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Note

Comparison of a novel high-throughput screening system with the Bottle assay for evaluating insecticide toxicity

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Insecticide toxicity is commonly evaluated for disease vectors by either the WHO test or Bottle assay. More recently, a high-throughput screening (HTS) system was developed for testing insecticide effects on mosquito behavior and mortality. We compared HTS with the Bottle assay to evaluate the toxicity of insecticides in a population of *Aedes aegypti* from Thailand. Both the HTS and Bottle assay system were determined to be equivalent. The two systems mainly differed (1) in reaction time, with mosquitoes reacting faster in the Bottle assay than HTS, (2) in knockdown and mortality at low doses. This information will guide the testing protocol for evaluating chemical effects on behavioral responses in various vector populations.

Keywords: Bottle assay, HTS, insecticide toxicity, mosquitoes, resistance.

Introduction

For decades, insecticides for use in vector-borne disease control have been evaluated based on their toxic effects; however, the rapid development of insecticide resistance in targeted populations has limited their efficacy. Therefore, evaluating the efficacy of insecticides against resistant vector populations has become the main focus for the development of novel compounds to be used in vector control strategies. The World Health Organization developed a bioassay system to detect resistance levels in adult mosquito populations.[1)] This system exposes insects to chemical impregnated filter paper placed inside a plastic cylinder. Standardization of diagnostic doses and the establishment of baseline data for susceptible populations has facilitated the monitoring of resistance and has guided the decision making process for the use of pesticides.[1)] Brogdon and MacAllister modified the WHO resistance test kit using insecticide-coated glass bottles with solutions of standard grade insecticides and synergists.[2)] The bottle assay answers the question: will an insecticide at a concentration that gives 100% mortality for a susceptible population kill test mosquitoes during the same time interval? This system has also been used to evaluate the diagnostic dose/time within different populations of mosquitoes[3–5)] and to determine the standardized diagnostic dose for new insecticides.[6)] Recently, Grieco *et al.* developed a high-throughput screening system (HTS) which exposes mosquitoes to insecticides *via* a treated nylon net placed inside a metal cylinder.[7)] This assay was designed to test the contact irritant and spatial repellent activity as well as toxic effects of chemicals to insects. Better knowledge of these effects at a specific level could have a role in improving vector control strategies by disrupting contact more efficiently between humans and vectors. This system has previously been used to evaluate behavioral responses and the mortality of *Aedes aegypti* in response to topical repellents and other standard compounds used for vector control.[7–8)] The results were reproducible but no further comparisons were made to evaluate HTS performance against more standard assays.

The current approach aimed to assess HTS as a potential new system for evaluating the effects of chemicals on insects. Therefore, this study compared HTS to the bottle assay system to evaluate the toxicity of alpha-cypermethrin (pyrethroid), malathion (organophosphate), bendiocarb and propoxur (carbamates) against a Thai population of *Ae. aegypti*, the primary vector of dengue. Therefore, the ultimate goal was to use HTS as a unique assay to compare resistant and susceptible populations of disease vectors by evaluating their level of resistance and their behavioral responses to contact irritant and spatial repellent chemicals.

Materials and Methods

1. *Mosquitoes*

Aedes aegypti were colonized at Kasetsart University, Bangkok, Thailand, from a population collected in Pu Teuy Village, Sai Yok District, Kachanaburi Province, Thailand (14°20′11″N, 98°59′45″E). F_1 or F_2 eggs from this colony were shipped to the Uniformed Services University of the Health Sciences (USUHS) (Bethesda, Maryland) to establish a colony. The colony was maintained at 28°C and 80%RH under a photoperiod of 12 : 12 (L : D) h. Baseline testing against DDT 4%, deltamethrin 0.05%, malathion 0.8%, propoxur 0.1% were performed with the WHO filter paper test and the population was determined to only be resistant to DDT (Chareonviriyaphap, unpublished data).

Females (4–7 days old) used in testing were from the F2–F4 generations, non-bloodfed, unmated and starved from 10% sucrose solution 24-h prior to conducting an assay.

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