

Research Article

Temephos resistance levels in populations of *Aedes aegypti* (Diptera: Culicidae) from Havana, Cuba

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Abstract

Aedes aegypti chemical control remains an indispensable alternative to prevent dengue, Zika, and Chikungunya outbreaks. Havana City requires constant surveillance due to its bioecological characteristics favor the proliferation of mosquito vectors of these diseases, which constitutes a high risk to the health of its inhabitants. The goal of this study was to determine temephos resistance levels in the populations of *Ae. aegypti* from five municipalities of Havana. The susceptibility of the larvae was evaluated by bioassays described by the World Health Organization. *Aedes aegypti* populations evaluated showed high resistance to temephos, with values that oscillated for the FR_{50} between 26,8 and 82,5 and for the FR_{90} between 16,6 and 42,5 respectively. The National Control Program of *Aedes aegypti* in Cuba must promote insecticide rotation policies to avoid or prevent the evolution of temephos resistance in Havana. In addition, an evaluation of the Abate doses applied by the operators in the municipalities studied must be carried out, since this could be influencing resistance development due to operational factors.

Introduction

The approach for the control of *Aedes aegypti* (L) since the last century is mainly based on environmental sanitation, through community participation and the application of chemical insecticides due to the presence of larval stages in a variety of natural and artificial containers [1,2].

Temephos (organophosphate) is the most widely used pesticide to control *Ae. aegypti* larvae stages [3-5]. Its intensive use has generated insecticide resistance development in

different *Ae. aegypti* populations associated with metabolic action enzymes in some countries such as Brazil [6], the Martinique Islands [7] and India [8].

The first report of temephos resistance in Cuba was in 1997, with the occurrence of a dengue outbreak in Santiago de Cuba [9]. Temephos resistance in *Ae. aegypti* from Cuba was characterized for the first time through the selection of a temephos-resistant reference strain and it was shown that the mechanism of metabolic action, based on the activity of the enzymes α and β esterases, glutathione s-transferase

and monooxygenases were responsible for resistance to this insecticide; although the highest level of correlation of resistance to temephos resulted with esterases, specifically A4 [10]. This esterase has also been identified, as associated with temephos resistance in *Ae. aegypti* strain from other Latin American countries [11–13].

In a study carried out in Havana, it was shown that only esterase enzymes play an important role in temephos resistance [14] and later it was possible to show that the reversal of temephos resistance was possible due to the mechanism involved in the appearance [15]. Temephos resistance is a phenomenon that has been evolving rapidly, which constitutes a threat to vector control, therefore it is considered to be an issue that needs greater attention in Cuba due to the continued use of this larvicide as part of the interventions carried out by the national control program of *Ae. aegypti* and *Aedes albopictus* were established in Cuba in 1981.

The objective of this work was to determine the temephos resistance status in *Ae. aegypti* populations belonging to five municipalities of Havana, Cuba after more than 40 years of insecticide use.

Methods

Study area

The selected study area to evaluate temephos resistance status in *Ae. aegypti* population was five municipalities (Playa, Diez de Octubre, Arroyo Naranjo, Marianao, and La Lisa) in Havana province (Figure 1). Playa is located north and west of the capital $23^{\circ} 05' 39''$ N $82^{\circ} 26' 56''$ W and covers a total area of 35 km². Diez de Octubre is located west of the capital at $23^{\circ} 05' 17''$ N $82^{\circ} 21' 35''$ W and covers a total area of 12.27 km². Arroyo Naranjo is located at $23^{\circ} 02' 37''$ N $82^{\circ} 19' 58''$ W and

covers a total area of 83 km². Marianao is located at $23^{\circ} 05' 00''$ N $82^{\circ} 26' 00''$ W and covers a total area of 22 km². La Lisa is located on the western outskirts of the capital at $23^{\circ} 01' 29''$ N $82^{\circ} 27' 47''$ W and covers a total area of 37.5 km².

Biological material collections were carried out using ovitraps. The collection was carried out in the field from January to June 2022 by personnel from the National Control Program during their routine activities following the sampling protocols established in Cuba for this program [16].

Ae. aegypti strains

Bora–Bora: *Aedes aegypti* susceptible strain collected in (French Polynesia), free of any detectable resistance mechanism, provided by the Institute Pasteur de Guadeloupe. This strain was used as a control strain in the bioassays performed.

Ae. aegypti colonies maintenance

Aedes aegypti colonies were maintained under the conditions prevalent in the insectarium of the Department of Vector Control in the Institute of Tropical Medicine “Pedro Kourí” (IPK); temperature of 25 ± 2 °C, relative humidity of $75 \pm 2\%$ and a photoperiod of 12:12 (Light and Dark) hours were maintained [17]. Larvae for the evaluation were obtained from the F1 generation from eggs collected in each municipality studied. For hatching, the eggs (contained in strips of paper) were placed in 29 x 20 x 4 cm plastic trays with one liter of dechlorinated water to which a minimum amount of larval diet was added, as a stimulus for microbial activity that guaranteed favorable conditions for the hatching of the larvae. Each tray was identified with the name of the strain (place and date of collection). Once the hatching of the first instar larvae was evidenced, and 48 hours after the activation of the eggs, the

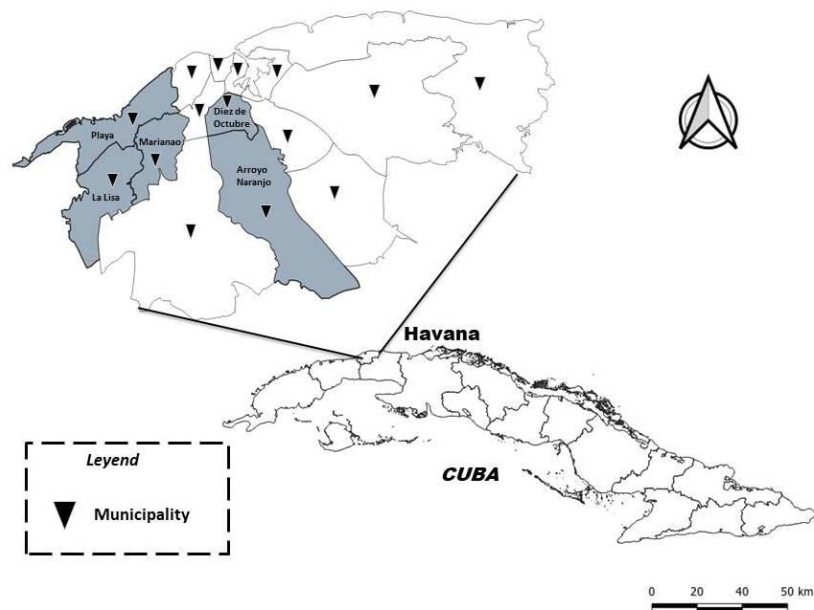


Figure 1: Municipalities from Havana province where *Ae. aegypti* larvae collection was carried out in this study.

paper was carefully removed, avoiding the elimination of recently emerged larvae due to their small size attached to the paper. *Aedes aegypti* colonies maintenance was performed following the established methodology [18]. The morphological characterization of the larvae was carried out in the Vector Control Department of the Pedro Kourí Institute of Tropical Medicine (IPK) using taxonomic keys [18].

Insecticide used

- Temefos 94% purity, supplied by Chemotécnica SA, Buenos Aires, Argentina

Larvae bioassays

The level of susceptibility to the insecticide temephos was evaluated in larval bioassays [19]. Five concentrations of the insecticide were used with four repetitions each and a control by concentration. Twenty-five late third instar and/or early fourth instar larvae of uniform size were placed in plastic cups containing 99 ml of tap water and 1 ml of prepared insecticide solution. Controls contained 99 ml of tap water and 1 ml of acetone. In total (not counting controls), 100 larvae were evaluated for each concentration, and 500 larvae were analyzed for each bioassay. Mortality was determined 24 h after insecticide application.

Data analysis

The results of the larval bioassays were analyzed using the Probit test implemented in the statistical program SPSS Version 21. The Resistance Ratio (RR_{50}) was calculated by comparing the value of lethal concentrations (LC_{50} and LC_{90} : lethal concentrations that cause 50 and 90% mortality) of field colonies with the Bora Bora strain. Populations were classified as resistant or susceptible using the following criteria: $RR_{50} \leq 5$: Susceptible, $5 < RR_{50} \leq 10$: Moderate resistance, and $RR_{50} > 10$: High resistance [20].

Results

Late third instar and/or early fourth instar larvae from F1 generation of 5 *Ae. aegypti* populations from Havana province were tested.

Values of the lethal concentrations (LC_{50} and LC_{90}), the resistance ratios (RR_{50} and RR_{90}), and the slope of the probit regression lines for the organophosphate temephos in these *Ae. aegypti* populations are shown below (Table 1 and Figure 2). The results showed that all populations showed high resistance ($RR_{50} > 10$) to temephos, with three municipalities (Arroyo Naranjo, Diez de Octubre, and Playa) showing values $26 < RR_{50} < 43$ (Table 1 and Figure 3). The Marianao population was the notable exception to be considered the highest resistant ($RR_{50} > 80$) of all colonies (Table 1 and Figure 3).

Although high resistance was found in the five municipalities of Havana province, it behaved at heterogeneous levels (Figures 2,3). Mosquito populations of La Lisa ($RR_{50} = 42.5$, $RR_{90} = 29.44$) and Arroyo Naranjo ($RR_{50} = 34.75$, $RR_{90} = 28.88$) municipalities showed similar resistance ratios (RR), although

they were higher than those obtained by Diez de Octubre ($RR_{50} = 30$, $RR_{90} = 16.94$) and Playa ($RR_{50} = 26.87$, $RR_{90} = 16.66$) the populations (Table 1 and Figure 3). The resistance ratios in these two *Ae. aegypti* populations (Diez de Octubre and Playa) were also similar to each other. The Temephos resistance level of the Marianao population ($RR_{50} = 82.5$, $RR_{90} = 42.5$) was heterogeneous in relation to the rest of the field colonies (Table 1 and Figure 3).

The slope values of the probit regression lines of the field colonies were higher than the Bora Bora strain. Marianao and Diez de Octubre populations showed the highest slope values (slope = 3.5 and 3.1) (Table 1 and Figure 2). This result confirms a homogeneous response to temephos resistance in *Ae. aegypti* population from both municipalities.

Discussion

The indiscriminate use of chemical insecticides causes resistance in mosquitoes of medical importance and is one of the factors that reduce the chances that vector control programs will be successful [21]. The emergence of resistance to insecticides is a complicated phenomenon involving physiological, genetic, ecological, and behavioral factors combined with insecticide application [22].

Temephos resistance has been reported in some regions of India [5,23], Colombia [24], Martinique [25], and Brazil [26–29]. However, susceptible mosquito populations have been detected in Malaysia [30], Cape Verde [31] and Thailand [32]. Recently, different levels of resistance to temephos were reported in *Ae. aegypti* populations from Peru [33].

The first report of temephos resistance in an *Ae. aegypti* population from Cuba occurred in 1997 and coincided with an outbreak of dengue in the municipality of Santiago de Cuba, located in the eastern part of the country [9]. Temephos resistance was also reported in *Ae. aegypti* populations from Guanabacoa and Playa municipalities during the dengue epidemic in Havana, in 2001–2002 [34,35]. Subsequently, resistance was confirmed in Boyeros municipality in 2006 [36]. A study carried out in 2008 reported high resistance to temephos in *Ae. aegypti* populations in 15 municipalities of Havana induced by its intensive use [14]. Besides *Ae. aegypti* has developed resistance worldwide induced by selective pressure due to mismanagement of temephos, causing variations in the susceptibility of mosquito populations, as reported in Colombia [37], Guadalupe Islands, Saint Martin [38], and Brazil [39].

The results of this study showed high resistance ($RR_{50} > 10$) to temephos in all populations evaluated with an increase of the resistance ratio in Marianao ($RR_{50} = 82.5$ and $RR_{90} = 42.5$) and Playa ($RR_{50} = 26.87$ and $RR_{90} = 16.66$), and a slight reduction in Arroyo Naranjo ($RR_{50} = 34.75$ and $RR_{90} = 28.88$), Diez de Octubre ($RR_{50} = 30$ and $RR_{90} = 16.94$) and La Lisa ($RR_{50} = 42.5$ and $RR_{90} = 29.44$) compared to the evaluation carried out in 2008 [14]. High levels of temephos resistance have been reported in Acre (Brazil) [40], Tamil Nadu (India) [41], Caldas (Colombia) [42], Pernambuco (Brazil) [43], Martinique [44] and Bahia (Brazil) [45]. Some studies have reported moderate levels of temephos



Table 1: Temephos resistance level expressed as resistance ratio (RR₅₀ and RR₉₀), calculated from the insecticide concentration that caused 50% (LC₅₀) and 90% (LC₉₀) of mortality in *Ae. aegypti* populations from 5 municipalities in Havana province, from January to June 2022.

<i>Ae. aegypti</i> colonies	^a LC ₅₀ (ppm)	^b RR ₅₀	^a LC ₉₀ (ppm)	^b RR ₉₀	^c b (±SD)	Susceptibility levels
Marianao	0.66 (0.4 - 1.2)	82.5	1.53 (0.94 - 12.4)	42.5	3.5 (±0.35)	High Resistance
Arroyo Naranjo	0.278 (0.169 - 0.419)	34.75	1.04 (0.648 - 2.786)	28.88	2.23 (±0.16)	High Resistance
Playa	0.215 (0.115 - 0.351)	26.87	0.6 (0.365 - 1.983)	16.66	2.87 (±0.2)	High Resistance
Diez de Octubre	0.24 (0.199-0.284)	30	0.61 (0.5-0.81)	16.94	3.1 (±0.18)	High Resistance
La Lisa	0.34 (0.177 - 0.618)	42.5	1.06 (0.593 - 6.473)	29.44	2.6 (±0.22)	High Resistance
Bora Bora	0.008 (0.006 - 0.01)		0.036 (0.024 - 0.069)		1.90 (±0.15)	Susceptible strain

Number of larvae evaluated: 500 in each colony. ^a Lethal concentration (LC₅₀ and CL₉₀) in mg /L, 95% confidence limits (CL) in parentheses. ^b Resistance ratio (RR₅₀ and RR₉₀): LC₅₀ or LC₉₀ strain to be evaluated / LC₅₀ or LC₉₀ Bora Bora strain. ^c Slope (b) of the Probit-log line, standard deviation (±SD) in parentheses.

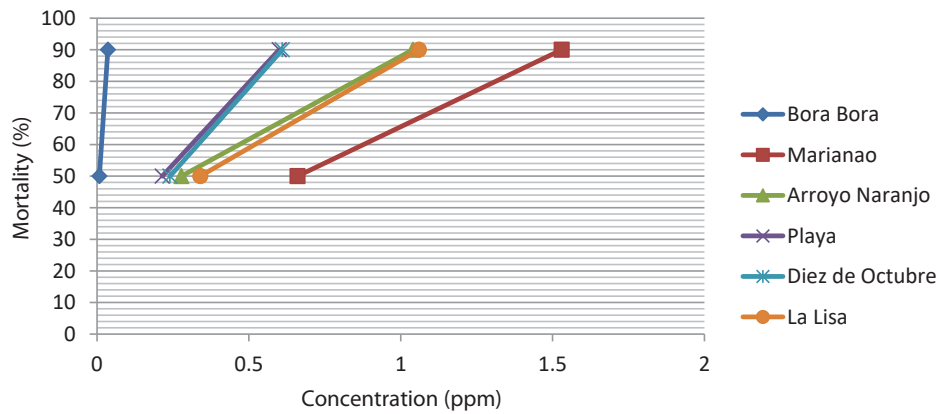


Figure 2: Linear regression of *Ae. aegypti* mortality after exposure to the organophosphate temephos in field colonies from Havana province compared to the susceptible Boa Bora strain.

Temephos resistance level

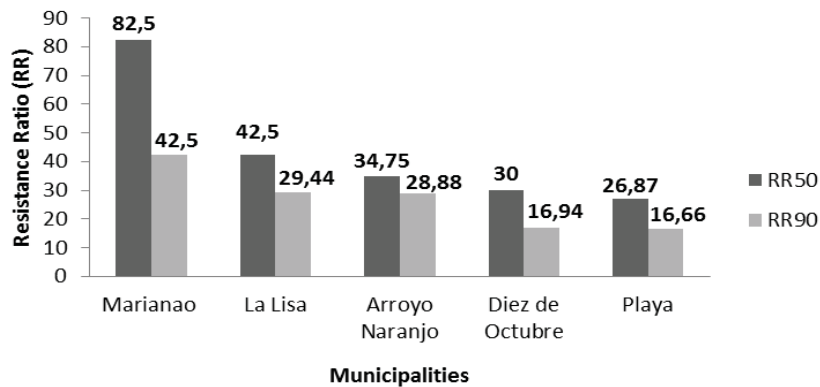


Figure 3: Resistance factor (RR₅₀ and RR₉₀) to organophosphate temephos of the *Ae. aegypti* colonies collected in five municipalities of Havana, 2022.

resistance Tocantins (Brazil) [46], Laos (Asia) [47], Paraná (Brazil) [48], Quindío (Colombia) [49], Delhi (India) [50], Sao Paulo and Northeast Region (Brazil) [51]. However, temephos susceptibility was reported in Malaysia [52], Santiago Island (Cape Verde) [53], and Phitsanulok province in Thailand [54]. The slope values obtained by the regression lines (Table 1) reflected that temephos resistance was more homogeneous in the populations of Marianao and Diez de Octubre. The National Vector Control Program applies larvicide monthly in all locations of the country throughout the year, the frequency of which can vary from one month to every 11 days in the case

of dengue outbreaks [14]. This explains that the different resistance values are not due to variation in the intensity and frequency of the temephos use, since the control actions carried out by the Campaign were homogeneous for all the municipalities studied. Contrariety these aspects were decisive in the results obtained in Peru [33].

However other factors that could influence the different resistance values of the municipalities are the geographical location of the studied areas and the operational factors related to the incorrect use of the insecticide. Marianao is a



highly urbanized municipality that shares land borders with several Havana municipalities where there is a constant flow of people and goods that favor the dispersal of mosquitoes from one place to another with different levels of resistance to insecticides, contrary to La Lisa which it is one of the most peripheral municipalities of the capital. This factor may have influenced the results of this study as reported in Peru [33].

It is known that as the slope is greater, resistance is more homogeneous in the population, that is, they have the same genes and are in the same proportions in individuals [55]. The second factor should be taken into account by the executives of the program in these municipalities, evaluating the quality of the control actions, since an excess or decrease in the doses of temephos applied by the operators could explain this heterogeneity in the resistance values found [22].

On the other hand, the high levels of temephos resistance in *Ae. aegypti* are of great concern for the control actions carried out by the National *Ae. aegypti* Control Program, which should be carried out more frequently, since some authors have reported a significant decrease in the residual effect of temephos in highly resistant mosquito populations (RR > 10) being effective for a period of 13 days while with susceptible populations (RR < 10) it has been effective for 18 days [14,56].

The results of this study demonstrate the urgent need to implement new integrated strategies, such as those using alternative insecticides *Bacillus thuringiensis var israelensis* (Bti) or pyriproxyfene (growth inhibitor) to avoid the continued increase of temephos resistance in these municipalities. A successful strategy carried out in the capital municipality of Boyeros showed how the resistance levels decreased in the *Ae. aegypti* populations when the temephos application was discontinued and replaced with Bti [2]. Similar results were obtained in Brazil using this biological control and using growth inhibitors [57–59]. It has also been shown that temephos susceptibility can be recovered because its metabolic resistance mechanisms are reversed when its use is discontinued [6,15]. Preferably, these strategies could be carried out by promoting insecticide rotation policies to preserve the effectiveness of insecticides [60].

The main limitation of this study was that the bioassays of susceptibility to temephos could not be carried out in the populations of the 15 municipalities of Havana because the capacity of our insectary is very limited to work with so many colonies. For this reason, the results could not be compared with studies previously carried out in other municipalities. The following studies will be aimed at determining temephos resistance status and its resistance mechanisms in the rest of the municipalities of Havana.

The recommendations for the National *Ae. aegypti* Control Program would be to apply strategies replacing temephos to the larvicide Bti for a certain period to eliminate selection pressure and reduce resistance levels in *Ae. aegypti* population from Havana province.

Conclusion

This study shows high levels of temephos resistance in

the 5 evaluated *Ae. aegypti* populations from Havana province. This finding confirms that the implementation of resistance management by the National *Ae. aegypti* Control Program in Cuba is crucial to reversing the evolution of temephos resistance by eliminating selection pressure for a given period that allows mosquito populations to recover susceptibility to this larvicide.

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