# 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 fénitrothion. Les larves de* C. quinquefasciatus *étaient sensibles au chlorpyrifos, au fénitrothion 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 fénitrothion (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 fénitrothion (0,06 mg/l) et les sélections successives des survivants à l'exposition au fénitrothion 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 aegypty *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 outbuildings 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 $LC_{50}$ 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 ($LC_{50}$) and exposure times ($LT_{50}$) 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 $LC_{50}$ 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 $LC_{95}$ 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 |
|---|---|---|---|---|---|
| A. aegypti 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 |
| B. thuringiensis[b] | 700 | 2/82 | 0.018±0.001 | poor | 0.039 |
| C. quinquefasciatus 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 |
| B. thuringiensis[b] | 1509 | 5/82 | 0.049±0.005 | good | 0.28 |
| | | | $LT_{50}$ | | $LT_{95}$ |
| A. aegypti 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] |
| C. quinquefasciatus 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 ITU/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 |
|---|---|---|---|---|---|
| A. aegypti 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 |
| A. aegypti larvae (wild-caught) | | | | | |
| Fenitrothion | 9/81 | 0.06 mg/l | 0.06 mg/l | 2198 | 85.9 |
| Temephos | 9/81 | 0.02 " | 0.02 " | 2067 | 97.2 |
| C. quinquefasciatus 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 |
| C. quinquefasciatus 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 Number tested | 0.0125 mg/l Percent kill | 0.02 mg/l Number tested | 0.02 mg/l Percent kill | 0.03 mg/l Number tested | 0.03 mg/l 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 $F_1$ and $F_2$ generations than in the parental generation, but all larvae of the $F_3$ 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 $F_1$ and $F_2$ generations were selected with 0.06 mg/l and larvae of the $F_3$, $F_4$ and $F_5$ 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 $F_5$ 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: Number tested | Percent kill | Surviving pupae | Fenitrothion 0.1 mg/l: 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 | 293 | 43.3 | 216 | 310 | 88.7 | 52 |
| $F_5$ | 3/82 | 557 | 11.7 | - | 772 | 24.0 | 463 |

(a) $LC_{50}$ of fenitrothion to $F_1$ 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 organophospate 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 ovipositing 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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