CHEMICAL CONTROL

Resistance of *Aedes aegypti* (L.) (Diptera: Culicidae) to Temephos in Brazil: A Revision and New Data for Minas Gerais State

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BioAssay: 6:7 (2011)

Resistência de *Aedes aegypti* (L.) (Diptera: Culicidae) a Temephos no Brasil: Revisão e Novos Dados para Minas Gerais

RESUMO – O dengue afeta milhares de pessoas nas regiões tropicais de todo o planeta. Os serviços de saúde no Brasil vêm desde 1967 usando o organofosforado temefós no combate às larvas do mosquito *Aedes aegypti*, mas vários trabalhos vêm mostrando um aumento da tolerância destes mosquitos ao temefós. Desde 1986 quando o dengue foi introduzido no Brasil, a doença tem sido detectada todos os anos principalmente em Minas Gerais, onde o maior surto epidêmico ocorreu em 1988 seguido de um novo surto no inicio de 2002. Objetivando verificar a susceptibilidade da população de *Ae. aegypti* ao temefós no município de Coronel Fabriciano, MG, bio-ensaios de resistência foram realizados com a concentração diagnóstica do temefós de 0,012 mg/L para as larvas F^1 dos mosquitos coletados com armadilhas de oviposição. Os ensaios 1, 2, 3 e 4 apresentaram taxas de mortalidade para a linhagem Rockefeller de 97%, 100%, 100% e 99% respectivamente. As taxas de mortalidade para a populacao natural foram de 5,12%, 3,37%, 0% e 2,66%. A mortalidade média para o experimento foi de 2,78% para a população natural e de 99% para os mosquitos Rockefeller. Os resultados indicam uma tendência para a ocorrência de resistência para larvas de *Ae. aegypti* expostas a concentrações de temefós. Comparações com resultados obtidos por outros autores em outras regiões do Brasil mostram que as taxas de mortalidade obtidas variam de zero to 100% com a mortalidade media para todos os valores obtidos de 39,29% ± 30,13. Os dados apresentados neste estudo servem como base para modificações no plano de manejo do controle do *Ae. aegypti* pelo Programa de Controle da Dengue no Brasil.

PALAVRAS-CHAVE: dengue, organofosforado, susceptibilidade, armadilha de oviposição

ABSTRACT – Dengue fever affects thousands of people in tropical regions of the world. Governmental health service in Brazil has used organophosphates like temephos against *Aedes aegypti* larvae since 1967 but today in several regions of Brazil authors have reported an increase of tolerance of *Ae. aegypti* to temephos. Since 1986 when dengue fever was introduced to Brazil, the disease has been detected every year. In Minas Gerais State, the major epidemics occurred in 1998 followed by a brand new epidemic peak at the beginning of 2002. Aiming to verify the susceptibility of *Ae. aegypti* population to temephos in the municipality of Coronel Fabriciano, Minas Gerais State, temephos resistance bioassays with diagnostic dose (0.012 mg/L) were performed with F^1 larvae of natural populations of *Ae. aegypti* collected with ovitraps. The bioassays 1, 2, 3 and 4 presented mortality rates for Rockefeller population of 97%, 100%, 100% and 99% respectively. Mortality rates for natural population were 5.12%, 3.37%, 0% and 2.66% for bioassays. The average mortality was 2.78% for natural population and 99% for Rockefeller mosquitoes. Results show that the exposure of *Ae. aegypti* larvae to different concentrations of temephos revealed resistance in several localities examined. Comparisons with the results obtained by authors for other regions in Brazil show that mortality obtained vary from 0.0 to 100% with mean of 39.29% ± 30.13. Results presented in this paper serve as parameter to possible changes in Brasil's Dengue Control Program aiming the insecticide resistance management.

KEYWORDS: dengue fever, susceptibility, organophosphates, ovitraps

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Dengue is the most important mosquito-borne viral disease affecting humans; its global distribution is comparable to that of malaria, and an estimated 2.5 billion people are living in areas at risk for epidemic transmission (Gubler & Clark 1995). Dengue fever affects thousands of people in tropical regions of the world. The main reasons for this situation are the unavailability of effective vaccines and the development of insecticide and drug resistance by the vector *Aedes (Stegomyia) aegypti* (L.) (Diptera: Culicidae) and virus pathogen.

Governmental health service in Brazil has used organophosphates like temephos against *Ae. aegypti* larvae and fenitrothion and malathion against adults since 1967 but today the effectiveness of temephos as main larvicide in Brazil for *Ae. aegypti* control programs have long been discussed (Lima *et al.* 2003). In several regions of Brazil authors have reported an increase of tolerance of *Ae. aegypti* to temephos and many papers suggest the occurrence of resistance(Braga *et al.* 2004, Carvalho *et al.* 2004, Beserra *et al.* 2007). The use of temephos in Brazil was intensified after 1986 epidemics (Braga *et al.* 2004). As a consequence of its massive use, some municipalities with high mosquito infestation or dengue incidence levels have substituted the organophosphates for the pyrethroid cypermethrin in the control of *Ae. aegypti* adults, though resistance to cypermethrin have already been reported (Cunha *et al.* 2005).

Half the world's population lives in countries endemic for dengue, underscoring the urgency to find solutions for dengue control (Guha-Sapir & Schimmer 2005). Since 1986 when dengue fever was introduced to Brazil, the disease has been detected every year and most of the notifications occur between January and March, a period of high rainfall and temperatures which provides suitable conditions for the development of mosquito *Ae. aegypti* (Ministério da Saúde, 2007). In Minas Gerais State, the outbreak of dengue fever occurred in 1993 with 3,863 notifications. In 1996, first cases were reported for the metropolitan region of Belo Horizonte and since then, the disease spread out throughout the State. The major epidemics occurred in 1998 followed by a brand new epidemic peak at the beginning of 2002. Since 2003, a significant reduction in notified cases of dengue fever has been observed. The Eastern region of Minas Gerais where is located the municipality of Coronel Fabriciano has presented the highest incidence levels for the state in the last five years (616.4 cases per 100,000 inhabitants) (SES-MG 2006). The present study aims to verify the susceptibility of *Ae. aegypti* population to temephos in the municipality of Coronel Fabriciano as well as to compare the results obtained in this study with data from previous studies concerning the resistance of *Ae. aegypti* in other regions.

Material and Methods

The study was conducted in the city of Coronel Fabriciano (19º30'52,21''S; 42º37'31,32''W), Minas Gerais state, Brazil, from June to August 2006. The municipality is extended in an area of 221 km^2 with 103,724 inhabitants, and has a historic in dengue notifications, with thousands of people affected by the illness in the last years. Dengue fever, being a vector-borne disease, is significantly influenced by weather. Thus, the warm temperature, high rainfall and the geographic position of the city has created an ideal location for breeding sites of *Ae. aegypti* populations assuring their survival for all the year.

Samples of *Ae. aegypti* were collected by using ovitraps prepared according to the procedure of Fay & Eliason (1966) using black plastic jars filled with hay infusion. The number of ovitraps installed followed a norm established by FUNASA (1999) and was based on the number of buildings in each municipality (an indirect measure of the population density): 60,000 buildings, 100 ovitraps; 60,000−120,000 buildings, 150 ovitraps; 120,000−500,000 buildings, 200 ovitraps; >500,000 buildings, 300 ovitraps. Field collection of eggs was done between July and August 2006. After five days exposed, the ovitraps were collected and taken to the laboratory where the presence of eggs in each ovitrap was then scored.

Positive ovitraps were immersed in dechlorinated water to induce larval hatching. After 24 hours, larvae were transferred to rectangular basins containing two liters of dechlorinated water. Dog food was supplied daily to feed the larvae. Pupae were transferred to another plastic jars and the resulting adults (*Ae. aegypti* or *Ae. albopictus*) were identified. Mosquitoes' identification followed the literature of Consoli & Oliveira (1994). Only *Ae. aegypti* mosquitoes were kept for bioassays. These were transferred to square plastic cages (60 cm per side) and fed on 10% solution of honey with distilled water and later three days old females were fed on chicken's blood (*Gallus gallus*)containing sodic heparin with an artificial feeder on the first two hours of photo-phase (Ahmed *et al.* 1999). Three days after the blood meal, eggs were collected for three days in small plastic cups containing wet filter paper. Paper strips containing the eggs were then allowed to dry in an insectary and served as the source of F1 mosquitoes for the bioassays.

Temephos resistance bioassays were performed with F1 larvae, according to the WHO recommended procedure and parameters (WHO 1981). The diagnostic concentration (DC), which corresponded to twice the concentration of the 99% lethal dose (LC_{99}) for the susceptible strain, was used. According to WHO standardization, the temephos DC for

Ae. aegypti is 0.012 mg/L. Twelve samples were used in each test. Each sample consisted of 20 larvae in 250 mL: 8 samples with 0.012 mg of Temephos/L (technical grade / 1 mL of a 3 mg/L alcoholic solution) and four samples with 1 mL of ethanol. Mortality was recorded 24 hours after the beginning of the test and it was based on mortality percentage formula by which the number of dead larvae is divided by the number of tested larvae (subtracting pupae) x 100 (Relcov 2005). Each bioassay was repeated three times. In all cases, the resistant/susceptible status of mosquito populations was evaluated according to WHO criteria (WHO, 1976). Mortality greater than 98% indicates susceptibility, mortality less than 80% defines resistance, and mortality between 80% and 98% is suggestive of an incipient altered susceptibility, indicating the need for surveillance of the corresponding population. The Rockefeller reference strain, which served as the susceptibility control in all assays (WHO 1981), was maintained continuously in the laboratory.

Results and Discussion

Ae. aegypti L3/L4 larvae from Coronel Fabriciano showed alterations in susceptibility when subjected to bioassays using the diagnostic temephos concentration (Table 1). The bioassays 1, 2, 3 and 4 presented mortality rates for Rockefeller population of 97%, 100%, 100% and 99% respectively thus corroborating its susceptibility to the insecticide and validating the results. Mortality rates for natural population of Coronel Fabriciano were 5.1%, 3.3%, 0% and 2.6% for bioassays. Mean mortality was 2.7% for natural population and 99% for Rockefeller mosquitoes. The results indicate occurrence of resistance. Based on the test results and the WHO classification for resistance (WHO, 1976), *Ae. aegypti* population from Coronel Fabriciano is highly resistant to temephos.

Table 2 presents a revision on mortality rates for *Ae. aegypti* natural populations submitted to diagnostic dose of temephos in several parts of Brazil. Mean mortality obtained for all the results from other authors resumed on table 2 in 39.2% ± 30.1. Carvalho *et al.* (2004) testing resistance to diagnostic dose of temephos in Federal District obtained mortality rates between 54.1% and 63.4%. Macoris *et al.* (2003) obtained 79% of mortalitity for insects from São Paulo. Lima *et al.* (2003), in the State of Espírito Santo observed 45.5% mortality. Several municipalities in Espírito Santo, Federal District, Rio de Janeiro and Sao Paulo State have shown mortality levels that require further surveillance of the mosquito populations. Whereas cities from Sergipe, Alagoas, Paraíba and Minas Gerais State, a low mortality was observed, indicating that the *Ae. aegypti* populations in such locations are already resistant. In the state of Rio de Janeiro, one of the most affected by dengue fever in the last years, mortality rates observed from manuscripts varied between 10.8% (São Gonçalo) and 74% (Campos de Goytacazes), an assumption that the seven populations analyzed showed resistance, with mortality rates less than 80%. The degree of resistance seemed to be higher in the capital (Rio de Janeiro) and neighboring cities, where the level of mortality did not exceed 35%. Campos dos Goytacazes, which is located at northern region of this state, had a mortality of 74%, a rate that indicates resistance but reveals a less drastic situation when compared with the more populated area in the state of Rio de Janeiro. Data also suggest a strong trend for

Table 1. Responses of *Aedes aegypti* larvae submitted to diagnostic concentration of temephos (0,012mg/L) in the city of Coronel Fabriciano, Minas Gerais State. Numbers indicate individuals used for each bioassay.

	Bioassay 1						Bioassay 2						Bioassay 3						Bioassay 4					
	D		L		P		D		L		P		D		L		P		D		L		P	
Control	R	N	R	N	R	N	R	N	R	N	R	N	R	N	R	N	R	N	R	N	R	N	R	N
1			20	20					19	19	1	1			20	18		2	20			18		2
2			20	19		1			20	18		2			20	18		2	20			17		3
3		1	20	19					19	18	1	2			20	16		4	19		1	20		
4			20	20					20	17		3			29	20	1		20			19		1
Test																								
5	19	1	1	19			20			20			20			18		2	20			19		1
6	20			20			20			17		3	20			17		3	19		1	18		2
7	19	2	1	17		1	20	1		15		4	20			20			20	1		17		2
8	20	1		17		2	20	1		16		3	20			18		3	20	2		18		
9	20			20			20	2		18			20			16		4	20	1		18		1
10	19	3	1	17			20	1		18		1	20			19		1	29		1	20		
11	20			19		1	20			19		1	20			20			20			17		3
12	18	1	2	19			20			20			20			17		3				19		1
% mortality	5,12						3,37						0						2,66					

[1]D=dead larvae; L=living larvae; P=pupae / R=Rockefeller strain; N=natural population.

Table 2. Mortality of *Aedes aegypti* larvae after submitted to diagnostic dose of temephos in municipalities from Brazil.

Region	State	Municipality	% mortality	Citation
Northern	AM	Manaus[1]	100.0	Pinheiro & Tadei, 2002
Northeastern	AL	Maceió	20.0	Braga & Lima, 2004
		Arapiraca	35.3	
	CE	Barbalha	5.3	Lima et al, 2006
		Crato	4.0	
		Fortaleza	0.0 – 28.0	
		Juazeiro do Norte	0.0 - 1.3	
	PB	Boqueirão[2]	4.8	Beserra et al, 2007
		Brejo dos Santos	5.6	
		Campina Grande	28.0	
		Itaporanga	4.0	
		Remigio	13.6	
	SE	Aracajú	27.5	Braga & Lima, 2004
		Barra dos Coqueiros	26.7	
		Itabaiana	7.1	
Federal District	FD	Ceilândia	66.4	Carvalho et al, 2004
		Planaltina	61.5	
		Sobradinho	44.4	
		N. Bandeirantes	56.4	
Southeastern	ES	Cariacica	93.8	Lima et al, 2003
		Vila Velha	45.5	
		Vitória	93.1	
	MG	Coronel Fabriciano	2.7	Present study
	RJ	Campos de Goytacazes	74.0	Lima et al, 2003
		Campos de Goytacazes	61.9	Braga & Lima, 2004
		Duque de Caxias	34.8	Lima et al, 2003
		Duque de Caxias	53.1	Braga & Lima, 2004
		Niterói	34.6	Lima et al, 2003
		Niteroi	44.0	Braga & Lima, 2004
		Nova Iguaçu	58.2	Lima et al, 2003
		Nova Iguaçu	24.6	Braga & Lima, 2004
		Rio de Janeiro	32.3	Lima et al, 2003
		São Gonçalo	23.5	Lima et al, 2003
		São Gonçalo	10.8	Braga & Lima, 2004
		São João de Meriti	44.0	Lima et al, 2003
		São João de Meriti	33.1	Braga & Lima, 2004
	SP	Santos	79.0	Macoris et al, 2003
		Campinas[3]	21.4	Campos & Andrade, 2003
Southern	PR	Curitiba	90.0	Luna et al, 2004
Centerwestern	MT	Cuiabá	100.0	Campos & Andrade, 2003

[1]Results refer to observations after 24hs and tests were made using plastic buks, tin cans and tires. Temephos used with formulation of 1ppm; [2]Data from Paraiba State refer to those collected in June 2004; [3]Natural F1 population LC_{95}

resistance to temephos in Fortaleza city and it's likely to be widespread throughout state of Ceará. Mosquito populations from municipalities have suffered a strong selection pressure by temephos in the last years. The authors still emphasize that temephos has been substituted by spores of *Bacillus thuringiensis* (Bti) in the cities of Fortaleza (2001), Juazeiro do Norte and Caucaia (2002) (Lima *et al.* 2006).

The prospects for dengue control are very much related to the ability of the affected countries to manage the problem of drug resistance. This will require a holistic approach since the evolution of drug resistance has originated from an interaction of various processes, several of which are outside the health sector and involve ecological and social factors. The evolution of resistance is characterized by common features: use of chemicals in inadequate doses, presence of high selection pressure on the local vector populations; high levels of transmissions through a very efficient vector; large scale uncontrolled population movements of non-immune labour forces, limited resources and operational difficulties that prevented the implementation of adequate control activities (Breckiling & Ekschmitt, 1999).

Genetic variability might explain the observed differences among *Ae. aegypti* populations when submitted to temephos test. In addiction, factors like migration and the amount of drug applied above the recommended levels might have impact over the populations and influence the raising of resistance. Migration events might take part when susceptible mosquitoes from treated areas escape from pesticide treatment and the entry of susceptible individuals into the treated area or the escape from insecticide exposure within the treated area, have been considered in most models of resistance management (Georghiou & Taylor 1977; Lenormand & Raymond, 1998). Resistant mosquitoes are likely to be migrating from neighboring regions like Rio de Janeiro and São Paulo, where resistance has long been detected, to Minas Gerais state.

The resistance management strategies require the description of both resistance gene frequencies and population densities, through time and space. The management must take into account gene flow or its geographic scale, the scale of the treated and untreated areas relative to the scale of gene flow and the fitness of resistance genotypes in the absence of insecticides (Lenormand & Raymond, 1998). An optimistic goal of management could be to maintain resistance genes at low and stable frequencies, especially when such genes are already present and no alternative pesticides are available.

Dengue control programs should provide, meaning of information for communities as well as for programme managers and policy makers (WHO, 2003). Even when vector control efforts may seem effective the real values of dengue cases and mosquito densities may be unknown or far from the official notifications. The Stegomyia indices (the House, Container, and Breteau indices, and various related derivations) are of some operational value for measuring the entomological impact of larval control interventions against the mosquito vectors of dengue virus, they are not proxies for adult vector abundance. Neither are they useful for assessing transmission risk because they do not take into consideration the epidemiologically important variables, including adult vector and human abundance, temperature, and seroconversion rates in the human population.

The current methods of mosquito control, focuses on suppression strategy designed to minimize costs or to improve sustainability by targeting only a subset of the breeding containers for control or elimination, specifically those container types that are responsible for the majority of adult production. Dengue transmission risk assessment should be made from operational tools which should provide useful information for planning and management of vector control programmes (WHO, 2003). Today, most dengue control efforts are based on suppression of *Ae. aegypti* and not on eradication (WHO, 2003).

The reasons for emergence of dengue as one of major public health problem in Brazil and worldwide are complex and not well understood. However, several important factors can be identified. First, effective mosquito control is virtually nonexistent. Considerable emphasis for the past 20 years has been placed on ultra-low-volume insecticide space sprays for adult mosquito control, a relatively ineffective approach for controlling *Ae. aegypti*. Second, major demographic changes have occurred, the most important of which have been uncontrolled urbanization and concurrent population growth. These demographic changes have resulted in substandard housing and inadequate water, sewer, and waste management systems, all of which increase *Ae. aegypti* population densities and facilitate transmission of *Ae. aegypti*-borne disease. Third, increased travel by airplane provides the ideal mechanism for transporting dengue viruses between population centers of the tropics, resulting in a constant exchange of dengue viruses and other pathogens. Lastly, the public health infrastructure is not ideal yet. Limited financial and human resources and competing priorities have resulted in “crisis of public health” with emphasis on implementing so-called emergency control methods in response to epidemics rather than on developing programs to prevent epidemic transmission (Gubler & Clark, 1995). This approach has been particularly detrimental to dengue control because, in most countries, surveillance is very inadequate; the system to detect increased transmission normally relies on reports by local physicians who often do not consider dengue in their diagnoses (Guber & Clark, 1995). As a result, an epidemic has often reached or passed the peak of transmission before it is detected.

The level of susceptibility in relation to the diagnostic concentration tests suggest that a system of constant monitoring of the *Ae. aegypti* population should be rapidly implemented. The results confirm the need for preventive strategies and alternative control methods that might diminish the selection of resistance. The selection process appears to have begun within this population. The results for temephos indicate that this product should no longer be utilized within several municipalities of Brazil like Coronel Fabriciano. They suggest that there is a need for immediate replacement of this insecticide by some other product of chemical or biological origin.

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Received: 19/XII/2007 Accepted: 28/VI/2009 Published: 30/VIII/2011 Available online: www.bioassay.org.br/ojs/index.php/bioassay/article/view/103