RESEARCH NOTE

EFFECT OF SUBLETHAL DOSAGES OF MALATHION ON THE ORAL SUSCEPTIBILITY OF *AEDES AEGYPTI* TO DENGUE-2 VIRUS INFECTION

HL Lee[1], Renee A Argubano[2] and Rohani Ahmad[1]

[1]Division of Medical Entomology, Institute for Medical Research, Kuala Lumpur, Malaysia;
[2]Municipal Government of Brooke's Point, Brooke's Point, Palawan, Philippines 5305

Dengue is an acute viral infection and a major public health problem today in many parts of the world, especially Southeast Asia. The vectors are *Aedes aegypti* and *Ae. albopictus* (WHO, 1995). In Malaysia, dengue has been reported since 1902 (Skae, 1902), but the first nationwide outbreak only occurred in 1973 (Wallace *et al*, 1980) and since then, outbreaks have been recurring yearly with increasing incidence rate. In the absence of specific treatment and an effective vaccine, the most important means of dengue control is the interruption of the disease transmission by the vectors such as the use of insecticides. Malathion, an organophosphate is the insecticide of choice in Malaysia since 1973 (Vector Borne Disease Control Program, 1986). The chemical is applied as an adulticide through thermal or ultra-low-volume (ULV) fogging, mostly during an outbreak. However, despite its long history of application, the effect of this chemical on the susceptibility of vectors to dengue infection remains unknown. Here, we report a study to determine the possible effect of sublethal dosages of malathion on the susceptibility of the vector to dengue infection.

Lab-bred 4-5 days old Malaysian strain of *Ae. aegypti* originated from the state of Selangor was used. This strain has been maintained in the insectarium since 1973 and is free from dengue infection. In this experiment, sublethal dosages were defined as those insecticide concentrations inducing mortality of < 50% after an exposure period of 30 minutes. The 30 minute exposure period was chosen to simulate field conditions since it was observed that adult mosquitos were generally killed 30 minutes after fogging with malathion at the recommended dosage. Adults were exposed for 30 minutes to 0.005%, 0.01%, 0.05% and 0.1% respectively of malathion-impregnated filter paper inside plastic tubes obtained from WHO insecticide test kits. These concentrations had been found to induce < 50% mortality in earlier trials. Each concentration and control was replicated 6 times. The surviving mosquitos were immediately transferred to clean paper cups and provided with pledgets soaked in 10% sucrose suspension. After a 12-hour holding period, surviving mosquitos were starved for 24 hours. They were then membrane-fed on a suspension containing 1 ml human blood mixed with 100 µl D-2 virus in C6/36 culture fluid. The D-2 virus originated from a human patient recently and has been maintained in a C6/36 cell line. After an incubation period of 10 days, surviving mosquitos were immobilized by chilling, pooled (< 30/pool) accordingly and homogenized in cell growth medium. The homogenate was inoculated into C6/36 clone of *Ae. albopictus* cell line and incubated for 4-7 days. The cells were harvested and transferred onto 12-welled slides for the preparation of smears. The presence of dengue-2 virus was determined by the peroxidase-antiperoxidase staining method (Maneekarn *et al*, 1993).

The vectorial capacity of the survived adults of *Ae. aegypti* exposed to sublethal dosages of malathion was not impaired (Table 1). Many pools of surviving adults after challenge with malathion were positive for D-2 virus and the minimum infection rates ranged from 26.8 to 41.1. It appeared that there was no apparent correlation of MIR to exposure to malathion dosages. It is thus obvious that as long as the vectors survived the initial sublethal effect of malathion, infected mosquito can continue to support the development of the virus. There has been a lack of information on the effect of sublethal dosages of insecticides on the mosquito susceptibility to viral agents and as such, no comparison can be made. On the other hand, studies reported by various workers showed that sublethal treatment with various insecticides has little effect on the

Table 1

Effect of sublethal dosages of malathion on oral susceptibility of *Aedes aegypti* to dengue-2 virus.

Test concentration of malathion (%)	Total no. mosquitos tested	Mortality (%)	No. pools available for virus detection	No. +ve pools	Minimum infection rate
0	360	15.0	7	6	29.3
0.005	360	42.7	7	6	36.1
0.01	360	42.5	8	6	36.1
0.05	360	45.8	7	4	26.8
0.1	360	46.9	8	6	41.1

susceptibility of culicine and anopheline mosquitos to malarial and filarial parasites (Mohan, 1955; Khalil *et al*, 1975; Prasittisuk and Curtis, 1982). However, in another study to investigate the effect of sublethal dosages of DDT on the vectorial capacity of mosquitos to *Brugia pahangi*, it was reported that there was a significant reduction of the infection rate of the susceptible strain and facilitated infection in the refractory strain (Gaaboub and Busvine, 1975).

The present findings are relevant to control programs against dengue vectors. Incomplete fogging and/or improper dosing of malathion and the subsequent exposure of vectors to sublethal dosages of the insecticide may account for one of the many factors contributing to the continued occurrence of dengue in spite of regular malathion application.

ACKNOWLEDGEMENTS

The authors thank the Director, Institute for Medical Research, Kuala Lumpur for permission to publish. This investigation received partial financial support from the SEAMEO-TROPMED Program in the Malaysian Centre at the Institute for Medical Research, Kuala Lumpur.

REFERENCES

Gaaboub IA, Busvine JR. DDT resistance status and the effect of DDT exposure in relation to the vectorial capacity of *Aedes aegypti* for *Brugia pahangi*. *Ann Trop Med Parasitol* 1975; 69 : 493-8.

Khalil HM, Rifaat MA, Sadex S, Gad AM. Effect of sublethal concentration of DDT, Abate and Sevin on the filarial cycle in *Culex pipens molestus*. *J Egyptian Public Health Assoc* 1975; 50 : 309-15.

Maneekarn N, Morita K, Tanaka M, *et al*. Application of polymerase chain reaction for identification of dengue virus isolated from patient sera. *Microbiol Immunol* 1993; 37 : 41-7.

Mohan BN. Sporogenic cycle of malaria parasites in resistant and non-resistant strains of mosquitoes after exposure to DDT. *Indian J Malaria* 1955; 9 : 287-90.

Prasittisuk C, Curtis CF. Absence of effects of insecticides in susceptibility of *Anopheles* to *Plasmodium yoelii nigeriensis*. *Southeast Asian J Trop Med Public Health* 1982; 13 : 127-32.

Skae FMT. Dengue fever in Penang. *Br Med J* 1902; 2 : 1581-2.

Vector Borne Diseases Control Programme, Malaysia. Guidelines for the prevention and control of dengue fever and dengue haemorrhagic fever, 1986; Ministry of Health, Malaysia.

Wallace HG, Lim TW, Rudnick A, Knudsen AB, Cheong WH, Chew V. Dengue haemorrhagic fever in Malaysia: the 1973 epidemic. *Southeast Asian J Trop Med Public Health* 1980; 11 : 1-13.

World Health Organization. Guidelines for dengue surveillance and mosquito control. Regional Office for the Eastern Pacific, Manila, 1995.