Universitat de les Illes Balears

DOCTORAL THESIS
2022

THE ROLE OF INSECTICIDES AND REPELLENTS IN PROTECTING HUMANS AND AS TOOLS IN THE FIGHT AGAINST VECTOR-BORNE DISEASE TRANSMISSION

Mara Moreno Gómez

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Doctoral Programme in Biomedical and Evolutionary Biotechnology

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THESIS PRESENTED AS A COMPENDIUM OF PUBLICATIONS

Thesis structure

This PhD thesis comprises an abstract, an introduction, four original publications, a discussion, and a conclusion. In each of the studies described herein, customised/non-standard methodologies were employed. As a result, it was necessary to detail their specific parameters, processes, and conditions to understand the research being performed. The materials and methods are fully described within each of the original publications.

List of publications

This thesis—entitled THE ROLE OF INSECTICIDES AND REPELLENTS IN PROTECTING HUMANS AND AS TOOLS IN THE FIGHT AGAINST VECTOR-BORNE DISEASE TRANSMISSION—is a compendium of four published papers. The references for these publications are as follows:

1.

AUTHORS: *Mara Moreno-Gómez[1], Rubén Bueno-Marí[2,3], Andrea Drago[4], and Miguel A. Miranda[5]*

TITLE: From the Field to the Laboratory: Quantifying Outdoor Mosquito Landing Rate to Better Evaluate Topical Repellents

AFFILIATIONS: [1]Henkel Ibérica S.A, Research and Development (R&D) Insect Control Department, Barcelona, Spain. [2]Laboratorios Lokímica, Departamento de Investigación y Desarrollo (I+D), Valencia, Spain. [3]Àrea de Parasitologia, Departament de Farmàcia i Tecnologia Farmacèutica i Parasitologia, Universitat de València, Burjassot, València, Spain. [4] Entoestudio srl, Ponte San Nicolo, Italy. [5]Applied Zoology and Animal Conservation Research Group, UIB, Palma de Mallorca, Spain

REFERENCE: https://doi.org/10.1093/jme/tjaa298 Volume 58, Issue 3, May 2021, Pages 1287–1297

JOURNAL: Journal of Medical Entomology

IMPACT FACTOR: 2.278 (2020)

JOURNAL RANK: JCR - Q1 (Insects Science)

PUBLISHER: Oxford Academy

Reviewed by two independent experts

2.

AUTHORS: *Mara Moreno-Gómez[1], Rubén Bueno-Marí[2,3], B Thomas Carr[4], Gary R. Bowman[5], Genevieve W. Faherty[6], Carlota Gobbi[7], Julie M. Palm[8], Petra Van Sloun[9], and Miguel Ángel Miranda[10]*

TITLE: Two New Alternatives to the Conventional Arm-in-Cage Test for Assessing Topical Repellents

AFFILIATIONS: [1]Henkel Ibérica S.A, Research and Development (R&D) Insect Control Department, Barcelona, Spain. [2]Laboratorios Lokímica, Departamento de Investigación y Desarrollo (I+D), Valencia, Spain. [3]Àrea de Parasitologia, Departament de Farmàcia i Tecnologia Farmacèutica i Parasitologia, Universitat de València, Burjassot, València, Spain. [4]Carr Consulting, Wilmette, Illinois, USA and Charles Sturt University, Wagga Wagga, NSW, Australia. [5]Reckitt Benckiser, Sydney NSW 2000, Australia. [6]Citrefine International Ltd. Moorfield Rd Yeadon, Leeds, UK. [7]Endura SpA, Products & Technology Development department, Bologna, Italy. [8]SC Johnson, Howe Street Racine, Wisconsin, USA. [9]Merck KGaA, Darmstadt, Germany. [10]Applied Zoology and Animal Conservation Research Group, UIB, Palma de Mallorca, Spain

REFERENCE: https://doi.org/10.1093/jme/tjab050 Volume 58, Issue 4, July 2021, Pages 1826–1838

JOURNAL: Journal of Medical Entomology

IMPACT FACTOR: 2.278 (2020)

JOURNAL RANK: JCR - Q1 (*Insects Science*)

PUBLISHER: Oxford Academy

Reviewed by two independent experts

3.

AUTHORS: *Mara Moreno-Gómez[1], Rubén Bueno-Marí[2,3] and Miguel Ángel Miranda[4]*

TITLE: A Three-Pronged Approach to Studying Sublethal Insecticide Doses: Characterising Mosquito Fitness, Mosquito Biting Behaviour, and Human/Environmental Health Risks

AFFILIATIONS: [1]Henkel Ibérica S.A, Research and Development (R&D) Insect Control Department, Barcelona, Spain. [2]Laboratorios Lokímica, Departamento de Investigación y Desarrollo (I+D), Valencia, Spain. [3]Àrea de Parasitologia, Departament de Farmàcia i Tecnologia Farmacèutica i Parasitologia, Universitat de València, Burjassot, València, Spain. [4]Applied Zoology and Animal Conservation Research Group, UIB, Palma de Mallorca, Spain

REFERENCE: https://doi.org/10.3390/insects12060546 2021, 12, 546

JOURNAL: Insects

SPECIAL ISSUE: Insecticides for Mosquito Control: Strengthening the Evidence Base

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JOURNAL RANK: JCR - Q1 (Entomology)

PUBLISHER: MDPI

Reviewed by two independent experts

4.

AUTHORS: *Mara Moreno-Gómez[1], Miguel Ángel Miranda[2], and Rubén Bueno-Marí [3,4]*

TITLE: To Kill or to Repel Mosquitoes? Exploring Two Strategies for Protecting Humans and Reducing Vector-Borne Disease Risks by Using Pyrethroids as Spatial Repellents

AFFILIATIONS: [1]Henkel Ibérica S.A, Research and Development (R&D) Insect Control Department, Barcelona, Spain. [2]Applied Zoology and Animal Conservation Research Group, UIB, Palma de Mallorca, Spain. [3]Laboratorios Lokímica, Departamento de Investigación y Desarrollo (I+D), Valencia, Spain. [4]Àrea de Parasitologia, Departament de Farmàcia i Tecnologia Farmacèutica i Parasitologia, Universitat de València, Burjassot, València, Spain.

REFERENCE: https://doi.org/10.3390/pathogens10091171 2021, 10(9), 1171

JOURNAL: Pathogens

SPECIAL ISSUE: Biology, Control and Zoonotic Role of Disease Vectors

IMPACT FACTOR: 3.492

JOURNAL RANK: JCR - Q2 (Microbiology)

PUBLISHER: MDPI

Reviewed by two independent experts

Annex 1 contains a standard form documenting that the article co-authors have agreed to the thesis being presented as a compendium of publications (page 133). By signing the form, the co-authors have indicated their approval of Ms. Mara Moreno's status as principal author and of the publications' inclusion in her doctoral thesis. Consequently, said articles cannot form part of any other doctoral thesis.

Supervisors' declaration

Universitat
de les Illes Balears

Drs. Miguel Ángel Miranda Chueca of University of Balearic Islands and Rubén Bueno Marí of Laboratorios Lokímica and University of Valencia

DECLARE:

That the thesis titles: The role of insecticides and repellents in protecting humans and as tools in the fight against vector- borne disease transmission presented by Mara Moreno Gómez to obtain a doctoral degree, has been completed under my supervision

For all intents and purposes, I hereby sign this document.

Signatures,

Dr. Miguel Ángel Miranda Chueca — Rubén Bueno Marí

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LIST OF ABBREVIATIONS AND ACRONYMS

AIC	arm-in-cage
AS	active substance
BCE	before common era
BP	biocidal product
BPR	Biocidal Products Regulation
BT	*Bacillus thuringiensis*
CAS	Chemical Abstract Services
CE	common era
CPT	complete protection time
DDT	dicloro-dipheny-trichloroethane
DEET	N,N-diethyl-meta-toluamide
ECHA	European Chemical Agency
EFF WG	Efficacy Working Group
EPA	Environmental Protection Agency
EU	European Union
FAO	Food and Agriculture Organization
HHRA	Human Health Risk Assessment
HLC	human landing catch
IRS	indoor residual spraying
ITN	insecticide-treated bednet
IVM	integrated vector management
KD	knockdown
LRC	landing rate count
PP	personal protection
PPP	personal protection product
PT	product type
PT18	product type 18
PT19	product type 19
RT	room test
SR	spatial repellent
TP	topical repellent
TFT	Transfluthrin
USA	United States of America
ULV	ultra-low volume
VBD	vector-borne disease
VBP	vector-borne pathogen
WHO	World Health Organization

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RESUM

ABSTRACT

Over the last century, vector-borne pathogens have spread to new areas, creating novel public health challenges. As major arthropod disease vectors, mosquitoes represent a significant, growing threat to human health due to their role in disease transmission.

Personal protection products such as insecticides and repellents are useful tools available to the general public that can decrease disease incidence and mosquito-related nuisances by reducing contact frequency between mosquitoes and their hosts. However, European Union regulations have become increasingly strict because biocide use generates toxicological risks for human and environmental health.

The doctoral research presented herein sought to 1) validate new laboratory and field methodologies and 2) explore alternative parameters and/or approaches for evaluating insecticides and repellents. To this end, four studies were conducted using *Aedes albopictus* (Skuse, 1894), *Culex pipiens* (Linnaeus, 1758), and human volunteers.

The first study estimated the landing rate outdoors in an area infested with the Asian tiger mosquito (*Ae. albopictus*) and replicated this rate in the laboratory. More specifically, 16 study participants were exposed to mosquitoes in a highly infested region of Italy. These field results were compared to laboratory results obtained in 1) a 30-m^3 room where 9 volunteers were exposed to different numbers of mosquitoes (15–50) and 2) a 0.064-m^3 AIC test cage containing 200 mosquitoes. The highest mosquito landing rate in the field was 26.8 landings/min. In the room test, a similar landing rate was achieved using 15–20 mosquitoes and an exposure time of 3 min. In the AIC test, the landing rate was 229 ± 48 landings/min. This study is the first to concomitantly measure and compare *Ae. albopictus* landing rates in the field and laboratory.

The second study assessed alternative laboratory methods for evaluating topical mosquito repellents and took place at three European testing laboratories. Each test utilised female *Ae. albopictus* and 30 study participants. First, a conventional AIC test and a sleeved AIC test were performed using exposed arm areas of 600 and 100 cm^2, respectively, and cage volumes of 0.040 and 0.064 m^3, respectively. Mosquito density was consistently 1 female/840 cm^3. Second, room-based testing (40 ± 5 mosquitoes in 25–30 m^3) was used as a proxy for field testing. A mosquito repellent (15% N,N-diethyl-m-toluamide) was employed at two doses: 1 and 0.5 g/600 cm^2. The two new methods showed themselves to be good alternatives to conventional AIC testing and yielded reproducible protection times across laboratories.

The third study used the room test to evaluate the effects of sublethal insecticide doses used in spatial treatments on mosquito fitness; protection against mosquito bites in humans; and toxicological risks to human and environmental health. This work employed a synthetic type I pyrethroid, prallethrin, at three sublethal doses (0.40 ± 0.01 mg/h, 0.80 ± 0.01 mg/h, and 1.60 ± 0.01 mg/h) and utilised colonies of *Ae. albopictus*

and *Cx. pipiens*. The results showed that sublethal doses reduced mosquito survival, influencing population size in the next generation. The doses also provided 100% protection to human hosts and presented limited risks to human and environmental health.

The fourth study explored how well sublethal doses of spatial repellents could protect people from bites outdoors. Two field experiments were performed in Italy in an area with high *Ae. albopictus* abundance using a synthetic type I pyrethroid, transfluthrin, in aerosol form. The first examined levels of protection for humans, and the second examined mosquito knockdown and mortality. Percent protection for humans remained high (>80%) at 5 h despite the absence of mosquito mortality at 1 h. This study showed that sublethal doses could be useful: they protected human hosts even when mosquito mortality was null.

RESUMEN

Los mosquitos representan una amenaza importante y creciente para la salud humana debido a su papel en la transmisión de enfermedades.

Productos de protección personal, como insecticidas y repelentes, pueden disminuir la incidencia de enfermedades y las molestias relacionadas con los mosquitos al reducir la frecuencia de contacto entre ellos y sus huéspedes. Sin embargo, la regulación de biocidas se ha vuelto cada vez más estricta debido a que su uso puede generar riesgos toxicológicos para la salud humana y medioambiental.

La presente tesis doctoral buscó 1) validar nuevas metodologías de laboratorio y de campo y 2) explorar parámetros y/o enfoques alternativos para evaluar insecticidas y repelentes. Para ello se llevaron a cabo cuatro estudios utilizando *Aedes albopictus* (Skuse, 1894), *Culex pipiens* (Linnaeus, 1758) y voluntarios humanos.

El primer estudio involucró a 16 participantes que evaluaron la tasa de aterrizaje de mosquitos en un área naturalmente infestada por *Ae. albopictus*. Los resultados obtenidos se compararon con estudios de laboratorio llevados a cabo en 1) una cabina de 30 m^3 donde 9 voluntarios fueron expuestos a diferente número de mosquitos (15–50) y 2) una jaula de 0,064 m^3 que contenía 200 mosquitos, test conocido como arm-in-cage (AIC). La tasa de aterrizaje de mosquitos más alta en el campo fue de 26,8 aterrizajes/min. El ensayo en la cabina logró una tasa similar utilizando 15 - 20 mosquitos y un tiempo de exposición de 3 min. En el AIC, la tasa fue de 229 ± 48 aterrizajes/min. Este estudio es el primero en medir y comparar tasas de aterrizaje en campo y laboratorio con *Ae. albopictus*.

El segundo estudio exploró métodos de laboratorio alternativos para evaluar repelentes de mosquitos de uso tópico. Se realizó en cuatro laboratorios europeos; se utilizaron hembras de *Ae albopictus* y 30 participantes por configuración. 1) Se comparó resultados de protección con el AIC convencional (áreas de brazo expuestas de 600 cm^2) versus el AIC con manguito (100 cm^2) y volúmenes de jaula de entre 0,040 y 0,064 m^3. La densidad de mosquitos fue de 1 hembra/840 cm^3. 2) se utilizaron cabinas de entre 25–30 m^3 con 40 ± 5 mosquitos. Se empleó 15% N,N-dietil-m-toluamida en dos dosis: 1 y 0,5 g/600 cm^2. Los dos nuevos métodos demostraron ser buenas alternativas al AIC convencional produciendo tiempos de protección reproducibles en todos los laboratorios.

El tercer estudio evaluó en laboratorio los efectos de dosis subletales de insecticidas utilizadas en tratamientos espaciales sobre 1) el estado físico de los mosquitos; 2) protección contra las picaduras de mosquitos en humanos; y 3) riesgos toxicológicos para la salud humana y ambiental. Este trabajo empleó un piretroide sintético tipo I, praletrina, en tres dosis subletales (0,40 ± 0,01 mg/h, 0,80 ± 0,01 mg/h y 1,60 ± 0,01

mg/h) y utilizó colonias de *Ae. albopictus* y *Cx. pipiens*. Las dosis subletales redujeron la supervivencia de los mosquitos, redujeron el tamaño de la población y brindaron una protección del 100% a los huéspedes humanos. Además, presentaron riesgos relativamente bajos para la salud humana y ambiental.

El cuarto estudio exploró cómo las dosis subletales de repelentes espaciales podrían proteger a las personas de las picaduras de mosquitos al aire libre. Se realizaron dos experimentos de campo en Italia en un área de alta abundancia de *Ae. albopictus* usando un piretroide tipo I sintético, transflutrina, en forma de aerosol. Primero examinó los niveles de protección para los humanos y el segundo evaluó la mortalidad de los mosquitos. El porcentaje de protección para humanos se mantuvo alto (>80%) a las 5 h a pesar de la ausencia de mortalidad de mosquitos después de 1 h de aplicación.

RESUM

Els mosquits representen una amenaça important i creixent per a la salut humana degut al seu paper en la transmissió de malalties.

Productes de protecció personal, com insecticides i repel·lents, poden disminuir la incidència de malalties i les molèsties relacionades amb els mosquits al reduir la freqüència de contacte entre ells i els seus hostes. Tot i això, la regulació de biocides s'ha tornat cada vegada més estricta pel fet de que el seu ús pot generar riscos toxicològics per a la salut humana i mediambiental.

Aquesta tesi busca 1) validar noves metodologies de laboratori i de camp i 2) explorar paràmetres i/o enfocaments alternatius per avaluar insecticides i repel·lents. Es van dur a terme quatre estudis utilitzant *Aedes albopictus* (Skuse, 1894), *Culex pipiens* (Linnaeus, 1758) i voluntaris humans.

El primer estudi va involucrar 16 participants per avaluar la taxa d'aterratge de mosquits en una àrea infestada per *Ae. albopictus*. Els resultats obtinguts es van comparar amb estudis de laboratori en 1) una cabina de 30 m3 on 9 voluntaris van ser exposats a diferent número de mosquits (15–50) i 2) una gàbia de 0,064 m3 que contenia 200 mosquits, test conegut com arm in cage (AIC). La taxa d'aterratge de mosquits més alta al camp va ser de 26,8 aterratges/min. L'assaig a la cabina va aconseguir una taxa similar utilitzant 15-20 mosquits i un temps d'exposició de 3 min. A l'AIC, la taxa va ser de 229 ± 48 aterratges/min. Aquest estudi és el primer a mesurar i comparar taxes d'aterratge a camp i laboratori amb *Ae. albopictus*.

El segon estudi va explorar mètodes de laboratori alternatius per avaluar repel·lents de mosquits dús tòpic. Quatre laboratoris europeus van repetir les probes amb d'*Ae albopictus* i 30 participants van particicpar per configuració. Es van comparar resultats de protecció amb l'AIC convencional (àrees de braç exposades de 600 cm2) versus 1) l'AIC amb mànega (100 cm2) i volums de gàbia d'entre 0,040 i 0,064 m3. La densitat de mosquits va ser de 1 femella/840 cm3. 2) cabines d'entre 25–30 m3 amb 40 ± 5 mosquits. Es va emprar 15% N,N-dietil-m-toluamida en dues dosis: 1 i 0,5 g/600 cm2. Els dos nous mètodes van demostrar ser bones alternatives a l'AIC convencional produint temps de protecció reproduïbles a tots els laboratoris.

El tercer estudi va avaluar en laboratori els efectes de dosis subletals d'insecticides utilitzades en tractaments espacials sobre l'estat físic dels mosquits; 2) protecció contra les picades de mosquits en humans; i 3) riscos toxicològics per a la salut humana i ambiental. Aquest treball va emprar un piretroide sintètic tipus I, praletrina, en tres dosis subletals (0,40 ± 0,01 mg/h, 0,80 ± 0,01 mg/hi 1,60 ± 0,01 mg/h) i va utilitzar colònies d'*Ae. albopictus* i *Cx. pipiens*. Les dosis subletals van reduir la supervivència dels mosquits, van reduir la mida de la població i van brindar una protecció del 100% als hostes humans. A més, van presentar riscos relativament baixos per a la salut humana i ambiental.

El quart estudi va explorar com les dosis subletals de repel·lents espacials podrien protegir les persones de les picades de mosquits a l'aire lliure. Es van fer dos experiments de camp a Itàlia en una àrea d'alta abundància d' *Ae. albopictus* usant un piretroide sintètic, tipus I, transflutrina, en forma d'aerosol. Primer es va examinar els nivells de protecció per als humans i segon es va avaluar la mortalitat dels mosquits. El percentatge de protecció per a humans es va mantenir alt (>80%) a les 5 h malgrat l'absència de mortalitat de mosquits després d'una hora de l' aplicació.

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INTRODUCTION

INTRODUCTION

Insects are the most diverse group of terrestrial eukaryotes in the world: they represent around 75% of the approximately one million terrestrial animal species described to date (Vélez, 2006). Consequently, they play key roles in most major biomes, particularly those in the tropics, where levels of insect species richness and specialisation are high; insects are, however, largely absent from marine and polar ecosystems (Danks, 2004). Their success seems related to their elevated reproductive rates, excellent dispersal abilities, highly specific niches, and capacity to adapt to new ecological challenges (Loxdale, Lushai, & Harvey, 2011).

Many insects pollinate plants or carry out biological control, providing major benefits to humans. However, certain species are pests: because of their abundance and/or diversity, they threaten crops and stored food products; destroy clothing, furniture, paper products (e.g., books), and buildings; and vector diseases, including some that are deadly to humans, livestock, crops, and wildlife (Resh, 2009).

Over the course of history, humans have developed different strategies for dealing with pest species. Among them, insecticide use has yielded great benefits, notably in agriculture, where it has promoted dramatic increases in crop production. Insecticides have also played a crucial role in limiting the spread of vector-borne diseases (VBDs) and have been widely used to control insect pests in domains such as forestry, horticulture, and human health (Gupta, 2017). However, limits on the use of ASs are a major topic of debate, especially in developed countries. Insecticides are now more strictly regulated because of past excessive, indiscriminate usage and because of new toxicological assessment methods. These stricter regulations have helped constrain the risks of insecticides for human and environmental health. As a result, certain ASs have been or will be phased out in the future, and the process of identifying, registering, and commercialising safe, effective ASs may take many years (ECHA, n.d.-a) (EPA, n.d.-c).

The terms “pesticides” and “biocides” are often confused and employed interchangeably. Both appear in this thesis, so their differences will be clarified here. Pesticides can be plant protection products or biocides. Plant protection products are used in the context of food production, whereas biocides are mainly used outside the context of food production. The word biocide is derived from the Latin roots “bio”, meaning “life”, and “caedo”, meaning “to kill”. European Union (EU) legislation defines a biocide as “a chemical substance or microorganism intended to destroy, deter, render harmless, or exert a controlling effect on any harmful organism” (ECHA, n.d.-b). The United States (US) Environmental Protection Agency (EPA) more commonly uses the word “pesticide”, derived from the Latin roots “pestis”, meaning “plague”, and “caedo”, meaning “to kill”. Its definition of biocide is slightly different: “a diverse group of poisonous substances including preservatives, insecticides, disinfectants, and pesticides used for the control of organisms that are harmful to human or animal health or that cause damage to natural or manufactured products” (EPA, n.d.-a).

1. From the Sumerians to the present day

The historical development of pesticides can be divided into five main phases: early pest management (prior to the year 1000 CE); the use of plant, animal, and/or mineral derivatives (1000–1850 CE); the use of inorganic products and industrial byproducts (1850–1940 CE); the use of synthetic organic compounds (1940–1970 CE); and the use of low-risk synthetic organic compounds (1970 CE–present) (J. Unsworth, Y. Nakagawa, 2019).

About 10,000 years ago, agriculture emerged in Mesopotamia, where the existence of rivers and the construction of canals made it possible to obtain abundant harvests of cereals, vegetables, and legumes (Kislev, Weiss, & Hartmann, 2004). However, the first recorded instance of pest management dates back to the Sumerians, who began to use sulphur powder to protect their crops from insects and mites around 4,500 BCE (Unsworth, 2010).

Around 1,200 BCE, the Chinese further developed pest control practices by using chemical substances. They also employed biological pest control: predatory ants were used to protect citrus groves from caterpillars and wood-boring beetles. Ropes or bamboo sticks were tied between branches to facilitate ant movement (Meyer, 2003). The Chinese continued to develop sophisticated chemical and biological pest control methods to protect crops and humans. The next documented advance happened around 750 BCE—a passage in Homer's Iliad describes wood ash being spread across the ground to control pests. In the 6th century BCE, the Greek philosopher and mathematician Pythagoras was credited with eliminating malaria in a Sicilian city by instructing its residents to drain local marshes, thus eliminating bodies of stagnant water and reducing the size of mosquito breeding grounds (Stent, 2006).

Examining the history of pest control, it would appear that 1) pest management advanced alongside improvements in agricultural practices and 2) societies with advanced writing systems were better able to disseminate techniques for controlling insects. From this perspective, it is unsurprising how little progress was seen in pest control during the

Middle Ages (5th to 15th century CE). Written languages existed in Europe, but only the wealthy were able to read or write. Without access to sources of knowledge, the general public frequently turned to divine or demonic explanations for the presence and control of insects (*Futur. Role Pestic. US Agric.*, 2000).

During the Renaissance (15th to 16th century), people began to view insects less as a punishment from God and more as members of the natural world. There was renewed interest in studying the natural history and behaviour of insects, which led to more innovative control practices. Manual labour was widely used in these early pest control efforts, but cultural, physical, and chemical practices were also present. For example, pests were killed by applying chemicals such as arsenic, mercury, and lead to crops (Unsworth, 2010).

In the 17th century, people started extracting nicotine sulphate from tobacco leaves and using it as an insecticide. In the 19th century, boosted by Europe's agricultural revolution (1750–1880), entomologists and chemists improved pest control techniques and introduced two organic compounds of plant origin: pyrethrum, extracted from the flowers of *Chrysanthemum cinerariaefolium*, and rotenone, extracted from three genera of tropical legumes: *Derris*, *Lonchocarpus*, and *Tephrosia* (Miller, 2002)

However, the real revolution in chemical pest control took place in 1939, when the Swiss chemist Paul Hermann Müller (1899- 1965) discovered the insecticidal properties of dichloro-diphenyl-trichloroethane (DDT). This synthetic organic insecticide was successfully used not only to control plant pests, but also to combat the lice infestations experienced by US troops in Europe. DDT also saved thousands of lives during World War II because it limited the populations of insects responsible for transmitting malaria and typhus to humans (Jarman & Ballschmiter, 2012). Müller received the Nobel Prize for his work in 1948.

The 1940s and 1950s marked the beginning of the pesticide era (Murphy, 2005). In the 1940s, manufacturers started to produce large quantities of synthetic pesticides, whose use quickly became widespread and indiscriminate (Daly H, Doyen JT, 1998). At that time, the environmental persistence of these compounds was seen as a boon: it resulted in lower costs because of the reduced application frequency. Organochlorines have two key features: they are insoluble in water but soluble in apolar liquids, such as ether and chloroform. Consequently, they are also soluble in oils and fats, which means DDT can accumulate in the fatty tissues of living organisms. Organochlorines are also highly stable (i.e., it takes years for them to degrade in nature) because their chemical bonds display low reactivity under normal conditions (Turusov, Rakitsky, & Tomatis, 2002).

At around the same time, the first organophosphates were developed for use as chemical weapons during World War II. More specifically, the military derived compounds from phosphoric acid; these could contain carbon, hydrogen, oxygen, sulphur, nitrogen, and/or phosphorus in their structures. The acute toxicity of organophosphorus insecticides is greater than that of organochlorine pesticides, although the former breaks down more rapidly. Consequently, they must be applied at greater frequencies to operate with the same degree of efficacy as organochlorine pesticides (Hurtado Clavijo & Gutiérrez de Salazar, 2005). The herbicide glyphosate and the insecticides disulphoton, malathion, and parathion are examples of well-known organophosphorus compounds (Espa & Mda, 2021).

Synthesis of carbamates began in 1957, the year in which the insecticidal properties of carbaryl became known (Haynes, Lambrech & Moorefiel, 1957). Carbamate insecticides are structurally and mechanistically similar to organophosphorus insecticides; however, carbamates differ in that they reversibly bind to acetylcholinesterase, allowing their rapid elimination from animal tissues (Silberman & Taylor, 2018).

In the 1960s, DDT was shown to interfere with reproduction in many piscivorous birds, posing a serious threat to biodiversity. In 1962, writer and marine biologist Rachel Carson published *Silent Spring*, an investigation into pesticide use practices. She denounced the (1907-1964) indiscriminate, widespread deployment of synthetic pesticides, which were accumulating in the food chain and presenting great risks to human, plant, and animal health. Her work launched the modern environmental movement and encouraged governmental regulation of agrochemicals, a subject that continues to inspire contentious debate (Rachel Carson, n.d.). The use of DDT is currently banned in more than 86 countries, but it is still employed in some developing nations to kill insect vectors of malaria and other tropical diseases (Lobe, 2006).

The environmental persistence of these second-generation insecticides meant reduced application costs. However, it also increased the likelihood that resistance would evolve in pest populations. In addition, both their persistence and broad-spectrum toxicity led to undesirable effects in non-target populations. Predators and parasites were especially vulnerable due to biomagnification—increases in compound concentration through the food chain. The resulting unintended devastation led to the emergence of secondary pests, species whose abundances had previously been limited by natural enemies. Biomagnification also threatened public health because people were experiencing repeated long-term exposure to residual pesticides via environmental and dietary contaminants (National Research Counci*l*., 2000 ; Banaszkiewicz, 2010).

In 1967, the first synthetic analogues of plant-derived pyrethrins, called pyrethroids (e.g., resmethrin), were produced in Great Britain (Unsworth, 2010). Since then, pyrethroids have achieved great commercial success thanks to their broad spectrum of activity against arthropods, the low doses they require, and the relatively low risk they present to field workers and the environment [ATSDR (Agency for Toxic Substances and Disease Registry), 2003].

Pesticide research continued. In the 1970s and 1980s, glyphosate came on the scene and swiftly became the world's best-selling herbicide. A third generation of pyrethroids was also produced, which included fenvalerate and permethrin. Other treatments emerged: the avermectins and the benzoylureas, and *Bacillus thuringiensis* (BT), currently the most commonly used biological pesticide worldwide. In the 1990s, research focused on identifying narrower-spectrum insecticides that displayed less environmental toxicity. Introduced in 1996, two examples are pyriproxyfen and buprofezin, whose ASs are insect growth regulators (Stent, 2006).

Since synthetic organic pesticides first appeared, strategies for developing new chemical tools have been evolving, a process that has led to safer, more environmentally friendly products. First, pesticides effective at lower doses were created: they can be used in vastly smaller quantities that still successfully control insect pests, fungi, mites, nematodes, and weeds. The result has been a reduced pesticide load in the environment. Second, more biodegradable pesticides were generated, leading to much lower ambient levels of residues. Third, narrow-spectrum insecticides were invented, helping to control pests without displaying toxicity in non-target species, such as humans and beneficial organisms (Umetsu & Shirai, 2020).

Over the history of agriculture, pesticides have become a vital tool for protecting plants and improving crop yields. Each year, approximately 45% of food production is lost to pests (Abhilash & Singh, 2009). Indeed, as the global economy expanded in the latter half of the 19th century, the use of synthetic pesticides also increased in the world's industrial and agricultural sectors, climbing from 2.3 million tonnes per year in 1990 to 4.2 million tonnes per year in 2019 (Sharma et al., 2019) (FAO, n.d.). Pesticides have allowed farmers in developing countries to increase agricultural yields and are sometimes viewed as the best, most reliable means of crop protection, limiting the threat posed by insects such as desert locusts, a common pest in parts of Africa. At times, pesticides provide the only way to ensure harvests (The European Parliament, 2021).

The Pesticide Manual shows that currently available ASs are mainly used in agriculture (68%); industrial and commercial activities (17%); households (8%); and governmental contexts (7%) (BCPC, 2015). In the EU in 2018, the most commonly sold pesticides were fungicides and bactericides (45%); herbicides (32%); and insecticides and acaricides (11%) (Eurostat, n.d.).

With respect to global insecticide usage, the Food and Agriculture Organization (FAO) database indicates that Asia is the continent with the highest consumption, followed by the Americas (Fig. 1) (FAO, n.d.).

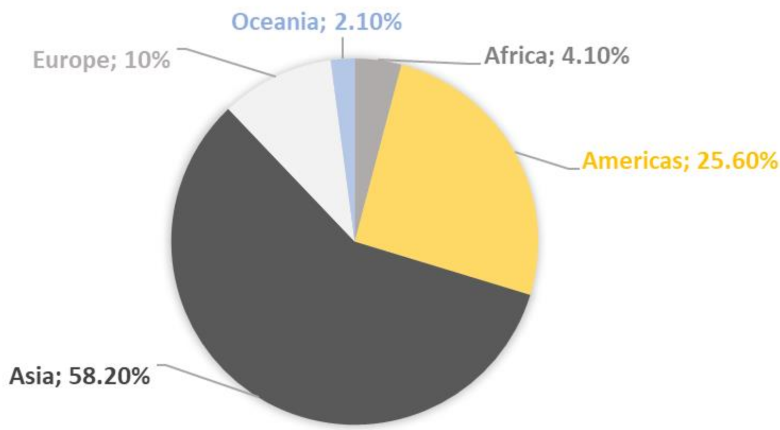

Figure 1. Relative insecticide use in 2019 by continent

(FAO, n.d.)

Across the world, annual insecticide use has climbed from 583,000 tonnes in 1900 to 698,000 tonnes in 2019 (Fig. 2) (FAO, n.d.).

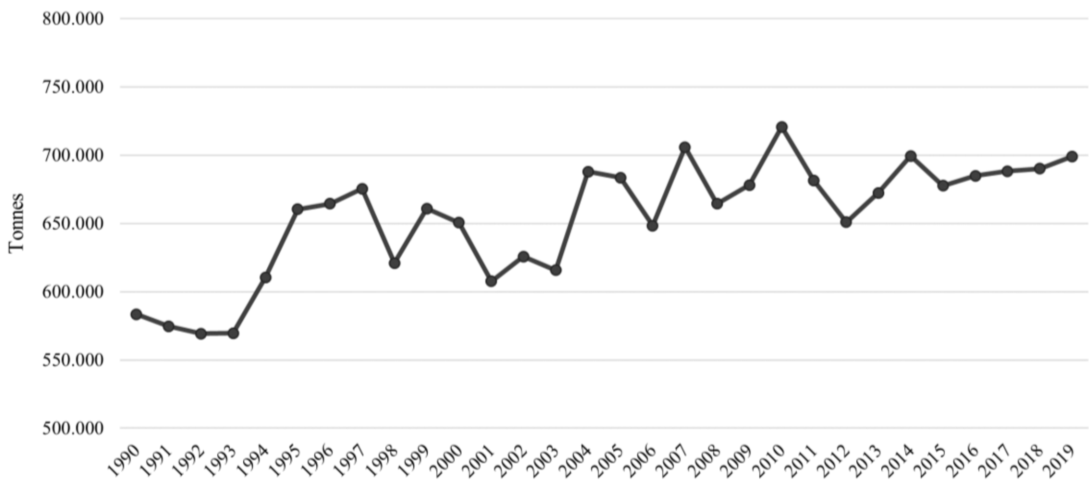

Figure 2. Annual global insecticide use in tonnes from 1900 to 2019

(FAO, n.d.)

Despite these trends, pesticides of all types are strictly regulated in most countries. In particular, the EU employs one of the world's strictest systems for authorising and controlling pesticide use. Directive 2009/128/EC (European Parliament, 2009) and the Biocidal Products Regulation (BPR; 528/2012) (The European parliament and the council of the european union, 2012) were passed with two key aims. The first is to reduce pesticide use, thus limiting its risks for human and environmental health. The second is to promote both integrated pest management systems as well as alternative pest control tools with a view to decreasing pesticide dependence.

2. Regulation of biocidal products in the European Union

In European Chemical Agency (ECHA) guidance on the BPR, a biocidal AS and a biocidal product (BP) are defined as follows (ECHA, n.d.-a):

- **AS**: *a substance or a micro-organism that has an action on or against harmful organisms.*
- **BP**: *any substance or mixture, consisting of, containing or generating one or more active substances, with the intention of destroying, deterring, rendering harmless, preventing the action of, or otherwise exerting a controlling effect on, any harmful organism by any means other than mere physical or mechanical action.*

In the EU, BPs are divided into 22 product types (PTs), which are grouped into 4 main categories (Table 1) (ECHA, n.d.-c).

Table 1. The 4 main categories and 22 product types for classifying biocidal products in the European Union

Main groups	Number	Product types
1. Disinfectants	PT1	Human hygiene
	PT 2	Disinfectants and algaecides not intended for direct application to humans or animals
	PT 3	Veterinary hygiene
	PT 4	Food and feed area
	PT 5	Drinking water
2. Preservatives	PT 6	Preservatives for products during storage
	PT 7	Film preservatives
	PT 8	Wood preservatives
	PT 9	Fibre, leather, rubber and polymerised materials preservatives
	PT 10	Construction material preservatives
	PT 11	Preservatives for liquid-cooling and processing systems
	PT 12	Slimicides
	PT 13	Working or cutting fluid preservatives
3. Pest Control	PT 14	Rodenticides
	PT 15	Avicides
	PT 16	Molluscicides, vermicides and products to control other invertebrates
	PT 17	Piscicides
	PT 18	Insecticides, acaricides and products to control other arthropods
	PT 19	Repellents and attractants
	PT 20	Control of other vertebrates
4. Other Biocidal products	PT 21	Antifouling products
	PT 22	Embalming and taxidermist fluids

In accordance with the BPR, regulatory authorities determine whether BPs and ASs comply with strict quality, human health, and environmental health standards before granting authorisation for commercial use. This process involves evaluating an AS's physicochemical properties, efficacy, and risks to human and environmental health. AS and BP approval must be granted before a product can be placed on the market. The evaluations are carried out by working groups focused on each of the elements mentioned above. Biocides are thus subject to a strict, complex, time-consuming, and expensive registration process. While this procedure helps limit toxicological risks to humans and the environment (European Parliament, 2009) (The European parliament and the Council of the European Union, 2012) (European Comission, 2018), it also dampens biocide innovation because not everyone can afford the high cost of registering new ASs or BPs (European Comission, 2014).

Indeed, over recent decades, the BPR has drastically reduced the quantities of ASs that can be used in BPs (European Comission, 2018). In 2003, prior to the regulation's passage, 953 ASs were in use; applications for continued usage under the BPR were only submitted for 369 of these ASs (The European Parliament and the Council of the

Euroepan Union, 2012). Among them, 104 were insecticides (PT18) and 42 were repellents or attractants (PT19) (Comission of the European Communities, 2003). According to the ECHA data for 2021, only 58 and 20 ASs are available for use in PT18 and PT19 biocides, respectively (ECHA, 2021).

In short, restrictions on AS usage and the complexity and cost of registering new ASs have generated roadblocks for the development and commercialisation of innovative technologies. At the same time, the world is facing a growing need for protective health measures, which means it is crucial to optimally exploit available tools and construct new approaches.

Evaluating the efficacy of insecticides and repellents in the European Union

BPs containing ASs that kill, repel, or attract one or more species of insects or other arthropods are available for indoor and/or outdoor use in different forms (e.g., as sprays, dusts, powders, liquids, gels, and/or baits). Some can only be employed by professionals because they require technical training, while others can be used by the general public. Personal protection products (PPPs) fall in the latter category and include insecticides and repellents. PPPs may play an important role in reducing interactions between humans and insects, thus minimising human exposure to insect bites as well as reducing disease transmission risks (Revay *et al.*, 2013; Lloyd *et al.*, 2013).

As explained earlier, in the EU, BPs are classified into 22 groups. Those of interest in this thesis belong to PT18, the insecticides, and PT19, the repellents. In the EU, an AS must be classified as both PT18 and PT19 to be authorised for both uses. This is currently the case for only three ASs: geraniol (CAS number 106-24-1), *Chrysanthemum cinerariaefolium* extract (CAS number 89997-63-7), and *Margosa* extract (CAS number 84696-25-3) (European Comission, 2018).

Insecticides are one of the main tools used to control vectors and protect public health (Mai, 2003). A variety of insecticide types are employed in public health contexts, such as organochlorines, organophosphates, and neonicotinoids. Among them, pyrethroids are the most commonly used worldwide because they are relatively less toxic to mammals, have a fast knockdown (KD) effect on target arthropods, and break down rapidly in the environment via photodegradation (WHO, 2005). Pyrethroids are neurotoxins—they interfere with the arthropod nervous system by blocking the closure of sodium channels. As a result, nerve impulses are prolonged, which leads to muscle paralysis and eventually death (Wilks, 2005; Espa & Mda, 2021).

Although ASs receive official classifications [i.e., they are assigned to PT18 (insecticides) versus PT19 (repellents)], they may actually have a dose-dependent effect that depends on usage. For example, certain pyrethroids can act as either insecticides or spatial repellents (SRs) (Bibbs & Xue, 2016). Examples of such compounds include metofluthrin (Kawada *et al.*, 2020), d-allethrin (Yap *et al.*1996), and transfluthrin (TFT) (Syafruddin *et al.*, 2020). SRs, also known as area repellents (ARs), are chemicals that are applied in a vaporised form within a given area. They curb contact between insect vectors and their human hosts, eliminating or reducing the risk of disease transmission. Multiple studies have demonstrated the efficacy of utilising certain pyrethroids at low doses and have underscored the potential benefits for public health and mosquito control efforts (Bibbs, Hahn, Kaufman, & Xue, 2018; Bibbs *et al.*, 2019). This approach contrasts with most regulatory frameworks, including those used in the EU for conventional insecticides. Indeed, in the EU, the efficacy of the insecticides employed in SRs is evaluated based on KD levels and mosquito mortality rather than on the degree of repellence or the reduction in biting pressure (ECHA, 2011). EU efficacy requirements stipulate that, in spatial treatments, insecticides must be used at a dose that kills 90% of exposed insects within 24 h (ECHA, 2011), a threshold known as the 90% lethal dose (LD90). Doses below LD90 are treated as ineffective and are therefore not authorised. However, achieving high levels of mortality requires the use of large doses, which is at odds with the constraints imposed by Human Health Risk Assessments (HHRAs), the results of which are also necessary for product authorisation (European Commission, 2002).

Thus, certain ASs could theoretically be employed as SRs if efficacy requirements shifted to focus on vector behaviour. It is essential to ask the following question: given that the greater objective is to promote human health, are high doses truly needed to obtain 100% levels of protection? Reconsidering the current evaluation framework could yield a new paradigm for disease control efforts. To date, there has been little willingness to move away from the status quo: achieving or surpassing established thresholds of insect mortality (Desneux et al., 2007). Given the current situation, it may be necessary to stimulate systematic change by nonetheless taking some initial steps in the above direction. Specifically, identifying new evaluation parameters and/or vector control paradigms for previously authorised ASs could help improve existing approaches and bypass the hurdles generated by the high cost of registering new ASs and/or new AS uses. Special attention should particularly be paid to improving protection levels outdoors, where personal protection options are often limited. A common strategy in these contexts is the use of topical repellents (TR), a product category associated with its own set of regulatory constraints.

When it comes to evaluating TR, the most frequently used methodology is the arm-in-cage (AIC) test (Fig 1.4, page 30), an approach initially described by the World Health Organization (WHO, 2009) and the EPA (EPA, 2010) in 2009 and 2010, respectively. Across the globe, the AIC test is used to evaluate TP under laboratory conditions. However, several studies have shown that this methodology could underestimate protection times under real-life outdoor conditions (Obermayr *et al.*, 2010; Colucci & Müller, 2018). One reason is that both sets of guidelines call for high mosquito densities. WHO guidelines stipulate that 3,125–3,900 mosquitoes should be used per m^3 (or 1 mosquito per 320 cm^3). In EPA guidelines, this figure is 881 mosquitoes per m^3 (or 1 mosquito per 1,160 cm^3). The aforementioned studies have demonstrated that higher mosquito densities are correlated with higher biting pressures and shorter protection times. However, they used different species in the field versus laboratory research, making it difficult to draw clear conclusions regarding the above relationships. Thus, to date, no study has used the same species in both the field and the laboratory with a view to recreating potential outdoor landing pressures under laboratory conditions. Consequently, although it has been recognised that the WHO's and EPA's recommended densities have the potential to underestimate protection times, EU authorities have been unable to establish new guidelines based on available data.

3. The rising incidence of vector-borne diseases and the spread of *Aedes albopictus*

The incidence of VBDs has increased worldwide in recent decades (Weaver & Reisen, 2010; Gould *et al.*, 2017). Among arthropod disease vectors, mosquitoes are increasingly one of the greatest threats to human health due to their role in disease transmission (Gould *et al.*, 2017; Gossner *et al.*, 2018). Worldwide, VBDs are intensifying, spreading across broader geographical areas, re-emerging, and/or expanding transmission seasons because of key factors such as social forces, demographics, and environmental changes, including climate change. Human populations in cities or large towns are at increased risk of mosquito-borne viruses because of global travel and trade, unplanned urbanisation, altered land use (e.g., deforestation), and/or inadequate waste management. VBD incidence is also climbing as a result of intrinsic factors, including vector competence, vectorial capacity, and insecticide resistance (Gould & Higgs, 2009; Wilke *et al.*, 2019; Nkemngo *et al.*, 2020).

The Asian tiger mosquito, *Aedes albopictus* (Skuse, 1894), is an excellent example of a vector that is now found across the world as a result of human activity (Lounibos, 2002; Powell & Tabachnick, 2013). It naturally occurs in Asia but successfully spread across Africa, Europe, Australia, the Americas, and the Middle East during the 20th century (Gratz, 2004; Paupy *et al.*, 2009). Laboratory studies have found that this species can transmit at least 26 diseases caused by arboviruses, including dengue, Zika, and chikungunya (Paupy *et al.*, 2009). No vaccines are currently available for many of the most prevalent VBDs (Gossner *et al.*, 2018), which poses a threat to human health. Indeed, *Ae. albopictus* has caused numerous local disease outbreaks (mainly of dengue and chikungunya) on several continents, including Europe (Grandadam *et al.*, 2011; Lourenço & Recker, 2014; Rudolf *et al.*, 2018) and the Americas (Moore & Mitchell, 1997; Ruiz-Moreno *et al.*, 2012; Kraemer *et al.*, 2015; Hennessey *et al.*, 2016).

Ae. albopictus is an anthropophilic and exophilic species that mainly bites during the day (Paupy *et al.*, 2009; Delatte *et al.*, 2010) Because people are increasingly participating in outdoor activities, they have a greater likelihood of encountering the mosquito across its expanded range (Greenberg & Schneider, 1997; Halasa *et al.*, 2013; Bell *et al.*, 2013) leading to a greater reliance on PPPs to prevent bites. Indeed, the demand for household repellents and insecticides has skyrocketed in recent years, notably during periods of increased mosquito activity (Chouhan & Deshmukh, 2020). Over the same period, vector control and management professionals have witnessed advances in the design and implementation of new methods for ensuring public health. These approaches complement the chemical control techniques currently deployed as part of integrated vector management (IVM) programmes and may take a biological, genetic, or physical form (Carson, 2002; Lobe, 2006). However, worldwide, chemical control (e.g., insecticides and repellents) remains the go-to strategy to protect human populations (Banaszkiewicz, 2010). Indeed, the use of insecticides in IVM programmes has increased in recent years, reducing human mortality from VBDs in many countries and, thus, playing a key role in efforts to improve public health (van den Berg *et al.*, 2012).

However, the spread of *Ae. albopictus* and the increasing incidence of VBDs have effects that extend beyond public health and the need for innovative pest control techniques. From a regulatory standpoint, BP efficacy should be evaluated under conditions as close to those experienced in real life as possible. For example, TR are normally used outdoors, so field testing of BPs would be the most appropriate approach to take. However, at the same time, concerns are growing about VBD risks, especially those related to mosquitoes (Seyler *et al.*, 2009; Rocklöv *et al.*, 2016). Consequently, in December 2018, the ECHA Efficacy Working Group (EFF WG) met with scientific representatives from the PPP industry (including the author of this thesis) and decided that field testing of BP efficacy should no longer be required under EU guidelines (ECHA, 2019). Even if field testing better represents real-use usage, it places study participants at greater risk.

4. The doctoral research as a thematic unit

Based on the above background, there was clearly a convergence of three challenges to be addressed in 2018: 1) a decline in the number of available ASs because of high registration costs concurrent with an increase in regulatory constraints because of toxicological concerns; 2) an increase in insecticide and repellent use in household and professional settings because of climbing VBD risks; and 3) a need for updated EU guidelines for evaluating BP efficacy given elements 1 and 2. There was thus a clear and pressing need to develop and implement new methodologies for assessing insecticides and repellents in the field and in the laboratory. It was essential to come up with better-grounded estimates of insecticide and repellent protection so that they could be incorporated into the new European guidelines for testing PPP efficacy. These challenges also underscored the necessity of exploring new ways to exploit available ASs—namely via techniques that strike a better balance between increasing protection against insect vectors and decreasing toxicological risks for human and environmental health.

As a response, the research described in this thesis was launched and took the form of four studies.

The first study assessed mosquito landing rates in the field, which were then compared to rates obtained in the laboratory using the conventional methodology (i.e., the AIC test) described in WHO (2009) and EPA (2010) guidelines and a novel methodology, the room test (RT). The study succeeded in replicating field landing rates using the RT test. In contrast, the landing rate in the AIC test greatly surpassed the landing rate in the field because of the high mosquito density required by test specifications. This work led to the publication of a first article: *"From the Field to the Laboratory: Quantifying Outdoor Mosquito Landing Rate to Better Evaluate Topical Repellents"* (*page 23).*

The next step was to conduct research whose aim was to adjust the mosquito densities and parameters recommended by WHO (2009) and EPA (2010) guidelines upstream of defining the methodology to be incorporated into the new EU guidelines. The second study thus characterised the protection afforded to human hosts using three tests: a conventional AIC test, an alternative AIC test (in which a sleeve was used), and an RT. This work was carried out between 2019 and 2020 as part of a collaboration among EU authorities, chemical industry representatives (including individuals from major repellent development companies: Citrefine, Endura, Henkel, Merck, Reckitt Benckiser, and SC Johnson), and four European BP assessment laboratories (BioGenius, i2LResearch, Henkel, and Tecnalia). The findings were published in the article *"Two New Alternatives to the Conventional Arm-in-Cage Test for Assessing Topical Repellents" (pag 39).*

As explained earlier, the passage of the BPR has drastically reduced AS number (European Commission, 2018). To explore strategies for dealing with this specific challenge, a third study was performed under laboratory conditions to assess the efficacy of prallethrin (a synthetic type I pyrethroid) when used as an SR. The objective was to determine whether there could be benefits to incorporating sublethal doses of insecticides in PPPs and prallethrin's potential utility in efforts aimed at reducing public health risks and improving vector control programmes. This work is detailed in the article *"A Three-Pronged Approach to Studying Sublethal Insecticide Doses: Characterising Mosquito Fitness, Mosquito Biting Behaviour, and Human/Environmental Health Risks" (p. 53).*

Since the third study examined the use of sublethal pyrethroid doses as SRs under laboratory conditions (i.e., indoors), the next step was to evaluate their use under field conditions. Thus, the fourth study evaluated the efficacy of transfluthrin (TFT; a synthetic type I pyrethroid) when applied in aerosol form in outdoors. This research is described in the article "*To Kill or to Repel Mosquitoes? Exploring Two Strategies for Protecting Humans and Reducing Vector-Borne Disease Risks by Using Pyrethroids as Spatial Repellents" (p. 77)*

The entirety of the research presented in this thesis focuses on a critical overarching concern: the need for adaptive and sustained approaches to current regulatory frameworks that will simultaneously improve vector control, limit pathogen transmission, and establish the appropriate balance between regulations, contexts of use, and risks to human and environmental health.

OBJECTIVES

OBJECTIVES

The doctoral research presented herein re-examined how compounds are evaluated and establishing a more robust set of globally accepted parameters and methodologies. Its broader aims are to improve vector control; reduce pathogen transmission; and limit the potential risks associated with compound use. Five objectives were thus defined:

Objective 1: To characterise the relationship between estimates of landing rate obtained in the field versus in the laboratory with a view to developing novel laboratory methods for evaluating repellents.

Objective 1 was achieved in the research described in the first publication: *"From the Field to the Laboratory: Quantifying Outdoor Mosquito Landing Rate to Better Evaluate Topical Repellents"*.

Objective 2: To validate an alternative AIC method for evaluating TR that could better estimate real-life protection times.

Objective 3: To validate an alternative room-based method for evaluating topical repellents under more realistic conditions.

Objectives 2 and 3 were attained in the research described in the second publication: *"Two New Alternatives to the Conventional Arm-in-Cage Test for Assessing Topical Repellents"*.

Objective 4: To assess indoors the utility of percent protection as an evaluation parameter and explore whether sublethal insecticide doses could be used as public health tools.

Objective 4 was accomplished in the research described in the third publication: *"A Three-Pronged Approach to Studying Sublethal Insecticide Doses: Characterising Mosquito Fitness, Mosquito Biting Behaviour, and Human/Environmental Health Risks"*.

Objective 5: To demonstrate that sublethal doses of PT18 ASs could be employed as SRs to protect humans under outdoors conditions.

Objective 5 was completed in the research described in the fourth publication: "*To Kill or to Repel Mosquitoes? Exploring Two Strategies for Protecting Humans and Reducing Vector-Borne Disease Risks by Using Pyrethroids as Spatial Repellents*".

ARTICLES PUBLISHED

From the Field to the Laboratory: Quantifying Outdoor Mosquito Landing Rate to Better Evaluate Topical Repellents

AUTHORS: *Mara Moreno-Gómez[1], Rubén Bueno-Marí[2,3], Andrea Drago[4], and Miguel A. Miranda[5]*

TITLE: From the Field to the Laboratory: Quantifying Outdoor Mosquito Landing Rate to Better Evaluate Topical Repellents

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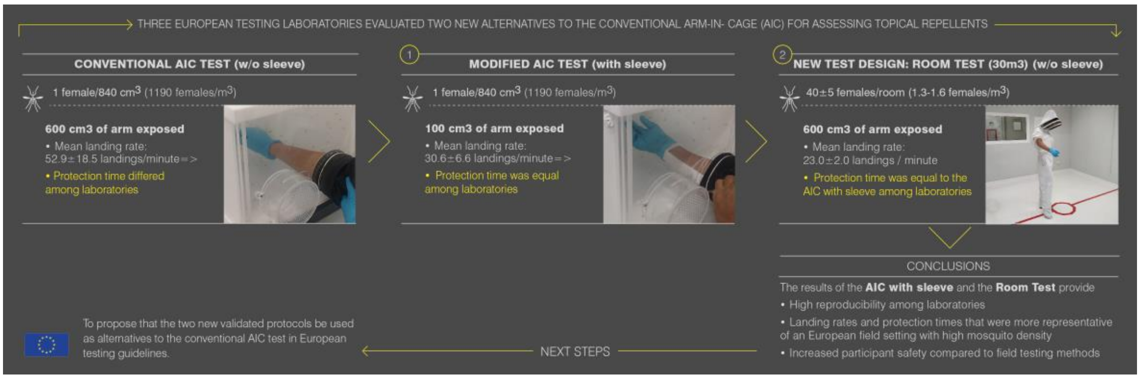
PUBLISHER: Oxford Academy

Two New Alternatives to the Conventional Arm-in-Cage Test for Assessing Topical Repellents



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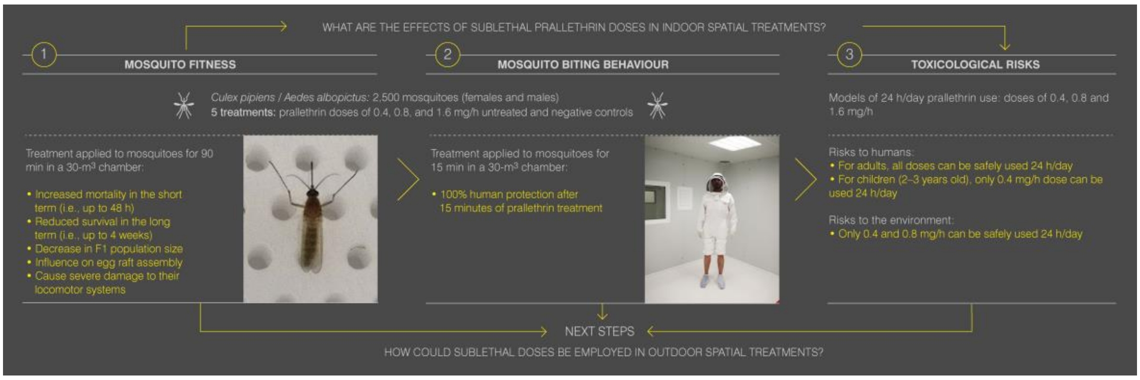
PUBLISHER: Oxford Academy

A Three-Pronged Approach to Studying Sublethal Insecticide Doses: Characterising Mosquito Fitness, Mosquito Biting Behaviour, and Human/Environmental Health Risks



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TITLE: A Three-Pronged Approach to Studying Sublethal Insecticide Doses: Characterising Mosquito Fitness, Mosquito Biting Behaviour, and Human/Environmental Health Risks

AFFILIATIONS: [1]Henkel Ibérica S.A, Research and Development (R&D) Insect Control Department, Barcelona, Spain. [2]Laboratorios Lokímica, Departamento de Investigación y Desarrollo (I+D), Valencia, Spain. [3]Àrea de Parasitologia, Departament de Farmàcia i Tecnologia Farmacèutica i Parasitologia, Universitat de València, Burjassot, València, Spain. [4]Applied Zoology and Animal Conservation Research Group, UIB, Palma de Mallorca, Spain

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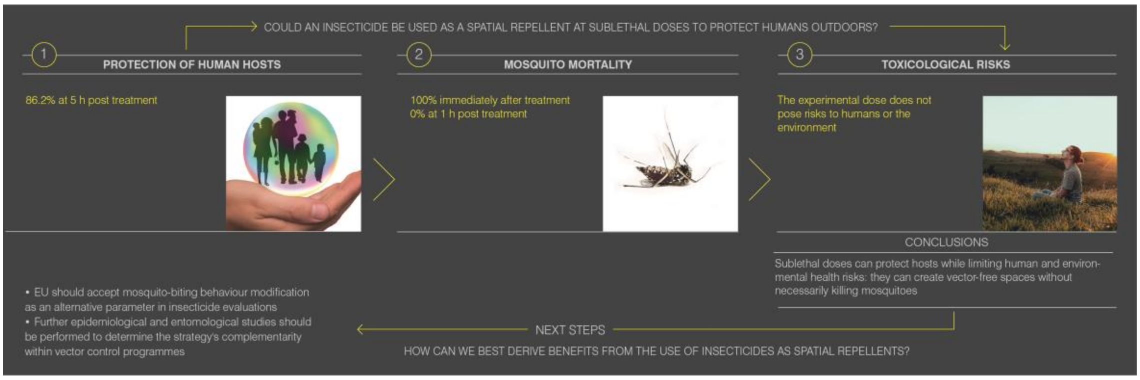
To Kill or to Repel Mosquitoes? Exploring Two Strategies for Protecting Humans and Reducing Vector-Borne Disease Risks by Using Pyrethroids as Spatial Repellents



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TITLE: To Kill or to Repel Mosquitoes? Exploring Two Strategies for Protecting Humans and Reducing Vector-Borne Disease Risks by Using Pyrethroids as Spatial Repellents

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GENERAL DISCUSSION

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The research presented in this thesis responds to an urgent need: we must develop innovative approaches for gauging the efficacy of mosquito-targeting BPs under laboratory and field conditions. The new evaluation methodologies and parameters presented herein can inform ongoing updates to EU guidelines for assessing insecticides and repellents. They can also help inspire novel uses and technologies aimed at reducing the potential risks of BPs for human and environmental health.

In study 1 (*From the Field to the Laboratory: Quantifying Outdoor Mosquito Landing Rate to Better Evaluate Topical Repellents*), the thesis's first research objective was achieved: to design a methodology that could successfully recreate the maximum field landing rate under laboratory conditions. In turn, this work made it possible to validate new laboratory methods that could simulate the conditions a person might encounter outdoors in an area with high mosquito density. The advantage of this approach is that it is safer than field testing for the study participants. This is especially important because of the increasing incidence of VBDs, notably those transmitted by mosquitoes (Gossner et al., 2018; Barrett, 2018). Finally, it was possible to clearly show that WHO guidelines for AIC testing result in highly unrealistic conditions for evaluating topical repellents (WHO, 2009).

One major challenge was identifying a suitable method/parameter that established a link between the laboratory and field tests. Two approaches were considered, including the human landing catch (HLC) method (WHO & WHO Pesticide Evaluation Scheme 2013), in which mosquitoes are captured, and the landing rate count (LRC) method, in which evaluators gently shake off any mosquitoes before biting occurs (Fellton, 1945; Vigilant *et al.*, 2020). It is important to note that, in the LRC method, a certain degree of mosquito disturbance is viewed as acceptable given the relative reduction in bites. Both methods were carefully assessed to determine which better fit with the study's objectives.

While the HLC method is a standard approach that is widely used to evaluate mosquito density and species occurrence within specific areas, the LRC method is primarily used during vector control programmes to evaluate the need for or the efficacy of adulticide treatments. The LRC method has also been employed in other contexts, such when seeking to determine 1) the habitat locations of larvae; 2) the vector potential of biting populations; and 3) the placement of light traps. Although the method has been used by mosquito control agencies, standard usage guidelines have yet to be established, and it is therefore not an officially recognised technique at present (Lloyd *et at.,* 2018; Vigilant *et al.*, 2020). That said, a customised version of the LRC method was employed here, under laboratory conditions, during the RT.

This research appears to be the first of its kind—no previous studies have measured the landing rates of a mosquito species in the field and then recreated those landing rates in the laboratory. Part of the challenge was successfully identifying the laboratory conditions needed to obtain the maximum landing rate observed outdoors in an area heavily infested with *Ae. albopictus*. To simulate the maximum field landing rate (26.8 landings/min; 5-min exposure period) in the laboratory, 15–20 mosquitoes were placed in a 30-m^3 room (density of 0.50–0.66 mosquitoes/m^3; 3-min exposure period) resulting in a landing rate of 30.4 ± 13.5 landings/min. In contrast, in the AIC test conducted according to WHO guidelines, the mean landing rate was 229 ± 48 landings/min, more than 8.5 times higher than the maximum field landing rate. These results clearly indicate that the conventional AIC test resulted in landing rates that far surpassed those seen in the field, which means that the method likely underestimates protection times. This discovery highlights how crucial it is to invest time and effort in fine-tuning methodologies when testing the efficacy of repellents intended to protect humans.

At present, neither WHO (2009) nor EU (ECHA, 2011) guidelines for evaluating mosquito repellents specify the minimum mosquito landing rate that must be achieved to validate a study plot when performing efficacy tests in the field. However, EPA guidelines recommend that at least one landing be observed per minute in experimental plots (EPA, 2010). Since this study found that the mean field landing rate was 9.5 landings/min, it seems reasonable to assume that the research area was characterised by high mosquito density and thus any repellent evaluation would take place under "challenging” conditions. Indeed, a landing rate of 26.8 landings/min translates into 134 attempted bites within a 5-min period. While it is important to note that landing rates were estimated using a limited data set (i.e., measurements obtained over two days at a single study site), it nonetheless remains unlikely that the average person, who is unaccustomed to field conditions, would be able to stand more than 1–2 min of such a high level of landing pressure.

In Europe, *Ae. albopictus* and *Cx. pipiens* are the standard mosquito species used in insecticide and repellent evaluations. Given that this study utilised the former, it would be useful to repeat this work in its entirety using *Cx. pipiens*.

The above results were presented in 2019 during EFFWG meetings; the doctoral student responsible for this thesis has been a scientific advisor to the group since 2017. During these meetings, it was underscored that current methodologies for evaluating TR need to be revamped, and it was further suggested that the key evaluation parameter be minimum landing rate rather than mosquito density guidelines (ECHA, 2018a; ECHA, 2018b; ECHA, 2018c) The latter remains the parameter of import in WHO and EPA guidelines (WHO, 2009; EPA, 2010).

However, before this step could be taken, it was necessary to more extensively examine AIC methodology, given its status as the international standard of reference. The discussions during the EFFWG meetings thus gave rise to the second study of this thesis.

Study 2 (*Two New Alternatives to the Conventional Arm-in-Cage Test for Assessing Topical Repellents*) was a natural expansion on the work done in study 1. More specifically, it examined two newly alternative tests—the sleeved AIC test and the RT. Its results suggest these methodologies could serve as viable alternatives to the conventional AIC test described in WHO (2009) and EPA (2010) guidelines.

The two alternative methods arrived at statistically equivalent estimates of complete protection time (CPT). Furthermore, even when the tests were performed in different laboratories, consistent CPTs were obtained. It is crucial that efficacy tests yield reproducible results, given that they will be carried out in a wide range of testing laboratories. Both alternative methods generated landing rates similar to the maximum field landing rate observed in the first study (26.8 landings/min). Moreover, this landing rate meets WHO criteria for validating AIC results; namely, the landing rate in the untreated area must be equal to 10 landings/30 sec or 20 landings/min. Consequently, these results indicate that these two methods could more reliably assess how TR will perform under outdoor conditions without exposing participants to the health risks associated with field testing. In contrast, the conventional AIC test resulted in landing rates that were higher (by 40–48%) and CPTs that were lower than those in the sleeved AIC and the RT. This finding implies that the former may underestimate the CPTs of TR under real-life conditions of use, as suggested by previous studies (Obermayr *et al.*, 2010; Colucci & Müller, 2018).

One of the greatest challenges faced during study 2 was research coordination, as the work was carried out in collaboration with EU authorities, industry representatives, and four testing laboratories. Indeed, all the parties involved had to agree upon each of the experimental parameters and conditions. The author of this thesis was responsible for the above task.

Before launching the study, different potential methodologies were discussed. The main reference source was Obermayr *et al.* (2010), which explores alternatives to conventional AIC testing. Notably, the researchers reduced the exposure area from 600 cm^2 to 100 cm^2, which reduced landing number. However, instead of using a sleeve, they used a customised "cage": the mosquitoes were located inside a box with a 100-cm^2 slot through which study participants inserted the treated part of their forearms. This "arm-to-cage" method requires fewer mosquitoes than does conventional AIC testing and is less uncomfortable for study participants (Obermayr *et al.*, 2010). Ultimately, however, the decision was made not to utilise this method in study 2 because it would have required the four laboratories to purchase new equipment to carry out an as-yet unvalidated test. Subsequently, the idea arose to use a sleeve that exposed a defined area of skin to the mosquitoes. The main difficulties were to create something that was comfortable but that fit well enough to prevent bites. The author of this thesis led the research at Henkel's biological laboratory in Barcelona to design a sleeve meeting these criteria.

Two main conclusions emerged from this research. First, the study confirmed the relationship discovered in past research: higher mosquito densities lead to higher landing rates and, consequently, to lower CPTs (Barnard *et al.*, 1998; Obermayr *et al.*, 2010; Colucci & Müller, 2018). Therefore, it seems logical to suggest that approaches for evaluating repellents, such as AIC testing, should focus on establishing a target mosquito landing rate rather than a target mosquito density, as stipulated in WHO (2009) and EPA (2010) guidelines. Secondly, sleeves could be used as a tool to help achieve target landing rates, increasing both CPT reproducibility among laboratories and the comfort of study participants.

The parameter "landing rate" and the testing methods, sleeved AIC test and the room test, were approved by EU authorities and were included in the new EU efficacy testing guidelines that were published in December 2021 (ECHA, 2021b).

Study 3 (*A Three-Pronged Approach to Studying Sublethal Insecticide Doses: Characterising Mosquito Fitness, Mosquito Biting Behaviour, and Human/Environmental Health Risks*) explored different parameters and methodologies that could serve as alternatives to those officially employed in the EU for evaluating insecticides. More specifically, this work addressed the utility of percent protection as an evaluation parameter and explore whether sublethal insecticide doses could be used as public health tools.

This study revealed that sublethal doses of the pyrethroid prallethrin (0.4, 0.8, and 1.6 mg prallethrin/h), 94.7% Technical Grade (CAS number 97 23031-36-9) negatively affected both female and male mosquitoes of the two study species, *Cx. pipiens* and *Ae. albopictus*. The insecticide influenced mortality, fitness, and egg-laying. Consequently, F1 population size decreased in all three treatment groups for both species. Mosquito behaviour was also altered. Biting was completely inhibited in as quickly as 15 min, offering 100% protection to human hosts. Furthermore, toxicological risk modelling showed that using sublethal doses for mosquito control poses limited risks to human and environmental health.

Study 3 also evaluated whether prallethrin affected female fecundity and/or egg viability in *Ae. albopictus*. However, it found no evidence to support this hypothesis, given that the ratio of larvae to females was similar across treatment groups. Additionally, the percentage of eggs that developed into third/fourth instar larvae was also generally consistent across groups (e.g., 43.36% in the negative control and 53.8% in the low dose group [0.4 mg/h]). Future research should expand on study 3 to determine whether more prolonged prallethrin exposure (i.e., longer than 90 min) would yield different results.

Throughout the world, pyrethroids are commonly used to control insects in households, professional settings, and IVM programmes (Benelli *et al.*, 2016). They are a control agent of choice because they are relatively less toxic to mammals, induce rapid knockdown (KD) in their targets, and break down quickly in the environment due to their photosensitivity (WHO & WHO Pesticide Evaluation Scheme, 2005).

Although pyrethroids are commonly classified and used as insecticides, some can also function as repellents, as this work has showed for prallethrin, when low doses or short exposure times are used. Examples include metofluthrin (Buhagiar *et al.*, 2017; Bibbs *et al.*, 2019), transfluthrin (Wagman *et al.*, 2015; Bibbs *et al.*, 2018), d-allethrin (Yap *et al.*, 1996), and prallethrin, the compound utilised in study 3. While prallethrin does not appear to be frequently employed in vector control programmes, people widely deploy it to control insects in their homes (Matsunaga *et al.*, 1987).

Many studies have explored how sublethal doses of certain pyrethroids affect fitness (Buhagiar *et al.*, 2017; Bibbs *et al.*, 2018; Bibbs *et al.*, 2019) and mosquito behaviour (Boonyuan *et al.*, 2011; Darbro *et al.*, 2017; Dye-Braumuller *et al.*, 2017); they have demonstrated the insecticides' efficacy at low doses. Study 3 addressed the biological, physiological, demographic, and behavioural effects on individual mosquitoes and mosquito populations when two species were exposed to indoor spatial treatments. It found that sublethal doses of prallethrin significantly increased short- and long-term mosquito mortality, leading to smaller population sizes in the next generation. Sublethal doses also provided 100% protection to human hosts and presented relatively low risks to human and environmental health.

Thus, in theory, sublethal doses of the compounds in household BPs could provide 100% protection to human hosts while limiting risks to public and environmental health. However, at present, the EU does not authorise this type of usage. Indeed, official efficacy requirements for SRs stipulate that the proscribed dose of a formulation/AS must kill 90% of exposed insects within 24 h, a threshold known as the 90% lethal dose (LD90). Doses that do not meet this threshold are considered to be ineffective and are therefore not authorised. Current efficacy guidelines thus centre on immediate mortality (i.e., up to 24 h post exposure); they do not take into account long-term mortality (i.e., beyond 48 h) and/or behavioural modifications (bite inhibition), which greatly limits their potential usefulness (ECHA, 2011).

These results showed here are promising from a perspective of human and environmental health. At the very least, they can be applied in the context of household BPs utilised indoors. However, certain aspects of this complex topic should be explored further. First, study 3 used two strains of mosquitoes that have been reared exclusively in the laboratory for several years. As a result, it is unknown how well the above findings reflect reality in wild mosquito populations. More research addressing this question is needed.

There are additional research directions that should be taken to explore the benefits and/or limitations of using sublethal pyrethroid doses. For example, it is important to consider how their deployment in outdoor domestic and professional settings plays out not only in terms of mosquito mortality and human protection but also in terms of human and environmental health. Although AS concentrations in the air are much lower outdoors, the environmental risks could be higher because the AS is directly applied to the environment. While sublethal doses could be employed as complementary tools within IVM programmes, their usefulness and consequences must be studied in greater depth. Therefore, before suggesting any modifications to EU guidelines, it was crucial to further explore the use of sublethal doses under outdoor conditions, work that was performed in study 4.

Study 4 (*To Kill or to Repel Mosquitoes? Exploring Two Strategies for Protecting Humans and Reducing Vector-Borne Disease Risks by Using Pyrethroids as Spatial Repellents*) demonstrated that PT18 ASs could be employed as SRs, creating vector-free spaces without necessarily killing mosquitoes; by preventing contact between humans and vectors, sublethal insecticide doses could provide an effective complementary tool within IVM programmes.

Study 4 evaluated how well a sublethal dose of a synthetic type I pyrethroid, transfluthrin (TFT 98.5% technical grade, CAS number 118712-89-3), functioned as a SR. At present, the EU has exclusively authorised TFT for use as an insecticide. In the first experiment, the insecticide's effects were assessed using host protection, a new approach. In the second experiment, the insecticide's effects were assessed using mosquito KD and mortality, the conventional approach. The third component of the study was a model that determined TFT's toxicological risks for human and environmental health under the conditions of use in the experiments.

In the first experiment, the presence of *Ae. albopictus* declined, and study participants experienced a high degree of protection (> 80% for up to 5 h), while in the area adjacent to the treatment zone, mean percent protection decreased more rapidly (~ 60% at 3 h). Interestingly, these results were not a consequence of mosquito mortality because, in the second experiment, mortality was zero 1 h post application in the treatment area. Taken together, these findings suggest sublethal doses of TFT may persist in the air or soil. While these TFT residues did not appear to knock down or kill mosquitoes, they still significantly protected hosts against bites within a defined area. The effects observed may have been mediated by pyrethroid-induced neuronal hyperexcitation, which can occur at levels much lower than those that cause KD and/or mortality (Lucas *et al.*, 2007). The modelling results revealed that this form of TFT usage did not pose a threat to the health of humans, vermivorous or insectivorous mammals, or the environment (target compartments examined: sewage treatment plants, aquatic habitats, soil, and groundwater).

The ability of certain pyrethroids to act as SRs has previously been explored. However, as stated previously, EU regulatory guidelines evaluate the insecticides in SRs based on levels of mosquito KD and mortality rather than on repellency or biting rates. Consequently, from the perspective of EU authorities, the TFT usage described in study 4 would only be effective immediately after application, and parameters based on behaviour modification (e.g., percent protection) would not be considered during the evaluation process (ECHA, 2011). Thus, based on current EU efficacy requirements (ECHA, 2011), if the SR were to be used outdoors, the same dose would have to be reapplied every hour to be considered effective. Such unnecessary insecticide use presents risks for human and environmental health. This regulatory constraint is limiting because, in the EU, TR are currently one of the few personal protection solutions that can be used outdoors. The WHO has recognised this issue and has issued evaluation guidelines in which the focus is placed on how well volatile pyrethroids repel insects and/or inhibit bites (WHO & WHO Pesticide Evaluation Scheme, 2013). They state that "*spatial repellency [refers to] a range of insect behaviours induced by airborne chemicals that result in a reduction in human vector contact and therefore personal protection. The behaviours can include movement away from a chemical stimulus, interference with host detection (attraction inhibition), and feeding response"* (WHO & WHO Pesticide Evaluation Scheme, 2013). Based on all of the above, it is recommended that the EU make regulatory changes to take better advantage of SRs. In particular, authorities should allow outdoor usage to be evaluated based on changes to vector behaviour, such as mosquito landing rates, instead focusing exclusively on toxicity/lethality (ECHA, 2011). The aforementioned WHO guidelines could serve as a good starting point to build a single set of global standards for deploying existing insecticidal compounds at the household level.

There are multiple arguments in favour of this change in approaches, including many that deserve to be explored before SR-based strategies can be globally implemented. First, this study and others have demonstrated that SRs can be highly beneficial: under outdoor conditions, SRs reduce the rate at which mosquitoes bite humans, thereby affecting a key factor underlying disease transmission and increasing levels of host protection (Alten *et al.*, 2003; Lucas *et al.*, 2007). Second, SR-based strategies could be deployed in the form of PPPs or in complement to vector control programmes based on the use of insecticide-treated bednets (ITNs) and indoor residual spraying (IRS); such an approach could boost protection for individuals, households, and/or communities via ASs with very low toxicity in mammals (Achee *et al.*, 2012; Bibbs & Kaufman, 2017). Study 4's findings demonstrate that SRs have effects that can be exploited as part of IVM. New or improved strategies could also be designed. For example, SRs can impair mosquito oviposition (Ogoma *et al.*, 2014;. Furthermore, Bibbs *et al.* (2018) discovered that sublethal doses of TFT could cause chorion collapse in *Ae. aegypti* eggs, rendering them nonviable. Study 3 also evaluated whether prallethrin affected female fecundity and/or egg viability in *Ae. Albopictus*, however, as mentioned earlier, additional studies are needed to explore this question further.

When examining the benefits of sublethal pyrethroid doses, we must also consider the potential risks. Chemical compounds have long been widely used to control insect pests (Gratz & Jany, 1994). Not surprisingly, insects have evolved genetic, enzymatic, and behavioural countering mechanisms as a result (Soderlund & Bloomquist, 1989; García *et al.*, 2009; Ranson, 2017). There is clear evidence that insects have developed resistance to the four major classes of insecticides used to protect public health (i.e., pyrethroids, carbamates, organophosphates, and organochlorines), with pyrethroid resistance predominating in insect vectors of major human and animal diseases (Moyes *et al.*, 2017). Developed in the 1970s, pyrethroids quickly became a very common form of chemical control due to their high toxicity in insects, low toxicity in mammals, and minimal cost (WHO & WHO Pesticide Evaluation Scheme, 2005). As a consequence, genes that confer resistance to insecticides have been spreading in vector species, notably those capable of transmitting the pathogens responsible for malaria and dengue fever (della Torre *et al.*, 2012). Research on malaria vectors in Africa confirms that resistance is increasing (Butler, 2011; Nkemngo et al., 2020). However, pyrethroids are currently the only class of insecticides approved for use in ITNs because other insecticide classes cannot safely and effectively serve in this capacity (IRAC, n.d.). The utilisation of ITNs, as well as that of long-lasting IRS (Zaim *et al.*, 2000), have been major contributors to pyrethroid resistance in malaria vectors (N'Guessan *et al.*, 2007; Kulkarni *et al.*, 2007; Matowo *et al.*, 2015). Similarly, ultra-low volume (ULV) insecticide applications have boosted levels of pyrethroid resistance in urban populations of *Ae. aegypti* (Macoris *et al.*, 2007; Marcombe *et al.*, 2013). While the above examples have been well documented, there may be additional selective forces operating in specific environments. For example, pyrethroid resistance in mosquitoes and other vectors may also emerge because of agricultural usage of pyrethroids (Nkya *et al.*, 2013; Nkya *et al.*, 2014). Indeed, most agricultural pesticides utilise the same active mechanisms as the pesticides used for vector control and, therefore, may select for resistance in mosquito populations occurring in agricultural regions (Diabate *et al.*, 2002; Yadouleton *et al.*, 2009). Likewise, household usage of insecticides could represent an as-yet unquantified cause of pyrethroid resistance (Gray *et al.*, 2018).

Continuous exposure to sublethal doses of pyrethroids might therefore prompt changes in mosquito sensitivity or susceptibility, resulting in the emergence of resistance or the development of behavioural shifts, whereby mosquitoes avoid contact with insecticides (via temporal, spatial, or trophic mechanisms; Carrasco *et al.*, 2019). Therefore, when designing SRs intended for outdoor usage, it is essential to make resistance management plans a key part of IVM programmes (Protopopoff *et al.*, 2013; Kleinschmidt *et al.*, 2018; Collins *et al.*, 2019). The EU already actively promotes the eight IVM principles set out in its directive on sustainable pesticide use (European Parliament, 2009) and has further strengthened its commitment via its Farm to Fork Strategy. These principles include 1) prevention; 2) monitoring; 3) threshold-based decision making; 4) the use of non-chemical pest control measures; 5) the selection of the most targeted pesticides (if pesticides are needed); 6) the use of the lowest necessary doses of pesticides (if pesticides are needed); 7) the use of anti-resistance strategies; and 8) the evaluation of the success of pest control measures (European Commission, 2021). The WHO and EPA also strongly encourage integrated pest and vector management: alternative measures, such as biological control or environmental management, are preferred in situations where they can be effective, but insecticides can be employed in a safe and targeted manner when there is no alternative (EPA, n.d.-b). To this end, the Insecticide Resistance Action Committee (IRAC) has produced a manual entitled "Prevention and management of insecticide resistance in vectors and pests of public health importance" with a view to fostering effective insecticide resistance management (IRAC, n.d.).

As underscored in study 4, household SR use is already authorised under certain sets of regulatory guidelines (WHO & WHO Pesticide Evaluation Scheme, 2013), which could serve as the basis for changes to other regulatory schemes, including those in the EU. However, additional epidemiological and entomological studies are needed to explore the idea that the use of pyrethroid-based SRs can reduce disease transmission (Maia *et al.*, 2018; Hul *et al.*, 2021) and be widely deployed as part of vector control efforts (Bibbs & Kaufman, 2017; Syafruddin *et al.*, 2020; Logan *et al.*, 2020). This need for additional research has been highlighted by the WHO: *"Phase III studies should be designed around epidemiological endpoints to demonstrate the public health value of the intervention. Entomological outcomes cannot be used on their own for this purpose, although they can be combined with epidemiological outcomes to evaluate a claimed entomological effect"* (WHO, 2017).

It is also important to recognise that the dynamic and complex nature of VBDs complicates predictions about how existing, re-emerging, or new diseases may affect human health. However, it seems reasonable to expect that new VBDs will emerge and that existing VBDs will further intensify, particularly viral diseases transmitted by *Aedes* mosquitoes closely associated with urban areas (Zelle *et al.*, 2013; Roiz *et al.*, 2018). Also of concern are pathogens transmitted by *Culex* mosquitoes (Barrett, 2018; Rochlin *et al.*, 2019) and other arthropods such as ticks (Relich & Grabowski, 2020; Millins *et al.*, 2021). This situation of unpredictability underscores that there is a critical need for sustained, adaptive approaches to reduce the risk of VBDs.

Chemical solutions for protecting public health will continue to play an important role in coming decades. The debate around insecticide use is contentious and dogmatic, which has led to profound polarisation. Opponents often hold an insidious belief that insecticide use is always undesirable. Furthermore, there is little recognition of the fact that, at least in the developed world, predictions are improving when it comes to the ecological and human health risks associated with insecticide use. This fact, combined with greater environmental awareness, is leading to improved decision making in relation to authorisation procedures and the health and environmental safety profiles of commercial insecticides.

The history of pesticide use and the results of this thesis research point to possible trajectories for future studies. Much work remains to be done. It is up to governmental authorities, industry stakeholders, researchers, and the international vector control community to tackle this challenge by developing and adopting promising innovations. Given that few new ASs are likely to become available for vector control within household and professional settings, we must reinvent how we deploy currently authorised compounds. The reinvention process means making optimal use of available tools, developing new approaches for evaluating household insecticides, and promoting a shift in current vector control programmes. For instance, greater emphasis could be placed on protecting humans rather than on killing mosquitoes. The use of sublethal doses could play an important role in this regard—the objective is not to employ them as exclusive, stand-alone tools, but rather as part of vector control programmes or as an alternative in situations where other options are limited (e.g., as a personal protection measure outdoors). By studying and authorising alternative parameters, methodologies, and/or modes of use that utilise currently approved ASs, we can better identify solutions for protecting people from VBDs while simultaneously limiting the risks to human and environmental health.

The story of humanity continues. Our future and that of the next generations is at stake as we confront the great challenges of the 21st century. Climate change, population growth, resource scarcity, and the fight against insect pests and the diseases they vector are all issues with tremendous environmental, economic, and social consequences. We can only identify solutions by adopting a collective approach that is firmly grounded in science, technology, and policy.

CONCLUSIONS

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1. The work described herein identified the laboratory conditions (i.e., 15–20 female *Ae. albopictus* in a 30-m^3 room) that could be used to recreate the maximum landing rate (26.8 landings/min) observed outdoors in an area highly infested with *Ae. albopictus.*

2. Both a parameter—landing rate—and a methodology—landing rate count—were identified as helpful in improving and refining the evaluation standards used for testing insecticides and repellents under laboratory conditions; notably, both led to a better simulation of outdoor conditions.

3. The conventional laboratory method described in WHO (2009) and EPA (2010) guidelines, the arm-in-cage test, had a higher *Ae. albopictus* landing rate (229 ± 47.87 landings/min), suggesting it might underestimate the real-life protection times of topical repellents.

4. Together with the room test, the sleeved arm-in-cage test successfully recreated the maximum *Ae. albopictus* landing rates observed in the field using colony-reared mosquito adults of the same species

5. Compared to the conventional arm-in-cage test, both the sleeved arm-in-cage test and the room test led to greater reproducibility in protection times across testing laboratories at both experimental repellent doses (0.5 and 1 g of 15% DEET). The two latter methods also facilitated mosquito counts and reduced study participant stress during testing.

6. The sleeved arm-in-cage test and the room test were found to be reliable for evaluating repellents under laboratory conditions, eliminating the need for field testing, which can present a health risk to study participants.

7. When sublethal doses of the pyrethroid prallethrin (i.e., 0.4, 0.8, and 1.6 mg/h) were used under laboratory testing conditions (i.e., in a 30-m^3 room for 90 min) on colony-reared adult *Ae. albopictus* and *Cx. pipiens*, there was an increase in short-term

mortality (i.e., over 48 h) and a decrease in long-term survival (i.e., up to 4 weeks). Mosquitoes also experienced severe locomotor damage and changes to their behaviour and egg laying.

8. Sublethal doses of prallethrin completely inhibited biting by colony-reared adult *Ae. albopictus* within 15 min in the room test, resulting in 100% protection for human hosts.

9. When sublethal doses of transfluthrin were deployed under outdoor conditions, the residues were found to persist in the air and/or soil. Under laboratory conditions, residues did not knock down or kill colony-reared adult *Ae. albopictus* for more than 1 h after application. However, in the field, residues did provide a high level of protection against adult *Ae. albopictus* when human hosts (86.2% at 5 h post application) remained within the treatment area.

10. The risk assessment models showed that sublethal doses of prallethrin and transfluthrin presented relatively low risks to human and environmental health under both indoor and outdoor conditions of use.

It always seems impossible,
until it's done.
NELSON MANDELA

BIBLIOGRAPHY

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Abdel-Mohdy, F. A., Fouda, M. M. G., Rehan, M. F., & Aly, A. S. (2008). Repellency of controlled-release treated cotton fabrics based on cypermethrin and prallethrin. *Carbohydrate Polymers, 73*(1), 92-97. https://doi.org/10.1016/j.carbpol.2007.11.006

Abhilash, P. C., & Singh, N. (2009). Pesticide use and application: An Indian scenario. *Journal of Hazardous Materials, 265*(1-3),1-12. https://doi.org/10.1016/j.jhazmat.2008.10.061

Achee, N. L., Bangs, M. J., Farlow, R., Killeen, G. F., Lindsay, S., Logan, J. G., Moore, S. J., Rowland, M., Sweeney, K., Torr, S. J., Zwiebel, L. J., & Grieco, J. P. (2012). Spatial repellents: From discovery and development to evidence-based validation. *Malaria Journal, 11*, article 164. https://doi.org/10.1186/1475-2875-11-164

Achee, N., Masuoka, P., Smith, P., Martin, N., Chareonviriyaphap, T., Polsomboon, S., Hendarto, J., & Grieco, J. P. (2012). Identifying the effective concentration for spatial repellency of the dengue vector Aedes aegypti. *Parasites and Vectors, 5*, article 300. https://doi.org/10.1186/1756-3305-5-300

Agency for Toxic Substances and Disease Registry (ATSDR) (2003). *Toxicological Profile for Pyrethrins and Pyrethroids. Toxicological Profile for Pyrethrins and Pyrethroids.* https://www.atsdr.cdc.gov/toxprofiles/tp155.pdf

Alten, B., Caglar, S. S., Simsek, F. M., Kaynas, S., & Perich, M. J. (2003). Field Evaluation of an Area Repellent System (Thermacell) Against Phlebotomus papatasi (Diptera: Psychodidae) and Ochlerotatus caspius (Diptera: Culicidae) in Sanliurfa Province, Turkey. *Journal of Medical Entomology, 40*(6), 930-934. https://doi.org/10.1603/0022-2585-40.6.930

Banaszkiewicz, T. (2010). Evolution of Pesticide Use. *Contemporary Problems of Management and Environmental Protection, 5*, 7-18. http://www.uwm.edu.pl/environ/vol05/vol_05_chapter01.pdf

Barnard, D. R., Posey, K. H., Smith, D., & Schreck, C. E. (1998). Mosquito density, biting rate and cage size effects on repellent tests. *Medical and Veterinary Entomology, 12*(1), 39-45. https://doi.org/10.1046/j.1365-2915.1998.00078.x

Barrett, A. D. T. (2018). West Nile in Europe: an increasing public health problem. *Journal of Travel Medicine, 25*(1), article 96. https://doi.org/10.1093/jtm/tay096

Bell, S., Tyrväinen, L., Sievänen, T., Pröbstl, U., & Simpson, M. (2013). Outdoor Recreation and Nature Tourism: A European Perspective. *Living Reviews in Landscape Research, 1*(2), 1-46. http://dx.doi.org/10.12942/lrlr-2007-2

Benelli, G., Jeffries, C. L., & Walker, T. (2016). Biological Control of Mosquito Vectors Past, Present, and Future. *Insects, 7*(4), article 52. https://doi.org/10.3390/insects7040052

Bibbs, C. S., & Kaufman, P. E. (2017). Volatile pyrethroids as a potential mosquito abatement tool: A review of pyrethroid-containing spatial repellents. *Journal of Integrated Pest Management, 8*(1), article 21. https://doi.org/10.1093/jipm/pmx016

Bibbs, C. S., & Xue, R. D. (2016). OFF! Clip-on repellent device with metofluthrin tested on Aedes aegypti (Diptera: Culicidae) for mortality at different time intervals and distances. *Journal of Medical Entomology, 53*(2), 480-483. https://doi.org/10.1093/jme/tjv200

Bibbs, C. S., Bloomquist, J. R., Hahn, D. A., Kaufman, P. E., & Xue, R. D. (2019). Gone in 60 seconds: Sub-lethal Effects of Metofluthrin Vapors on Behavior and Fitness of Resistant and Field Strains of Aedes aegypti (Diptera: Culicidae). *Journal of Medical Entomology, 56*(4), 1087-1094. https://doi.org/10.1093/jme/tjz048

Bibbs, C. S., Hahn, D. A., Kaufman, P. E., & Xue, R. D. (2018). Sublethal effects of a vapour-active pyrethroid, transfluthrin, on Aedes aegypti and Ae. albopictus (Diptera: Culicidae) fecundity and oviposition behaviour. *Parasites and Vectors, 11*, article 486. https://doi.org/10.1186/s13071-018-3065-4

Boonyuan, W., Kongmee, M., Bangs, M. J., Prabaripai, A., & Chareonviriyaphap, T. (2011). Host feeding responses of Aedes aegypti (L.) exposed to deltamethrin. *Journal of Vector Ecology, 36*(2), 361-372. https://doi.org/10.1111/j.1948-7134.2011.00177.x

Buhagiar, T. S., Devine, G. J., & Ritchie, S. A. (2017). Effects of sublethal exposure to metofluthrin on the fitness of Aedes aegypti in a domestic setting in Cairns, Queensland. *Parasites and Vectors, 10*, article 274. https://doi.org/10.1186/s13071-017-2220-7

Buist, H., Craig, P., Dewhurst, I., Hougaard Bennekou, S., Kneuer, C., Machera, K., Pieper, C., Court Marques, D., Guillot, G., Ruffo, F., & Chiusolo, A. (2017). Guidance on dermal absorption. *EFSA Journal, 15*(6), article 4873. https://doi.org/10.2903/j.efsa.2017.4873

Burg, W., Bremmer, H. J., & van Engelen, J. G. M. (2007). *RIVM rapport 320104007 Do-It-Yourself Products Fact Sheet*. https://www.rivm.nl/bibliotheek/rapporten/320104007.pdf

Butler, D. (2011). Mosquitoes score in chemical war. *Nature, 475*, article 19. https://doi.org/10.1038/475019a

Camann, D. E., Majumdar, T. K., & Geno, P. W. (2000). *Evaluation of saliva and artificial salivary fluids for removal of pesticide residues from human skin*. Southwest Research Institute.

Carrasco, D., Lefèvre, T., Moiroux, N., Pennetier, C., Chandre, F., & Cohuet, A. (2019). Behavioural adaptations of mosquito vectors to insecticide control. *Current Opinion in Insect Science, 34*, 48-54. https://doi.org/10.1016/j.cois.2019.03.005

Carson, R. (2002). *Silent Spring*. Penguin.

Choi, D. B., Grieco, J. P., Apperson, C. S., Schal, C., Ponnusamy, L., Wesson, D. M., & Achee, N. L. (2016). Effect of Spatial Repellent Exposure on Dengue Vector Attraction to Oviposition Sites. *PLoS Neglected Tropical Diseases, 10*(7), article e0004850. https://doi.org/10.1371/journal.pntd.0004850

Chouhan, N., & Deshmukh, R. (2020). *Mosquito repellent market by type (coil, spray, cream & oil, vaporizer, mat, and others) and distribution channel (hypermarket/supermarket, independent stores, online, and others): global opportunity analysis and industry forecast, 2020–2027*. Allied Market Research. https://www.alliedmarketresearch.com/mosquito-repellent-market

Collins, E., Vaselli, N. M., Sylla, M., Beavogui, A. H., Orsborne, J., Lawrence, G., Wiegand, R. E., Irish, S. R., Walker, T., & Messenger, L. A. (2019). The relationship between insecticide resistance, mosquito age and malaria prevalence in Anopheles gambiae s.l. from Guinea. *Scientific Reports, 9*, article 8846. https://doi.org/10.1038/s41598-019-45261-5

Colucci, B., & Müller, P. (2018). Evaluation of standard field and laboratory methods to compare protection times of the topical repellents PMD and DEET. *Scientific Reports, 8*(1), article 12578. https://doi.org/10.1038/s41598-018-30998-2

Daly, H., Doyen J. T., & Purcell, A. H. (1998). *Introduction to insect biology and diversity* (2.nd ed., pp. 279-300). Oxford University Press.

Danks, H. V. (2004). Seasonal adaptations in Arctic insects. *Integrative and Comparative Biology, 44*(2), 85-94. https://doi.org/10.1093/icb/44.2.85

Darbro, J. M., Muzari, M. O., Giblin, A., Adamczyk, R. M., Ritchie, S. A., & Devine, G. J. (2017). Reducing biting rates of Aedes aegypti with metofluthrin: investigations in time and space. *Parasites and Vectors, 10*, article 69. https://doi.org/10.1186/s13071-017-2004-0

Delatte, H., Desvars, A., Bouétard, A., Bord, S., Gimonneau, G., Vourc'h, G., & Fontenille, D. (2010). Blood-feeding behavior of aedes albopictus, a vector of chikungunya on la réunion. *Vector-Borne and Zoonotic Diseases, 10*(3), 249-258. https://doi.org/10.1089/vbz.2009.0026

Della Torre, A., Morou, E., Vontas, J., Pavlidi, N., Ranson, H., & Kioulos, E. (2012). Insecticide resistance in the major dengue vectors Aedes albopictus and Aedes aegypti. *Pesticide Biochemistry and Physiology, 104*(2), 126-131. https://doi.org/10.1016/j.pestbp.2012.05.008

Delmaar, J. E., & Schuur, A. G. (2017). *ConsExpo Web. Consumer Exposure Models documentation* [RIVM Report 2017-0197]. National Institute for Public Health and the Environment. https://doi.org/10.21945/RIVM-2017-0197

Desneux, N., Decourtye, A., & Delpuech, J. M. (2007). The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology, 52*, 81-106. https://doi.org/10.1146/annurev.ento.52.110405.091440

Diabate, A., Baldet, T., Chandre, F., Akogbeto, M., Guiguemde, T. R., Darriet, F., Brengues, C., Guillet, P., Hemingway, J., Small, G. J., & Hougard, J. M. (2002). The role of agricultural use of insecticides in resistance to pyrethroids in Anopheles gambiae s.l. in Burkina Faso. *American Journal of Tropical Medicine and Hygiene, 67*(6), 617-622. https://doi.org/10.4269/ajtmh.2002.67.617

Dye-Braumuller, K. C., Haynes, K. F., & Brown, G. C. (2017). Quantitative Analysis of Aedes albopictus Movement Behavior Following Sublethal Exposure to Prallethrin. *Journal of the American Mosquito Control Association, 33*(4), 282-292. https://doi.org/10.2987/17-6673.1

Espa, I., & Mda, T. (2021). *Clasificación del Modo de Acción de Insecticidas y Acaricidas*. IRAC.

European Chemicals Agency (ECHA) (2011). *Product type 18 – insecticides, acaricides and products to control other arthropods. Product type 19 – repellents and attractants (only concerning arthropods).* [CA-Dec12-Doc.6.2.a – Final]. https://bit.ly/3EPpYit

European Chemicals Agency (ECHA) (2015). *Biocides Human Health Exposure Methodology*. https://bit.ly/2XXvm2u

European Chemicals Agency (ECHA) (2017). *Default human factor values for use in exposure assessments for biocidal products (revision of HEEG opinion 17 agreed at the Human Health Working Group III on 12 June 2017)* [Recommendation no. 14 of the BPC Ad hoc Working Group on Human Exposure]. Helsinki. https://bit.ly/3zPag2R

European Chemicals Agency (ECHA) (2018a). *Minutes of the meeting of the efficacy working group of the biocidal products committee. 17 January 2018. WG-I-2018*. https://bit.ly/3ibv7ro

European Chemicals Agency (ECHA) (2018b). *Minutes of the meeting of the efficacy working group of the biocidal products committee. 12-13 September 2018. WG-V-2018*. https://bit.ly/2XU0E9N

European Chemicals Agency (ECHA) (2018c). *ECHA. Minutes of the meeting of the efficacy working group of the biocidal products committee. 3-4 December 2018 WG-VII-2018*.

European Chemicals Agency (ECHA) (2019). *Minutes of the meeting of the efficacy working group of the biocidal products committee. 27-29 March 2019*. https://bit.ly/2XVxWWL

European Chemicals Agency (ECHA) (2021a). List of active substances and suppliers. https://echa.europa.eu/es/information-on-chemicals/active-substance-suppliers

European Chemicals Agency (ECHA) (2021b). *Guidance on the Biocidal Products Regulation, Volume II Efficacy - Assessment and Evaluation (Parts B+C),* (Vol. IV). https://bit.ly/35eLKyM

European Chemicals Agency (ECHA) (n. d.-a). *ECHA: BPR legislation*. https://echa.europa.eu/regulations/biocidal-products-regulation/understanding-bpr

European Chemicals Agency (ECHA) (n. d.-b). *Regulations*. https://echa.europa.eu/regulations/biocidal-products-regulation

European Chemicals Agency (ECHA) (n. d.-c). *ECHA Product types*. https://echa.europa.eu/regulations/biocidal-products-regulation/product-types

European Commission (2002). *Technical Notes for Guidance. Human Exposure to Biocidal Products. Guidance on Exposure Estimation*. https://bit.ly/3kJmVjA

European Commission (2003). Commission Regulation (EC) No 2032/2003 of 4 November 2003 on the second phase of the 10-year work programme referred to in Article 16(2) of Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market, and amending Regulation (EC) No 1896/2000. *Official Journal of the European Union,* L307/1, 24 November 2003

European Commission (2014). *Report on the fees payable to Members States Competent Authorities pursuant to Article 80(2) of the Biocidal Product Regulation* [CA-Nov14-Doc.7.2]. https://news.wko.at/news/oesterreich/Anhang-CA-Nov14-Doc.7.2---Report-on-fees.pdf

European Commission (2018). Commission Regulation (EU) 2018/1472 of 28 September 2018 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council and the Annex to Commission Regulation (EU) No 231/2012 as regards Cochineal, Carminic acid, Carmines (E 120). *Official Journal of the European Union,* L247/1, 3 October 2018.

European Commission (2021). *Farm to Fork targets - Progress.* https://bit.ly/2ZBmlMX

European Parliament (2009). Directiva 2009/128/CE. *Diario Oficial de la Unión Europea,* L309/71, 24 de noviembre de 2009.

European Parliament (2021). *The use of pesticides in developing countries and their impact on health and the right to food* [EP/EXPO/DEVE/FWC/2019-01/LOT3/R/06]. https://bit.ly/3idEyqb

European Parliament and the Council of the European Union (2012). Reglamento (UE) n.º 528/2012, de 22 de mayo de 2012, relativo a la comercialización y el uso de los biocidas. *Diario Oficial de la Unión Europea,* L167/1, 27 de junio de 2012.

Eurostat (2020, 3 June). *Sales of pesticides in the EU.* https://ec.europa.eu/eurostat/web/products-eurostat-news/-/ddn-20200603-1

FAO (n.d.). Pesticides Use. http://www.fao.org/faostat/en/#data/RP/visualize

Fellton, H. L. (1945). The Mosquitoes of New Jersey and Their Control. *American Journal of Public Health and the Nations Health, 35*(10), 1094. shttps://doi.org/10.2105/AJPH.35.10.1094-a

García, G. P., Flores, A. E., Fernández-Salas, I., Saavedra-Rodríguez, K., Reyes-Solis, G., Lozano-Fuentes, S., Bond, J. G., Casas-Martínez, M., Ramsey, J. M., García-Rejón, J., Domínguez-Galera, M., Ranson, H., Hemingway, J., Eisen, L., & Black IV, W. C. (2009). Recent rapid rise of a permethrin knock down resistance allele in Aedes aegypti in México. *PLoS Neglected Tropical Diseases, 3*(10), article e531. https://doi.org/10.1371/journal.pntd.0000531

Gossner, C. M., Ducheyne, E., & Schaffner, F. (2018). Increased risk for autochthonous vector-borne infections transmitted by aedes albopictus in continental europe. *Eurosurveillance, 23*(24), article 1800268. https://doi.org/10.2807/1560-7917.es.2018.23.24.1800268

Gould, E. A., & Higgs, S. (2009). Impact of climate change and other factors on emerging arbovirus diseases. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 103*(2), 109-121. https://doi.org/10.1016/j.trstmh.2008.07.025

Gould, E., Pettersson, J., Higgs, S., Charrel, R., & de Lamballerie, X. (2017). Emerging arboviruses: Why today? *One Health, 4*, 1-13. https://doi.org/10.1016/j.onehlt.2017.06.001

Grandadam, M., Caro, V., Plumet, S., Thiberge, J. M., Souarès, Y., Failloux, A. B., Tolou, H. J., Budelot, M., Cosserat, D., Leparc-Goffart, I., & Desprès, P. (2011). Chikungunya virus, Southeastern France. *Emerging Infectious Diseases, 17*(5), 910-913. https://doi.org/10.3201/eid1705.101873

Gratz, N. G. (2004). Critical review of the vector status of Aedes albopictus. *Medical and Veterinary Entomology, 18*(3), 215-227. https://doi.org/10.1111/j.0269-283x.2004.00513.x

Gratz, N. G., & Jany, W. C. (1994). What role for insecticides in vector control programs? *American Journal of Tropical Medicine and Hygiene, 50*(6), 11-20. https://doi.org/10.4269/ajtmh.1994.50.6_Suppl.TM05006S0011

Gray, L., Florez, S. D., Barreiro, A. M., Vadillo-Sánchez, J., González-Olvera, G., Lenhart, A., Manrique-Saide, P., & Vazquez-Prokopec, G. M. (2018). Experimental evaluation of the impact of household aerosolized insecticides on pyrethroid resistant Aedes aegypti. *Scientific Reports, 8*, article 12535. https://doi.org/10.1038/s41598-018-30968-8

Greenberg, M., & Schneider, D. (1997). Neighborhood quality, environmental hazards, personality traits, and resident actions. *Risk Analysis, 17*(2), 169-175. https://doi.org/10.1111/j.1539-6924.1997.tb00856.x

Gupta, R. C. (ed.) (2017). *Reproductive and Developmental Toxicology*. Academic Press.

Halasa, Y. A., Shepard, D., Fonseca, D. M., Farajollahi, A., Healy, S., Gaugles, R., Bartlet-Healy, K., Strickman, D. A., & Clark, G. G. (2013). Quantifying impact of mosquitoes on quality of life. *American Journal of Tropical Medicine and Hygiene, 9*(3), article e89221. https://dx.doi.org/10.1371%2Fjournal.pone.0089221

Haynes, H. L., Lambrech, J. A., & Moorefield, H. H. (1957). Insecticidal properties and characteristics of 1-naphthyl N-methyl-carbamate. *Contributions. Boyce Thompson Institute for Plant Research, 18*(11), 507-513.

Hennessey, M., Fischer, M., & Staples, J. E. (2016). Zika Virus Spreads to New Areas - Region of the Americas, May 2015-January 2016. *American Journal of Transplantation, 16*(3), 1031-1034. https://doi.org/10.1111/ajt.13743

Hul, N. van, Braks, M., & Bortel, W. van (2021). *A systematic review to understand the value of entomological endpoints for assessing the efficacy of vector control interventions*. EFSA Supporting Publications. https://www.efsa.europa.eu/sites/default/files/2021-07/en-9984.pdf

Hurtado Clavijo, C. M., & Gutiérrez de Salazar, M. (2005). Organophosphorates: acute intoxication practical issues. *Revista Facultad de Medicina de La Universidad Nacional de Colombia, 53*(4), 244-258.

Insecticide Resistance Action Committee (IRAC) (n.d.). *Prevention and management of insecticide resistance in vectors and pests of public health importance*. https://bit.ly/3zNZ32C

Jarman, W. M., & Ballschmiter, K. (2012). From coal to DDT: The history of the development of the pesticide DDT from synthetic dyes till Silent Spring. *Endeavour, 36*(4), 131-142. https://doi.org/10.1016/j.endeavour.2012.10.003

Kawada, H., Nakazawa, S., Shimabukuro, K., Ohashi, K., Kambewa, E. A., & Pemba, D. F. (2020). Effect of metofluthrin-impregnated spatial repellent devices combined with new long-lasting insecticidal nets (Olyset® plus) on pyrethroid-resistant malaria vectors and malaria prevalence: field trial in South-Eastern Malawi. *Japanese Journal of Infectious Diseases, 73*(2), 124-131. https://doi.org/10.7883/yoken.jjid.2019.311

Kislev, M. E., Weiss, E., & Hartmann, A. (2004). Impetus for sowing and beginning of agriculture: Ground collecting of wild cereals. *Proceedings of the National Academy of Sciences of the United States of America, 101*(9), 2692-2695. https://doi.org/10.1073/pnas.0308739101

Kleinschmidt, I., Bradley, J., Knox, T. B., Mnzava, A. P., Kafy, H. T., Mbogo, C., Ismail, B. A., Bigoga, J. D., Adechoubou, A., Raghavendra, K., Cook, J., Malik, E. M., Nkuni, Z. J., Macdonald, M., Bayoh, N., Ochomo, E., Fondjo, E., Awono-Ambene, H. P., Etang, J., … & Donnelly, M. J. (2018). Implications of insecticide resistance for malaria vector control with long-lasting insecticidal nets: a WHO-coordinated, prospective, international, observational cohort study. *The Lancet Infectious Diseases, 18*(6), 640-649. https://doi.org/10.1016/S1473-3099(18)30172-5

Kraemer, M. U., Sinka, M. E., Duda, K. A., Mylne, A. Q., Shearer, F. M., Barker, C. M., Moore, C. G., Carvalho, R. G., Coelho, G. E., van Bortel, W., Hendrickx, G., Schaffner, F., Elyazar, I. R. F., Teng, H. J., Brady, O. J., Messina, J. P., Pigott, D. M., Scott, T. W., Smith, D. L., … & Hay, S. I. (2015). The global distribution of the arbovirus vectors Aedes aegypti and Ae. albopictus. *ELife, 4*, article e8347. https://doi.org/10.7554/elife.08347

Kulkarni, M. A., Malima, R., Mosha, F. W., Msangi, S., Mrema, E., Kabula, B., Lawrence, B., Kinung'hi, S., Swilla, J., Kisinza, W., Rau, M. E., Miller, J. E., Schellenberg, J. A., Maxwell, C., Rowland, M., Magesa, S., & Drakeley, C. (2007). Efficacy of pyrethroid-treated nets against malaria vectors and nuisance-biting mosquitoes in Tanzania in areas with long-term insecticide-treated net use. *Tropical Medicine and International Health, 12*(9), 1061-1073. https://doi.org/10.1111/j.1365-3156.2007.01883.x

Lloyd, A. M., Farooq, M., Diclaro, J. W., Kline, D. L., & Estep, A. S. (2013). Field evaluation of commercial off-the-shelf spatial repellents against the Asian tiger mosquito, Aedes Albopictus (Skuse), and the potential for use during deployment. *U.S. Army Medical Department Journal,* 80-86.

Lloyd, A.M., C.R. Connelly, and D.B. Carlson (2018). Florida Coordinating Council on Mosquito Control. Florida Mosquito Control: The state of the mission as defined by mosquito controllers, regulators, and environmental managers. Vero Beach, FL: University of Florida, Institute of Food and Agricultural Sciences, Florida Medical Entomology Laboratory. https://fmel.ifas.ufl.edu/media/fmelifasufledu/7-15-2018-white-paper.pdf

Lobe, J. (2006, Sept.). HEALTH: WHO Urges DDT for Malaria Control Strategies. *Inter Press Service*. http://www.ipsnews.net/2006/09/health-who-urges-ddt-for-malaria-control-strategies

Logan, J., Chen-Hussey, V., O'Halloran, L., Greaves, C., Due, C., & Macdonald, M. (2020). *An Expert Review of Spatial Repellents for Mosquito Control*. https://bit.ly/3obZF00

Lounibos, L. P. (2002). Invasions by insect vectors of human disease. *Annual Review of Entomology, 47*, 233-266. https://doi.org/10.1146/annurev.ento.47.091201.145206

Lourenço, J., & Recker, M. (2014). The 2012 Madeira dengue outbreak: epidemiological determinants and future epidemic potential. *PLoS Neglected Tropical Diseases, 8*(8), article e3083. https://doi.org/10.1371/journal.pntd.0003083

Loxdale, H. D., Lushai, G., & Harvey, J. A. (2011). The evolutionary improbability of 'generalism' in nature, with special reference to insects. *Biological Journal of the Linnean Society, 103*(1), 1-18. https://doi.org/10.1111/j.1095-8312.2011.01627.x

Lucas, J. R., Shono, Y., Iwasaki, T., Ishiwatari, T., Spero, N., & Benzon, G. (2007). U.S. Laboratory and field trials of metofluthrin (SumiOne®) emanators for reducing mosquito biting outdoors. *Journal of the American Mosquito Control Association, 23*(1), 47-54. https://doi.org/10.2987/8756-971x(2007)23[47:ulafto]2.0.co;2

Macoris, M. D. L. D. G., Andrighetti, M. T. M., Otrera, V. C. G., De Carvalho, L. R., Caldas, A. L., & Brogdon, W. G. (2007). Association of insecticide use and alteration on Aedes aegypti susceptibility status. *Memorias do Instituto Oswaldo Cruz, 102*(8), 895-900. https://doi.org/10.1590/s0074-02762007000800001

Maia, M. F., Kliner, M., Richardson, M., Lengeler, C., & Moore, S. J. (2018). Mosquito repellents for malaria prevention. *Cochrane Database of Systematic Reviews, 2*(2), article CD011595. https://doi.org/10.1002/14651858.cd011595.pub2

Marcombe, S., Paris, M., Paupy, C., Bringuier, C., Yebakima, A., Chandre, F., David, J. P., Corbel, V., & Despres, L. (2013). Insecticide-driven patterns of genetic variation in the dengue vector Aedes aegypti in Martinique Island. *PLoS ONE, 8*(10), article e77857. https://doi.org/10.1371/journal.pone.0077857

Matowo, J., Kitau, J., Kaaya, R., Kavishe, R., Wright, A., Kisinza, W., Kleinschmidt, I., Mosha, F., Rowland, M., & Protopopoff, N. (2015). Trends in the selection of insecticide resistance in Anopheles gambiae s.l. mosquitoes in northwest Tanzania during a community randomized trial of longlasting insecticidal nets and indoor residual spraying. *Medical and Veterinary Entomology, 29*(1), 51-59. https://doi.org/10.1111/mve.12090

Matsunaga, T., Makita, M., Higo, A., Nishibe, I., Dohara, K., & Shinjo, G. (1987). Studies on prallethrin, a new synthetic pyrethroid, for indoor applications : I. The insecticidal activities of prallethrin isomers. *Medical Entomology and Zoology, 8*(3), 219-223. https://doi.org/10.7601/mez.38.219

Meesters, J. A. J., Nijkamp, M. M., Schuur, A. G., & te Biesebeek, J. D. (2018). *Cleaning Products Fact Sheet* [RIVM Report 2016-0179]. National Institute for Public Health and the Environment. http://dx.crossref.org/10.21945/RIVM-2016-0179

Meyer, J. R. (2003). *Pest control tactics*. NC State University. https://projects.ncsu.edu/cals/course/ent425/text19/tactics1.html

Miller, G. (2002). *Living in the Environment* (12th ed.). Wadsworth; Thomson Learning.

Millins, C., Leo, W., MacInnes, I., Ferguson, J., Charlesworth, G., Nayar, D., Davison, R., Yardley, J., Kilbride, E., Huntley, S., Gilbert, L., Viana, M., Johnson, P., & Biek, R. (2021). Emergence of lyme disease on treeless Islands, Scotland, United Kingdom. *Emerging Infectious Diseases, 27*(2), 538-546. https://doi.org/10.3201/eid2702.203862

Moore, C. G., & Mitchell, C. J. (1997). Aedes albopictus in the United States: Ten-Year Presence and Public Health Implications. *Emerging Infectious Diseases, 3*(3), 329-334. https://doi.org/10.3201/eid0303.970309

Moyes, C. L., Vontas, J., Martins, A. J., Ng, L. C., Koou, S. Y., Dusfour, I., Raghavendra, K., Pinto, J., Corbel, V., David, J. P., & Weetman, D. (2017). Contemporary status of insecticide resistance in the major Aedes vectors of arboviruses infecting humans. *PLoS Neglected Tropical Diseases, 11*(7), article e0005625. https://doi.org/10.1371/journal.pntd.0005625

Murphy, G. (2005). *Resistance Management - Pesticide Rotation*. Ontario Ministry of Agriculture, Food and Rural Affairs.

N'Guessan, R., Corbel, V., Akogbéto, M., & Rowland, M. (2007). Reduced efficacy of insecticide-treated nets and indoor residual spraying for malaria control in pyrethroid resistance area, Benin. *Emerging Infectious Diseases, 13*(2), 199-206. https://doi.org/10.3201/eid1302.060631

National Research Council (2000). *The Future Role of Pesticides in US Agriculture*. The National Academies Press. https://doi.org/10.17226/9598

Nkemngo, F. N., Wondji, C. S., Mugenzi, L. M. J., Terence, E., Niang, A., Wondji, M. J., Tchoupo, M., Nguete, N. D., Tchapga, W., Irving, H., Ntabi, J. D. M., Agonhossou, R., Boussougou-Sambe, T. S., Akoton, R. B., Koukouikila-Koussounda, F., Pinilla, Y. T., Ntoumi, F., Djogbenou, L. S., Ghogomu, S. M., Ndo, C., … & Wondji, C. S. (2020). Multiple insecticide resistance and Plasmodium infection in the principal malaria vectors Anopheles funestus and Anopheles gambiae in a forested locality close to the Yaoundé airport, Cameroon. *Wellcome Open Research, 5*, article 146. https://doi.org/10.12688/wellcomeopenres.15818.2

Nkya, T. E., Akhouayri, I., Poupardin, R., Batengana, B., Mosha, F., Magesa, S., Kisinza, W., & David, J. P. (2014). Insecticide resistance mechanisms associated with different environments in the malaria vector Anopheles gambiae: A case study in Tanzania. *Malaria Journal, 13*, article 28. https://doi.org/10.1186/1475-2875-13-28

Nkya, T. E., Akhouayri, I., Kisinza, W., & David, J. P. (2013). Impact of environment on mosquito response to pyrethroid insecticides: Facts, evidences and prospects. *Insect Biochemistry and Molecular Biology, 43*(4), 407-416. https://doi.org/10.1016/j.ibmb.2012.10.006

Obermayr, U., Rose, A., & Geier, M. (2010). A novel test cage with an air ventilation system as an alternative to conventional cages for the efficacy testing of mosquito repellents. *Journal of Medical Entomology, 47*(6), 1116-1122. https://doi.org/10.1603/me10093

Ogoma, S. B., Lorenz, L. M., Ngonyani, H., Sangusangu, R., Kitumbukile, M., Kilalangongono, M., Simfukwe, E. T., Mseka, A., Mbeyela, E., Roman, D., Moore, J., Kreppel, K., Maia, M. F., & Moore, S. J. (2014). An experimental hut study to quantify the effect of DDT and airborne pyrethroids on entomological parameters of malaria transmission. *Malaria Journal, 13*, article 131. https://doi.org/10.1186/1475-2875-13-131

Ogoma, S. B., Mmando, A. S., Swai, J. K., Horstmann, S., Malone, D., & Killeen, G. F. (2017). A low technology emanator treated with the volatile pyrethroid transfluthrin confers long term protection against outdoor biting vectors of lymphatic filariasis, arboviruses and malaria. *PLoS Neglected Tropical Diseases, 11*(4), e0005455. https://doi.org/10.1371/journal.pntd.0005455

Ogoma, S. B., Ngonyani, H., Simfukwe, E. T., Mseka, A., Moore, J., & Killeen, G. F. (2012). Spatial repellency of transfluthrin-treated hessian strips against laboratory-reared Anopheles arabiensis mosquitoes in a semi-field tunnel cage. *Parasites and Vectors, 5*, article 54. https://doi.org/10.1186/1756-3305-5-54

Pates, H. V., Lines, J. D., Keto, A. J., & Miller, J. E. (2002). Personal protection against mosquitoes in Dar es Salaam, Tanzania, by using a kerosene oil lamp to vaporize transfluthrin. *Medical and Veterinary Entomology, 16*(3), 277-284. https://doi.org/10.1046/j.1365-2915.2002.00375.x

Paupy, C., Delatte, H., Bagny, L., Corbel, V., & Fontenille, D. (2009). Aedes albopictus, an arbovirus vector: From the darkness to the light. *Microbes and Infection, 11*(14-15), 1177-1185. https://doi.org/10.1016/j.micinf.2009.05.005

Powell, J. R., & Tabachnick, W. J. (2013). History of domestication and spread of Aedes aegypti--a review. *Memórias do Instituto Oswaldo Cruz, 108*(Suppl. 1), 11-17. https://doi.org/10.1590/0074-0276130395

Protopopoff, N., Matowo, J., Malima, R., Kavishe, R., Kaaya, R., Wright, A., West, P. A., Kleinschmidt, I., Kisinza, W., Mosha, F. W., & Rowland, M. (2013). High level of resistance in the mosquito *Anopheles gambiae* to pyrethroid insecticides and reduced susceptibility to bendiocarb in north-western Tanzania. *Malaria Journal, 12*, article 149. https://doi.org/10.1186/1475-2875-12-149

Ranson, H. (2017). Current and future prospects for preventing malaria transmission via the use of insecticides. *Cold Spring Harbor Perspectives in Medicine, 7*(11), article a026823. https://doi.org/10.1101/cshperspect.a026823

Relich, R. F., & Grabowski, J. M. (2020). Tick-Borne Viruses of North America. *Clinical Microbiology Newsletter, 42*(10), 79-86. http://dx.doi.org/10.1016/j.clinmicnews.2020.05.001

Resh, V. H. (2009). *Encyclopedia of Insects*. *Encyclopedia of Insects* (2nd ed.). Academic Press.

Revay, E. E., Junnila, A., Xue, R. D., Kline, D. L., Bernier, U. R., Kravchenko, V. D., Qualls, W. A., Ghattas, N., & Müller, G. C. (2013a). Evaluation of commercial products for personal protection against mosquitoes. *Acta Tropica, 125*(2), 226-230. https://doi.org/10.1016/j.actatropica.2012.10.009

RMS: the Netherlands (2014). *Product assessment report of a biocidal product for national authorisation applications* [Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products. Case Number in R4BP: BC-NH027167-40]. https://echa.europa.eu/documents/10162/6f1a5175-f1eb-4e6b-4b49-8cea13efcf5a

Rochlin, I., Faraji, A., Healy, K., & Andreadis, T. G. (2019). West Nile Virus Mosquito Vectors in North America. *Journal of Medical Entomology, 56*(6), 1475-1490. https://doi.org/10.1093/jme/tjz146

Rocklöv, J., Quam, M. B., Sudre, B., German, M., Kraemer, M. U. G., Brady, O., Bogoch, I. I., Liu-Helmersson, J., Wilder-Smith, A., Semenza, J. C., Ong, M., Aaslav, K. K., & Khan, K. (2016). Assessing Seasonal Risks for the Introduction and Mosquito-borne Spread of Zika Virus in Europe. *EBioMedicine, 9*, 250-256. https://doi.org/10.1016/j.ebiom.2016.06.009

Roiz, D., Wilson, A. L., Scott, T. W., Fonseca, D. M., Jourdain, F., Müller, P., Velayudhan, R., & Corbel, V. (2018). Integrated Aedes management for the control of Aedes-borne diseases. *PLoS Neglected Tropical Diseases, 12*(12), article e0006845. https://doi.org/10.1371/journal.pntd.0006845

Rudolf, I., Blažejová, H., Straková, P., Šebesta, O., Peško, J., Mendel, J., Šikutová, S., Hubálek, Z., Kampen, H., & Schaffner, F. (2018). The invasive Asian tiger mosquito *Aedes albopictus* (Diptera: Culicidae) in the Czech Republic: Repetitive introduction events highlight the need for extended entomological surveillance. *Acta Tropica, 185*, 239-245. https://doi.org/10.1016/j.actatropica.2018.05.020

Ruiz-Moreno, D., Vargas, I. S., Olson, K. E., & Harrington, L. C. (2012). Modeling Dynamic Introduction of Chikungunya Virus in the United States. *PLoS Neglected Tropical Diseases, 6*(11), article e1918. https://doi.org/10.1371/journal.pntd.0001918

Seyler, T., Grandesso, F., Strat, Y. L., Tarantola, A., & Depoortere, E. (2009). Assessing the risk of importing dengue and chikungunya viruses to the European Union. *Epidemics, 1*(3), 175-184. https://doi.org/10.1016/j.epidem.2009.06.003

Sharma, A., Kumar, V., Shahzad, B., Tanveer, M., Sidhu, G. P. S., Handa, N., Kohli, S. K., Yadav, P., Bali, A. S., Parihar, R. D., Dar, O. I., Singh, K., Jasrotia, S., Bakshi, P., Ramakrishnan, M., Kumar, S., Bhardwaj, R., & Thukral, A. K. (2019). Worldwide pesticide usage and its impacts on ecosystem. *SN Applied Sciences, 1*, article 1446. https://doi.org/10.1007/s42452-019-1485-1

Shirai, Y., Funada, H., Kamimura, K., Seki, T., & Morohashi, M. (2002). Landing sites on the human body preferred by aedes albopictus. *Journal of the American Mosquito Control Association, 18*(2), 97-99.

Silberman, J., & Taylor, A. (2018). *Carbamate Toxicity*. StatPearls.

Soderlund, D. M., & Bloomquist, J. R. (1989). Neurotoxic actions of pyrethroid insecticides. *Annual Review of Entomology, 34*, 77-96. https://doi.org/10.1146/annurev.en.34.010189.000453

Stent, V. (2006, 24 January). *The history of pest control*. Ezine Articles. http://ezinearticles.com/?The- History-of-Pest-Control&id=133689

Syafruddin, D., Asih, P. B. S., Rozi, I. E., Permana, D. H., Hidayati, A. P. N., Syahrani, L., Zubaidah, S., Sidik, D., Bangs, M. J., Bøgh, C., Liu, F., Eugenio, E. C., Hendrickson, J., Burton, T., Baird, J. K., Collins, F., Grieco, J. P., Lobo, N. F., & Achee, N. L. (2020). Efficacy of a spatial repellent for control of malaria in Indonesia: A cluster-randomized controlled trial. *American Journal of Tropical Medicine and Hygiene, 103*(1), 344-358. https://doi.org/10.4269/ajtmh.19-0554

Turner, J. A. (ed.) (2015). *A World Compendium: The Pesticide Manual* (17th ed.). British Crop Production Council.

Turusov, V., Rakitsky, V., & Tomatis, L. (2002). Dichlorodiphenyltrichloroethane (DDT): Ubiquity, persistence, and risks. *Environmental Health Perspectives, 110*(2), 125-128. https://doi.org/10.1289/ehp.02110125

Umetsu, N., & Shirai, Y. (2020). Development of novel pesticides in the 21st century. *Journal of Pesticide Science, 45*(2), 54-74. https://doi.org/10.1584/jpestics.d20-201

United States Environmental Protection Agency (EPA) (2010). *EPA product Performance test Guidelines* [OPPTS 810.3700]. *Insect Repellents to be applied to human skin*.

http://nepis.epa.gov

United States Environmental Protection Agency (EPA) (n.d.-a). *EPA Pesticides*. https://www.epa.gov/minimum-risk-pesticides/what-pesticide

United States Environmental Protection Agency (EPA) (n.d.-b). *Integrated Pest Management (IPM) Principles*. https://www.epa.gov/safepestcontrol/integrated-pest-management-ipm-principles

United States Environmental Protection Agency (EPA) (n.d.-c). *Regulatory and Guidance Information by Topic: Pesticides*. https://www.epa.gov/regulatory-information-topic/regulatory-and-guidance-information-topic-pesticides

Unsworth, J. (2010, 10 May). *History of Pesticide Use*. International Union of Pure and Applied Chemistry. https://bit.ly/3zL8Wyj

Unsworth, J., Nakagawa, Y., Harris, C., & Kleter, G. (2019). The Battelle for a Sustainable Food Supply. In *Sustainable Agrochemistry* (Ed. V. Silvio, pp. 13-84). Springer.

Van den Berg, H., Zaim, M., Yadav, R. S., Soares da Silva, A., Ameneshewa, B., Mnzava, A., Hii, J., Dash, A. P., & Ejov, M. (2012). Global trends in the use of insecticides to control vector-borne diseases. *Environmental Health Perspectives, 120*(4), 577-582. http://dx.doi.org/10.1289/ehp.1104340

Vélez, S. (2006). Evolution of the Insects [Review of the book *Evolution of the Insects,* by D. Grimaldi & M. S. Engel]. *Ecoscience, 13*(2), 290. http://dx.doi.org/10.2980/1195-6860(2006)13[290:EOTI]2.0.CO;2

Vigilant, M., Battle-Freeman, C., Braumuller, K. C., Riley, R., & Fredregill, C. L. (2020). Harris county public health mosquito and vector control division emergency response to hurricane harvey: Vector-borne disease surveillance and control. *Journal of the American Mosquito Control Association, 36*(2), 15-27. https://doi.org/10.2987/19-6890.1

Wagman, J. M., Achee, N. L., & Grieco, J. P. (2015). Insensitivity to the spatial repellent action of transfluthrin in Aedes aegypti: a heritable trait associated with decreased insecticide susceptibility. *PLoS Neglected Tropical Diseases, 9*(4), article e0003726. https://doi.org/10.1371/journal.pntd.0003726

Weaver, S. C., & Reisen, W. K. (2010). Present and future arboviral threats. *Antiviral Research, 85*(2), 328-345. https://doi.org/10.1016/j.antiviral.2009.10.008

Wilke, A. B. B., Caban-Martinez, A. J., Ajelli, M., Vasquez, C., Petrie, W., & Beier, J. C. (2019). Mosquito Adaptation to the Extreme Habitats of Urban Construction Sites. *Trends in Parasitology, 35*(8), 607-614. https://doi.org/10.1016/j.pt.2019.05.009

Wilks, M. F. (2005). Pyrethroids: mechanisms of toxicity, features and management. *Clinical Toxicology, 43*, 423-425.

World Health Organization (2003). *Draft: guidelines on the management of public health pesticides: report of the WHO Interregional Consultation, Chiang Mai, Thailand 25-28 February 2003* [WHO/CDS/WHOPES/2003.7]. World Health Organization. https://bit.ly/3ALv10V

World Health Organization (2009). *Guidelines for efficacy testing of mosquito repellents for human skin* [WHO/HTM/NTD/WHOPES/2009.4]. World Health Organization. https://bit.ly/2Y0ExPq

World Health Organization (2017). *How to design vector control efficacy trials: guidance on phase III vector control field trial design provided by the Vector Control Advisory Group* [WHO/HTM/NTD/VEM/2017.03]. World Health Organization. https://bit.ly/2WlqiUM

World Health Organization & WHO Pesticide Evaluation Scheme (2005). *Safety of pyrethroids for public health use* [WHO/CDS/WHOPES/GCDPP/2005.10; WHO/PCS/RA/2005.1]. World Health Organization. https://bit.ly/3m1lNaA

World Health Organization & WHO Pesticide Evaluation Scheme (2013). *Guidelines for Efficacy Testing of Spatial Repellents*. World Health Organization. https://bit.ly/3kMq4iV

Yadouleton, A. W. M., Asidi, A., Djouaka, R. F., Brama, J., Agossou, C. D., & Akogbeto, M. C. (2009). Development of vegetable farming: A cause of the emergence of insecticide resistance in populations of Anopheles gambiae in urban areas of Benin. *Malaria Journal, 8*, article 103. https://doi.org/10.1186/1475-2875-8-103

Yap, H. H., Lim, M. P., Chong, N. L., & Lee, C. Y. (1996). Efficacy and sublethal effects of mosquito coils on Aedes aegypti and Culex quinquefasciatus (Diptera: Culicidae). In *Proceedings of the Second International Conference on Urban Pests* (pp. 177-184). K. B. Wildey.

Zaim, M., Aitio, A., & Nakashima, N. (2000). Safety of pyrethroid-treated mosquito nets. *Medical and Veterinary Entomology, 14*(1), 1-5. https://doi.org/10.1046/j.1365-2915.2000.00211.x

Zelle, H., Marrama, L., Sudre, B., van Bortel, W., & Warns-Petit, E. (2013). Dengue: A concern for Europe? *Tropical Medicine and International Health, 18*(Suppl 1), 9-10. https://doi.org/10.1111/tmi.12161

ANNEXES

Annex 1. Document of agreement between the co-authors of articles when the thesis is presented as a compendium of publications

Dr Miguel Ángel Miranda as co-author of the following articles

1- From the Field to the Laboratory: Quantifying Outdoor Mosquito Landing Rate to Better Evaluate Topical Repellents. Mara Moreno-Gómez, Rubén Bueno-Marí and Andrea Drago *Journal of Medical Entomology* Volume 58, Issue 3, May 2021, Pages 1287–1297 https://doi.org/10.1093/jme/tjaa298

2- Two New Alternatives to the Conventional Arm-in-Cage Test for Assessing Topical Repellents. Mara Moreno-Gómez, Rubén Bueno-Marí, B Thomas Carr, Gary R. Bowman, Genevieve W. Faherty, Carlota Gobbi, Julie M. Palm and Petra Van Sloun *Journal of Medical Entomology* Volume 58, Issue 4, July 2021, Pages 1826–1838 https://doi.org/10.1093/jme/tjab050

3- A Three-Pronged Approach to Studying Sublethal Insecticide Doses: Characterising Mosquito Fitness, Mosquito Biting Behaviour, and Human/Environmental Health Risks, Mara Moreno-Gómez and Rubén Bueno-Marí *Insects* June 2021, *12*, 546. https://doi.org/10.3390/insects12060546

4- To Kill or to Repel Mosquitoes? Two Strategies for Protecting Humans and Reducing Vector-Borne Disease Risks. Mara Moreno-Gómez and Rubén Bueno-Marí Pathogens September 2021, *10*(9), 1171; https://doi.org/10.3390/pathogens10091171

I DECLARE:

Accepts that Ms. Mara Moreno presents the cited articles as the principal author and as a part of her doctoral thesis and that said articles cannot, therefore, form part of any doctoral thesis.

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Dr Rubén Bueno Marí as co-author of the following articles

1- From the Field to the Laboratory: Quantifying Outdoor Mosquito Landing Rate to Better Evaluate Topical Repellents. Mara Moreno-Gómez, Andrea Drago and Miguel A. Miranda. *Journal of Medical Entomology* Volume 58, Issue 3, May 2021, Pages 1287–1297 https://doi.org/10.1093/jme/tjaa298

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Dr Andrea Drago as co-author of the following article:

1- From the Field to the Laboratory: Quantifying Outdoor Mosquito Landing Rate to Better Evaluate Topical Repellents. Mara Moreno-Gómez, Rubén Bueno-Marí and Miguel A. Miranda- doi: 10.1093/jme/tjaa298

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Ponte San Nicolò- Padova - Italy, 27.04.2021

Universitat de les Illes Balears

Dr Petra Van Sloun as co-author of the following articles:

1- Two New Alternatives to the Conventional Arm-in-Cage Test for Assessing Topical Repellents. Mara Moreno-Gómez, Rubén Bueno-Marí, B Thomas Carr, Gary R. Bowman, Genevieve W. Faherty, Carlota Gobbi, Julie M. Palm and Miguel Ángel Miranda.
doi: 10.1093/jme/tjab050

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i.V. Dr. Petra Van Sloun
Head of Product Compliance EU – Surface Solutions
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Darmstadt, Germany, 28 April 2021

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Mr. B Thomas Carr as co-author of the following article:

1- Two New Alternatives to the Conventional Arm-in-Cage Test for Assessing Topical Repellents. Mara Moreno-Gómez, Rubén Bueno-Marí, Genevieve W. Faherty, Carlota Gobbi and Julie M. Palm, Petra Van Sloun and Miguel Ángel Miranda.
doi: 10.1093/jme/tjab050

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Signature

B. Thomas Carr

Palma de Mallorca, 27-April-2021

Universitat de les Illes Balears

Dr Gary R. Bowman as co-author of the following articles:

1- Two New Alternatives to the Conventional Arm-in-Cage Test for Assessing Topical Repellents. Mara Moreno-Gómez, Rubén Bueno-Marí, B Thomas Carr, Genevieve W. Faherty, Carlota Gobbi and Julie M. Palm, Petra Van Sloun and Miguel Ángel Miranda. doi: 10.1093/jme/tjab050

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Signature

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1- Two New Alternatives to the Conventional Arm-in-Cage Test for Assessing Topical Repellents. Mara Moreno-Gómez, Rubén Bueno-Marí, B Thomas Carr, Carlota Gobbi and Julie M. Palm, Petra Van Sloun and Miguel Ángel Miranda.
doi: 10.1093/jme/tjab050

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Kirby, Vermont, USA, 27 April, 2021

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Dr Julie M. Palm as co-author of the following articles:

1- Two New Alternatives to the Conventional Arm-in-Cage Test for Assessing Topical Repellents. Mara Moreno-Gómez, Rubén Bueno-Marí, B Thomas Carr, Gary R. Bowman, Genevieve W. Faherty, Carlota Gobbi and Petra Van Sloun and Miguel Ángel Miranda. doi: 10.1093/jme/tjab050

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Palma de Mallorca, [date] April, 28 2021

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Dr Carlotta Gobbi as co-author of the following article:

1- Two New Alternatives to the Conventional Arm-in-Cage Test for Assessing Topical Repellents. Mara Moreno-Gómez, Rubén Bueno-Marí, B Thomas Carr, Gary R. Bowman, Genevieve W. Faherty, Julie M. Palm and Miguel Ángel Miranda.
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Ravenna, 28/04/2021

Annex 2. Article 4: Supplementary material

Table S1. Parameters used in the Scenario 1 ConsExpo model

Parameter		Value
AS concentration (% [w/w])		0.104
Potential body exposure (mg/min)[1]		45.20
Potential hand exposure (mg/min)[1]		64.7
Potential inhalation exposure (mg/m^3)[1]		35.9
Inhalation/respiration rate (m^3/h)[2]		1.25
Application duration (s)[3]		9
Dermal absorption (%)[4]	TFT	50
Body mass (kg)[2]		60

[1] Default BHHEM value (European Chemicals Agency, 2015)

[2]Recommendation no. 14 of the Biocidal Products Committee (BPC) Ad hoc Working Group on Human Exposure—default values when assessing human exposure to biocidal products (ECHA, 2017)

[3]Recommended spray duration based on mode of use

[4]Default value based on EFSA guidance on dermal absorption (Buist *et al.*, 2017)

Table S2. Parameters used in the Scenario 2 ConsExpo model

Parameters		Value
AS concentration (% [w/w])		0.104
Vapour pressure (kPa)	TFT	9 x 10^{-7}
Molar mass (g/mol)	TFT	371
Parameters in the exposure to vapour: evaporation model	Product amount (g)[1]	12.3
	Emission duration (h)[1]	24
	Exposure duration (min)[1]	720
	Room volume (m^3)[2]	30
	Ventilation rate (1/h)[2]	0.5
	Mass transfer coefficient (m/hr)[3]	10
	Release area (m^2)[2]	12
Body mass (kg)[4]	Adult	60
	Toddler	10

[1]Product-specific information

[2]Pest Control Products Fact Sheet (Delmaar *et al.*, 2017)

[3]Cleaning Products Fact Sheet—2018 update (Meesters *et al.*, 2018)

[4]Recommendation no. 14 of the BPC Ad hoc Working Group on Human Exposure—default values when assessing human exposure to biocidal products (ECHA, 2017)

Table S3. Parameters used in the Scenario 3 ConsExpo model

Parameters		Value
AS concentration (% [w/w])		0.104
Dislodgeable residues[1] (mg/cm²)	Outdoors	0.0574
Skin surface area (cm²)[2]	Adult	9,550
	Toddler	2,419.2
Surface contamination (%)		100
Dermal absorption (%)[3]		70
Body mass (kg)[4]	Adult	60
	Toddler	10

[1]Default values from ECHA Technical Notes for Guidance Part 2 (p. 257; in cases of non-professional use and residential exposure to biocides) (European Commission, 2002)

[2]Based on recommendation no. 14 of the BPC Ad hoc Working Group on Human Exposure (ECHA, 2017), where skin surface areas were calculated as follows:

- adults: 820 cm² for hands + 2,270 cm² for arms + 1,130 cm² for feet + 5,330 cm² for legs = 9,550 cm²
- toddlers: 230.4 cm² for hands + 681.6 cm² for arms + 288 cm² for feet + 1,219.2 cm² for legs = 2,419.2 cm²

[3]Default value from EFSA guidance on dermal absorption (Buist *et al.*, 2017)

[4]Recommendation no. 14 of the BPC Ad hoc Working Group on Human Exposure—default values when assessing human exposure to biocidal products (ECHA, 2017)

Table S4. Parameters used in the Scenario 4 ConsExpo model

Parameters		Value
AS concentration (% [w/w])		0.104
Dislodgeable residues[1] (mg/cm2)	Outdoors	0.0574
Surface area of fingertips (cm²)[2]		4
Efficiency of removal by saliva from skin (%)[3]		50
Number of hand-to-mouth contacts (per day)[3]		36
Oral absorption (%)[4]		100
Body mass (kg)[5]	Toddler	10

[1]Default values from ECHA Technical Notes for Guidance Part 2 (p. 257; in cases of non-professional use and residential exposure to biocides) (European Commission, 2002)

[2]According to the Do-It-Yourself Products Fact Sheet (RIVM report 320104007/2007; assuming a fingertip surface area of 1 cm²) and utilising four fingers as a worst-case situation (Burg *et al.*, 2007)

[3]Camann *et al.*, 2000. Evaluation of Saliva and Artificial Salivary Fluids for Removal of Pesticide Residues from Human Skin. Final Report to EPA by ManTech (Contract 68-D5-0049) (Camann *et al.*, 2000)

[4]TFT Assessment Report NL (2014) (RMS: the Netherlands, 2014)

[5]Recommendation no. 14 of the BPC Ad hoc Working Group on Human Exposure—default values when assessing human exposure to biocidal products (ECHA, 2017)

Annex 3. International Congresses: Oral presentations and Awards

- **March 10–14, 2019**. Spatial repellents, insecticides, and their role in human protection. 9th Annual International Conference of the European Mosquito Control Association (EMCA), La Rochelle, France. Theme: Mosquito Control Without Borders.
- **October 28–30, 2019**. Mosquito biting pressure as a parameter linking field and laboratory testing. Awarded 1st prize in the category Microbiology, Parasitology, and Vectors. 11th Annual Conference of the Spanish Society for Tropical Medicine and International Health (SEMTSI), Ávila, Spain. Theme: Global Health Challenges.