SUSCEPTIBILITY OF *AEDES ALBOPICTUS* FROM CHINA TO INSECTICIDES, AND MECHANISM OF DDT RESISTANCE[1]

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ABSTRACT. *Aedes albopictus* collected from urban districts of China (12 strains) has developed high levels of DDT resistance, while 2 strains collected from rural areas remain susceptible. The mechanism of DDT resistance was due to intensified activity of DDT-dehydrochlorinase, but the penetration of DDT into the mosquito body is also different. This may possibly have had some influence on the resistance levels of some strains, but it seems unlikely that was the main mechanism of DDT resistance.

INTRODUCTION

Aedes albopictus (Skuse) is an important vector of arboviruses, especially dengue, and also a nuisance mosquito. It is presumed to have originated in southeast Asia, and is now widely distributed in an area from Madagascar westward through the Pacific islands, to the USA (Rai 1986).

After the 1980 dengue fever incidence in some coastal areas of the People's Republic of China, a national co-operative group was set up to determine the susceptibility of vectors to insecticides. The present paper reports the susceptibility of *Aedes albopictus* to insecticides and the mechanism of DDT resistance.

MATERIALS AND METHODS

Susceptibility determination: The insecticides tested were DDT, lindane, malathion, fenitrothion, temephos, trichlorfon, deltamethrin, permethrin, Sumithrin, resmethrin and tetramethrin. Acetone solutions of each insecticide were prepared and 5–6 different dilutions of each were used. Distilled water was added to each dilution to make a volume of 200 ml which was placed in an enamel bowl. The quantity of acetone was adjusted to equal 1% on a volume basis; a 1% acetone solution was used as control. Tests at each concentration were duplicated.

One hundred early fourth-instar larvae, from colonies which had been maintained in our laboratory for 2–4 generations, were immersed for 24 h in the various insecticides solutions. A larva which was unable to swim to the surface of the water was used as the criterion for death. Mortalities were corrected by Abbott's formula and data were subjected to probit analysis (Finney 1952). Temperature and relative humidity for mosquito rearing and also insecticidal testing were 25–26°C and 80%, respectively.

Activity of DDT-dehydrochlorinase determination: Fourth-instar larvae were immersed in 200 ml of 8 ppm DDT solution for 6 h, after which all larvae were removed and washed with detergent solution, distilled water, acetone and again distilled water to remove DDT from the body surface. The larval samples were then homogenized individually with 1 ml distilled water and the homogenate transferred to a test tube. The homogenizer was washed 3 times with 2 ml distilled water and the wash combined with the homogenate, which was then extracted with 4 ml petroleum ether by shaking for 2 min and discarding the water layer. The petroleum ether layer was quantified and stored in a refrigerator, or immediately analyzed.

Newly emerged female mosquitoes were placed in contact with filter papers impregnated with 4 g DDT/m^2. After 6 hours the mosquitoes were anaesthetized with ether, and then treated in the same manner as the larvae; 30–50 mosquito larvae or adult of each strain were used to determine the activity of DDT-dehydrochlorinase.

One to 5 microliters of extracts were injected into the gas chromatography column to determine the DDT and DDE content in the mosquito tissue (McDonald and Wood 1979). The gas chromatograph used was a Perkin-Elmer F-17 with an ECD detector; stationary phase: 1.5% OV-17 + 2.5% QF-1, support chromosorb: WHP 80/100, glass column, 2 m × 2.5 mm, column temperature: 200°C, detector temperature: 250°C, carrier gas: nitrogen at high purity, 99.99%.

RESULTS

Fourteen geographical strains of *Aedes albopictus* were collected and their susceptibilities to insecticides determined (Table 1). Their susceptibility to DDT varied, two rural strains (Jiangsu and Yunnan) were more susceptible than strains

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Table 1. Susceptibility of *Aedes albopictus* to insecticides.

Location city/(province)	LC_{50} value (ppm)										
	Lindane	DDT	Trichlorfon	Malathion	Fenitrothion	Temephos	Permethrin	Deltamethrin	Sumithrin	Resmethrin	Tetramethrin
Chengzhou (Hunan)	0.597	12.048	0.218	0.194	0.019	0.017					
Fuzhou (Fujian)	0.356	13.566	0.121	0.209	0.019	0.018					
Zhengzhou (Henan)	0.275	20.408	0.085	0.234	0.023	0.017					
Chengdu (Sichuan)	0.403	5	0.184	0.165							
Shiping (Yunnan)	0.138	0.036	0.093	0.185	0.013	0.009	0.003	0.0019	0.0465	0.0273	
Yixin (Jiangsu)	0.101	0.016	0.105	0.161	0.020	0.005	0.004	0.0007	0.0945	0.0309	
Fushan (Guangdong)	0.366	13.940	0.153	0.174	0.027						
Guangzhou (Guangdong)	0.175	0.865	0.115	0.133	0.022	0.007	0.004	0.0007	0.0364	0.0352	
Haikou (Hainan)	0.328	1.222	0.189	0.238	0.022	0.010	0.018	0.0012	0.1384	0.0226	
Shanghai	0.079	1.359	0.088	0.140	0.018	0.005	0.014	0.0007	0.1341	0.0299	
Xi'an (Shaanxi)	0.229	4.682	0.262	0.186	0.016	0.005	0.663	0.0030	0.0246		
Guiyang (Guizhou)	0.176	3.918	0.278	0.222	0.024	0.009	0.008	0.0013	0.0028		
Beihai (Guangxi)		4.186					0.007	0.0020	0.0809		0.614
Nanning (Guangxi)		3.212	0.155	0.157	0.019	0.017	0.013	0.0035	0.0704	0.0289	0.817
Wuming (Guangxi)							0.009	0.0019	0.0469		0.922

collected from urban districts. The susceptibilities to lindane and organophosphate insecticides differed little, while the susceptibility of the Xi'an strain to permethrin decreased.

The activity of DDT-dehydrochlorinase was expressed as:

Degradation rate %
= (DDE/DDT penetrated) × 100

The total DDT (penetrated DDT), metabolized DDT (DDE) and the degradation rate of mosquito larvae and adults of 5 strains of *Aedes albopictus* are shown in Table 2. There was significant correlation between the LC_{50} value and activity of DDT-dehydrochlorinase of both larvae and adult mosquitoes, but the total amount of DDT penetrated into the mosquito was not significantly related to the LC_{50}. To further show the relationship between the LC_{50} and activity of DDT-dehydrochlorinase, 14 pairs of data are shown in Table 3. The LC_{50} and activity of DDT-dehydrochlorinase were plotted as the abscissa (X) and the ordinates (Y), respectively, but with the X and Y axes changed in different ways, after which a series of regression analyses were calculated. It was found that the maximum correlation coefficient arose when the reciprocal of the X and Y axes was plotted. In this case the relationship between the LC_{50} and activity of DDT-dehydrochlorninase was a hyperbola.

The frequency distribution of activity of DDT-dehydrochlorinase of the susceptible strain (Yixin) was concentrated in the low region of the activity spectrum that is positive skewed. In contrast, the resistant strain (Shanghai) was negatively skewed and so occupied the high region, while the strain having a medium level of resistance (Nanning) exhibited a more normal frequency distribution in the middle of the spectrum (Fig. 1).

DISCUSSION

Since the 1950s DDT and HCH have been used as the main insecticides for vector control in China, with HCH being more popular than DDT for sanitary purposes in residential areas. At the end of the 1970s, HCH was banned for agricultural and medical purposes. DDT is still used for malaria control, but only as a residual spraying agent on the inside surface of the wall of houses and animal shelters, and use is confined to the southern part of China where the main vector of malaria (*Anopheles minimus* Theobald) has not yet developed resistance to DDT.

Table 2. Regression coefficients (r) between the LC_{50} value of DDT of 5 strains of *Aedes albopictus* and other parameters.

Location	Jiangsu	Yunan	Guangzhou	Hainan	Shanghai	r-value
LC_{50} value of DDT (ppm)	0.015	0.036	0.865	1.197	1.492	
Degradation rate of larvae %	4.70	6.99	31.84	58.71	66.30	0.99**
DDT in the body nM/larva	1.031	0.069	0.368	0.059	0.088	−0.56
Total DDT in the body nM/larva	1.082	0.074	0.473	0.143	0.261	−0.21
DDE in the body nM/larva	0.051	0.005	0.151	0.084	0.173	0.83
Degradation rate of adult %	2.18	6.00	26.04		81.44	0.96**
DDT in the body nM/adult	0.180	0.057	0.038	0.038	0.023	−0.72
Total DDT in the body nM/adult	0.184	0.061	0.051	0.038	0.091	−0.48
DDE in the body nM/adult	0.004	0.004	0.013		0.074	0.89

** $P = 0.01$.

Table 3. Fourteen paired comparisons of the LC_{50} and DDT-ase activity from 8 strains of *Aedes albopictus*.

Location	LC_{50} of DDT (ppm)	DDT-ase activity (%)
Yixin (Jiangsu) (1)	0.015	4.70
Yixin (Jiangsu) (2)	0.016	4.42
Shiping (Yunnan) (1)	0.036	6.99
Shiping (Yunnan) (2)	0.036	6.73
Guangzhou (Guangdong) (1)	0.856	27.63
Guangzhou (Guangdong) (2)	0.865	31.84
Haikou (Hainan) (1)	1.197	57.24
Haikou (Hainan) (2)	1.222	58.71
Shanghai (1)	1.492	66.30
Shanghai (2)	1.359	64.94
Shanghai (3)	1.492	75.25
Nanning (Guangxi)	3.212	62.40
Guiyang (Guizhou)	3.912	60.13
Xi'an (Shaanxi)	4.682	68.83

Generally, *Aedes albopictus* was neglected in mosquito control programs in China; that is until 1980 when its importance as a dengue vector was shown in outbreaks in some coastal areas of Guangxi and Guangdong provinces. Aerial spraying with dichlovos, fenitrothion and some pyrethroids was the most popular method for controlling *Aedes albopictus* and *Aedes aegypti* (Linn.), with particular attention to public places such as schools, hospitals, hotels and theaters as well as gardens and parks. DDT was not used to control either *Aedes* species at that time. From the 1950s to 1970s, the nation-wide eradication Four Pest Movement (to eradicate mosquitoes, house flies, rodents and roaches) was undertaken. This movement was led by the National Committee on Patriotic and Hygiene Campaign which usually issued the orders for taking unified action. This then produced an extravagant use of insecticides, especially DDT in urban areas. The large scale and intensified use of DDT may have been responsible for *Aedes albopictus* developing resistance to DDT.

The present survey shows that *Aedes albopictus* collected from urban areas possesses a higher level of DDT resistance than strains collected from rural districts. The LC_{50} value of *Aedes albopictus* from Japan was reported as 0.02 ppm by Takeshi and Mizutani (1962) and 0.082 ppm for material collected in Singapore (Ho et al. 1981). Comparing these values with the present ones shows that *Aedes albopictus* strains collected from rural areas of China remain susceptible to DDT. Strains collected from urban dis-

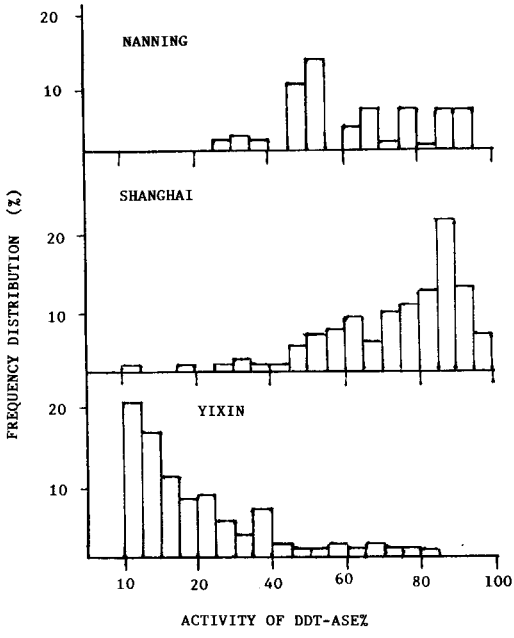

Fig. 1. Frequency distribution of DDT-dehydrochlorinase activity in 3 strains of *Aedes albopictus*.

tricts have developed a high level of DDT resistance, but they are still susceptible to lindane and organophosphate insecticides.

Although no general cross resistance to pyrethroids has been found, the difference in susceptibility to Sumithrin was 49.4 times greater in *Aedes albopictus* from Guiyang (0.0028) than from Haikou (0.138), and *Aedes albopictus* from Shiping (0.0034) was 194.8 times more susceptible to permethrin than the strain from Xi'an (0.662). These 2 pyrethroid insecticides have never been used on a large scale in an operational mosquito control program.

The results clearly show that all DDT-resistant strains of *Aedes albopictus* have higher levels of DDT-dehydrochlorinase activity than susceptible strains, and that with both larvae and adults, there is a significant correlation between the LC_{50} value and activity of DDT-dehydrochlorinase. However, the penetration of DDT into the mosquito body differs between some of the strains. This may have some influence on the resistance levels of some strains, but it seems unlikely that this is a main mechanism of DDT resistance.

There are 2 factors concerning the mechanism of DDT resistance, of which the first is the intensified activity of DDT-dehydrochlorinase; the second is the kdr gene. The kdr gene is related to DDT resistance and usually expresses a cross-resistance to pyrethroids. *Aedes albopictus* collected from Haikou, Shanghai and Xi'an has a high LC_{50} value to Sumithrin and permethrin; in spite of this it also has a high DDT-dehydrochlorinase activity.

From the theoretical point of view, the relationship between the reaction velocity of enzyme and concentration of substrate can best be fitted by a hyperbola. The degradation rate of DDT is directly in proportion to the reaction velocity of the enzyme so that the Y-axis value (activity of DDT-dehydrochlorinase) cannot reach 100%; it can only approach a definite limit, that is the asymptote of a hyperbola.

Using biochemical methods to determine the activity of the detoxification enzyme means that the frequency distribution of this activity can be plotted. From such plots showing the dynamics of the development of DDT resistance in *Aedes albopictus* populations, alternative control strategies can be planned to suppress the development of resistance at an early stage.

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