization (WHO), Geneva,

presumably the results of tests by WHO and PAHO workers on *Aedes aegypti* samples sent to them as eggs from Suriname.

This paper is a summary of susceptibility tests carried out at the Centraal Laboratorium, Paramaribo, between August 1979 and May 1982.

Materials and methods

Mosquitoes in laboratory cultures descended from *A. aegypti* larvae caught in auto tires on the Gemenelandsweg in August 1979, and *C. quinquefasciatus* larvae caught in a ditch on the Toekomstweg in April 1980, were used for tests of all the insecticides except *Bacillus thuringiensis*, which was tested against mosquitoes in laboratory cultures descended from *A. aegypti* larvae caught at the Gemenelandsweg and the Cultuurtuin in January 1982, and *C. quinquefasciatus* females caught in a house on the Zonnebloemstraat in April 1982. Wild-caught *A. aegypti* from each of the 20 zones into which the city is divided for mosquito control work were tested in September 1981 with WHO diagnostic dosages of fenitrothion and temephos. Fourth instar larvae (4-5 days old when laboratory-reared) and sugar-fed females 3-10 days old were used in the tests. Temperatures in the culture room, where the tests were conducted, were 26-29°C (mean 27°C) and relative humidities were 75-95 % (mean 85 %).

Impregnated papers and standard solutions of all insecticides were kindly supplied by the WHO via their representative in Suriname, except for the *Bacillus thuringiensis* H 14, which was a wettable powder formulation (Bactimos, Batch 339, LRB 676) with a relative potency of 6 000 ITU/mg, kindly supplied by the manufacturer, Biochem Products of Brussels, in January 1982. Standard test methods as given in the documents WHO/VBC/81.805, 806 and 807 were used throughout, except that in the experiments on selection for fenitrothion resistance more than 25 larvae per test vessel had to be used, because of shortage

of insecticide. Glass beakers of 400 ml were used for the tests with larvae. Tests for goodness-of-fit of the lines and confidence limites of the LC₅₀ were carried out according to the instructions in Swaroop (1966).

Results and discussion

The World Health Organization (in WHO/VBC/81.807 and other documents) recommend first measuring the baseline susceptibility of the mosquito population before it is subjected to

insecticidal pressure, and from the results obtained establishing a diagnostic dose for each insecticide, that would be expected to kill all susceptible individuals. For mosquito populations that have already been attacked with insecticides, such as *A. aegypti* and *C. quinquefasciatus* in Paramaribo, the WHO (1980) has produced a tentative list of diagnostic dosages, based on studies such as that of Coosemans *et al.* (1978) on normal variations in the susceptibility of *A. aegypti* to organophosphates.

Median lethal concentrations (LC₅₀) and exposure times (LT₅₀) are shown in Table I and the results of diagnostic dose tests in Table II. Larvae and adults of both species were resistant to DDT and dieldrin, adults of both species were resistant to propoxur. Larvae of both species were susceptible to *Bacillus thuringiensis* H-14, though the LC₅₀ values obtained were higher than most of those given in the latest WHO data sheet on this microbial insecticide (WHO/VBC/79.750, Rev. 1, 1982). *C. quinquefasciatus* larvae were susceptible to chlorpyrifos, fenitrothion and temephos, but a few adults survived diagnostic dosages of fenitrothion and malathion. *A. aegypti* larvae were susceptible to chlorpyrifos, but the LC₉₅ values for fenitrothion and temephos were higher than normal

TABLE I

Susceptibility of laboratory-reared *Aedes aegypti* and *Culex quinquefasciatus* of Paramaribo stock to insecticides, 1979-1982.

Species and insecticide	Number tested	Date mo/yr	LC ₅₀ mg/l	Goodness of fit	LC ₉₅ mg/l
<i>A. aegypti</i> larvae					
DDT	385	8/79	0.74±0.15 ^(a)	poor	4.80
Dieldrin	253	8/79	0.12±0.06	good	1.75
Chlorpyrifos	352	8/79	0.0018±0.0003	poor	0.005
Fenitrothion	379	8/79	0.029±0.005	good	0.077
Temephos	306	8/79	0.0054±0.0006	poor	0.035
<i>B. thuringiensis</i> ^(b)	700	2/82	0.018±0.001	poor	0.039
<i>C. quinquefasciatus</i> larvae					
DDT	490	6/80	1.00±0.10	poor	2.40
Dieldrin	652	6/80	0.44±0.04	poor	1.05
Chlorpyrifos	456	6/80	0.0012±0.0001	good	0.003
Fenitrothion	432	6/80	0.017±0.001	poor	0.034
Temephos	430	6/80	0.0018±0.0004	poor	0.009
<i>B. thuringiensis</i> ^(b)	1509	5/82	0.049±0.005	good	0.28
			LT ₅₀		LT ₉₅
<i>A. aegypti</i> female adults					
DDT 4.0%	281	9/79	>24 h	-	>24 h
Dieldrin 4.0%	383	9/79	>24 h	-	>24 h
Fenitrothion 1.0%	395	9/79	16.2±2.0 min	poor	40.0 min
Malathion 5.0%	513	9/79	8.6±0.6 min	poor	16.8 min
Propoxur 0.1%	536	12/80	8 h	-	>24 h ^(c)
<i>C. quinquefasciatus</i> female adults					
DDT 4.0%	88	6/80	>24 h ^(d)	-	-
Dieldrin 4.0%	85	6/80	>24 h ^(e)	-	-
Fenitrothion 1.0%	399	12/80	20.0±2.4 min	poor	30 min
Malathion 5.0%	614	12/80	15.9±2.1 min	poor	54 min
Propoxur 0.1%	451	12/80	2.2±0.2 h	poor	5.4 h

(a) ± 95% confidence limits
 (b) Potency 6000 IPU/mg
 (c) 72% dead in 24 h
 (d) 22% dead in 24 h
 (e) 15% dead in 24 h

TABLE II

Mortalities of *Aedes aegypti* and *Culex quinquefasciatus* produced by WHO diagnostic dosages of insecticides, Paramaribo, 1980-81.

Species and insecticide	Date mo/yr	Diagnostic dosage (WHO)	Dosage used	Number tested	Percent kill
<i>A. aegypti</i> larvae (lab-reared)					
Chlorpyrifos	11/80	0.01 mg/l	0.01 mg/l	90	98.9
Fenitrothion	11/80	0.06 "	0.06 "	79	97.9
Temephos	11/80	0.02 "	0.02 "	104	99.0
<i>A. aegypti</i> larvae (wild-caught)					
Fenitrothion	9/81	0.05 mg/l	0.06 mg/l	2198	85.9
Temephos	9/81	0.02 "	0.02 "	2067	97.2
<i>C. quinquefasciatus</i> larvae (lab-reared)					
Chlorpyrifos	6/80	0.01 mg/l	0.005 mg/l	45	100.0
Fenitrothion	6/80	0.125 "	0.125 "	18	100.0
Temephos	6/80	0.02 "	0.01 "	124	100.0
<i>C. quinquefasciatus</i> adult females (lab-reared)					
DDT 4.0%	6/80	4 h	24 h	88	22.0
Dieldrin 4.0%	6/80	1 h	24 h	85	15.0
Fenitrothion 1.0%	12/80	2 h	2 h	86	98.8
Malathion 5.0%	12/80	1 h	1 h	60	95.6
Propoxur 0.1%	12/80	2 h	2 h	68	52.9

for *A. aegypti* (Coosemans *et al.*, 1978), and in the diagnostic-dose tests some laboratory-reared and wild-caught larvae survived fenitrothion or temephos. No diagnostic dosages for *A. aegypti* adults have been proposed by the WHO, but all the females we tested were killed by less than one hour's exposure to fenitrothion or malathion papers.

Two attempts were made to select *A. aegypti* larvae for resistance to organophosphates. Larvae surviving diagnostic dosages were reared to adults, which were allowed to breed freely with each other, and larvae of the next generation were selected with the same or higher dosages. In the first attempt (Table III), survival after exposure to

TABLE III

Selection for temephos resistance in *A. aegypti* from a Paramaribo culture, December 1980-March 1981. The ancestry of the larvae tested is shown by the connecting lines.

Generation	Concentration of temephos					
	0.0125 mg/l		0.02 mg/l		0.03 mg/l	
	Number tested	Percent kill	Number tested	Percent kill	Number tested	Percent kill
Parental	303	85	106	99	95	100
F ₁	167	23	58	52	-	-
F ₂	-	-	144	49	141	94
F ₃	-	-	145	100	104	100

0.02 mg/l temephos was higher in the F₁ and F₂ generations than in the parental generation, but all larvae of the F₃ generation were killed by this dosage. Many of the larvae which had survived the 24-hour exposure to the insecticide died before pupating, and only 4 females of each generation lived long enough to lay eggs. In the second attempt, wild-caught larvae which had survived 0.06 mg/l fenitrothion were cultured in the laboratory (Table IV). Larvae of the F₁ and F₂ generations were selected with 0.06 mg/l and larvae of the F₃, F₄ and F₅ generations with 0.1 mg/l. The mortality produced by 0.06 mg/l fell from 83.9% in the parental generation to 11.7% in the F₅ generation. This is a case of true heritable resistance as defined by the WHO Expert Committee on Insecticides (WHO, 1976, Annex 1) and Coosemans *et al.* (1978).

It was not surprising to find that both species were resistant to DDT and dieldrin, because resistance to both compounds in both species is now world-wide, (WHO, 1980). DDT has been used against both species in Paramaribo, dieldrin against *A. aegypti* only, and resistance to dieldrin in *C. quinquefasciatus* may have resulted either from the use of HCH against it, or from the use of dieldrin against *A. aegypti*, though the breeding sites are generally different. Resistance to propoxur is probably a result of the extensive use of this compound as a household aerosol by the public, since it has not been used by the government in any of its vector control campaigns.

TABLE IV

Selection for fenitrothion resistance in *Aedes aegypti* larvae collected in Paramaribo, September 1981. The ancestry of the larvae tested is shown by the connecting lines.

Generation	Date mo/yr	Fenitrothion 0.06 mg/l			Fenitrothion 0.1 mg/l		
		Number tested	Percent kill	Surviving pupae	Number tested	Percent kill	Surviving pupae
Parental	9/81	2198	83.9	275	-	-	-
F ₁ (a)	10/81	1541	80.5	219	192	100.0	0
F ₂	11/81	2205	65.8	406	103	99.0	0
F ₃	12/81	1404	32.2	664	2010	48.3	508
F ₄	1/82	295	43.5	216	310	88.7	52
F ₅	3/82	557	11.7	-	772	24.0	463

(a) LC_{50} of fenitrothion to F₁ larvae = 0.029 mg/l, LC_{95} = 0.074 mg/l.

The detection of fenitrothion resistance in *A. aegypti* is a matter for concern, but it does not mean that this compound can no longer be used. The WHO Expert Committee on vector control state that « in some Caribbean countries and other tropical areas of the Americas, the appearance of low-level resistance to organophosphate compounds has not resulted in operational difficulties, and the severe outbreaks of dengue which have occurred in central America and the Caribbean

have been due to inadequacy of control measures rather than to organophosphate resistance » (WHO, 1980). How rapidly the insecticide becomes ineffective will depend on the selection pressure for resistance, which is determined by how long and how often the insecticide is used, how many of the breeding sites are treated, and the dosage. In focal treatment (adding insecticide directly to the breeding site water), the development of resistance may be delayed by using dosages high enough to kill the heterozygous resistant as well as the susceptible larvae. In perifocal treatment, however, (residual spraying around actual or potential breeding sites to kill emerging adults and females coming to oviposit), both the weathering rate and the duration of the mosquitoes' contact with the insecticide deposit are uncertain, so it is impossible to specify how much the dosage should be increased to kill the heterozygotes.

Conclusions

At the time this survey was concluded, it was still proposed to carry out another 5-year campaign against *A. aegypti* throughout the coastal region of Suriname, using temephos as a focal treatment against larvae, fenitrothion perifocal residual spray against emerging adults and ovipo-

siting females and malathion ULV spray against adults. It would still be possible to begin the campaign with these compounds, but it will also be necessary to monitor the development of resistance regularly, and to have alternative insecticides field-tested and ready for use. These could include *B. thuringiensis* H 14 and insect growth regulators as larvicides and pyrethroids as adulticides. Since cross-resistance between organophosphates is known (WHO, 1980), it is better not to use organophosphates against both larvae and adults anyway. Finally, it will be more important than ever to use non-chemical methods of control as well, such as removing or destroying potential breeding sites, not only to reduce insecticide costs, but also to reduce selection for resistance.

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