# GLUTATHION S-TRANSFERASE ACTIVITY AND DDT-SUSCEPTIBILITY OF MALAYSIAN MOSQUITOS

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**Abstract.** Comparative DDT-susceptibility status and glutathion s-transferase (GST) activity of Malaysian *Anopheles maculatus, Culex quinquefasciatus* and *Aedes aegypti* was investigated to ascertain the role of this enzyme in DDT resistance. The standardised WHO dose-mortality bioassay tests were used to determine DDT susceptibility in these mosquitos, whilst GST microassay (Brogdon and Barber, 1990) was conducted to measure the activity of this enzyme in mosquito homogenate. It appeared that DDT susceptibility status of Malaysian mosquitos was not correlated with GST activity.

## INTRODUCTION

In Malaysia, vector-borne diseases such as malaria, dengue and filariasis still remain as public health concern at present. Vector control agencies continue to depend on the use of chemical insecticides for the control of several vectors and nuisance mosquitos. The prolonged use of these agents may induce high levels of resistance in mosquitos and the emergence of insecticide resistance may hamper control programs. Thus, it is important to detect and characterize any possible developing resistance so that future control strategies can be developed by optimizing the use of current insecticides. At present, detection of resistance is mainly based on WHO standard bioassay procedures.

DDT [1,1,1-trichloro-2, 2-bis (p-chlorophenyl) ethane] today is still widely used in Malaysia in malaria control programs since the initiation of MEP more than 2 decades ago. There is therefore a need to study the possible emergence of DDT resistance in mosquito. It is known that DDT-dehydrochlorination is a major route of detoxication in some significant insect pest species, notably the house fly and several species of mosquito (Sternburg *et al,* 1954; Lipke and Kearns, 1959; 1960; Kimura and Brown, 1964; Kimura *et al,* 1965). The reaction is enzyme catalyzed, and elevated levels of the enzyme may lead to substantial resistance in the organism concerned (Lipke and Kearns, 1960). Multiple forms of GST are also found in insects (Usai and Fukami, 1977; Clark *et al,* 1985), where they have been implicated in resistance to organophosphate (OP) (Usui and Fukami, 1977; Oppenoorth *et al,* 1979; Motoyama and Dauterman, 1980; Motoyama *et al,* 1980). Evidence suggests that DDT-dehydrochlorinase (DDT-deh) resistance and glutathion s-transferase (GST) enzymes are closely linked (Sawicki and Farnham, 1969; Oppenoorth *et al,* 1979; Clark *et al,* 1986; Brogdon and Barber, 1990) and that DDT-deh may be a GST (Clark and Shamaan, 1984).

A microplate-based assay similar to other resistance detection assays (Hemingway *et al,* 1987; ffrench-Constant *et al,* 1987, 1988; Brogdon *et al,* 1984a, b; Brogdon, 1989; ffrench-Constant and Bonning, 1989) should be a simple method which could detect elevated GST levels in individual insects, aiding efforts to establish the correlation between GST activity and DDT-deh resistance in mosquitos. Hence the basic objectives in this study are to detect GST in mosquitos using enzyme microassay technique and to investigate the role of GST in DDT resistance in mosquito.

## MATERIALS AND METHODS

### Mosquitos

A wild synthetic strain of *Culex quinquefasciatus* adults originated from Kuala Lumpur was selected for resistance to DDT by exposure to WHO insecticide impregnated paper at the diagnostic dosage of 4% (World Health Organization, 1986). Other mosquito used were lab-bred *Cx. quinquefasciatus* (Penang and London strain); *Aedes aegypti* (Liverpool and Selangor strain) and *Anopeles maculatus* collected from Kuala Milot, Sg Tamu and Kota Tinggi, Malaysia.

**Bioassay**

**Adult mosquitos:** The WHO (1981) standard adult bioassay procedures were followed with some modification. Blood-fed, less than 7 days old adult female mosquitos were exposed to WHO insecticide-impregnated paper at the diagnostic dosage of 4% DDT. 25 mosquitos were tested in each exposure tube. The mosquitos were exposed for 1 or 24 hours and the mortality was recorded. Any survivors were immediately assayed for GST activity (see below).

**Larval mosquitos:** Larval bioassays were performed by using the WHO method (World Health Organization, 1981) for determining larval susceptibility. Bioassay was conducted in 200-ml disposable paper cups. Fifteen early fourth-instar larvae were assayed in each paper cup. The pre-test range of DDT concentrations was determined prior to bioassay. All test results were pooled and analysed. The survivors were used in the micro-assays of GST.

**Microassay of glutathion s-transferase** (Brogdon and Barber, 1990)

Single freshly-killed adults and live larvae were homogenized in wells of a plastic spot plate with 100 µl of 0.05 M solution of potassium phosphate buffer (80 ml of 9.5% $Na_2HPO_4$ and 19.6 ml of 9.1% of $KH_2PO_4$, pH7.4) using a glass rod. Each homogenate was diluted to a final volume of 500 µl with the same buffer, transferred into micro-centrifuge tubes and centrifuged at 8,000 rpm for 5 minutes. Fifty µl of the clear homogenate was then pipetted into a well of microplate. Using this technique, 8 replicates of aliquots of homogenate from a single adult mosquitos/larvae were used for assay. 50 µl aliquot of glutathion [0.03g of glutathion (GSH) in 50 ml potassium phosphate buffer] and 50 µl of 1-chloro-2, 4-dinitrobenzene (CDNB) (0.01 g of CDNB in 0.5 ml acetone and 50 ml of potassium phosphate buffer) were then added into each wells. The reaction was allowed to continue for 30 minutes and the absorbance was read with an immunoassay reader at 414 nm.

## RESULTS AND DISCUSSION

In the selection of a DDT-resistance strain of *Cx. quinquefasciatus* adults, an average of 24.55% (Range : 0-60%) mortality in F-0 *Cx. quinquefasciatus* adults was observed for DDT susceptibility (Table 1). In F-1 adults, the average mortality decreased to 10.20% (0-16%). By F-2 generation, 0% mortality was recorded in all test replicates; thus indicating the existence of DDT resistance in adults. However, in F-3 generation, an increase in mortality was recorded among the adult *Cx. quinquefasciatus* at 17.3% (8-32%). The increased mortality could be due to the incomplete selection of DDT susceptibility of *Cx. quinquefasciatus* adults in the early generations. Diagnostic dosage of DDT (4%) supplied by WHO may not be sufficiently high to completely eliminate the heterozygotes. In this case, higher dosage of DDT might be needed in order to effect complete selection.

The average GST optical density (OD) for lab-selected *Cx. quinquefasciatus* was 0.023 (0.001-0.056). When compared to the *An. maculatus* from KM strain, Sg Tamu and Kota Tinggi which were highly susceptible to DDT, the average GST OD were 0.020 (0.002 - 0.039), 0.025 (0.008 - 0.069) and 0.024 (0.003 - 0.077) respectively, while for the lab-bred *Cx. quinquefasciatus* (Penang and London strain), the average GST OD were 0.018 (0.008 - 0.082) and 0.028 (0.015 - 0.056) respectively. The results indicated that the correlation of adult mortality with mean GST activity was not significant ($r = -0.22$, $t = 0.45$, $p = 0.68$).

In mosquito larvae, resistance to DDT was observed in both *Ae. aegypti* and *Cx. quinquefasciatus* with resistance ratio ranging from 1.4 - 4.2. However, although the GST activity of these larvae was significantly different from that of the lab-

Table 1

Mortality of blood-fed *Cx. quinquefasciatus* adults exposed to 4% DDT (corrected by Abbot's formula).

| F - 0 | F - 1 | F - 2 | F - 3 |
|---|---|---|---|
| 60.0% | 10.0% | 0.0% | 32.0% |
| 50.0% | 15.0% | 0.0% | 8.0% |
| 21.0% | 15.8% | 0.0% | 12.0% |
| 0.0% | 0.0% | 0.0% | |
| > 11.0% | | | |
| 5.3% | | | |
| $\bar{x}$ = 24.55% | $\bar{x}$ = 10.2% | $\bar{x}$ = 0.0% | $\bar{x}$ = 17.3% |

Table 2

Adult GST activity in relation to DDT susceptibility.

| Species | DDT test Mor (%) | GST Activity Total repl | Std D | A GST OD | Range |
|---|---|---|---|---|---|
| * *An. maculatus* (KM Strain) | 100 | 144 | 0.007 | 0.020 | 0.002 - 0.039 |
| * *An. maculatus* (Sg Tamu) | 100 | 80 | 0.009 | 0.025 | 0.008 - 0.069 |
| * *An. maculatus* (Kota Tinggi) | 100 | 80 | 0.020 | 0.024 | 0.003 - 0.077 |
| @ *Cx. quinquefasciatus* (Lab selected) | 82.7 | 224 | 0.014 | 0.023 | 0.001 - 0.056 |
| @ *Cx. quinquefasciatus* (Penang) | 44.4 | 48 | 0.013 | 0.026 | 0.008 - 0.082 |
| @ *Cx. quinquefasciatus* (London) | 75.0 | 32 | 0.012 | 0.020 | 0.015 - 0.056 |

* = 1 hour continuous exposure to 4% DDT-impregated paper.
@ = 24 hours continuous exposure to 4% DDT-impregnated paper.

Mor - Mortality
Total repl - Total replication
Std D - Standard deviation
A GST OD - Average GST optical density

strain of *Cx. quinquefasciatus* ($p < 0.05$, ANOVA); in practice it was difficult to differential GST activity since overlapping of the results occurred (Table 3).

In conclusion, although GST activity was detected in all mosquitos, the results indicated that correlation of GST activity and DDT susceptibility in Malaysian mosquitos was difficult. As such, other enzyme systems that may play a role in DDT-detoxification should also be investigated.

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Table 3

Larval GST activity in relation to DDT susceptibility.

| Species | DDT test LC50 (mg/l) | RR | GST activity Total repl | Std D | A GST OD | Range |
|---|---|---|---|---|---|---|
| *Cx. quinquefasciatus* (Lab strain) | 0.56 | 1 | 80 | 0.0067 | 0.023 | 0.010 - 0.045 |
| | | | @ 80 | 0.0089 | 0.020 | 0.002 - 0.041 |
| | | | # 56 | 0.0078 | 0.021 | 0.004 - 0.037 |
| *Cx. quinquefasciatus* (Penang) | 0.23 | 2.4 | 80 | 0.0055 | 0.026 | 0.013 - 0.038 |
| | | | # 152 | 0.0092 | 0.020 | 0.003 - 0.036 |
| *Ae. aegypti* (Liverpool) | 0.97 | 4.2 | 80 | 0.0053 | 0.035 | 0.025 - 0.046 |
| *Ae. aegypti* (Selangor) | 0.32 | 1.4 | 80 | 0.0049 | 0.021 | 0.004 - 0.029 |

@ = assays of survivors after exposure to 0.3 mg/l
# = assays of survivors after exposure to 0.5 mg/l

RR - Resistance Ratio
Total repl - Total Replication
Std D - Standard Deviation
A GST OD - Average GST Optical Density

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