รายงานวิจัยฉบับสมบูรณ์

พฤติกรรมการหลีกหนีสารเคมีฆ่าแมลงของยุงพาหะนำโรค มาลาเรียชนิด *Anopheles minimus* ในประเทศไทย

(Pesticide Avoidance Behavior in *Anopheles minimus*, a Malaria Vector in Thailand)

โดย

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สัญญาเลขที่ PDF/67/2540

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สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย

กิตติกรรมประกาศ

การวิจัยครั้งนี้สำเร็จลุล่วงไปด้วยดีด้วยความร่วมมือของหลายฝ่าย โดยเฉพาะสำนักงานกองทุนสนับสนุนการวิจัย (สกว.) ที่ได้สนับสนุนเงินทุนในการทำวิจัยโครงการวิจัยหลังปริญญาเอก ตามโครงการเลขที่ PDF/67/2540 โดยมีนายธีรภาพ เจริญวิริยภาพ เป็นหัวหน้าโครงการ และมี นางสาวคุภาภรณ์ รัตนธรรม เป็นที่ปรึกษาโครงการ ซึ่งผู้วิจัยขอขอบคุณมา ณ โอกาสนี้ นอกจากนี้ผู้วิจัยขอขอบคุณกองมาลาเรีย กรมควบคุมโรคติดต่อ กระทรวงสาธารณสุข ที่อำนวยความสะดวกในการสำรวจพื้นที่ที่ใช้ในการวิจัยในหลาย ๆ จังหวัด และ ขอขอบคุณกองการเกษตรและสหกรณ์ สำนักงานทหารพัฒนา กองบัญชาการทหารสูงสุด บ้านพุเตย ตำบลท่าเสา อำเภอไทรโยค จังหวัดกาญจนบุรี ที่อำนวยความสะดวกเรื่องที่พักและดูแลความสะดวกเรียบร้อยในระหว่างปฏิบัติงาน สุดท้ายนี้ผู้วิจัยและคณะขอขอบคุณ นิสิต นักศึกษาและผู้ร่วมงานทุกคนที่มีส่วนร่วมให้งานวิจัยครั้งนี้สำเร็จไปได้ด้วยดี

ABSTRACT

Project Code: PDF/67/2540

Project Title: Pesticide Avoidance Behavior in Anopheles minimus, a Vector of Malaria in Thailand

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Project Period: 3 years

Objectives: Survey insecticide resistance (physiological resistance) in Anopheles minimus, a vector of malaria in Thailand
Determine the pesticide avoidance behavior (behavioral resistance) in Anopheles minimus, a vector of malaria in Thailand

Methodology: Anopheles minimus populations
Physiological resistance was detected using World Health Organization test (1981)
Behavioral resistance was determined using an improved ERE chamber (Chareonviriyaphap, 2000)
Survival analysis with the log-rang test

Results

1.Physiological resistance: All wild-caught populations used in this study were found to be completely susceptible to insecticides commonly used in malaria control in Thailand, namely DDT, deltamethrin and lambdacyhalothrin (See included tables). Those Anopheles minmus populations were collected from Tak, Mae-Hong-Sorn, Kanchanaburee, Trat, Nakhon Ratchseema and Chantaburee Provinces.

2.Behavioral resistance: Due to the availability of the specimens, only 2 populations were used in this study. The first population was a wild-caught collected from Ban Pu-Teuy, Kanchanaburee Province and the second population was a laboratory-reared colony obtained from Malaria Division, CDC. Both exhibited strong avoidance behavior (behavioral resistance) to all 3 compounds, DDT, deltamethrin and lambdacyhalothrin.

Discussion and Conclusion

This finding showed that both young and wild-caught populations of An. minimus female demonstrated tremendous irritancy responses to DDT, deltamethrin and lambdacyhalothrin and most specimens took off from the treated chambers without receiving a letal dose, indicating strong natural behavioral avoidance to all 3 compounds. In the present study, the wild population showed much quicker escape responses to the chambers treated with DDT and lambdacyhalothrin than that of deltamethrin; whereas a young colony exhibited stronger responses to 2 pyrethroids than DDT. The comparatively weaker response to deltamethrin by test specimens from the wild population than a young colony was unclear. However, age composition and physiological status of wild specimens could play a role in this result.

Suggestion

Clearly, more field research is needed on the behavioral responses of vector populations from different geographical areas in Thailand. Chemically-induced avoidance behaviors by malaria vector mosquitoes should be defined using standardized methods (e.g., excito-repellency boxes and **experimental huts**) to determine the exact impact of chemicals on malaria transmission and malaria control.

บทคัดย่อ

ชื่อเรื่อง	พฤติกรรมการหลีกหนีสารเคมีฆ่าแมลงของยุงพาหะนำโรคมาลาเรีย ชนิด Anopheles minimus ในประเทศไทย
รหัสโครงการ	PDF 67/2540
หน่วยงาน	คณะศิลปศาสตร์และวิทยาศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ กำแพงแสน นครปฐม 73140
ระยะเวลาที่ทำการวิจัย	3 ปี
วัตถุประสงค์	สำรวจการต้านทานสารเคมีเชิงสรีรในยุงมินิมัส สำรวจการต้านทานสารเคมีเชิงพฤติกรรมในยุงมินิมัส

ขอบเขตการวิจัย

วิธีการที่ได้ข้อมูล: จับยุง Anopheles minimus ที่ได้จากพื้นที่และห้องปฏิบัติการมาทดสอบการต้านทานสารเคมีเชิงสรีระโดยใช้ World Health Organization Susceptibility Test (1981) และใช้ An Improved Excito-Repellency Escape Chamber (2000) เพื่อทดสอบการต้านทานต่อสารเคมีในเชิงพฤติกรรม

วิธีการเก็บตัวอย่าง: ใช้คนเป็นเหยื่อในการเก็บยุง โดยแบ่งออกเป็นสองกลุ่ม ๆ ละสามถึงสี่คน นั่งจับยุงตั้งแต่เวลา 1800-0600 น. จากนั้นนำยุงที่ได้ไปเก็บไว้ในกระบอกเลี้ยงยุงให้น้ำหวาน แยกชนิดของยุงในตอนเช้าและทำการทดสอบ

วิธีการทดสอบ: WHO Susceptibility Test (1981) โดยใช้ความเข้มข้นที่ระดับวินิจฉัย (4%DDT, 0.025%deltamethrin และ 0.1%lambdachalothrin) ทำการทดสอบ 3 ครั้งต่อหนึ่งการทดลองต่อหนึ่งสารเคมี แต่ละครั้งใช้ยุง 100 ตัว นำยุงที่ผ่านการทดสอบไปเลี้ยงและบันทึกจำนวนการตายและการอยู่รอดหลังจาก 24 ชั่วโมง

An Improved Excito-Repellency Escape Chamber (2000): เปรียบเทียบการหลีกหนีต่อสารเคมี 3 ชนิดทั้งสัมผัสโดยตรงและสัมผัสโดยอ้อม (ดูรายละเอียดในเล่ม) โดยทำการทดลอง 4 ซ้ำ ใช้ยุงครั้งละ 100 ตัว ต่อหนึ่งสารเคมี

วิธีการวิเคราะห์ข้อมูลและแปรผล: ใช้ Survival Analysis ในการวิเคราะห์หาการเปลี่ยนแปลงที่เกิดขึ้นของประชากรยุงหลังจากเริ่มการทดลองจนสิ้นสุด ใช้ Log Rank test เปรียบเทียบความแตกต่าง

ผลการทดลอง

จากการศึกษาไม่พบว่ายุงกันปล่องชนิดมินิมัสจากพื้นที่ต่าง ๆ ที่จับได้แสดงการต้านทานต่อสารเคมีในเชิงสรีระ (Physiological resistance) ที่ใช้ในการทดลอง แต่พบว่ายุงกันปล่องมินิมัสจากกองมาลาเรีย และ จากอำเภอไทรโยคแสดงการต้านทานต่อสารเคมีในเชิงพฤติกรรม (Behavioral resistance) อย่างรุนแรง

สรุปและวิจารณ์

จากการศึกษาวิจัยครั้งนี้ทำให้ทราบว่าสารเคมีทั้ง 3 ชนิด (DDT, deltamethrin และ lambdacyhalothrin) มีประสิทธิภาพสูงในการควบคุมยุงกันปล่องโดยเฉพาะ DDT ยังคงมีประสิทธิภาพสูงกว่าสารไพรีทรอยด์ทั้งชนิด 2 ทั้งในการขับและไล่ยุงกันปล่อง เพราะฉะนั้นในการสรุปการเปลี่ยนแปลงการใช้สารเคมีควบคุมยุงพาหะจำเป็นต้องทำอย่างละเอียดและรอบครอบ

แนะนำ

เพื่อให้การทดสอบสมบูรณ์ได้มาตราฐานควรจะมีการทำ Standardization ของชุดอุปกรณ์ที่ใช้ในการทดสอบ และหามาตรการควบคุมปัจจัยต่าง ๆ (confounding factors) ที่จะมีผลกระทบต่อผลการทดลอง นอกจากนี้ควรทำการทดลองเปรียบเทียบกับยุงในหลายประชากร และ ที่สำคัญควรจะทำการศึกษาโดยใช้กระท่อมทดลอง

Executive Summary

The study was designed to determine toxicity responses of DDT, deltamethrin and lambdacyhalothrin to several wild-caught Anopheles minimus populations using the WHO Susceptibilty Test. In addition, the behavioral responses of 2 test populations of An. minimus females to 2 g/m^2 DDT, 0.0625 g/m^2 deltamethrin and 0.0369 g/m^2 lambdacyhalothrin using an improved excito-repellency escape chamber were performed. One test population was colonized in 1993 and referred to as "a young colony". The second field test population was collected from Ta-Soa County, Tri-Yok District, Kanchanaburi Province, West of Thailand and referred to as "a wild population". Results showed that females of both young and wild test populations rapidly escaped from direct contact with DDT, deltamethrin, and lambdacyhalothrin. Lambdacyhalothrin exhibited the strongest irritant effect to female mosquitoes followed by DDT and deltamethrin. Fewer females escaped from test chambers without direct contact with treated surfaces, indicative of non-contact repellent action, but the response was significantly different from the controls ($P<0.05$). This suggested that both contact iritancy and non-contact repellency are involved in An. minimus escape responses. Experimental hut studies that include monitoring of house-entering populations of An. minimus are needed for a meaningful assessment of non-contact repellent actions.

เนื้อหางานวิจัย

บทนำ

Anopheles minimus จัดเป็นพาหะนำโรคมาลาเรียซึ่งพบแพร่หลายในประเทศต่างๆ แถบทวีปเอเชียและเอเชียอาคเนย์รวมทั้งประเทศไทยด้วย ในประเทศไทย An. minimus ได้ถูกจัดเป็นยุงพาหะชนิดซับซ้อน ที่นำเชื้อมาลาเรียและแพร่กระจายอยู่ทั่วไป ยุงชนิดนี้มีแหล่งที่อยู่อาศัยบริเวณชายป่าหรือชายเขาที่มีน้ำไหลรินๆ และมีนิสัยชอบกัดกินคนภายในบ้าน (Endophagic) ต่อมาการเปลี่ยนแปลงของสภาพนิเวศวิทยาทำให้แหล่งที่อยู่อาศัยของยุงชนิดนี้ลดน้อยลงโดยเฉพาะอย่างยิ่งสภาพแวดล้อมต่างๆ ที่เปลี่ยนไปอันเนื่องมาจากการทำลายป่า รวมทั้งการใช้สารเคมีชนิดต่างๆ ทั้งทางด้านการเกษตรและทางด้านการแพทย์โดยเฉพาะอย่างยิ่งการใช้ดีดีทีควบคุมมาลาเรียเป็นเวลานาน ทำให้ยุงชนิดนี้มีการเปลี่ยนแปลงด้านพฤติกรรมและมีการตอบสนองต่อการเปลี่ยนแปลงที่เกิดขึ้นโดยเข้ามาอยู่ใกล้ชิดมนุษย์มากขึ้นจนกลายเป็นยุงพาหะที่มีความสำคัญในบางพื้นที่ของประเทศไทย

อนึ่งถึงแม้ว่าดีดีทีถูกนำเข้ามาใช้ควบคุมมาลาเรียเป็นเวลาช้านานแล้วและสามารถลดปัญหาไข้มาลาเรียได้ตามลำดับ ปัญหาที่เกิดขึ้นในปัจจุบันนี้ได้แก่ประชาชนมีการยอมรับการพ่นดีดีทีน้อยลงไปตามลำดับ ทำให้ต้องหามาตรการอื่นขึ้นมาทดแทนหรือเสริมโดยเฉพาะการใช้สารในกลุ่มไพรีทรอยด์สังเคราะห์ เช่น การใช้มุ้งที่ชุบด้วย permethrin หรือการพ่นฝาผนังบ้านด้วย deltamethrin แต่ปัญหาที่เกิดขึ้นในขณะนี้ก็คือยังไม่มีหลักฐานการศึกษาเกี่ยวกับบทบาทและกลไกการตอบสนองโดยอาศัยพฤติกรรมการหลีกเลี่ยง (avoidance behavior[1]) ของยุงพาหะนำโรคมาลาเรียชนิดนี้ต่อสารฆ่าแมลงอย่างแท้จริง และการวิเคราะห์ข้อมูลทางด้าน behavioral response ยังไม่มีการศึกษามากนักเพราะฉะนั้นจึงมีความจำเป็นอย่างยิ่งที่จะต้องให้ความสนใจและศึกษาในเรื่องของบทบาท และ กลไกการตอบสนองของสารเคมีฆ่าแมลงโดยเฉพาะเรื่อง avoidance behavior ในยุงพาหะนำโรคชนิดนี้ให้มากขึ้น บทบาทและกลไกที่แท้จริงด้าน avoidance behavior ของยุงพาหะนำโรคมาลาเรียต่อสารเคมีฆ่าแมลงยังเป็นที่ถกเถียงกันอยู่ในวงการกีฏวิทยาทางการแพทย์เพราะถือว่าเป็นงานที่ซับซ้อน มีความหลากหลายในตัวเอง ต้องใช้อุปกรณ์ที่มีความจำเพาะสูงและต้องอาศัยวิธีการวิเคราะห์ผลข้อมูลที่ถูกต้องและแม่นยำ พร้อมทั้งต้องใช้ยุงจาก หลายประชากรเพื่อประโยชน์ในการเปรียบเทียบพฤติกรรมดังกล่าว

ในขณะที่ยังไม่มีการศึกษาและรายงานเกี่ยวกับกลไกหรือบทบาทที่แน่นอนด้าน avoidance behavior ของยุงพาหะนำโรคมาลาเรียที่มีต่อดีดีที ทั้งๆ ที่มีการใช้ดีดีทีมานานนับสิบปีแล้ว ปัจจุบันนี้สารไพรีทรอยด์สังเคราะห์ในรูปแบบของมุ้งชุบด้วยสาร permethrin และการพ่นตามฝาผนังบ้านด้วย deltamethrin ก็ได้ถูกนำเข้ามาเพื่อใช้ในการควบคุมยุงพาหะนำโรคมาลาเรียควบคู่ไปกับดีดีที เพราะฉะนั้นจึงควรทำการศึกษาให้แน่ชัดว่าไพรีทรอยด์สังเคราะห์มีบทบาทอย่างไรต่อยุงพาหะมาลาเรียโดยเฉพาะในด้าน avoidance behavior เพราะไพรีทรอยด์สังเคราะห์ซึ่งกำลังใช้เสริมหรือ ทดแทนดีดีทีในการควบคุมไข้มาลาเรียมีคุณสมบัติที่เด่นชัดทางด้าน excito-repellency ด้วย

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[1]บางคนเรียกว่า Behavioral resistance (The term "behavioral resistance" can be defined as a population-based change in a species'genetics, resulting from selective pressure of insecticide use, that increase the frequency of insecticide avoidance behavior).

เพื่อให้เข้าใจปรากฏการณ์เกี่ยวกับบทบาทและกลไกที่แท้จริงด้าน avoidance behavior ผู้วิจัยได้ออกแบบกล่องบ้านจำลอง (experimental escape chamber[2]) ซึ่งสามารถใช้ศึกษาพฤติกรรมการตอบสนองของยุงพาหะนำโรคมาลาเรียที่มีต่อดีดีทีและไพรีทรอยด์สังเคราะห์ โดยจะให้ขาส่วนปลายที่เรียกว่า tarsi ของยุงพาหะมีโอกาสได้สัมผัสกับสารเคมีโดยตรง (physical contact with chemical) หรือที่เรียกว่า "การศึกษาทางตรง" ส่วน "การศึกษาทางอ้อม" จะกระทำโดยที่ tarsi ของยุงพาหะที่ทำการศึกษาไม่มีโอกาสที่จะสัมผัสกับสารเคมี (without physical contact[3]) กล่องจำลองหลังนี้จะเลียนแบบบ้านจำลองหลังเล็กๆ ซึ่งมีทางเข้า (entrance) และทางออก (exit) ของยุงอย่างละหนึ่งช่องทาง ภายในกล่องจะมีกล่องชั้นในซึ่งทำด้วยตาข่ายและสามารถเลื่อนเข้าออกได้เพื่อใช้ศึกษาในกรณีของ "การศึกษาทางอ้อม" ซึ่งบ้านจำลองหลังเล็กๆ หลังนี้จะช่วยอธิบายเงื่อนงำถึงบทบาทและกลไกที่แท้จริงทางด้าน avoidance behavior ของยุงพาหะนำโรคมาลาเรียที่มีต่อดีดีที และไพรีทรอยด์สังเคราะห์ (อาทิ เช่นยุงพาหะจะใช้เวลาในการเข้ากัดกินเหยื่อนานเพียงไร พอที่จะทำการแพร่ระบาดของโรคมาลาเรียได้หรือไม่ หากมีการพ่นบ้านจำลองด้วยสารเคมี) และการศึกษาครั้งนี้จะทำการประเมินประสิทธิภาพของสารไพรีทรอยด์สังเคราะห์ทั้ง permethrin และ deltamethrin ที่มีต่อยุงพาหะนำโรคมาลาเรียโดยเฉพาะ An. minimus ซึ่งจะนำมาใช้เป็นมาตรการเสริมหรือทดแทนการใช้ดีดีทีซึ่งมีแนวโน้มที่จะเลิกใช้ในอนาคตอันใกล้นี้

อนึ่งเป็นที่ทราบกันดีแล้วว่าได้มีการใช้สารเคมีฆ่าแมลงจำนวนมากในประเทศไทยทั้งด้านการเกษตรและทางด้านการแพทย์ซึ่งจะมีผลโดยตรงและโดยอ้อมต่อยุงพาหะนำโรคที่มีแหล่งพักอาศัยในพื้นที่ทำการเกษตรและเข้ามากัดคนภายในบ้าน ปัญหายุงพาหะนำโรคด้านทานต่อสารเคมี (physiological resistance และ biochemical resistance) ชนิดต่างๆ ในประเทศไทยยังคงได้รับความสนใจน้อยมากและเป็นปัญหาสำคัญที่ควรจะได้รับการศึกษาให้แน่ชัดเพื่อที่จะแก้ไขสถานการณ์ได้ทันเวลาหากพบว่ายุงพาหะนำโรคมีการต้านทานต่อสารเคมี พร้อมกันนี้ควรจะมีการศึกษาถึงกลไกของการสร้างความต้านทานต่อสารเคมี (mechanism of resistance) แต่ละชนิดให้แน่ชัดเพื่อที่จะหาทางแก้ไขได้ตรงประเด็นมากขึ้น และเพื่อให้ทราบสภาวะการต้านทานต่อสารฆ่าแมลงในยุงพาหะนำโรคในประเทศไทย การศึกษาครั้งนี้จะทำการสำรวจการต้านทานต่อสารฆ่าแมลงของยุงพาหะนำโรคมาลาเรียในพื้นที่ต่างๆ ของประเทศไทย หากปรากฏว่ามีการต้านทานสารฆ่าแมลงเกิดขึ้น ทีมวิจัยโครงการปัจจุบันจะสามารถเชื่อมโยงงานออกไปในอนาคตโดยจะทำการศึกษากลไกการต้านทาน (physiological resistance) ต่อสารฆ่ายุงพาหะชนิดนั้นๆ เพื่อที่จะรวบรวมเป็นข้อมูลพื้นฐานในการศึกษาทางด้านระบาดวิทยาที่ถูกต้องและหามาตรการการป้องกันที่มีประสิทธิภาพต่อไป

วิธีการทดลอง

1. World Health Organization Susceptibility Test (1980)

1.ภาคสนาม (Field collection)

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[2]Experimental escape chamber (Chemically clean box) ได้ถูกออกแบบโดยกลุ่มนักวิจัยจาก Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, Bethesda, Maryland และกลุ่มวิศวกรจาก Walter Reed Army Institute of Research (WRAIR), Washington DC. ในปี 2536 เพื่อใช้ทำการศึกษา avoidance behavior ของยุงพาหะนำโรคมาลาเรียที่มีต่อสารเคมีฆ่าแมลงเพื่อใช้ในการควบคุมมาลาเรีย อย่างไรก็ตาม เพื่อให้เหมาะสมกับการใช้งานในประเทศไทยผู้วิจัยได้นำมาดัดแปลงปรับปรุงบางอย่าง

[3]ผู้วิจัยและคณะได้ออกแบบ experimental escape chamber เพื่อใช้ในการศึกษา non-contact condition ขึ้นเป็นครั้งแรก

เพื่อที่จะให้ได้ตัวเต็มวัย An. minimus ตามจำนวนที่ต้องการ ทำการเก็บตัวอย่างยุงด้วยวิธีใช้คนเป็นเหยื่อล่อ (human-landing collection) โดยจะใช้คนจำนวน 8 คน ซึ่งจะแบ่งการทำงานออกเป็น 2 กลุ่มโดยกลุ่มแรก (4 คน) จะเริ่มปฏิบัติงานตั้งแต่ 18.00 น. ถึง 24.00 น และกลุ่มที่ 2 (4 คน) จะเริ่มปฏิบัติงานตั้งแต่ 00.00 น. ถึง 06.00 น. แต่ละกลุ่มจะมีเวลาพัก 10 นาทีของปลายชั่วโมงที่ทำการจับยุง ยุงที่เก็บได้ทั้งหมดจะถูกเก็บไว้ในกล่อง ให้น้ำตาลและนำไปเก็บไว้ในที่ที่อุณหภูมิและความชื้นเหมาะสมเพื่อทำการแยกชนิดในตอนเช้า หลังจากนั้นยุงตัวเต็มวัยตัวเมีย An. mimimus จะนำมาทดสอบการต้านทานต่อสารฆ่าแมลง

2.ห้องปฏิบัติการชั่วคราวในท้องที่ (Temporary Laboratory)

การทดสอบการต้านทานต่อสารฆ่าแมลงในยุงพาหะนำโรคมาลาเรียจะดำเนินการโดยวิธีการที่แนะนำโดยองค์การอนามัยโลก (World Health Organization/Vector Control/Biology/ 81.806)โดยวิธีการนี้จะใช้ความเข้มข้นของสารเคมี (Organochlorine, Organophosphate, Carbamate และ Pyrethroids) ที่ระดับวินิจฉัย (diagnostic dose) ของแต่ละสารเคมี โดยจะสั่งซื้อกระดาษที่ชุบสารเคมีผ่าน US Army Center for Health Promotion and Preventive Medicine (USACHPPM) ข้อมูลที่ได้จากการทดสอบด้วยยุงจากท้องที่ (treatment) จะนำมาเปรียบเทียบกับข้อมูลมาตรฐาน (ยุงที่ไม่ต้านทานต่อสารเคมีฆ่าแมลงและต้องเป็นตัวแทนประชากรที่ดีตัวอย่างเช่นไม่สูญเสียยีนโฟว เป็นต้น) เพื่อให้การทดสอบดำเนินไปอย่างมีประสิทธิภาพจะต้องทำการทดลอง 3 ซ้ำแต่ละซ้ำจะใช้ยุงประมาณ 20-25 ตัวต่อหนึ่งความเข้มข้น วิธีการโดยนำยุง An. minimus ไปสัมผัสต่อกระดาษที่ชุบสารเคมีและกระดาษที่ไม่ชุบสารเคมี (control) ในภาชนะปิดรูปทรงกระบอกเป็นเวลา 1 ชั่วโมงหลังจากนั้นจะนำไปพักไว้ใน holding tube ให้น้ำตาลซึ่งมีความเข้มข้นประมาณ 10% เป็นเวลา 24 ชั่วโมงและนับจำนวนตาย[4]

การวิเคราะห์ข้อมูล (Data analysis)

จะต้องทำการทดลองใหม่หากพบว่าอัตราการตายของยุงเปรียบเทียบ (กลุ่มควบคุม) เกิน 20% แต่ถ้าอัตราการตายของกลุ่มควบคุมน้อยกว่า 20% จะต้องปรับอัตราการตายให้เท่ากับศูนย์โดยใช้วิธีของ Abbott's formula (Finney, 1971) ส่วนการวิเคราะห์ข้อมูลนั้นจะใช้กราฟตามวิธีการของ Probit และความชันของเส้นกราฟจะบอกให้เราทราบถึงระดับของการต้านทานต่อสารเคมีโดยการเปรียบเทียบกับกลุ่มควบคุม (World Health Organization, 1975)

2.An Improved Excito Repellency Escape Chamber (2000)

1. จับยุง An. minimus โดยใช้คนเป็นเหยื่อหรืออาจจะใช้ควายหรือวัวเป็นเหยื่อล่อหากพบว่า An. minimus ในพื้นที่นั้นๆ เข้ากัดกินควายหรือวัวเป็นอาหาร แต่ในการศึกษาครั้งนี้ยุงที่ใช้ในการทดสอบเป็นยุงที่เก็บได้จากการใช้คนเป็นเหยื่อ

2. การทดลองในห้องปฏิบัติการจะใช้ยุงตัวเมียที่ยังไม่ได้กินเลือดอายุประมาณ 3 ถึง 5 วันส่วนในท้องที่จะใช้ยุงตัวเมียที่ทำการจับได้ในคืนนั้นและก่อนการทดลองจะงดให้น้ำหวานแต่จะเอาสำลีชุบน้ำหมาดๆวางบนกล่องยุงจะทำการทดลองในช่วงเช้า (7.00-12.00) เท่านั้น

3. กระดาษชุบสารเคมีชนิดต่างๆ ตามความเข้มข้นที่ต้องการจะสั่งซื้อโดยตรงจาก US Army Center for Health Promotion and Preventive Medicine (USACHPPM)

4. จะต้องทำการทดลองอย่างน้อย 3 ซ้ำ เพราะฉะนั้นแต่ละชุดการทดลอง (treatment) ซึ่งประกอบด้วยการทดสอบโดยทางตรง 2 กล่องและการทดสอบโดยทางอ้อม 2 กล่อง จะใช้ยุงประมาณ 100 ตัวในแต่ละชุดการทดลอง และเพื่อไม่ให้เกิดการลำเอียงในการทดสอบ จะทำการสุ่มใช้กล่องบ้านจำลองทั้ง 4 กล่องในแต่ละครั้ง อย่างไรก็ตามจะต้องใช้แอลกอฮอล์ชุบสำลีเช็ดทำความสะอาดกล่องบ้านจำลองก่อนที่จะนำมาใช้ในแต่ละครั้ง หลังจากบุกล่องบ้านจำลองด้วยกระดาษชุบสารเคมีซึ่งสั่งซื้อจาก USA CHPPM และที่ไม่ได้ชุบสารเคมี (ชุบน้ำมัน) ใน

[4]การทดลองแต่ละครั้งจะต้องทำการบันทึกความชื้นและอุณหภูมิ

กล่องควบคุมเป็นที่เรียบร้อยแล้ว นำยุงใส่เข้าไปในกล่องและปิดประตูทางเข้าและทางออกเป็นเวลา 3 นาทีหลังจากนั้นนับจำนวนยุงที่บินหนีออกจากกล่องทั้ง 4 ทุกๆ 1 นาที จนครบ 30 นาที ตามวิธีการของ Chareonviriyaphap, (1997) จึงหยุดการทดลอง (จะมีกล่องกระดาษสำหรับรับยุงที่บินหนีออกมาจากกล่องบ้านทดลอง) เพื่อที่ให้เห็นภาพชัดเจนมากขึ้นการทดลองที่สมบูรณ์แบบจะแสดงให้เห็นดังตารางดังต่อไปนี้[5]

Papers lining the exposure chambers	With or Without contact with test papers	
	With	Without
With Insecticide(Treatment Chamber)	X	X
Without Insecticide (Control Chamber)	X	X

5.ยุงที่ใช้เสร็จแล้วในการทดสอบจะถูกนำมาเลี้ยงไว้ในสภาพที่เหมาะสม (ยุงที่หนีออกจากกล่องบ้านจำลองและยุงที่ยังคงเหลืออยู่ในกล่องบ้านจำลอง) นับจำนวนที่ตายหรือสลบในขณะนั้น (บันทึกอุณหภูมิและความชื้นทุกครั้งในการทดลอง)

การวิเคราะห์ข้อมูล

ข้อมูลที่ได้จะถูกนำมาเก็บไว้ในฐานข้อมูลโดยใช้โปรแกรม excel หรือ double helix express และนำไปวิเคราะห์หาค่า ET-50 (เวลาที่ประชากรของยุงหนีไป 50% หรือ ที่เราเรียกว่า escape time of 50%) และ ET-75 (เวลาที่ประชากรของยุงหนีไป 75% หรือที่เราเรียกว่า escape time at 75%)โดยวิธี survival analysis จากโปรแกรม SAS การวิเคราะห์วิธีนี้จะทำให้เราทราบถึงความเป็นไปได้หรือแนวโน้มว่ายุงได้เข้ากัดกินเหยื่อ (มนุษย์) สำเร็จหรือไม่ และสามารถบอกให้ทราบถึงความแตกต่างของการตอบสนองของสารเคมี ที่เกิดขึ้นระหว่างประชากรของยุงที่เรานำมาเปรียบเทียบ

[5] See Roberts, D.R., T. Chareonviriyaphap, H.H. Harlan & P. Hakeih 1995. Methods of testing and analyzing responses of malaria vectors to insecticides. J. Amer. Mos. Cont. Asso. Submitting.

ผลการทดลองที่ 1 (Physiological Resistance)

1. DDT (4%) exposure time 1 ชั่วโมง

สถานที่ทดสอบ อำเภอบ่อไร่ จังหวัดตราด

ยุงพาหะ An. minimus

ระยะ ตัวเต็มวัย เพศเมีย

อุณหภูมิระหว่างทดสอบ	Exposure tube	Holding tube
	27-28 °C	27-28 °C
ความชื้นสัมพัทธ์	75-95%	75-95%

Replicates	Replicate 1	Replicate 2	Replicate 3	Raplicate 4	Replicate 5	Total test	Total control
Test/Time(hr)	24	24	24	24	24	24	24
No exposed	20	20	20	20	20	100	100
No knockdown (at the end of exposure)	20	20	20	20	20	100	100
% knockdown (at the end of exposure)	100	100	100	100	100	100	100
No dead (at the end of exposure)	20	20	20	20	20	100	100
% dead (at the end of exposure)	100	100	100	100	100	100	100

2. Permethrin (0.25%) exposure time 1 ชั่วโมง

สถานที่ทดสอบ อำเภอบ่อไร่ จังหวัดตราด

ยุงพาหะ An. minimus

ระยะ ตัวเต็มวัย เพศเมีย

อุณหภูมิระหว่างทดสอบ	Exposure tube	Holding tube
	28-29 °C	27-28 °C
ความชื้นสัมพัทธ์	75-85%	75-85%

Replicates	Replicate 1	Replicate 2	Replicate 3	Raplicate 4	Replicate 5	Total test	Total control
Test/Time(hr)	24	24	24	24	24	24	24
No exposed	20	20	20	20	20	100	100
No knockdown (at the end of exposure)	20	20	20	20	20	100	100
% knockdown (at the end of exposure)	100	100	100	100	100	100	100
No dead (at the end of exposure)	20	20	20	20	20	100	100
% dead (at the end of exposure)	100	100	100	100	100	100	100

3. Deltamethrin (0.025%) exposure time 1 ชั่วโมง

สถานที่ทดสอบ อำเภอบ่อไร่ จังหวัดตราด

ยุงพาหะ An. minimus

ระยะ ตัวเต็มวัย เพศเมีย

อุณหภูมิระหว่างทดสอบ	Exposure tube	Holding tube
	26-27 °C	27-28 °C
ความชื้นสัมพัทธ์	75-85%	75-85%

Replicates	Replicate 1	Replicate 2	Replicate 3	Raplicate 4	Replicate 5	Total test	Total control
Test/Time(hr)	24	24	24	24	24	24	24
No exposed	20	20	20	20	17	97	100
No knockdown (at the end of exposure)	20	20	20	20	17	97	100
% knockdown (at the end of exposure)	100	100	100	100	100	100	100
No dead (at the end of exposure)	20	20	20	20	17	97	100
% dead (at the end of exposure)	100	100	100	100	100	100	100

4. Lambda-Cyhalothrin (0.1%) exposure time 1 ชั่วโมง

สถานที่ทดสอบ อำเภอบ่อไร่ จังหวัดตราด

ยุงพาหะ An. minimus

ระยะ ตัวเต็มวัย เพศเมีย

อุณหภูมิระหว่างทดสอบ	Exposure tube	Holding tube
	28-29 °C	27-28 °C
ความชื้นสัมพัทธ์	75-95%	75-95%

Replicates	Replicate 1	Replicate 2	Replicate 3	Raplicate 4	Replicate 5	Total test	Total control
Test/Time(hr)	24	24	24	24	24	24	24
No exposed	20	20	20	20	20	100	100
No knockdown (at the end of exposure)	20	20	20	20	20	100	100
% knockdown (at the end of exposure)	100	100	100	100	100	100	100
No dead (at the end of exposure)	20	20	20	20	18	98	100
% dead (at the end of exposure)	100	100	100	100	100	100	100

5. 0.25% Etophenpox exposure time 1 ชั่วโมง

สถานที่ทดสอบ อำเภอบ่อไร่ จังหวัดตราด

ยุงพาหะ An. minimus

ระยะ ตัวเต็มวัย เพศเมีย

อุณหภูมิระหว่างทดสอบ	Exposure tube	Holding tube
	27-28 °C	27-28 °C
ความชื้นสัมพัทธ์	75-85%	75-85%

Replicates	Replicate 1	Replicate 2	Replicate 3	Raplicate 4	Replicate 5	Total test	Total control
Test/Time(hr)	24	24	24	24	24	24	24
No exposed	20	20	20	20	20	100	100
No knockdown (at the end of exposure)	20	20	20	20	20	100	100
% knockdown (at the end of exposure)	100	100	100	100	100	100	100
No dead (at the end of exposure)	20	20	20	20	20	100	100
% dead (at the end of exposure)	100	100	100	100	100	100	100

6. Deltametrin (0.025%) exposure time 1 ชั่วโมง

สถานที่ทดสอบ ตำบลป่างหมู อำเภอเมือง จังหวัดแม่ฮ่องสอน

ยุงพาหะ An. minimus

ระยะ ตัวเต็มวัย เพศเมีย

อุณหภูมิระหว่างทดสอบ	Exposure tube	Holding tube
	28-29 °C	25-29 °C
ความชื้นสัมพัทธ์	75-95%	75-95%

Replicates	Replicate 1	Replicate 2	Replicate 3	Raplicat 4	Replicate 5	Total test	Total control
Test/Time(hr)	24	24	24	24	24	24	24
No exposed	20	20	20	20	13	93	100
No knockdown (at the end of exposure)	20	20	20	20	13	93	100
% knockdown (at the end of exposure)	100	100	100	100	100	100	100
No dead (at the end of exposure)	20	20	20	20	13	93	100
% dead (at the end of exposure)	100	100	100	100	100	100	100

7. Etophenpox (0.25%) Exposure time 1 ชั่วโมง

สถานที่ทดสอบ่ ตำบลปางหมู อำเภอเมือง จังหวัดแม่ฮ่องสอน

ยุงพาหะ An. minimus

ระยะ ตัวเต็มวัย เพศเมีย

อุณหภูมิระหว่างทดสอบ Exposure tube 28-29 °C Holding tube 25-29 °C

ความชื้นสัมพัทธ์ 75-95% 75-95%

Replicates	Replicate 1	Replicate 2	Replicate 3	Raplicat 4	Replicate 5	Total test	Total control
Test/Time(hr)	24	24	24	24	24	24	24
No exposed	20	20	20	20	20	100	100
No knockdown (at the end ofexposure)	20	19	20	20	20	99	100
% knockdown (at the end of exposure)	100	100	100	100	100	100	100
No dead (at the end of exposure)	20	20	20	20	20	100	100
% dead (at the end of exposure)	100	100	100	100	100	100	100

8. Permethrin (0.25%) exposure time 1 ชั่วโมง

สถานที่ทดสอบ ตำบลปางหมู อำเภอเมือง จังหวัดแม่ฮ่องสอน

ยุงพาหะ An. minimus

ระยะ ตัวเต็มวัย เพศเมีย

อุณหภูมิระหว่างทดสอบ	Exposure tube	Holding tube
	29 °C	28 °C
ความชื้นสัมพัทธ์	75-85%	75-85%

Replicates	Replicate 1	Replicate 2	Replicate 3	Raplicat 4	Replicate 5	Total test	Total control
Test/Time(hr)	24	24	24	24	24	24	24
No exposed	20	20	20	20	20	100	100
No knockdown (at the end of exposure)	20	20	20	20	20	100	100
% knockdown (at the end of exposure)	100	100	100	100	100	100	100
No dead (at the end of exposure)	20	20	20	20	20	100	100
% dead (at the end of exposure)	100	100	100	100	100	100	100

9. Deltametrin (0.025%) exposure time 1 ชั่วโมง

สถานที่ทดสอบ อำเภอแม่ละมาด จังหวัดตาก

ยุงพาหะ An. minimus

ระยะ ตัวเต็มวัย เพศเมีย

อุณหภูมิระหว่างทดสอบ	Exposure tube	Holding tube
	27 °C	26 °C
ความชื้นสัมพัทธ์	85%	85%

Replicates	Replicate 1	Replicate 2	Replicate 3	Raplicat 4	Replicat5	Total test	Total control
Test/Time(hr)	24	24	24	24	24	24	24
No exposed	20	20	20	20	18	98	100
No knockdown (at the end of exposure)	20	20	20	20	18	98	100
% knockdown (at the end of exposure)	100	100	100	100	100	100	100
No dead (at the end of exposure)	20	20	20	20	18	98	100
% dead (at the end of exposure)	100	100	100	100	100	100	100

10. Permethrin (0.25%) Exposure time 1 ชั่วโมง

สถานที่ทดสอบ อำเภอแม่ละมาด จังหวัดตาก

ยุงพาหะ An. minimus

ระยะ ตัวเต็มวัย เพศเมีย

อุณหภูมิระหว่างทดสอบ	Exposure tube	Holding tube
	26 °C	26 °C
ความชื้นสัมพัทธ์	80%	80%

Replicates	Replicate 1	Replicate 2	Replicate 3	Raplicat 4	Replicate 5	Total test	Total control
Test/Time(hr)	24	24	24	24	24	24	24
No exposed	20	20	20	20	20	100	100
No knockdown (at the end of exposure)	20	20	20	20	20	100	100
% knockdown (at the end of exposure)	100	100	100	100	100	100	100
No dead (at the end of exposure)	20	20	20	20	20	100	100
% dead (at the end of exposure)	100	100	100	100	100	100	100

11. DDT (4%) exposure time 1 ชั่วโมง

สถานที่ทดสอบ บ้านพุเตย ตำบลท่าเสา อำเภอไทรโยค จังหวัดกาญจนบุรี

ยุงพาหะ An. minimus

ระยะ ตัวเต็มวัย เพศเมีย

อุณหภูมิระหว่างทดสอบ	Exposure tube	Holding tube
	27-28 °C	27-28 °C
ความชื้นสัมพัทธ์	75-85%	75-85%

Replicates	Replicate 1	Replicate 2	Replicate 3	Raplicat 4	Replicate 5	Total test	Total control
Test/Time(hr)	24	24	24	24	24	24	24
No exposed	20	20	20	20	20	100	100
No knockdown (at the end of exposure)	20	20	20	20	20	100	100
% knockdown (at the end of exposure)	100	100	100	100	100	100	100
No dead (at the end of exposure)	20	20	20	20	20	100	100
% dead (at the end of exposure)	100	100	100	100	100	100	100

12. Permethrin (0.25%) exposure time 1 ชั่วโมง

สถานที่ทดสอบ บ้านพุเตย ตำบลท่าเสา อำเภอไทรโยค จังหวัดกาญจนบุรี

ยุงพาหะ An. minimus

ระยะ ตัวเต็มวัย เพศเมีย

อุณหภูมิระหว่างทดสอบ	Exposure tube	Holding tube
	28-29 °C	27-28 °C
ความชื้นสัมพัทธ์	75-85%	75-85%

Replicates	Replicate 1	Replicate 2	Replicate 3	Raplicat 4	Replicate 5	Total test	Total control
Test/Time(hr)	24	24	24	24	24	24	24
No exposed	20	20	20	20	20	100	100
No knockdown (at the end of exposure)	20	20	20	20	20	100	100
% knockdown (at the end of exposure)	100	100	100	100	100	100	100
No dead (at the end of exposure)	20	20	20	20	20	100	100
% dead (at the end of exposure)	100	100	100	100	100	100	100

13. Deltametrin (0.025%) exposure time 1 ชั่วโมง

วันที่ทดสอบ วันที่ 11 มิถุนายน พ.ศ. 2541 0900 น.

สถานที่ทดสอบ บ้านพุเตย ตำบลท่าเสา อำเภอไทรโยค จังหวัดกาญจนบุรี

ยุงพาหะ An. minimus

ระยะ ตัวเต็มวัย เพศเมีย

อุณหภูมิระหว่างทดสอบ	Exposure tube	Holding tube
	26-27 °C	27-28 °C
ความชื้นสัมพัทธ์	75-85%	75-85%

Replicates	Replicate 1	Replicate 2	Replicate 3	Raplicate 4	Replicate 5	Total test	Total control
Test/Time(hr)	24	24	24	24	24	24	24
No exposed	20	20	20	20	17	97	100
No knockdown (at the end of exposure)	20	20	20	20	17	97	100
% knockdown (at the end of exposure)	100	100	100	100	100	100	100
No dead (at the end of exposure)	20	20	20	20	17	97	100
% dead (at the end of exposure)	100	100	100	100	100	100	100

14. Lambda-Cyhalothrin (0.1%) exposure time 1 ชั่วโมง

สถานที่ทดสอบ บ้านพุเตย ตำบลท่าเสา อำเภอไทรโยค จังหวัดกาญจนบุรี

ยุงพาหะ An. minimus

ระยะ ตัวเต็มวัย เพศเมีย

อุณหภูมิระหว่างทดสอบ	Exposure tube	Holding tube
	28-29 °C	27-28 °C
ความชื้นสัมพัทธ์	75-95%	75-95%

Replicates	Replicate 1	Replicate 2	Replicate 3	Raplicate 4	Replicate 5	Total test	Total control
Test/Time(hr)	24	24	24	24	24	24	24
No exposed	20	20	20	20	20	100	100
No knockdown (at the end of exposure)	20	20	20	20	20	100	100
% knockdown (at the end of exposure)	100	100	100	100	100	100	100
No dead (at the end of exposure)	20	20	20	20	18	98	100
% dead (at the end of exposure)	100	100	100	100	100	100	100

15. Etophenpox (0.25%) exposure time 1 ชั่วโมง

สถานที่ทดสอบ บ้านพุเตย ตำบลท่าเสา อำเภอไทรน้อย จังหวัดกาญจนบุรี

ยุงพาหะ An. minimus

ระยะ ตัวเต็มวัย เพศเมีย

อุณหภูมิระหว่างทดสอบ	Exposure tube	Holding tube
	27-28 °C	27-28 °C
ความชื้นสัมพัทธ์	75-85%	75-85%

Replicates	Replicate 1	Replicate 2	Replicate 3	Raplicate 4	Replicate 5	Total test	Total control
Test/Time(hr)	24	24	24	24	24	24	24
No exposed	20	20	20	20	20	100	100
No knockdown (at the end of exposure)	20	20	20	20	20	100	100
% knockdown (at the end of exposure)	100	100	100	100	100	100	100
No dead (at the end of exposure)	20	20	20	20	20	100	100
% dead (at the end of exposure)	100	100	100	100	100	100	100

ผลการทดลองที่ 2 (Behavioral Avoidance หรือ Behavioral Resistance)

Table 1.Mortalities of Anopheles minimus females after a 24-h holding period following exposures in contact trials of excito-repellency tests.

Condition	Population	Chemical	Number		%Mortality		
				#Test	Escaped(%)	Escaped	not escaped
CONTACT							
		Young colony	DDT	100	67(67)	0	1
			DDT-C	99	18(18)	1	2
			Del	126	107(85)	3	2
			Del-C	127	10(8)	0	1
			Lam	177	132(75)	5	7
			Lam-C	171	32(19)	1	0
		Wild population	DDT	100	67(67)	1	0
			DDT-C	100	17(17)	2	1
			Del	98	53(54)	1	0
			Del-C	94	10(10)	0	1
			Lam	96	91(95)	1	0
			Lam-C	97	18(19)	0	0
NON-CONTACT							
		Young colony	DDT	101	25(24)	1	0
			DDT-C	96	11(11)	2	1
			Del	124	35(28)	0	0
			Del-C	125	17(14)	1	1
			Lam	174	27(16)	1	0
			Lam-C	174	8(5)	0	0
		Wild population	DDT	98	24(24)	0	0
			DDT-C	97	10 (10)	0	0
			Del	98	24(24)	0	0
			Del-C	100	11(11)	0	1
			Lam	99	19(19)	0	0
			Lam-C	95	15(16)	0	0

Codes for chemicals and doses DDT=2g/m^2DDT; Del=0.0625 g/m^2 deltamethrin, Lam=0.0369 g/m^2 lambdacyhalothrin

DDT-C, Del-C and Lam-C are codes for controls (without insecticides).

Table 2.Time in minutes for 50 (ET_{50}) and 75% (ET_{75}) of Anopheles minimus females to escape from exposure chambers (in excito-repellency tests) treated with DDT, deltamethrin and lambdacyhalothrin

Population/	DDT[1]		Del[2]		Lam[3]	
Colony	ET_{50}[4]	ET_{75}[4]	ET_{50}	ET_{75}	ET_{50}	ET_{75}
Young	16	--[5]	6	15	6	29
Wild	15	--	26	--	4	10

[1]DDT at 2 g/m^2

[2]Deltamethrin at 0.0625 g/m^2

[3]Lamdacyhalothrin at 0.0369 g/m^2

[4]Survival analysis was used to estimate the time in minutes for 50 and 75% of test populations to take off from the exposure chambers.

[5]Very few mosquitoes (<75%) escape from exposure chambers, so that the ET_{75} estimates could not be calculated for a 30- min exposure period.

Table 3.Comparison of escape responses between 2 populations of Anopheles minimus females in contact vs. non-contact trials by insecticides.

Insecticides	Contact trial	Non contact trial
DDT	Wild vs. Young	Wild vs. Young
Del	Wild vs. Young*	Wild vs. Young
Lam	Wild vs. Young*	Wild vs. Young

DDT= 2 g/m^2 DDT

Del=0.0625 g/m^2 deltamethrin

Lam=0.0369 g/m^2 lambdacyhalothrin

The * identifies results of log-rank tests with statistically significant (0.05 level of probability) differences in pattern of escape behavior

Table 4.Comparison of escape responses between contact vs. non-contact, contact vs. control and non-contact vs. control for 2 test populations

Population	contact vs. non-contact	contact vs. control	non-contact vs. control
Young			
	DDT*	DDT*	DDT*
	Del*	Del*	Del*
	Lam*	Lam*	lam*
Wild			
	DDT*	DDT*	DDT*
	Del*	Del*	Del*
	Lam*	Lam*	Lam*

DDT= 2 g/m^{2}DDT

Del=0.0625 g/m^2 deltamethrin

Lam=0.0369 g/m^2 lambdacyhalothrin

The * identifies results of log-rank tests with statistically significant (0.05 level of probability) differences in pattern of escape behavior

Table 5. Mortalities of unfed, sugar fed, early blood fed and late blood fed Anopheles minimus females after a 24-h holding period following exposures in contact trials with DDT, deltamethrin and lambdacyhalothrin of excito-repellency tests.

Chemicals	Physiological conditions	Number(%)		% Mortality	
		Tested	Escaped (%)	Escaped	Not escaped
CONTACT					
DDT					
DDT	Unfed	200	142(71)	3.5	3.4
DDT-C	Unfed	200	26(13)	3.8	0
DDT	Sugar fed	200	114(57)	0.8	1.1
DDT-C	Sugar fed	200	23(11.5)	0	0
DDT	Early blood fed	200	98(49)	0	0
DDT-C	Early blood fed	200	16(8)	0	0
DDT	Late blood fed	200	100(50)	0	0
DDT-C	Late blood fed	200	17(8.5)	0	0
Deltamethrin					
Del	Unfed	200	177(88.5)	4.0	8.7
Del-C	Unfed	200	19(9.5)	0	0.5
Del	Sugar fed	200	138(59)	0.14	0
Del-C	Sugar fed	200	3(1.5)	0	0.5
Del	Early blood fed	200	121(60.5)	4.1	2.5
Del-C	Early blood fed	200	10(5)	0.1	2.1
Del	Late blood fed	200	174(87)	0	0
Del-C	Late blood fed	200	15(7.5)	0	0
Lambdacyhalothrin					
LAM	Unfed	200	178(89)	1.1	22.7
LAM-C	Unfed	200	41(20.5)	0	0
LAM	Sugar fed	200	168(84)	2.4	15.6
LAM-C	Sugar fed	200	18(9)	0	0.6
LAM	Early blood fed	200	174(87)	14.9	38.5
LAM-C	Early blood fed	200	17(8.5)	11.8	2.2
LAM	Late blood fed	200	168(84)	7.7	43.8
LAM-C	Late blood fed	200	10(5)	0.2	5.3

Codes for chemicals and doses DDT=2g/m^2 DDT; Del=0.0625 g/m^2 deltamethrin, Lam=0.0369 g/m^2 lambdacyhalothrin

DDT-C, Del-C and Lam-C are codes for controls (without insecticides).

Table 6. Mortalities of unfed, sugar fed, early blood fed and late bloodfed Anopheles minimus females after a 24-h holding period following exposures in noncontact trials with DDT, deltamethrin and lambdacyhalothrin of excito-repellency tests.

Chemicals	Physiological conditions	Number(%)		% Mortality	
		Tested	Escaped (%)	Escaped	Not escaped
NONCONTACT					
DDT					
DDT	Unfed	200	26(13)	0	0.5
DDT-C	Unfed	200	27(13.5)	0.1	0.1
DDT	Sugar fed	200	53(26.5)	0	0
DDT-C	Sugar fed	200	28(14)	0	0.6
DDT	Early blood fed	200	31(15.5)	0	0.6
DDT-C	Early blood fed	200	9(4.5)	0	0
DDT	Late blood fed	200	45(22.5)	0	0
DDT-C	Late blood fed	200	17(8.5)	0	0
Deltamethrin					
Del	Unfed	200	28(14)	0	0.6
Del-C	Unfed	200	9(4.5)	0	1
Del	Sugar fed	200	19(9.5)	0	0
Del-C	Sugar fed	200	8(4)	0	0
Del	Early blood fed	200	23(11.5)	8	5
Del-C	Early blood fed	200	7(3.5)	0	5.7
Del	Late blood fed	200	42(21)	0	0
Del-C	Late blood fed	200	8(4)	0	0
Lambdacyhalothrin					
LAM	Unfed	200	47(23.5)	0	0
LAM-C	Unfed	200	16(8)	0	0
LAM	Sugar fed	200	10(5)	0	0.5
LAM-C	Sugar fed	200	4(2)	0	0
LAM	Early blood fed	200	40(20)	15.0	4.3
LAM-C	Early blood fed	200	12(6)	0	0
LAM	Late blood fed	200	27(13.5)	7.4	4.6
LAM-C	Late blood fed	200	16(8)	0	3.8

Codes for chemicals and doses DDT=2g/m^2 DDT; Del=0.0625 g/m^2 deltamethrin, Lam=0.0369 g/m^2 lambdacyhalothrin

DDT-C, Del-C and Lam-C are codes for controls (without insecticides).

Table 7. Comparison of escape patterns (in excito-repellency test) of different physiological conditions by DDT, deltamethrin and lambdacyhalothrin for laboratory Anopheles minimus colony in contact and noncontact trials

Condition	Physiological conditions	DDT (p-value)	Deltamethrin (p-value)	Lambdacyhalothrin (p-value)
CONTACT	UF vs. SF	0.0029*	0.0001*	0.0001*
	UF vs. EBF	0.0001*	0.0001*	0.0033*
	UF vs. LBF	0.0001*	0.9130	0.0003*
	SF vs. EBF	0.0719	0.4494	0.0104*
	SF vs. LBF	0.2543	0.0001*	0.0598
	EBF vs. LBF	0.5175	0.0001*	0.5072
NONCONTACT	UF vs. SF	0.0008*	0.1563	0.0001*
	UF vs. EBF	0.4364	0.4597	0.3053
	UF vs. LBF	0.0093*	0.0613	0.0066*
	SF vs. EBF	0.0101*	0.4977	0.0001*
	SF vs. LBF	0.4584	0.0011*	0.0034*
	EBF vs. LBF	0.0686	0.0093*	0.0864

DDT:2 g/m^2 DDT, Del:0.025% Deltamethrin, Lam:0.1% Lambdacyhalothrin, C: Control

UF: Unfed, SF: Sugarfed, EBF:Early bloodfed, LBF:Late bloodfed

Table 8. Time in minutes for 50 (ET50) and 75% (ET75) of Anopheles minimus females to escape from exposure chambers (in excito-repellency test) treated with DDT, deltamethrin and lambdacyhalothrin

Physiological conditions	DDT[1]		Deltamethrin[2]		Lambdacyhalothrin[3]	
	ET_{50}[4]	ET_{75}[4]	ET_{50}	ET_{75}	ET_{50}	ET_{75}
Unfed	16	-	6	14	2	5
Sugar fed	25	-	16	-	6	14
Early blood fed	-	-	18	-	3	10
Late blood fed	-	-	6	13	3	10

1DDT at 2 g/m2

2Deltamethrin at 2 g/m2

3Lambdacyhalothrin at 2 g/m2

4Survival analysis was used to estimate the time in minutes for 50 and 75% of test populations to escape from exposure chambers.

5Very few specimens escaped from exposure chambers, so the ET50 and ET75 estimates could not be calculated for a 30-min exposure period.

Table 9. Comparison of escape patterns (in excito-repellency test) of different physiological conditions by DDT, deltamethrin and lambdacyhalothrin for laboratory Anopheles minimus colony in contact vs. control, non-contact vs. controland contact vs. noncontact.

Chemicals	Physiological conditions	Contact vs. Control (p-value)	Noncontact vs. Control (p-value)	Contact vs. Noncontact (p-value)
DDT	Unfed	0.0001*	0.8089	0.0001*
	Sugar fed	0.0001*	0.0030*	0.0001*
	Early blood fed	0.0001*	0.0002*	0.0001*
	Late blood fed	0.0001*	0.0001*	0.0001*
Deltamethrin	Unfed	0.0001*	0.0009*	0.0001*
	Sugar fed	0.0001*	0.0307*	0.0001*
	Early blood fed	0.0001*	0.0021*	0.0001*
	Late blood fed	0.0001*	0.0001*	0.0001*
Lambdacyhalothrin	Unfed	0.0001*	0.0001*	0.0001*
	Sugar fed	0.0001*	0.0994	0.0001*
	Early blood fed	0.0001*	0.0001*	0.0001*
	Late blood fed	0.0001*	0.0395*	0.0001*

[1]DDT at 2 g/m^2

[3]Lamdacyhalothrin at 0.0369 g/m^2

[4]Survival analysis was used to estimate the time in minutes for 50 and 75% of test populations to take off from the exposure chambers.

[5]Very few mosquitoes (<75%) escape from exposure chambers, so that the ET_{75} estimates could not be calculated for a 30- min exposure period.

Figure 1. An improved excito-repellenct test chamber used to study the pesticide avoidance behavior of Anopheles minimus in this study

Figure 2. Escape probability of unfed, sugerfed, early bloodfed and late bloodfed female Anopheles minimus in exposure chambers in contact and noncontact with DDT.

Figure 3. Escape probability of unfed, sugerfed, early bloodfed and late bloodfed female Anopheles minimus in exposure chambers in contact and noncontact with deltamethrin.

Figure 4. Escape probability of unfed, sugerfed, early bloodfed and late bloodfed female Anopheles minimus in exposure chambers in contact and noncontact with lambdacyhalothrin

Figure 5. Escape probability of unfed, sugerfed, early bloodfed and late bloodfed female Anopheles minimus in exposure chambers in noncontact and control with DDT.

Figure 6. Escape probability of unfed, sugerfed, early bloodfed and late bloodfed female Anopheles minimus in exposure chambers in noncontact and control with deltamethrin.

Figure 7. Escape probability of unfed, sugerfed, early bloodfed and late bloodfed female Anopheles minimus in exposure chambers in noncontact and control with lambdacyhalothrin

Figure 8.Escape probability of Anopheles minimus females remaining in exposure chambers in contact vs. non-contact trials with 2 g/m^2 DDT, 0.0625 g/m^2 deltamethrin and 0.0369 g/m^2 lambdacyhalothrin.

Figure 9.Escape probability of Anopheles minimus females remaining in exposure chambers in contact vs. control trialswith 2 g/m^2 DDT, 0.0625 g/m^2 deltamethrin and 0.0369 g/m^2 lambdacyhalothrin.

Figure 10.Escape probability of Anopheles minimus females remaining in exposure chambers in non-contact vs. control trials with 2 g/m^2 DDT, 0.0625 g/m^2 deltamethrin and 0.0369 g/m^2 lambdacyhalothrin.

FIGURE 1

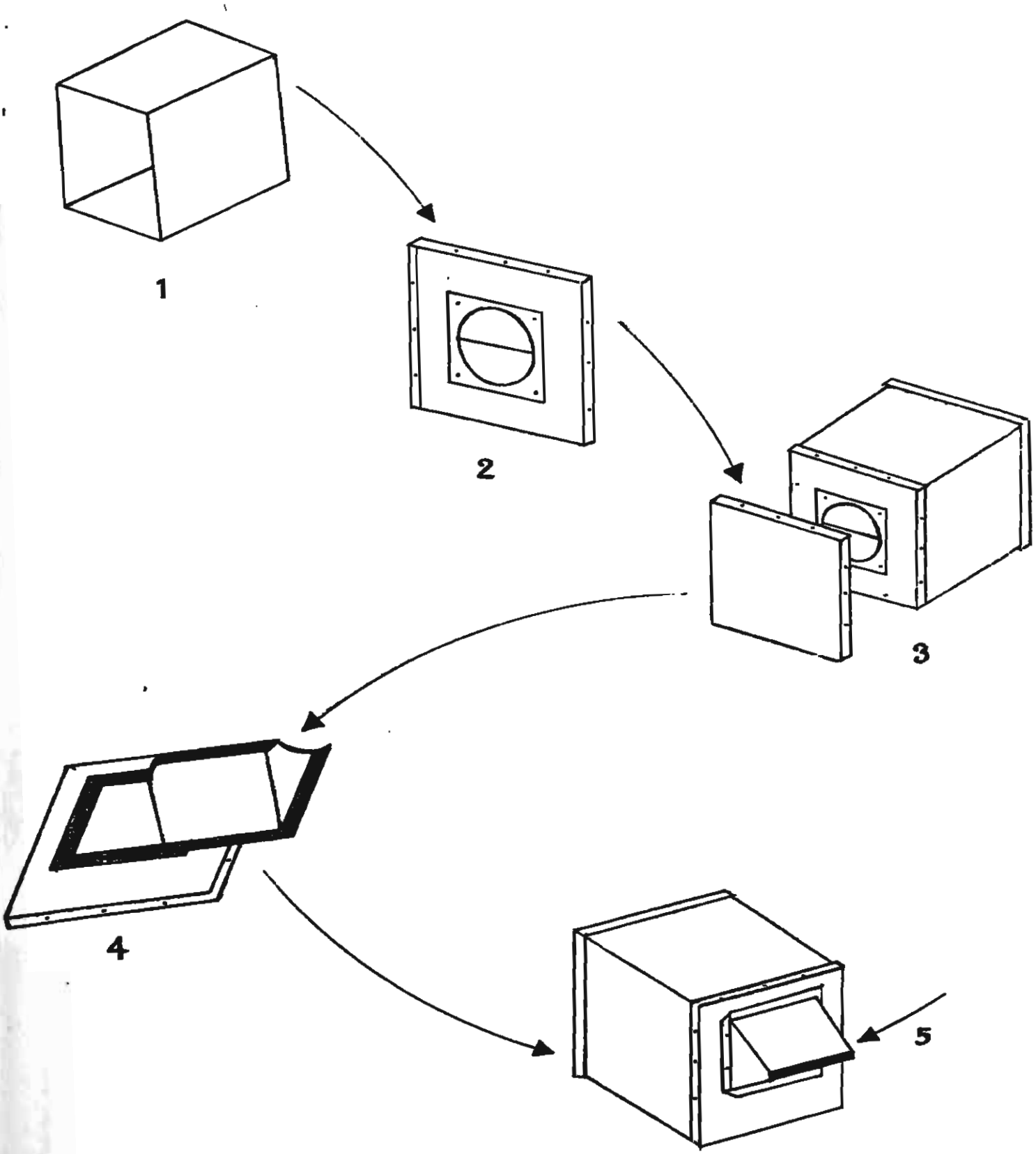
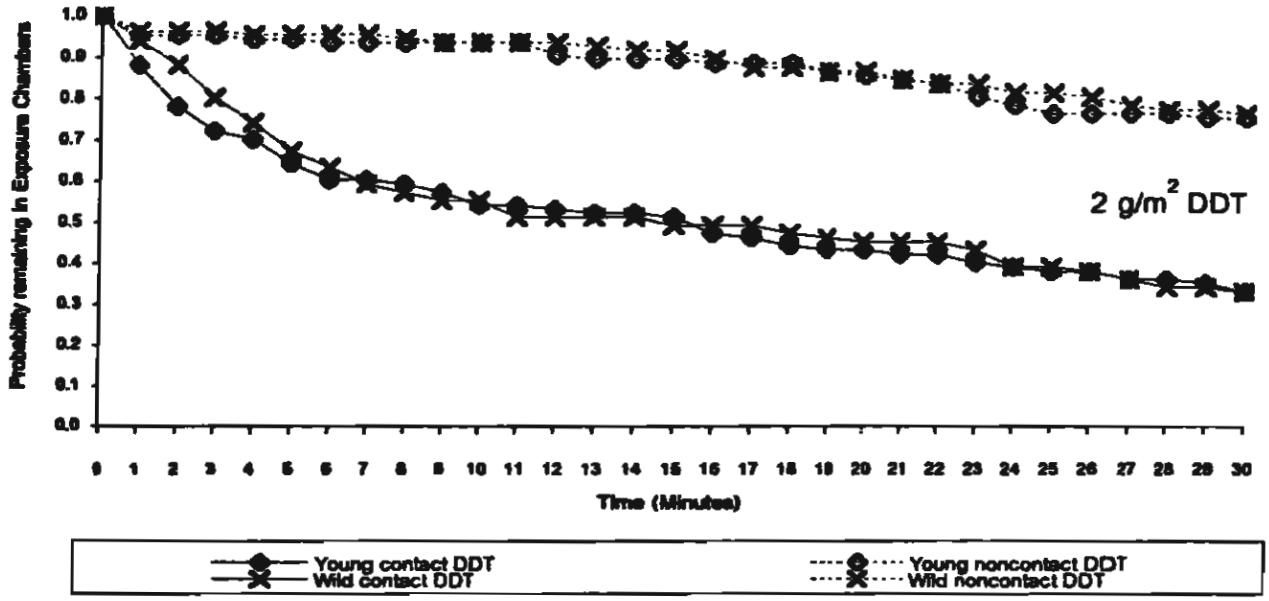
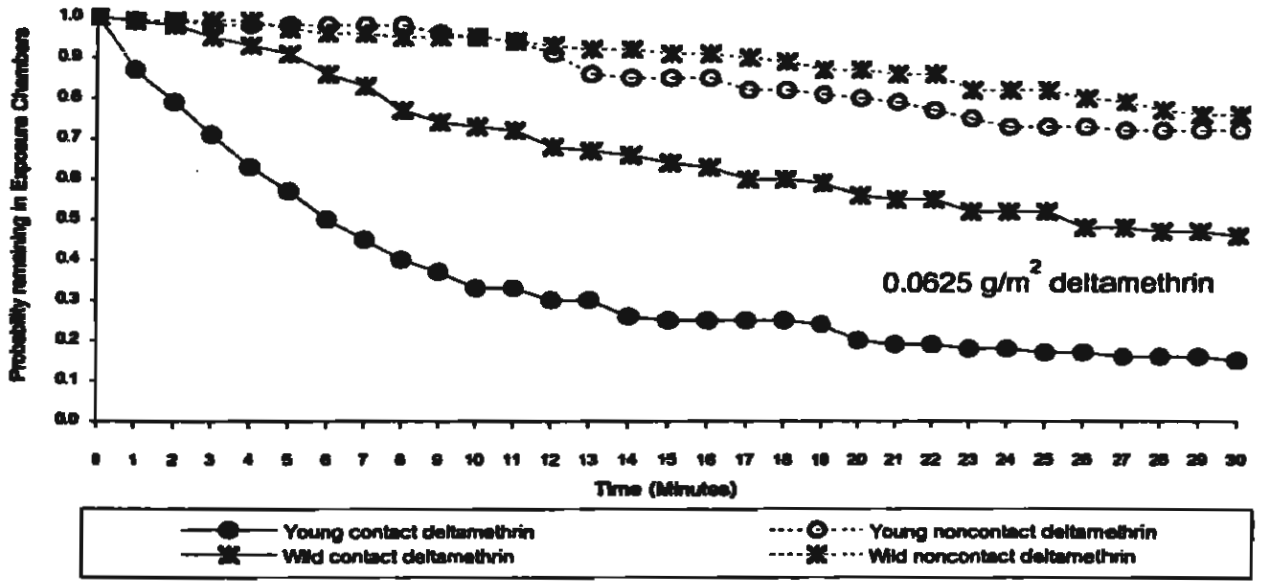


FIGURE 2


Probability remaining in Exposure Chambers
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time (Minutes)
2 g/m2 DDT
Young contact DDT
Wild contact DDT
Young noncontact DDT
Wild noncontact DDT


Probability remaining in Exposure Chambers
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time (Minutes)
0.0625 g/m2 deltamethrin
Young contact deltamethrin
Wild contact deltamethrin
Young noncontact deltamethrin
Wild noncontact deltamethrin

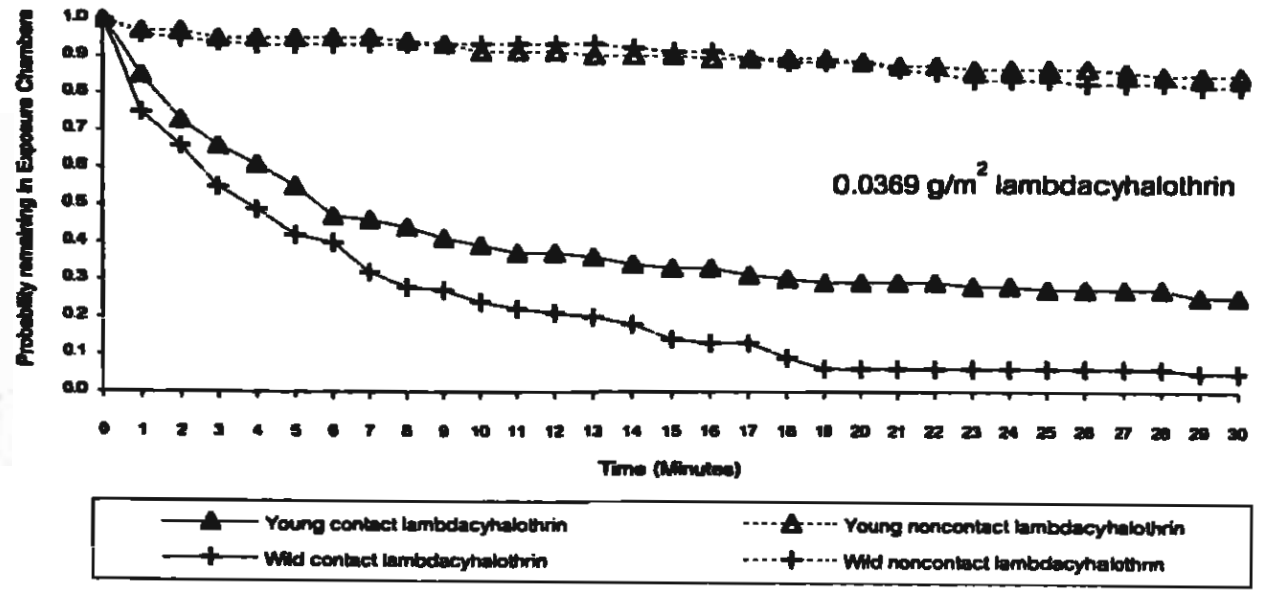
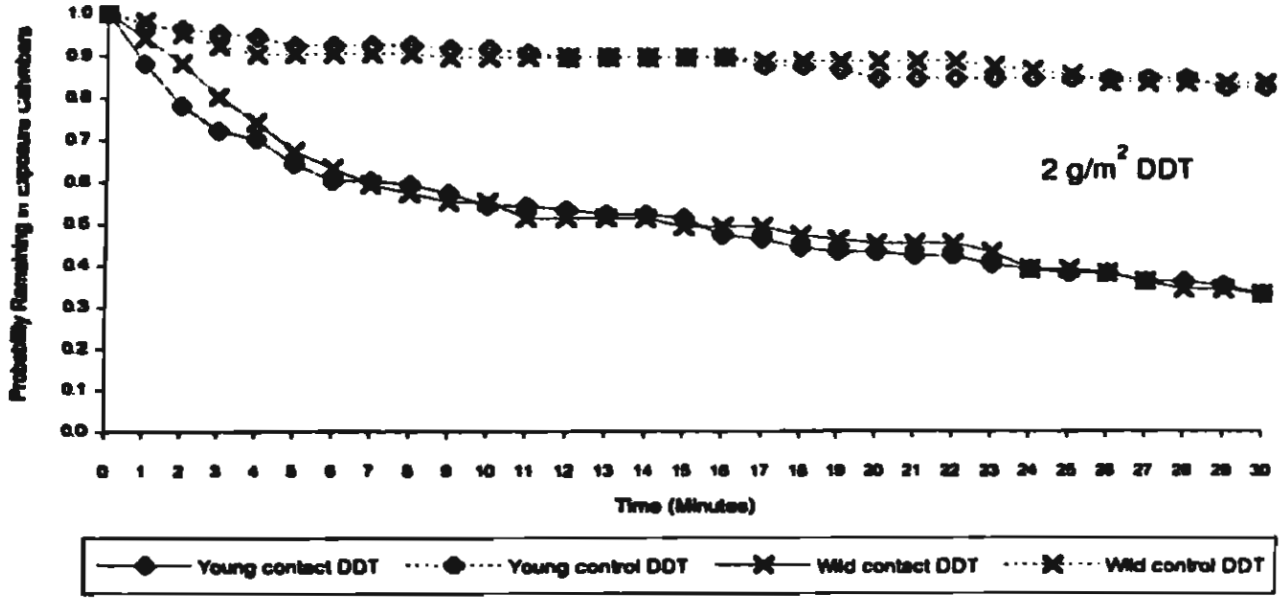
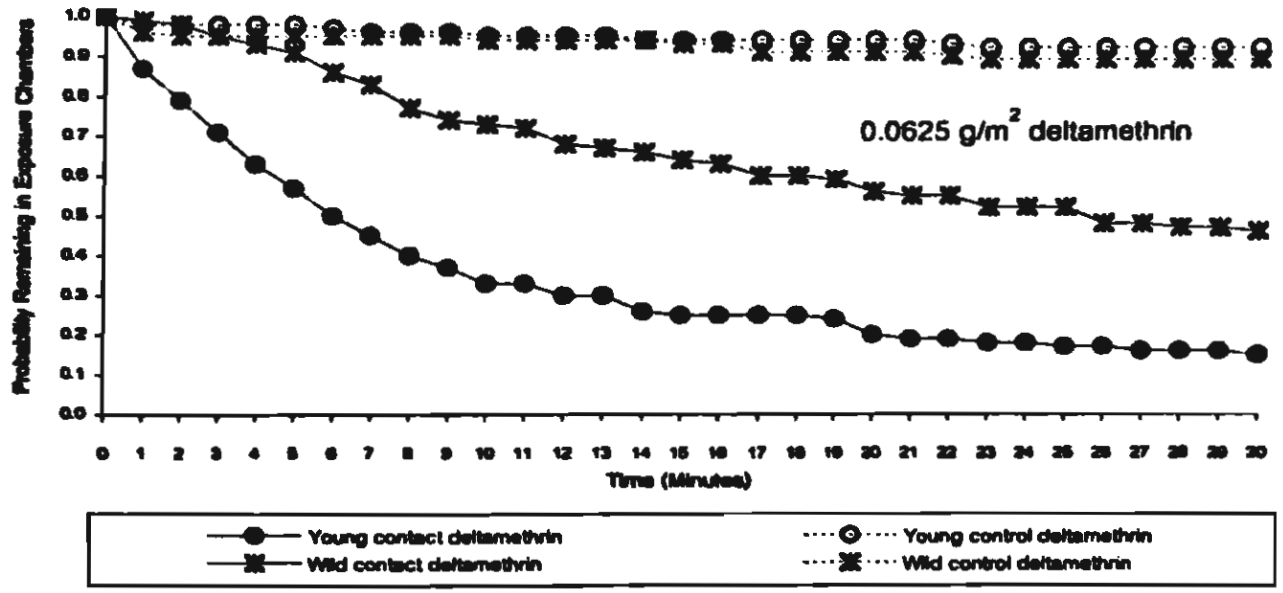

Probability remaining in Exposure Chambers
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time (Minutes)
0.0369 g/m2 lambdacyhalothrin
Young contact lambdacyhalothrin
Wild contact lambdacyhalothrin
Young noncontact lambdacyhalothrin
Wild noncontact lambdacyhalothrin

FIGURE 3


Probability Remaining in Exposure Chambers
2 g/m² DDT
Time (Minutes)
Young contact DDT
Young control DDT
Wild contact DDT
Wild control DDT


Probability Remaining in Exposure Chambers
0.0625 g/m² deltamethrin
Time (Minutes)
Young contact deltamethrin
Young control deltamethrin
Wild contact deltamethrin
Wild control deltamethrin

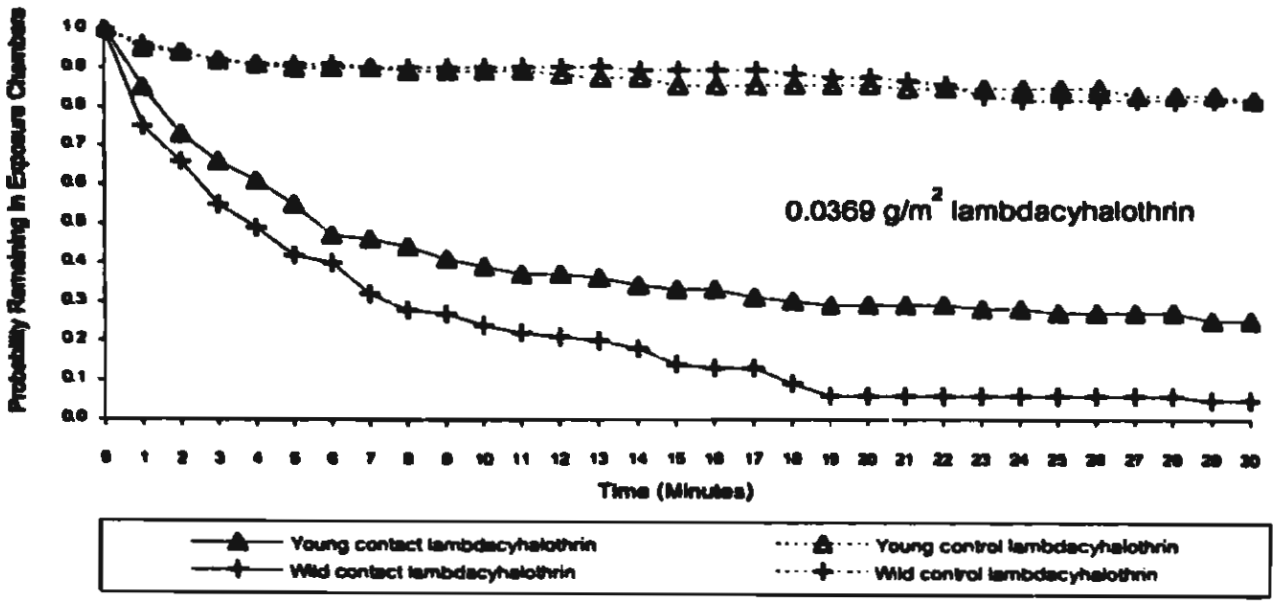
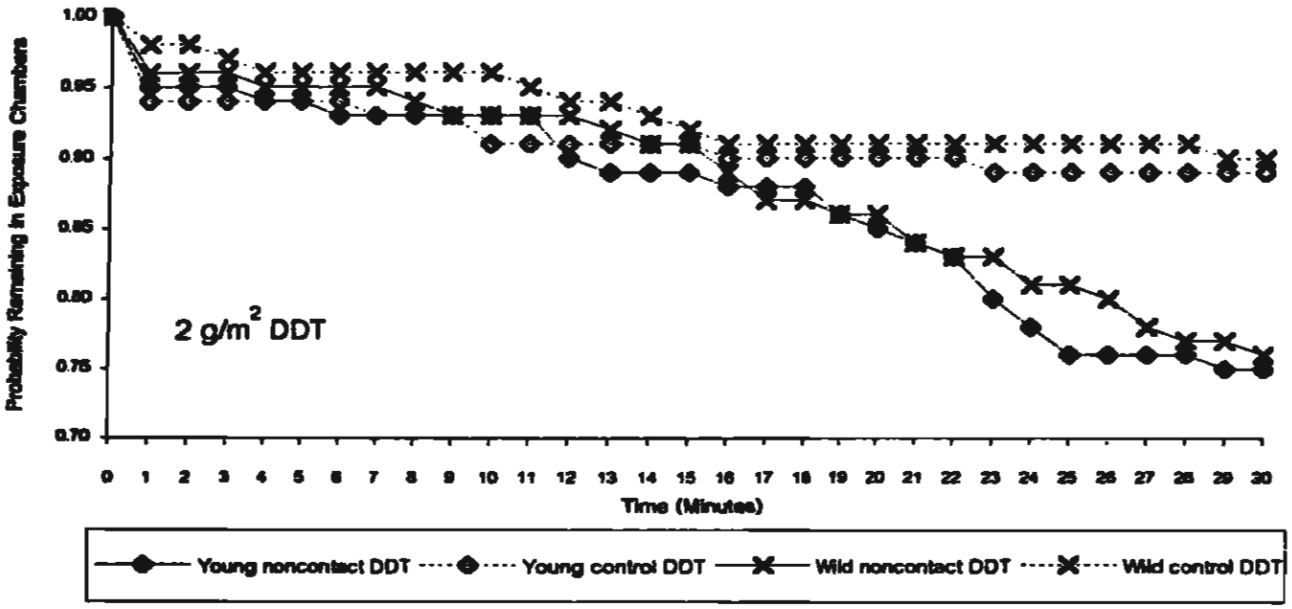
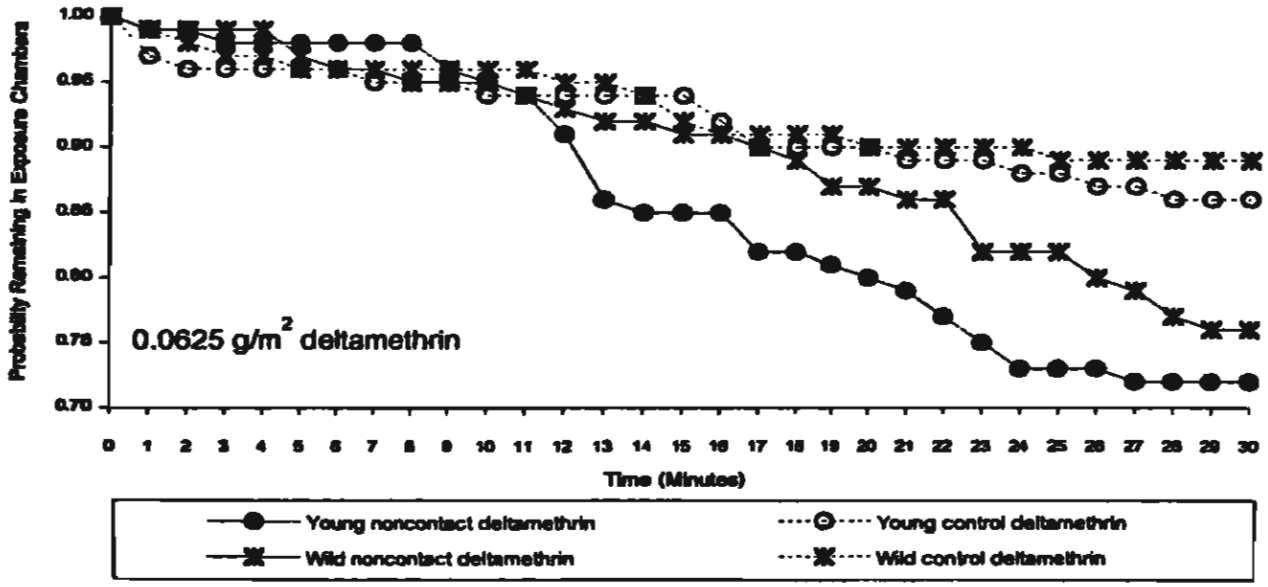

Probability Remaining in Exposure Chambers
0.0369 g/m² lambdacyhalothrin
Time (Minutes)
Young contact lambdacyhalothrin
Young control lambdacyhalothrin
Wild contact lambdacyhalothrin
Wild control lambdacyhalothrin

FIGURE 4


Probability Remaining in Exposure Chambers
1.00
0.95
0.90
0.85
0.80
0.75
0.70
2 g/m2 DDT
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time (Minutes)
Young noncontact DDT
Young control DDT
Wild noncontact DDT
Wild control DDT


Probability Remaining in Exposure Chambers
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.0625 g/m2 deltamethrin
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time (Minutes)
Young noncontact deltamethrin
Young control deltamethrin
Wild noncontact deltamethrin
Wild control deltamethrin

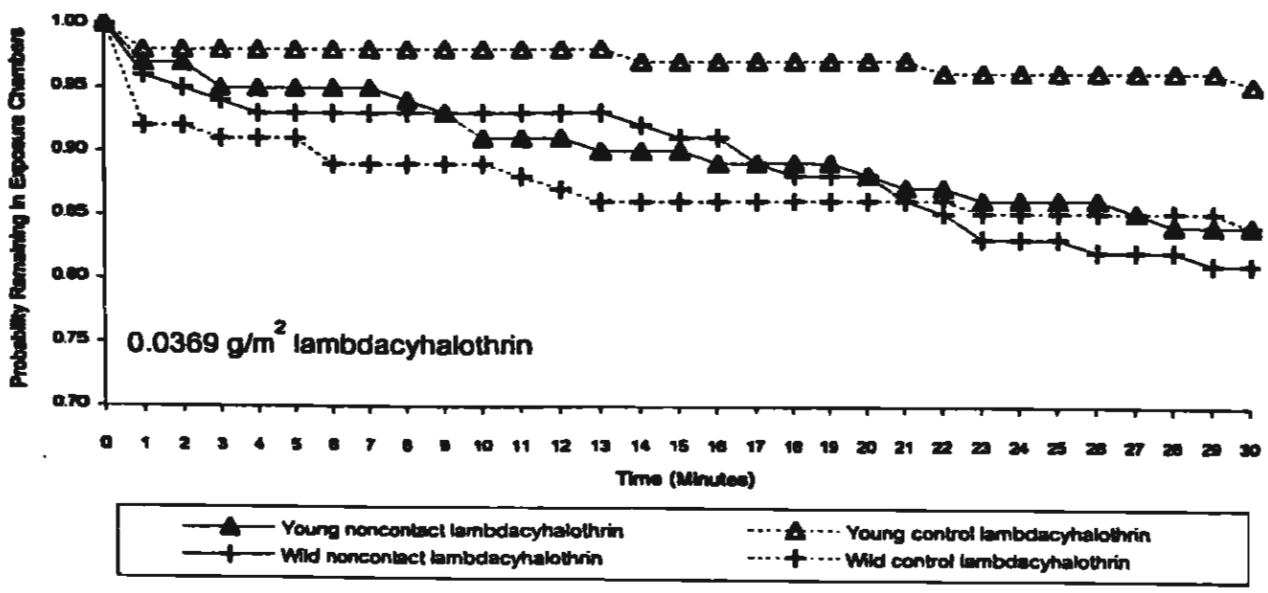

Probability Remaining in Exposure Chambers
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.0369 g/m2 lambdacyhalothrin
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time (Minutes)
Young noncontact lambdacyhalothrin
Young control lambdacyhalothrin
Wild noncontact lambdacyhalothrin
Wild control lambdacyhalothrin

FIGURE 5

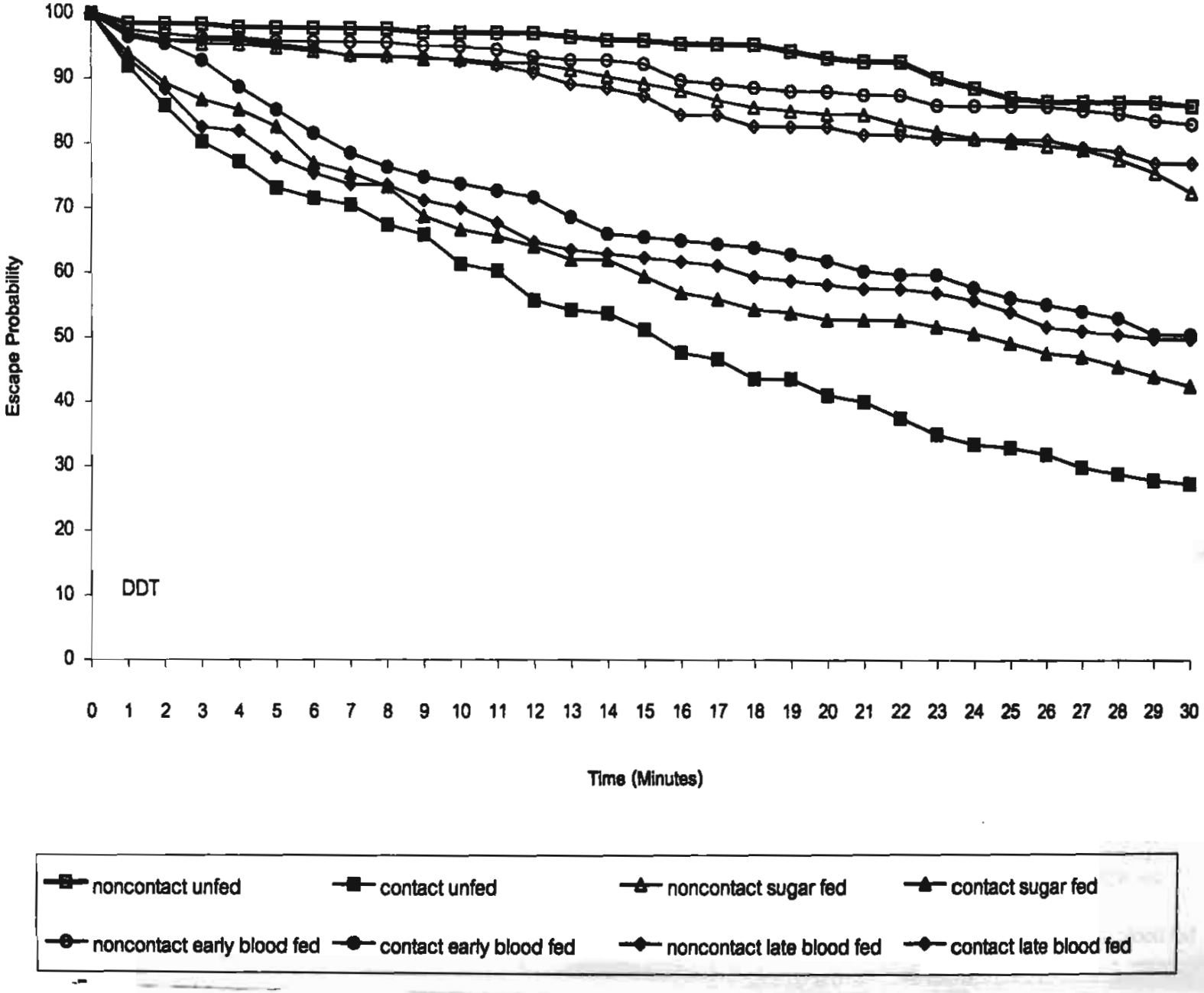

Escape Probability
100
90
80
70
60
50
40
30
20
10
0
DDT
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time (Minutes)
noncontact unfed
contact unfed
noncontact sugar fed
contact sugar fed
noncontact early blood fed
contact early blood fed
noncontact late blood fed
contact late blood fed

FIGURE 6

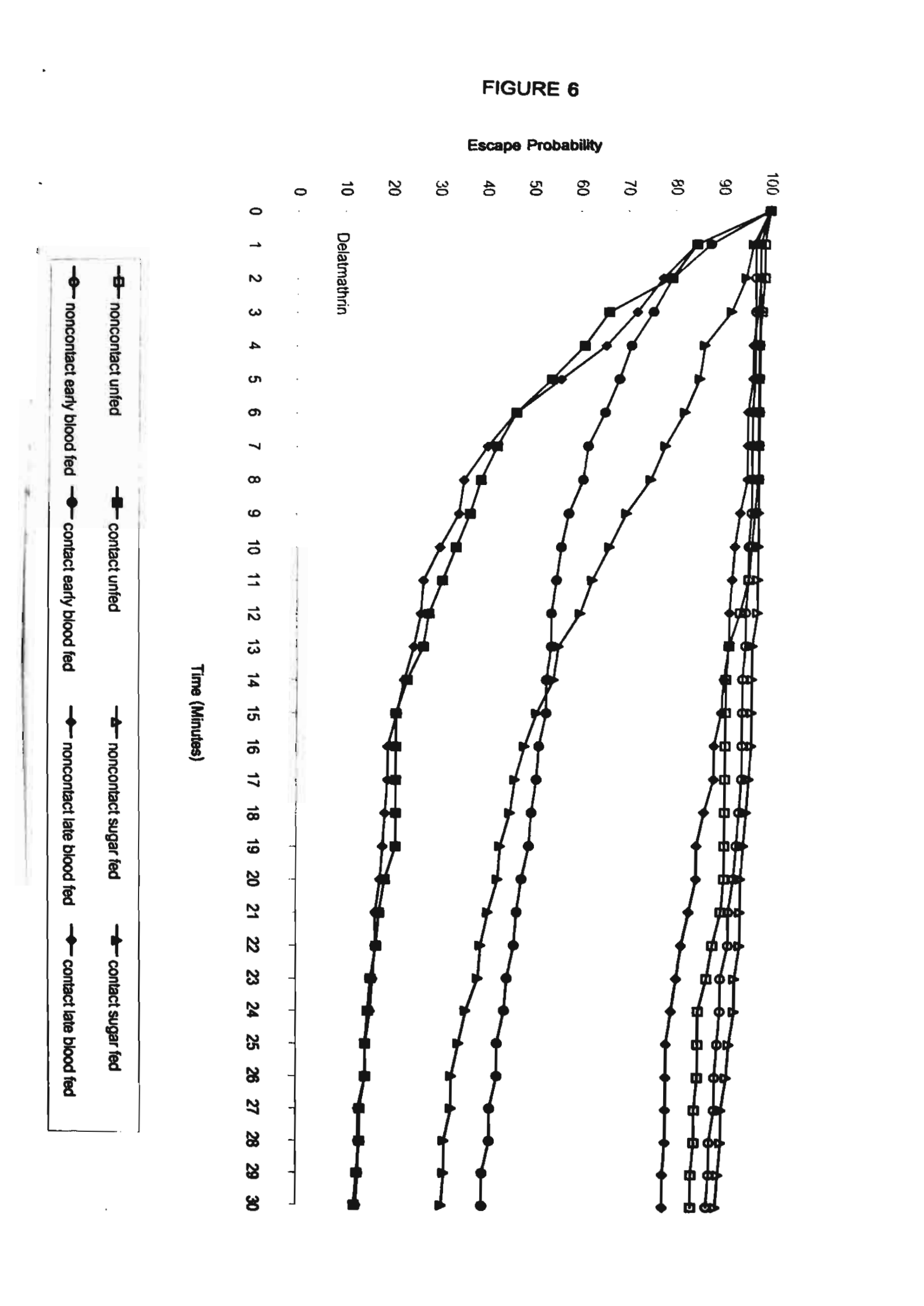

Escape Probability
100
90
80
70
60
50
40
30
20
10
0
Deltamethrin
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time (Minutes)
noncontact unfed
contact unfed
noncontact sugar fed
contact sugar fed
noncontact early blood fed
contact early blood fed
noncontact late blood fed
contact late blood fed

FIGURE 7

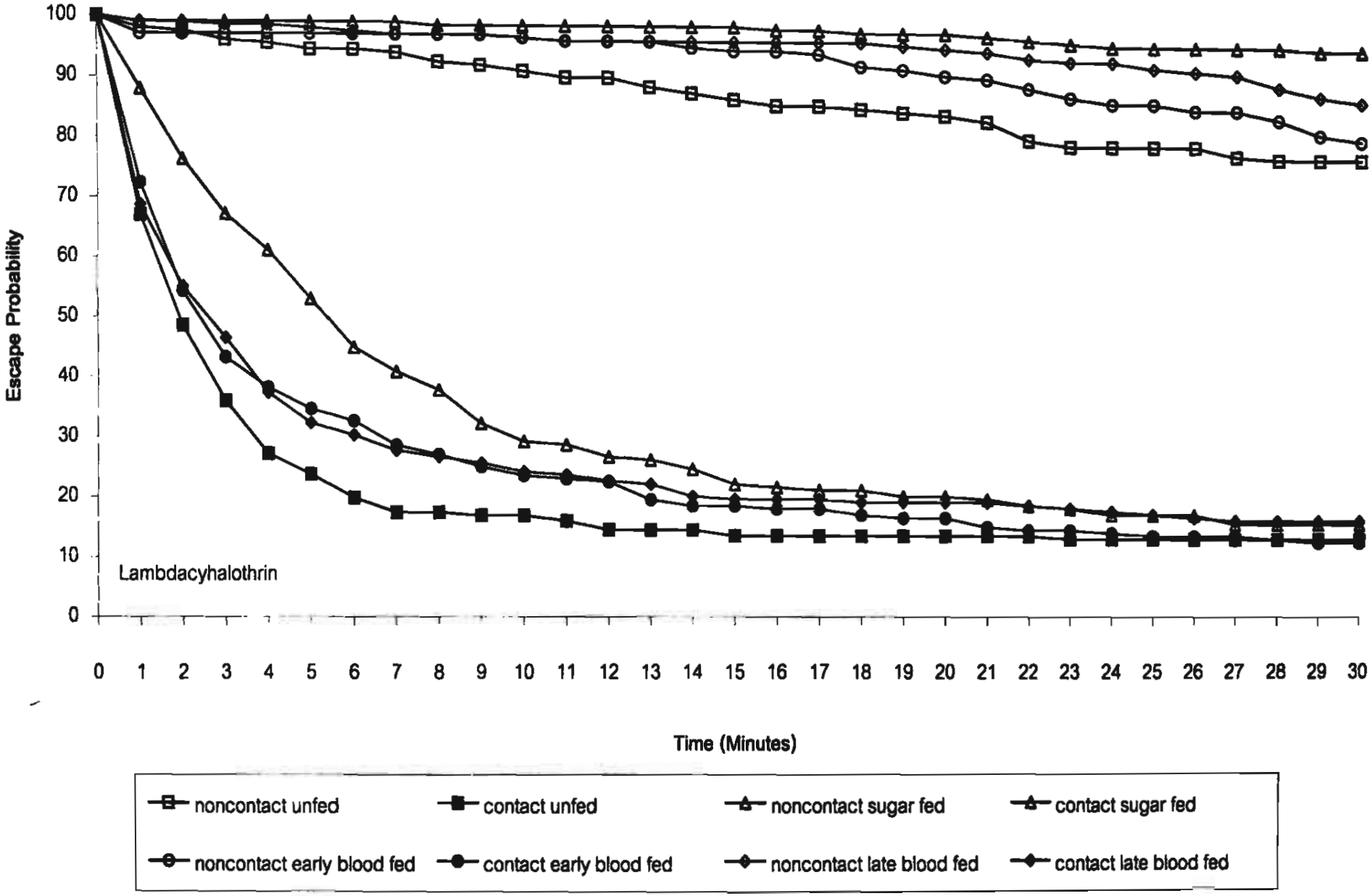

Escape Probability
100
90
80
70
60
50
40
30
20
10
0
Lambdacyhalothrin
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time (Minutes)
noncontact unfed
contact unfed
noncontact sugar fed
contact sugar fed
noncontact early blood fed
contact early blood fed
noncontact late blood fed
contact late blood fed

FIGURE 8

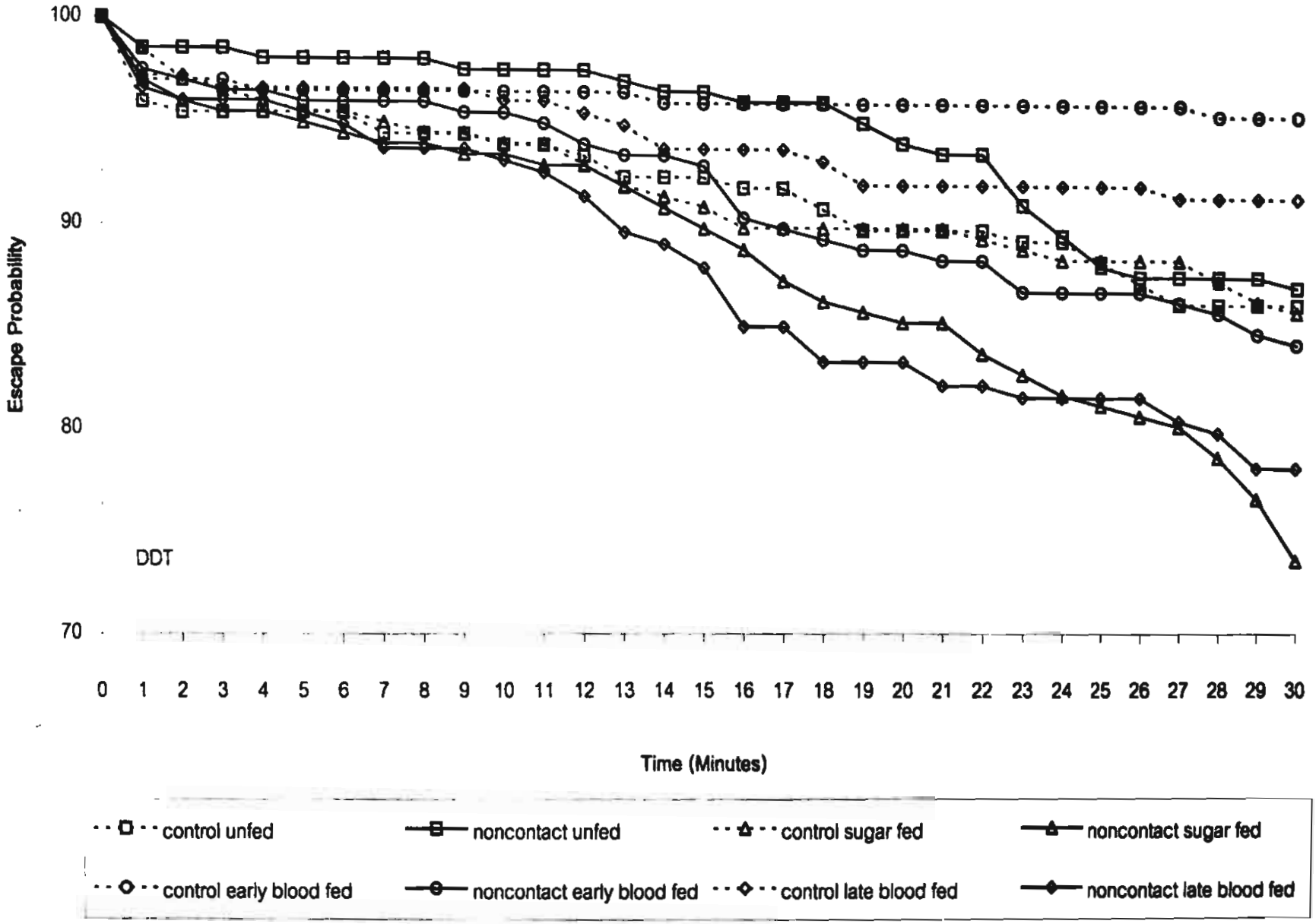

100
90
80
70
Escape Probability
DDT
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time (Minutes)
control unfed
noncontact unfed
control sugar fed
noncontact sugar fed
control early blood fed
noncontact early blood fed
control late blood fed
noncontact late blood fed

FIGURE 9

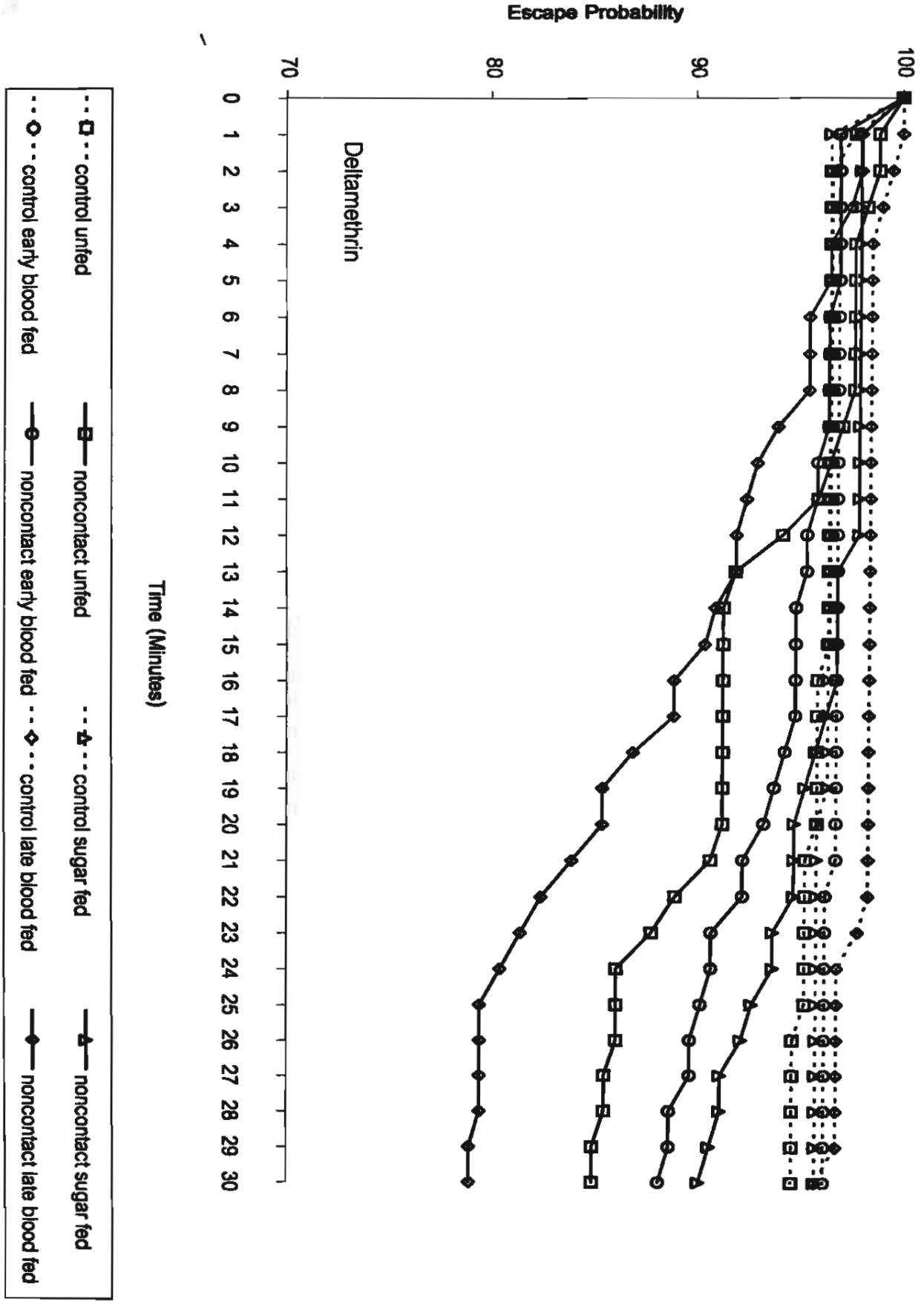
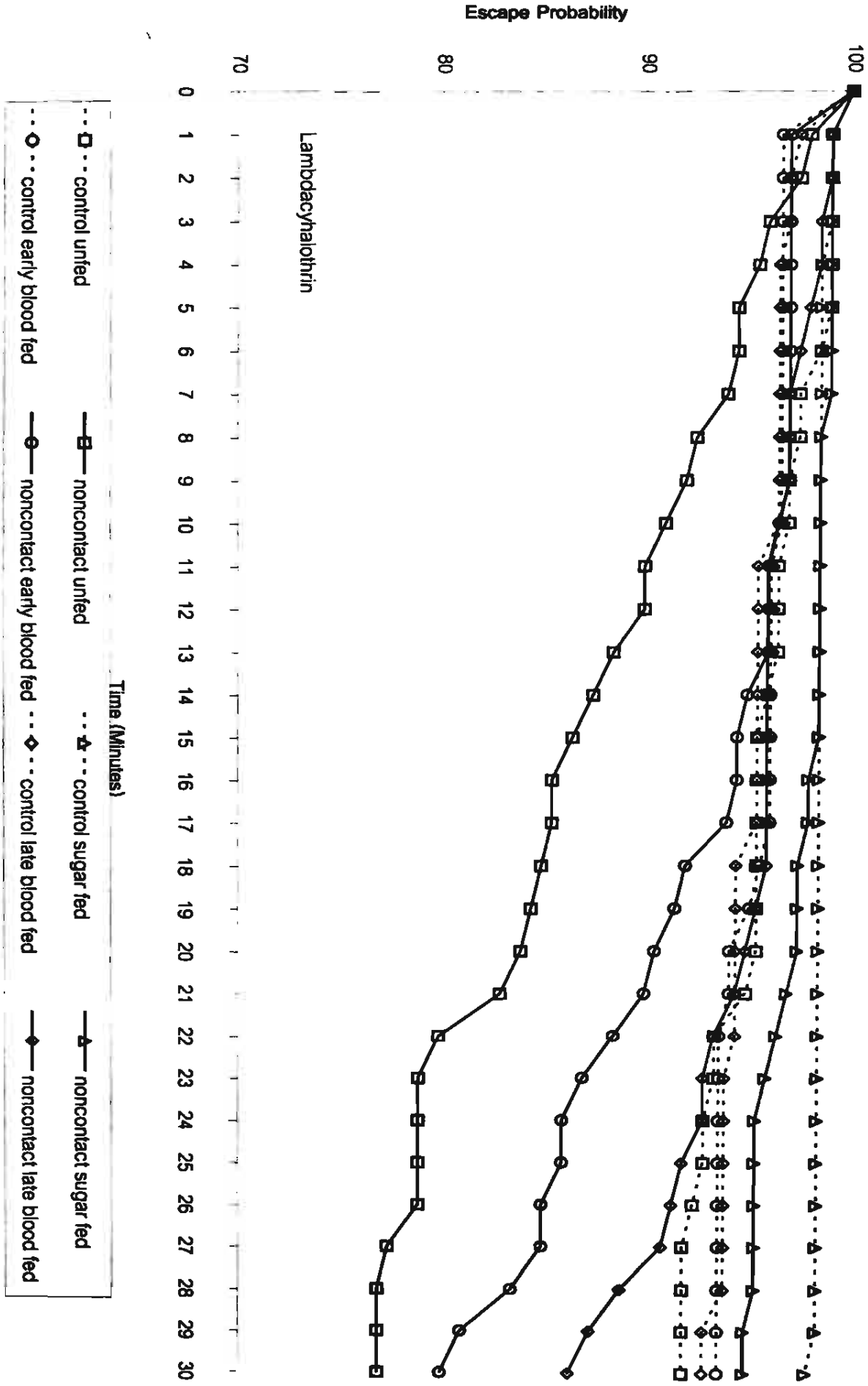

Escape Probability
100
90
80
70
Deltamethrin
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time (Minutes)
control unfed
noncontact unfed
control sugar fed
noncontact sugar fed
control early blood fed
noncontact early blood fed
control late blood fed
noncontact late blood fed

FIGURE 10


Escape Probability
100
90
80
70
Lambdacyhalothrin
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time (Minutes)
control unfed
noncontact unfed
control sugar fed
noncontact sugar fed
control early blood fed
noncontact early blood fed
control late blood fed
noncontact late blood fed

วิจารณ์

ในขณะที่วัคซีนและยากินกันมาลาเรียยังไม่ได้นำออกมาใช้ การควบคุมโรคมาลาเรียโดยทั่วไปยังคงเน้นการควบคุมยุงพาหะเป็นหลักดังได้อธิบายในวารสาร (Chareonviriyaphap et al. 2000) จากการศึกษาครั้งนี้พบว่ายุง Anopheles minimus ซึ่งถือว่าเป็นยุงพาหะนำโรคมาลาเรียที่สำคัญและอยู่ใกล้ชิดมนุษย์มากขึ้นเนื่องจากการเปลี่ยนแปลงของสภาพแวดล้อม รวมทั้งการใช้สารเคมีในการควบคุม จากการศึกษายุงชนิดนี้จากหลายพื้นที่ (จันทบุรี ตราด ตาก นครราชสีมา แม่ฮ่องสอน กาญจนบุรี) พบว่าไม่ได้มีการต้านทานเชิงสรีระต่อสาร DDT, Deltamethrin และ Lambdacyhalothrin ที่กำลังใช้อยู่ในปัจจุบัน ทำให้ทราบว่าสารเคมีที่ใช้ในการควบคุมยุงกันปล่องนำโรคมาลาเรียในปัจจุบันยังคงมีประสิทธิภาพดี ดังนั้นในการพิจารณาใช้สารเคมีตัวใดควรคำนึงถึง ความเหมาะสม อันตรายต่อสัตว์เลือดอุ่น มนุษย์ พิษตกค้าง และราคา เป็นต้น นอกเหนือไปจากบทบาทหน้าที่ในการฆ่าหรือไล่ยุง นอกจากนี้ข้อมูลที่ได้ทำให้ทราบว่าสารเคมีบางตัวที่กำลัง (หรือจะยกเลิกใช้) โดยหน่วยงานที่เกี่ยวข้องควรจะมีการทบทวนใหม่อีกครั้งหนึ่ง โดยเฉพาะในช่วงภาวะเศรษฐกิจถดถอยอย่างปัจจุบันและภาวะไข้มาลาเรียที่กำลังระบาดมากขึ้นเรื่อย ๆ ในช่วง 4-5 ปีที่ผ่านมา

ในการตรวจสอบสารเคมีทั้งสามชนิดพบว่าสารเคมีทั้งสามชนิดมีฤทธิ์ในการขับและไล่ยุง (excito-repellency) ได้เป็นอย่างดี ไม่ได้ฆ่ายุงตามที่หลายคนเข้าใจ (toxicity) นั่นไม่ได้หมายความว่าสารเคมีทั้งสามชนิดไม่มีประสิทธิภาพ ในทางกลับกันการขับและไล่ยุงพาหะที่เข้ามาหาเหยื่อในบ้านช่วยลดอัตราการที่ยุงเข้ามาดูดเลือดเหยื่อ ซึ่งแน่นอนว่าสามารถลดการระบาดของโรคได้ด้วย อย่างไรก็ตามจะต้องมีการศึกษาเพื่อเติมให้ชัดเจนว่า excito-repellency มีบทบาทในการลดการแพร่เชื่อจริงหรือไม่ อีกแนวทางหนึ่งที่มีความจำเป็นอย่างยิ่งคือ ควรจะมีการศึกษากระท่อมทดลอง (experimental hut) ที่พ่นด้วยสารเคมีดังกล่าวเปรียบเทียบ ซึ่งการใช้กระท่อมทดลองได้ทีการศึกษากันบ้างแต่ผู้วิจัยมีความเห็นว่าการ<u>ออกแบบโครงสร้างของกระท่อม หน้าต่างกับดัก ประตูกับดักและวิธีการเก็บตัวอย่าง และการวิเคราะห์ข้อมูลมีความสำคัญอย่างยิ่ง</u> ซึ่งการศึกษาที่ผ่านมาอาจจะมองข้ามจุดนี้ไป จากการศึกษาครั้งนี้ผู้วิจัยพบว่า DDT เป็นสารเคมีที่มีประสิทธิภาพมากที่สุดในการขับและไล่ยุง ดังนั้นพฤติกรรมการตอบสนองของยุงพาหะต่อสารเคมีมีความสำคัญอย่างยิ่งต่อบทบาทการถ่ายทอดเชื้อปรสิตมาลาเรียมาสู่คน

เอกสารอ้างอิง

Ayurakit-Kosol, L. and M.E. Griffith. 1963. Progress toward malaria eradication in Thailand. Proc. 9th Pacific Sci. Congr., 17, 122-136.

Bondareva, N.I., M.M. Artem'ev and G.V. Gracheva. 1986. Susceptibility and irritability caused by insecticides to malaria mosquitoes in USSR. Part 1. Anopheles pulcherrimus. Med. Parazitol. Parazit. Bolezni 6: 52-55.

Chareonviriyaphap, T., D.R. Roberts, R.G. Andre, H.H. Harlan, S. Manguin, and M.J. Bangs. 1997. Pesticide avoidance behavior in Anopheles albimanus, a malaria vector in the Americas. J. Amer. Mosq. Control Assoc. 13(2) 171-183.

Chareonviriyaphap, T., B. Aum-Aong and S. Ratanatham. 1999. Current insecticide resistant pattern in mosquito vectors in Thailand. Southeast Asian J. Trop. Med. Public Health 30: 130-141.

Chareonviriyaphap, T. and B. Aum-Aong. 2000. Improved excito-repellency escape chamber for behavioral tests on mosquitoes. Mekong Malaria Forum 5: in press.

Davidson, G. 1953. Experiments on the effect of residual insecticides in houses against Anopheles gambiae and Anopheles funestus. Bull. Entomol. Res. 44: 231-254.

Evans, R.G. 1993. Laboratory evaluation of the irritancy of bendiocarb, lambdacyhalothrin and DDT to Anopheles gambiae. J. Am. Mosq. Control Assoc. 9: 285-293.

Mantel, N. and W. Haenzel. 1959. Statistic aspects of the analysis of data from retrospective studies of diseases. J. Natl. Cancer Inst. 22: 719-748.

Nutsathapana, S., P. Sawasdiwongphorn, U. Chiprarop and J.R.Cullen. 1986. The behavior of Anopheles minimus Theobald (Diptera: Culicidae) subjected to differing levels of DDT selection pressure in northern Thailand. Bull. Entomol. Res. 76: 303-312.

Pell, J.K., M.A. Spinney and K.J. Ward. 1989. Observations on the behavior of adult Anopheles gambiae encountering residual deposits of lambdacyhalothrin compared with the other major classes. Fourth Annu. Conf. Soc. Vector Eco. Eur. Region 18 pp.

Poolsuwan, S.1995. Malaria in prehistoric Southeast Asia. Southeast Asian J. Trop. Med. Public Health 26: 3-22.

Prasittisuk, M. 1995. Comparative study of pyrethroids impregnated bednets with DDT residual spraying for malaria control in Thailand. Ph.D. Thesis. Mahidol University Bangkok Thailand.

Quinones, M.L. and M.F. Suarez. 1989. Irritability to DDT of natural populations of the primary malaria vectors in Colombia. J. Am. Mosq. Control Assoc. 5: 56-59.

Reid, J.A. 1968. Anopheles mosquitoes of Malaya and Borneo. Studies of the Institute for Medical Research, Malaysia, no. 31, 520pp.

Rawlings, P. and G. Davidson. 1982. The dispersal and survival of Anopheles culicifacies in a Sri Lankan village under malathion spraying. Bull. Entomol. Res. 72: 139-144.

Ree, H.I. and K.P. Loong. 1989. Irritability of Anopheles farauti, Anopheles maculatus and Culex quinquefasciatus to permethrin. Jpn. J. Sanit. Zool. 40: 47-51.

Roberts, D.R., W.D. Alecrim, A.M. Tavares and K.M. Mc. Neil. 1984. Influence of physiological condition on the behavioral response of Anopheles darlingi to DDT. Mosq. News 4: 357-361.

Roberts, D.R. and R.G. Andre. 1994. Insecticide resistant issues in vectors. Amer. J. Trop. Med. Hyg. 50 (Suppl): 21-34.

Roberts, D.R., T. Chareonviriyaphap, H.H. Harlan and P. Hshieh. 1997. Methods of testing and analyzing excito-repellency responses of malaria vectors to insecticides. J. Amer. Mosq. Control Assoc. 13(1) 13-17.

Roberts, D.R., W.D. Alecrim, P. Hshish, J.P. Grieco, M. Bangs, R.G. Andre, and T. Chareonviriyaphap. 2000. A probability model of vector behavior: Effects of DDT repellency, irritancy, and toxicity in malaria control. J. Vector Ecol. 25(1) 48-61.

Rutledge, L.C., N.M. Echana, and R.K. Gupta. 1999. Responses of male and female mosquitoes to repellents in the World Health Organization insecticide irritability test system. J. Amer. Mosq. Control Assoc. 15(1) 60-64.

Sucharit, S., N. Komalamisra, S. Leemingsawat, C. Apiwathnasorn and S. Thongrungkiat. 1988. Population genetic studies on the Anopheles minimus complex in Thailand. Southeast Asian J. Trop. Med. Pub. Health 19: 717-723.

Suwonkerd, W., B. Aum-Aong, K. Rimwangtrakul, S. Wongkattiyakul, B. Kattiyamongkool, U. Chitprarop and M. Takagi. 1990. A field study on the response of Anopheles dirus to DDT and fenetrothion sprayed to huts in Phetchabun province, Thailand. Trop. Med. 32: 1-5.

Threlkeld, S.F.H. 1985. Behavioral responses in Drosophila melanogaster associated with permethrin and ectiban, pp. 29-36. In. Proceedings of the thirty-second annual meeting, Canadian Pest Management Society, Charlottertown, Prince Island, 24-26 June, 1985. Can. Pest. Manage. Soc.

ผลงานวิจัย (Output)

Publications

1.Insecticide resistance patterns in mosquito in Thialand. *Southeast Asian J Trop. Med Publ. Health* 1999: 30: 131-141. (Senior author)

2.Status of Malaria in Thailand. *Southeast Asian J Trop Med Publ Health* 2000: 31 (2): 1-14. (Senior author)

3.An improved excito-repellency escape chamber. *Mekong Malaria Forum* 2000: 5(2): 82-86. (Senior author)

4 Insecticide-induced behavioral responses of Anopheles minimus (Diptera: Culicidae), a malaria vector in Thailand. *Journal of the American Mosquito Control Association* 2000: (Accepted as a minor revision) (Senior author)

5. A probability model of vector behavior: Effects of DDT repellency, irritancy, and toxicity in malaria control. *Journal of Vector Ecology* 2000: 25(1) 48-61. (Co-author)

6.Influence of physiological conditions on behavioral responses in Anopheles minimus. *Journal of Medical Entomology* (Submitted) (Senior author)

7.กล่องทดสอบพฤติกรรมการหลีกหนีสารเคมีฆ่าแมลง *วารสารโรคติดต่อ* กรมควบคุมโรคติดต่อ 2543: 28 (in press) (Senior author)

สิทธิบัตร

1.An improved Excito-Repellency Escape Chamber (Processing)

เสนอผลงานที่ประชุมนานาชาติ

1.Workshop on Insecticide Resistance. Aral Presentation *on Mosquito Resistance to Insecticides in Thailand* 5-8 August 1997: Catelina Hotel, Salatiga, Indonesia WHO Fund.

2.International Meeting of American Mosquito Control Association. Aral Presentation on *Pesticide Avoidance Behavior in Anopheles minmus, a Vector of Malaria in Thailand.* 12-16 March 2000: Atlantic City USA. Kasetsart University Fund.

3.2000 International Mosquito Control Meeting. Aral Presentation on *Mosquito Ecology and Behavioral Response to Insecticides in Thailand.* Fort de France, Matinique, Central America 28 February-3 March 2000: WHO Fund.

4.Evaluation of some insecticide used in malaria control progarm. 9-11 June 2000 Chaengmai TRF Fund.

สำเนา

สำนักงานกองทุนสนับสนุนการวิจัย
THE THAILAND RESEARCH FUND

ที่ นร 6808/23343

1 มิถุนายน 2543

เรื่อง การจดสิทธิบัตรผลงานวิจัย

เรียน ศ.ดร. ประสิทธิ์ ประพิณมงคลการ
ผู้อำนวยการสถาบันทรัพย์สินทางปัญญา แห่งจุฬาลงกรณ์มหาวิทยาลัย

ด้วยสำนักงานกองทุนสนับสนุนการวิจัย (สกว.) ได้ให้การสนับสนุนโครงการวิจัย เรื่อง "พฤติกรรมการหลีกหนีสารเคมีฆ่าแมลงของยุงพาหะนำโรคมาลาเรียชนิด *Anopheles minimus* complex ในประเทศไทย" กับมหาวิทยาลัยเกษตรศาสตร์ ในขณะนี้โครงการดังกล่าวได้มีผลงานวิจัยในโครงการเรื่อง "ชุดอุปกรณ์สำหรับตรวจหาการต้านทานสารเคมีฆ่าแมลงเชิงพฤติกรรมในยุงพาหะนำโรค" โดยมี ดร. ธีรภาพ เจริญวิริยภาพ เป็นหัวหน้าโครงการซึ่งเชื่อว่าจะจดสิทธิบัตรได้ และประสงค์จะจดสิทธิบัตรผลงานดังกล่าวในประเทศไทย ตามข้อถือสิทธิบัตรที่ ดร. ธีรภาพ เจริญวิริยภาพ ได้จัดทำร่างดังเอกสารแนบ

สกว. ใคร่ขอความอนุเคราะห์จากสถาบันทรัพย์สินทางปัญญา แห่งจุฬาลงกรณ์มหาวิทยาลัย ซึ่งมีความเชี่ยวชาญและมีประสบการณ์ในด้านนี้ เป็นผู้ดำเนินการจดสิทธิบัตรแทน สกว. และมหาวิทยาลัยเกษตรศาสตร์ ทั้งนี้ ขอได้โปรดแจ้งขอบเขตและขั้นตอนที่จะดำเนินงาน รวมทั้งงบประมาณที่ต้องใช้ให้ สกว. ทราบ เพื่อพิจารณาต่อไปด้วย จักเป็นพระคุณยิ่ง

จึงเรียนมาเพื่อโปรดพิจารณา

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ภาคผนวก

CURRENT INSECTICIDE RESISTANCE PATTERNS IN MOSQUITO VECTORS IN THAILAND

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Abstract. Chemical pesticides are still commonly used in Thailand for control of agricultural pests and disease vectors. Organophosphates, carbamates and synthetic pyrethroids are commonly used for agricultural purposes, whereas synthetic pyrethroids have become more popular and predominate for public health use. The genetic selection of insecticide resistance (whether physiological, biochemical or behavioral) in pests and disease vectors has been extensively reported worldwide (Brown and Pal, 1971). The long-term intensive use of chemical pesticides to control insect pests and disease vectors is often cited as the reason behind the development of insecticide resistance in insect populations. Unfortunately, reliable information on vector resistance patterns to pesticides in Thailand is sparse because of a remarkable shortage of carefully controlled, systematic studies. This review gathers useful information on what is presently known about disease vector resistance to chemical pesticides in Thailand and provides some possible management strategies when serious insecticide resistance occurs.

INTRODUCTION

Over the 20th century, insecticides of natural and synthetic origins have increased in importance and overall volume of uses as agricultural and public health needs have demanded. Years of routine use have led in some cases to high levels of chemical resistance by certain pests and disease vectors (Georghiou and Saito, 1983).

Resistance can be broadly defined as"the developed ability in a strain of insects to tolerate doses of insecticides which prove lethal to the majority of individuals in a normal population of the same species" (WHO, 1975). This ability is brought about by selection of individuals in a population with a genetically inheritable capacity to withstand insecticides, and not due to the action of the insecticide on a given individual insect. Therefore, the development of resistance is dependent on genetic variability already present in a target population or spontaneously arising during the period of selection (Oppenoorth, 1984). Development of physiological resistance by mosquitos, the most important group of medically important arthropods, was first reported in 1947 when *Aedes. taeniorhynchus* was shown to be resistant to DDT in Florida after only 4 years of use (Brown, 1986). The following 40 years of intensive use of organic insecticides to control insect pests and disease vectors has led to the extensive selection of insecticide resistance in more than 450 species (Georghiou, 1986). Resistance to insecticides has been reported in over 500 species of arthropods, including at least 109 mosquito species found resistant to organochlorines, primarily DDT and dieldrin (Roberts and Andre, 1994).

There are 2 principal types of responses to insecticides, one is physiological and the other is behavioral (avoidance). Physiological resistance, sometimes referred to as biochemical resistance, is the ability of mosquito to survive the effect of insecticide by mechanisms such as detoxifying enzymes. Behavioral avoidance is the ability of a mosquito to avoid the insecticide-treated surface by either direct contact irritancy or noncontact repellency or the combination of both, referred to as excito-repellency (Chareonviriyaphap *et al*, 1997).

Common resistance mechanisms in arthropods include: reduced sensitivity of altered acetylcholinesterases to organophosphates and carbamates; the *kdr* (knockdown resistance) insensitivity to DDT and pyrethroids; reduced neuronal sensitivity to chlorinated cyclodienes; increased metabolism by hydrolysis of organophosphates, carbamates and pyrethroids; increased activity of mixed function oxidases in DDT,

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organophosphate, carbamate and pyrethroid resistance; enhanced metabolism by glutathion-*S*-transferase in DDT and organophosphate resistance; enhanced metabolism by DDT-ase; and reduced cuticular penetration of DDT, organophosphate, carbamate, pyrethroids and chlorinated cyclodienes (Kerkut and Gilbert, 1985; Georghiou, 1986). An arthropod may possess more than one mechanism at work to avoid the adverse effects of various toxic compounds.

VECTOR-TRANSMITTED DISEASES IN THAILAND

Despite years of vector control and public health activities, several vector-borne diseases remain major health threats in Thailand, principally malaria, dengue fever and dengue hemorrhagic fever (DHF), lymphatic filariasis and Japanese encephalitis. All are transmitted by various species of mosquitos, some of which are capable of conveying more than one pathogen. The distribution of these important endemic vector-borne diseases in Thailand is presented in Fig 1. Malaria and lymphatic filariasis are more prevalent along the borders of eastern Myanmar, western Cambodia and northern Malaysia borders. All 4 dengue virus serotypes are widespread throughout the country, while Japanese encephalitis occurs mainly in the rice-growing areas of Thailand (Rattanarithikul and Panthusiri, 1994). Table I lists mosquito species known or suspected to act as important vectors of diseases in Thailand.

Malaria parasites are only transmitted by *Anopheles* mosquitos. Of the 74 *Anopheles* species recognized in Thailand, only 3 species are considered to be important malaria vectors. These are *Anopheles dirus*, *Anopheles minimus* and *Anopheles maculatus* (Malaria Division, 1994). All 3 taxa represent individual complexes; of which the respective sibling species are not easily separated from one another (Rattanarithikul and Panthusiri, 1994). *Anopheles dirus* is a forest and forest-fringe inhabiting mosquito, whereas *An. minimus* and *An. maculatus*, are associated with low hill zones and generally have closer contact with humans along the margin of villages. *Anopheles minimus* is commonly found along the quiet edges of slow moving streams and *An. maculatus* is often present at the margin of hilly forest zones, especially in rubber-plantation areas. All 4 human malaria parasites have been reported and can potentially be transmitted by all 3 malaria vectors. *Plasmodium falciparum* and *Plasmodium vivax* are common in Thailand, whereas *Plasmodium malariae* and *Plasmodium ovale* are considered rare occurrences (Rattanarithikul and Panthusiri, 1994).

Dengue is one of the most important arthropod-borne viral diseases in the world and commonly occurs throughout Southeast Asia (Gubler, 1988). Only 2 species of *Aedes* mosquitos, *Aedes aegypti*, an urban species, and *Aedes albopictus*, primarily a rural species, are known to be important dengue virus vectors in Thailand (Rattanarithikul and Panthusiri, 1994). *Aedes aegypti* is more prevalent around human dwellings and is a principal vector in the urban zones, whereas *Ae. albopictus* is considered to be an important vector in the rural areas. For larval habitats, *Ae. aegypti* prefers clean water found in many types of domestic containers inside or near human dwellings, whereas *Ae. albopictus* is more likely to be found in natural containers and outdoor man-made habitats (*eg* tree hole, leaf axils) containing a greater amount of organic debris (Rattanarithikul and Panthusiri, 1994).

Japanese encephalitis is an important mosquito-borne virus in Thailand. A number of different species in the genera *Culex* are responsible for Japanese encephalitis virus (JEV) transmission. The most important naturally infected vector of JEV in Thailand is *Culex tritaeniorhynchus*, followed by slightly less efficient vectors, *Culex fuscocephala*, *Culex gelidus*, *Culex vishnui* and *Culex pseudovishnui* (Burke and Leake, 1988). These mosquitos typically breed in pools associated with wet rice cultivation areas throughout the country. JEV transmission normally occurs as periodic epidemics in the rice-growing areas. Pigs are considered to be the major viral amplifying host and can circulate the virus without obvious disease symptoms. Besides pigs, there are several natural vertebrate reservoir hosts that can circulate JEV, primarily *Ardeidae* (egrets, night herons) birds. The potential roles of other animals as reservoir hosts remain unknown.

Lymphatic filariasis is also present in Thailand, consisting of at least 2 distinct species, *Wuchereria bancrofti* and *Brugia malayi* (Phothikasikorn, 1991). *W. bancrofti* is widely distributed along the western Thai-Myanmar border, whereas *B. malayi* is more prevalent in the south and along the Thai-Malaysia border area (Guptavanij and Harinasuta, 1977). The main vectors of *B. malayi* in the south of Thailand are *Mansonia* species, in particular, *Mansonia uniformis*. *Culex quinquefasciatus* is considered to be the principal vector of urban *W. bancrofti*. *Aedes* species such as *Ae. neveus* along with some *Anopheles* species have been implicated as either secondary or possible vectors of lymphatic filariasis in Thailand (Rattanarithikul and Panthusiri, 1994).

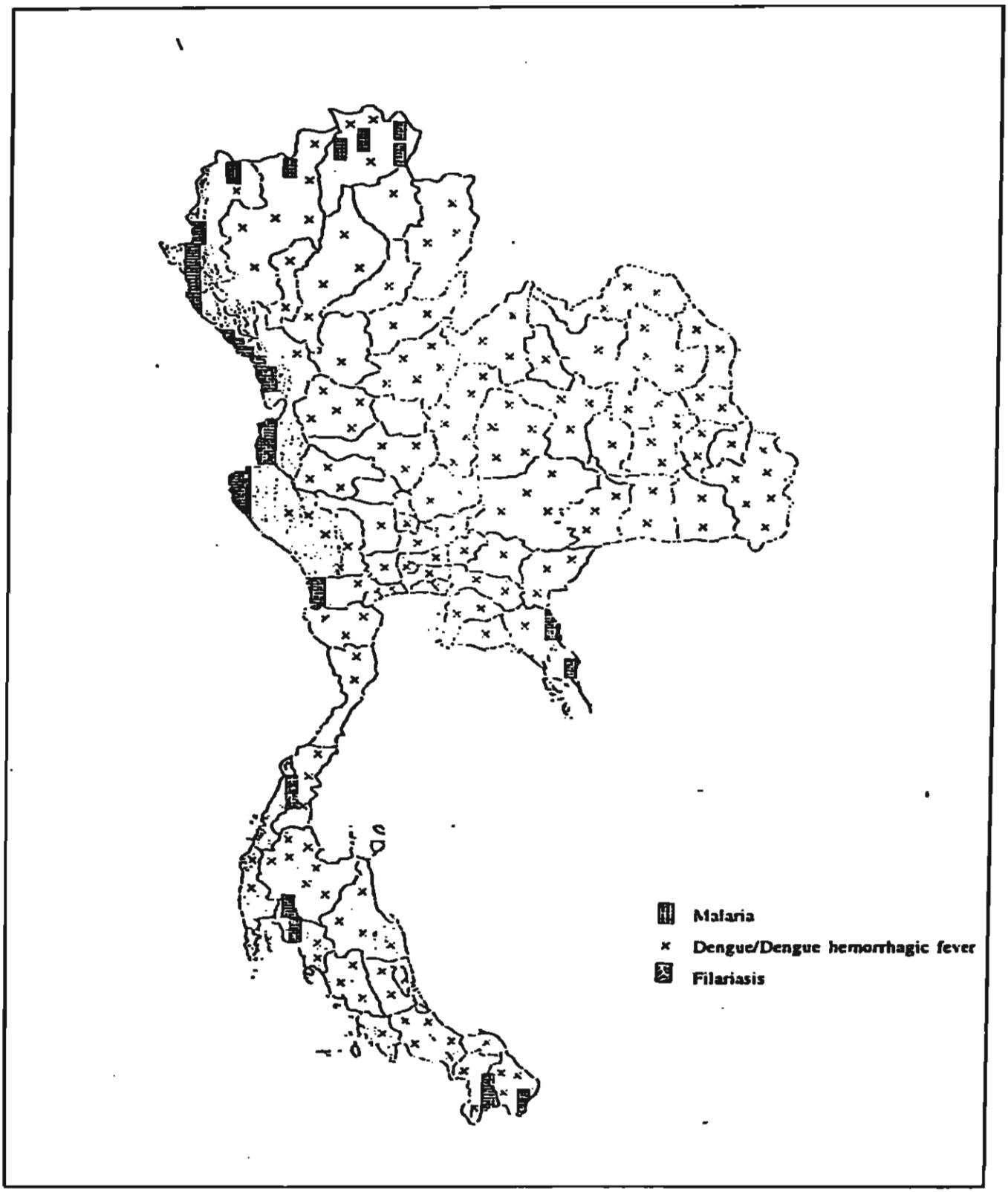

Fig 1–Distribution of malaria, DF/DHF and filariasis in 1997 in Thailand.

Table 1
Confirmed, secondary and suspected vectors of human diseases in Thailand.

Diseases	Vectors	References
Malaria	Main vector *(Anopheles)*	
	1. *An. dirus*	(Rosenberg *et al*, 1990)
	2. *An. minimus*	(Harrison, 1980)
	3. *An. maculatus*	(Malaria Division, 1995)
	Secondary vectors	
	1. *An. sundaicus*	(Scanlon *et al*, 1968)
	2. *An. aconitus*	(Gould *et al*, 1967)
	3. *An. psuedowillmori*	(Green *et al*, 1991)
	Suspected vectors	
	1. *An. barbirostris*	(Harrison and Scanlon, 1975)
	2. *An. philippinensis*	(Rosenberg *et al*, 1990)
	3. *An. campestris*	(Malaria Division, 1995)
	4. *An. culicifacies*	(Harrison, 1980)
Dengue (Type 1-4)	Main vector *(Aedes)*	
	1. *Ae. aegypti*	(Watts *et al*, 1987)
	2. *Ae. albopictus*	(Gould *et al*, 1968, 1970; Chan *et al*, 1971)
Japanese encephalitis	Main vector *(Culex)*	
	1. *Cx. tritaeniorhynchus*	(Gould *et al*, 1974)
	2. *Cx. gelidus*	(Gould *et al*, 1974)
	3. *Cx. fuscocephala*	(Gould *et al*, 1974)
	4. *Cx. pseudovishnui*	(Mourya *et al*, 1991)
	5. *Cx. vishnui*	(Gould *et al*, 1974)
Lymphatic filariasis	Several *Anopheles, Aedes, Mansonia,* and *Culex* mosquitos	(Rattanarithikul and Panthuiri, 1994)

INSECTICIDES USED IN PUBLIC HEALTH AND OTHER SECTORS IN THAILAND

In Thailand, many chemical compounds, including organochlorines, organophosphates, carbamates, synthetic pyrethroids and so-called biorational pesticides, have been used in both agricultural practices and public health control programs. This review concentrates on public health insecticide usage and their impact on disease vectors. For years, DDT was used for malaria control as an indoor residual spray in Thailand. DDT was withdrawn for all agricultural uses beginning 1983 and has been decreasing overtime for malaria control use (Table 2). Although, DDT importation was stopped in 1995, remaining stocks of DDT were still be used in some malaria problem areas of Thailand. The reasons for the removal of DDT from malaria control in Thailand was because of reported vector resistance and perceived adverse impact on the environment. However, the true impact of DDT on mosquito vectors in terms of behavioral effects and disease transmission remain poorly studied. Synthetic pyrethroids (*eg.* permethrin and deltamethrin) are the current insecticides of choice for malaria control in Thailand. These pyrethroids have been used for the impregnation of bed nets and/or as indoor residual house spray in many parts of the country. Temephos (Abate®), an organophosphate, is regularly used in containers for the control of *Ae. aegypti* larvae. Ultra-low-volume (ULV) applications of fenitrothion and malathion are used during the peak period of adult *Aedes* populations, especially during the rainy seasons (June to November each year). *Bacillus thuringiensis israelensis (Bti)*, a safe and commonly used biopesticide, is being used for the control of *Ae. aegypti* larvae in indoor containers. Types of compounds, currently used in public health control programs in Thailand, are presented in Table 3.

Thailand remains principally an agricultural country. Rice, along with several tropical products such as palm, rubber and oranges, represent major commercial and export crops. Many of these crop-growing areas (*ie*, rice fields, rubber plantations and palm) are also suitable habitats for various disease vectors. To control the wide variety of crop pests and disease vectors, several major groups of pesticides are being used concurrently. Quantity and cost value of insecticides imported to Thailand each year from 1978-1997 are relatively high, reaching a peak in 1996 with 14,398 imported tons, costing an estimated 1.57 billion baht (US$=62 million based on the old rate of 1US$=25 Baht) seen in Table 4. Table 5 illustrates yearly quantity (in tons) of pesticides by classes imported for use in Thailand from 1985 to 1997. Overall, use of organophosphates and synthetic pyrethroids has been increasing over time, along with some carbamates, as the pesticides of choice compared to organochlorines.

CURRENT RESISTANT STATUS OF MOSQUITO VECTORS IN THAILAND

Arthropod-borne diseases are an ever increasing cause of death and suffering worldwide. Thailand is endemic for several serious vector-borne diseases, including malaria, dengue fever and DHF, lymphatic filariasis and Japanese encephalitis. Increases in human population and demographic movement of the people in many parts of the country have led to great deforestation, irrigation and urbanization. Many of these environmental changes have favored conditions for increasing vector transmission of diseases. Past efforts to control these diseases in Thailand have focused on the routine use of chemical insecticides. In the malaria control program, the application of indoor residual insecticides to control *Anopheles* mosquitos has become less acceptable to local people and vector resistance to many commonly applied pesticides is now regarded as a major impediment to disease control. Resistance in some disease vectors has been documented with several major groups of pesticides and the present knowledge of vector resistance to chemical insecticides are shown in Table 6.

In Thailand, information on vector resistance to insecticides is limited due to a remarkable shortage of comprehensive and carefully designed studies. This paper provides some useful background

Table 2
Quantity (tons) of DDT imported for use in Thailand for agricultural and public health purposes.

Year	Agricultural use (tons)		Public health use (tons)	
	AI	Formulation	AI	Formulation
1977	227	859	1,350	1,800
1978	597	1,683	999	1,322
1979	300	953	570	1,484
1980	378	1,487	390	520
1981	83	264	225	720
1982	14	36	594	986
1983	Banned	Banned	345	460
1984			522	696
1985			399	600
1986			485	647
1987			468	623
1988			387	516
1989			414	552
1990			492	656
1991			430	574
1992			418	557
1993			346	462
1994			254	339
1995*			161	215

AI = Active ingredient; *Stop purchasing

Table 3
Historical use of chemical insecticides used in mosquito control programs in Thailand.

Class of compounds	Name of pesticides	Use	Date-present
1. Organochlorine	DDT	Malaria	1949
		Dengue	1950
2. Organophosphate	Temephos	Malaria	unknown[a]
	Fenitrothion	Malaria	1983[a]
		Dengue	unknown
	Malathion	Dengue	unknown
3. Carbamate	Propoxur	Dengue	unknown[b]
	Pirimiphosmethyl	Dengue	1990[a]
	Bendiocarb	Malaria	unknown[a]
4. Pyrethroids	Permethrin	Malaria	1992
	Deltamethrin	Malaria	1994
	Lambda-cyhalothrin	Malaria	1990[a]
	Etofenprox	Malaria	1991[a]
5. Biopesticide	*Bacillus thuringiensis isrealensis (Bti)*	Dengue	1990[a]
		Malaria	1989[a]
	Bacillus sphearicus (Bs)	Dengue	1986[a]
		Malaria	1986[a]

a = small scale
b = indoor use

Table 4
Quantity and approximate value of all insecticides imported for use in Thailand from 1978-1997.

Year	Quantity (tons)*	Value (million baht)
1978	10,809	514
1979	10,571	679
1980	10,045	785
1981	6,625	792
1982	5,588	692
1983	6,718	631
1984	8,233	884
1985	7,284	855
1986	8,299	928
1987	6,673	765
1988	8,034	1,137
1989	9,068	1,206
1990	9,356	1,472
1991	7,233	1,244
1992	7,903	1,386
1993	7,330	1,193
1994	7,708	1,150
1995	7,708	1,644
1996	14,398	1,570
1997	12,151	1,761

*organochlorines, organophosphates, carbamates, synthetic pyrethroids and insect growth inhibitors

Table 5
Yearly quantity (tons) of pesticides by class imported for use in Thailand.

Years	Organochlorines (tons)	Organophosphates (tons)	Cabamates (tons)	Synthetic pyrethroids (tons)
1985	622	4,190	917	132
1986	510	4,492	1,060	161
1987	835	5,381	998	133
1988	624	5,463	1,550	226
1989	-	-	-	-
1990	-	-	-	-
1991	-	-	-	-
1992	-	-	-	-
1993	444	4,814	1,386	314
1994	462	5,352	967	348
1995	697	6,157	1,605	409
1996	657	6,231	1,232	231
1997	543	6,123	1,231	342

- Not available

information on the status of insecticide resistance. DDT has been used in malaria control for decades as a safe and effective insecticide with a long residual life. For many years, chemical companies have been developing synthetic pyrethroid pesticides as alternatives or replacements for DDT. These synthetic pyrethroids have shown great promise for insect control due to their fairly low mammalian toxicity and outstanding potency at low doses that rapidly immobilize and kill insects (Prasittisuk, 1994). However, overtime, physiological resistance to these compounds has been detected in numerous arthropods, including *Anopheles* species (WHO, 1992; Malaria Division, 1985-1998). Increasing resistance to pyrethroids is of particular concern because Thailand has been extensively using synthetic pyrethroids, such as permethrin and deltamethrin for malaria control.

Malaria vector resistance to insecticides has been monitored, based on the results of standard World Health Organization contact susceptibility tests using discriminating dosages (WHO, 1981a). Tests are regularly conducted by the 5 Regional Malaria Zones, located throughout the country. Results are reported to the Malaria Division, Department of Communicable Disease Control, Ministry of Public Health, Thailand. In 1985, there was no evidence of insecticide resistance in mosquito vectors from any region of Thailand. In 1986, development of physiological resistance to DDT was detected in *An. aconitus* from the north (Table 6) where DDT was commonly used for malaria control. A year later, development of resistance to DDT was found in field collected mosquitos of *An. philippinensis*, *An. nivipes* and *Anopheles aconitus* from the same northern region. Between 1990 and 1997, the development of physiological resistance to DDT had been detected in all 3 primary malaria vectors, *An. dirus*, *An. minimus* and *An. maculatus*, mostly from the northern part of Thailand (Table 6; Fig 2). This apparent rise and spread of DDT resistance in these *Anopheles* mosquitos could be attributed to the rapid and increased use of DDT either for malaria control or use of other related organochlorines for agricultural needs. Resistance and inherent environmental problems associated with DDT use resulted in a change to the synthetic pyrethroids for impregnation of bednets and intradomicillary spraying programs beginning in 1992. Unfortunately, development of insecticide resistance to permethrin was shown in a population of *An. minimus* from northern Thailand, approximately 1 year after its introduction into the program (Malaria Division, 1997). In general, malaria vector resistance to DDT and permethrin is more prevalent in areas of the northern Thailand (Fig 2). This might be a reflection of more monitoring of the resistance status by entomology teams in the areas. Furthermore, mosquito population numbers remain quite stable in these northern areas compared to the other parts of the country. Malaria teams in other parts of the country often encounter very low seasonal mosquito populations resulting in incomplete insecticide susceptibility monitoring. Therefore, careful and complete monitoring of *Anopheles* vectors resistant to insecticides, especially synthetic pyrethroids, should be of a major emphasis of public health activities.

Table 6
Development of insecticide resistance in mosquitos vectors in Thailand detected from 1985-1995 (based on WHO standard diagnostic concentrations of insecticides).

Mosquitos	Insecticides	Regions	Locations[a]	Date
An. dirus	DDT (4%)	North	Lampang, Mae Mao, Ban Dong	1995
	DDT (4%)	North	Lampang, Maung, Ban Lang	1995
An. minimus	Permethrin (0.25%)	North	Phrae, Rong Kwang, Huay Rong	1992
An. minimus	DDT (4%)	North	Uttaradit, Ban Khok, Ban Khok	1990
An. maculatus	DDT (4%)	North	Uthaitanee, Ban Rai, Ban Rai	1990
	DDT (4%)	North	Phrae, Rong Kwang,Pai Ton	1994
An. aconitus	DDT (4%)	North	Chiangrai, Chiang Khlong, Vieng	1986
	DDT (4%)	North	Phayao, Pong, Ngim	1986
	DDT (4%)	North	Phayao, Chiang Kham, Rom Yen	1986
	DDT (4%)	North	Chiang Rai, Turng, Hngaoa	1986
	DDT (4%)	North	Chiang Rai, Chiang Khlong, Vieng	1987
	DDT (4%)	North	Phayao, Chiang Kham, Rom Yen	1987
	DDT (4%)	North	Lampang, Tung, Mai Mog	1991
	DDT (4%)	North	Phayao, Chiang Kham, Rom Yen	1991
	DDT (4%)	North	Chieng Rai, Turng, Hngaoa	1991
	DDT (4%)	North	Chiangrai, Chiang Khong, Chiang Khong	1992
	DDT (4%)	North	Chiangrai, Chiang Khong, Vaing	1994
	DDT (4%)	North	Phayao, Chiang Kham, Rom Yen	1995
	DDT (4%)	North	Phayao, Chiang Kham, Rom Yen	1995
An. culicifacies	DDT (4%)	North	Chiang Mai, Chom Tong, Ban Pae	1991
	DDT (4%)	North	Chiang Mai, Chom Tong, Ban Pae	1994
An. nivipes	DDT (4%)	North	Chiang Rai, Thoeng, Hngaoa	1987
	DDT (4%)	North	Nan, Thung Chang, Pou	1987
	DDT (4%)	North	Nan, Sa, Eye La Nai	1987
	DDT (4%)	North	Phrae, Song, Sa Aead	1987
	DDT (4%)	North	Chiang Rai, Thoeng, Hngaoa	1988
	DDT (4%)	North	Phayao, Chaing Kham, Rom Yen	1988
	DDT (4%)	North	Uttaradit, Ta Pa, Nam Mun	1989
	DDT (4%)	North	Chiang Rai, Thoeng, Hngaoa	1989
	DDT (4%)	North	Chiang Rai, Thoeng, Hngaoa	1990
	DDT (4%)	North	Chiang Rai, Thoeng, Hngaoa	1991
	DDT (4%)	North	Chiang Rai, Thoeng, Hngaoa	1992
	DDT (4%)	North	Chiang Rai, Thoeng, Hngaoa	1994
	DDT (4%)	North	Phayao, Chaing Kham, Rom Yen	1994
An. philippinensis	DDT (4%)	North	Chiangrai, Thoeng, Hngaoa	1987
	DDT (4%)	North	Phayao, Chiang Kham, Rom Yen	1987
Ae. aegypti	Malathion (1.0 ppm)	Northeast	Si Sa Ket[b]	1992
	Malathion	Northeast	Ubon Ratchathani[b]	1992
	Malathion	Central	Bangkok[b]	1992
	Malathion	Northeast	Udon Thani[b]	1993
	Temephos	Central	Bangkok[b]	1986
	(0.2 ppm)	North	Phayao[b]	1990
	Temephos	Central	Bangkok[b]	1992
	Fenitrothion (0.05 ppm)	North	Lampang[b]	1990
Cx. quinquefasciatus	Malathion (1.0 ppm)	Central	Bangkok[b]	1986
	Malathion	South	Pattani[b]	1991
	Malathion	Central	Ratchaburi[b]	1991
	Malathion	Northeast	Nakhon Ratchasima[b]	1992
	Temephos (0.2 ppm)	Central	Bangkok[b]	1986
	Temephos	Northeast	Nakhon Ratchasima[b]	1992
	Temephos	North	Phitsanulok[b]	1993
	Temephos	Central	Suphan Buri[b]	1995

[a]In some areas, routine programs for susceptibility testing was not possible due to the shortage of mosquitos.
[b]Information on District not available.

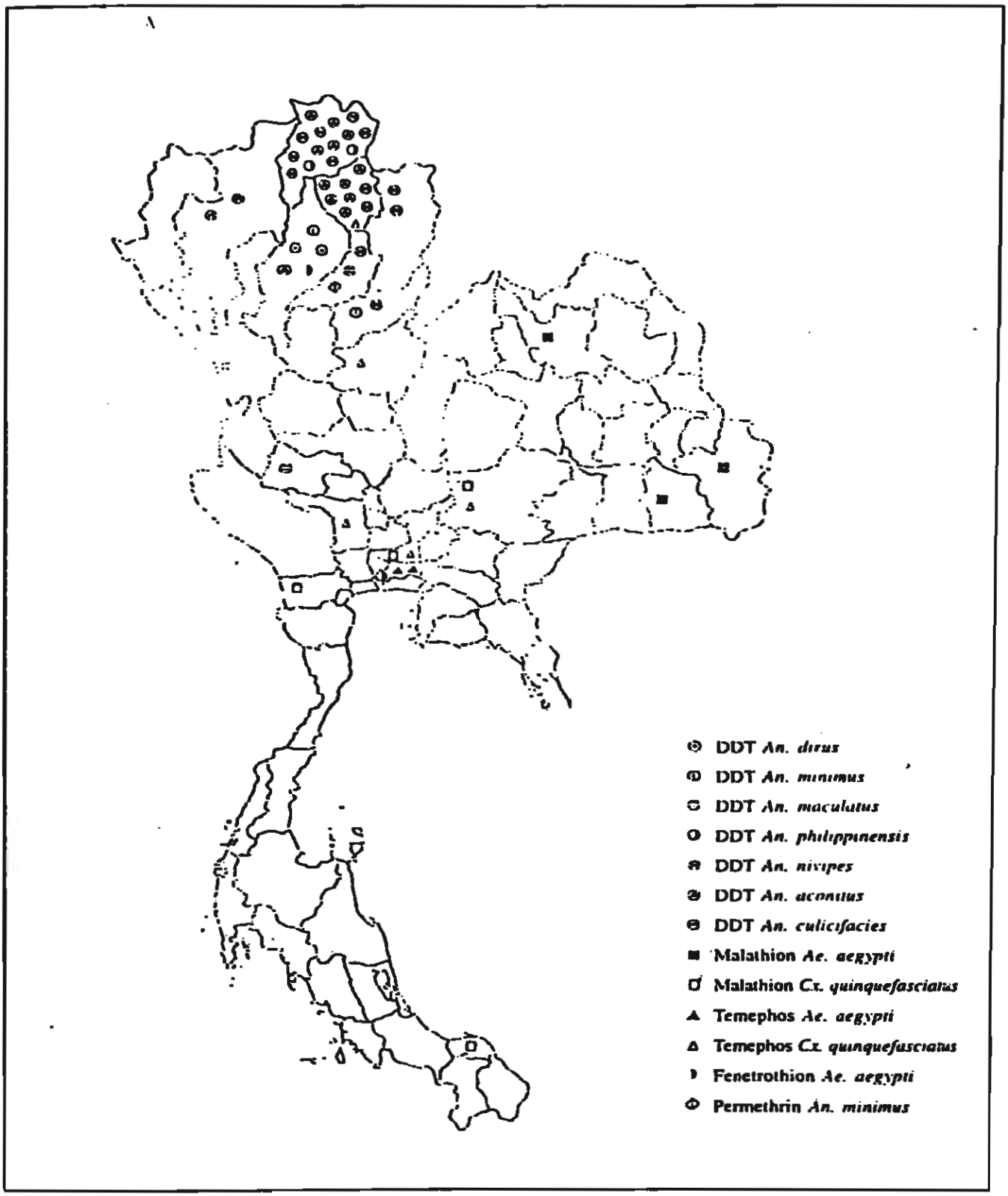

Fig 2–Distribution of mosquito vectors resistance to chemical insecticides in Thailand.

Evidence of resistance to malathion, temephos and fenitrothion in *Ae. aegypti* and *Cx. quinquefasciatus* has been observed, based on data from 1985-1998 the WHO susceptibility tests for larvae (WHO, 1981b). In 1986, there was evidence of the development of resistance to temephos and malathion in Bangkok mosquito strains of *Ae. aegypti* and *Cx. quinquefasciatus*. A few years later, development of physiological resistance to other organophosphates and carbamates was detected in *Ae. aegypti* and *Cx. quinquefasciatus* populations of the south, north, northeast and central parts of the country where those chemicals were commonly used for mosquito control.

Insecticide resistance in mosquito vectors could have arisen from the common use of the same insecticides by other sectors such as agriculture, forestry and from general public health use (operational and household uses). In agriculture, most pesticides are toxic to disease vectors and other insects. Mosquito vectors are normally found resting and breeding in agricultural habitats, where they are exposed to those insecticides. Resistance development might possibly be related to the foraging habits of female in search of bloodmeals. In host seeking behaviors, female mosquitos may spend more time in pesticide treated areas either indoors or outdoors closed to preferred hosts. Additionally, household products, mainly organophosphates, carbamates and synthetic pyrethroids, are commonly used in homes and may be an important cause of insecticide resistance, especially in the house-haunting mosquito, *Ae. aegypti*.

Efforts to control resistance patterns in insects have been proposed to prevent or slow the development of insecticide resistance and to manage the impact of resistance to new insecticides (Brattsten *et al.* 1986). Various countermeasures have been proposed for avoiding the development of insecticide resistance in mosquitos (Georghiou, 1980; Brown, 1981; Leeper *et al.* 1986; Plapp, 1986; Croft, 1990). Methods include varying the doses of insecticide applied, using restricted rather than wide area applications, applying insecticides only when vector-borne transmission occurs, using of less persistent insecticides, rotation of insecticides, and protecting the natural enemies of vectors, all to help minimize the selection pressure from insecticides. Use of an integrated control approach including the application of bacterial toxins and use of biological control organisms has been encouraged (Brown, 1981; WHO, 1986, Roberts and Andre, 1994; Chareonviriyaphap, 1995).

In conclusion, insecticide resistance monitoring should be detected and evaluated as early as possible and should be increased in both periodicity and geographical coverage in Thailand to include as many known vectors as possible. Detection of incipient or operationally unacceptably high levels of physiological resistance will alert public health authorities to take appropriate steps to counter potential reduced control efforts. Moreover, control programs should remain aware of cross-resistance to many related insecticides that may arise from the wide use of the same groups of synthetic compounds against mosquito populations and agricultural pests.

ACKNOWLEDGEMENTS

The authors would like to thank LCDR Michael Bangs from the US Naval Medical Research Unit No. 2. Special thanks go to Dr Pompimol Rongnoparat from Armed Forces Research Institute Medical Sciences (AFRIMS), Bangkok, Thailand for her valuable advice. This work was supported by The Thai Research Fund (TRF), Bangkok, Thailand.

REFERENCES

Brattsten LB, Holyoke CWJr, Leeper JR, Raffa KF. Insecticide resistance: challenge to pest management and basic research. *Science* 1986; 231: 1255-60.

Brown AWA, Pal R. Insecticide resistance in arthropods. 2nd ed. *WHO Monogr Ser* 1971; 38: 491 pp.

Brown AWA. Countermeasures for insecticide resistance. *Entomol Soc Am Bull* 1981; 27: 198-202.

Brown AWA. Insecticide resistance in mosquitoes: a pragmatic review. *J Am Mosq Control Assoc* 1986; 2: 123-40.

Burke DS, Leake CJ. Japanese encephalitis. The arboviruses: epidemiology and ecology. Vol III. Monath T, ed. Florida: CRC Press 1988; 63-92.

Chan YC, Ho BC, Chan KL. *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) in Singapore City. 5. Observation in relation to dengue haemorrhagic fever. *Bull WHO* 1971; 44: 651-8.

Chareonviriyaphap T. Pesticide avoidance behavior in *Anopheles albimanus*. Bethesda, Maryland: Uniformed Services University of the Health Sciences (USUHS), 1995. PhD Thesis.

Chareonviriyaphap T, Roberts DR, Andre RG, Harlan HJ, Manguin S, Bangs MJ. Pesticide avoidance behavior in *Anopheles albimanus*, a malaria vector in the Americas. *J Am Mosq Control Assoc* 1997; 13: 171-83.

Croft BA. Developing a philosophy and program of pesti-

cide resistance management. Roush RT, Tabashnik BE, eds. Pesticide Resistance in Arthropods. New York and London: Chapman and Hall 1990; 277-96.

Georghiou GP. Implications of the development of resistance to pesticide: basic principles and consideration of countermeasures. Proceeding of seminar and workshop. Pest Pesticide Management. Caribbean, 1980: 116-29.

Georghiou GP. Strategies and tactics for resistance management. In : Pesticide Resistance. Washington DC: National Academy Press, 1986.

Georghiou GP, Saito T, eds. Pest resistance to pesticides. New York/London: Plenum. 1983: 809 pp.

Gould DJ, Edelman R, Grossman RA, Nisalak A, Sullivan MF. Study of Japanese Encephalitis virus in Chiangmai valley, Thailand. *Am J Epidemiol* 1974; 100: 49-56.

Gould DJ, Esah S, Pranith U. Relation of *Anopheles aconitus* to malaria transmission in the central plain of Thailand. *Trans R Soc Trop. Med Hyg* 1967; 61: 441-2.

Gould DJ, Mount GA, Scanlon JE, Ford HR, Sullivan MF. Ecology and control of dengue vectors on the island in the Gulf of Thailand. *J Med Entomol* 1970; 7:499-508.

Gould DJ, Yuill TM, Moussa MA, Simasathien P, Rutledge LC. An insular outbreak of dengue hemorrhagic fever. III. Identification of vectors and observations on vector ecology. *Am J Trop Med Hyg* 1968; 17: 609-18.

Green CA, Rattanarithikul R, Pongparit S, Sawadwongporn P, Baimai V. A newly-recognized vector of human malaria parasites in the Oriental region. *Anopheles* (Cellia) *pseudowillmori* (Theobald, 1910). *Trans R Soc Trop Med Hyg* 1991; 85: 35-6.

Gubler DJ. Dengue. In: Boca Raton, ed. The arboviruses: epidemiology and ecology, Vol II. Florida: CRC Press, 1988: 223-60.

Guptavanij P, Harinasuta C. The periodicity of *Brugia malayi* in South Thailand. *Southeast Asian J Trop Med Public Health* 1977; 8: 185-9.

Harrison BA. Medical entomology studies: XIII. The Myzomyia series of *Anopheles* (Cellia) in Thailand, with emphasis on intra-interspecific variations (Diptera:Culicidae). *Contrib Am Entomol Inst* 1980; 17: 1-195.

Harrison BA, Scanlon JE. Medical entomology studies: II. The subgenus *Anopheles* in Thailand (Diptera: Culicidae). *Contrib Am Entomol Inst* 1975; 12: 1-307.

Kerkut GA, Gilbert LI. Comparative Insect Physiology Biochemistry and Pharmacology, Vol 12. Insect Control. Pergamon Press, 1985; 849 pp.

Leeper JR, Roush RT, Reynolds HT. Prevention or managing resistance in arthropods. In : Pesticide Resistance Strategies and Tactics for Management. Washington, DC : National Academy Press, 1986; 335-46.

Malaria Division, Department of Communicable Disease Control (CDC), Ministry of Public Health, Thailand. Annual Malaria Report. 1985-1998.

Mourya DT, Mishra RC, Soman RS. Transmission of Japanese encephalitis virus in *Culex pseudovishnui* and *Culex tritaeniorhynchus* mosquitoes. *Indian J Med Res* 1991; 93: 250-2.

Oppennoorth FJ. Biochemistry of insecticide resistance. *Pestic Biochem Physiol* 1984; 22: 187-93.

Phothikasikorn J. The susceptibility of some mosquitoes to *Wuchereria bancrofti*. Bangkok, Thailand: Mahidol University, 1991. MSc Thesis.

Plapp FWJr. Genetics and biochemistry of insecticide resistance in arthropods: propose for the future. In: Pesticide Resistance: Strategies and Tactics for Management. Washington DC: National Academy Press, 1986; 74-6.

Prasittisuk M. Comparative study of pyrethroids impregnated mosquito nets with DDT residual spraying for malaria control in Thailand. Bangkok, Thailand: Mahidol University, 1994. PhD Thesis.

Rattanarithikul R, Panthusiri P. Illustrated keys to the medically important mosquitoes of Thailand. *Southeast Asia J Trop Med Public Health* 1994; 25 (suppl): 1-66.

Roberts DR, Andre RG. Insecticide resistance issues in vectors. *Am J Trop Med Hyg* 1994; 50 (suppl): 21-34.

Rosenberg R, Andre RG, Somchit L. Highly efficient dry season transmission of malaria in Thailand. *Trans R Soc Med Hyg* 1990; 89: 22-8.

Scanlon JE, Peyton EL, Gould DJ. An annotated checklist of the *Anopheles* of Thailand. *Thai Natl Sci Pap Fauna Ser* 1968; 2: 1-35.

Watts DM, Burke DS, Harrison BA, Whitmire RE, Nisalak A. Effect of temperature on the vector efficiency of *Aedes aegypti* for dengue two virus. *Am J Trop Med Parasitol* 1987; 54: 78-91.

WHO. Manual on practical entomology in malaria. Part II. Method and Technique. Geneva: WHO Offset Publication 1975; 13.

WHO. Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate and carbamate insecticide-diagnostic test. Geneva, Switzerland, WHO/VBC/81.806, 1981a: 6 pp.

WHO. Instructions for determining the susceptibility or resistance of larval mosquitoes to insecticides. Geneva, Switzerland WHO/VBC/81.807, 1981b: 6 pp.

WHO. Resistance of disease vectors to pesticides. *WHO Tech Rep Ser* 1986; 737: 86-7.

WHO. Vector resistance to pesticides. Fifteenth Report of the WHO Committee on Vector Biology and Control. 1992; 35: 143-8.

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Improved excito-repellency escape chamber for behavioural tests on mosquitoes

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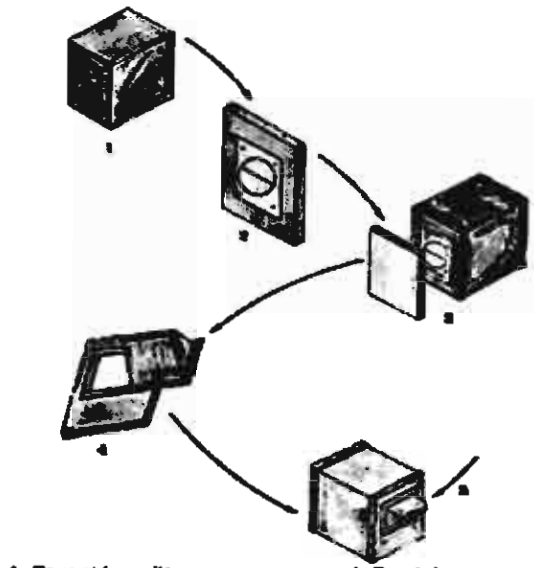
The behavioural responses of malaria vectors to insecticides are important components of malaria control strategies. Several attempts have been made using various types of excito-repellency test boxes to assess the behavioural responses of mosquito vectors to insecticides. However, no test system has yet been fully accepted. The new test system described herein provides additional improvements on the capacity to test behavioural responses, with and without physical contact with insecticide residues. A behavioural response with contact is defined as irritancy and a response without contact is repellency. The test system has already been used in a preliminary study of behavioural responses of an Anopheles minimus population to DDT, deltamethrin, and lambdacyhalothrin. This test system provided highly reproducible results.

Excito-repellency tests to study the irritant effect of insecticides on mosquitoes have been developed (Rachou et al, 1963; WHO, 1970). Investigations have been conducted on malaria vectors using modified WHO excito-repellency test boxes (Bondareva et al, 1986; Ree and Loong, 1989; Pell et al). The chamber reproduces local housing conditions, with walls, an entrance, and an exit window. Unfortunately, no method for behavioural studies has been fully accepted, compounding the difficulties of excito-repellency testing, data analysis, and interpretation (Roberts et al, 1984; Evan, 1993). Until recently, methods have not been available for testing non-contact repellency (Roberts et al, 1997). In 1997,

Chareonviriyaphap et al used an improved experimental escape chamber, providing information on both contact irritability and non-contact repellency for behavioural response tests. However, this prototype test system was cumbersome, sometimes difficult to use, and required much time to attach the test papers to the inner walls, especially under field conditions. To help avoid these problems, an improved collapsible metal excito-repellency test chamber was developed as described below.

Figure 1. Excito-repellency escape chamber



1. Four side walls
2. Rear Plexiglas® inner door
3. Rear outer door cover
4. Front door
5. Removable exit portal (escape funnel)

Figure 1 shows the exterior of the stainless steel collapsible excito-repellency escape chamber (EREC) (34cm x 32cm x 32cm) with the front panel and escape funnel at one end. The box has four side walls (1), a rear Plexiglas® inner door (2), a rear outer door cover (3), a front door (4), and a removable exit portal (escape funnel) (5). The side walls are composed of two parts, the wall itself and the test paper holder. The wall is constructed of a stainless steel sheet (thickness 0.7mm) which has an aluminium sliding rib on each end and a socket - a surface for the test paper holder - in the middle. The test paper holder has two sides; a sheet of fine iron mesh is permanently attached on one side, and a panel to hold test papers to secure the panel on top is on the opposite side. There is a 0.8cm gap between the test papers and screen to prevent mosquitoes from making physical contact with the test paper surface in the exposure windows during the non-contact repellency trials. The test paper holder is convenient and functions similarly under contact and non-contact conditions, depending on the purpose of the test. The holder simply has to be inverted to provide the proper conditions. There is a spring mechanism on one side of the test paper holder to secure it tightly when putting the holder into the socket. The front door is constructed of a stainless steel frame with a stainless steel sheet affixed on the front side. The steel sheet has a trough for sliding the exit funnel into place. There are two screws at one end that secure the funnel to the front panel. The inner rear door is constructed of a stainless steel frame and a transparent Plexiglas® door which is attached to the frame. The Plexiglas® door seals the

chamber and at the same time allows the investigator to look inside the exposure chamber before and after a test is conducted. There is a self-sealing portal (diameter 15.5cm) made of dental dam for placing test specimens inside the chamber and for removing the specimens from the chamber after each test. The outer rear door is constructed of stainless steel and is used to shut off all light inside the chamber when the test is being conducted. The last component is a removable exit funnel attached to the outside of the chamber. The escape funnel gap is a horizontal slit 20.5cm long and 1.5cm wide.

To assemble the collapsible excito-repellency chamber, all four side walls are put together by sliding the appropriate aluminium tongue and groove elements together to connect the four side walls of the chamber. The front door and the inner rear door are screwed to each side of the adjoining walls. To change the test papers, the nuts holding the transparent Plexiglas® to the metal frame are unscrewed, the Plexiglas® door is removed and the test paper holder is taken out of the chamber. When the test papers have been removed or replaced, the holder and Plexiglas® door are put back in place.

For a complete test, 25 mosquitoes were introduced into each of the four chambers using a mouth aspirator. After the mosquitoes were put in the chamber, the outer rear door was secured shut. A 6cm3 paper receiving cage was connected to the exit window for collecting escaping specimens. At the start of a test, a three-minute resting period was used to permit mosquitoes to adjust to test chamber conditions (Busvine, 1964; Chareonviriyaphap et al, 1997). After three minutes, the escape funnel was opened to initiate the observation period. Numbers escaping from the exposure chamber into receiving cage were recorded at one-minute intervals (Chareonviriyaphap et al, 1997). Tests were performed as described by Chareonviriyaphap et al (1997). A typical test involved four chambers, one allowing contact with the insecticide, the other preventing direct contact. The two chambers are paired with two other chambers without insecticide.

Preliminary results of behavioural responses indicated that female An. minimus, a population recently colonised by the Malaria Division, CDC, Ministry of Public Health, Thailand, demonstrate a dramatic avoidance response to 4% DDT, 0.025% deltamethrin, and 0.1% lambda-cyhalothrin under contact conditions. Most specimens (>98%) quickly escaped the exposure chamber without receiving a lethal dose of insecticide. In our study, test specimens show a certain degree of non-contact repellency to synthetic pyrethroids and DDT. Non-contact repellency to DDT may also play a significant role in decreasing human-vector contact as shown by studies on Anopheles culicifacies in India (Shalaby, 1966).

In conclusion, this test system provides the following desirable characteristics:

- Exposure chambers are made from stainless steel and can be chemically treated between uses with different doses and types of insecticides.

- The test paper holder has two sides, for the dual purposes of exposure with or without insecticide contact. A sheet of fine iron screening is used to prevent contact with the test paper.
- Mosquitoes are easily transferred to the exposure chambers and are easily removed from the chambers after the test is completed.
- The whole chamber system can be disassembled for transport.
- Highly reproducible test results are obtained.

The authors would like to thank LCDR Michael Bangs of U.S. Naval Medical Research Unit No. 2 (NAMRU-2), Jakarta, Indonesia for his valuable suggestions on E-R chamber modifications. Special thanks go to Dr. Pornpimol Rongnoparat of the Armed Forces Research Institute Medical Centre (AFRIMS), Bangkok, Thailand for her critical reading. This study was supported in full by the Thai Research Fund, Bangkok, Thailand. Insecticide papers were supported by Dr. D.R. Roberts from the Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA. Readers who desire more information on the boxes are kindly asked to contact the principal author (see Author contacts, page 105)

References

Bondareva, N.L.; Artem'ev, M.M.; and Gracheva, G.V. Susceptibility and irritability caused by insecticides to malaria mosquitoes in the USSR. Part 1. Anopheles pulcherrimus. Medit. Parazitol. Parazit. Bolezni 1986; 6: 52-55.

Chareonviriyaphap, T.; Roberts, D.R.; Andre, R.G.; Harlan, H.; and Bangs, M.J. Pesticide avoidance behavior in Anopheles albimanus, Wiedemann. Am. J. Mos. Control Assoc. 1997; 13: 171-183.

Evan, R.G. Laboratory evaluation of the irritancy of bendiocarb, lambda-cyhalothrin, and DDT to Anopheles gambiae. J. Am. Mosq. Control Assoc. 1993; 9: 285-293.

Pell, J.K.; Spinney, M.A.; and Ward, K.J.
Observations on the behavior of

Rachou, R.G.; Moura Lima, M.; Duret, J.P.; and Kerr, J.R. Experiences with the excito-repellency test box-model OPS. Proc. of the 50th Ann. Meet of N. J. Mosq. Exter. Assoc. and the 19th Ann. Meet of Am. Mosq. Control Assoc. 1963; pp. 442-447.

Roberts, D.R.; Chareonviriyaphap, T.; Harlan, H. H.; and Hshieh, P. Methods for testing and analyzing excito-repellency responses of malaria vectors to insecticides. Am. J. Mos. Con. Assoc. 1997; 13 (1): 13-17.

Roberts, D.R.; Alecrim, W.D.; Tavares, A.M.; and McNeill, K.M. Influence of physiological condition on the behavioral response of Anopheles darlingi to DDT. Mos. News 1984; 4: 357-361.

Shalaby, A.M. Observations

Observations on the behavior of adult Anopheles gambiae encountering residual deposits of lambda-cyhalothrin compared with the other major classes. Fourth Ann. Conf. Soc. Vector Ecol. (Eur. Region) 1989; p. 18.

Ree, H.I. and Loong, K.P. Irritability of Anopheles farauri, Anopheles maculatus, and Culex quinquefasciatus to permethrin. Jap. J. Sanit. Zool. 1989; 40: 47-51.

Shidaby, A.M. Observations on some responses of Anopheles culicifacies to DDT in experimental huts in Gujarat State, India. Ann. Entomol. Res. 1966; 59: 33-46.

World Health Organization. Insecticide resistance and vector control (17th report of the WHO Expert Committee on Insecticides). Instructions for determining the irritability of adult mosquitoes to insecticide. WHO Tech. Rep. Ser. 1970; **433**: 158-163.

rnal of Vector Ecology 25(1): 48-61

A Probability Model of Vector Behavior: Effects of DDT Repellency, Irritancy, and Toxicity in Malaria Control[1]

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Received 27 May 1999; Accepted 11 February 2000

ABSTRACT: A probability model of how DDT residues may function within a malaria control program is described. A step-wise organization of endophagic behaviors culminates in a vector acquiring a human blood meal inside the house. Different vector behaviors are described, epidemiologically defined, temporally sequenced, and quantified with field data. Components of vector behavior and the repellent, irritant, and toxic actions of insecticide residues are then assembled into a probability model. The sequence of host-seeking behaviors is used to partition the total impact of sprayed walls according to the three chemical actions. Quantitatively, the combined effect of repellency and irritancy exert the dominant actions of DDT residues in reducing man-vector contact inside of houses. These relationships are demonstrated with published and unpublished data for two separate populations of *Anopheles darlingi*, for *Anopheles gambiae* and *Anopheles funestus* in Tanzania, and *Anopheles punctulatus* in New Guinea.

Keyword Index: Probability model, malaria control, vector behavior, DDT, *Anopheles*.

INTRODUCTION

Efficient vectors of human malaria typically move a house, enter, and bite indoors. These activities are interrupted by intervals of resting. The acts of entering and exiting a house involve finding and entering openings in a physical barrier (the house wall), so they are distinct from movement or flight from one location to another. House entering and indoor biting (endophagic) behaviors are epidemiologically important because they influence the likelihood that a mosquito will bite a human and imbibe an infectious blood meal or transmit malaria. Endophagic behaviors are equally important in malaria control to the extent that an insecticide may prevent vectors from entering a sprayed house or stimulate vectors to exit a house before they bite.

Practically every important vector of malaria exhibits insecticide avoidance behavior (Elliott and de Zulueta 1975 and Lockwood et al. 1984). Yet the epidemiological significance of these chemically-induced behaviors is controversial. In this paper we describe a model for defining the importance of repellent and irritant actions of insecticide residues. The term excito-repellent will be used to describe all chemically-

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induced behaviors, including both repellent and irritant actions (Roberts and Andre 1994). A repellent is a chemical that causes insects to make oriented movements of avoidance without tarsal contact with the chemical. An irritant is a chemical that causes insects to make oriented movements of avoidance after tarsal contact.

The sequential nature of mosquito host-seeking behaviors is used to partition the separate contributions of repellency, irritancy/repellency, and toxicity that comprise the overall impact of DDT-sprayed houses. A series of mathematical formulas are described that explicitly isolate and quantify these actions. The formulas are used with field data from experimental hut studies on four important malaria vectors to show that repellency is the primary action of DDT residues in preventing human-vector contact.

The probability model is a product of many years of work with vectors in the Americas, originating with studies in 1978-1981 on the behavior and ecology of *Anopheles darlingi* in the Amazon Basin of Brazil (Roberts and Alecrim 1991). Also, this model has benefited from recent studies on *Anopheles pseudopunctipennis* (Fernandez-Salas et al. 1993 and Fernandez-Salas et al. 1994), *Anopheles albimanus* (Chareonviriyaphap et al. 1997), *An. albimanus*, *Anopheles vestitipennis*[7], and *An. vestitipennis* (Grieco et al. 2000). Others studies contributed to this model by documenting repellent and irritant actions of DDT residues with *An. albimanus* (Hecht and Hernandez 1960, Mancera and Hernandez 1960, Duret 1964, and Rachou et al. 1966), *An. pseudopunctipennis* (Downs and Bordas 1951, Hecht and Hernandez 1960, Loyola et al. 1990, Loyola et al. 1991, and Cases et al. 1998), and *An. darlingi*[8] (Charlwood and Paraluppi 1978, Roberts et al. 1984).

MATERIALS AND METHODS

Components of Indoor Host-Seeking Behaviors of Malaria Vectors

The primary scenarios of host-seeking activities that occur within the peridomestic environment and inside houses are listed in Figures 1 and 2. All 10 scenarios might be represented within natural host-seeking populations of a vector species. The sequence of directed movements at sunset and early evening, followed by variable levels of indoor biting activity through the remainder of the night have been documented for *An. darlingi* populations in Brazil (Roberts et al. 1987), Colombia (Elliott 1972), and Suriname[8]. Although the patterns of biting activity were not identical, the sequence of activities was essentially the same. Symbols employed in Figures 1 and 2 and in the probability model are:

- c = control house (unsprayed),
- t = treated house (sprayed),
- a = average number of mosquitoes during a post-spray interval (can indicate numbers entering, exiting, or biting indoors),
- a'= average number of mosquitoes during a pre-spray interval (can indicate numbers entering, exiting, or biting indoors),
- h = index of reduced numbers entering, biting indoors, or exiting a treated house,
- r = resting for a prolonged period (2-3 days),
- r'= not resting for a prolonged period (temporary period of minutes to hours),
- re= resting at site through oogenesis (time required for egg development),
- o = outside of house (outdoors),
- i = inside of house (indoors),
- f = fed (blood engorged),
- u = unfed (not blood engorged),
- b = biting,
- b'= not biting,
- s = surviving for 24 hours after exiting,
- s'= not surviving (death) 24 hours after exiting the house, and
- m= movement from one site to another.

Endophily characterizes a mosquito that rests indoors until oogenesis is completed (r_{ii}), and then departs to lay eggs. Exophilic mosquitoes rest outdoors during oogenesis (r_{oo}). Endophagy characterizes indoor biting (b_i), and exophagy characterizes biting out of doors (b_o).

A malaria-susceptible mosquito that moves into the peridomestic environment, enters a house (m_i), bites a human indoors (b_i), and subsequently departs (m_o) for an outdoor resting site (r_{oo}) is epidemiologically

[7]Bangs, M. 1999. The susceptibility and behavioral responses of *Anopheles albimanus* Weidemann and *Anopheles vestitipennis* Dyar and Knab (Diptera: Culicidae) to insecticides in northern Belize. Dissertation submitted to the Uniformed Services University of the Health Sciences, Bethesda, MD, 448 pp.

[8]Rozendaal, J. A. 1990. Epidemiology and control of malaria in Suriname. ICG Printing b.v., Dorbrecht, 170 pp.

Figure 1. Patterns of outdoor host-seeking behaviors of *Anopheles* mosquitoes, illustrating the primary schemes of exophagic behavior, with or without house entering. Symbols are indicative of: *r* = resting for prolonged period (1-3 days); *r'* = not resting for prolonged period; *re* = resting at site through oogenesis (time required for egg development); *u* = unfed (unengorged); *o* = outdoors; *b* = biting; *m* = moving; *i* = indoors; *f* = fed (engorged).

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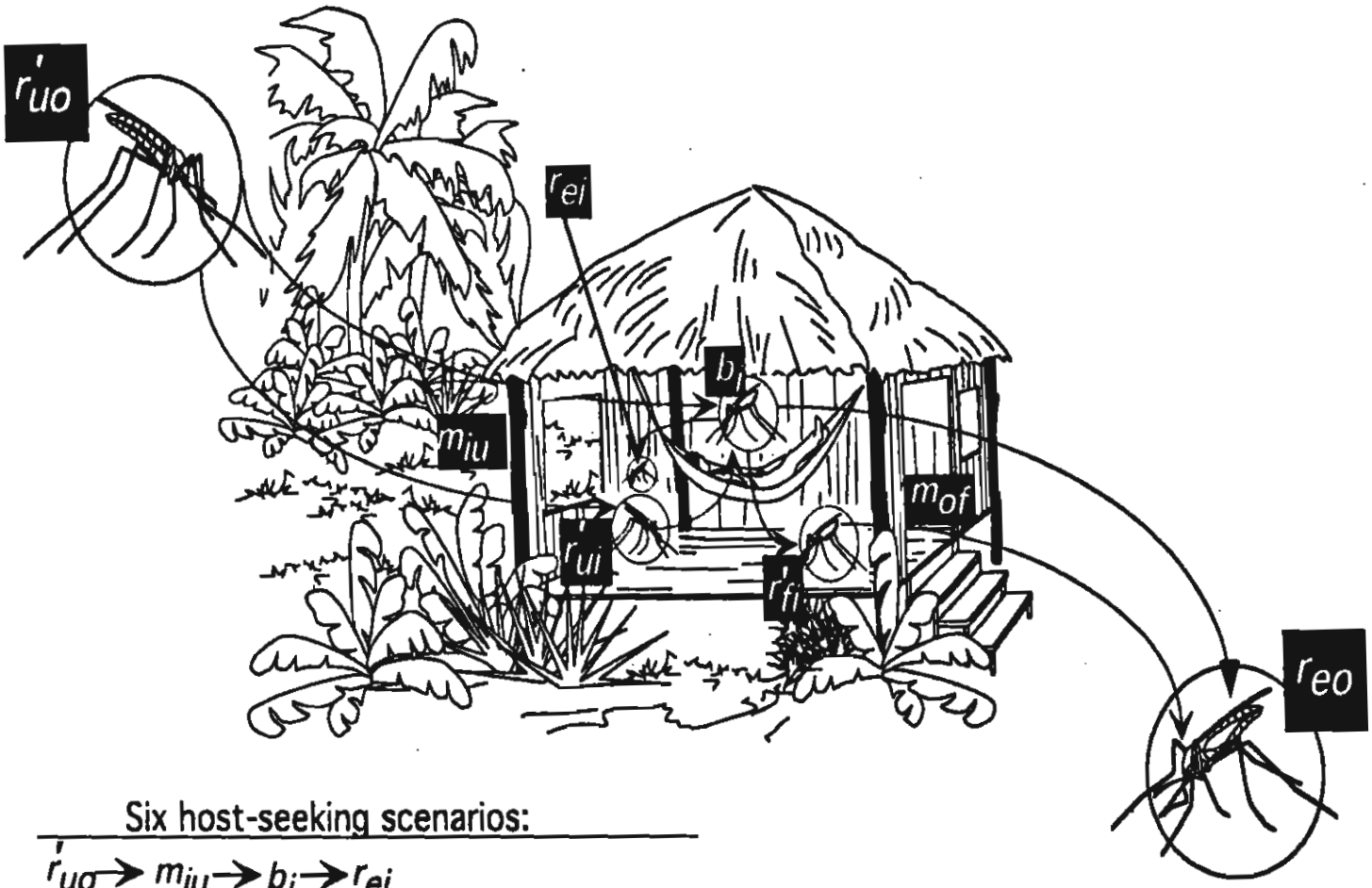

Six host-seeking scenarios:

$r'_{uo} \rightarrow m_{iu} \rightarrow b_i \rightarrow r_{ei}$

$r'_{uo} \rightarrow m_{iu} \rightarrow b_i \rightarrow m_{of} \rightarrow r_{eo}$

$r'_{uo} \rightarrow m_{iu} \rightarrow b_i \rightarrow r'_{fi} \rightarrow m_{of} \rightarrow r_{eo}$

$r'_{uo} \rightarrow m_{iu} \rightarrow r'_{ui} \rightarrow b_i \rightarrow r_{ei}$

$r'_{uo} \rightarrow m_{iu} \rightarrow r'_{ui} \rightarrow b_i \rightarrow m_{of} \rightarrow r_{eo}$

$r'_{uo} \rightarrow m_{iu} \rightarrow r'_{ui} \rightarrow b_i \rightarrow r'_{fi} \rightarrow m_{of} \rightarrow r_{eo}$

Epidemiological Descriptors

b_i, r_{eo} =	endophagic and exophilic
b_i, r_{ei} =	endophagic and endophilic

Figure 2. Patterns of indoor host-seeking behaviors of *Anopheles* mosquitoes, illustrating the primary schemes of endophagic behavior. Symbols are indicative of: r' = not resting for prolonged period; re = resting at site through oogenesis (time required for egg development); u = unfed (unengorged); o = outdoors; b = biting; m = moving; i = indoors; f = fed (engorged).

portant. In the context of human malaria, a mosquito bites some nonhuman host outdoors (b_o), then rests vors through oogenesis (r_{io}) is not epidemiologically portant. The former is exhibiting endophagic (b_i) and ophilic (r_{oo}) behaviors, and the latter is exhibiting phagic (b_o) and endophilic (r_{ii}) behaviors. Behavioral aracterizations of vectors should reflect the mix and tive frequency of host-seeking scenarios (Fig. 1 and pressed by vector populations under field conditions.

ta Sources

A behavioral study on *An. darlingi* populations was d to develop basic probability statistics (Roberts et 1987, Roberts and Alecrim 1991). The study was ducted along the Ituxi River in Amazonas, Brazil in 9-1981 and was used here because the primary hor has the complete data set. Comparative data e also extracted from studies conducted on *An. rlingi* in Suriname[8], *An. gambiae* and *An. funestus* in nzania (Smith and Webley 1969), and *An. punctulatus* rian Jaya (West New Guinea)[9].

Anopheles darlingi females captured in entrance exit traps were examined for physiological condition, ., using Detinova's (1962) descriptions of Sella's ges of blood digestions and egg development. Based observations by Roberts et al. (1983), specimens cribed as being in Sella's stages 4 through 7 probably 24 hours or more before they were captured. We l refer to these specimens as a group of "old" fed cimens. Specimens marked as "recent" or "late" fed n Sella's stages 2 and 3 will be grouped as having fed night they were captured.

ethods of Calculating Probabilities

Data from biting collections and entrance and exit p collections were compiled for the two houses for veral days before one of the houses was sprayed with DT. Discrete probabilities (p) were developed by viding the average number captured during an interval time by total number collected for the whole night. As example, consider that, over several nights of llecting, an average of 80, 50, 20, 10, 5, and 4 osquitoes/night were captured in entrance traps during t two-hour intervals from 6:00 P.M. to 6:00 A.M. The m of average values is 169 and the estimated discrete obability for the first two-hour interval is 80/169, or 47. The estimated discrete probability for the second ur was 50/169, or 0.296. These values suggest that the obabilities for mosquitoes to enter the house from 6:00 to 8:00 P.M. is 0.47 and from 8:00-10:00 P.M. is 0.296. Alternatively, cumulative probabilities (*cump*) would be 80/169, or 0.47 for the first interval; (80+50)/169, or 0.769 for the second interval; and so forth until the estimated *cump* for the last interval (4:00 to 6:00 A.M.) would equal 1.0.

The entrance and exit traps were identical in dimensions and design and were employed in equal number simultaneously in identical sampling intervals. The traps functioned as unbiased and equal estimators of population movements into or out of the house. Consequently, we adjusted patterns of entering unsprayed houses by subtracting the average number of unfed females that exited by window traps (m_{oo}) from the average number of unfed females that entered (m_{io}) through window traps for each time interval during the night. This "adjusted" value corrected for background movement of unfed females into and out of the house that seemed to vary according to the numbers of unfed females that entered during a collection interval. In our opinion, the adjustment provided a more refined estimate of numbers of unfed females that both entered the house, remained longer indoors and possibly took a blood meal indoors (m_{io}, b_i). These adjusted values were then converted to *cump* values.

The *cump* values were used to estimate times for 50% or 75% of the population to enter, bite and exit unsprayed houses. Smoothing splines were used to plot the *cump* data points (with *cump* values on the y-axis; time on the x-axis; *cump* values plotted by the midpoint of each time interval). Estimates of time for 50% or 75% of mosquitoes to enter, bite, or exit were obtained by drawing lines from the 0.5 and 0.75 values on the y-axis. A line was dropped to the x-axis from points intersecting the graphical plot of *cump* values. Estimates of time were read from the x-axis.

Since entrance and exit trap collections were continued for 24-hour periods, values for p and *cump* for entering and escaping houses were arrayed for both 12 and 24-hour intervals. However, emphasis was placed on defining activities during the 12 hours of darkness. Biting collections were conducted from 6:00 P.M. to 6:00 A.M. The series of *cump* values for house-entering covered the same 6:00 P.M. to 6:00 A.M. interval of time; but we used a 30-min. offset because entrance and exit trapping started at 6:30 P.M.

The impact of DDT on numbers entering the treated house (t) was estimated by dividing the average number entering a sprayed house (a) for each time interval/night

[9]Sloof, R. 1964. Observations on the effect of residual DDT house spraying on behaviour and mortality in species of the *Anopheles punctulatus* group. A. W. Sythoff, Leyden, Holland, 144 pp.

with the average number that entered before the house was sprayed (a'), or a_t/a'_t. For the control house, the formula a'_c/a_c, was used to measure the natural change in numbers entering houses from the pre-spray to post-spray intervals of time. The two calculations were combined to adjust for natural difference between the treated and control houses under pre-spray conditions and for natural change in mosquito populations from the pre- to post-spray time intervals. Thus, the formula for estimating proportions entering the sprayed house is $(a_t/a'_t)(a'_c/a_c)$, or $(a_t x a'_c)/(a'_t x a_c)$. We converted this to an index of reduced numbers entering the sprayed house with the formula:

Index of Reduced Numbers =
$1-[(a_t x a'_c)/(a'_t x a)] = h.$ (Formula 1)

This formula was also used with numbers exiting the experimental houses.

The Probability Model

In general, probabilities employed in this model are conditional simply because a mosquito cannot enter a house unless it has first moved near the house, cannot bite indoors unless it has first moved into the house, and cannot escape engorged until it has first taken a blood meal inside the house. Reduced house entering (see formula 3) was estimated with entrance trap data from the Ituxi River study. Alternatively, investigators, using indirect measures, in the Suriname, Tanzania, and New Guinea studies developed estimates of reduced house entering. Measures of reduced indoor biting (see formula 5) were obtained from biting collections conducted simultaneously in the treated and control houses, and by proportions of females captured in exit traps that were blood engorged. Reduced survival (see formula 8) was estimated by holding females captured in exit traps from both treated and control houses for 24 hours and recording numbers that died at end of the holding period.

All parameters of this model were standardized with data from the control house. To standardize data, we assume that the maximum possible number of females will enter and bite indoors and that the smallest possible numbers of specimens will subsequently die. As a method of standardization, we assume that $p(m_{uic}) = 1$, and $p(b_{ic}) = 1$. We also assume that $p(s_{fc}) = 1$, whether the mosquito remains indoors throughout oogenesis or departs the house after biting. Finally, we assume that

$p(m_{uic}) = p(b_{ic}) = p(m_{fnc}) = p(s_{fc}) = 1,$
and that $p(s'_{fc}) = 1 - p(s_{fc}) = 0.$

To examine the effect of a repellency action that prevents mosquitoes from entering the sprayed house, let m_{uit} be the event that the unfed mosquito enters the sprayed house (m'_{uit} for not entering), then the probability of entering will be denoted by $p(m_{uit})$. We assume the probability that an unfed mosquito will enter the control house is $p(m_{uic}) = 1$. It follows that the probability of reduced entry for the sprayed house is,

$p(m_{uic}) - p(m_{uit}) = 1 - p(m_{uit}).$ (Formula 2)

Thus, the percent reduction in numbers entering the sprayed house is,

$(1-p(m_{uit}))*100.$ (Formula 3)

Discrete and cumulative probabilities for entering the unsprayed house can be used with h values to estimate discrete and cumulative probabilities for entering the sprayed house (remember, h is a measure of reduced entering, biting, or exiting for the sprayed house). Using the earlier example, the probability of entering the control house $p(m_{uic})$ during the 6:00 to 8:00 P.M. is 0.47. Lets suppose that the index of reduced numbers, h, entering the sprayed house from 6:00 to 8:00 P.M. is 0.95. As a consequence, the index of entering the house is 0.05, or $1-h$. The discrete probability for entering the sprayed house from 6:00 to 8:00 P.M. is $(p*(1-h))$, or $0.47 * 0.05 = 0.0235$. Estimates of discrete probabilities for all time intervals during the night can be summed to give a standardized, total probability ($cump$) for unfed females to move into the sprayed house, that is, $p(m_{uit})$.

Impact of irritant/non contact repellent actions on mosquitoes after they enter the house is defined by numbers biting indoors. Let b_{it} be the event that a mosquito will bite in the sprayed house (b'_{it} for not biting), then the probability that the mosquito will bite in the sprayed house is denoted by $p(b_{it}, m_{uit}) = p(b_{it}|m_{uit}) * p(m_{uit})$. We denote the probability of the mosquito entering and biting in the control house as $p(b_{ic}, m_{uic}) = p(b_{ic}|m_{uic}) * p(m_{uic}) = 1$. Given these relationships, the probability of reduced biting in the sprayed house is

$[p(b_{ic}|m_{uic}) * p(m_{uic})] - [p(b_{it}|m_{uit}) * p(m_{uit})] =$
$1 - [p(b_{it}|m_{uit}) * p(m_{uit})].$ (Formula 4)

Consequently, the percent reduction in numbers that enter the sprayed house and subsequently bite indoors can be defined as,

$[1 - p(b_{it}|m_{uit}) * p(m_{uit})]*100.$ (Formula 5)

To define the reduction in numbers escaping after

feeding indoors, let m_{oft} be the event that the mosquito will escape from the sprayed house (m'_{oft} for not escaping). The probability a mosquito entered, took a blood meal indoors, and escaped from the sprayed house is $p(m_{oft},b_{it},m_{uit}) = p(m_{oft}|m_{uit},b_{it}) * p(b_{it}|m_{uit}) * p(m_{uit})$. Alternatively, the probability that the mosquito will escape from the control house can be denoted by $p(m_{ofc},b_{ic},m_{uic}) = 1$. The probability of entering, biting indoors, and escaping the sprayed house is

$$p(m_{ofc},b_{ic},m_{uic}) - p(m_{oft},b_{it},m_{uit}) = 1-[p(m_{oft}| m_{uit},b_{it}) * p(b_{it}|m_{uit}) * p(m_{uit})]. \quad \text{(Formula 6)}$$

If mosquitoes die indoors, then formula 6 can be used to capture this element of insecticide-vector interaction. Alternatively, numbers dead inside the house can be included in formulas 7 and 8. In the Ituxi River study, too few females entered and fed in the sprayed house and no indoor deaths were observed. Additionally, engorged females that were marked and released indoors rapidly escaped and none were found dead as a result of DDT exposure. As a consequence, the probability of moving out of doors from the treated house was treated as unity (i.e., $p(m_{oft},b_{it},m_{uit}) = 1.0$).

Let $p(s_{ft},m_{oft},b_{it},m_{uit})$ be the event that the mosquito will survive for 24 hours after entering, feeding, and escaping from the sprayed house ($p(s'_{ft},m_{oft},b_{it},m_{uit})$ for not surviving), then the probability is

$$p(s_{ft},m_{oft},b_{it},m_{uit}) = [p(s_{ft}|m_{uit},b_{it},m_{oft}) * p(m_{oft}|m_{uit},b_{it}) * p(b_{it}|m_{uit}) * p(m_{uit})].$$

We assume that all mosquitoes entering the control house will survive and this probability is denoted by $p(s_{fc}|m_{ofc},b_{ic},m_{uic}) = 1$. Using these relationships, the probability of reduced survival (which is equal to probability of increased mortality) after entering and escaping the sprayed house can be obtained by

$$p(s_{fc},m_{ofc},b_{ic},m_{uic}) - p(s_{ft},m_{oft},b_{it},m_{uit}) = 1 - p(s_{ft},m_{oft},b_{it},m_{uit}) = 1 - [p(s_{ft}|m_{uit},b_{it},m_{oft} * p(m_{oft}|m_{uit},b_{it}) * p(b_{it}|m_{uit}) * p(m_{uit})]. \quad \text{(Formula 7)}$$

The percent reduction in probabilities of 24-hour survival of specimens that have taken a blood meal inside the treated house is defined as

$$[1-(p(s_{ft}|m_{uit},b_{it},m_{oft}) * p(m_{oft}|m_{uit},b_{it}) * p(b_{it}|m_{uit}) * p(m_{uit}))]*100. \quad \text{(Formula 8)}$$

Overall epidemiological impact of DDT residues is described by proportional reductions in house entering, indoor biting, and subsequent survival. Parameters that compose this total effect are quantified with formulas 3, 5, and 8. The singular impact of toxicity, $p(s'_{ft})$, in relation to the overall impact of repellent and irritant actions of DDT can be defined as

$$[p(b_{it}|m_{uit})*p(m_{uit})]-[p(s_{oft}|b_{it},m_{uit})*p(b_{it}|m_{uit}) *p(m_{uit})] =p(b_{it}|m_{uit})*p(m_{uit})[1- p(s_{ft}|b_{it},m_{oft})].$$

To calculate percent reduction due to mortality,

$$p(b_{it}|m_{uit})*p(m_{uit})[1- p(s_{ft}|b_{it},m_{fmt})]*100. \quad \text{(Formula 9)}$$

We used this probability model to quantify the actions of DDT residues on four important vectors of malaria, with emphasis on the use of formulas 1, 3, 5, 8, and 9. In the Ituxi River study, the numbers of females entering, biting, and escaping the sprayed house were small, e.g., in a single night after spraying DDT, 386 females were captured in entrance and exit traps in the control house and only six females were captured in an equal number of trap collections in the sprayed house. For this reason, no standard statistics, e.g., means or standard deviations, are used to describe parameters of the probability model. Regardless, the magnitude of differences between treatment and control are amply defined by probability values, and the probability values are based on actual numbers captured.

As stated above, the numbers of specimens entering the treated house were so small that no direct estimates of biting rates and mortality were possible. Consequently, we used Rozendaal's estimate[8] of reduced biting in the sprayed house. For mortality, we used Rozendaal's estimate that 95% of all escaping *An. darlingi* females died after entering the sprayed house. We also performed comparative calculations using an estimated mortality of 22%, which was the mortality rate reported by Smith and Webley (1969) for *An. gambiae* mosquitoes.

RESULTS

Pre-Spray Observations in Experimental Houses

Data in TABLES 1 and 2 show that *An. darlingi* females of varying physiological conditions routinely entered and exited houses. Unfed females entered the house in the early evening, whereas, blood-fed females entered later at night. The "old" fed females also entered the house late at night (data not shown).

Data in TABLE 2 show that females of varying physiological conditions exit houses. Only during the sunrise period did the numbers of fed females represent the majority of exiting specimens.

TABLE 1. Summary statistics for 151 entrance trap and 208 exit trap collections of *Anopheles darlingi* females in two experimental, unsprayed houses along the Ituxi River, Amazonas, Brazil. Collections were conducted for two-hour intervals throughout the night and, for selected sampling regimes, two- to six-hour sampling periods through daylight hours. Numbers represent the average number collected per trap per collection by physiological condition of the specimen.

	Entrance Trap Collections				Exit Trap Collections			
Time intervals	Unfed	Recent* fed	Late** fed	(n)	Unfed	Recent* fed	Late** fed	(n)
6:30-8:30 P.M	15	0.1	0	21	9.6	0.38	0.97	29
8:30-10:30 P.M	15	0	0	21	7.66	0.93	0.07	29
10:30 P.M..-00:30 A.M.	13	0.1	0.1	18	7.22	0.87	0.22	23
00:30-2:30 A.M.	8.6	0	0.4	17	6.25	0.42	0.58	24
2:30-4:30 A.M.	5.8	0.1	0.4	17	3.52	0.2	0.4	2
4:30-6:30 A.M.	7.1	0.1	0.1	18	11.65	1.4	5.13	23
6.30-8:30 A.M.	0.9	0	0	18	2.04	0.38	0.67	24
8:30 A.M.-12:30 P.M.	0.1	0	0	12	0.5	0	0.22	18
12:30-6:30 P.M	1.8	0.1	0	9	3.31	0.85	0.69	13

*Blood engorged female with red blood visible in the abdomen.

**Blood engorged female with black blood visible in the abdomen; but without the level of oogenesis as apparent in a Sella stage 2 female (Detinova 1962).

TABLE 2. Discrete (*p*) and cumulative probabilities (*cump*) of unfed and blood-fed *Anopheles darlingi* females entering or exiting two experimental houses along the Ituxi River, Amazonas, Brazil. Probabilities derived from collections for two-hour intervals throughout the night and, for selected sampling regimes, two- to six-hour sampling periods through daylight hours. Specimens that fed during the night of capture (specified as exiting fed) are represented by specimens that were recently fed*, late fed**, or were in Sella's stages*** 2 or 3 (numbers in parentheses are *cump*).

	Probabilities**** of		
Time Intervals	Entering Unfed	Exiting Unfed	Exiting Fed
6:30-8:30 P.M.	0.22(0.22)	0.19(0.19)	0.06(0.06)
8:30-10:30 P.M.	0.22(0.44)	0.14(0.33)	0.04(0.10)
10:30 P.M.-00:30 A.M.	0.20(0.64)	0.14(0.47)	0.05(0.15)
00:30-2:30 A.M.	0.12(0.76)	0.12(0.59)	0.08(0.23)
2:30-4:30 A.M.	0.09(0.85)	0.07(0.66)	0.03(0.26)
4:30-6:30 A.M.	0.11(0.96)	0.23(0.89)	0.47(0.73)
6.30-8:30 A.M.	0.01(0.97)	0.04(0.93)	0.13(0.86)
8:30 A.M.-12:30 P.M.	0.00(0.97)	0.01(0.94)	0.04(0.91)
12:30-6:30 P.M.	0.03(1.00)	0.06(1.00)	0.09(1.00)

* Blood engorged female with red blood visible in the abdomen.

** Blood engorged female with black blood visible in the abdomen; but without the level of oogenesis as apparent in a Sella stage 2 female.

*** Stages of blood digestion and egg development, see Detinova (1962).

**** Probability = Average number collected by time interval/Total number collected over 24 hours.

he majority of exiting specimens.

Of fed specimens exiting the house, roughly 3% had fed more than 24 hours before exiting. The "old" fed specimens also had a peak exit time at sunrise; 33% exited from 4:30 to 6:30 A.M. and another peak in exiting occurred during the afternoon. Proportionately, the highest number of specimens that fed during the night of collecting exited during late morning, with peak numbers exiting from 4:30-6:30 A.M.

Two compilations of probability (p) values and cumulative probabilities (*cump*) of house-escaping and house-entering activities were developed. The first set of statistics was for a 24-hour period (TABLE 3). The second set of statistics was developed for direct comparison with the 6:00 P.M. to 6:00 A.M. observations on paired indoor/outdoor landing collections. Graphical representations (not shown) of *cump* values (from TABLE 3) showed that 50% of all females that would enter the house over 24 hours, entered within the first 3.6 hours, and 75% entered within the first 6.8 hours. Of all unfed females that exited the house during a full 24-hour period, 50% had exited within the first 5.6 hours and 75% had exited within the first 9.5 hours. Of all females that fed during the night in which they were collected, 50% exited within the first 9.8 hours and 75% within the first 12 hours of a 24-hour period.

Graphical representations (not shown) of *cump* values for indoor biting were also used to show that highest indoor landing rates occurred early in the evening, with declining rates through the remainder of the night. Time adjusted *cump* values (from 6:00 P.M. to 6:00 A.M.) were also calculated for house entering as defined by entrance trap collection data. However, numbers that entered the house were further "adjusted" (as described in M&M) to give a more realistic estimate of numbers of unfed females that both entered the house and took a blood meal indoors. Using this adjustment, the *cump* for times of house entry were calculated and analyzed graphically (not shown). The *cump* values showed that 50% of unfed females that would subsequently bite indoors had entered the house within the first 2.67 hours, compared to an average of 3.64 hours for 50% of the females to bite. For 75% of the females to enter and bite, 4.37 and 6.8 hours were required, respectively. Based on these statistics, the average female that fed indoors must have rested in the house at least one hour before taking a blood meal.

Behavior in a DDT-Sprayed House

Summary statistics from systematic collections in each of the two houses after one of the two houses was sprayed were arrayed (not shown) according to the data format presented in TABLES 1 and 2.

Data in TABLE 4 show that the early evening surge of house entering by unfed females did not occur in the sprayed house (h=1.0 or h*100 =100% reduction). DDT residues also eliminated the peak exiting activity near sunrise.

As described in materials and methods for formula 3, the estimates of h were multiplied by the discrete p values in TABLE 3 to obtain adjusted probabilities for entering and exiting the sprayed house. The cumulative adjusted probabilities are presented in TABLE 5. Overall, 95% fewer unfed *An. darlingi* females entered the sprayed house compared to the unsprayed house. Cumulative adjusted probability for blood-engorged females to exit the sprayed house was only 0.038.

Summary probability values for *An. darlingi* (Ituxi River populations), along with comparable data for other vector populations[4,9], (Roberts and Alecrim 1991, Smith and Webley 1969) are presented in TABLE 6. Normally, the impact of insecticide on biting can be determined by comparing the relative mix of fed and unfed specimens in control versus sprayed houses. Unfortunately, so few specimens were collected in the sprayed house in the Ituxi River study that we could not quantify the reduction in biting by populations that entered the houses. As an alternative, we used Rozendaal's estimate of 0.56 as an estimated probability for biting in the sprayed house, so $p(b_u|m_u) = 0.56$.

Likewise, mortality determinations were not possible because too few specimens escaped from the sprayed house. For comparison, we used two different estimates of mortality. First, we used Rozendaal's (Rozendaal 1990[5]) estimate of 95 % mortality, equating to a $p(s') = 0.95$ or $p(s) = 0.05$; and we also used the estimate of 22% mortality from the study with *An. gambiae* (Smith and Webley 1969), equating to $p(s') = 0.22$ or $p(s) = 0.78$.

When formula 8 is used with *An. darlingi* (Ituxi River-Brazil) data in TABLE 6, we see that DDT residues reduced joint probabilities of house entering, biting indoors, exiting, and subsequently surviving (using 95% mortality) by 99.81%, that is $(1-0.0019)$*100. This probability is reduced by 97% (versus 99.81%) when a mortality of 22% is substituted into the equation. Using formula 8, the joint probabilities for entering, biting, and surviving were reduced in the sprayed house by 98.1% for *An. darlingi* (Suriname), by 72% for *An. gambiae*, by 88% for *An. funestus*, and by 66% for *An. punctulatus*.

Reduced house entry as a result of repellency, calculated on the basis of $(1-p(m_u))$*100, showed that repellency actions were responsible for 95, 32, 60, 63, and 50% of the overall effect of DDT sprayed walls against *An. darlingi* (Ituxi River), *An. darlingi*

TABLE 3. Indexes (*h* values)* of reduced numbers of unfed and blood-fed** *Anopheles darlingi* females that entered or exited a DDT-sprayed house along the Ituxi River, Amazonas, Brazil. Estimates are presented for two-hour intervals.

Time Intervals	Indexes (*h* values) of Reduced Numbers of Females		
	Entering Unfed	Exiting Unfed	Exiting Fed
6:30-8:30 P.M.	1.00	0.98	0.96
8:30-10:30 P.M.	0.95	0.98	0.89
10:30 p.m.-00:30 A.M.	0.95	0.998	1.00
00:30-2:30 A.M.	0.83	0.99	1.00
2:30-4:30 A.M.	1.00	1.00	-0.06
4:30-6:30 A.M.	0.97	1.00	1.00
6.30-8:30 A.M.	0.74	1.00	1.00

*Index of reduced numbers $= 1-[(a_t x a'_c)/(a'_t x a_c)] = h$, with t representing the sprayed house, c the control house and a representing the average number entering a sprayed house and a' representing the average number that entered before the house was sprayed.

**Specimens classified as recent fed (red blood visible in the abdomen), late fed (black blood in abdomen), or in Sella's stage 2 or 3 (stages of blood digestion or egg development, see Detinova (1962).

TABLE 4. Estimates of probabilities (*p*) and cumulative probabilities (*cump*) of *Anopheles darlingi* females entering and exiting a DDT-sprayed house. These probabilities are estimated from (*a*) the discrete, normal probability (*p*) of a behavioral event occurring at a specified time in an unsprayed house (see TABLE 2 for relevant values), and (*b*) the index of numbers (*1-h*) entering the sprayed house (see TABLE 3 for estimates of *h*). So, *(p * (1-h))* equates to the probability that the behavioral event will occur in the sprayed house during a specific interval of time. The value for *(p * (1-h))* represents a discrete probability, the *cump* is obtained by summing discrete probabilities over the whole night (values in parentheses). Data compiled from studies of unfed and blood-fed *An. darlingi* females that entered or exited sprayed and unsprayed experimental houses along the Ituxi River, Amazonas, Brazil.

Time Intervals	Discrete and Cumulative Probabilities, *p* and (*cump*)		
	Entering Unfed	Exiting Unfed	Exiting Fed
6:30-8:30 P.M.	0.0(0.0)	0.004(0.004)	0.0024(0.0024)
8:30-10:30 P.M.	0.011(0.01)	0.003(0.007)	0.0044(0.0068)
10:30 P.M.-00:30 A.M.	0.01(0.02)	0.00(0.007)	0.00(0.0068)
00:30-2:30 A.M.	0.02(0.04)	0.001(0.008)	0.00(0.0068)
2:30-4:30 A.M.	0.0(0.04)	0.00(0.008)	0.0318(0.0386)
4:30-6:30 A.M.	0.003(0.04)	0.00(0.008)	0.00(0.0386)
6.30-8:30 A.M.	0.0026(0.05)	0.00(0.008)	0.00(0.0386)

BLE 5. Probabilities for vector populations to enter a DDT-sprayed house, $p(m_{ss})$; bite indoors after entering, $p(b_s|m_{ss})$; survive 24 hours after exiting the house with a full blood meal, $p(s_p|b_s,m_{ss})$; and joint probability of entering, biting, escaping, and surviving, $p(s_{ep},m_{ep},b_s,m_{ss})$.

	Probabilities				
dy populations	Enter House	Bite Indoors	Survive 24-hours	Survive after Entering, Biting, and Escaping	Data Source
opheles darlingi uxi River study)					Unpublished data and References (Roberts et al.
% mortality:	0.05	0.56*	0.05*	0.0019	1987, Roberts and Alecrim
% mortality:	0.05	0.56*	0.78**	0.03	1991 and Rozendaal[a])
opheles darlingi uriname)	0.68 [a]	0.56 [a]	0.05 [a]	0.02	Rozendaal[8]
opheles gambiae	0.4 [a]	0.9 [b]	0.78 [a]	0.28	Smith & Webley 1969
opheles funestus	0.37 [a]	0.82 [b]	0.41 [a]	0.12	Smith & Webley 1969
opheles punctulatus	0.5 [a]	0.85 [a]	0.76 [a]	0.32	Sloof[9]

[a]Numbers of females captured exiting house were not sufficient for estimating reduction in biting due to irritant/non contact repellency. Estimate of 0.56 was taken from Rozendaal's study[8].

[b]Value extracted from estimates of mortality from Smith and Webley (1969) and Sloof[9].

[c]Values extracted directly from published study.

[d]Value derived by comparing ratio of numbers fed and unfed in sprayed and unsprayed houses.

Suriname), *An. gambiae*, *An. funestus*, and *An. punctulatus* populations, respectively. Finally, formula was used to show that toxic actions of DDT residues ccounted for <10% of the total impact of DDT residues r *An. darlingi* (Ituxi River), *An. gambiae* (Tanzania), d *An. punctulatus* (Irian Jaya) populations. Toxicity ccounted for 36.2% of the total effect of DDT on *An. arlingi* populations in Suriname and for 18.3% of total ffect on *An. funestus* populations in Tanzania. However, is important to note that repellency and irritancy gether, $[1 - p(b_s|m_{ss}) * p(m_{ss})]*100$, accounted for 1.9% of the overall DDT impact on *An. darlingi* opulations in Suriname.

DISCUSSION

The probability model described in this report olates and quantifies the repellent, irritant/repellent, nd toxic actions of DDT residues on vector behavior. his model challenges the idea that residual spraying serts control over malaria by killing mosquitoes and educing vector population longevity. This model also challenges conventional approaches to collecting and interpreting malaria control data.

The critical importance of endophagic behavior is defined by the fact that mosquitoes have options of multiple hosts outdoors; but are generally limited to a human host once they enter a house. Field data show that repellent actions of DDT may prevent mosquitoes from entering and biting inside of houses. As a consequence, a truly endophagic vector may exhibit predominantly exophagic behavior when a house is sprayed with DDT.

Studies of vector ecology and malaria epidemiology are commonly conducted in areas covered by routine house spray programs. Under such conditions, vector studies based on indoor or paired indoor/outdoor collections in DDT-sprayed houses may show that the vectors are exophagic. Epidemiological studies of malaria in populations covered by house spray programs may reveal age-sex distributions of cases indicative of outdoor transmission. Such results might be interpreted as indicating that residual spraying is not useful because vectors bite outside and malaria cases are acquired out

of doors. This is a reasonable interpretation if DDT only functions by killing vectors and reducing vector population longevity. However, in both types of studies, the indicators of exophagic behavior may result from repellent and/or repellent/irritant actions of DDT residues. In other words, out of doors biting and disease transmission can be chemically induced. As a consequence, the results would belie the true endophagic nature of the vector and the true potential for indoor transmission. It is critically important to reexamine claims of exophagic behaviors because if exophagy and out of doors transmission are chemically induced, then elimination of residual spraying will allow a return of natural endophagic behavior, malaria transmission will move indoors and become more efficient, and malaria rates will increase.

In the Ituxi River study, repellent action reduced the probabilities that *An. darlingi* females would enter the house by 95%. In other words, the repellent effect was the dominant action of DDT residues. The females that still entered the sprayed house were often stimulated to leave without feeding. Rozendaal[8] showed that for females that entered the house, biting was 44 % less than for a comparable population in the control house. We propose that reduced biting and rapid exit were due primarily to irritant, and secondarily, to repellent actions of DDT.

Roberts and Alecrim (1991) determined that biting in the sprayed house along the Ituxi River was reduced by 96.4% (this effect includes reduced house entry by *An. darlingi* females). Similar levels of reduced endophagic behavior were documented for the sprayed house two months later, and there was significant suppression of endophagic behaviors one year after the house had been sprayed. The toxic (killing) properties of DDT residues produced an additional, albeit small, overall impact.

The Ituxi River study is just one of many studies against just one of many vectors that show dominant excito-repellent effects of DDT residues. For *An. darlingi*, excito-repellency tests by Charlwood and Paraluppi (1978), Roberts et al. (1984), and Rozendaal[8], and experimental hut studies by Roberts and Alecrim (1991) and Rozendaal[8] have all shown powerful excito-repellent responses to DDT residues. Even earlier investigations suggested a similar phenomenon, e.g., de Bustamente et al. (1952) and Elliott (1972).

A high frequency of strong behavioral responses of malaria vectors to DDT residues was suggested by statistics extracted from field studies on populations of *An. gambiae* and *An. funestus* in Tanzania (Smith and Webley 1969) and *An. punctulatus* in Irian Jaya (West New Guinea)[9]. In each case, when formulas 3, 5, 8, and 9 were used with probability data, the joint action of repellency and irritancy were the dominant functions of DDT residues. Rozendaal's study in Suriname[8] showed that the probability of *An. darlingi* females entering, biting indoors and surviving was 0.02 in the sprayed house. However, the joint action of repellent and irritant actions accounted for 62% of the overall DDT impact.

We propose that reported changes in mosquito host-seeking activities after houses are sprayed with DDT are primarily a result of the vector's natural behavioral responses to DDT. Repellent actions that reduce numbers of females entering houses explain the findings of larger numbers biting outdoors relative to numbers biting inside sprayed houses. This is a change in relative proportions only, and may not result in an increase in absolute numbers biting outside.

Contact irritancy, and secondarily, non-contact repellency, can account for a change in time of peak indoor biting activity. As shown for *An. darlingi* populations, some malaria vectors enter houses in the early evening. However vectors will not remain indoors in the presence of contact irritant and non-contact repellent actions of DDT. Therefore, early evening biting may still occur; but biting later at night will be eliminated. In the Ituxi River study, one year after the experimental house was sprayed with DDT, biting occurred in the sprayed house only in the early evening, while biting in the control house continued throughout the night (Roberts and Alecrim 1991). Similar behavioral responses have been documented for *An. albimanus* and *An. vestitipennis* in Belize[5] (Grieco et al. 2000).

The primary goal for spraying houses is to reduce the indoor transmission of malaria. Presumably, outdoor transmission is less affected by this control measure. Given these relationships, a better quantification of indoor versus outdoor behavior patterns is needed. In particular, it is important to systematically sample populations as they enter and exit houses. Beyond this, the probability model could be expanded to include probabilities for movement of vector populations from sylvatic to peridomestic environments, and ensuing probabilities for biting humans, versus other vertebrates, outdoors. Through this process, we could begin to better understand the dynamics of malaria transmission on a more quantitative basis and more accurately predict the outcome of a house spray program.

The probability model described in this report may not be descriptive of behavioral responses of all vector species in all environmental settings. However, the model and underlying concepts can be used to evaluate the frequent reports of changes in peak biting times and the relationships of indoor to outdoor biting in the presence of insecticide residues. Indeed, it provides a