Journal of the American Mosquito Control Association, 41(3):160–162, 2025

SCIENTIFIC NOTE

DETERMINING DISCRIMINATING CONCENTRATIONS OF TRANSFLUTHRIN AND PERMETHRIN USING THE WHO BOTTLE ASSAY AGAINST *AEDES ALBOPICTUS* IN THAILAND

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ABSTRACT. The spread of vector-borne diseases by *Aedes albopictus* necessitates precise monitoring of insecticide resistance to maintain the effectiveness of control strategies. This study aimed to establish discriminating concentrations (DCs) for high-volatility transfluthrin and low-volatility permethrin pyrethroids widely used in vector control programs in Thailand against *Ae. albopictus* using the World Health Organization (WHO) bottle bioassay guidelines. Non-blood-fed, 3–5-day-old female mosquitoes were exposed to serial concentrations of transfluthrin and permethrin, and knockdown and mortality were recorded at 1 h and 24 h, respectively. A probit analysis was conducted to determine dose-response relationships. The results demonstrated a clear dose-dependent response to transfluthrin and permethrin. The established DCs were 1.712 µg/bottle and 22.74 µg/bottle for transfluthrin and permethrin, respectively. These findings provide essential reference points for monitoring insecticide resistance in *Ae. albopictus* populations of Thailand and offer critical guidance for optimizing vector control strategies to ensure the efficacy of pyrethroids in disease prevention programs.

KEY WORDS *Aedes albopictus*, bottle bioassay, discriminating concentrations, insecticide resistance, pyrethroids

Aedes albopictus (Skuse) transmits several important arboviruses, including all 4 dengue virus serotypes, which pose a high risk for causing dengue hemorrhagic fever, causing significant morbidity and mortality in Thailand (Fried et al. 2010, Ahebwa et al. 2023, Perez et al. 2025). Vector control primarily focuses on insecticides, but widespread use has led to resistance, which reduces their effectiveness (Chareonviriyaphap et al. 2013, Saeung et al. 2020). *Aedes* mosquitoes, vectors of arboviruses, have developed increasing resistance to pyrethroids, which are widely used in public health and household insecticides (Juntarajumnong et al. 2012, Lissenden et al. 2021, Zulfa et al. 2022). Resistance monitoring relies on laboratory bioassays, particularly the World Health Organization (WHO) tube and bottle bioassays (WHO 2022a, 2022b, 2022c). The WHO bottle bioassay, adapted from the Centers for Disease Control and Prevention (CDC) method (CDC 2012), is especially useful for volatile insecticides such as transfluthrin and metofluthrin but is also applicable to nonvolatile compounds such as permethrin and deltamethrin. However, the applicability of standardized discriminating concentrations (DCs) recommended by the WHO to distinguish between susceptible and resistant mosquito strains across different geographic regions remains uncertain (Campos et al. 2020). Logistical constraints may also limit the use of a single bioassay method, potentially affecting susceptibility monitoring accuracy. This study aims to determine DCs for transfluthrin and permethrin against *Ae. albopictus* in Thailand, using the WHO bottle bioassay.

Pyrethroid-susceptible *Ae. albopictus* strain, originally collected in 1996 from Chanthaburi Province, Thailand, and maintained without insecticide pressure at Kasetsart University since 2013, was used in this study. Technical-grade transfluthrin (99.0% purity), permethrin (94.0% purity), and analytical-grade acetone (99.9%) were used to prepare separate stock solutions for both pyrethroids at 100 µg/ml. Preliminary experiments were conducted to determine serial dilution ranges. Clean bottles were randomly assigned to control (acetone; $n = 2$) or treatment (insecticide; $n = 4$) groups and coated, following WHO guidelines (WHO 2022b, 2022c; Corbel et al. 2023). The coated bottles were uncapped and air-dried overnight at 25 ± 2°C and 60 ± 10% RH in the absence of light to prevent pyrethroid degradation. Only 3–5-day-old, non-blood-fed female adults were tested. Before exposure, a total of 150 female mosquitoes per insecticide concentration (25 mosquitoes per bottle) were starved for 2 h (WHO 2022b, 2022c). The mosquitoes were introduced inside treated bottles and exposed for 60 min. Afterwards, mosquitoes were transferred to holding cups with cotton wool soaked in a 10% sugar solution, knockdown was recorded, and mortality was assessed at 24 h post-exposure. Abbott's correction formula was applied for 5–20% control mortality; each test with >20% control mortality was discarded and repeated (Abbott 1925). Mortality data were analyzed using a chi-square goodness-of-fit test ($P < 0.05$), and LC_{50}/LC_{99} values with 95% fiducial

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Table 1. Mean 24-h post-exposure mortality of susceptible *Ae. albopictus* to serial concentrations of transfluthrin and permethrin under laboratory conditions.

Chemical	Concentration (µg/bottle)	No. tested	Mean mortality (%)
Transfluthrin	1.5	95	100.00
	1.0	100	99.00
	0.5	100	85.00
	0.25	100	47.00
	0.125	95	25.55
	0.065	100	18.00
	0.03125	93	10.75
Permethrin	11.50	100	100.00
	8.0	99	83.94
	5.0	99	61.67
	2.5	98	34.72
	1	97	11.65
	0.6	73	6.67

limits were estimated via maximum likelihood analysis and log-probit regression in Jamovi (version 2.6) (Jamovi 2025). The final diagnostic concentration was defined as twice the LC_{99} value of the susceptible test population (WHO 2022c).

Results confirmed the dose-dependent toxicity of transfluthrin (highly volatile) and permethrin (low volatility) against *Ae. albopictus*, with location-specific DCs observed in Thailand. *Aedes albopictus* were exposed to 7 and 6 concentrations of transfluthrin and permethrin, respectively, to assess dose-response relationships. Mortality rates at 24 h post-exposure followed a dose-dependent pattern, ranging from 10.75% at 0.03115 µg/bottle to 100% at 1.5 µg/bottle for transfluthrin, and from 6.67% at 0.60 µg/bottle to 100% at 11.50 µg/bottle for permethrin (Table 1). The probit analysis for transfluthrin yielded a chi-square value of 8.198 (df = 3; $P = 0.085$), indicating an acceptable fit. The LC_{50} and LC_{99} values were estimated at 0.270 µg/bottle and 0.856 µg/bottle, respectively, resulting in a DC of 1.712 µg/bottle. For permethrin, probit analysis yielded a chi-square value of 6.31 (df = 3; $P = 0.176$), also indicating a good fit. The LC_{50} and LC_{99} were estimated at 4.227 µg/bottle and 11.37 µg/bottle, resulting in a DC of 22.74 µg/bottle.

To standardize susceptibility testing, the WHO has established universal DCs for major mosquito vectors (WHO 2022b, 2022c; Corbel et al. 2023), ensuring consistency across bioassay methods, such as the WHO tube test and bottle bioassay (Althoff and Huijben 2022). In this study, the DC for transfluthrin against *Ae. albopictus* (1.712 µg/bottle) was notably lower than the WHO-recommended DC of 3 µg/bottle (WHO 2022b, 2022c). Similarly, the DC for permethrin was about twofold lower than the CDC-recommended 43 µg/bottle (at 10-min diagnostic time) for the CDC bottle bioassay (CDC 2021). Although goodness-of-fit tests indicate that both probit models fit the data well (Table 2), model fit does not equate to relative efficacy. The lower LC_{99} for transfluthrin and permethrin, relative to WHO and CDC thresholds, indicate sufficient efficacy against the laboratory-susceptible *Ae. albopictus* colony used in this study.

It is important to note that the CDC bottle bioassay protocol differs from that of the WHO, where the CDC protocol uses a fixed insecticide concentration with variable exposure times, whereas the WHO protocol standardizes exposure time (60 min) while varying the insecticide concentration and doubling the LC_{99} to produce a DC. While the diagnostic concentrations established in this study provide valuable local reference for assessing insecticide susceptibility, it is important to recognize that variations can occur even among laboratory-maintained susceptible colonies (Gloria-Soria et al. 2019, Ross et al. 2019). The *Ae. albopictus* colony used in this study differs from those utilized in the development of WHO and CDC bottle bioassay guidelines, and such differences in genetic and generational background, rearing history, and laboratory conditions can contribute to variability in insecticide susceptibility. While localized susceptibility testing improves the relevance of insecticide resistance monitoring, we acknowledge that it may not always be feasible in all settings. In such cases, standardized DCs remain a critical reference, but integrating them with locally derived DCs can provide more accurate insecticide resistance monitoring.

This study shows significant variation in the susceptibility of *Ae. albopictus* to transfluthrin and permethrin, with observed DCs differing from WHO- and CDC-recommended values. These discrepancies highlight the importance of localized insecticide susceptibility assessments, which could improve intervention outcomes, reduce environmental impact, and help mitigate resistance development. In an illustrative scenario evaluating transfluthrin resistance of a field

Table 2. Probit analysis of susceptible *Aedes albopictus* mortality to establish LC_{50}, LC_{99}, and DC (P value = 0.05).

Chemical	Chi-square	df	Sig.	Lethal concentration	WHO bottle bioassay (µg/bottle)
Transfluthrin	8.198	3	0.085	LC_{50}	0.270
				LC_{99}	0.856
				DC	1.712
Permethrin	6.31	3	0.176	LC_{50}	4.227
				LC_{99}	11.370
				DC	22.74

population of *Aedes albopictus* from northeastern Thailand, mortality from the locally derived DC of transfluthrin (1.712 μg/bottle) might be around 95%, indicating possible resistance. In contrast, the use of WHO's DC (3 μg/bottle) could result to ≥98% or complete mortality, thereby classifying the population as "susceptible" despite evidence of reduced sensitivity as suggested by the locally derived DC. Reliance on standardized or recommended DCs alone might mask the early emergence of insecticide resistance. This could potentially delay the implementation of alternative control measures and inadvertently increase selection pressure. Locally derived DCs from a known susceptible mosquito population allow sensitive detection of shifts in susceptibility, enabling more precise insecticide utilization and timely intervention. While these DCs were established using a laboratory-susceptible colony of *Ae. albopictus*, they offer essential baselines for subsequent assessments of field-collected mosquitoes. We recommend incorporating parallel WHO bottle bioassays of field populations using both standard (WHO/CDC) and locally derived DCs accompanied by topical application bioassays into existing insecticide resistance monitoring protocols to validate local thresholds and tailor mosquito vector control measures to regional ecological conditions.

ACKNOWLEDGMENTS

This research was funded by Kasetsart University, Bangkok, Thailand, through the Graduate School Fellowship Program and the Kasetsart University Research and Development Institute (KURDI) (Grant No. FF [KU] 51.68). The main author would like to express gratitude to the study committee, who tirelessly devoted their time and effort to this study.

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