VNIVERSITAT DE VALÈNCIA

FACULTAD DE FARMACIA
Departamento de Biología Celular y Parasiología

TESIS DOCTORAL INTERNACIONAL

EVALUACIÓN DE LA EFECTIVIDAD DE UNA NUEVA PINTURA INSECTICIDA CONTRA LOS VECTORES DE LA MALARIA

EVALUATION OF THE EFFICACY OF A NEW INSECTICIDE PAINT AGAINST MALARIA VECTORS

Beatriz Mosqueira Marín

Directores

Prof. Dr. Dr.h.c. Santiago Mas Coma
Catedratico
Departamento de Biología Celular y Parasitología,
Facultad de Farmacia, Universidad de Valencia

Dr. Pierre Carnevale
Director de Investigación de Clase Excepcional
Institut de Recherche pour le Développement, France

VALENCIA, 2015

AREA DE PARASITOLOGIA
DEPARTAMENTO DE BIOLOGIA CELULAR Y PARASITOLOGIA
FACULTAT DE FARMACIA, UNIVERSITAT DE VALENCIA
Av. Vicent Andrés Estellés s/n
46100 Burjassot, Valencia
Tel.: 963544298
Fax: 963544769

El Prof. Dr. Dr.h.c. SANTIAGO MAS-COMA, Catedrático de Parasitología del Departamento de Biología Celular y Parasitología de la Facultad de Farmacia de la Universitat de Valencia, y el Dr. PIERRE CARNEVALE, Director de Investigación de Clase Excepcional del l'Institut de Recherche pour le Développement, por la presente:

CERTIFICAN:

Que Doña BEATRIZ MOSQUEIRA MARIN ha realizado el trabajo experimental titulado "Evaluación de la efectividad de una nueva pintura insecticida contra los vectores de la malaria" en el Departamento antedicho en la Facultad de Farmacia de la Universitat de València bajo su dirección y con el fin de optar al Grado de Doctor.

Y para que así conste a los efectos oportunos, firman la presente en Valencia, a 22 de Mayo de 2015.

Fdo.:
Prof. Dr. Dr.h.c. Santiago Mas-Coma

Fdo.:
Dr. Pierre Carnevale

A MIS PADRES, HERMANOS Y HERMANA,
A MIS HIJOS,
A MI COMPAÑERO

The results appearing in this thesis have been or will be published in the following articles (please refer to the **Annex**):

Mosqueira, B., Duchon, S., Chandre, F., Hougard, J.-M., Carnevale, P., & Mas-Coma, S. (2010a). Efficacy of an insecticide paint against insecticide-susceptible and -resistant mosquitoes, Part 1: Laboratory evaluation. *Malaria Journal*, *9*(1).

Mosqueira, B., Chabi, J., Chandre, F., Akogbeto, M., Hougard, J.-M., Carnevale, P., & Mas-Coma, S. (2010b). Efficacy of an insecticide paint against malaria vectors and nuisance in West Africa, Part 2: Field evaluation. *Malaria Journal*, *9*, 341.

Mosqueira, B., Chabi, J., Chandre, F., Akogbeto, M., Hougard, J.-M., Carnevale, P., & Mas-Coma, S. (2013). Proposed use of spatial mortality assessments as part of the pesticide evaluation scheme for vector control. *Malaria Journal*, *12*, 366.

Mosqueira, B., Soma, S.D., Namountougou, M., Poda, S., Diabaté, A., Ali, O., Fournet, F., Baldet, T., Carnevale, P., Dabiré, K.R., & Mas-Coma, S. (2015). Pilot study on the combination of an organophosphate-based insecticide paint and pyrethroid-treated long lasting nets against pyrethroid-resistant malaria vectors in Burkina Faso. *Acta Tropica*, *148*, 162–169.

Mosqueira, B., Poda, S., Namountougou, M., Soma, S.D., Ali, O., Diabaté, A., Fournet, F., Baldet, T., Carnevale, P., Mas-Coma, S., & Dabiré, K.R. Testing of the house-proof approach against malaria vectors resistant to pyrethroids using an organophosphate-based paint applied on windows and doors under real life conditions in Vallée du Kou, Burkina Faso (West Africa). In prep.

Mosqueira, B. (2015). Evaluation of the efficacy of a new insecticide paint against malaria vectors. PhD (Directors S. Mas-Coma & P. Carnevale), Faculty of Pharmacy, University of Valencia, 182 pp. + Annexes.

ABSTRACT

Malaria continues to be a serious public health concern worldwide. An estimated 3.3 billion people in 97 countries and territories are at risk of malaria, and 1.2 billion are at high risk (WHO, 2014). Mosquitoes are the main malaria parasite vector; and control on a large scale is essentially achieved using Long-Lasting Insecticide-Treated Nets (LLINs) and, to a lesser extent, Indoor Residual Spraying (IRS).

All recommended LLINs and most IRS interventions consist of the use of pyrethroids. However, the increased resistance of malaria vectors to pyrethroids reported in many African countries—along with operational constraints to LLINs and IRS, such as inadequate use, or the need of specialized equipment—suggest the need to study the potential of different products and strategies. To this end, a new product has been tested: the insecticide paint Inesfly 5A IGR, which contains two organophosphates (OPs), chlorpyrifos (1.5%), and diazinon (1.5%); plus an Insect Growth Regulator (IGR), pyriproxyfen (0.063%).

Inesfly 5A IGR was tested in a series of laboratory studies performed in France and several African countries, following Phase I of the WHO Pesticide Evaluation Scheme (WHOPES) procedures in Côte d'Ivoire. The first tests showed the importance of the porosity of the materials to which the paint is applied.

The next Phase I tests were performed in the laboratory of the Laboratoire de Lutte contres des Insectes Nuisibles (LIN) in France. The mosquitoes used were two strains of the urban pest mosquito *Culex quinquefasciatus* (Say, 1823): one susceptible to OPs, and one resistant to them (a homozygote for the ace-1R gene resistant to OPs and carbamates). One year after treatment with insecticide paint (IP), delayed mortality was still 93–100%—even for OP-resistant females, and even on non-porous surfaces such as hard plastic or softwood. On porous surfaces such as cement, death rates were still low 12 months after treatment, regardless of dose or resistance status. Fecundity, fertility and adult emergence were reduced right after treatment even at the lower dose ($p<10–3$), since females exposed to the higher dose during bioassays did not survive in enough numbers. A reduction in fecundity was still observed 9 months after treatment at both doses ($p<10–3$), and adult emergence was reduced at the higher dose ($p<10–3$).

Phase II field tests were performed in experimental huts in the field in Bénin, against populations of *Anopheles gambiae* (Giles, 1902) and *Cx. quinquefasciatus*. Both populations were resistant to pyrethroids with high knockdown resistance (*kdr*) frequencies. In the field, 6 months after treatment with Inesfly 5A IGR, mortality rates of both pyrethroid-resistant mosquito strains were still 90–100% on porous cement surfaces. Nine months after treatment, mortality rates in experimental huts treated with 2 layers of insecticide paint were still about 90–93% against pyrethroid-resistant *An. gambiae*, and 55% against resistant *Cx. quinquefasciatus*.

A parallel study assessed spatial mortality, with the goal of developing a model to assess the killing effect of insecticide products at a distance. This test, performed both in the laboratory and in the field, obtained high long-term mortality (96–100%) for 12 months in the field against pyrethroid-susceptible *An. gambiae* and *Cx. quinquefasciatus*. The mosquitoes never came in direct contact with treated surfaces, being kept at a distance of at least 1 metre overnight. Suggestions to include these tests as part of WHOPES were published accordingly.

Based on the good results obtained during Phase I and II studies, and in an effort to support LLIN-based public health initiatives, two pilot studies were performed in the field to assess the efficacy of Inesfly 5A IGR in combination with pyrethroid-treated LLINs. The studies took place in houses in two villages in the Kou Valley, in Burkina Faso, VK1 and VK3. In the VK1 village, house interiors were treated with 1 or 2 layers of insecticide paint on different surfaces: walls alone, or walls plus ceiling. In the VK3 village, only windows and doors were treated, with only 1 layer of paint.

The VK1results showed that where houses were treated and LLINs were used, the combination yielded a long-term mortality rate of 80% over 12 months against *Anopheles coluzzii* (Coetzee & Wikerson, 2013), the local pyrethroid-resistant malaria vector. But at VK3, treating windows and doors alone yielded a killing efficacy of 80% for only 2 months against *An. coluzzii*. In entomological terms, these pilot studies provided useful information to conclude that treating walls and ceilings, not just doors and windows, is needed for the forthcoming large-scale WHOPES Phase III evaluation.

In terms of preparation for the WHOPES Phase III study, 32 sites met the four socio-epidemiological criteria for inclusion: villages had at least 100 children, from 6 months

to 14 years old (to ensure at least 30 evaluable subjects at the end of the study); villages were at least 1–2 km from each other; villages could be accessed by road; and residents had expressed an interest in participating.

The Phase III study will assess what impact the combination of treatments—insecticide paint applied to house interiors, and pyrethroid-treated LLINs—may have on reducing the incidence of malaria, in a West African area where malaria is holoendemic, and vectors are resistant to pyrethroids with high *kdr* frequencies. The specific targets of the study will be children aged 6 months to 14 years.

Key Words: *Anopheles gambiae*, *Anopheles coluzzii*, *Culex quinquefasciatus*, insecticide-treated nets, long-lasting insecticide nets, indoor residual spraying, pyrethroids, organophosphates, chlorpyrifos, diazinon, pyriproxyfen, insect growth regulator, WHOPES, experimental huts, insecticide resistance, malaria vector control, pest, vector control, Côte d'Ivoire, France, Bénin, Burkina Faso, West Africa.

RESUMEN

La malaria continúa siendo un problema de salud pública global. Se calcula que en el mundo hay 3.300 millones de personas en 97 países y territorios que corren el riesgo de padecer el paludismo, y que para 1.200 millones ese riesgo es elevado (WHO, 2014). El control de la malaria a gran escala está altamente basado en al control del vector, principalmente mediante el uso de mosquiteras tratadas con insecticidas de larga duración (LLINs), y en menor medida, el Rociamiento Residual Intradomiciliario.

Todos los tratamientos recomendados de LLINs y la mayoría del Rociamiento Residual Intradomiciliario se basan en el uso de piretroides. La creciente resistencia de los vectores de malaria a los piretroides detectada en muchos países de África, así como las limitaciones operativas de los LLINs y el Rociamiento Residual Intradomiciliario, como el uso inadecuado y la necesidad de equipo especializado, respectivamente, señalan la conveniencia de investigar el potencial de productos y estrategias diferentes frente a los vectores del parásito de la malaria. Con este propósito, se ha estudiado la pintura insecticida Inesfly 5A IGR™, que contiene dos organofosfatos (OPs), clorpírifos (1.5%) y diazinón (1.5%) y un IGR piriproxifeno (0.063%), en una serie de proyectos realizados en Costa de Marfil, Francia, Benín y Burkina Faso: Las primeras pruebas con Inesfly 5A IGR™ se realizaron en el laboratorio siguiendo los protocolos de Fase I del Plan de Evaluación de Pesticidas de la OMS (WHOPES) y mostraron la importancia de la porosidad de los materiales en la eficacia a largo plazo. Las siguientes pruebas se realizaron en el laboratorio en el Insituto LIN en Francia (Fase I) con cepas resistentes y susceptibles a los OPs del mosquito plaga urbano *Culex quinquefaciatus* (Say, 1823). La cepa resistente era homozigota para el gen de resistencia ace-1R involucrado en la resistencia a los OPs.

En el laboratorio, un año tras el tratamiento con Inesfly 5A IGR™, la mortalidad diferida se matuvo elevada, 93–100%, incluso contra las cepa resistente a los OPs sobre superficies no porosas como el plástico y la madera. En superficies porosas como el cemento, las tasas de mortalidad fueron bajas 12 meses tras el tratamiento independientemente de la dosis y el nivel de resistencia. Las tasas de fecundidad, fertilidad y emergencia de adultos se vieron reducidas justo después del tratamiento a la dosis menor ($p < 10–3$), ya que a la dosis mayor las hembras expuestas a los bioensayos no sobrevivieron en suficiente número. Nueve meses tras el tratamiento,

la tasa de fecundidad se redujo en ambas dosis ($p < 10–3$), y la tasa de emergencia se redujo en la dosis mayor ($p < 10–3$).

Las evaluaciones en Fase II se realizaron en casas experimentales en el terreno en Bénin contra poblaciones del principal vector del parásito de la malaria, *Anopheles gambiae* Giles, 1902, y *Cx. quinquefasciatus*. Ambas poblaciones, *An. gambiae* y *Cx. quinquefasciatus*, son resistentes a los piretrinoides con elevadas frecuencias de *kdr*. En el terreno, seis meses tras el tratamiento con Inesfly 5A IGR™, las tasas de mortalidad se mantuvieron a 90–100% en superficies no porosas de cemento, en poblaciones de mosquitos resistentes a pirotrides. Nueve meses después del tratamiento, las tasas de mortalidad en las casas experimentales tratadas con dos capas de pintura insecticida todavía ascendía al 90–93% en *An. gambiae* y un 55% en *Cx. quinquefasciatus*, ambos resistentes a piretroides.

La evaluación de la mortalidad espacial ha sido el tema de un estudio paralelo para desarrollar un modelo de valoración sobre el efecto letal que pueden tener los productos insecticidas en la distancia, tanto en el laboratorio como sobre el terreno, a estos tests les hemos denominado evaluación de la mortalidad espacial. Durante 12 meses, una elevada mortalidad espacial se observó a largo plazo (96–100%) contra poblaciones de *An. gambiae* y *Cx. quinquefasciatus* susceptibles a los piretrinoides colocados a distancias de un metro durante la noche, sin entrar en contacto directo con las superficies tratadas. Recomendaciones para añadir la evaluación de la mortalidad espacial a la batería de tests comúnmente realizados en los protocolos WHOPES fueron publicadas.

Basado en los buenos resultados obtenidos en los estudios de las Fases I y II, y con objeto de apoyar las iniciativas de salud pública basadas en LLINs, se realizaron dos estudios piloto para evaluar la eficacia de Inesfly 5A IGR™ combinada con LLINs en casas reales tratadas con pirotroides en las aldeas VK1 y VK3 en Burkina Faso. En la aldea VK1, el interior de las casas fue tratado con una o dos capas de pintura sobre un número de superficies diferentes (paredes *versus* paredes y techo). En la otra aldea, VK3, únicamente las ventanas y puertas fueron tratadas con una capa. En la aldea VK1, donde se trató el interior de las casas, la combinación de Inesfly 5A IGR™ y LLINs resultó en una mortalidad a largo plazo de 12 meses, con una tasa de

mortalidad de 80% durante las capturas de mosquitos contra poblaciones de mosquitos *Anopheles coluzzii* Coetzee & Wikerson, 2013, el vector local de la malaria resistente a los piretrinoides con altas frecuencias de *kdr*. Por otra parte, la aplicación de Inesfly 5A IGR™ en el exterior de ventanas y puertas en VK3 logró una eficacia letal de 80% durante apenas 2 meses contra *An. coluzzii* resistentes a piretroides. Entomológicamente, estos estudios piloto aportaron una información valiosa concluyendo que el interior de las casas, y no únicamente puertas y ventanas, deberá ser tratado en la siguiente evaluación a gran escala del estudio de Fase III. En términos socio-epidemiológicos, en preparación para el estudio de Fase III, treinta y dos localizaciones/aldeas cumplieron los requisitos del estudio: al menos 100 niños por aldea de edades comprendidas entre los 6 meses y los 14 años (para asegurar 30 sujetos evaluables al final des estudio), las aldeas estaban al menos a 1–2 km de distancia, las aldeas eran accesibles por carretera y sus habitantes mostraron un interés en participar.

La Fase III de este estudio se llevará a cabo para valorar el impacto de la combinación de la pintura insecticida de organofosforados junto con LLINs tratadas con piretroides en la reducción real de la incidencia de malaria en niños de edades comprendidas entre los 6 meses y los 14 años en África del Oeste donde la malaria es holoendémica y los vectores son resistentes a piretroides.

Palabras Clave: *Anopheles gambiae*, *Anopheles coluzzii*, *Culex quinquefasciatus*, mosquiteras tratadas con insecticida, mosquiteras tratadas con insecticida de larga duración, rociamiento residual intradomiciliario, piretroides, organoforfados, clorpírifos, diazinón, piriproxifeno, inhibidor del crecimiento del los insectos, WHOPES, casas experimentales, resistencia a los insecticidas, vector de la malaria, mosquito plaga, control vectorial, Costa de Marfil, Francia, Benín, Burkina Faso, África del Oeste.

ACKNOWLEDGEMENTS

This sequence of studies was made possible thanks to the funding and support of the following institutions.

The first project, at the Institut Pierre Richet (IPR) in Bouaké, Côte d'Ivoire, was supported by Project Number 3005/1999 of the Dirección General de Cooperación para el Desarrollo, Presidencia de Gobierno of the Generalitat Valenciana, Valencia, Spain.

The Phase I WHOPES study at the Laboratoire de Lutte contre des Insectes Nuisibles (LIN) de l'Institut de Recherche pour le Développement (IRD), in Montpellier, France, was funded by Project Number 07-CAP3–1513 of the Agencia Española de Cooperación Internacional para el Desarrollo (AECID), Ministry of Foreign Affaires, Madrid, Spain; and by the Red de Investigación de Centros de Enfermedades Tropicales (RICET), Project Number RD06/0021/0017 "Redes Temáticas de Investigación Cooperativa," Fondo de Investigación Sanitaria (FIS), Ministry of Health, Madrid, Spain.

The Phase II WHOPES study, at the Centre de Recherche Entomologique de Cotonou (CREC) in Bénin, was funded by Project Number 07-CAP3–1513 of the Agencia Española de Cooperación Internacional para el Desarrollo (AECID); the Ministry of Foreign Affairs, Madrid, Spain; and by the Red de Investigación de Centros de Enfermedades Tropicales (RICET), Project Number RD06/0021/0017 "Redes Temáticas de Investigación Cooperativa," Fondo de Investigación Sanitaria (FIS), Ministry of Health, Madrid, Spain.

The Spatial Mortality assessments that took place at the Laboratoire des Insectes Nuisibles (LIN) de l'Institut de Recherche pour le Développement (IRD), in Montpellier, France, and at the Centre de Recherche Entomologique de Cotonou (CREC) in Bénin, were funded by Project Number 11-CAP2–1558 of the Agencia Española de Cooperación Internacional para el Desarrollo (AECID), Ministry of Foreign Affaires, Madrid, Spain; by Project Number ISCIII-RETIC RD12/0018/0013, Red de Investigación de Centros de Enfermedades Tropicales – RICET, of the Program of "Redes Temáticas de Investigación Cooperativa" RETICS/FEDER, Fondo de Investigación Sanitaria (FIS), Ministry of Health and Consumption, Madrid, Spain; and

by Project Number PROMETEO/2012/042 of the program of Ayudas para Grupos de Investigación de Excelencia, Generalitat Valenciana, Valencia, Spain.

The pre-Phase III pilot studies (in the Bama area at sites VK1 and VK3), and the selection of villages for the Phase III study in the Orodara region, were carried out at the Institut de Recherche en Science de la Santé (IRSS)/Centre Muraz in Bobo Dioulasso, Burkina Faso. These activities were funded by Project Number 11-CAP2–1558 of the Agencia Española de Cooperación Internacional para el Desarrollo (AECID), Ministry of Foreign Affaires, Madrid, Spain; by Project Number ISCIII-RETIC RD12/0018/0013, Red de Investigación Colaborativa en Enfermedades Tropicales – RICET, of the Program of "Redes Temáticas de Investigación Cooperativa" RETICS/FEDER, Fondo de Investigación Sanitaria (FIS), Ministry of Health and Consumption, Madrid, Spain; and by Project Number PROMETEO/2012/042 of the program of Ayudas para Grupos de Investigación de Excelencia, Generalitat Valenciana, Valencia, Spain.

The ultimate goal of this work is to reduce the incidence of malaria in areas where the disease is endemic. The work of all the following people contributes to making that goal possible; and I value and respect their expertise and dedication. From an academic perspective, I offer them my deepest appreciation.

First and foremost, to my thesis directors, Prof. Dr. Santiago MAS-COMA, Director of the Parasitology and Cellular Biology Department of the Faculty of Pharmacy of the University of Valencia; and Dr. Pierre CARNEVALE, research director at the Institut de Recherche pour le Développement (IRD), France. These two men provided the scientific means and knowledge for this project. Neither its conception nor its implementation would have been possible without their guidance, vast expertise and constructive support. Dr. Pierre CARNEVALE was also director of the Institute Pierre Richet (IPR) in Côte d'Ivoire, where we performed our first Phase I assays; and I am indebted to him for his hospitality and indispensable mentorship.

I thank Dr. Jean-Marc HOUGARD, research director at the IRD in France, for not only managing the practical aspects of the study, but for paving the way and giving important factual counsel. Dr. Hougard's kind encouragement has been a continuous source of motivation.

I appreciated the ideas and suggestions of Frédéric DARRIET, research assistant at the IRD, which rendered the studies more solid. I have every respect for his honest approach to science.

I thank my friends and colleagues Mahama TOURÉ, Nestor KESSI BLI, and Dr. Raphaël N'GUESSAN for their many hours of work, dedication and guidance during those first tests in Côte d'Ivoire.

Heartfelt thanks Stephan DUCHON, Luc FINOT, Dr. Fabrice CHANDRE and Dr. Vincent CORBEL of the IRD for their support and assistance on every aspect of the Phase I and Phase II studies, from administrative issues to technical questions. Their help has been an important driving force for the projects.

I would also like to mention my high regard for the work done by colleagues and staff member at CREC during the Phase II evaluations and distance tests, including Joseph CHABI, Lazare HOUNKANRIN, Honoré FONTECLOUNON and René GBELISSA.

Thanks to the leader of the Ladji station, Yanclo PIERRE, and the entire "mosquito collection team" at Ladji, for all their work: Jean KOUWASSODÉ, Adolphe KOUWASSODÉ, Hugues AHOUANDJINOU, Pierre NOUVOESSI, Antoine KOULIHO, and Abdou laye AVOCE.

I thank Dr. Martin AKOGBETO, director of CREC, for his accessibility, and for providing the necessary resources.

My most sincere gratitude for giving wings to this endeavour goes to Dr. Roch DABIRÉ, Dr. Abdoulaye DIABATE, Dr. Thierry BALDET, Dr. Florence FOURNET, Ali OUARI, Dr. Franck REMOUÉ, Dr. Jeremy ROUAMBA, and Dr. Noël ROUAMBA.

I would also like to thank the members of the IRSS/Centre Muraz Team in Burkina Faso, including Ouari ALI, Dieudonné SOMA, Moussa NAMOUNTOUGOU and Serge PODA.

Once again, I would like to express my appreciation for the hospitality, professionalism and friendship shown to me by everyone at the international institutes where I spent many years performing these studies: the Institute Pierre Richet (IPR) in Côte d'Ivoire, the Laboratoire de Lutte contre les Insectes Nuisibles (LIN) de l'Institut de Recherche pour le Développement (IRD) in France, the Centre de Recherche Entomologique de

Cotonou (CREC) in Bénin, and the at the Institut de Recherche en Science de la Santé (IRSS)/Centre Muraz in Burkina Faso.

I am forever grateful to Prof. Dr. Santiago MAS-COMA, the DEPARTMENT OF PARASITOLOGY and all PROFESSORS of the XIV edition of the “MASTER INTERNACIONAL DE ENFERMEDADES PARASITARIAS TROPICALES” of the UNIVERSITY OF VALENCIA for opening a new world for me. Particularly, Dr. Patricio ARTIGAS at the Department of Parasitology of the University of Valencia gave me invaluable support with all the administrative aspects of this PhD.

Finally, on a personal note, my deep appreciation goes to my long-time friends Marta, Eva and Juan for their friendship and boundless generosity. I thank Rafael, Jorge and Thibaud for their reliable presence and pursuing their passion in science while lighting up others’.

I thank my parents, José and Elisa; my brothers, Sergio and Nacho; and my sister, Irene. Their example taught me the strength to question, and the need to search for answers. I admire them all profoundly.

A most special recognition to my partner, Thierry, and our two sons, Hector and Gaspar. We are together, and I cannot think of anything more exciting and beautiful.

Thank you.

TABLE OF CONTENTS

ABBREVIATIONS AND ACRONYMS

ace-1^R G119S: G199S mutation in *ace-1^R*

ace-1^R: acetylcholinesterase-1 resistance

ACT: Artemisinin-based Combination Therapies

AECID: Agencia Española de Cooperación Internacional para el Desarrollo

ANOVA: Analysis of Variance

CDC: Centers for Disease Control and Prevention

CI: Confidence Interval

CREC: Centre de Recherche Entomologique de Cotonou, Bénin

DDT: Dichloro-diphenyl-trichloroethane

EIR: Entomological Inoculation Rate

ELISA: Enzyme-Linked ImmunoSorbent Assay

EMCs: Early morning collections

F (G119S): Allelic frequency of the mutation *ace-1^R* G119S

F (kdr): Allelic frequency of the mutation *knockdown resistance*

FIS: Fondo de Investigación Sanitaria

GHS: Global Harmonized System

GIS: Geographic Information System

GPS: Global Positioning System

GST: Glutathione S-transferase

H1, H2, H3, H4, H5 and H6: Experimental Huts 1 to 6

IGR: Insect Growth Regulator

Inesfly 5A IGR: Trademarked Insecticide Paint containing OPs and Insect Growth Regulators

IP: Insecticide Paint

IPR: Institut Pierre Richet in Bouaké, Côte d'Ivoire

IRD: Institut de Recherche pour le Développement

IRS: Indoor Residual Spraying

IRSS: Institut de Recherche en Science de la Santé, Burkina Faso

ITN: Insecticide-Treated Net

JHs: Juvenile Hormones

Kdr: Knockdown resistanceL1014F *kdr*: Leucine-to-Phenylalanine substitution at position 1014 termed West Africa knockdown resistance

L1014S *kdr*: Leucine-to-Serine substitution at position 1014 termed East Africa knockdown resistance

LIN: Laboratoire des Insectes Nuisibles in IRD, Montpellier, France.

LLIN: Long-Lasting Insecticidal Net

LSM: Larval Source Management

M & S Forms: M & S molecular forms of *Anopheles gambiae*

MCHIP: Maternal and Child Health Integrated Program

OP: Organophosphates

p (HW): Value for Hardy-Weinberg equilibrium hypothesis

PCR: Polymerase Chain Reaction

PNLP: Programme National de Lutte contre le Paludisme

R: Resistant

RDT: Rapid diagnostic test

RICET: Red de Investigación de Centros de Enfermedades Tropicales

RP: Regular Paint

S: Susceptible

SINE: Short Interspersed Transposable Element

T (number): Time in months since treatment, e.g. T0 = 0 months after treatment.

USAID: United States Agency for International Development

WHO: World Health Organization

WHOPES: WHO Pesticide Evaluation Scheme

CHAPTER 1: INTRODUCTION

Malaria continues to be a serious public health concern worldwide. An estimated 3.3 billion people in 97 countries and territories are at risk of malaria, and 1.2 billion are at high risk (WHO, 2014). In 2013, globally there were an estimated 198 million cases of malaria (range 124–283 million), and an estimated 584,000 deaths (range 367,000–755,000). Of all malaria deaths, 90% occur in Africa (WHO, 2014). Children are particularly at risk: in 2013, an estimated 437,000 African children died of malaria before their fifth birthday. Globally, the disease caused an estimated 453,000 under-five deaths in 2013 (WHO, 2014). In addition to the devastating impact on human health, malaria also imposes an enormous economic burden, estimated at 1.3 % of economic growth per year in sub-Saharan Africa (WHO, 2013).

Consequently, the challenge of malaria prevention is no small matter. Current strategies rely mostly on controlling mosquitoes—the main malaria parasite vector—using insecticides. Other control strategies include early detection of malaria cases; prompt use of drug treatments; prophylaxis targeted to at-risk groups, such as pregnant women; improving people's dwellings, or otherwise modifying their environment; education about transmission and protection; and the development of vaccines. Another promising intervention strategy may involve genetic control of mosquitoes. But this approach is still being evaluated (McGraw & O'Neill, 2013), and its efficacy remains to be proven.

Of all these disease-control strategies, identifying which are most sustainable in the long term will depend on several factors. These include mosquitoes' resistance to insecticides, the availability of medical treatment, the resistance of malaria to available drugs, and the difficulties of developing a vaccine. A combination of such strategies will most increase the chances of successfully reducing the burden of malaria.

So far, the main strategy for preventing malaria on a large scale is controlling the mosquitoes that transmit the parasite (WHO, 2014). At present, this is mainly achieved by the use of Long-Lasting Insecticide-Treated Nets (LLINs) and, to a lesser extent, Indoor Residual Spraying (IRS) (WHO, 2013).

All currently recommended LLINs are treated with pyrethroids. This class of insecticidal chemicals has a rapid knockdown effect; high potency at low dosages; and relative safety (Zaim, Aitio, & Nakashima, 2000). IRS coverage also mostly uses pyrethroids (about 75%), with DDT the second most widely used insecticide; carbamates and organophosphates (OPs) represented only small percentages of global usage (WHO, 2012). Although pyrethroids are more expensive than DDT, their use is preferred because of their lower human toxicity and environmental impact. However, since pyrethroids are so vital to the control of malaria mosquito vectors, raised levels of pyrethroid resistance is (Ranson *et al*., 2011; Dabiré *et al*., 2012) a serious concern.

The main mechanism that confers resistance to pyrethroids and DDT in many insect species is termed *knockdown resistance (kdr)*. This phenomenon results from reduced sensitivity of the insects' nervous systems, caused by genetic mutations in the population. Although there is as yet no consensus on whether *kdr*-based adaptations significantly reduce the operational efficacy of insecticides (Hemingway, 2014), it is recommended that other potential strategies are explored to address the problem of pyrethroid resistance (Beier *et al*., 2008; The malERA Consultative Group on Vector Control, 2011).

As a result of this need, this study evaluates the strategy of combining the use of an organophosphate-insecticide paint with pyrethroid-treated LLINs. This approach follows the lead of many institutions, donors and malaria-control programs efforts to promote LLINs, while at the same time evaluating other tools. Since organophosphates and pyrethroids have different mechanisms—the first inhibits acetylcholinesterase, while the second disturbs the gated sodium channel—combining both may help to both preserve the efficacy of LLINs, and reduce the development of insecticide resistance in mosquito populations (WHO, 2011).

Central to this research is a new product called Inesfly 5A IGR. This insecticide paint is composed of two organophosphates (OPs): chlorpyrifos (1.5%) and diazinon (1.5%), and one Insect Growth Regulator (IGR), pyriproxyfen (0.063%). Seven studies were conducted to assess the efficacy of this product. The first

laboratory assessments, in Côte d'Ivoire, were stopped by civil war; but they provided evidence that surface porosity was a major issue. A porous material with a higher ratio of pores per volume absorbs fluids, allowing them to pass through it more easily—rather than retaining them on the surface for maximum insecticidal effect.

The Côte d'Ivoire studies tested 4 surfaces of varying porosity: adobe, cement, wood, and metal. The results showed that right after treatment with Inesfly 5A IGR, adobe—the most porous substance—yielded the fewest mortalities (similar to control) against the main malaria vector, *Anopheles gambiae* (Giles, 1902). Surfaces made of less-porous materials, especially metal, yielded higher mortality rates.

Based on those observations, the next evaluations took into account the porosity of the treated materials. Phase I laboratory tests were performed at the LIN Laboratory in France, and Phase II field tests in experimental huts in Bénin. These studies used novel approaches to assess spatial mortality, particularly to investigate the effect of the organophosphates at the chosen distance of 1 metre. The objective was to study the efficacy of the insecticide paint against mosquitoes at this distance. Both in the laboratory and in the field, a model was developed to ensure that caged mosquitoes never came closer than 1 metre to a treated surface.

Once the entomological efficacy of the insecticide paint was well defined, a pilot study (pre-Phase III) took place in houses in two villages in the Bama area (Kou Valley, or Vallée du Kou) of Burkina Faso. These villages—known as VK1 and VK3—were identified as being areas of pyrethroid resistance, with high *kdr* frequency. The goal of the study was to evaluate the combination of Inesfly 5A IGR and LLINs.

In the VK1 village, the house interiors were variously treated with 1 or 2 layers of insecticide paint. In VK3, only the outside of window and door openings was treated, with just 1 layer of paint. The goal was to test if treating the smaller area, with a smaller amount, sufficed in terms of mosquito mortality. The study also monitored resistance on the local mosquito populations, to assess the potential impact on the development of insecticide resistance (Enayati & Hemingway,

2010). Finally, the study examined the dead mosquitoes to determine the origin of their blood meals. Understanding the blood-feeding behaviour of malaria parasite vectors is a key component of malaria control. (Killeen, 2014; Killeen *et al.*, 2014).

As part of the recommended preparation for the WHOPES Phase III study, 32 sites in the Orodara region in Burkina Faso were selected for future research. The selection was based on the study's four socio-epidemiological criteria (Rogier *et al.*, 2009):

- villages had at least 100 children, from 6 months to 14 years old (to ensure at least 30 evaluable subjects at the end of the study);
- villages were at least 1–2 km from each other;
- villages could be accessed by road;
- village residents had expressed an interest in participating.

The preparation work included the geo-referencing of all habitations in the villages where the Phase III study will be conducted; and assessing the residents' level of knowledge about malaria, and their preventative practices. Also assessed were the number of sleeping units per village, and the number of children.

Table 1, below, summarizes the different Inesfly 5A IGR studies performed, along with the locations and sites, the methods used, and the specific malaria vectors: the mosquitoes used or present in the study area.

#	Site	Methods	Mosquitoes Used
1	Laboratory evaluations at IPR in Bouaké, Côte d'Ivoire; WHOPES Phase I	30-minute WHO bioassays	Susceptible *An. gambiae* Kisumu and wild pyrethroid-resistant *An. gambiae* from Yao, reared in the IPR insectarium from field-collected larvae
2	Laboratory evaluations at LIN in Montpellier, France; WHOPES Phase I	30-minute WHO bioassays	Susceptible *Cx. quinquefasciatus* S-Lab and OP-resistant *Cx. quinquefasciatus* SR
		IGR efficacy on fertility and fecundity	OP-resistant *Cx. quinquefasciatus* SR that survived the previous WHO bioassays
3	Field evaluations in Ladji, Bénin; WHOPES Phase II experimental huts	Early morning collections	Wild pyrethroid-resistant *An. gambiae* and *Cx. quinquefasciatus* from Ladji
		Mosquito release experiments	Pyrethroid-resistant *An. gambiae* from Ladji, reared in the CREC insectarium from field-collected larvae
		30-minute WHO bioassays	Susceptible *An. gambiae* Kisumu and *Cx. quinquefasciatus* S-Lab
		Distance tests in huts	Susceptible *An. gambiae* Kisumu and *Cx. quinquefasciatus* S-Lab
4	Spatial mortality assesments; recommendations for WHOPES	Phase I, laboratory, CREC, Bénin	Susceptible *An. gambiae* Kisumu and *Cx. quinquefasciatus* S-Lab
		Phase II, experimental huts, Ladji, Bénin	Susceptible *An. gambiae* Kisumu and *Cx. quinquefasciatus* S-Lab
5		Early morning collections	Wild pyrethroid-resistant *An. coluzzii* from Bama VK1

	Real-world evaluations in Bama VK1, Burkina Faso; pre-Phase III pilot study inside houses	30-minute WHO bioassays	Susceptible *An. gambiae* Kisumu and pyrethroid-resistant *An. coluzzii* from Bama VK1, reared in the IRSS insectarium from field-collected larvae
		Resistance monitoring	Wild pyrethroid-resistant *An. coluzzii* from Bama VK1, captured during EMCs
		Blood meal origin	Wild pyrethroid-resistant *An. coluzzii* from Bama VK1, captured during EMCs
6	Real-world evaluations in Bama VK3, Burkina Faso; pre-Phase III pilot study, Doors and Windows	Early morning collections	Wild pyrethroid-resistant *An. coluzzii* from Bama VK3
		30-minute WHO bioassays	Susceptible *An. gambiae* Kisumu and wild pyrethroid-resistant *An. coluzzii* from Bama VK3, reared in the IRSS insectarium from field-collected larvae
		Spatial mortality assessments	Susceptible *An. gambiae* Kisumu and wild pyrethroid-resistant *An. coluzzii* from Bama VK3, reared in the IRSS insectarium from field-collected larvae
		Resistance status	Wild pyrethroid-resistant *An. coluzzii* from Bama VK3, captured during EMCs
7	Various villages in the Orodara region; preparation for the WHOPES Phase III study	Preliminary surveys, geo-referencing and mapping	Main malaria vectors in the area are mostly from the *An. gambiae* complex, and are resistant to pyrethroids

The design and performance of these studies constituted the main research on which this dissertation is based. The Phase I and Phase II WHOPES protocols provided an insight into the effect of the use of on entomological parameters, both

in the laboratory and in the field. The two pilot studies performed in village settings helped to evaluate the potential of the new strategy of combining Inesfly 5A IGR insecticide paint with pyrethroid-treated LLINs. The results obtained from the seven studies detailed above provide a basis for designing the forthcoming large-scale Phase III evaluation, to assess the impact of this strategy on the incidence of malaria in children. This will take place in an area of holoendemic malaria in south-western Burkina Faso, where the local mosquito population is highly resistant to pyrethroids, and have high *kdr* frequency.

Following this brief introductory chapter (**Chapter 1**), this research work is examined in the following six chapters.

Chapter 2 provides some background, including an overview of malaria and of vector control strategies, with an emphasis on the testing of Inesfly 5A IGR.

Chapter 3 describes the materials and methods of past studies.

Chapter 4 covers the results obtained from this study, along with an analysis.

Chapter 5 discusses the results from the perspectives of the research goals.

Chapter 6 presents the conclusions from this study, along with some future research prospects.

Chapter 7 lists the work cited in this paper (References).

Annex: consists on the articles published by the Doctorate student during the Doctoral training and research work in the domain of malaria vector control.

CHAPTER 2: BACKGROUND

2.1. Malaria

Human malaria is caused by five species of a protozoan parasite belonging to genus *Plasmodium*. The main four species are *Plasmodium falciparum* (the most deadly), *P. vivax*, *P. malariae*, and *P. ovale*. A fifth species, *P. knowlesi*, has recently been shown to cause an emerging public health problem in Maylasia (Waugh, 2015).

The malaria parasite life cycle involves two hosts, as illustrated in Figure 1 (at the end of this chapter). When a female *Anopheles* mosquito ingests mature and infective gametocytes from an infected human, the parasite develops within the mosquito for 8–35 days until the infective sporozoites form, and accumulate in the mosquito's salivary glands. The malaria parasite's process of multiplication is known as sporogonic development, and its duration depends on the species of parasite, the species of mosquito, and the temperature. For example, it takes about 12 days for a *P. falciparum* parasite to develop in a *An. gambiae* mosquito at 25℃.

When a person is bitten by a female *Anopheles* mosquito with sporozoites in its salivary glands, the malaria parasite is transmitted. After an incubation period (of varying length, depending on factors such as the type of parasite, temperature and humidity, and the host's immune system), the person usually experiences symptoms of malaria, including fever, chills, and a flu-like illness. However, there are no pathognomonic features for malaria.

Mosquitoes have four main life stages: egg, larva, pupa in water, and adult. Most females, including *Anopheles*, require a blood meal in order for the eggs in her ovaries to develop. The eggs mature while the mosquito female digests the blood; the gravid female will then search for a suitable site to lay her eggs. This process of blood-feeding, egg maturation and oviposition is termed the gonotrophic cycle, and it is repeated several times throughout the 3–4 week life cycle of a female. For *An. gambiae*, the length of the gonotrophic cycle is estimated at 2–3 days (Clements, 1992; Mouchet *et al*., 2004), depending on external temperature. The

relationship between the gonotrophic cycle, the female lifespan and the sporogonic development is represented in Figure 2 (at the end of this chapter).

The age structure of any given adult mosquito population is a major determinant of its vectorial capacity (ability to transmit the malaria parasite). Even a small shift in adult age can have large consequences for parasite transmission. Only a small percentage of females in each population live long enough for sporogonic development to take place; so the older the mosquito population, the higher its capacity for infectivity. For this reason, as shown in Figure 3 (at the end of this chapter), any strategies are useful control tools if they:

- interfere with the development cycle, at any point from egg-laying to adult emergence;
- lengthen the gonotrophic cycle;
- shorten the longevity of the individual mosquito.

The genus *Anopheles* (Meigen, 1918) currently includes 467 formally named species (Harbach, 2013), and more than 50 unnamed members of species complexes. Approximately 70 of these species have the capacity to transmit human malaria parasites (Service & Townson, 2002); and 41 are considered to be dominant species that are capable of transmitting malaria parasites at a level of major concern to public health (Sinka *et al*., 2010, 2012). Sub-Saharan Africa suffers from the highest malaria parasite transmission levels in the world, and consequently from the most morbidity and mortality (Fontenille & Simard, 2004; Guerra *et al*., 2008; Hay *et al*., 2009; Hay *et al*., 2010).

Anopheles gambiae s.l. is the most effective vector of malaria currently known (Coluzzi, 1999; Gillies & de Meillon, 1968), and the dominant one in West and Central Africa. Until recently, the two reproductive units in *An. gambiae* were known as the “M” and “S” molecular forms. In light of new genomic evidence, the *An. gambiae* form “M” is now officially known as *Anopheles coluzzii* (Coetzee & Wikerson, 2013; Coetzee *et al*., 2013).

2.2. Vector Control Strategies

2.2.1. Overview of vector control tools: LLINs and IRS

Between 2000 and 2013, an expansion of malaria interventions—mostly based on vector-control strategies using LLINs and IRS—has taken place (WHO, 2014). Those are the cornerstones of malaria vector control, and international public health efforts are being made to increase their coverage. In the period from 2008 to 2010, 254 million LLINs were supplied to countries in sub-Saharan Africa (WHO, 2013). The National Malaria Control Program (Programme National de Lutte contre le Paludisme, or PNLP), a 2010 initiative of the Ministry of Health in Burkina Faso, distributed more than 8 million LLINs to around 16 million of the most at-risk people: pregnant women, and children under 5 years old (MCHIP/USAID/PNLP, 2013). Even though the national LLIN campaign did not fully meet its target of universal coverage by distributing 1 LLIN for every 2 people, a 2011 study (conducted by Zollner *et al.*) concluded that it had still achieved a high level of coverage; and had fostered equity by allocating nets according to the number of family members (2015).

However, the initiative of distributing LLINs, while worthy, appears to be insufficient to provide protection around the world. Global malaria transmission still occurs in 97 countries, putting about 1.2 billion people at high risk (WHO, 2014). In sub-Saharan Africa, for WHO to achieve universal access, some 780 million people would need to have LLINs, with approximately 150 million being delivered every year (WHO, 2012). Even that large number may be underestimated: if 1 LLIN covers 2 people, then 150 million LLINs will only cover 300 million people—not the full 780 million at risk.

The problem of cost is secondary, but needs to be taken into account. An estimated US$ 5.1 billion would be needed every year between 2011 and 2020 to achieve universal access (WHO, 2012). But as of 2011, available funding was only US$ 2.3 billion—less than half the amount needed (WHO, 2012).

Regarding IRS, coverage reached 58 million people in Africa in 2012; this represented some 8% of the global human population at risk, as reported by National Malaria Control Programmes (WHO, 2013). Pyrethroids were estimated

to account for about 75% of IRS coverage, while DDT was the second most widely used insecticide (WHO, 2012). The estimated effective duration (at least 80% of mosquito mortality) range from 3 to 6 months for pyrethroids, and about 2 to 6 months for carbamates and OPs. In the case of DDT, the effective duration is more than 6 months (WHO, 2015).

How have malaria control interventions reduced the burden of malaria? Between 2000 and 2013, an expansion of interventions helped to decrease global incidence by 30%, and by 34% in Africa alone (WHO, 2014). This success was mainly due to the increase in coverage of LLINs and IRS; but other interventions—such as access to rapid diagnostic tests (RDTs), and to artemisinin-based combination therapies (ACTs)—have also increased around the world. The 2014 World Malaria Report found that: 1) the volume of RDT sales to the public and private sectors of endemic countries increased from 46 million in 2008, to 319 million in 2013; 2) the number of patients tested by microscopic examination increased to 197 million in 2013, with India accounting for over 120 million slide examinations; and, 3) globally, 392 million courses of ACTs were procured by endemic countries in 2013, up from 11 million in 2005.

However, during the same period 53 countries reported mosquito resistance to at least one insecticide. Of these, 41 reported resistance to two or more insecticide classes. The most commonly reported resistance is to pyrethroids, the most frequently used insecticide in malaria vector control (WHO, 2014). This expansion of resistance to pyrethroids has been reported for more than 15 years now among malaria mosquito vectors (Chandre *et al*., 1999; Diabaté *et al*., 2004; Dabiré *et al*., 2012).

Whether or not pyrethroid resistance compromises efficacy of malaria control tools continues to be a matter of debate. Studies in West Africa have consistently shown that pyrethroid-treated nets remain effective against *An. gambiae* populations resistant to pyrethroids through the *kdr* mechanism (Darriet *et al*., 1998, Darriet *et al*., 2000a; WHO, 2004, Dabiré *et al*., 2006), with a corresponding reduction in malaria incidence rate (Henry *et al*., 2005). By contrast, an entomological study examining the effectiveness of using ITNs at two sites in

Bénin has given clear evidence of pyrethroids failing to control an *An. gambiae* population that contains *kdr* resistance at high levels (N'Guessan *et al*., 2007). In Bioko Island, Equatorial Guinea, indoor residual spraying with pyrethroids failed to reduce the population density of *kdr*-resistant *An. gambiae* (Sharp *et al*., 2007). Likewise, a recent study performed in Burkina Faso reports the reduced efficacy of pyrethroid-based vector control tools against *kdr*-resistant *An gambiae* (Toé *et al*., 2014).

In addition to the problem of widespread pyrethroid resistance among malaria vectors (Ranson *et al*., 2011; Dabiré *et al*., 2012), there is evidence that ITNs are often incorrectly used—rendering them less effective (Winch *et al*., 1994; Kroeger *et al*., 1997; Simon *et al*., 2002). As well, several studies suggest that both treated and non-treated bednets (Alaii *et al*., 2003; Binka & Adongo, 1997; D'Alessandro, 2001) ranked low among household expenditure priorities, regardless of intervention status; and that re-treatment rates are low (Cham *et al*., 1997; Snow *et al*., 1999). In terms of adequate use, obstacles for many households might include the need for the day-to-day organization of placing bednets in small houses, with rooms that serve multiple purposes (Toé *et al*., 2009; MCHIP/USAID/PNLP, 2013).

Culex quinquefasciatus, the most common mosquito in tropical urban areas, is a great nuisance. The insect has developed a resistance to the pyrethroids used to impregnate nets (Chandre *et al*., 1998; Corbel *et al*., 2007). This resistance may hamper malaria control efforts, since many people may be reluctant to use LLINs—which protect against malaria—if the nets do not also protect against nuisance insects (Winch *et al*., 1994; Aikins *et al*., 1994; Van Bortel *et al*., 1996, Guillet *et al*., 2001a; Samuelsen *et al*., 2004).

There are also known obstacles to the use of IRS, such as the need for special equipment to apply the product, and trained personnel to do the work. The task usually requires both government support, and strong community participation (Munguambe *et al*., 2011). Residents often dislike the application process, since it leaves a white residue on walls, smells bad, and obliges people to leave their homes during the procedure (Najera & Zaim, 2001). Other reasons for people to dislike IRS include: embarrassment about moving poor-quality possessions out

of the house during the process; ignorance of the reason for using IRS; uncertainty about its effectiveness; fear of its potential side effects (people may have heard rumours of the chemicals affecting fertility); belief that the spray process is politically motivated (Kaufman *et al*., 2012).

As a result, although LLINs and IRS remain currently key in malaria vector control, there is a growing need to find alternative mosquito control strategies that can be added to the list of tools to rationally choose from (Beier *et al*., 2008) while assessing and monitoring insecticide resistance (Enayati & Hemingway, 2010).

2.2.2. Presentation of an insecticide paint: Inesfly 5A IGR

Inesfly 5A IGR is a chemical cocktail composed of microcapsules containing two organophosphates (OPs): chlorpyriphos (1.5%) and diazinon (1.5%) and an insect growth regulator (IGR), pyriproxyfen (0.063%). The product consists of a white vinyl paint with an aqueous base; its active ingredients are Ca CO^3 + resin microcapsules ranging in size from one to several hundred micrometres.

The microencapsulation of the paint is expected to present advantages, allowing a gradual release of these active ingredients that increases stability (Perlatti *et al*., 2013).

In mosquitoes, pyriproxyfen mimics the action of juvenile hormones (JHs), though their mode of action is not well understood. Pyriproxyfen and other IGRs inhibit maturation in a large spectrum of insects during their developmental stages (Dhadialla *et al*., 1998); and are traditionally used in aquatic habitats to prevent mosquito larvae and pupae from developing into adults (Dhadialla *et al*., 1998). Several studies have also shown that pyriproxyfen may also impact the fertility of adult mosquito females, by absorption through tarsal contact (Loh & Yap, 1989; Kamal & Khater, 2010).

The reasoning behind the microencapsulation of OPs rather than pyrethroids is the search for other alternatives to pyrethroids (Beier *et al*., 2008; The malERA Consultative Group on Vector Control, 2011).

The main ingredients in Inesfly 5A IGR are the OPs, which—like carbamates—operate on a different target from pyrethroids. Instead of acting on hormones, OPs inhibit acethylcholinesterase, an enzyme that terminates nerve impulses by catalyzing the hydrolysis of the neurotransmitter acetylcholine (Aldridge, 1950). However, OPs also inhibit many other enzymes that confer different mechanisms of neurotoxicity besides acetylcholine. Chlorpyrifos has been shown to induce a glutamate-mediated excitotoxicity, and diazinon to induce apoptotic neuronal death (Rush *et al*., 2010). By having different OPs in Inesfly 5A IGR, the hope is to broaden the spectrum of action.

As with pyrethroids, OPs and carbamates are used in many parts of the world for vector and insect pest resistance (Vaughan *et al*., 1998; Karunaratne & Hemingway, 2001; Rodriguez *et al*., 2001), including populations of *Culex* (Magnin, Marboutin & Pasteur, 1988; Chandre *et al*., 1997) and *An. gambiae* (N'Guessan *et al*., 2003) from Côte d'Ivoire.

However, a 2003 study showed that a single G119S mutation in the *ace-1* gene of *An. gambiae* mosquitoes renders them insensitive to acetylcholinesterase inhibitors such as OPs and carbamates (Weill *et al*., 2003). The same mutation, conferring cross-resistance to OPs and carbamates, has also been found in both *An. gambiae* and *An. coluzzii* populations in West Africa, at various frequencies depending on the location. This mutation was not detected in *An. gambiae* populations in south Bénin, and was only weakly detected in their populations in south-western Burkina Faso (Djogbenou *et al*., 2008).

Studies in experimental huts in Côte d'Ivoire and The Gambia suggested that bednets treated with the OP pirimiphos-methyl were effective in killing mosquitoes, and that efficacy lasted longer than pyrethroids (Miller *et al*., 1991; Kolaczinski *et al*., 2000). Similarly, various studies have shown an efficacy when using OP-impregnated bednets even in the presence of insensitive acetylcholinesterase (Kolaczinski *et al*., 2000; Guillet *et al*., 2001b; Corbel *et al*., 2003; N'Guessan *et al*., 2003; Asidi *et al*., 2005).

Recent studies have shown that other organophosphates (chlorpyrifos-methyl and pirimiphos-methyl) are also effective, and potentially safe enough to be considered as a possible alternative for treating mosquito nets, either alone or in

combination (Darriet *et al*., 2003; Asidi *et al*., 2005; N'Guessan *et al*., 2010), as well as IRS (Fuseini *et al*., 2011). Likewise, a long lasting micro-encapsulated formulation of pirimiphos-methyl has been shown as an interesting alternative for indoor residual spraying in Zambia (Chanda *et al*., 2013), Tanzania (Oxborough *et al*., 2014) and Bénin (Rowland *et al*., 2013), in areas of high vector resistance to pyrethroids and carbamates.

Beyond its chemical properties, the Inesfly 5A IGR paint offers a different operational approach. Since no special equipment is needed, and the appearance of homes is improved, this could motivate residents to accept the insecticidal treatment. Applying paint could also have a public health value.

In Chile, Honduras and Paraguay, a study on triatomine control compared a slow-release paint containing malathion (an organophosphate), to fumigant cans containing dichlorvos (also an organophosphate) and cypermethrin (a pyrethroid). Results 6 months post-treatment showed an efficient control when insecticide paints were used indoors and in the peridomicilium, keeping reinfestation near zero. Final results in Chile, from two years post-treatment, confirmed the superiority of the slow-release paint compared to the fumigant approaches (Oliveira Filho, 1996).

Inesfly 5A IGR has been evaluated previously under experimental conditions in South America against the main Chagas disease vector, *Triatoma infestans* (Klug, 1834; Hemiptera, Reduviidae: Triatominae) (Amelotti *et al*., 2009; Dias & Jemmio, 2008; Maloney *et al*., 2013; Gorla *et al*., 2015). Results showed high mortalities and a long residual activity of the treatment, even in areas where local populations of triatomines are resistant to pyrethroids (Lardeux *et al*., 2010; Gorla *et al*., 2015). In addition, the paint was well accepted and tolerated by people exposed to it (Dias & Jemmio, 2008). Toxicology studies performed so far support the product's safety (International Center of Training and Medical Investigations, 2003; National Center of Tropical Diseases, 2004; Spanish Ministry of Health and Consumer Affairs, 1996). Inesfly 5A IGR is considered a Category 5 substance by the Globally Harmonized System of Classification and Labelling of Chemicals

(GHS)—a standardized and internationally accepted system for classifying and labelling chemicals.

2.2.3. Testing of Inesfly 5A IGR against malaria vectors

The objective of the first tests was to gain insight into the insecticidal potential of Inesfly 5A IGR, in terms of entomological parameters that could be measured objectively using WHOPES Protocols. The Phase I and Phase II studies were both performed with the expertise and support of the team from the WHO-reference centre, the Laboratoire de Lutte contre les Insects Nuisibles (LIN). The Phase I study was performed at the LIN laboratory itself, in Montpellier, France.

Once the entomological profile of Inesfly 5A IGR was assessed, during the Phase I and II studies, a long-term strategy was developed on how to further evaluate the product's potential for increasing protection to users It was decided to combine the OP-insecticide paint with LLINs—an approach is expected to offer many advantages in terms of mode of action and operational coverage. These advantages include:

- combining different insecticides that operate on different targets may help to reduce the development of resistance in vector populations (WHO, 2011; 2012);
- the lethal effect of OPs, coupled with the excito-repellent effect of pyrethroids, may broaden the scope of action by killing mosquitoes that were not deterred or repelled by the pyrethroids in LLINs;
- the paint may provide protection before and after regular sleeping hours, when people are not yet under treated nets;
- the paint may kill indoor resting (endophilic) mosquitoes as well as blood-feeding (endophagic) vector mosquitoes.

As well, insecticide paint differs from IRS in several respects. While IRS provides similar benefits to Inesfly 5A IGR, it needs special equipment to apply, and leaves a sticky white residue on walls (Najera & Zaim, 2001). By contrast, residents can apply the paint themselves; and the effect is one of home improvement. In fact, in the VK1 and VK3 villages in Burkina Faso where the studies were performed,

most home owners regarded the embellishing effect of the paint as an added benefit.

The pyriproxifen in Inesfly 5A IGR could confer an additional angle of attack against mosquito females, once the OP effect diminishes over time. In the lab, studies of pyriproxyfen have shown that its effect on the fecundity (number of eggs laid), fertility (percent of eggs that hatch) and adult emergence of exposed adult females lasts even once the lethal effect of OPs diminished over time even against OP-resistant mosquitoes (Mosqueira *et al*., 2010a).

Two pilot studies to assess the combination of Inesfly 5A IGR and LLINs were performed in Burkina Faso—where 32 villages were also pre-selected in preparation for the large-scale WHOPES Phase III study. These villages met the following study criteria: at least 100 children from 6 months to 14 years of age (to ensure 30 evaluable children at the end of the study), minimal distances of 1–2 km from each other, access by road and willingness to participate.

To summarize, the results obtained during the seven studies already performed provide a solid basis for designing the forthcoming large-scale Phase III evaluation. Its goal is to assess the impact of the new strategy—the combination of OP-insecticide paint, and pyrethroid-treated LLINs—on the incidence of malaria in children, aged 6 months to 14 years old. The study will take place in areas of holoendemic malaria and high pyrethroid resistance in south-western Burkina Faso.

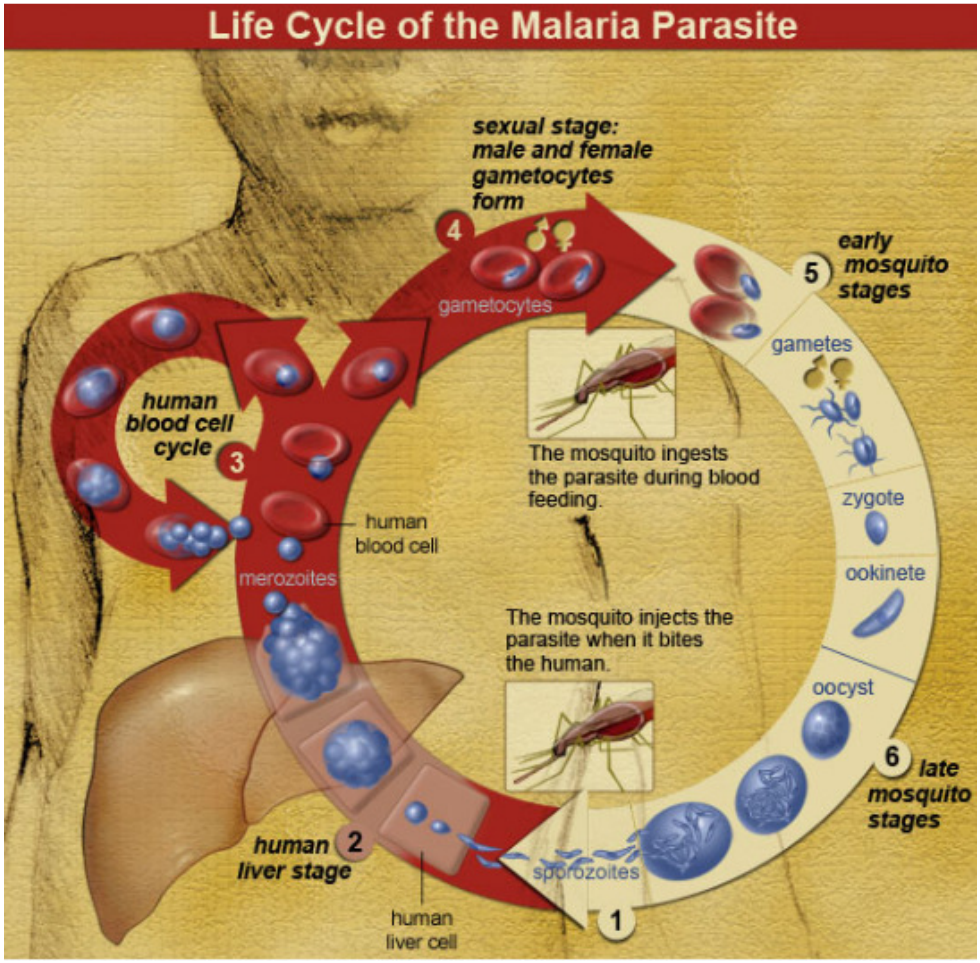

Figure 1: The life cycle of the malaria parasite (NIAID)

An illustratation of the stages of the malaria parasite. (1.) A female *Anopheles* mosquito carrying malaria-causing parasites feeds on a human, and injects the parasites (in the form of sporozoites) into the person's bloodstream. The sporozoites travel to the liver and invade liver cells. (2.) Over 5–16 days*, the sporozoites grow, divide and produce tens of thousands of haploid forms (called merozoites) in each liver cell. Some species are able to remain dormant for extended periods in the liver, causing relapses weeks or months later. (3.) The merozoites exit the liver cells and re-enter the bloodstream, beginning a cycle of invasion of red blood cells, asexual replication, and release of newly formed merozoites from the red blood cells repeatedly over 1–3 days*. This multiplication can result in thousands of parasite-infected cells in the host's bloodstream,

leading to illness and complications of malaria that can last for months if not treated. (4.) Some of the merozoite-infected blood cells leave the cycle of asexual multiplication. Instead of replicating, the merozoites in these cells develop into sexual forms of the parasite, called male and female gametocytes, which circulate in the bloodstream. (5.) When a mosquito bites an infected human, it ingests the gametocytes. In the mosquito gut, the infected human blood cells burst, releasing the gametocytes, which develop further into mature sex cells called gametes. Male and female gametes fuse to form diploid zygotes, which develop into actively moving ookinetes that burrow into the mosquito midgut wall and form oocysts. (6.) The growth and division of each oocyst produces thousands of active haploid forms called sporozoites. After 8–15 days*, the oocyst bursts, releasing sporozoites into the body cavity of the mosquito. From there they travel to, and invade, its salivary glands. This allows the cycle of human infection to begin anew when the mosquito next takes a human blood meal and injects the sporozoites.

* The timing depends on the specific parasite species.

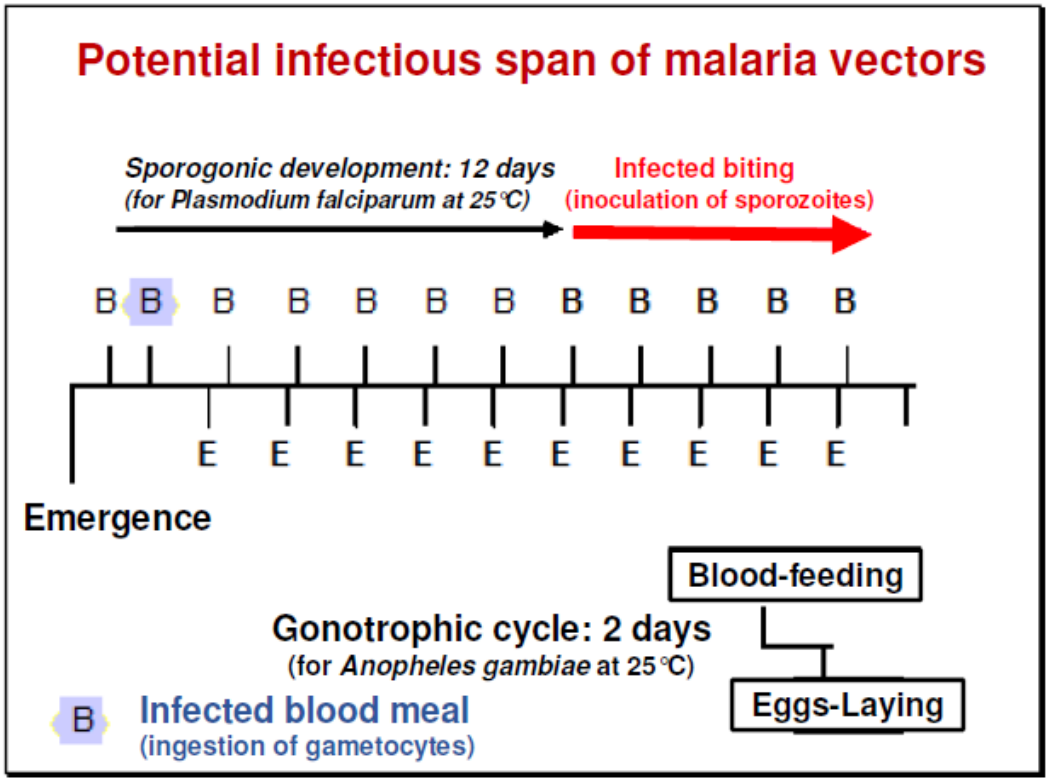

Figure 2: The link between the gonotrophic cycle, longevity, and sporogonic development (IRD)

The sporogonic development of *Plasmodium falciparum* in *Anopheles gambiae* mosquitoes at 25℃ is about 12 days. Assuming a mean longevity of about 20 days for a female, and a lapse of 2 days between blood-feeding and egg-laying (gonotrophic cycle), the number of potentially infective bites is 6–7. If the female becomes infected on the second night (as shown above), 5–6 bites would be infective during its lifespan.

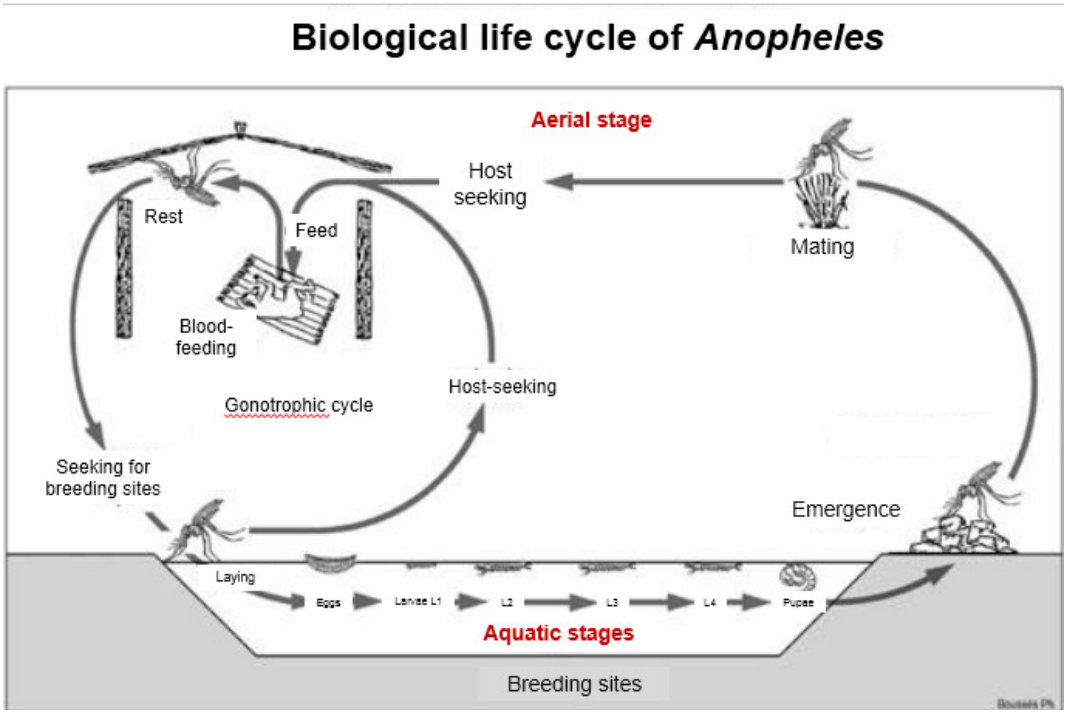

Figure 3: The biological life cycle of *Anopheles* (P. Boussès, IRD)

The most useful mosquitoes control tools are strategies that a) interfere with the development cycle of the mosquito (from the moment eggs are laid to the emergence of adult mosquitoes); b) lengthen the gonotrophic cycle; or c) shorten the insects' longevity.

CHAPTER 3: MATERIALS AND METHODS

3.1. WHOPES Phase I at IPR, Côte d'Ivoire

The efficacy of Inesfly 5A IGR was studied under laboratory conditions at IPR in Côte d'Ivoire using 30-minute WHO bioassay cones (WHO, 1998). Treated surfaces were painted at 1 kg/6 m^2 (manufacturer's recommended dose to leave surfaces completely white). Paint was applied undiluted with a regular brush and left to dry for 48 hours. The mosquitoes used were 3–5-day-old pyrethroid-susceptible *An. gambiae* Kisumu females; the insecticide-susceptible reference strain, reared at the IPR insectarium; and *An. gambiae* females bred at IPR's insectarium from field-collected larvae at Yao, where *An. gambiae* populations were resistant to pyrethroids.

The choice of surfaces treated was based on the most commonly used material for housing construction in the area: metal (many roofs are made of this material), cement and adobe (walls are commonly made with one or the other material). There was one treatment and one control arm for each kind of surface. After a 30-minute exposure, mosquitoes were introduced to the huts, housed in 150-ml plastic cups provided with honey-juice. Tests were done in four repeats, using 15 females per cone. Females were left at a temperature of 27 ± 1°C and a relative humidity of 80%, for 24-hour delayed mortality assessments. Because of the first Ivorian civil war (2002–2007), tests were carried out only 3 times in 2001: 2 days after treatment, 1 month and 2 months after treatment.

3.2. WHOPES Phase I at LIN, France

3.2.1. Delayed mortality using 30-minute WHO bioassays

The paint's efficacy was tested against laboratory strains of the pest mosquito *Cx. quinquefasciatus*, both susceptible and resistant to OPs (though at the time of the study, there was no laboratory strain of *An. gambiae* that was specifically resistant to OPs). Tests consisting of 30-minute WHO bioassay cones (WHO, 1998) were performed on two laboratory strains of *Cx. quinquefasciatus*. One, *Cx. quinquefasciatus* S-Lab, is an insecticide-susceptible reference strain

(Georghiou, Metcalf, & Gidden, 1966); the other, *Cx. quinquefasciatus* SR, is homozygote for the *ace-1*^{R} resistant gene involved in the resistance to OPs and carbamates, but has the same genetic background as S-Lab (Berticat *et al.*, 2002).

Unfed, 3–5 day-old females bred at the LIN insectariums were placed in forced contact with four different surfaces: softwood and hard plastic (non-porous materials), and ready-mixed cement and ready-mixed stucco (porous materials). There were two treatment arms and two controls arms for each kind of surface. Treated surfaces were painted at two doses, 1 kg/6 m^2 (manufacturer's recommended dose to leave surfaces completely white), and 1 kg/12 m^2. For each kind of surface, there were two kinds of control: one control was left untreated; the other was painted at 1 kg/6 m^2 with the same paint, but without the insecticides and the IGR. Paint was applied undiluted with a regular brush and left to dry for 48 hours.

After a 30-minute exposure, mosquitoes were introduced, housed in 150-ml plastic cups provided with honey-juice. Tests were done in four repeats using 15 females per cone (as shown in Figure 4, at the end of this chapter). Females were left at a temperature of 27 ± 1°C and a relative humidity of 80%, for 24-hour delayed mortality assessments. Tests were done at intervals of 6 months for 1 year. When not tested, surfaces were stored in aluminium foil at a temperature of 27 ± 1°C and a relative humidity of 80%. Delayed mortality was analyzed using Epi-Info 6. Where values were <5, Fisher exact tests were used. Because bioassay tests are subject to variations, a 99% confidence interval was applied.

3.2.2. IGR efficacy on fecundity, fertility and larval development

Females were 4–5 day-old to increase the probability of having females fertilised by male mosquitoes. LIN-reared *Cx. quinquefasciatus* OP-resistant females were exposed to treated or control surfaces for 30 minutes. Females alive 24 hours after a 30-minute exposure, were put in cages and allowed to blood-feed overnight. Females that had been well blood-fed were put in a new cage and given honey-juice every two days. At T0, 50 blood-fed females were tested per

surface. At T9, 30 blood-fed females were tested per surface. At T0 and T9, blood-feeding took place about 36 hours after previous exposure to control or treated surfaces.

Efficacy was measured in terms of fecundity (number of eggs laid), fertility (% hatching) and larval development (% pupation and % emergence). Tests were not done using susceptible *Cx. quinquefasciatus* S-Lab, because they all died during the 30-minute exposure. Tests were carried out on the most porous surface, cement, because not enough females of the *Cx. quinquefasciatus* OP-resistant survived exposure to other surfaces. Eggs were counted with a dissecting microscope and placed in plastic measuring containers with 2L of water for hatching. Water loss due to evaporation was replaced daily. Larvae were fed every two days. The mean number of eggs was compared between treated and non-treated surfaces using a student T test. Differences in % hatching, % pupation, and % emergence were analyzed using Epi-Info 6. Where values were <5, Fisher exact tests were used.

3.3. WHOPES Phase II at Ladji, Bénin

3.3.1. Study site

Ladji (6◦23N-2◦25) is a large village located by Nokoué Lake in southern coastal Bénin, which floods during the rainy season—creating ideal breeding sites for *An. gambiae*. The local population of *An. gambiae* is composed entirely of the M molecular form and shows resistance to pyrethroids and DDT, *kdr* is present at a high frequency, but is susceptible to organophosphates and carbamates, the *ace-1^R* mutation was absent (Corbel *et al*., 2007).

Pest mosquito *Cx. quinquefasciatus* is also present all year round and shows high resistance to DDT and pyrethroids with high *kdr* frequency (Corbel *et al*., 2007). The *ace-1^R* mutation conferring cross resistance to organophosphates and carbamates was absent (Corbel *et al*., 2007).

3.3.2. Insecticide paint

Inesfly 5A IGR was applied in the interior of four experimental huts. Two huts were used as control huts: one with no paint, and one with the same base paint used by Inesfly 5A IGR but with no insecticide.

The four treated huts had Inesfly 5A IGR treated with 1 or two layers of insecticide paint at 1 kg commercial product/6 sq m—that is, 0.51 g a.i. per sq m. Based on the huts' dimensions, 3.4 kg of paint was applied on walls per layer, and 1.0 kg on ceilings. Huts treated with two layers had the first layer diluted in 20% water following recommendations of the manufacturer. The overall random disposition of huts was as follows:

- H1: Control 1 (no paint);
- H2: one layer of insecticide paint on walls;
- H3: one layer of insecticide paint on walls and ceiling;
- H4: two layers of insecticide paint on walls;
- H5: Control 2 (Inesfly 5A IGR paint with no insecticide);
- H6: two layers of insecticide paint on walls and ceiling.

In all huts, the paint was applied with a regular brush.

3.3.3. Early morning collections (EMC)

Inesfly 5A IGR was evaluated in 6 experimental huts for over 12 months from September 2003 to September 2004 at the WHOPES Ladji station (as shown in Figures 5 and 6, at the end of this chapter). Mosquito collections were performed following WHO testing procedures (WHO, 1996). Experimental huts were built similarly to those used in Cote d'Ivoire by Darriet *et al*. (2002). Team members working in mosquito collection were informed about the study both in writing and verbally (though they were all literate); and were given the time to think it over before giving Informed Consent. All team members were provided with intact non-treated bednets to protect them. Ethical authorization for this research was

obtained from the Ministry of Health. Confirmed *P. falciparum* parasitaemia would be treated as per Bénin's Ministry of Health's recommendations. Before treating huts, mosquitoes were collected for several nights to check that there was no difference between huts in terms of their attractiveness to mosquitoes.

Though generally done, in this study it was even more important since treatments could not be rotated. To reduce the effect of variation in individual attractiveness to mosquitoes, sleepers rotated between huts on successive study nights. Mosquito collections were performed for thirteen weeks during the first 3 months; and for 6 weeks minus/plus 3 weeks on time points 6, 9 and 12 months after treatment. Following WHOPES Phase II procedures, four entomological criteria were evaluated: (i) deterrent effect, (ii) excito-repellent effect, (iii) blood-feeding inhibition, and (iv) mosquito mortality rate.

3.3.4. Mosquito release experiments

On two occasions, mosquito bednets were removed and malaria-free females were released. Mosquitoes used were malaria-free 5-day-old unfed *An. gambiae* females bred at CREC's insectarium from field-collected larvae at Ladji (as shown in Figure 7, at the end of this chapter). Females were released in batches of 100 females per hut at 21:00, just after volunteers entered huts. The next morning, females were collected as per Early Morning Collections. Two replicates were performed at the start of the evaluation (T0). Volunteeers had no bednets on those two occasions, allowing the assessment of blood-feeding in the absence of a physical barrier.

3.3.5. Residual efficacy tests

Thirty-minute standard WHO cone bioassays (WHO, 1998) were carried out using 3–5-day-old unfed females of *Cx. quinquefasciatus* S-Lab and *An. gambiae* Kisumu—both reference strains susceptible to all insecticides—reared at the CREC insectarium (as shown in Figure 8, at the end of this chapter). Tests were performed every 3 months after treatment. Females were introduced into transparent plastic cones. Immediate mortality was assessed 30 and 60 minutes after the beginning of the exposure. Delayed mortality was assessed 24 hours later at the laboratory.

3.3.6. Distance tests

Unfed females of *An. gambiae* Kisumu and *Cx. quinquefasciatus* S-Lab—between 3 and 5 days old, reared at the CREC insectarium, and susceptible to all insecticides—were introduced into four 150-ml cups, with 15 females per cup per hut. Mosquito netting was placed at both ends to allow air to go through. Honey-soaked cotton was introduced to ensure that females did not die from starvation. Tubes containing females were placed horizontally inside experimental huts overnight, from 19:00 to 7:00 h, at a distance of 1 metre from two perpendicular walls.

The following morning, females were taken to the insectarium for mortality assessment after 24 hours at 80 ± 10% relative humidity and 27 ± 2°C temperature. Tests were performed every 3 months after treatment.

3.3.7. Statistical analysis

χ2 analysis were run to test whether differences were statistically significant. EMC and Mosquito release experiments: The Statcalc application of Epi-Info 6 (USD, Inc., Snellville, U.S.A.) was used to analyse differences in exophily, blood-feeding and mortality rates among huts; to analyse differences in entry rates, ANOVA was used. When mortality rates in control huts were between 5 and 20% Abbott's mortality correction formula was applied (Abbott, 1925). Residual efficacy and distance tests: Immediate and delayed mortality were analyzed using Epi-Info 6. Where values were <5, Fisher exact tests were used. Because bioassay tests are subject to variations, a 99% confidence interval was applied.

3.4. Spatial mortality assesments: Recommendations for WHOPES

3.4.1. Laboratory tests using distance boxes

Two identical wooden boxes were built, one for control and one for treatment. Each wooden box was 50 cm wide × 50 cm high × 100 cm long, with two horizontal slits of 4 cm × 50 cm in the middle of each side to allow air to flow

through. Wood was chosen as a material most readily available and easy to work with. One end of the box was left open, and mosquitoes were placed inside in 150 ml tubes. The other end was closed by a cement surface 50 cm × 50 cm (cement was chosen, as it was the material experimental huts were made of). The box used as control had a cement surface with no paint. The box used for treatment had a cement surface with of one layer of Inesfly 5A IGR insecticide-paint at 1 kg/6 sq m. Boxes were placed in a closed room at 80 ± 10% relative humidity and 27 ± 2°C temperature.

Unfed females of *An. gambiae* Kisumu and *Cx. quinquefasciatus* S-Lab, 3 to 5 days old—reared at the Centre de Recherche Entomologique de Cotonou (CREC) insectarium, and susceptible to all insecticides—were used. Mosquitoes were introduced in four 150 ml tubes, with mosquito netting at both ends to protect them from scavengers but allow air to flow through. Honey juice-soaked cotton was put in each tube to prevent starvation. Four tubes of 15 females each gave a total of 60 females per surface per test. Tubes were placed horizontally at the edge of the box, 1 metre from the cement surface, overnight from 19:00 to 07:00 (as shown in Figure 9, at the end of this chapter).

The following morning, females were taken to the insectarium for delayed mortality assessments after 24 hours at 80 ± 10% relative humidity and 27 ± 2°C temperature. Distance testing was done after treatment (T0) under laboratory conditions.

3.4.2. Field tests in experimental huts in Bénin

Inesfly 5A IGR was evaluated in six experimental huts at the Ladji station in Cotonou (south of Bénin) (Figures 5 & 6). Experimental huts were built in the West African style (Darriet *et al.*, 2002), and were treated with 1 or 2 layers of insecticide paint, at 1 kg commercial product/6 sq m—that is, 0.51 g a.i. per sq m. Based on the huts' dimensions, 3.4 kg of paint was applied on walls per layer, and 1.0 kg on ceilings. Huts treated with two layers had the first layer diluted in 20% water following recommendations of the manufacturer. The overall random disposition of huts was:

- H1: Control 1 (no paint);
- H2: Control 2 (two layers of control paint on walls and ceiling);
- H3: one layer of insecticide paint on walls;
- H4: one layer of insecticide paint on walls and ceiling;
- H5: two layers of insecticide paint on walls;
- H6: two layers of insecticide paint on walls and ceiling.

Unfed females of *An. gambiae* Kisumu and *Cx. quinquefasciatus* S-Lab, 3 to 5 days old, reared at the CREC insectarium, and susceptible to all insecticides, were used.

A total of 60 females were introduced into four tubes of 150 ml, with 15 females per tube. Mosquito netting was placed at both ends to allow air through. Honey-soaked cotton was introduced to ensure that females did not die from starvation. Tubes containing females were placed inside the hut, on the floor, horizontally from 19:00 to 07:00, at a distance of 1 metre from two perpendicular walls inside the hut and 1.90 metre from the ceiling (as shown in Figure 10, at the end of this chapter).

The following morning, females were taken to the insectarium for delayed mortality assessment after 24 hours at 80 ± 10% relative humidity and 27 ± 2°C temperature. Tests were performed again 12 months after treatment.

Results from laboratory and field distance tests were analyzed using Epi-Info 6. When values were <5, Fisher exact tests were used.

3.5. Pre-Phase III pilot study at Bama VK1, Burkina Faso

3.5.1. Study site and mosquitoes

The study was conducted in the Kou Valley, a rice growing area in south-western Burkina Faso, West Africa. It is located at 30 km in the north of Bobo-Dioulasso (lat. 11° 23' 14" N and long. 4° 24' 42" W) and is composed of 7 villages with a total of 4,470 residents in 2013. The study was conducted specifically at the

Bama VK1 village (as shown in Figure 11, at the end of this chapter). Irrigation has existed in this area since 1972, and is now semi-permanent with two crops grown every year: from February to June during the dry season, and from July to November during the rainy season. Numerous studies have been conducted in the Kou Valley, including the first to show the impact of deltamethrin-impregnated bednets on malaria transmission (Robert & Carnevale, 1991).

The study area was chosen because of the high abundance of perennial populations of malaria vectors (Robert *et al*., 1985; Baldet *et al*., 2003) and the high frequency of the L1014F *kdr* mutation (about 90%), rendering local malaria vector populations highly resistant to pyrethroids and DDT (Dabiré *et al*., 2008; Dabiré *et al*., 2009). Both *An. gambiae* (former *An. gambiae* S form) and *An. coluzzii* (former *An. gambiae* M form) coexist in sympatry in the study area, but *An. coluzzii* is preponderant in the rice field habitats. As part of the necessary background information, the exact species were determined molecularly (Santolamazza *et al*., 2008). The study was performed continuously for 6 months, from June to December 2013, and then again in June 2014, 12 months after treatment.

3.5.2. Insecticide paint and LLINs

Inesfly 5A IGR was applied on plastic sheetings at 1 kg commercial product/6 sq m. that is 0.51g a.i. per sq m. The paint was applied with no need of special equipment, just a regular brush and gloves. Polypropylene plastic sheeting was bought at the local market and consisted of big plastic rolls cut and fit into the study houses. The plastic sheeting was used to homogenize test surfaces as some houses were made of adobe and some of cement. The plastic sheeting was then placed on the superior two thirds of interior house walls and ceilings. The lower part of all walls was left untreated for up to 1 metre for all houses to reduce direct exposure to both, babies and young toddlers.

The LLINs in this study were all PermaNet 2.0, a trademarked product made of multifilament polyester netting (100 denier), factory-impregnated with deltamethrin at 55 mg/m^2 in a wash-resistant binder system. These had been distributed locally by the Programme National de Lutte contre le Paludisme

(PNLP), an initiative of Burkina Faso's Ministry of Health, in 2013. All nets were checked prior to the study, and were found to be intact and correctly used by the owners.

3.5.3. Early morning collections (EMCs)

Inesfly 5A IGR was evaluated in 14 village houses at VK1. The 14 houses at VK1 were chosen based on owners' wish to participate and equivalence in dimensions. The control houses consisted on plastic sheetings with no paint, but with intact LLINs. For the treated houses, paint was applied on plastic sheetings with one or two layers of insecticide paint. Huts treated with two layers had the first layer diluted in 20% water following recommendations of the manufacturer. The ceilings of certain houses were also covered with painted plastic sheeting per the configuration below. The different configurations were treated in duplicate. Configurations were designed to allow the evaluation of a potential volume effect and dose effect.

1) 2 x control sheeting with no paint + LLIN.
2) 2 x regular paint 1 layer + insecticide paint 1 layer on walls only + LLIN.
3) 2 x regular paint 1 layer + insecticide paint 1 layer on walls & ceiling + LLIN.
4) 2 x insecticide paint: 1 layer on walls only + LLIN.
5) 2 x insecticide paint: 1 layer on walls & ceiling + LLIN.
6) 2 x insecticide paint: 2 layers on walls only + LLIN.
7) 2 x insecticide paint: 2 layers on walls & ceiling + LLIN.

Mortality was the entomological indicator evaluated during this Phase II performed under field conditions. As there was no verandah, the excito-repellent effect generally assessed in experimental huts following the Phase II WHOPES protocols, could not be implemented. Similarly, although house dimensions were similar, the number and size of openings (windows and doors) were too different to reliably evaluate the deterrent effect and blood-feeding inhibition.

Before any treated sheetings were applied, mosquito collections took place for one full week just with LLINs to ensure there that there was no difference between huts in attractiveness to mosquitoes. Between June and December 2013 and again in June 2014, mosquito collections were performed nightly at VK1.

The study was approved by the Ethics Committee of the Institut de Recherche en Sciences de la Santé at Centre Muraz. Sixteen volunteers, 18 or older, were recruited from residents of VK1 (2 volunteers also served as back-ups). After being informed about the study and discussing it, these volunteers provided an Informed Consent in writing or with a finger print if illiterate. The volunteers received training on mosquito collection procedures. At the first suspicion of malaria, volunteers were provided with the curative treatment recommended by the National Malaria Control Program in Burkina Faso. Furthermore, all houses were checked and had intact well used LLINs.

Volunteers rotated houses each night to avoid bias while avoiding contamination between houses. The lower part of doors were covered with cloth to reduce the number of scavengers from entering houses. Houses were broomed every morning and every evening to remove scavengers that made it in through other openings. There was one volunteer sleeping per house.

Mosquito collections were performed following WHOPES testing procedures (WHO, 1996) for mortality rates except for the time when windows were closed. Volunteers would enter their houses at 18:00 hours, one volunteer per house, and sleep under LLINs until 5:30 hours, when they would be awaken to close the windows (that had been left open during the night as it is commonly done in the area). Once windows were closed at 5:30 hours, the volunteers proceeded to collect mosquitoes within the house. After classifying mosquito females as dead or alive, alive females were taken to the insectarium for delayed mortality assessment after 24 hours at 80 ± 10% relative humidity and 27 ± 2°C temperature.

All mosquitoes were then conserved in silica gel at –20°C to identify the species, analyse the resistance status and determine the source of blood meal.

3.5.4. Residual efficacy tests using 30-minute WHO bioassays

Thirty-minute standard WHO cone bioassays (WHO, 1998) were carried out using 2–4 days old unfed females of *An. gambiae* Kisumu, the reference strain susceptible to all insecticides reared at the IRSS/Centre Muraz insectarium. The local malaria vector population—identified molecularly as pyrethroid-resistant *An. coluzzii*—was reared at the insectarium from field-collected larvae to the adult stage, and was also tested in parallel to *An. gambiae* Kisumu.

For each house, 10 females were introduced in 5 cones placed on five sides of the house (four walls and the ceiling) for 30 minutes. Cones were not placed on LLINs. Females were taken to the insectarium for delayed mortality assessment after 24 hours at 80 ± 10% relative humidity and 27 ± 2°C temperature. Tests were performed monthly at T0, T1, T3, T6 and T12 after treatment.

3.5.5. Molecular analysis on resistance

The detection of *kdr* resistance genes was performed following protocols developed for the L1014F *kdr* mutation (Martinez-Torres *et al*., 1998), for the L1014S *kdr* mutation (Ranson *et al*., 2000), as well as the detection of the *ace-1*^{R}G119S mutation (Weill *et al*., 2004). Testing took place each month for 5 months after treatment on *An. coluzzii* females collected in control houses and houses treated with 1 or 2 layers of insecticide paint on walls and ceiling.

3.5.6. Determination of blood meal source

Blood meal identification was performed using a direct enzyme-linked immunosorbent assay (ELISA) (Beier *et al*., 1988). The choice of antibodies tested was based on the animals that are more frequent in the study area. Six antibodies were tested: human, dog, sheep, donkey, cattle and pig. These antibodies, marked with peroxidase, were kept at + 4°C. Blood-fed *Anopheles* females collected during EMCs from June to December 2013 in control houses and houses treated with 1 or 2 layers of insecticide paint on walls and ceiling were tested. A total of 425 females identified molecularly as *An. coluzzii* were

tested from each of those 3 configurations (> 140 per configuration) to determine the source of the blood meal.

3.5.7. Statistical analysis

Results on mortality were compiled and analyzed using Epi-Info Version 6 to test for any significant difference in mortality rates between the different configurations via Chi square tests. A 95% confidence interval was applied. When mortality rates in control huts were between 5 and 20% Abbott's mortality correction formula was applied (Abbott, 1925). Because bioassay tests are subject to variations, a 99% confidence interval was applied.

The source of the blood meal in engorged *An. coluzzii* collected from the three configurations were compared by chi-2 test.

The allelic frequency of each mutation (*kdr* and *ace-1^R*) was calculated with the formula F(R) = (2RR + RS)/2n where n is the total sample size. The frequency of *kdr* and *ace-1^R* in *An. coluzzii* collected from the three configurations (control, houses treated with 1 or 2 layers of insecticide paint on walls and ceiling) were compared by chi-2 test. The genotypic frequencies at the *kdr* and *ace-1^R* loci were compared to Hardy-Weinberg expectations using the exact test procedures implemented in GENEPOP (version 4) software (Raymond & Rousset, 1996).

3.6. Pre-Phase III pilot study at Bama VK3

3.6.1. Study site and mosquitoes

The study was conducted in the VK3 village in the Kou Valley (11° 23' 14" N; 4° 24' 42" W), a rice growing area of south-western Burkina Faso located at 30 km in the north of Bobo-Dioulasso (as shown in Figure 12, at the end of this chapter). As in the case of VK1, malaria vectors are abundant throughout the year and the frequency of the L1014F *kdr* mutation is high in the area, inducing high resistance level of local malaria vectors populations to pyrethroids and DDT (Dabiré *et al.*, 2008; Dabiré *et al.*, 2009). *Anopheles coluzzii* (former *An. gambiae* form M) was the only *An. gambiae s.l.* species present in the area during the study, from August to December 2013, confirming previous results obtained in the VK3 village

during the same season. As in the VK1 study, the identification of *Anopheles* species among the *An. gambiae* complex was performed using a Short INterspersed Elements (SINE)-PCR approach (Santolamazza *et al*., 2008).

3.6.2. Insecticide paint and LLINs

Inesfly 5A IGR was applied on metallic doors and windows of the selected houses. As in the VK1 study, the LLINs used during this study were intact PermaNet 2.0, made of polyester netting impregnated with deltamethrin in a wash-resistant binder system (distributed by the National Malaria Control Program campaign in 2013).

3.6.3. Early morning collections (EMCs)

The design was evaluated in 20 inhabited houses selected for the study in VK3. The control consisted on houses free of paint and LLINs (sleepers could use any tool to protect against nuisance following their own regimen). For the treated houses, paint was applied on doors and windows with one layer of insecticide paint at 1 kg commercial product/6 sq m. (that is. 0.51g a.i. per sq m.) The 20 houses at VK3 were selected according to their similarities in terms of size (the mean was 3.5 × 5 metres), and included in the study based on informed consent from home-owners. Houses were randomly allocated to the control arm or the treatment arm.

1) 10 houses with LLINs and/or other methods based on their own choice
2) 10 houses with LLINs + insecticide paint at 1 layer on doors and windows

The design allowed the assessment of the killing effect and the long-term residual efficacy of such configurations. Like in VK1, as there was no verandah, the excito-repellent effect generally assessed in experimental huts following the Phase II WHOPES protocols, could not be implemented. Similarly, although window and door dimensions were comparable, they were not identical and remained opened for different amounts of time, making it difficult to reliably evaluate the deterrent effect and blood-feeding inhibition. Thus, only mortality was assessed in this pilot study.

Before even randomizing the treatment arms, mosquito collections took place for 1 full week just with LLINs to ensure there that there was no difference between huts in attractiveness to mosquitoes. Between August and December 2013 (T0-T4), mosquito collections were performed monthly during four consecutive days.

The study was approved by the Ethics Committee of IRSS at Centre Muraz. During collections, 20 volunteers of at least 18 years old were recruited from residents at VK3.

After being informed about the study and discussing it, these volunteers provided an Informed Consent in writing (or with a fingerprint if illiterate). The volunteers received training on mosquito collection procedures.

Mosquito collections were performed following WHOPES testing procedures (WHO, 1996) for mortality rates, except for the fact that inhabited houses were used rather than experimental huts. Volunteers would enter their houses at 18:00 hours, one volunteer per house, and sleep under LLINs until 5:30 hours, when they would be woken to close the windows (these were left open during the night, as is common in the area). Before entering houses every evening and every morning, after collection, houses would be cleaned to eliminate scavengers. At the first suspicion of malaria, volunteers were provided with the curative treatment recommended by the National Malaria Control Program in Burkina Faso. Volunteers rotated houses each night to avoid bias in mosquito collections. Early in the morning, mosquito females were collected inside each house and classified as dead or alive, unfed or blood-fed. (Although the full blood-feeding inhibition assessment could not be performed, these females were collected for the blood-origin studies.) Live mosquitoes were put in observation for delayed mortality assessment after 24 hours at 80 ± 10% relative humidity and 27 ± 2°C temperature. All mosquitoes were then conserved in silica gel at –20°C to study the species, the resistance status and the source of blood meal.

3.6.4. Residual efficacy tests using 30-minute WHO bioassays

The WHO protocol for evaluation of residual efficacy was followed (WHO, 1998) using 2–4 day-old unfed females of *An. gambiae* Kisumu, a reference strain susceptible to all insecticides reared at the IRSS/Centre Muraz insectarium. The

local malaria vector population at VK3, identified molecularly as *An. coluzzii* and resistant to pyrethroids, was reared at the insectarium from field-collected larvae to the adult stage; it was also tested in parallel to *An. gambiae* Kisumu.

For each house, 10 females were introduced in 5 cones placed on two sides of the paint-treated surface (or control) for 30 minutes respectively on metallic doors and windows. Cones were not placed on LLINs. Females were taken to the insectarium for delayed mortality assessment after 24 hours at 80 ± 10% relative humidity and 27 ± 2°C temperature. Tests were performed monthly at T0, T1, T3 and T6 after treatment.

3.6.5. Spatial Mortality Assesments

The effect of mortality was also assessed at a distance. Mosquito females were placed at distances of 1 metre and never came in direct contact with the treated surface.

Unfed females of *An. gambiae* Kisumu and *An. coluzzii* from VK3 raised at the insectarium from field-collected larvae were used. A total of 60 females were introduced into four tubes of 150 ml, with 15 females per tube. Mosquito netting was placed at both ends to allow air through. Honey-soaked cotton was introduced to ensure that females did not die from starvation. The protocol followed was the same described by Mosqueira *et al*. (2013), except females were exposed for 30 minutes only, instead of 12 hours. Females were taken to the insectarium for delayed mortality assessment after 24 hours at 80 ± 10% relative humidity and 27 ± 2°C temperature. Tests using *An. gambiae* Kisumu were performed from T0 to T4; tests using *An. coluzzii* from VK3 were tested from T0-T3 because of some difficulties at T4 in rearing the local population from field-collected larvae at the insectarium.

3.6.6. Molecular analysis on resistance

The detection of the L1014F *kdr* mutation was performed following Martinez-Torres *et al*. (1998) and the *ace-1*^{R}G119S mutation by Weill *et al*. (2004) to

analyse of the resistance status at the time of the study. Testing took place after treatment on *An. coluzzii* females collected in the 2 arms of the study houses.

3.6.7. Statistical analysis

To evaluate efficacy of the insecticide paint, results were compiled and analyzed using Epi Info Version 6 to test for any significant difference in mortality rates between the different configurations via Chi square tests.

A 95% confidence interval was applied. When mortality rates in control huts were between 5 and 20% Abbott's mortality correction formula was applied (Abbott, 1925). Because bioassay tests are subject to variations, a 99% confidence interval was applied. The allelic frequency of each mutation (*kdr* and *ace-1^R*) was calculated with the formula F(R) = (2RR + RS)/2n where n is the total sample size. The frequency of *kdr* and *ace-1^R* in *An. coluzzii* collected in the 2 arms of the study houses were compared by chi-2 test. The genotypic frequencies at the *kdr* and *ace-1^R* loci were compared to Hardy-Weinberg expectations using the exact test procedures implemented in GENEPOP (version 4) software (Raymond & Rousset, 1996).

3.7. Selection of villages for WHOPES Phase III

3.7.1. Pre-selection of study sites

The Orodara region is holoendemic for malaria and its main vectors, *An. gambiae* and *An. coluzzi* are resistant to pyrethroids through the L1014F *kdr* mechanism (Dabiré *et al*., 2009). The number of villages needed per arm will be 12–15 to allow for a more powerful statistical analysis (Rogier *et al*., 2009). Cluster-randomized trials with fewer than five clusters per arm are inadvisable, because parametric tests may be unreliable with such small numbers and because nonparametric tests require at least four clusters per arm to achieve statistical significance (Rogier *et al*., 2009). The minimum distance between villages—the buffer zone—must be 1–2 km. This distance was selected as the radius because the active dispersal (due to appentential flight) of *An. gambiae* is estimated to be less than 500 metres (Costantini *et al*., 1996; Midega *et al*., 2007; Zhu *et al*., 2015). As well, villages needed to be accessible during the rainy season. The

number of children aged 6 months to 14 years old needs to be defined (Hayes & Bennett, 1999), but the size of the villages selected should allow for at least 30 evaluable children per village at the end of the study.

Owing to heterogeneity between communities, the communities will be stratified in terms of their size, location, coverage of household protection measures, and entomological and parasitoclinical parameters.

Within each stratum, communities will be randomly allocated to the intervention or control arms: villages will be randomized to: 1) LLINs allone or, 2) combination of the OP insecticide paint and LLINs (Inesfly 5A IGR + LLINs).

In the selected villages, each house with no exception will be treated to either LLINs or Inesfly 5A IGR + LLINs. The paint will be applied, as for the trial at VK1, on plastic sheeting at 1Kg/6m^2 on walls and ceilings of local houses. The lower part will be left untreated up to 1 metre to avoid contact with babies and young toddlers.

The present preparatory phase focused on mapping the communities, geo-referencing the communities and habitations, and assessing the residents' level of knowledge and practices about malaria. During the present preparatory phase, the entomological, parasitological and clinical parameters of malaria are not yet evaluated. These evaluations will be performed at baseline and post-treatment during the Phase III study.

A complete list of villages in the Orodara region, and estimates of the number of residents, was obtained from the Orodara Sanitary District. Some 34 villages were found to meet the main criteria of the Phase III study: at least 100 children from 6 months to 14 years of age (to ensure 30 evaluable children at the end of the study), minimal distances of 1–2 km from each other, and access by road. These 34 villages were visited for preliminary surveys and geo-referencing.

3.7.2. Preliminary surveys and geo-referencing

All the habitations in the 34 villages were geo-referenced using a Garmin etrex-type GPS; and a questionnaire was provided to residents. It included questions

on demographic information (name, age, sex, family status, and ethnic group); number of children per family aged 6 months to 14 years; geomapping information on the number of homes or habitations, and the number of sleeping units; and sociological information on malaria perception, prevention and treatment. People were also asked if they would be interested in participating in the study involving insecticide paint and LLINs. All the questions, and the information about the study, were presented in French and/or Dioula, the main local langage. The French version of the questionnaire appears below.

1	Date de l'enquête				
2	Formation sanitaire				
3	Village				
4	Non de l'enquêteur				
5	Nom de l'enquêté				
6	Qualité de l'enquêté	CC	EpCC	autre	
7	Age de l'enquêté		ans		
8	Sexe de l'enquêté		M		F
9	Identifiant concession				
10	Notez si vous avez repeint l'identifiant concession		Oui	Non	
11	Nouvelle concession (non recensée par Zinc) --> Donner un identifiant concession		Oui	Non	
12	Latitude (noter seulement si nouvelle concession)				
13	Longitude (noter seulement si nouvelle concession)				
14	Nom du chef de la concession				
15	Ethnie du chef de la concession				

16	Nombre total de pièces à peindre (où les gens peuvent dormir)	

17	Homme	Femme
Adultes >15 ans		
Enfants 0–4 ans		
Enfants 5–14 ans		
Enfants < 6 mois		
Total 6 mois-14 ans		

18	Est-ce que dans votre village les moustiques vous dérangent?			Oui		non
19	Si oui, à quel moment les moustiques vous dérangent ? (entourer la ou les réponses)	Mai-juin	Juillet-sept	Oct-Nov	Déc-février	Mars-Avril
20	Le paludisme est-il une maladie fréquente dans votre village ? (cocher)					
	Oui		Non		Je ne sais pas	
21	Selon vous, quelles sont les causes du paludisme ?					
	Moustiques		Eaux sales		Pluie/ Fraîcheur	
	Aliments trop gras et/ou trop sucrés		Premiers fruits		Autres (préciser) :	

22	Que faites-vous pour vous protéger (vous et votre famille) du paludisme ?			
	Rien		Utiliser les bombes aérosols	
	Fumer les noix de karité		Utiliser les moustiquaires	
	Utiliser les spirales		Bien se couvrir la nuit	
	Eviter les premiers fruits		Eviter les aliments trop gras et/ou trop sucrés	
	Fumer les plantes		Autres (préciser) :	
23	Quand y a-t-il eu distribution de moustiquaires imprégnées dans votre village pour la dernière fois ? (année)			
24	Est-ce que vous avez une moustiquaire imprégnée ?	Oui	Non	
25	Quels sont les signes du paludisme selon vous ?			
	Fièvre/Corps chaud		Maux de tête	
	Courbatures		Vomissements	
	Autres (préciser) :			
26	Que pensez-vous du fait d'ajouter de l'insecticide à la peinture des maisons pour lutter contre les moustiques ?			
	Je ne sais pas		C'est une bonne chose	
	Ce n'est pas une bonne chose		Cela pourrait rendre malade	

	La peinture ne peut pas être utilisée dans toutes les maisons			Autre (préciser) :		
27	Seriez-vous d'accord qu'une telle peinture soit utilisée dans votre maison ?					
	Oui		Non		NSP	

3.7.3. Mapping and cartography of data collected

Results on the questionnaire and the geo-reference were entered in an Excel database. This database was then converted into a database Dbase IV format and transferred to MapInfo for the creation of a Geographic Information System (GIS) and Study Maps. The resulting study area in the Orodara region (Kenedougou Province) is represented in as shown in Figure 13.

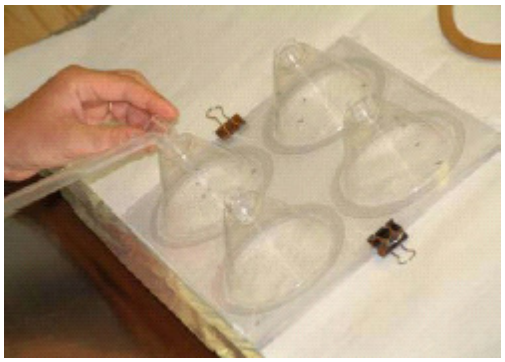

Figure 4: Mosquitoes in 30-minute WHO bioassay cones

The tests used 15 females per cone, and were done in four repeats.

Figure 5: Experimental WHOPES Phase II Station at Ladji

In Cotonou, Bénin, 6 experimental huts stand by Lake Nokoué. With almost perennial populations of *Anopheles gambiae* and *Culex quinquefasciatus*, the site allows entomological parameters to be measured, to assess the efficacy of insecticide products against pest and malaria mosquitoes.

Figure 6: View of a WHOPES Phase II experimental hut at Ladji

In Cotonou, Bénin, experimental huts allow mosquitoes to enter through the slits of four wooden windows. The insects are collected from the main room and (if they try to escape) from the veranda, wrapped in blue plastic.

Figure 7: Insectarium at the CREC institute in Cotonou, Bénin

Anopheles gambiae nymphs are put into plastic cups inside cages covered with cloth. Once the females emerge as adults and are 3–5 days old, they are ready for testing.

Figure 8: 30-minute WHO cone bioassays at Ladji

In the WHOPES Phase II experimental hut in Cotonou, Bénin, females of *Culex quinquefasciatus* S-Lab and *Anopheles gambiae* Kisumu were put in contact with treated walls for 30 minutes to estimate the insecticide's residual efficacy.

Figure 9: Spatial mortality assessments in the CREC laboratory, Bénin

Four plastic cups, with 15 mosquito females in each, were placed overnight at a distance of 1 metre from either the treated or the control surface.

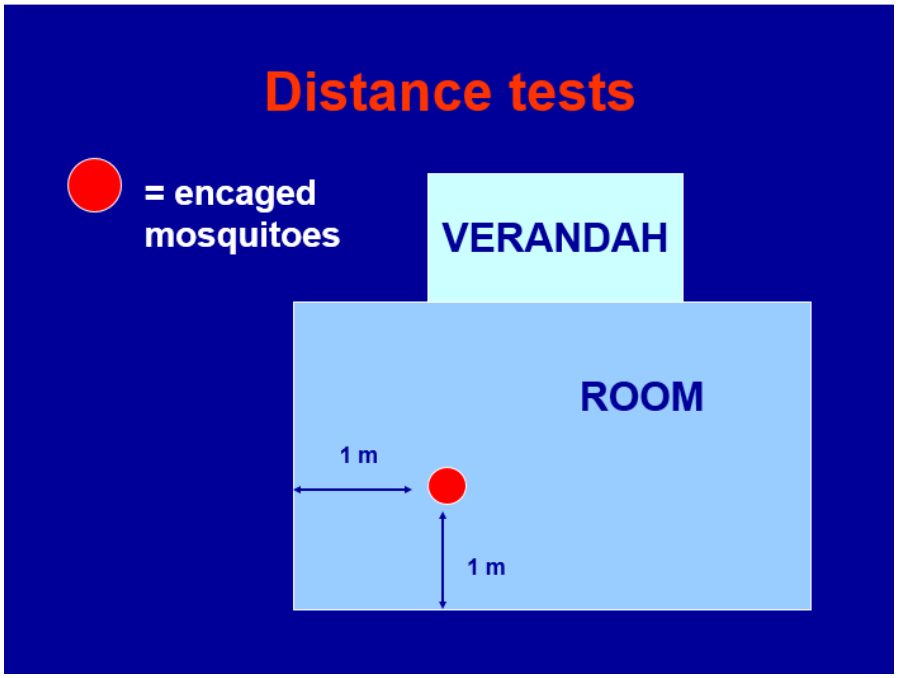

Figure 10: Distance tests of mosquito placement inside experimental huts

Caged mosquitoes (represented by the red dot) were placed at distances of one metre (100 cm) from two perpendicular walls, and left there overnight.

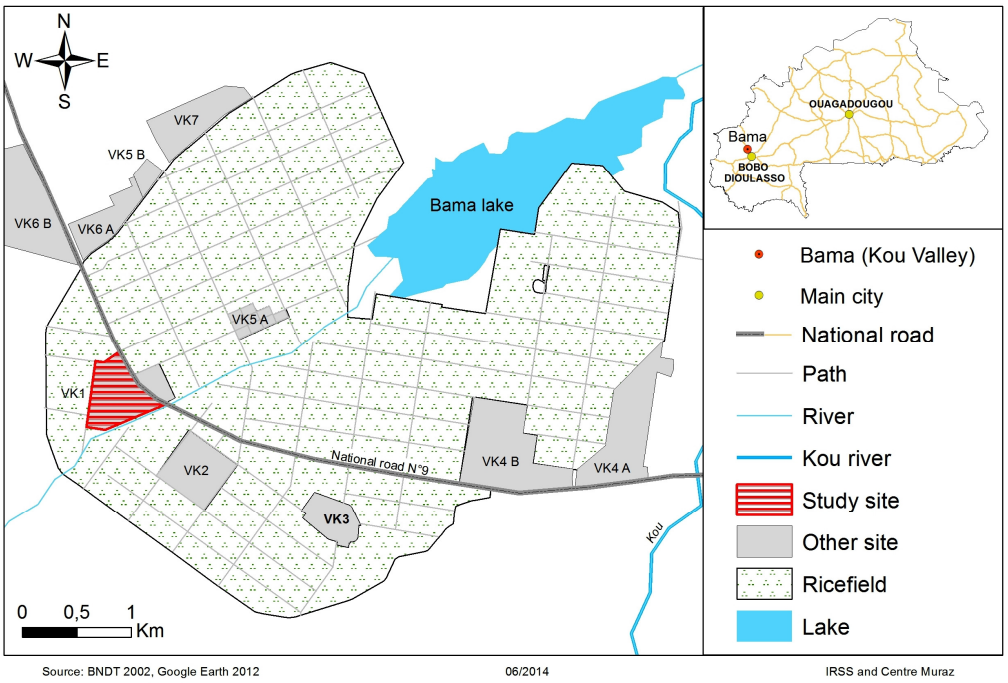

Figure 11: Location of Bama VK1 at Kou Valley, in south-western Burkina Faso. Source: BNDT.

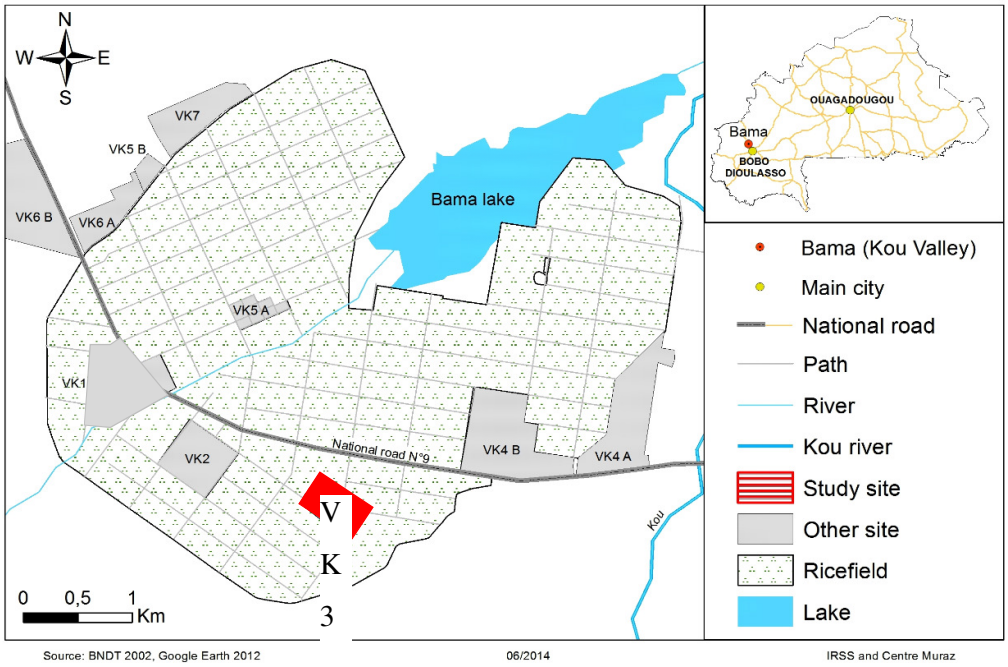

Figure 12: Location of VK3 at Kou Valley (Source: BNDT)

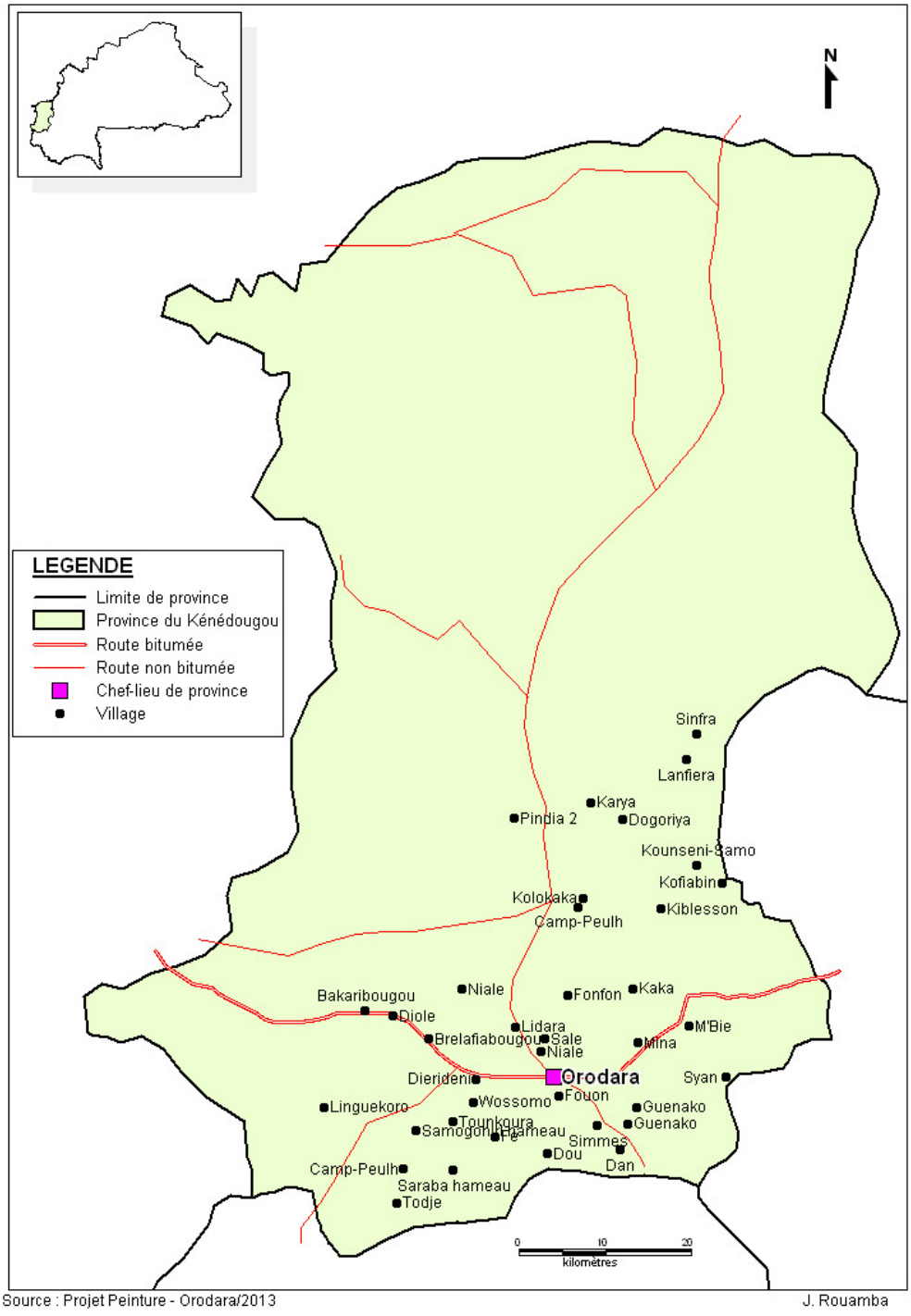

Figure 13: Study area in the Orodara region of Burkina Faso (Source: Dr. J. Rouamba)

This map of Kenedougou Province shows the 34 geo-referenced villages for the Phase III study.

CHAPTER 4: RESULTS

4.1. WHOPES Phase I at IPR, Côte d'Ivoire

Mortality on adobe treated surfaces was almost zero after only 1 month after treatment. At the time when the last tests were done (2 months after treatment), mortality was still high on metal and cement treated surfaces. The fact that those big differences were clearly observed between adobe and the other two surfaces, cement and metal, even shortly after treatment, suggested that porosity was an important factor to consider. As a result, the issue of porosity and layers was raised and taken into account on all the subsequent studies.

4.2. WHOPES Phase I at LIN, France

4.2.1. Delayed mortality using 30-minute WHO bioassays

After treatment, at T0, delayed 24-hour mortality was 98–100% (compared to control, $p < 10^{-3}$) for both, susceptible S-Lab and OP-resistant *Cx. quinquefasciatus* on non-porous surfaces and porous surfaces treated at 1 kg/6 m^2. While non-porous surfaces performed equally well regardless the dose and the resistance status, porous surfaces, cement and stucco, treated at the lower dose 1 kg/12 m^2 performed less optimally against OP-resistant mosquitoes yielding mortalities of 87% ($p < 10^{-3}$) and 15% ($p < 10^{-2}$) respectively.

Six months after treatment (T6), efficacy dropped on cement surfaces treated at both doses, on both resistant and susceptible mosquitoes. On stucco surfaces, only OP-resistant *Cx. quinquefasciatus* experienced a drop. Twelve months after treatment (T12), mortality at 24 hours was 90–100% (compared to control, $p < 10^{-3}$) even against resistant mosquitoes at the lower dose, on the non-porous surfaces. This is shown in Table 2, at the end of this chapter—as are all the Tables referred to below.

4.2.2. IGR efficacy on fecundity, fertility and larval development

At T0, a 46% reduction in the number of eggs laid was shown at 1 Kg/12 m^2 ($p < 10^{-3}$) (Table 3). At T9, a 38–40% reduction in the number of eggs laid was shown

at both doses, 1 Kg/6 m^2 and 1 Kg/12 m^2 ($p < 10–^3$) (Table 4). At T0, 50.3% of eggs in the control groups hatched versus 41.3% at 1 Kg/12 m^2 ($p < 10–^3$). At T9, differences in %hatching were no longer significative. The % of pupation was not significantly different between control and treated surfaces at any timepoint or dose. Regarding emergence, an increased mortality from the nymph to the adult stage was shown 0 months after treatment ($p < 10–^3$), and 9 months after treatment only at the higher dose ($p < 10–^3$) (Tables 3 and 4, respectively). No differences were found on the duration of the larval development which lasted 12–13 days. No IGR effect was observed 12 months after treatment.

4.3. WHOPES Phase II at Ladji, Bénin

4.3.1. Early morning collections (EMC)

As expected in the case of OPs, no deterrent or excito-repellent effect was not observed against local populations of *An. gambiae* or *Cx. quinquefasciatus*. For ethical reasons, non-treated but intact and well fixed bednets had been placed in all experimental huts. As a result, blood-feeding rates could not be adequately evaluated using EMCs. Instead, blood-feeding inhibition rates were tested using malaria-free *An. gambiae* release experiments as exposed below.

For the first 3 months of treatment, mortality rates were 100% after treatment for all treated huts against both, local populations of *An. gambiae* and *Cx. quinquefasciatus*, and differences were significant compared to control. Six months after treatment (T6), mortality rates against *Cx. quinquefasciatus* were of 90–100% for all treated huts (Table 6). Due to seasonal factors, there is no data on *An. gambiae* for that time point. By nine months after treatment (T9), mortality rates in huts treated with two layers were still 90–93% against *An. gambiae* and 54–57% against *Cx. quinquefasciatus* (Tables 5 and 6, respectively). Twelve months after treatment (T12), mortality against *Cx. quinquefasciatus* was still higher compared to control in huts treated with one layer ($p < 0.05$) and two layers ($p < 10–3$) and (Table 6).

4.3.2. Mosquito release experiments

At the start of the evaluation (T0), blood-feeding in treated huts went from 2 to 13%, whereas control huts yielded blood-feeding rates of 68.5 and 76.1% (as shown in Figure 14, at the end of this chapter). Differences between treated and control huts were significantly different ($p < 10–3$).

4.3.3. Residual efficacy tests using 30-minute WHO bioassays

For the first 3 months, in huts treated with one layer, mortality rates of 98–100% were observed against both *An. gambiae* Kisumu and *Cx. quinquefasciatus* S-Lab (Tables 7 and 8). Six months after treatment, for *An. gambiae*, mortality rates started dropping to values of 79.4 and 59.7%. For *Cx. quinquefasciatus* values of 98–100% continued to be observed 6 and 9 months after treatment. At nine months after treatment, mortality rates dropped to 14.7% against *An. gambiae* in the house treated with just one layer just on walls (Table 7). In huts treated with two layers, mortality rates of 98–100% were observed for both *An. gambiae* and *Cx. quinquefasciatus* for up to nine months (Tables 7 and 8). Twelve months after treatment mortality rates were of 70–80% against pyrethroid-susceptible *An. gambiae* and *Cx. quinquefasciatus*.

4.3.4. Distance tests

For up to 6 months, huts treated with one layer yielded mortalities of 90–100% against *An. gambiae* Kisumu (Table 9) and *Cx. quinquefasciatus* S-Lab (Table 10) at a distance of 1 metre.

By 12 months after treatment, a volume effect was observed in the hut treated with one layer just on walls (35.6% for *An. gambiae* and 60% *Cx. quinquefasciatus*) compared to the one treated on both walls and ceiling (98.4% for *An. gambiae* and 96.2% *Cx. quinquefasciatus*), but differences were still significant with respect to control ($p < 10–6$) for both. Huts treated with two layers yielded mortalities 100% against pyrethroid-susceptible *An. gambiae* and *Cx. quinquefasciatus* for 12 entire months at a distance of 1 metre (Tables 9 and 10).

4.4. Spatial mortality assessments: Recommendations for WHOPES

4.4.1. Laboratory tests using distance boxes

After treatment, at T0, distance boxes yielded 100% mortality rates against both *An. gambiae* Kisumu and *Cx. quinquefasciatus* S-Lab. Results using distances of 1 metre were similar to the ones observed in experimental huts (Table 11). Compared to control, mortality rates were significantly different for the treated surface ($p<10–^{6}$).

4.4.2. Field tests in experimental huts in Bénin

Under field conditions at T0, all huts, regardless of the surface treated and the number of layers, yielded 100% mortality against both *An. gambiae* Kisumu and *Cx. quinquefasciatus* S-Lab (Table 12). Twelve months after treatment, mortality rates observed at the huts where a larger volume was treated with one or two layers of paint were 98.4% for *An. gambiae* Kisumu and 96.2% for *Cx. quinquefasciatus* S-Lab (Table 12). Mortality rates in the hut treated on only walls with one layer of insecticide paint was lower than in the other three huts: 36% mortality against susceptible *An. gambiae* Kisumu and 60% against susceptible *Cx. quinquefasciatus* S-Lab ($p < 10–^{6}$), though still higher than control ($p < 10–^{6}$).

4.5. Pre-Phase III pilot study at Bama VK1, Burkina Faso

4.5.1. Early morning collections (EMC)

No difference in house attractiveness was found prior to treatment. *Anopheles coluzzii* was the only *An. gambiae s.l.* species present in the study area, as established from the molecular analysis performed during the study. Between June and December 2013 and June 2014, a total of 3,903 *An. coluzzii* females were collected in all houses combined. Full collections started 1 month after treatment (Table 13).

For the first 6 months, the mortality rates observed in houses treated with the insecticide paint were 97–100%. Globally, 6 months after treatment, all houses treated with the insecticide paint, with 1 or 2 layers, on walls or on walls and ceiling, presented 100% mortality rates against local populations of *An. coluzzii* from VK1 whether they were blood-fed or not and were statistically significantly different from control ($p<0.001$). By T12, mortalities were still high and significantly different from control ($p<0.001$), but rates had slightly decreased to 69.5–82.2%.

The highest mortality rates 12 months after treatment were observed in houses treated with 2 layers of insecticide paint and a larger number of surfaces (82.2%). No statistically significant differences were found between treated houses at T12. Mortality rates observed in control houses with no insecticide paint but with LLINs ranged from 5.2 to 9.5%, throughout the study (Table 13).

4.5.2. Residual efficacy tests using 30-minute WHO bioassays

At T0, 30-minute standard WHO cone bioassays on *An. gambiae* Kisumu and local populations of *An. coluzzii* from VK1, yielded mortality rates of 98–100% in all houses treated with insecticide paint (Table 14) regardless of the configuration. Mortality in control houses was lower and significantly different from treated houses.

At T6, mortality rates were 100% against both *An. gambiae* Kisumu and local populations of *An. coluzzii* from VK1, in all treated houses. At T12, mortality rates were still 98–100% in all houses against *An. gambiae* Kisumu. In the case of the local *An. coluzzii* from VK1, 12 months after treatment mortality rates were 97% in houses treated with 2 layers of insecticide paint on walls and ceiling, but slightly lower mortalities were observed in the other configurations. Differences between treated houses with different configuations were not statistically significant ($p>0.05$).

Mortality rates observed in control houses with LLINs only ranged from 1.7% to 10.9% (Table 14). Again, cones were only placed on walls and ceiling, not on LLINs.

4.5.3. Molecular analysis on resistance

Allelic frequency of the L1014F and L1014S *kdr* mutations

All houses contained pyrethroid treated LLINs. Also, because the *Anopheles* females collected in treated houses were dead and around 89% to 94% of the females were alive in control houses, no comparisons could be done between dead and alive mosquitoes within each given configuration. Thus, comparisons were done overtime between control houses with LLINs and treated houses with LLINs and 1 or 2 layers of insecticide paint.

Overall, *An. coluzzii* females at VK1 were pyrethroid-resistant: the allelic frequency of the L1014F *kdr* mutation was high, ranging from 60 to 98% (Table 15) with no significant difference between alive specimens collected from the control and dead specimens collected from the treated houses during the period tested, up to 5 months after treatment. Similarly, no increasing or decreasing trends were identified on the allelic frequency overtime.

The L1014S *kdr* mutation was not found in the samples collected in control houses with LLINs and was weakly detected in the heterozygous form in houses treated with 1 layer at T2, T3, T4 and T5, and in houses treated with 2 layers, and at T5, though only in the heterozygote form (Table 15).

Allelic frequency of the mutation *ace-1^R*

The *Ace1^R* mutation was detected at low allelic frequencies and was heterozygous. It was only randomly found at T0 and T5 in the control houses at frequencies of 8.3 and 4.0%, respectively (Table 16) and at no point in the treated houses.

4.5.4. Determination of the blood meal source

There were no statistical differences between control houses, houses treated with 1 insecticide paint layer, and houses with 2 insectide paint layers (Table 17).

The averages of all houses combined from T0 to T6, showed about 27% of *An. coluzzii* females collected during EMCs at VK1 had fed on humans and about

16% had fed on both humans and other animals. All in all, the rate of zoophily was high (58%). Of the females having blood-fed on other animals (non human), about 45% of them had not blood-fed on any of the domestic animals chosen as the most typical blood meal sources in the area. Of the identified domestic animals (cattle, sheep, donkey, pig, dog), cattle remained the most common blood meal source followed by donkey (Table 17).

4.6. Pre-Phase III pilot study at Bama VK3, Burkina Faso

4.6.1. Early morning collections (EMCs)

No difference in house attractiveness was found during the blank collections. Mosquito collections began one week after the treatment. Overall 1,856 *An. gambiae* s.l. were collected from August to December 2013. A sub-sample of 165 mosquitoes (50±10 per arm for resistance gene characterization) were molecularly analyzed for their species identification within the *An. gambiae* complex. They were all identified as *An. coluzzii*.

Mortality rates in the houses with Insecticide Paint and LLINs was over 80% for 2 months, but decreased, progressively, to less than 30% at T4. Mortality observed in the control houses with no insecticide paint but with LLINs ranged from 8.6–15.7% (Table 18).

4.6.2. Residual efficacy tests using 30-minute WHO bioassays

The mortality rates obtained after the 30-minute standard WHO cone bioassays with *An. gambiae* Kisumu reached 100% on all treated surfaces (windows and doors made of metal) during the 3 first months after paint application (T0-T2). From the painted doors (Table 19A), the residual efficacy was still superior to 80% (85%) in T3 but fell down to 62% at T4. With the painted windows the mortality rates were relatively higher and rates were still 80% at T4 (Table 19A).

The mortality rates obtained with the local *An. coluzzii* from VK3 were between 100–90% from T0 to T2 and decreased significantly under 80% to reach 39% and 53% respectively for painted doors and windows at T3 (Table 19B).

4.6.3. Spatial mortality assesments

From T0 to T1, the distant killing effect went from 90% to 75% against *An. gambiae* Kisumu placed during 30 minutes at one metre from the insecticide painted doors and windows. At T2, this mortality decreased drastically to 30%. By T3 and T4, mortality further decreased to 20%.

In the case of *An. coluzzii*, at T0, 80% of *An. coluzzii* were killed. By T1, 77% of exposed individuals were killed. By T2, spatial mortality decreased to 25%, and by T3 to 15% (Table 20).

4.6.4. Molecular analysis on resistance

The allelic frequency of the L1014F *kdr* mutation in *An. coluzzii* females collected during EMCs at VK3 was high averaging 94% (Table 21) without any difference between control and treated houses at the time of the study.

Within the specimen analyzed by PCR no individual was detected sharing the *ace-1*^{R} mutation as well as from mosquitoes collected in control and paint treated houses (Table 21).

4.7. Selection of villages for WHOPES Phase III

4.7.1. Pre-selection of study sites

From the review of the villages listed by the Orodara Sanitary District, up to 34 were visited for preliminary surveys and geo-referencing.

4.7.2. Preliminary surveys and geo-referencing

In each of the 34 geo-referenced villages (as shown in Figure 15, at the end of this chapter), the habitations were numbered (Figure 16) and the information was collected for each individual residing in those habitations. Based on the results obtained, 2 out of the 34 villages were taken out. The reason for taking those 2 villages out is that they were much smaller (102 residents) and much bigger (844 residents) than the other villages selected and could not be paired to any other village for randomization. The other villages ranged from 139 to 654 residents

(Table 22). As a result, a total of 32 villages that fit the main criteria - at least 100 children from 6 months to 14 years of age (to ensure 30 evaluable children at the end of the study), minimal distances of 1–2 km from each other, and access by road – and that allowed pairing during the randomization process were selected.

The main roads and paths to arrive to the villages was also traced using GPS. In total, 11,890 people were registered in the 32 villages were the survey was carried (Table 22). Of those, a total of 5,492 children from 6 months to 14 years old were registered, 48.3% were girls and 51.7% were boys.

Information on the number of sleeping units was also collected. A total of 7,046 sleeping units were recorded. Of those, more than half of the homes consisted on 1–6 sleeping units, meaning there was a greater possibility to place bednets than if a single-spaced house is used (as shown in Figure 17, at the end of this chapter).

With regard to the question on malaria perception, almost every person receiving the questionnaire declared being bothered by mosquitoes, especially from May to November, corresponding to the onset of the rainy season in the region (as shown in Figure 18, at the end of this chapter).

With regard to transmission, most frequently the people surveyed attributed malaria to mosquitoes and/or rain: 89% of the people surveyed, associated malaria with both mosquitoes and rain. Only 11% did not associate malaria with rain, only with mosquitoes; there was a clear overlap of people associating malaria with both mosquitoes and rain.

Malaria was considered “frequent” by 97.3% of the people. It should be noted that malaria symptoms are not specific and are similar to other diseases.

Regarding practices on protection against malaria, 88% of residents slept under bednets during the rainy season, when the questionnaires were taken, between May and June 2013. Out of those, 92.8% declared those bednets were impregnated with insecticide (pyrethroids). According to the people surveyed, the bednets had been distributed by the Malaria National Control Program, of the Ministry of Health, between 2010 and 2011 (91.3%).

The main malaria evoking symptom in the surveyed villages was fever, followed by vomiting, headaches and joint pain (Table 23). Again, to be noted that those symptoms are not malaria specific. The grand majority of people interviewed were tentatively “favourable” to adding an insecticide paint to the walls of their homes to fight against mosquitoes (Table 24).

4.7.3. Mapping and cartography of collected data

The distribution of the 34 geo-referenced villages is represented in Figure 19 (at the end of this chapter), along with information on the number of children aged from 6 months to 14 years of age, the number of homes/habitations and preliminary information on minimal distances between villages.

Culex % Delayed mortality 24 h (N = 60)	Control 1 No paint	Control 2 Control paint	Cement 1Kg/ 6m^2	Cement 1Kg/ 12m^2	Stucco 1Kg/ 6m^2	Stucco 1Kg/ 12m^2	Softwood 1Kg/ 6m^2	Softwood 1Kg/ 12m^2	Plastic 1Kg/ 6m^2	Plastic 1Kg/ 12m^2
			Porous Surfaces				Non-Porous Surfaces			
T0 OP-Susceptible	0.5	0.4	100†	100†	100†	100†	100†	100†	100†	100†
T0 OP-Resistant	**2**	**2.2**	**100†**	**15.7†**	**100†**	**87.3†**	**100†**	**100†**	**100†**	**100†**
T6 OP-Susceptible	2.2	2.9	3.1	1.7	100†	96.7†	100†	100†	100†	100†
T6 OP-Resistant	**1.6**	**3.3**	**0**	**0**	**31.7†**	**3.3**	**100†**	**100†**	**100†**	**100†**
T12 OP-Susceptible	0	2.1	2	0	91.4†	20.3†	100†	100†	100†	100†
T12 OP-Resistant	**1.5**	**1**	**4.1**	**5.3**	**20.3†**	**5.3**	**100†**	**93.2†**	**100†**	**100†**

Control 1 = No paint; Control 2 = Paint without insecticide at 1 Kg/6 m2. Culex = *Cx. quinquefasciatus* ; T0 = 0 months after treatment, T6 = 6 months after treatment, T12 = 12 months after treatment, N = sample size per surface tested; (-) females had already died during the first hour. † = significant differences from control ($P < 0.05$).

Table 2: Delayed 24-hour mortality rates

Mortality of susceptible *Culex quinquefasciatus* S-Lab, and OP-resistant *Culex quinquefasciatus*, after a 30-minute exposure (using WHO bioassay cones) to two types of surfaces: control surfaces (no treatment), and surfaces treated with Inesfly 5A IGR.

T0, Cement (N = 50) OP-resistant *Culex*	Egg Number	% Egg Hatching	% Pupation	% Emergence
C1/No paint	2,104	51.8	39.6	79.5
C2/Paint, no insecticide	2,473	48.8	40.0	85.9
Insecticide at 1 Kg/6 m²	No survivors			
Insecticide at 1 Kg/12 m²	800†	41.3†	45.5	52.7†

Control 1 = No paint; Control 2 = Paint without insecticide at 1 Kg/6 m2; *Culex* = *Cx. quinquefasciatus*; T0 = 0 months after treatment; N = sample size per surface tested. † = significant differences from control ($P < 0.05$).

Table 3: IGR effect on *Culex quinquefasciatus*, *T0*

An examination of Insect Growth Regulator on the fecundity, fertility and larval development of OP-resistant *Culex* females, exposed to treated surfaces for 30 minutes, immediately (T0) after treatment.

T9, Cement (N = 30) OP-resistant *Culex*	Egg Number	% Egg-Hatching	% Pupation	% Emergence
C1/No paint	1,908	75.8	56.3	87.8
C2/Paint, no Insecticide	2,002	73.1	60.0	84.4
Insecticide at 1 Kg/6 m²	1,216†	77.5	64.6	65.9†
Insecticide at 1 Kg/12 m²	1,156†	70.9	59.9	86.6

Control 1 = No paint; Control 2 = Paint without insecticide at 1 Kg/6 m^2; Culex = *Cx. quinquefasciatus*; T9 = 9 months after treatment; N = sample size per surface tested. † = significant differences from control ($P < 0.05$)

Table 4: IGR effect on *Culex quinquefasciatus*, *T9*

An examination of Insect Growth Regulator at on the fecundity, fertility and larval development of OP-resistant *Culex* females, exposed to treated surfaces for 30 minutes, 9 months (T9) after treatment.

EMC *An. gambiae*		Untreated bednet (control)	Untreated bednet + 2 layers control paint on walls and ceiling	Untreated bednet + 1 layer IP on walls	Untreated bednet + 1 layer IP on walls and ceiling	Untreated bednet + 2 layers IP on walls	Untreated bednet + 2 layers IP on walls and ceiling
T0–T3	% Overall Mortality	0[a]	0[a]	100[b]	100[b]	100[b]	100[b]
T9	% Overall Mortality	0[a]	0.9[a]	34.6[a]	79.7[b]	90.2[b]	93.1[b]

IP = Insecticide Paint. T0–T3 and T9 = 0–3 and 9 months after treatment; values in the same row sharing a letter superscript do not differ significantly (P ≥ 0.05)

Table 5: Mortality of *Anopheles gambiae* females

Overall mortality of mosquitoes collected in the field during EMCs from experimental huts in Bénin.

EMC *Cx. quinquefasciatus*		Untreated bednet (control)	Untreated bednet + 2 layers control paint on walls and ceiling	Untreated bednet + 1 layer IP on walls	Untreated bednet + 1 layer IP on walls and ceiling	Untreated bednet + 2 layers IP on walls	Untreated bednet + 2 layers IP on walls and ceiling
T0–T3	% Overall Mortality	0^{a}	0^{a}	100^{b}	100^{b}	100^{b}	100^{b}
T6	% Overall Mortality	0^{a}	2.2^{a}	92.9^{b}	95.7^{c}	100^{d}	99.5^{d}
T9	% Overall Mortality	0^{a}	2.1^{a}	20.8^{b}	40.1^{c}	56.7^{d}	54.5^{d}
T12	% Overall Mortality	0^{a}	1.2^{a}	5.7^{b}	5.3^{b}	15.6^{c}	21.6^{d}

IP = Insecticide Paint. T0–T3, T6, T9 and T12 = 0–3, 6, 9 and 12 months after treatment; values in the same row sharing a letter superscript do not differ significantly ($P \geq 0.05$)

Table 6: Mortality of *Culex quinquefasciatus* females

Overall mortality of mosquitoes collected in the field during EMCs from experimental huts in Bénin.

% Mortality *An. gambiae* Kisumu using WHO test cones	Untreated bednet (control)	Untreated bednet + 2 layers control paint on walls and ceiling	Untreated bednet + 1 layer IP on walls	Untreated bednet + 1 layer IP on walls and ceiling	Untreated bednet + 2 layers IP on walls	Untreated bednet + 2 layers IP on walls and ceiling
T0	12.5[a]	14.1[a]	100[b]	100[b]	100[b]	100[b]
T3	0[a]	3.3[a]	100[b]	100[b]	100[b]	100[b]
T6	0[a]	1.8[a]	79.4[b]	59.7[c]	100[d]	100[d]
T9	0[a]	3.4[a, b]	14.7[b]	44.6[c]	100[d]	98.5[d]
T12	1.7[a]	6.1[a, b]	0[a]	12.9[b]	80.6[c]	71.9[c]

IP = Insecticide Paint. T0, T3, T6, T9 and T12 = 0, 3, 6, 9 and 12 months after treatment; values in the same row sharing a letter superscript do not differ significantly ($P \geq 0.05$)

Table 7: Delayed 24-hour mortality of *Anopheles gambiae* Kisumu

Mortality of mosquitoes after a 30-minute exposure to treated walls and control walls, in experimental huts in the field in Bénin.

% Mortality *Cx. quinquefasciatus* S-Lab using WHO test cone	Untreated bednet (control)	Untreated bednet + 2 layers control paint on walls and ceiling	Untreated bednet + 1 layer IP on walls	Untreated bednet + 1 layer IP on walls and ceiling	Untreated bednet + 2 layers IP on walls	Untreated bednet + 2 layers IP on walls and ceiling
T3	5.5[a]	6.2[a]	100[b]	100[b]	100[b]	100[b]
T6	13.8[a]	10.3[a]	100[b]	98.3[b]	100[b]	100[b]
T9	1.6[a]	3.3[a]	72.6[b]	49.2[c]	100[d]	98.4[d]
T12	1.6[a]	0[a]	5[a]	8.1[a]	70[b]	72.4[b]

IP = Insecticide Paint. T0, T3, T6, T9 and T12 = 0, 3, 6, 9 and 12 months after treatment; values in the same row sharing a letter superscript do not differ significantly (P ≥ 0.05)

Table 8: Delayed 24-hour mortality of *Culex quinquefasciatus* S-Lab

Mortality of mosquitoes after a 30-minute exposure to treated walls and control walls, in experimental huts in the field in Bénin.

% Mortality *An. gambiae* Kisumu	Untreated bednet (control)	Untreated bednet + 2 layers control paint on walls and ceiling	Untreated bednet + 1 layer IP on walls	Untreated bednet + 1 layer IP on walls and ceiling	Untreated bednet + 2 layers IP on walls	Untreated bednet + 2 layers IP on walls and ceiling
T0	0^{a}	3.4^{a}	100^{b}	100^{b}	100^{b}	100^{b}
T6	0^{a}	0^{a}	91.8^{b}	100^{b}	100^{b}	100^{b}
T12	1.5^{a}	3^{a}	35.6^{b}	98.4^{c}	100^{c}	100^{c}

IP = Insecticide Paint. T0, T6 and T12 = 0, 6 and 12 months after treatment; values in the same row sharing a letter superscript do not differ significantly ($P \geq 0.05$)

Table 9: Delayed 24-hour mortality of *Anopheles gambia*e Kisumu

Mortality of mosquitoes after an overnight exposure at a distance of one metre from two perpendicular walls, in experimental huts in the field in Bénin.

% Mortality *Cx. quinquefasciatus* S-Lab	Untreated bednet (control)	Untreated bednet + 2 layers control paint on walls and ceiling	Untreated bednet + 1 layer IP on walls	Untreated bednet + 1 layer IP on walls and ceiling	Untreated bednet + 2 layers IP on walls	Untreated bednet + 2 layers IP on walls and ceiling
T0	8.3[a]	0[a]	100[b]	100[b]	100[b]	100[b]
T6	13.8[a]	10.3[a]	100[b]	98.3[b]	100[b]	100[b]
T12	1.8[a]	3[a]	60[b]	96.2[c]	100[c]	100[c]

IP = Insecticide Paint. T0, T6 and T12 = 0, 6 and 12 months after treatment; values in the same row sharing a letter superscript do not differ significantly ($P \geq 0.05$)

Table 10: Delayed 24-hour mortality of *Culex quinquefasciatus* S-Lab

Mortality of mosquitoes after an overnight exposure at a distance of one metre from two perpendicular walls, in experimental huts in the field in Bénin.

Cement tested at a distance of 1 m at T0	Control	One layer IP at 1 kg/6 sq m in distance box, Phase I	One layer IP at 1 kg/6 sq m in experimental huts, Phase II
An. gambiae Kisumu	0[a]	100[b]	100[b]
Cx. quinquefasciatus S-Lab	0[a]	100[b]	100[b]

IP, Insecticide Paint. T0, 0 months after treatment. Values in the same row sharing a letter superscript do not differ significantly ($P \geq 0.05$).

Table 11: Delayed 24-hour mortality of *Anopheles gambiae* Kisumu and *Culex quinquefasciatus* S-Lab

Comparison of Phase I and Phase II spatial mortality rates of two mosquito types, after overnight exposure to control and treated surfaces. Exposure was at a distance of one metre in distance boxes, both in the laboratory and in experimental huts in the field in Bénin.

Phase II: Cement tested at a distance of 1 m at T0 and T12	Timepoint	Control 1 No paint	Control 2 two layers of control paint on walls and ceiling	One layer IP on walls at 1 kg/6 sq m	One layer IP on walls and ceiling at 1 kg/6 sq m	Two layers IP on walls at 1 kg/6 sq m	Two layers IP on walls and ceiling at 1 kg/6 sq m
An. gambiae Kisumu	T0	0^{a}	3.4^{a}	100^{b}	100^{b}	100^{b}	100^{b}
	T12	1.5^{a}	3^{a}	35.6^{b}	98.4^{c}	100^{c}	100^{c}
Cx. quinquefasciatus S-Lab	T0	8.3^{a}	0^{a}	100^{b}	100^{b}	100^{b}	100^{b}
	T12	1.8^{a}	3^{a}	60^{b}	96.2^{c}	100^{c}	100^{c}

IP, Insecticide Paint. T0 and T12, 0 and 12 months after treatment. Values in the same row sharing a letter superscript do not differ significantly ($P \geq 0.05$).

Table 12: Spatial long-term mortality rates in control and treated huts.

Delayed 24-hour mortality of *Anopheles gambiae* Kisumu and *Culex quinquefasciatus* S-Lab, after overnight exposure at a distance of 1 metre from two perpendicular walls in experimental huts in the field in Bénin.

% Mortality in *Anopheles coluzzii*, collected via EMCs	T1	T3	T6	T12
C (LLINs)	9.5[a]	5.2[a]	8.9[a]	7.6[a]
RP/1 layer + IP/1 layer walls + LLINs	100[b]	100[b]	100[b]	78.6[b]
RP/1 layer+ IP/1 layer walls + ceiling + LLINs	100[b]	100[b]	100[b]	69.5[b]
IP/1 layer walls + LLINs	100[b]	100[b]	100[b]	78.9[b]
IP/1 layer walls + ceiling + LLINs	100[b]	100[b]	100[b]	79.9[b]
IP/2 layers walls + LLINs	100[b]	99.9[b]	100[b]	78.5[b]
IP/2 layers walls + ceiling + LLINs	100[b]	100[b]	100[b]	82.2[b]

Averages taken for each configuration, 2 houses per configuration. C= Control with LLINs only; RP= Regular Paint; IP = Insecticide Paint; T= Time in months since treatment. EMCs = Early Morning Collections. Numbers in the same column sharing a letter superscript do not differ significantly (P> 0.05).

Table 13: Mortality rates on local populations of *Anopheles coluzzii* at VK1 using EMCs

	***Anopheles gambiae* Kisumu (A)**					***Anopheles coluzzii* VK1 (B)**				
% Mortality using WHO test cones	T0	T1	T3	T6	T12	T0	T1	T3	T6	T12
C (LLINs)	10.9[a]	7.9[a]	6.1[a]	5.6[a]	6.9[a]	1.7[a]	2.6[a]	2.9[a]	2.1[a]	2.1[a]
RP+ IP/1 layer walls + LLINs	100[b]	100[b]	100[b]	100[b]	99.0[b]	100[b]	100[b]	100[b]	98.9[b]	90.9[b]
RP+ IP/1 layer walls + ceiling + LLINs	100[b]	100[b]	98.1[b]	100[b]	99.0[b]	100[b]	100[b]	100[b]	99.0[b]	91.3[b]
IP/1 layer walls + LLINs	100[b]	100[b]	98.0[b]	100[b]	99.0[b]	100[b]	100[b]	100[b]	100[b]	85.0[b]
IP/1 layer walls + ceiling + LLINs	100[b]	100[b]	100[b]	100[b]	98.1[b]	100[b]	100[b]	100[b]	100[b]	81.8[b]
IP/2 layers walls + LLINs	100[b]	100[b]	100[b]	100[b]	100[b]	100[b]	100[b]	100[b]	98.8[b]	88.9[b]
IP/2 layers walls + ceiling + LLINs	100[b]	100[b]	100[b]	100[b]	100[b]	100[b]	100[b]	100[b]	100[b]	97.0[b]

Averages taken for each configuration, 2 houses per configuration. C= Control with LLINs only; RP= Regular Paint; IP = Insecticide Paint; T= Time in months since treatment. Numbers in the same column sharing a letter superscript do not differ significantly (P> 0.05)

Table 14: Residual efficacy tests on mosquitoes using 30-minute WHO bioessay cones

Treat-ments	Month	n	SS	RS	RR	F(L1014F *kdr*)	p (HW)	SS	RS	RR	F(L1014S *kdr*)	p (HW)
C (LLINs)	T0	30	7	3	20	0.717	0.0001	30	0	0	0	–
	T1	30	0	0	30	0.98	-	30	0	0	0	–
	T2	30	11	2	17	0.6	0	30	0	0	0	–
	T3	31	8	3	20	0.694	0	31	0	0	0	–
	T4	30	7	0	23	0.767	0	30	0	0	0	–
	T5	25	5	0	20	0.8	0	25	0	0	0	–
IP/1 layer walls + ceiling + LLINs	T0	30	3	0	27	0.9	0.0001	30	0	0	0	–
	T1	28	1	0	27	0.964	-	30	0	0	0	–
	T2	30	4	3	23	0.817	0.002	27	3	0	0.05	1
	T3	31	6	1	24	0.79	0	30	1	0	0.016	–
	T4	29	3	6	20	0.793	0.066	24	5	0	0.086	1
	T5	30	4	7	19	0.75	0.048	23	7	0	0.117	1
IP/2 layers walls + ceiling + LLINs	T0	30	4	0	26	0.867	0	30	0	0	0	–
	T1	31	0	0	31	0.98	-	30	0	0	0	–
	T2	30	4	0	26	0.867	0	30	0	0	0	–
	T3	31	9	0	22	0.71	0	31	0	0	0	–
	T4	29	3	0	26	0.897	0.0001	29	0	0	0	–
	T5	30	4	10	16	0.7	0.378	20	10	0	0.167	0,563

C= Control with LLINs only; IP = Insecticide Paint; n= number of mosquitoes tested; T= Time in months since treatment; *F (kdr)* = frequency of the *kdr* mutation; p (HW) = value for Hardy-Weinberg equilibrium hypothesis; "–" = non-determinable.

Table 15: Distribution of the frequency of L1014F and L1014S *kdr* mutations in *Anopheles coluzzii* collected from EMCs in VK1

Treatment	Month	n	Genotypes			F (G119S)	[95%CI]	p (HW)
			119G 119G	119G 119S	119S 119S			
C (LLINs)	T0	30	25	5	0	0.083	[0.00–0.18]	1
	T1	30	30	0	0	0	-	-
	T2	30	30	0	0	0	-	-
	T3	30	30	0	0	0	-	-
	T4	30	30	0	0	0	-	-
	T5	23	21	2	0	0.04	[0.00–0.12]	1
IP/1 layer walls + ceiling + LLINs	T0	30	30	0	0	0	-	-
	T1	30	30	0	0	0	-	-
	T2	30	30	0	0	0	-	-
	T3	30	30	0	0	0	-	-
	T4	30	30	0	0	0	-	-
	T5	30	30	0	0	0	-	-
IP/2 layers walls + ceiling + LLINs	T0	30	30	0	0	0	-	-
	T1	30	30	0	0	0	-	-
	T2	30	30	0	0	0	-	-
	T3	30	30	0	0	0	-	-
	T4	30	30	0	0	0	-	-
	T5	24	24	0	0	0	-	-

C= Control with LLINs only; IP = Insecticide Paint; n= number of mosquitoes tested; T= Time in months since treatment; F (G119S) = allelic frequency of the *ace-1^R* mutation; p (HW) = value for Hardy-Weinberg equilibrium hypothesis; "–" = non-determinable.

Table 16: Allelic frequency and genotype of the *ace-1^R* mutation in *Anopheles coluzzii* collected from EMCs in VK1

Treatment	Total	Humans		Animals								Mixed	
		n	%	Cattle	Sheep	Donkey	Pig	Dog	Others	n	%	n	%
C (LLINs)	141	35	24.8[a]	16	8	18	7	5	39	93	66.0[a]	13	9.2[a]
IP/1 layer walls + ceiling + LLINs	143	51	35.7[a]	21	4	3	5	4	33	70	49.0[a]	22	15.4[a]
IP/2 layers walls + ceiling + LLINs	141	28	19.9[a]	30	3	9	0	2	38	82	58.2[a]	31	22.0[a]
TOTAL	425	114	26.8	67	15	30	12	11	110	245	57.6	66	15.5

C= Control with LLINs only; IP = Insecticide Paint; n= numbers of mosquitoes tested; T0-T6 = Period from June to December 2013 when collected *Anopheles coluzzii* were pooled and randomly tested for blood-feeding source. Proportions in the same column sharing a letter superscript do not differ significantly (P> 0.05).

Table 17: Analysis of blood from *Anopheles coluzzii* females collected during EMCs at VK1

% Mortality in *Anopheles coluzzii* collected via EMCs	T0	T1	T2	T3	T4
C (LLINs)	8.6[a]	11.9[a]	11.8[a]	15.7[a]	10.8[a]
IP/1 layer doors + windows + LLINs	100[b]	81.1[b]	60.6[b]	39.4[b]	28.3[a]

Averages taken for each configuration, 10 houses per configuration. C= Control with LLINs only; IP = Insecticide Paint; T= Time in months since treatment. EMCs = Early Morning Collections. Numbers in the same column sharing a letter superscript do not differ significantly (P> 0.05).

Table 18: Mortality rates on local populations of *Anopheles coluzzii* at VK3 using EMCs

	***Anopheles gambiae* Kisumu (A)**					***Anopheles coluzzii* VK3 (B)**				
% Mortality using WHO test cones	T0	T1	T2	T3	T4	T0	T1	T2	T3	T4
C (LLINs)	5[a]	5[a]	3[a]	10[a]	7[a]	8[a]	7[a]	2[a]	2[a]	ND
IP/1 layer on doors	100[b]	100[b]	100[b]	85[b]	62[b]	100[b]	100[b]	90[b]	39[b]	ND
IP/1 layer on windows	100[b]	100[b]	100[b]	95[b]	80[b]	100[b]	100[b]	90[b]	53[b]	ND

Averages taken for each configuration, 10 houses per configuration. C= Control with LLINs only; IP = Insecticide Paint; T= Time in months since treatment; ND = Not Done because of insufficient numbers reared in the insectarium. Numbers in the same column sharing a letter superscript do not differ significantly (P> 0.05).

Table 19: Residual efficacy tests on VK3 mosquitoes

Tests on *Anopheles gambiae* Kisumu and *Anopheles coluzzii*, using WHO bioassay cones placed on doors and windows.

	Anopheles gambiae Kisumu (a)					*Anopheles coluzzii* VK3 (b)				
% Mortality during spatial mortality assessments	T0	T1	T2	T3	T4	T0	T1	T2	T3	T4
C (LLINs)	10.9[a]	7.9[a]	6.1[a]	5.6[a]	6.9[a]	1.7[a]	2.6[a]	2.9[a]	2.1[a]	ND
IP/1 layer doors + windows + LLINs	90[b]	75[b]	30[a]	20[a]	20 [a]	80[b]	77[b]	25[a]	15[a]	ND

Averages taken for each configuration, 10 houses per configuration. C= Control with LLINs only; IP = Insecticide Paint; T= Time in months since treatment; ND = Not Done because of insufficient numbers reared in the insectarium. Numbers in the same column sharing a letter superscript do not differ significantly (P> 0.05).

Table 20: Spatial mortality assessments on VK3 mosquitoes

Mortality assessments on *Anopheles gambiae* Kisumu and *Anopheles coluzzii*, using test tubes placed 1 metre away from treated surfaces for 30 minutes.

Treatments	Kdr L1014F							Ace-1^R (G119S)				
	n	SS	RS	RR	F(L1014F)	[95%CI]	p (HW)	SS	RS	RR	*F*(G119S)	p (HW)
C (LLINs)	52	1	3	48	0.952	[0.89–1.01]	0.01	52	0	0	0	–
IP/1 layer doors + windows + LLINs	50	1	4	44	0.929	[0.86–1.00]	<0.05	49	0	0	0	–

C= Control with LLINs only; IP = Insecticide Paint; n= number of mosquitoes tested; F (L1014F) = allelic frequency of the *kdr* mutation *ace-1^R*; F (G119S) = allelic frequency of the *ace-1^R* mutation; p (HW) = value for Hardy-Weinberg equilibrium hypothesis; "–" = non-determinable.

Table 21: Allelic frequency and genotype of the L1014F *kdr* and *ace-1^R* mutations in *Anopheles coluzzii* collected from EMCs in VK3

Villages or Sectors	Village Population 2013
Koflabin	139
Simmin	139
Dou	140
Kokouna	160
Koua	168
Linguekoro	214
Lanfièra	214
Samogohiri Hameau	218
Niallé-Sallé	233
Camp peulh	251
Kiblesson	268
Fon-Fon	278
Saraba Hameau	293
Kaka	301
Fe	303
Kariya	335
Dogoriya	374
Karya	385
Kolokaka	447
Todjè	451
Fon	462
Guenako	474
Kounseni-Samo	503

Mina	533
Diolé	535
Sinfra	536
Bakaribougou	548
Tounkoura	551
Syan	556
Diéridéni	590
Lidara	637
Nialé	654

Table 22: Village populations for the WHOPES Phase III study

In the 32 Orodara-region villages selected for the Phase III study, roughly half the residents consisted of children aged 6 months to 14 years old.

Malaria-like symptoms reported	%
Fever	70.5
Joint pain	31.8
Headaches	39.1
Vomiting	65.4

Table 23: Malaria-like symptoms reported

Responses to questionnaires by residents of the 34 geo-referenced villages for the WHOPES Phase III study in the Orodara region.

Perceptions	%
No feedback	98.8
Favourable	97.5
Could make you sick	0.2

Table 24: Perceived feedback reported during questionnaires by the residents of the 34 geo-referenced villages on their interest in participating on a WHOPES Phase III study involving insecticide paint

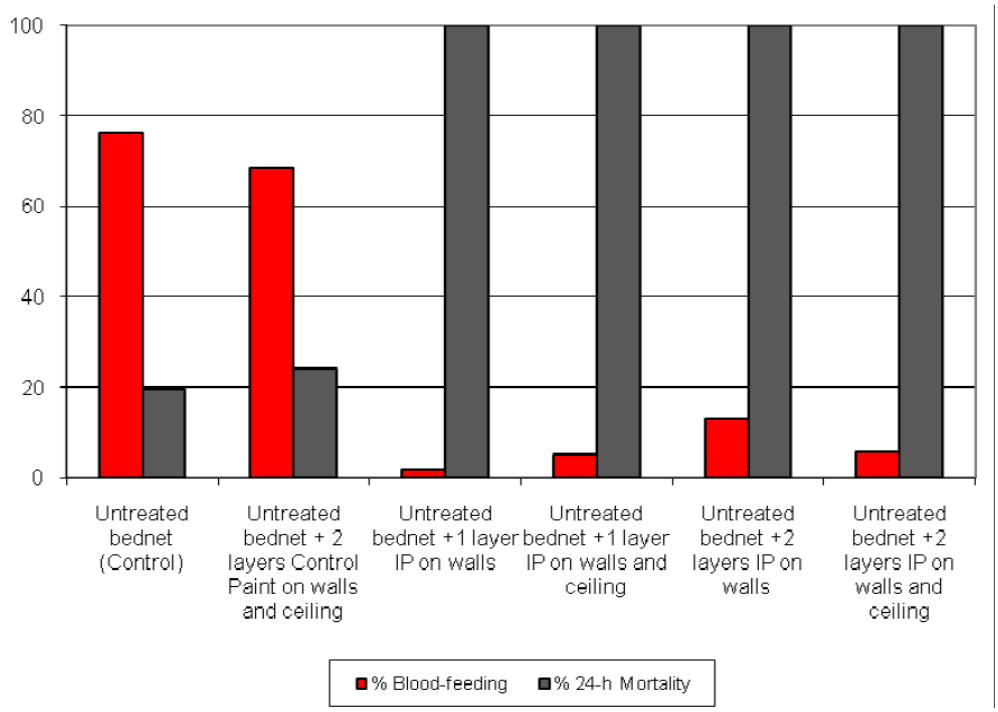

Figure 14: Delayed 24-hour mortality and blood-feeding rates

Malaria-free females of local *Anopheles gambiae*, reared at the CREC insectarium from field-collected larvae, were released into treated experimental huts each night at 21:00 hours, and collected the next day over a period of 5–7 hours. Bednets had been withdrawn, and entry of other mosquito into the huts was blocked. The averages from 2 repeats were N>30 each.

Figure 15: Team members from IRSS/Centre Muraz

The IRSS team went to the 34 geo-referenced villages in the study area (the Orodara region), taking questionnaires and collecting information. That research formed the basis of the 32 villages selected for the WHOPES Phase III study.

Figure 16: Habitations numbered and geo-referenced

The IRSS team identified a number of homes in the Orodara region suitable for the WHOPES Phase III study.

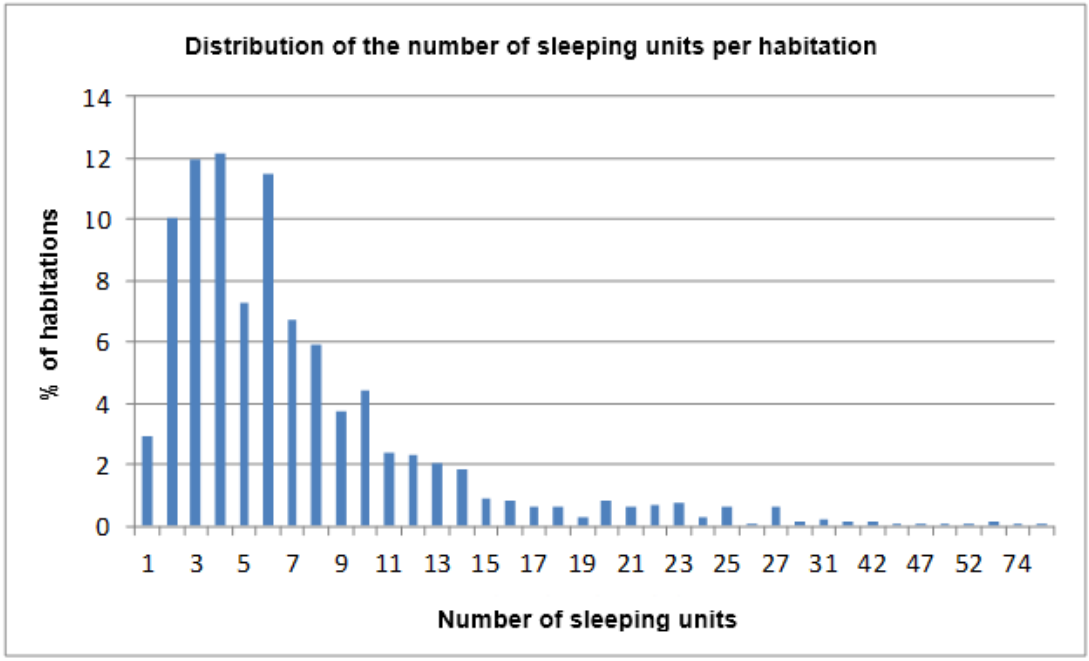

Figure 17: Distribution of sleeping units per habitation

The IRSS team identified the number of beds in the geo-referenced villages of the Orodara region for the WHOPES Phase III study.

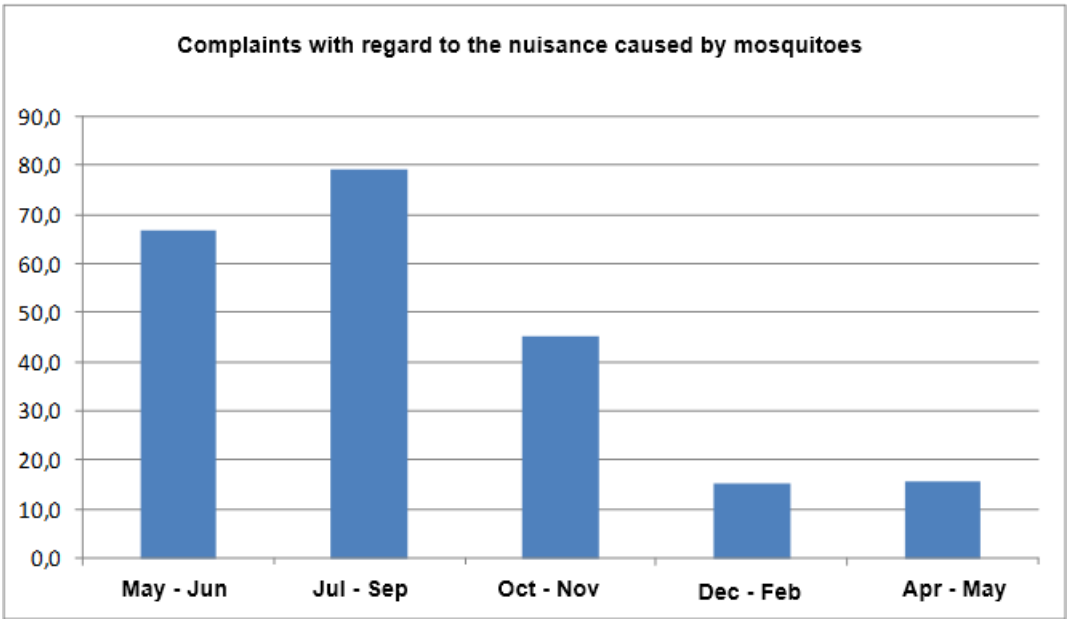

Figure 18: Complaints about mosquito nuisance

Residents responded to the questionnaire issued by the IRSS team on the seasonal level of nuisance caused by mosquitoes, in the 34 geo-referenced villages for the WHOPES Phase III study (Orodara region).

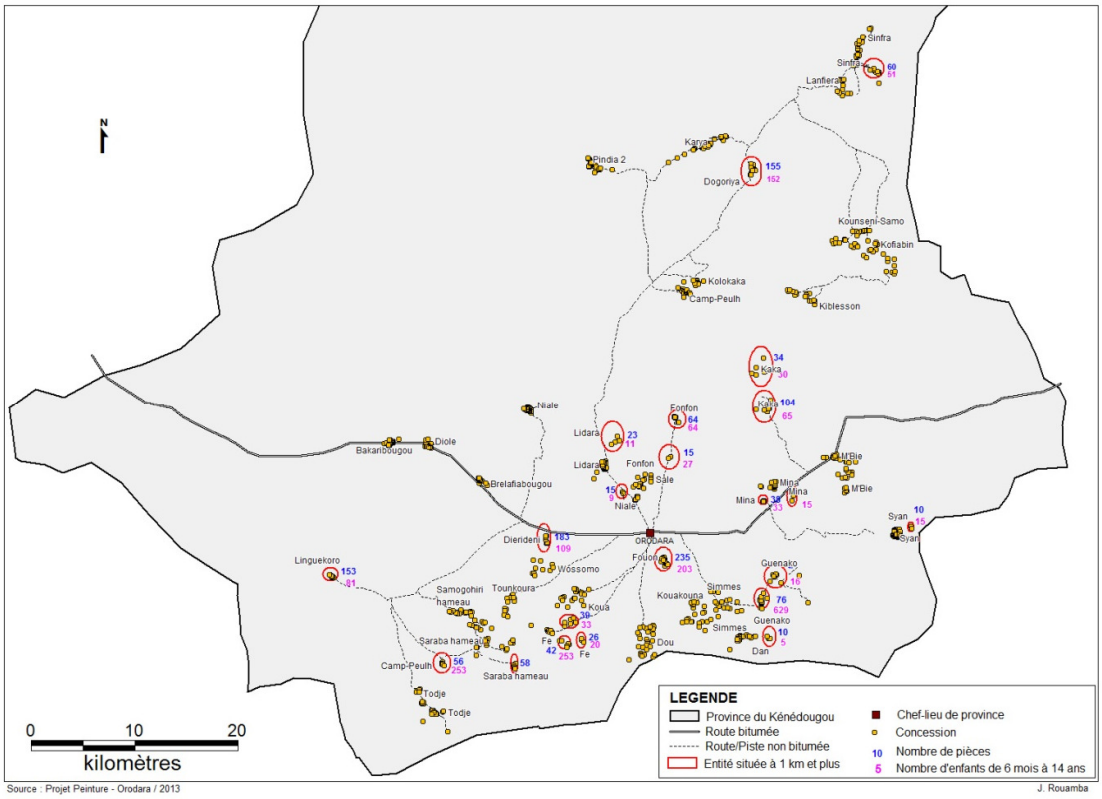

Figure 19: Distribution of the 32 villages for the WHOPES Phase III study

Of the 34 geo-referenced villages in the Orodara region of Burkina Faso, 32 were selected for inclusion in the upcoming WHOPES study. This map presents preliminary information on the number of homes in the villages, the number of children, and the distances between villages—all important criteria for the study.

CHAPTER 5: DISCUSSION

A series of studies was performed to evaluate the potential of the insecticide paint Inesfly 5A IGR as a malaria parasite vector control tool, as follows.

- The Phase I and Phase II WHOPES studies served to gain an insight on the effect of Inesfly 5A IGR on entomological parameters, both in the laboratory and in the field.
- The two pilot studies performed in a village setting helped to evaluate the potential of the strategy of combining Inesfly 5A IGR and LLINs.
- Thirty-two villages were selected as part of the preparation work for the WHOPES Phase III study, in an area of holoendemic malaria and high pyrethroid resistance in south-western Burkina Faso.

The discussion of these studies, outlined below, has been broken down into the different phases.

The WHOPES Phase I laboratory evaluations (Côte d'Ivoire and France)

The first laboratory Phase I tests were performed at the IPR institute in Côte d'Ivoire. The IPR had an insectarium where strains of *An. gambiae* could be reared, and optimal laboratories to perform experiments. The institute had also built Phase II experimental stations in an area of pyrethroid resistance (Darriet *et al*., 2000a; Darriet *et al*., 2002). This made the IPR a suitable place to test whether the new tool, Inesfly 5A IGR, was effective against pyrethroid-resistant mosquitoes—since such resistance was becoming widespread, and its operational impact was not known.

Indeed, the first evidence of pyrethroid resistance in *Anopheles* mosquitoes was discovered in *An. gambiae* in Bouake, Côte d'Ivoire, by researchers from IPR (Elissa *et al*., 1993). The pyrethroid resistance in *An. gambiae* due to the L1014F *kdr* mutation was then described among several *An. gambiae* populations throughout West Africa, thanks to a research network supported by WHO and coordinated by IPR (Chandre *et al*., 1994). IPR has also conducted several studies in the savannah environment of Côte d'Ivoire, which showed that

pyrethroid-treated nets still achieve good control of *kdr*-resistant *An. gambiae*— both in Phase II experimental huts (Darriet *et al*., 1998; 2000a), and in Phase III field trials (Henry *et al*., 2005).

The types of surfaces to be treated during those first tests at IPR were chosen based on the most frequently used material for construction in West African rural areas: metal, cement and adobe. Corrugated roofs are made of metal, and walls are commonly made of cement or adobe. Those initial tests alerted researchers to the important issue of porosity, which was considered more carefully during the next study: the WHOPES Phase I at LIN in France.

During that French study, the effectiveness of the insecticides and IGR was tested on porous (cement and stucco) and non-porous (softwood and hard plastic) surfaces. The mosquitoes used during the WHOPES Phase I tests were laboratory strains of *Cx. quinquefasciatus*, both susceptible and resistant to OPs. (At the time of the study, there was no laboratory strain of *An. gambiae* specifically resistant to OPs.) *Culex* is the most common mosquito in tropical urban areas, and constitutes a great nuisance owing to the numerous bites it inflicts on people. This nuisance is an important factor to consider, as it will encumber malaria control if not considered when planning interventions (Winch *et al*., 1994; Aikins *et al*., 1994; Van Bortel *et al*., 1996; Samuelsen *et al*., 2004). Studies show that people are reluctant to use measures against malaria (essentially ITNs) if they are not also effective against nuisance bites. Adherence to vector control measures may be further complicated by the fact that *Cx. quinquefasciatus* has become resistant to the most common insecticides used for impregnation of nets (Chandre *et al*., 1998, Corbel *et al*., 2007).

This tendency for people to avoid using nets unless mosquitoes actually bother them with bites is an issue that needs to be addressed, since malaria transmission can occur even if mosquito densities are low (Thomson *et al*., 1996). In West Africa, nuisance is of great significance as it constitutes the main motivator for people to use malaria control (ITNs) in tropical towns and villages (Desfontaine *et al*., 1990; Guillet *et al*., 2001a). The pest mosquito species *Cx. quinquefasciatus* obviously has a role to play in the success of vector control

strategies; and based on the availability of both OP-susceptible and OP-resistant laboratory strains of *Cx. quinquefasciatus*, it was decided to test surfaces treated with Inesfly 5A IGR against this species. The results were encouraging: 100% of OP-susceptible females, exposed to treated surfaces using traditional bioassays, died after 24 hours. This was the case on all surfaces, porous and non-porous, at both the higher and lower doses (1 kg/6 m^2 and 1 kg/12 m^2). The killing rate was significant (87–100%), even against OP-resistant *Cx. quinquefasciatus* females, on all surfaces except cement treated at the lower dose (1 kg/12 m^2) (Mosqueira *et al*. 2010a).

One year after initial treatment, mortality rates were still quite high (93–100%) on non-porous surfaces such as softwood and hard plastic, at both doses; this was the case against both OP-resistant and OP-susceptible females. However, the lethal effect of porous surfaces such as cement and adobe had disappeared by 6 months after treatment, for both resistant and susceptible mosquitoes (Mosqueira *et al*. 2010a). It seems clear that long-term efficacy is an issue of the porosity of materials, rather than the dose applied or the pH of materials: the paint's active principles are kept in an acid pH within its microcapsule, making it more resistant to alkalinity than conventional paints.

To study whether efficacy depended more on porosity than dose, a parallel study was performed: cement surfaces were painted with a control layer and an insecticide paint layer at 1 kg/6 m^2. These performed as well as two insecticide paint layers at 1 kg/6 m^2, even though the latter had twice the dose (Mosqueira *et al*., unpublished data). It seemed that the first layer of non-insecticide "primer" paint acted as a screen to reduce the porosity of the surface, and prevented any rediction in the bioavailability of the insecticide in the second layer.

Many studies have already shown that the residual efficacy of most IRS depend on the nature of the treated surfaces (Najera & Zaim, 2001). The impact of porosity on long-term efficacy was recently confirmed for a number of different insecticide formulations (such as Bendiocarb WP, lambda-cyhalothrin CS, and deltamethrin WG) on different types of wall surfaces, such as cement, wood and mud (Etang *et al*., 2011).

Similarly, other studies against the main vector of Chagas disease in South America, *Triatoma infestans*, had tested the efficacy and the residual effect of Inesfly 5A IGR on surfaces such as wood, cement block, and adobe bricks. The insecticide paint yielded longer and higher mortality rates against triatomines than other conventional products (Amelotti *et al*., 2009; Dias & Jemmio, 2008; Maloney *et al*., 2013; Gorla *et al*., 2015). In those studies, porosity was also an issue: cement surfaces performed worse than wood and even adobe (Amelotti *et al*. 2009).

The IGR in the paint, pyriproxyfen, is a juvenile hormone usually used as a larvicide; but it may also have an impact on the fecundity and fertility of adult mosquito females through tarsal contact. Indeed, pyriproxyfen can affect the development and production of eggs (fecundity) and reduce their hatching (fertility) (Loh & Yap, 1989; Kamal & Khater, 2010). A recent study showed that *An. gambiae* females exposed once to pyriproxyfen may experience an irreversible sterilizing effect (Koama *et al*., 2015).

In semi-field conditions, an experimental evaluation has recently shown the potential of black cloth treated with pyriproxyfen to control *Anopheles arabiensis* (Patton, 1905) in cow-baited huts (Lwetoijera *et al*., 2014a). An important recent study, performed in the field, showed the high sterilizing efficacy of pyriproxyfen-impregnated bednets against wild pyrethroid-resistant *An. gambiae* population in western Kenya (Kawada *et al*., 2014). Another recent study also found complete sterilization in wild pyrethroid-resistant *An. gambiae* that came into contact with pyriproxyfen-treated nets while seeking a blood meal (Ngufor *et al*., 2014a).

In the Phase I study, the effect of IGR pyriproxyfen was studied on OP-resistant *Cx. quinquefasciatus* females that survived a 30-minute exposure to cement-treated surfaces. Mosquito females were exposed to treated surfaces about 36 hours before blood-feeding, at 0 and 9 months after treatment. At T0, a reduction in both fecundity and fertility was observed: the number of eggs laid per female (fecundity) had been reduced almost by 46% in the treated group. Compared to control, significant differences were also found in fertility (a 20% reduction in hatched eggs) and emergence: a 36% reduction in adult emergence. At T9, 9

months after treatment, a 38–40% reduction in the number of eggs laid (fecundity) was still observed, but it was less marked than at T0. The reduction in fertility (% egg hatching) was no longer observed.

As in previous studies, the timing of pyriproxyfen exposure is important. A few studies showed that pyriproxyfen had a larger impact on fecundity when mosquito females were exposed to pyriproxyfen before blood-feeding (Itoh *et al*. 1994). On the other hand, the fertility effect of pyriproxyfen on egg-hatching and production of viable offspring (Itoh *et al*., 1994; Dell Chism & Apperson, 2003; Sihunincha *et al*., 2005; Ohashi *et al*., 2012; Harris *et al*., 2013) and adult emergence (Itoh *et al*., 1994; Sihunincha *et al*., 2005) seemed higher when mosquito females had blood-fed before exposure. A recent study showed that the sterilizing effect on both fecundity and fertility of *An. gambiae* can be achieved during a relatively large window of time—from 24 hours before, to 24 hours after, a blood meal—and at a relatively low concentration of pyriproxyfen (Mbare *et al*., 2014).

The observation that the effect on fecundity was longer-lasting than the effect on fertility may be due to the reduced bio-availability of pyriproxyfen over time, as the product degrades. Mbare *et al*. (2014) showed that low doses of pyriproxyfen affected fecundity but not fertility. This was consistent with the study comparing T9 to T0. The exact effect of the timing of pyriproxyfen exposure (before or after blood-feeding) remains to be studied. This can be done by varying the blood-feeding time in the protocol used during the Phase I tests, against both anophelines and culicines.

A major challenge is the study of a potential auto-dissemination effect of pyriproxyfen. Research on *Aedes* mosquitoes, vectors of dengue and Chikungunya, among other diseases, showed that female adults contaminated from resting sites can render oviposition sites unproductive by horizontal dissemination of pyriproxyfen, in the case of *Aedes aegypti* (Linnaeus, 1762; Itoh *et al*., 1994; Devine *et al*., 2009) and *Aedes albopictus* (Skuse, 1895; Caputo *et al*., 2012). Similar observations have recently been made of the malaria vector, *An. arabiensis* (Lwetoijera *et al*., 2014b). However, a recent study suggested that for use in an auto-dissemination approach, *An. gambiae* females would need to be exposed to pyriproxifen when already gravid and close to oviposition—so that

sufficient pyriproxifen could be delivered to aquatic habitats (Mbare *et al*., 2014). More studies are needed to fully understand the transfer of pyriproxyfen between resting and oviposition sites, and its potential use in malaria vector control (Devine & Killeen, 2010; Mbare *et al*., 2014). As well, the potential horizontal dissemination of insecticide paint to natural mosquito breeding habitats also remains to be studied.

Studies on preliminary Phase I results in the laboratory suggest that sterilizing adult female mosquitoes using pyriproxyfen could form part of a malaria control strategy. This would take advantage of the lack of reported resistance to pyriproxyfen in mosquitoes, providing an additional angle of attack once the residual lethal effect of the OPs has decreased (Mosqueira *et al*., 2010a). This would target residual mosquito populations (Killeen *et al*., 2014). Pyriproxyfen, like larvicides, would provide the added desirable feature of potentially, targetting different developmental cycles (White *et al*., 2011), thereby having an effect on both indoor and outdoor biting mosquitoes (Fillinger & Lindsay, 2011).

To summarize: after the WHOPES Phase I tests, two key results gave grounds for optimism about the potential of insecticide paint as a tool for malaria vector and pest mosquito control. These results were the high long-term killing rates against OP-resistant mosquitoes; and the effect of IGR on mosquito fecundity, fertility and adult emergence.

The WHOPES Phase II field evaluations (Bénin)

Tests performed in experimental huts against two local mosquito populations—*An. gambiae* malaria parasite vectors, and *Cx. quinquefasciatus* pest mosquitoes resistant to pyrethroids—showed mortality rates as high as 100% up to 3 months (T3) for both species. Nine months after treatment (T9), mortality rates in huts treated with two layers of insecticide paint were still 90–93% against *An. gambiae*, and 54–57% against *Cx. quinquefasciatus*. The differences in long-term effect between the two species might be explained both by the larger body size of *Cx. quinquefasciatus* relative to *An. gambiae*, and by their different intrinsic ability to metabolize insecticides (Brown & Pal, 1973).

On the other hand, the long-term efficacy on mosquito mortality (against pyrethroid- and DDT-resistant *An. gambiae* with high *kdr* frequencies) was similar to the 9-month residual activity obtained with another OP, a microencapsulated formulation of chlorpyrifos-methyl. This was applied by IRS in experimental huts following WHOPES Phase II in the same study area of Ladji in Cotonou (N'Guessan *et al*., 2010).

Several studies show that the microencapsulation of OPs yields a longer-term efficacy than traditional emulsifiable concentrate (EC). This could provide more prolonged mosquito control (N'Guessan *et al*., 2010; Chanda *et al*., 2014; Oxborough *et al*., 2014; Rowland *et al*., 2014).

The mosquito release experiments performed just after treatment with Inesfly 5A IGR showed that mosquitoes were killed quickly enough to prevent their blood-feeding. In treated huts, in the absence of the physical barrier provided by bednets, only 2 to 13% of females had blood-fed; whereas in the control huts, blood-feeding was 72%—a number similar to the 83% obtained in a study in Côte d'Ivoire of huts with no bednets (Darriet *et al*., 2000b). These experiments provided an important indication that Inesfly 5A IGR succeeded in killing a high percentage of mosquitoes before they had the chance to bite. The greatest advantage was found when *Anopheles* females had not had the opportunity to blood-feed before they died, which would give them personal protection. This protection under field conditions will be studied during Phase III.

A volume effect was observed during EMCs and spatial mortality assessments. When a larger number of surfaces were treated (walls and ceilings versus walls only) huts with just 1 layer of paint on walls and ceiling performed as well as huts with 2 layers of paint on the walls. However, another study (Ngufor *et al*., 2014b) did not observe such an effect in their Phase II evaluation (assessing OP-treated wall lining in the Kou Valley, Burkina Faso). The reason for this may be because that study ran for only 6 weeks; and in that time, mortality was still high at both configurations (walls versus walls and ceiling). The longer residual efficacy of a larger treated volume was not yet noticeable; but the volume effect became evident as months passed after the initial treatment.

To summarize, the WHOPES Phase II tests in the field gave three additional reasons for further exploring the potential of the insecticide paint Inesfly 5A IGR:

- a 9-month efficacy was observed against pyrethroid-resistant *An. gambiae* and *Cx. quinquefasciatus*;
- mortality occurred prior to blood-feeding, even in the absence of a physical barrier after treatment;
- mortality occurred at distances of 1 metre against insecticide-susceptible mosquitoes exposed overnight for up to 12 months after treatment.

The killing effect observed at distances of 1 metre prompted the question of how best to study spatial mortality in a systematic way, both in the laboratory and in the field. That subject is discussed next.

The spatial mortality assessments (France and Bénin)

The objective of such study was to propose the use of spatial mortality tests as part of the WHOPES in the light of results obtained in the laboratory using distance boxes(Phase I); and in the field using experimental huts (Phase II). Consequently, it was decided to test spatial mortality at distances of 1 metre from cement-treated surfaces, both in the laboratory and in the field, in order to confirm the preliminary results obtained on experimental huts at Ladji (Cotonou) during Phase II evaluation.

The distance of 1 metre was proposed as an initial test measurement. However, the nature of the insecticide—applied via vapour pressure—and the size of the dwellings to be treated may also play a role in deciding the most appropriate distance. If large halls in schools, airports or hospitals are treated, it may be useful to test for efficacy at greater distances.

At T0, the results obtained in the laboratory and in the field were similar: 100% mortality rates were observed on surfaces treated with 1 layer of insecticide paint, at the recommended dose of 1 kg/6 sq m, against susceptible *An. gambiae* Kisumu and *Cx. quinquefasciatus* S-Lab. The uniformity of results suggests that distance boxes could be a useful and simple approach in the laboratory, for

testing the lethal efficacy of insecticide products at a distance during Phase I evaluations.

At T12, spatial mortality after overnight exposure at distances of 1 metre remained high, 96–98%, against susceptible *An. gambiae* Kisumu and *Cx. quinquefasciatus* S-Lab, in experimental huts with 1 layer of insecticide paint on both walls and ceiling (larger volume). But huts treated with 1 layer on the walls alone performed less well after 12 months: 36% mortality against susceptible *An. gambiae* Kisumu, and 60% against susceptible *Cx. quinquefasciatus* S-Lab.

Because it can be argued that even highly endophilic pest or vector mosquitoes are not always in contact with an insecticide-treated surface before contacting a human or animal host, it is desirable both to have a distance effect, and to be able to evaluate it. For this reason, the spatial mortality efficacy of insecticide products should be evaluated, in addition to the contact bioassays currently done and recommended by WHOPES (Najera & Zaim, 2001; Mosqueira *et al*., 2013).

Another factor that has also been studied for some time is the repellent (rather than the killing) effect of insecticides at a distance. Recent findings emphasize the need to study the spatial repellency of insecticides, in particular of pyrethroids, in addition to traditional contact irritancy tests (Grieco *et al*., 2007). Spatial repellence may also be an effective tool in the fight against vector-borne disease transmission (Achee *et al*., 2012).

As with IRS and LLINs, for OP-based paint to be effective there must be coverage of at least 80% of houses in a community that are potential resting places for mosquitoes. Living in the only treated house in the neighbourhood will do little to protect residents (Lengeler & Sharp, 2003). A coverage of above 80% provides both personal protection and a community effect (Lengeler, 2004; Teklehaimanot *et al*., 2007). The distance from the treated clusters is also important (Hawley *et al*., 2003; Kroeger *et al*., 2006). However, despite evidence for the community effect of vector control strategies, little is known about the exact mechanism. Interestingly, a community effect of ITNs against malaria parasite transmission has also been observed in areas where the malaria vector is largely exophagic and zoophilic (Charlwood *et al*., 2005). The impact of IRS or OP-based paints in combination with LLINs requires future study.

To summarize, models were developed to test the spatial mortality efficacy of insecticides, both in the laboratory and in the field. A recommendation has been made to add spatial mortality assessments to the battery of assays regularly performed by WHOPES (Mosqueira *et al*., 2013).

The pre-Phase III pilot study evaluations at VK1 and VK3 (Burkina Faso)

The next step was to formally test the combination strategy in a village setting, in preparation for the large-scale Phase III study on the potential impact of this strategy. The specific focus was on malaria incidence in children in the same region of south-western Burkina Faso. The Kou Valley (Vallée du Kou) was chosen, based on the abundance of perennial populations of malaria vectors there (Robert *et al*., 1985; Baldet *et al*., 2003), as well as on the high level of pyrethroid resistance with high *kdr* frequency (Dabiré *et al*., 2008; Dabiré *et al*., 2009).

The team was also drawn by the residents' interest in the OP paint, and the efforts that home-owners had previously made (when their economic situation allowed it).to paint the interiors of their homes, including windows and doors. From that standpoint, the villages termed VK1 and VK3 presented an optimal profile for performing the pilot studies on the efficacy of combining an OP-based paint and LLINs. As indicated earlier, the interiors of VK1 houses were treated with Inesfly 5A IGR at 1 or 2 layers of paint; whereas at VK3, only the edges of windows and doors were treated, with just 1 layer. The goal was to test whether treating a smaller surface, through which mosquitoes had to pass, would suffice in terms of mortality. At both sites, PermaNet 2.0 LLINs were used by the occupants, and were checked to make sure they were intact.

Identifying and understanding the bio-ecology of the malaria vector in the study area is important (Ferguson *et al*., 2010; The malERA Consultative Group on Vector Control, 2011; Sinka *et al*., 2012). During the study period in both VK1 and VK3, local wild populations were genomically identified as exclusively *An. coluzzii*—considered a highly anthropophilic mosquito (Besansky *et al*., 2004; Takken & Verhulst, 2013), though this species can also feed on a wide range of

other animals if they are more readily available (Gillies & de Meillon, 1968; Lefevre *et al*., 2009).

At VK1, 6 months after treatment, mortality rates were 100% against pyrethroid-resistant *An. coluzzii* populations—regardless of the number of paint layers, or the configuration of surfaces treated: walls, or walls + ceiling (Mosqueira *et al*., 2015). After 6 months, however, houses with 2 layers of paint and a larger number of surfaces treated were shown to have a higher long-term efficacy. These results—established using EMCs—were supported by long-term residual tests using WHO cone tests. Mortality rates in all treated houses remained at 98.9–100% for 6 months, against both insecticide-susceptible *An. gambiae* Kisumu and pyrethroid-resistant *An. coluzzii* populations.

Results obtained 12 months after treatment seemed to confirm that, in the long term, houses with two paint layers and a larger number of treated surfaces performed best (Mosqueira *et al*., 2015). The results obtained using EMCs are consistent with previous Phase II studies performed in the experimental huts in Bénin: huts treated with two layers of the same paint, on a larger number of surfaces, had a longer-lasting efficacy. The only difference was that the mortality rates observed in Bénin were lower than the ones observed in VK1. This is probably linked to the higher porosity of the cement surfaces in the experimental huts, compared to the less-porous plastic sheeting placed in VK1 houses (Mosqueira *et al*., 2010a).

At the VK3 village, the strategy consisted of a combination of LLINs and Inesfly 5A IGR applied to windows and doors, rather than to walls and ceiling. The concept of the “house-proof” system for malaria prevention was actually the basis for the experimental proof of the mosquito malaria theory (Manson, 1900). The concept was recently reviewed in order to assess the hypothesis that improved housing can reduce malaria by decreasing entry of mosquitoes (Tusting *et al*., 2015). More particularly, a randomized-controlled trial in The Gambia showed that the use of window screens and closed eaves led to a reduction in the number of mosquitoes entering houses, and a reduction in the prevalence of anaemia in children; but it did not show a reduction in malaria prevalence (Kirby *et al*., 2009). Other studies have shown that the closure of eaves, and netting over windows,

can be effective in preventing mosquito entry into houses (Majori *et al*., 1987; Lwetoijera *et al*., 2013). However, there is little evidence as to how much that screening can reduce malaria infection.

In the VK3 study, even the insecticide paint applied on windows and doors did not prevent the entry of mosquitoes. This was probably due to the absence of a deterrent effect of these OPs (Mosqueira *et al*., 2010b). The treatment of windows and doors combined with LLINs yielded a high level of killing efficacy compared to control houses; but the effect only lasted for about 2 months (Mosqueira *et al*., in prep).

The reason for this short-lasting efficacy may be that the size of the treated surface was insufficient to ensure a sustained protection beyond 2 months. This is consistent with repeated observations on the importance of having a volume effect (Mosqueira *et al*., 2010b; Mosqueira *et al*., 2013; Mosqueira *et al*., 2015). Another possibility is the degradation that insecticides may undergo (despite microencapsulation), when exposed to high levels of heat and sunlight (Najera & Zaim, 2001). This is especially the case with metallic doors and windows.

The mortality rates observed in control houses without insecticide paint in the VK1 and VK3 area were low. These results are consistent with recent findings in the nearby VK7 village (also in the Bama area). Those studies measured the efficacy of pyrethroid-treated LLINs (PermaNet 2.0, distributed by the PNLP and similar to the ones in VK1 and VK3) against local populations of *An. gambiae* s.l. (mainly referring to *An. coluzzii*) that were highly resistant to pyrethroids (Toe *et al*., 2014). Mortality rates for the PermaNet 2.0 were about 20% (Toe *et al*., 2014), similar to the observed rates in these studies.

One potential concern of the combination LLINs/paint strategy was the risk of resistance development. This was assessed briefly during the studies at VK1 and VK3. Tests showed that the allelic frequency of the L1014F *kdr* mutation did not vary significantly during the testing period. This was likely because baseline frequencies are so high anyway in both villages. The L1014S *kdr* mutation revealed in Burkina Faso in recent years (Dabiré *et al*., 2009) was only studied at VK1. At VK1, the distribution of the allelic frequencies of the L1014S *kdr* mutation

remained low and heterozygous, and appeared 3 months after treatment in houses treated with insecticide paint and LLINs but not in control houses with LLINs alone.

The above results provide only some indication but the relatively small samples analyzed and the absence of homozygous individuals made it impossible for the current study to demonstrate any differential selection of the L1014S *kdr* mutation between the LLIN and combination treatments.

With regard to the *ace-1*^{R} mutation, *An. coluzzii* in VK1 and VK3 were considered to be susceptible to OPs as the distribution of the *ace-1*^{R} mutation is still low thus far (less than 10% overall) and in the heterozygous form at both VK1 and VK3.

Longer term and large data should be obtained during the Phase III study.

The anticipation is that the combined strategy will select less for resistance over time. Per Ngufor *et al*. (2014b), combining organophosphate treated wall linings and LLINs selected less for resistance to *kdr* and *ace-1*^{R} when compared to the individual single interventions.

Assessing the impact that vector control tools have on blood-feeding inhibition may yield misleading information as it cannot distinguish females entering houses to feed on humans, from females that have blood-fed outside (on either humans or animals, or both) and then enter the houses to complete their blood-feeding and/or to rest. Analysis done at VK1 on the source of blood meals showed that in VK1 an average of 58% of the *An. coluzzii* collected in houses had blood-fed on other animals (non-human) versus about 27% on humans, and about 16% had blood-fed on both other animals and humans.

In terms of the rate of zoophily or anthropophily, there were no differences between control and treated houses. It is worth noting that of the 58% of females that blood-fed on other non-human animals, about 45% obtained their blood meals from animals not identified as any of the five chosen domestic animal antibodies (Mosqueira *et al*., 2015).

The surprisingly relatively low rate of anthropophily of *An. coluzzii* in this particular rice-field area had already been highlighted in previous studies and may be

explained by the large mosquito densities and extensive live-stocking activities (Robert, 1989; Baldet *et al*., 2003), rendering other vertebrates more readily available for blood-feeding (Lefevre *et al*., 2009) even if less preferred (Besansky *et al*., 2004; Lefevre *et al*., 2009; Takken & Verhulst, 2013). In this anthropo-zoophilic context, the insecticide paint consisting on OPs could have provided a more optimal coverage by decreasing the longevity of both, malaria vectors having blood-fed outside on humans or other animals and entering houses to rest, as well as malaria vectors entering houses to blood-feed (Killeen *et al*., 2014).

Based on the results obtained at VK1 on the high proportion of mosquitoes collected in village houses, it is recommended that future Phase II studies also implement assays on the blood meal origin of engorged females collected during EMCs in experimental huts. Such tests may help to put into perspective further results on personal protection with regard to blood-feeding inhibition. This is particularly important when testing insecticides with no deterrent effect, and/or in areas of high pyrethroid resistance, since the blood-feeding inhibition may be underestimated.

Several other studies (mostly Phase II) have assessed the efficacy of combining LLINs with OP-based paint. The results support these findings, as outlined below.

A study performed in experimental huts on *An. arabiensis* in Tanzania tested several IRS compounds used concomitantly with LLINs to evaluate whether the combination in households would have synergistic or redundant effects. The study showed that IRS with DDT or pyrethroids did not add any value to the use of LLINs alone; but it did show that IRS with OPs such as pirimiphos-methyl conferred modest enhancements by slightly increasing mosquito mortality (Okumu *et al*., 2013). This study points out that combining LLINs and non-pyrethroid IRS may be justified as a means for managing insecticide resistance, but it does not specify the resistance status of the *An. arabiensis* used in the study.

Another study performed on pyrethroid-resistant *An. gambiae* suggested that the combination of OP-based wall lining with LLINs was more advantageous than the pyrethroid-based wall lining alone (Ngufor *et al*., 2014b).

Besides the cited Phase II evaluations, an observational, randomized trial performed in northern Tanzania in a region of pyrethroid-resistant *An. gambiae* reported significant added protection from combining non-pyrethroid IRS (carbamate) and LLINs, compared to LLINs alone (West *et al*., 2014). That effect was likely attributable to IRS providing added protection to LLIN users, as well as compensating for inadequate net use (West *et al*., 2014).

To summarize, the pilot studies at VK1 and VK3 showed that treating only windows and doors was not efficient in the long term. Treating a larger number of surfaces (walls and ceiling) with Inesfly 5A IGR, combined with the use of LLINs, yielded a one-year efficacy against pyrethroid-resistant *An. coluzzii*. Given the results obtained at VK1, the chosen strategy will be the combination of the insecticide paint Inesfly 5A IGR applied onto the interior of houses on walls and ceiling and LLINs.

Based on these study results, and those obtained by other research groups, on the potential benefits of combining OP-based tools (such as insecticide paint) with LLINs in controlling malaria vectors—particularly those with pyrethroid resistance—it was decided to go ahead with further evaluations. The next step was the selection of the villages where Phase III would take place, based on the results obtained in the previous studies.

The selection of Phase III villages (Orodara region, Burkina Faso)

The Orodara region is holoendemic for malaria and its main vectors, *An. gambiae* and *An. coluzzi* are resistant to pyrethroids through the L1014F *kdr* mutation (Dabiré *et al*., 2009).

The full list of villages in the Orodara District were reviewed and 34 villages were found to meet the main criteria needed for the Phase III study: at least 100 children from 6 months to 14 years of age (to ensure 30 evaluable children at the end of the study), minimal distances of 1–2 km from each other, and access by road. These 34 villages were visited for preliminary surveys and geo-referencing.

The surveys included assessing the level of knowledge and practices on malaria in the study area, the number of sleeping units per site/village and the number of children per site/village.

Based on those surveys, out of those 34 villages, 32 villages were selected. The other 2 villages were much smaller and much bigger than the other villages selected and could not be paired to any other village for randomization, and were taken out of the group as outliers.

Surveys (performed during the rainy season) showed that for the most part, houses comprised more than one sleeping unit; and that the villages selected had roughly 50 children within the study age range. Residents mentioned being bothered by mosquitoes during the rainy season, and mostly attributed malaria to mosquito bites—despite their use of impregnated bednets to prevent malaria

These findings were consistent with reports obtained by Toe *et al*., 2009 also in south-western Burkina Faso, where residents mentioned suffering from mosquito bites, and used bednets mostly during the rainy season. Toe *et al*., 2009, also noted however, that malaria was mainly perceived as an ordinary disease, not necessarily serious. This familiarity surrounding the perception of malaria, combined with the logistical obstacles surrounding bednet use—such as the need to reorganize the house to place the nets—result in poor sustained year-round motivation to use bednets (Toe *et al*., 2009).

It is hoped that the insecticide paint will find a high degree of acceptability among local residents. No particular day-to-day measures are needed by users, the paint can be easily applied, and its use may lead to home improvement. In the current study, people were tentatively favourable to the idea of painting their houses with an insecticide paint “to fight against mosquitoes.”

(It should be emphasized that residents were asked about their willingness to fight against *mosquitoes*, rather than to fight against *malaria*. It still not fully known whether the combination of Inesfly 5A IGR and LLINs will, in fact, help fight malaria—that is an issue to explore during Phase III. However, there is plenty of evidence that this strategy helps to kill mosquitoes.)

In terms of residents' perception of how the malaria parasite is transmitted, 89% of the people surveyed associated malaria with both mosquitoes and rain. A much smaller number (11%) associated malaria only with mosquitoes, not with rain.
In VK1 and VK3, the area of south-western Burkina Faso where the pre-Phase III studies were performed, most owners had chosen painting their homes and volunteers saw the study's paint as an added benefit towards home improvement. Nevertheless, a strong communication plan with advice from experienced and local socio-anthropologists must be implemented prior and during the intervention to maximize acceptability and participation by residents. Such community sensitization approaches has been shown to be crucial in making vector control interventions such as IRS genuinely participative, acceptable and sustainable (Munguambe *et al*., 2011; Kaufman *et al*., 2012).

Likewise, an economical component should consider overall operational cost and feasibility (inclusive of retreatment) per year of protection taking into account the duration of efficacy and the local epidemiological context. The associated cost of Inesfly 5A IGR use versus other standard treatments using pyrethroids has been studied in Bolivia in the fight against Chagas disease vectors (Gorla *et al*., 2015). Gorla *et al*. (2015) showed the inflation-adjusted cost of the vector control intervention using Inesfly 5A IGR for an average house with 300–320 square metres is about US$86, while the average for a pyrethroid-based IRS intervention is about US$51. Taking into account that African houses are generally smaller and that there are Inesfly 5A IGR manufacturing facilities in Ghana (West Africa), the cost may be lower, but it has to be studied along with the acceptability. It should be noted that acceptability is also linked to efficacy. In the case of the study in Bolivia, Inesfly 5A IGR proved to be more efficient in reducing house reinfestation by Triatomines and was perceived by the population as a house embellisher which resulted in the owners taking better care of their homes further reducing reinfestations (Gorla *et al*., 2015). Insecticide paints have been used for some time concomitantly with home improvement as a control method for Chagas disease with good results (Oliveira Filho 1996; Rozendaal, 1997). Likewise, further research should evaluate the protective effect of specific house features and incremental housing improvements associated with socio-economic development to control malaria in sub-Saharan regions (Tusting *et al*., 2015).

To summarize, 32 villages were selected in the Orodara region for the Phase III study, meaning 16 villages will be randomized per arm (LLINs versus Inesfly 5A IGR and LLINs).

The selected villages meet requirements in terms of number of residents, including children; distances between communities; road access; and potential willingness to participate.

As a whole, the results obtained during these seven studies allowed to gain a better understanding of the benefits and limitations of Inesfly 5A IGR as a potential malaria vector control tool: the combination of Inesfly 5A IGR and LLINs presents several advantages in terms of insecticide efficacy and operational use: the combination of different insecticides (pyrethroids in LLINs and OPs in the paint) may help reduce the pressure for resistance development in the target vector (WHO, 2011; 2012). The lethal effect of OPs combined with the excito-repellent effect of pyrethroids may broaden the scope of action of the active materials. At high coverage rates, it may provide a mass protective effect against the whole vector population.

The IGR can provide an additional angle of attack once the efficacy of the insecticide diminishes over time. The paint may provide indoor protection to users even outside of regular sleeping hours, when they are not under the LLIN: the paint may kill resting mosquitoes as well as indoor blood-feeding mosquitoes, as seen in the VK1pilot studies in Burkina Faso. While IRS with non-pyrethroids may provide similar benefits, the application of the paint requires no special equipment and may lead to a perceived improvement of people's homes that needs to be studied.

The combination of Inesfly 5A IGR and LLINs will be further tested during a large-scale Phase III evaluation. This randomized controlled trial will study the impact of Inesfly 5A IGR and LLINs on clinical malaria incidence rate in children aged 6 months to 14 years in a holoendemic malaria area against pyrethroid-resistant malaria vectors with high *kdr* frequencies in south-western Burkina Faso. The Phase III study will also include entomological as well as socio-anthropological

and economical surveys to assess the strategy's perception and acceptability by the population and the cost-effectiveness of the intervention.

CHAPTER 6: CONCLUSIONS

A series of seven studies was conducted, in a logical sequence, to assess the efficacy of the organophosphate-insecticide paint Inesfly 5A IGR against malaria disease vectors. The first studies were conducted in the laboratory (Phase I evaluations), followed by studies in experimental huts in the field (Phase II evaluations). Spatial mortality assessments were developed and performed to assess the lethal effect of Inesfly 5A IGR on mosquitoes at distances of 1 metre in the laboratory and in the field.

As a next step, Inesfly 5A IGR was combined with Long-Lasting Insecticide-Treated Nets (LLINs), and was tested in houses in a West African village setting. In preparation for the Phase III evaluation, 32 more village sites were selected in south-western Burkina Faso. The conclusions drawn from these seven studies are outlined below.

The WHOPES Phase I laboratory evaluations (in Côte d'Ivoire and France) revealed the following facts.

- A high mortality was observed on non-porous surfaces against both OP-susceptible and OP-resistant pest mosquito *Cx. quinquefasciatus*, for up to 12 months.
- As previously observed at the IPR institute, mosquito mortality rates were higher on the tested non-porous surfaces (softwood and hard plastic) than on the porous surfaces (cement and stucco).
- Lack of long-term efficacy was the result of the porosity of materials, rather than the pH of materials, or the dose of insecticide applied: Cement surfaces painted with a control layer and an insecticide paint layer at 1 kg/6 m^2, performed as well as two insecticide paint layers at the same coverage—even though the latter had twice the dose.
- Non-porous treated surfaces in the laboratory had a long-lasting efficacy of at least 12 months, even against OP-resistant mosquitoes.
- The effect of pyriproxyfen on the fecundity, fertility and adult emergence of exposed mosquito females obtained during laboratory evaluations may

afford an added tool in reducing overall pest mosquito and malaria vector population densities when the lethal effect of OPs diminishes over time.

- A similar experiment would need to be carried in the field to further evaluate the potential benefits of pyriproxyfen as an additional angle of attack against mosquito females having survived exposure to the insecticides.

The WHOPES Phase II field evaluations (in Bénin) revealed the following facts.

- The lethal effect of the insecticide paint Inesfly 5A IGR observed in the field against local populations of *An. gambiae* and pyrethroid-resistant *Cx. quinquefasciatus* was encouraging. A residual efficacy of 9 months was observed from early mosquito collections and 30-minute bioassays.
- The mosquito-release experiments showed that the killing rate was not only high, but also quick enough to prevent blood-feeding even in the absence of a physical barrier (bednets).
- Mosquito females left overnight at distances of 1 metre continued to die in significant numbers for up to 12 months after treatment.
- As observed during the laboratory Phase I evaluations, a critical question continues to be the porosity of materials. Porous surfaces such as cement and adobe benefitted from 2 layers of paint. In the field, huts treated with only 1 layer were more effective when treated with a larger volume of paint.

The Spatial Mortality Assessments (in France and Bénin) revealed the following facts.

- Phase I and Phase II WHOPES tests, both in the laboratory and in the field, showed that spatial mortality assessments provided additional information on the efficacy of insecticides that is not available from the contact bioassays that are currently recommended by WHOPES.
- As a result, it is proposed that spatial mortality assessments be added to the battery of Phase I and Phase II WHOPES tests.

- Distance boxes were used to evaluate an insecticide's lethal effect in the laboratory. In the field, exposing mosquitoes at a fixed distance from treated surfaces in experimental huts provided valuable information (with little added effort or cost).

The pre-Phase III village evaluations at VK1 and VK3 (in Burkina Faso) revealed the following facts.

- The results of the studies supported those of the previous Phase II studies performed in experimental huts in Bénin, in terms of the efficacy on mosquito mortality of the insecticide paint Inesfly 5A IGR, used in combination with pyrethroid-treated Long-Lasting Insecticide-treated Nets (LLINs) in houses in a village setting in Burkina Faso.
- The study at VK1 concluded that the combination of LLINs with Inesfly 5A IGR-treated house surfaces yielded a1-year killing efficacy against the local populations of *An. coluzzii*—a mosquito highly resistant to pyrethroids, but susceptible to OPs.
- The study at VK3 concluded that painting only doors and windows conferred no protection after 2 months against the local populations of *An. coluzzii*, which were also highly resistant to pyrethroids but susceptible to OPs.
- At VK3, spatial mortality assessments were performed by placing mosquitoes 1 metre away from treated doors and windows for 30 minutes. This yielded high mortality rates for about 1 month.
- Preliminary results provided no evidence of differential selection of the L1014S *kdr*, L1014S *kdr* or *ace-1*^{R} mutations between LLINs alone, and combination treatments. More detailed long-term monitoring is needed. Results suggested a large degree of zoophily in the study area, despite the anthropophilic character of *An. coluzzii*. It is suggested that, in the future, WHOPES field studies (Phase II) assess blood meal origin systematically, to better estimate personal protection provided by vector control tools.

- The ease of applying the insecticide paint makes it more feasible for communities to use, since no special equipment is needed. Home-owners can apply the paint themselves, taking charge of their home improvement.
- Initial interviews conducted with residents indicated their willingness to use the insecticide paint. A more thorough sociological assessment on acceptance will be performed during Phase III.

The selection of Phase III villages (in the Orodara region of Burkina Faso) revealed the following facts.

- The Orodara region was chosen for the upcoming large-scale Phase III study because malaria is holoendemic there; and its main vectors, *An. gambiae* and *An. coluzzi*, are highly resistant to pyrethroids through the *kdr* mutation.
- From May 25 to June 30, 2013, all residents of the 34 geo-referenced villages were registered, and 32 villages meeting the requirements of Phase III were selected.
- In interviews, the majority of people questioned said that malaria and mosquito nuisance were “frequent.” Most (89%) of residents associated malaria with both mosquitoes and rain. A smaller number (11%) associated malaria only with mosquitoes, not with rain.
- Most residents slept under pyrethroid-impregnated bednets.
- The main symptom of malaria in the villages was fever, followed by vomiting, headaches and joint pain.
- Most people interviewed, 97.5%, were at least tentatively “favourable” to the concept of painting the walls of their homes with an insecticide, to fight against mosquito-born disease.

These seven studies provided useful information in preparation for the forthcoming large-scale Phase III evaluation. It will assess the impact of the combination of OP-insecticide paint and pyrethroid-treated LLINs on reducing the incidence of malaria. The study will focus particularly on children aged 6 months

to 14 years of age, living in villages of an area of West Africa where malaria is holoendemic, and mosquito vectors are resistant to pyrethroids.

Conclusiones

Se han dirigido siete estudios con una secuencia lógica para evaluar la eficacia de la pintura insecticida de organofosforados Inesfly 5A IGR™. Los primeros estudios se realizaron en el laboratorio, seguidos de estudios realizados en casas experimentales en el terreno. Se realizaron evaluaciones sobre mortalidad espacial para estudiar el efecto de la pintura Inesfly 5A IGR™ a distancias de un metro tanto en el laboratorio como en el terreno. Como siguiente paso, se combinó el uso de la pintura Inesfly 5A IGR™ con mosquiteras tratadas con insecticidas de larga duración (LLINs), y se probó en una aldea real. Para preparar la Fase III se seleccionó una lista de treinta y seis localizaciones/aldeas en el Sudoeste de Burkina Faso. A continuación se muestran las conclusiones de estos siete estudios:

WHOPES Fase I. Evaluaciones de laboratorio en Costa de Marfil y Francia:

En superficies no porosas se ha observado una alta mortalidad tanto de *Cx. quinquefasciatus* sensibles como de los resistentes a OPs de hasta 12 meses.

Como ya se había observado en el Instituto IPR en Costa de Marfil, las tasas de mortalidad eran más altas en las superficies tratadas no porosas (madera y plástico duro) que en las porosas (cemento y estuco).

La eficacia a largo plazo ha resultado ser una cuestión de porosidad de los materiales tratados más que del pH de los materiales o de las dosis aplicadas.

Se ha demostrado que la eficacia depende más de la porosidad que de la dosis aplicada: las superficies de cemento pintadas con una capa de control y una capa de pintura insecticida de 1 Kg/6 m^2 han tenido los mismos buenos resultados que aquellas tratadas con dos copas de pintura de 1Kg/6 m^2, aunque la segundas llevaran el doble de dosis.

La aplicación sobre superficies no porosas ha tenido una eficacia a largo plazo de por lo menos 12 meses incluso con mosquitos resistentes a OP en las pruebas de laboratorio.

El efecto del piriproxifen en la fecundidad, fertilidad y emergencia de los estadios adultos de las hembras adultas expuestas podría ofrecer un ángulo de ataque adicional para reducir la densidad los mosquitos vectores de la malaria y los mosquitos plaga cuando el efecto letal de los organofosforados disminuye con el tiempo.

WHOPES Fase II. Evaluaciones sobre el terreno en Benín:

El efecto letal de la pintura insecticida observado en el terreno con poblaciones locales de *An. gambiae* y *Cx. quinquefasciatus* resistentes a piretroides ha sido alentador. Se ha observado además una eficacia residual de nueves meses en las recolecciones de mosquitos y los bioensayos.

En los experimentos en los que se soltaron mosquitos, la mortalidad no solamente fue elevada, sino que además fue lo bastante rápida como para evitar que se alimenten de sangre incluso en ausencia de barreras físicas como mosquiteras.

Las hembras que se colocaron durante la noche a distancias de un metro siguieron muriendo incluso 12 meses después de manera significativa.

Tal y como se observó en las evaluaciones de la Fase I de laboratorio, la cuestión abierta y crucial siguió siendo la porosidad de los materiales. Las superficies porosas como el cemento se trataron con dos capas. De igual modo, las casas que se pintaron en el terreno con una sola capa, se trataron con mayor volumen de pintura.

Evaluaciones de Mortalidad Espacial en Francia y Benín.

Los ensayos de WHOPES Fases I y II realizados en el laboratorio y sobre el terreno han demostrado que las evaluaciones de mortalidad espacial ofrecen información adicional ignorada en los bioensayos sobre la eficacia de contacto recomendados actualmente por WHOPES.

De este modo se propuso añadir ensayos de evaluación sobre la eficacia de mortalidad espacial de los insecticidas, a la batería de pruebas WHOPES de las Fases I y II en el laboratorio y sobre el terreno.

Se propuso una herramienta que consistía en colocar cajas a una cierta distancia en el laboratorio para evaluar el efecto letal de la pintura en la distancia.

En el terreno la exposición de mosquitos a distancias fijas de las superficies tratadas ha conseguido información valiosa con poco esfuerzo y gasto añadido.

Pre-Fase Piloto III. Evaluaciones en aldeas reales VK1 y VK3 en Burkina Faso.

Los resultados de los ensayos de la pre-fase piloto III sobre la eficacia de la pintura Inesfly 5A IGR™ combinada con mosquiteras tratadas con piretroides en casas reales de una aldea, han apoyado los estudios de la Fase II realizados en casas experimentales.

El estudio de la aldea VK1 concluyó que el uso combinado de Inesfly 5A IGR™ con LLINs conseguía una eficacia de mortalidad de un año contra *An. coluzzii* muy resistentes a piretroides pero sensibles a OPs.

El estudio de la aldea VK3 concluyó que la pintura aplicada únicamente en puertas y ventanas no ofrece protección más allá de 2 meses contra *An. coluzzii* muy resistentes a piretroides pero sensibles a OPs.

En VK3 se realizaron evaluaciones sobre mortalidad espacial colocando los mosquitos a un metro de distancia de las puertas y ventanas tratadas y se observaron altas tasas de mortalidad durante un mes, lo que indica la conveniencia de su uso como estrategia para combatir las picaduras en el exterior.

La facilidad de aplicación de la pintura la hace asequible al uso en las comunidades. Los usuarios no tienen necesidad de un equipo especial y pueden aplicar la pintura ellos mismos, responsabilizándose así de la mejora de sus hogares.

Selección de aldeas para la Fase III en la región de Orodara en Burkina Faso

La región de Orodara fue elegida para el futuro estudio a gran escala de Fase III porque la malaria en esa zona es holoendémica y sus principales vectores, *An. gambiae* and *An. coluzzi*, son resistentes a piretroides por el mecanismo *kdr*.

Entre el 25 de mayo y el 30 de junio de 2013, se realizó un registro de todos los habitantes de las 34 aldeas geo-referenciadas y 32 aldeas fueron seleccionadas. Las entrevistas llevadas a cabo concluyeron lo siguiente:

La malaria y la molestia de los mosquitos era considerada “frecuente” por la mayoría de la gente entrevistada: 89% de los entrevistados atribuyen la malaria a ambos, los mosquitos y la lluvia. 11% de los entrevistados atribuyen la malaria a los mosquitos y no a la lluvia.

La mayoría dormían bajo mosquiteras impregnadas con insecticidas (piretroides).

Los principales síntomas relacionados con malaria en las aldeas pre-seleccionadas eran fiebre, seguida de vómitos, dolores de cabeza y dolor en las articulaciones.

La gran mayoría de la gente entrevistada, el 97.5%, se mostró “favorable”a pintar las paredes de sus casas con pintura insecticida para luchar contra los mosquitos, pero necesitarían más información.

Estos siete estudios han aportado una información muy provechosa para la preparación de la siguiente evaluación a gran escala de la Fase III. El objetivo del estudio de la Fase III es evaluar el impacto de la combinación de la pintura insecticida OP y las LLINs tratadas con piretroides, en la reducción real de la incidencia de malaria en niños entre 6 meses y 14 años de edad en una zona de Africa del Oeste donde la malaria es holoendémica y los mosquitos vectores son resistentes a los piretroides.

CHAPTER 7: REFERENCES

References

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, *18*, 265–267.

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*, 164.

Aikins, M. K., Pickering, H., & Greenwood, B. M. (1994). Attitudes to malaria, traditional practices and bednets (mosquito nets) as vector control measures: A comparative study in five West African countries. *American Journal of Tropical Medicine and Hygiene*, *97*, 81–86.

Alaii, J. A., van den Borne, H. W., Kachur, S. P., Mwenesi, H., Vulule, J. M., Hawley, W. A., Meltzer, M. I., Nahlen, B. L., & Phillips-Howard, P. A. (2003). Perceptions of bednets and malaria prevention before and after a randomized controlled trial of permethrin-treated bednets in western Kenya. *American Journal of Tropical Medicine and Hygiene*, *68*(4 Suppl), 142–148.

Aldridge, W. N. (1950). Some properties of specific cholinesterase with particular reference to the mechanism of inhibition by diethyl p-nitrophenyl thiophosphate (E 605) and analogues. *Biochemical Journal*, *46*(4), 451–460.

Amelotti, I., Catalá, S. S., & Gorla, D. E. (2009). Experimental evaluation of insecticidal paints against *Triatoma infestans* (Hemiptera: Reduviidae), under natural climatic conditions. *Parasites & Vectors*, *2*(1), 30.

Asidi, A. N., N'Guessan, R., Koffi, A. A., Curtis, C. F., Hougard, J.-M., Chandre, F., Corbel, V., Darriet, F., Zaim, M., & Rowland, M. W. (2005). Experimental hut evaluation of bednets treated with an organophosphate (chlorpyrifos-methyl) or a pyrethroid (lambdacyhalothrin) alone and in

combination against insecticide-resistant *Anopheles gambiae* and *Culex quinquefasciatus* mosquitoes. *Malaria Journal*, *4*, 25.

Baldet, T., Diabaté, A., & Guiguemdé, T. R. (2003). Malaria transmission in 1999 in the rice field area of the Kou Valley (Bama), (Burkina Faso). *Santé*, *13*(1), 55–60.

Beier, J. C., Perkins, P. V., Wirtz, R. A., Koros, J., Diggs, D., Gargan, T. P., & Koech, D. K. (1988). Bloodmeal identification by direct enzyme-linked immunosorbent assay (ELISA), tested on *Anopheles* (Diptera: Culicidae) in Kenya. *Journal of Medical Entomology*, *25*(1), 9–16.

Beier, J. C., Keating, J., Githure, J. I., Macdonald, M. B., Impoinvil, D. E., & Novak, R. J. (2008). Integrated vector management for malaria control. *Malaria Journal*, *7 Suppl 1*, S4.

Berticat, C., Boquien, G., Raymond, M., & Chevillon, C. (2002). Insecticide resistance genes induce a mating competition cost in *Culex pipiens* mosquitoes. *Genetics Research*, *79*, 41–47.

Besansky, N.J., Hill, C.A., & Costantini, C. (2004). No accounting for taste: host preference in malaria vectors. *Trends in Parasitology*, 20, 249–251.

Binka, F. N., & Adongo, P. (1997). Acceptability and use of insecticide impregnated bednets in northern Ghana. *Tropical Medicine & International Health*, *2*(5), 499–507.

Brown, A.W.A. & Pal, R. (1973) Insecticide Resistance in Arthropods. 2nd edn. World Health Organization Monograph, 38, 491 pp.

Caputo, B., Ienco, A., Cianci, D., Pombi, M., Petrarca, V., Baseggio, A., Devine, G.J., Della Torre, A. (2012). The “Auto-Dissemination” approach: a novel concept to fight *Aedes albopictus* in urban areas. *PLoS Neglected Tropical Diseases*, 6:e1793.

Cham, M. K., Olaleye, B., D’Alessandro, U., Aikins, M., Cham, B., Maine, N., Williams, L. A., Mills, A., & Greenwood, B. M. (1997). The impact of

charging for insecticide on the Gambian National Impregnated Bednet Programme. *Health Policy and Planning*, *12*(3), 240–247.

Chanda, E., Chanda, J., Kandyata, A., Phiri, F. N., Muzia, L., Haque, U., & Baboo, K. S. (2013). Efficacy of ACTELLIC 300 CS, pirimiphos methyl, for indoor residual spraying in areas of high vector resistance to pyrethroids and carbamates in Zambia. *Journal of Medical Entomology*, *50*(6), 1275–1281.

Chandre, F., Darriet, F., Doannio, J. M. C., Riviere, F., Pasteur, N., & Guillet, P. (1997). Distribution of organophosphate and carbamate resistance in *Culex pipiens quinquefasciatus* (Diptera: Culicidae) from West Africa. *Journal of Medical Entomology*, *34*, 664 – 671.

Chandre, F., Darriet, F., Darder, M., Cuany, A., Doannio, J. M. C., Pasteur, N., & Guillet, P. (1998). Pyrethroid resistance in *Culex quinquefasciatus* from West Africa. *Medical and Veterinary Entomology*, *12*, 359 – 366.

Chandre, F., Darrier, F., Manga, L., Akogbeto, M., Faye, O., Mouchet, J., & Guillet, P. (1999). Status of pyrethroid resistance in *Anopheles gambiae* sensu lato. *Bulletin of the World Health Organization*, *77*(3), 230–234.

Charlwood, J. D., Alcantara, J., Pinto, J., Sousa, C. A., Rompao, H., Gil, V., & Rosario, V. E. (2005). Do bednets reduce malaria transmission by exophagic mosquitoes? *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *99*, 901 – 904.

Clements, A.N. (1992). The biology of mosquitoes. Vol. 1 Development, nutrition and reproduction. Chapman & Hall. 509 pp.

Coetzee, M., Hunt, R. H., Wilkerson, R., Torre, A. D., Coulibaly, M. B., & Besansky, N. J. (2013). *Anopheles coluzzii* and *Anopheles amharicus*, new members of the *Anopheles gambiae* complex. *Zootaxa*, *3619*(3).

Coluzzi, M. (1999). The clay feet of the malaria giant and its African roots: hypotheses and inferences about origin, spread and control of *Plasmodium falciparum*. *Parassitologia*, *41*(1-3), 277–283.

Corbel, V., Darriet, F., Chandre, F., & Hougard, J. M. (2002). Insecticide mixtures for mosquito net impregnation against malaria vectors. *Parasite*, *9*(3), 255–259.

Corbel, V., Hougard, J.-M., N'Guessan, R., & Chandre, F. (2003). Evidence for selection of insecticide resistance due to insensitive acetylcholinesterase by carbamate-treated nets in *Anopheles gambiae* s. s. (Diptera: Culicidae) from Côte d'Ivoire. *Journal of Medical Entomology*, *40*(6), 985–988.

Corbel, V., Raymond, M., Chandre, F., Darriet, F., & Hougard, J.-M. (2004). Efficacy of insecticide mixtures against larvae of *Culex quinquefasciatus* (Say) (Diptera: Culicidae) resistant to pyrethroids and carbamates. *Pest Management Science*, *60*(4), 375–380.

Corbel, V., N'Guessan, R., Brengues, C., Chandre, F., Djogbenou, L., Martin, T., Akogbeto, M., Hougard, J.-M., & Rowland, M. (2007). Multiple insecticide resistance mechanisms in *Anopheles gambiae* and *Culex quinquefasciatus* from Bénin, West Africa. *Acta Tropica*, *101*, 207 – 16.

Costantini, C., Li, S. G., Della Torre, A., Sagnon, N., Coluzzi, M., & Taylor, C. E. (1996). Density, survival and dispersal of *Anopheles gambiae* complex mosquitoes in a West African Sudan savanna village. *Medical and Veterinary Entomology*, *10*(3), 203–219.

Curtis, C. F., & Mnzava, A. E. P. (2000). Comparison of house spraying and insecticide-treated nets for malaria control. *Bulletin of the World Health Organization*, *78*, 1389 – 1400.

D'Alessandro, U. (2001). Insecticide treated bed nets to prevent malaria. *British Medical Journal*, *322*(7281), 249–250.

Dabiré, R. K., Diabaté, A., Baldet, T., Paré-Toé, L., Guiguemdé, R. T., Ouédraogo, J.-B., & Skovmand, O. (2006). Personal protection of long lasting insecticide-treated nets in areas of *Anopheles gambiae* s. s. resistance to pyrethroids. *Malaria Journal*, *5*, 12.

Dabiré, K. R., Diabaté, A., Djogbenou, L., Ouari, A., N'Guessan, R., Ouédraogo, J.-B., Hougard, J.-M., Chandre, F., Baldet, T. (2008). Dynamics of multiple insecticide resistance in the malaria vector *Anopheles gambiae* in a rice growing area in South-Western Burkina Faso. *Malaria Journal*, *7*, 188.

Dabiré, K. R., Diabaté, A., Namountougou, M., Toé, K. H., Ouari, A., Kengne, P., Bass, C., Baldet, T. (2009). Distribution of pyrethroid and DDT resistance and the L1014F *kdr* mutation in *Anopheles gambiae* s.l. from Burkina Faso (West Africa). *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *103*(11), 1113–1120.

Dabiré, K. R., Diabaté, A., Namountougou, M., Djogbenou, L., Wondji, C., Chandre, F., Simard, F., Ouédraogo, J.-B., Martin, T., Weill, M., Baldet, T. (2012). Trends in Insecticide Resistance in Natural Populations of Malaria Vectors in Burkina Faso, West Africa: 10 Years' Surveys. In F. Perveen (Ed.), *Insecticides - Pest Engineering*. InTech.

Darriet, F., Guillet, P., N'Guessan, R., Doannio, J. M. C., Koffi, A. A., Konan, L., & Carnevale, P. (1998). [Impact of resistance of *Anopheles gambiae* s. s. to permethrin and deltamethrin on the efficacy of impregnated mosquito nets]. *Médecine Tropicale*, *58*, 349 – 354.

Darriet, F., N'guessan, R., Koffi, A. A., Konan, L., Doannio, J. M., Chandre, F., & Carnevale, P. (2000a). Impact of pyrethrin resistance on the efficacy of impregnated mosquito nets in the prevention of malaria: results of tests in experimental cases with deltamethrin SC. *Bulletin de La Société de Pathologie Exotique*, *93*(2), 131–134.

Darriet, F., N'Guessan, R., & Carnevale, P. (2000b). Evaluation in test huts of the protective effects of untreated mosquito nets against *Anopheles gambiae* s. s. bites. *Cahiers Sante*, *10*, 413 – 417.

Darriet, F., N'Guessan, R., Hougard, J. M., Traore-Lamizana, M., & Carnevale, P. (2002). Un outil experimental indispensable a l'evaluation des insecticides: les cases-pieges. *Bulletin de la Societe de Pathologie Exotique*, *95*, 299 – 303.

Darriet, F., Corbel, V., & Hougard, J. M. (2003). Efficacy of mosquito nets treated with a pyrethroid-organophosphorous mixture against *Kdr*- and *Kdr*+ malaria vectors (*Anopheles gambiae*). *Parasite*, *10*(4), 359–362.

Dell Chism, B., & Apperson, C. S. (2003). Horizontal transfer of the insect growth regulator pyriproxyfen to larval microcosms by gravid *Aedes albopictus* and *Ochlerotatus triseriatus* mosquitoes in the laboratory. *Medical and Veterinary Entomology*, *17*, 211 – 220.

Desfontaine, M., Gelas, H., Cabon, H., Goghomou, A., Kouka-Bemba, D., Carnevale, P. (1990). Évaluation des pratiques et des coûts de lutte antivectorielle à l'échelon familial en Afrique Centrale. II. Enquête dans la ville de Douala (Cameroun), *Annales de la Société Belge de Médecine Tropicale*, 70(2), 137-144.

Devine, G. J., Zamora Perea, E., Killeen, G. F., Stancil, J. D., Clark, S. J., & Morrison, A. C. (2009). Using adult mosquitoes to transfer insecticides to *Aedes aegypti* larval habitats. *Proceedings of the National Academy of Sciences*, *106*, 11530 – 11534.

Devine, G. J., & Killeen, G. F. (2010). The potential of a new larviciding method for the control of malaria vectors. *Malaria Journal*, *9*, 142.

Dhadialla, T. S., Carlson, G. R., & Le, D. P. (1998). New insecticides with ecdysteroidal and juvenile hormone activity. *Annual Review of Entomology*, *43*, 545–569.

Djogbénou, L., Chandre, F., Berthomieu, A., Dabiré, R., Koffi, A., Alout, H., & Weill, M. (2008). Evidence of introgression of the ace-1(R) mutation and of the ace-1 duplication in West African *Anopheles gambiae* s. s. *PLoS One*, 14, 3(5), e2172.

Diabaté, A., Brengues, C., Baldet, T., Dabiré, K. R., Hougard, J. M., Akogbeto, M., Kengne, P., Simard, F., Guillet, P., Hemingway, J., & Chandre, F. (2004). The spread of the Leu-Phe *kdr* mutation through Anopheles

gambiae complex in Burkina Faso: genetic introgression and de novo phenomena. *Tropical Medicine & International Health*, *9*(12), 1267–1273.

Dias, J. C. P., & Jemmio, A. (2008). [About an insecticidal paint for controlling *Triatoma infestans*, in Bolivia]. *Revista Da Sociedade Brasileira De Medicina Tropical*, *41*(1), 79–81.

Elissa, N., Mouchet, J., Riviere, F., Meunier, J.Y., & Yao, K. (1993). Resistance of *Anopheles gambiae* s.s. to pyrethroids in Côte d'Ivoire. *Annales de la Societe belge de medecine tropicale*, 73(4), 291-4.

Enayati, A., & Hemingway, J. (2010). Malaria management: past, present, and future. *Annual Review of Entomology*, *55*, 569–591.

Etang, J., Nwane, P., Mbida, J., Piameu, M., Manga, B., Souop, D., & Awono-Ambene, P. (2011). Variations of insecticide residual bio-efficacy on different types of walls: results from a community-based trial in south Cameroon. *Malaria Journal*, *10*(1), 333.

Ferguson, H. M., Dornhaus, A., Beeche, A., Borgemeister, C., Gottlieb, M., Mulla, M. S., Gimnig, J. E., Fish, D., & Killeen, G. F. (2010). Ecology: a prerequisite for malaria elimination and eradication. *PLoS Medicine*, *7*(8), e1000303.

Fillinger, U., & Lindsay, S. W. (2011). Larval source management for malaria control in Africa: myths and reality. *Malaria Journal*, *10*, 353.

Fontenille, D., & Simard, F. (2004). Unravelling complexities in human malaria transmission dynamics in Africa through a comprehensive knowledge of vector populations. *Comparative Immunology*, *Microbiology and Infectious Diseases*, *27*(5), 357–375.

Fuseini, G., Ebsworth, P., Jones, S., & Knight, D. (2011). The efficacy of ACTELLIC 50 EC, pirimiphos methyl, for indoor residual spraying in Ahafo, Ghana: area of high vector resistance to pyrethroids and organochlorines. *Journal of Medical Entomology*, *48*(2), 437–440.

Georghiou, G. P., Metcalf, R. L., & Gidden, F. E. (1966). Carbamate-resistance in mosquitoes: selection of *Culex pipiens fatigans* Wied (= *Culex quinquefasciatus*) for resistance to Baygon. *Bulletin of the World Health Organization*, *35*, 691 – 708.

Gillies, M. T., & de Meillon, B. (1968). *The Anophelinae of Africa South of the Sahara (Ethiopian zoogeographical region).* 2nd ed. Johannesburg: The South African Institute for Medical Research.

Gorla, D.E., Vargas Ortiz, R., Catalá, S. S. (2015). Control of rural house infestation by *Triatoma infestans* in the Bolivian Chaco using a microencapsulated insecticide formulation. *Parasites & Vectors*, 8, 255.

Grieco, J. P., Achee, N. L., Chareonviriyaphap, T., Suwonkerd, W., Chauhan, K., Sardelis, M. R., & Roberts, D. R. (2007). A new classification system for the actions of IRS chemicals traditionally used for malaria control. *PLoS ONE*, *2*, e716.

Guerra, C. A., Gikandi, P. W., Tatem, A. J., Noor, A. M., Smith, D. L., Hay, S. I., & Snow, R. W. (2008). The limits and intensity of *Plasmodium falciparum* transmission: implications for malaria control and elimination worldwide. *PLoS Medicine*, *5*(2), e38.

Guillet, P., N'Guessan, R., Darriet, F., Traore-Lamizana, M., Chandre, F., & Carnevale, P. (2001a). Combined pyrethroid and carbamate "two-in-one" treated mosquito nets: field efficacy against pyrethroid-resistant *Anopheles gambiae* and *Culex quinquefasciatus*. *Medical and Veterinary Entomology*, *15*(1), 105–112.

Guillet, P., Alnwick, D., Cham, M. K., Neira, M., Zaim, M., Heymann, D., & Mukelabai, K. (2001b). Long-lasting treated mosquito nets: a breakthrough in malaria prevention. *Bulletin of the World Health Organization*, *79*(10), 998.

Hamel, M.J., Otieno, P., Bayoh, N., Kariuki, S., Were, V., Marwanga, D., Laserson, K.F., Williamson, J., Slutsker, L., & Gimnig, J. (2011). The

combination of indoor residual spraying and insecticide-treated nets provides added protection against malaria compared with insecticide-treated nets alone. *The American Journal of Tropical Medicine and Hygiene*, 85(6): 1080-6.

Harbach, R.E. (2013). *Anopheles* Classification. Mosquito Taxonomic Inven-tory, http://mosquito-taxonomic-inventory.info/ltemgtanophelesltemgt-classification (accessed 1.09.13).

Harris, C., Lwetoijera, D. W., Dongus, S., Matowo, N. S., Lorenz, L. M., Devine, G. J., & Majambere, S. (2013). Sterilising effects of pyriproxyfen on *Anopheles arabiensis* and its potential use in malaria control. *Parasites & Vectors*, *6*(1), 144.

Hawley, W. A., Phillips-Howard, P. A., Feiko, O., Kuile, T., Terlouw, D. J., Vulule, J. M., Ombok, M., Nahlen, B. L., Gimnig, J. E., Kariuki, S. K., Kolczak, M. S., & Hightower, A. W. (2003). Community-wide effects of permethrin-treated bed nets on child mortality and malaria morbidity in Western Kenya. *The American Journal of Tropical Medicine and Hygiene*, *68* (suppl 4), 121 – 127.

Hay, S. I., Guerra, C. A., Gething, P. W., Patil, A. P., Tatem, A. J., Noor, A. M., Kabaria, C. W., Manh, B. H., Elyazar, I. R., Brooker, S., Smith, D. L., Moyeed, R. A., & Snow, R. W. (2009). A world malaria map: *Plasmodium falciparum* endemicity in 2007. *PLoS Medicine*, *6*(3), e1000048.

Hay, S. I., Okiro, E. A., Gething, P. W., Patil, A. P., Tatem, A. J., Guerra, C. A., & Snow, R. W. (2010). Estimating the global clinical burden of *Plasmodium falciparum* malaria in 2007. *PLoS Medicine*, *7*(6), e1000290.

Hayes, R.J. & Bennett, S. (1999). Simple sample size calculation for cluster-randomized trials. *International Journal of Epidemiology*, 28, 319-26.

Hemingway, J. (2014). The role of vector control in stopping the transmission of malaria: threats and opportunities. *Philosophical Transactions of the Royal Society of London. Series B*, *Biological Sciences*, *369*(1645), 20130431.

Henry, M.-C., Assi, S.-B., Rogier, C., Dossou-Yovo, J., Chandre, F., Guillet, P., & Carnevale, P. (2005). Protective efficacy of lambda-cyhalothrin treated nets in *Anopheles gambiae* pyrethroid resistance areas of Côte d'Ivoire. *The American Journal of Tropical Medicine and Hygiene*, *73*(5), 859–864.

International center of training and medical investigations. (2003). *Toxicity studies on Inesfly 5A IGR. CIDEIM*, *Cali (Colombia); 2003 [http://www.cideim.org.co].*

Itoh, T., Kawada, H., Abe, A., Eshita, Y., Rongsriyam, Y., & Igarashi, A. (1994). Utilization of bloodfed females of *Aedes aegypti* as a vehicle for the transfer of the insect growth regulator pyriproxyfen to larval habitats. *Journal of the American Mosquito Control Association*, *10*, 344 – 347.

Kamal, H. A., & Khater, E. I. M. (2010). The biological effects of the insect growth regulators; pyriproxyfen and diflubenzuron on the mosquito *Aedes aegypti*. *Journal of the Egyptian Society of Parasitology*, *40*(3), 565–574.

Karunaratne, S. H., & Hemingway, J. (2001). Malathion resistance and prevalence of the malathion carboxylesterase mechanism in populations of mosquito vectors of disease in Sri Lanka. *Bulletin of the World Health Organization*, *79*(11), 1060–1064.

Kaufman, M.R., Rweyemamu, D., Koenker, H., Macha, J. (2012). "My children and I will no longer suffer from malaria": a qualitative study of the acceptance and rejection of indoor residual spraying to prevent malaria in Tanzania. *Malaria Journal*, 11: 220.

Kawada, H., Dida, G. O., Ohashi, K., Kawashima, E., Sonye, G., Njenga, S. M., Mwandawiro, C., & Minakawa, N. (2014). A Small-Scale Field Trial of Pyriproxyfen-Impregnated Bed Nets against Pyrethroid-Resistant *Anopheles gambiae* s. s. in Western Kenya. *PLoS ONE*, *9*(10), e111195.

Killeen, G. F. (2014). Characterizing, controlling and eliminating residual malaria transmission. *Malaria Journal*, *13*, 330.

Killeen, G. F., Seyoum, A., Gimnig, J. E., Stevenson, J. C., Drakeley, C. J., & Chitnis, N. (2014). Made-to-measure malaria vector control strategies: rational design based on insecticide properties and coverage of blood resources for mosquitoes. *Malaria Journal*, *13*, 146.

Kirby, M., Ameh, D., Bottomley, C., Green, C., Jawara, M., Milligan, P.J., Snell, P.C., Conway, D.J., & Lindsay, S.W. (2009). Effect of two different house screening interventions on exposure to malaria vectors and on anaemia in children in The Gambia: a randomised controlled trial. *Lancet* 374: 998-1009.

Koama, B., Namountougou, M., Sanou, R., Ndo, S., Ouattara, A., Dabiré, R. K., Malone, D., & Diabaté, A. (2015). The sterilizing effect of pyriproxyfen on the malaria vector *Anopheles gambiae*: physiological impact on ovaries development. *Malaria Journal*, *14*(1).

Kolaczinski, J. H., Fanello, C., Hervé, J. P., Conway, D. J., Carnevale, P., & Curtis, C. F. (2000). Experimental and molecular genetic analysis of the impact of pyrethroid and non-pyrethroid insecticide impregnated bednets for mosquito control in an area of pyrethroid resistance. *Bulletin of Entomological Research*, *90*(2), 125–132.

Kroeger, A., Meyer, R., Mancheno, M., Gonzalez, M., & Pesse, K. (1997). Operational aspects of bednet impregnation for community-based malaria control in Nicaragua, Ecuador, Peru and Colombia. *Tropical Medicine & International Health*, *2*(6), 589–602.

Kroeger, A., Lenhart, A., Ochoa, M., Villegas, E., Levy, M., Alexandrer, N., & McCall, P. J. (2006). Effective control of dengue vectors with curtains and water container covers treated with insecticide in Mexico and Venezuela: cluster randomized trials. *British Medical Journal*, *332*, 1247 – 1252.

Lardeux, F., Depickère, S., Duchon, S. & Chavez, T. (2010). Insecticide resistance of *Triatoma infestans* (Hemiptera, Reduviidae) vector of Chagas disease in Bolivia. *Tropical Medicine and International Health*, 15(9), 1037–48.

Lefèvre, T., Gouagna, L.C., Dabiré, K.R., Elguero, E., Fontenille, D., Renaud, F., Costantini, C., Thomas, F. (2009). Beyond nature and nurture: phenotypic plasticity in blood-feeding behavior of *Anopheles gambiae* s.s. when humans are not readily accessible. *American Journal of Tropical Medicine and Hygiene*, 81(6), 1023-9.

Lengeler, C., & Sharp, B. (2003). *Indoor residual spraying and insecticide-treated nets. In: Reducing malaria's burden: evidence of effectiveness for decision makers. In Global Health Council. Washington D.C; 2003:17-24*.

Lengeler, C. (2004). Insecticide-treated bed nets and curtains for preventing malaria. Cochrane Database of Systematic Reviews 2004, Issue 2. Art. No.: CD000363. DOI: 10.1002/14651858.CD000363.pub2.

Loh, P. Y., & Yap, H. H. (1989). Laboratory studies on the efficacy and sublethal effects of an insect growth regulator, pyriproxyfen (S-31183) against *Aedes aegypti* (Linnaeus). *Tropical Biomedicine*, (6), 7–12.

Lwetoijera, D.W., Kiware, S.S., Mageni, Z.D., Dongus, S., Harris, C., Devine, G.J., & Majambere, S. (2013). A need for better housing to further reduce indoor malaria transmission in areas with high bed net coverage. *Parasites and Vectors*, 6: 57.

Lwetoijera, D. W., Harris, C., Kiware, S. S., Killeen, G. F., Dongus, S., Devine, G. J., & Majambere, S. (2014a). Comprehensive Sterilization of Malaria Vectors Using Pyriproxyfen: A Step Closer to Malaria Elimination. *The American Journal of Tropical Medicine and Hygiene*, *90*(5), 852–855.

Lwetoijera, D.W., Harris, C., Kiware, S., Dongus, S., Devine, G.J., McCall, P.J., Majambere, S. (2014b). Effective autodissemination of pyriproxyfen to breeding sites by the exophilic malaria vector *Anopheles arabiensis* in semi-field settings in Tanzania. *Malaria Journal*, 13:161.

Magnin, M., Marboutin, E., & Pasteur, N. (1988). Insecticide resistance in *Culex quinquefasciatus* (Diptera: Culicidae) in West Africa. *Journal of Medical Entomology*, *25* (2), 99–104.

Majori, G., Sabatinelli, G., Coluzzi, M. (1987). Efficacy of permethrin-impregnated curtains for malaria vector control. Med Vet Entomol., 1:185–192.

Maloney, K. M., Ancca-Juarez, J., Salazar, R., Borrini-Mayori, K., Niemierko, M., Yukich, J. O., Naquira, C., Keating, J. A., & Levy, M. Z. (2013). Comparison of insecticidal paint and deltamethrin against *Triatoma infestans* (Hemiptera: Reduviidae) feeding and mortality in simulated natural conditions. *Journal of Vector Ecology*, *38*(1), 6–11.

Manson, P. (1900). Experimental proof of the mosquito malaria theory. *British Medical Journal*, 2:949 –51.

Martinez-Torres, D., Chandre, F., Williamson, M. S., Darriet, F., Bergé, J. B., Devonshire, A. L., Guillet, P., Pasteur, N., & Pauron, D. (1998). Molecular characterization of pyrethroid knockdown resistance (*kdr*) in the major malaria vector *Anopheles gambiae* s. s. *Insect Molecular Biology*, *7*(2), 179–184.

Mbare, O., Lindsay, S. W., & Fillinger, U. (2014). Pyriproxyfen for mosquito control: female sterilization or horizontal transfer to oviposition substrates by *Anopheles gambiae* sensu stricto and *Culex quinquefasciatus*. *Parasites & Vectors*, *7*, 280.

McGraw, E. A., & O'Neill, S. L. (2013). Beyond insecticides: new thinking on an ancient problem. *Nature Reviews Microbiology*, *11*, 181 – 193.

MCHIP/USAID/PNLP. (2013). *Rapport sur la mise en œuvre du programme de lutte contre le paludisme au Burkina Faso. MCHIP/USAID 2013*.

Midega, J. T., Mbogo, C. M., Mwnambi, H., Wilson, M. D., Ojwang, G., Mwangangi, J. M., Nzovu, J. G., Githure, J. I., Yan, G., & Beier, J. C. (2007). Estimating dispersal and survival of *Anopheles gambiae* and *Anopheles funestus* along the Kenyan coast by using mark-release-recapture methods. *Journal of Medical Entomology*, *44*(6), 923–929.

Miller, J. E., Lindsay, S. W., & Armstrong, J. R. (1991). Experimental hut trials of bednets impregnated with synthetic pyrethroid or organophosphate

insecticide for mosquito control in The Gambia. *Medical and Veterinary Entomology*, *5*(4), 465–476.

Mosqueira, B., Duchon, S., Chandre, F., Hougard, J.-M., Carnevale, P., & Mas-Coma, S. (2010a). Efficacy of an insecticide paint against insecticide-susceptible and resistant mosquitoes - Part 1: Laboratory evaluation. *Malaria Journal*, *9*(1).

Mosqueira, B., Chabi, J., Chandre, F., Akogbeto, M., Hougard, J.-M., Carnevale, P., & Mas-Coma, S. (2010b). Efficacy of an insecticide paint against malaria vectors and nuisance in West Africa - Part 2: field evaluation. *Malaria Journal*, *9*, 341.

Mosqueira, B., Chabi, J., Chandre, F., Akogbeto, M., Hougard, J.-M., Carnevale, P., & Mas-Coma, S. (2013). Proposed use of spatial mortality assessments as part of the pesticide evaluation scheme for vector control. *Malaria Journal*, 12, 366.

Mosqueira, B., Soma, S.D., Namountougou, M., Poda, S., Diabaté, A., Ali, O., Fournet, F., Baldet, T., Carnevale, P., Dabiré, K.R., Mas-Coma, S. (2015). Pilot study on the combination of an organophosphate-based insecticide paint and pyrethroid-treated long lasting nets against pyrethroid resistant malaria vectors in Burkina Faso. *Acta Tropica*, 148, 162–169.

Mosqueira, B., Poda, S., Namountougou, M., Soma, S.D., Ali, O., Diabaté, A., Fournet, F., Baldet, T., Carnevale, P., Mas-Coma, S. Dabiré, K.R. Testing of the house-proof approach against of *Anopheles coluzzii* resistant to pyrethroids using an organophosphate-based paint applied on windows and doors under real life conditions in Vallee du Kou, Burkina Faso (West Africa). In prep.

Mouchet, J., Carnevale, P., Coosemans, M., Julvez, J., Manguin, S., Richard-Lenoble, D. & Sircoulon, J. (2004). Biodiversité du paludisme dans le monde. Paris: John Libbey Eurotext Limited. Doi: 10.1016/j.medmal.2004.11.001.

Munguambe, K., Pool, R., Montgomery, C., Bavo, C., Nhacolo, A., Fiosse, L., Sacoor, C., Nhalungo, D., Mabunda, S., Macete, E., Alonso, P. (2011). What drives community adherence to indoor residual spraying (IRS) against malaria in Manhiça district, rural Mozambique: a qualitative study. *Malaria Journal*, 10: 344.

Najera, J. A., & Zaim, M. (2001). Malaria vector control. Insecticides for indoor residual spraying. *In World Health Organ. Geneva; 2001. WHO/CDS/WHOPES/2001.3*.

National Center of Tropical Diseases. (2004). *CENETROP. Santa Cruz de la Sierra (Bolivia). 2004 [http://www.cenetrop.org.bo].*

Ngufor, C., N'Guessan, R., Fagbohoun, J., Odjo, A., Malone, D., Akogbeto, M., Rowland, M. (2014a). Olyset Duo(R) (a pyriproxyfen and permethrin mixture net): an experimental hut trial against pyrethroid resistant *Anopheles gambiae* and *Culex quinquefasciatus* in Southern Benin. *PLoS One*, 9:e93603.

Ngufor, C., Tchicaya, E., Koudou, B., N'Fale, S., Dabire, R., Johnson, P., Randon, H., & Rowland, M. (2014b). Combining organophosphate treated wall linings and long-lasting insecticidal nets for improved control of pyrethroid resistant *Anopheles gambiae*. *PLoS ONE* 9(1): e83897. doi:10.1371/journal.pone.0083897.

N'Guessan, R., Darriet, F., Guillet, P., Carnevale, P., Traore-Lamizana, M., Corbel, V., Koffi A. A., & Chandre, F. (2003). Resistance to carbosulfan in *Anopheles gambiae* from Ivory Coast, based on reduced sensitivity of acetylcholinesterase. *Medical and Veterinary Entomology*, *17*(1), 19–25.

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.

N'Guessan, R., Boko, P., Odjo, A., Chabi, J., Akogbeto, M., & Rowland, M. (2010). Control of pyrethroid and DDT-resistant *Anopheles gambiae* by

application of indoor residual spraying or mosquito nets treated with a long-lasting organophosphate insecticide, chlorpyrifos-methyl. *Malaria Journal*, *9*, 44.

Ohashi, K., Nakada, K., Ishiwatari, T., Miyaguchi, J., Shono, Y., Lucas, J. R., & Mito, N. (2012). Efficacy of pyriproxyfen-treated nets in sterilizing and shortening the longevity of *Anopheles gambiae* (Diptera: Culicidae). *Journal of Medical Entomology*, *49*(5), 1052–1058.

Okumu, F. O., Mbeyela, E., Lingamba, G., Moore, J., Ntamatungiro, A. J., Kavishe, D. R., Kenward, M. G., Turner, E., Lorenz, L. M., Moore, S. J. (2013). Comparative field evaluation of combinations of long-lasting insecticide treated nets and indoor residual spraying, relative to either method alone, for malaria prevention in an area where the main vector is *Anopheles arabiensis*. *Parasites & Vectors*, *6*, 46.

Oliveira Filho, A. M. (1996). [Use of new tools for controlling triatomines in different entomologic situations in the American continent]. *Revista Da Sociedade Brasileira De Medicina Tropical*, *30*(1), 41–46.

Oxborough, R. M., Kitau, J., Jones, R., Feston, E., Matowo, J., Mosha, F. W., & Rowland, M. W. (2014). Long-lasting control of *Anopheles arabiensis* by a single spray application of micro-encapsulated pirimiphos-methyl (Actellic® 300 CS). *Malaria Journal*, *13*, 37.

Perlatti, B., De Souza Bergo, P.L., Das Graças Fernandes da Silva, M.F., Batista Fernandes, J., & Rossi Forim, M. (2013). Polymeric Nanoparticle-Based Insecticides: A Controlled Release Purpose for Agrochemicals. Agricultural and Biological Sciences:"Insecticides - Development of Safer and More Effective Technologies" ISBN 978-953-51-0958-7.

Ranson, H., Jensen, B., Vulule, J. M., Wang, X., Hemingway, J., & Collins, F. H. (2000). Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan *Anopheles gambiae* associated with resistance to DDT and pyrethroids. *Insect Molecular Biology*, *9*(5), 491–497.

Ranson, H., N'guessan, R., Lines, J., Moiroux, N., Nkuni, Z., & Corbel, V. (2011). Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? *Trends in Parasitology*, *27*(2), 91–98.

Raymond, M. & Rousset, F. (1996). GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, *86*: 248-9.

Robert, V., Gazin, P., Boudin, C., Molez, J.F., Ouedraogo, V., & Carnevale, P. (1985). The transmission of malaria in a wooded savannah area and a rice-growing area around Bobo Dioulasso (Burkina Faso). *Annales de la Societe belge de medecine tropicale*, 65 (2), 201-14.

Robert, V. (1989). La transmission du paludisme humain: la zone des savanes d'Afrique de l'Ouest. *PhD Thesis*, *Université de Paris 6*, *1989 ; 325 p.*

Robert, V., & Carnevale, P. (1991). Influence of deltamethrin treatment of bed nets on malaria transmission in the Kou valley, Burkina Faso. *Bulletin of the World Health Organization*, 69(6), 735-40.

Rodríguez, M. M., Bisset, J., de Fernandez, D. M., Lauzán, L., & Soca, A. (2001). Detection of insecticide resistance in *Aedes aegypti* (Diptera: Culicidae) from Cuba and Venezuela. *Journal of Medical Entomology*, *38*(5), 623–628.

Rogier, C., Henry, M. C., Rowland, M., Carnevale, P., Chandre, F., Corbel, V., Curtis, C., Hougard, J.-M., & WHOPES, WHO Pesticide Evaluation Scheme. (2009). [Guidelines for phase III evaluation of vector control methods against malaria]. *Médecine Tropicale*, *69*(2), 173–184.

Rowland, M., Boko, P., Odjo, A., Asidi, A., Akogbeto, M., (2013). A new long-lasting indoor residual formulation of the organophosphate insecticide Pirimiphos Methyl for prolonged control of pyrethroid-resistant mosquitoes: An experimental hut trial in Benin. *PLoS ONE*, 8(7): e69516.

Rozendaal, J. A. (1997). *Vector control. Methods for use by individuals and communities.* Geneva: World Health Organization.

Rush, T., Liu, X.Q., Hjelmhaug, J., Lobner, D. (2010). Mechanisms of chlorpyrifos and diazinon induced neurotoxicity in cortical culture. Neuroscience, 166:899–906.

Samuelsen, H., Toé, L. P., Baldet, T., & Skovmand, O. (2004). Prevention of mosquito nuisance among urban populations in Burkina Faso. *Social Science & Medicine*, *59*(11), 2361–2371.

Santolamazza, F., Mancini, E., Simard, F., Qi, Y., Tu, Z., & della Torre, A. (2008). Insertion polymorphisms of SINE200 retrotransposons within speciation islands of *Anopheles gambiae* molecular forms. *Malaria Journal*, *7*, 163.

Service, M. W., & Townson, H. (2002). *The Anopheles vector.* In: Gilles HM, Warrell DA, editors. Essential Malariology. Fourth edition ed. London: Arnold.

Sharp, B. L., Ridl, F. C., Govender, D., Kuklinski, J., & Kleinschmidt, I. (2007). Malaria vector control by indoor residual insecticide spraying on the tropical island of Bioko, Equatorial Guinea. *Malaria Journal*, *6*(1), 52.

Sihuincha, M., Zamora-Perea, E., Orellana-Rios, W., Stancil, J. D., López-Sifuentes, V., Vidal-Oré, C., & Devine, G. J. (2005). Potential use of pyriproxyfen for control of *Aedes aegypti* (Diptera: Culicidae) in Iquitos, Perú. *Journal of Medical Entomology*, *42*(4), 620–630.

Simon, J. L., Larson, B. A., Zusman, A., & Rosen, S. (2002). How will the reduction of tariffs and taxes on insecticide- treated bednets affect household purchases? *Bulletin of the World Health Organization*, *80*(11), 892–899.

Sinka, M. E., Bangs, M. J., Manguin, S., Coetzee, M., Mbogo, C. M., Hemingway, J., Patil, A. P., Temperley, W. H., Gething, P. W., Kabaria, C. W., Okara, R. M., Van Boeckel, T., Godfray, H. C. J., Harbach, R. E., & Hay, S. I. (2010). The dominant *Anopheles* vectors of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and bionomic précis. *Parasites & Vectors*, *3*, 117.

Sinka, M. E., Bangs, M. J., Manguin, S., Rubio-Palis, Y., Chareonviriyaphap, T., Coetzee, M., Mbogo, C. M., Hemingway, J., Patil, A. P., Temperley, W. H., Gething, P. W., Kabaria, C. W., Burkot, T. M., Harbach, R. E., & Hay, S. I. (2012). A global map of dominant malaria vectors. *Parasites & Vectors*, *5*, 69.

Snow, R. W., McCabe, E., Mbogo, C. N., Molyneux, C. S., Some, E. S., Mung'ala, V. O., & Nevill, C. G. (1999). The effect of delivery mechanisms on the uptake of bed net re-impregnation in Kilifi District, Kenya. *Health Policy and Planning*, *14*(1), 18–25.

Spanish Ministry of Health and Consumer Affairs. (1996). *Report on the study of the toxicity and irritability of Inesfly 5A. Health Institute Carlos III Madrid.*

Takken. W., & Verhulst, N.O. (2013). Host preferences of blood-feeding mosquitoes. *Annual Review of Entomology*, 58, 433-453.

Teklehaimanot, A., Sachs, J. D., & Curtis, C. (2007). Malaria control needs mass distribution of insecticidal bed nets. *Lancet*, *369*, 2143 – 2146.

The malERA Consultative Group on Vector Control. (2011). A Research Agenda for Malaria Eradication: Vector Control. *PLoS Medicine*, *8*(1), e1000401.

Thomson, M., Connor, S., Bennett, S., D'Alessandro, U., Milligan, P., Aikins, M., Langerock, P., Jawara, M., & Greenwood, B. (1996). Geographical perspectives on bednet use and malaria transmission in The Gambia, West Africa. *Social Science & Medicine*, *43*(1), 101–112.

Toé, L. P., Skovmand, O., Dabiré, K. R., Diabaté, A., Diallo, Y., Guiguemdé, T. R., Doannio, J. M. C., Akogbeto, M., Baldet, T., & Gruénais, M.-E. (2009). Decreased motivation in the use of insecticide-treated nets in a malaria endemic area in Burkina Faso. *Malaria Journal*, *8*, 175.

Toé, K. H., Jones, C. M., N'Fale, S., Ismail, H. M., Dabiré, R. K., & Ranson, H. (2014). Increased Pyrethroid Resistance in Malaria Vectors and Decreased Bed Net Effectiveness, Burkina Faso. *Emerging Infectious Diseases*, *20*(10).

Tusting, L.S., Ippolito, M.M., Willey, B.A., Kleinschmidt, I., Dorsey, G., Gosling, R.D., & Lindsay S.W. (2015). The evidence for improving housing to reduce malaria: a systematic review and meta-analysis. *Malaria Journal*, 14, 209.

Van Bortel, W., Barutwanayo, M., Delacollette, C., & Coosemans, M. (1996). [Motivation to acquire and use impregnated mosquito nets in a stable malaria zone in Burundi]. *Tropical medicine & international health*, *1*(1), 71–80.

Vaughan, A., Chadee, D. D., & French-Constant, R. (1998). Biochemical monitoring of organophosphorus and carbamate insecticide resistance in *Aedes aegypti* mosquitoes from Trinidad. *Medical and Veterinary Entomology*, *12*(3), 318–321.

Waugh, S. (2015). Apropos: *Plasmodium knowlesi* malaria an emerging public health problem in Hulu Selangor, Selangor, Malaysia (2009-2013): epidemiologic and entomologic analysis. *Parasites & Vectors*, *8*(1), 79.

Weill, M., Lutfalla, G., Mogensen, K., Chandre, F., Berthomieu, A., Berticat, C., Pasteur, N., Philips, A., Fort, P., & Raymond, M. (2003). Comparative genomics: Insecticide resistance in mosquito vectors. *Nature*, *423*(6936), 136–137.

Weill, M., Malcolm, C., Chandre, F., Mogensen, K., Berthomieu, A., Marquine, M., & Raymond, M. (2004). The unique mutation in ace-1 giving high insecticide resistance is easily detectable in mosquito vectors. *Insect Molecular Biology*, *13*(1), 1–7.

West, P.A., Protopopoff, N., Wright, A., Kivaju, Z., Tigererwa, R., Mosha, F.W., Kisinza, W., Rowland, M., & Kleinschmidt, I. (2014). Indoor residual spraying in combination with insecticide-treated nets compared to insecticide-treated nets alone for protection against malaria: a cluster randomised trial in Tanzania. *PLoS Med*, 15, 11(4):e1001630.

White, M. T., Griffin, J. T., Churcher, T. S., Ferguson, N. M., Basáñez, M.-G., & Ghani, A. C. (2011). Modelling the impact of vector control interventions on *Anopheles gambiae* population dynamics. *Parasites & Vectors*, *4*, 153.

WHO. (1996). Evaluation and testing of insecticides. *World Health Organization*, *Geneva; Report of the WHO Informal Consultation WHO/HQ*, *Geneva*, *7-11 October 1996. Document CTD/WHOPES/IC/96.1*.

WHO. (1998). Test Procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces. *World Health Organization*, *Geneva; 1-43. Report of the WHO Informal Consultation. Document WHO/CDS/CPC/MAL/1998.12*.

WHO. (2004). Report of the Seventh WHOPES Working Group Meeting. *WHO/CDS/WHOPES/2004.8*, *World Health Organization*, *Geneva*.

WHO. (2006). Indoor residual spraying: use of indoor residual spraying for scaling up global malaria control and elimination. World Health Organization, Geneva.

WHO. (2011). The Technical Basis for Coordinated Action against Insecticide Resistance: Preserving the Effectiveness of Modern Malaria Vector Control. *World Health Organization 2011. http://whqlibdoc.who.int/publications/2011/9789241501095_eng.pdf*.

WHO. (2012). Global plan for insecticide resistance management in malaria vectors. *Geneva*, *World Health Organization*, *2012 (http:// www.who.int/malaria/publications/atoz/gpirm/en/index.html*.

WHO. (2013). World Malaria Report 2013. Geneva: World Health Organization 2013. *http://www.who.int/malaria/publications/world_malaria_report_2013/report/en/.*

WHO. (2014). World Malaria Report 2013. Geneva: World Health Organization 2014. *http://www.who.int/malaria/publications/world_malaria_report_2014/report/en/.*

WHO. (2015). Quality Control. WHO specifications for public health pesticides 2015. http://www.who.int/whopes/quality/en/.

Winch, P. J., Makemba, A. M., Kamazima, S. R., Lwihula, G. K., Lubega, P., Minjas, J. N., & Shiff, C. J. (1994). Seasonal variation in the perceived risk of malaria: implications for the promotion of insecticide-impregnated bed nets. *Social Science & Medicine*, *39*(1), 63–75.

Zaim, M., Aitio, A., & Nakashima, N. (2000). Safety of pyrethroid-treated mosquito nets. *Medical and Veterinary Entomology*, *14*, 1 – 5.

Zhu, L., Qualls, W. A., Marshall, J. M., Arheart, K. L., DeAngelis, D. L., McManus, J. W., Traore, S. K., Doumbia, S., Schlein, Y., Muller, G. C., & Beier, J. C. (2015). A spatial individual-based model predicting a great impact of copious sugar sources and resting sites on survival of *Anopheles gambiae* and malaria parasite transmission. *Malaria Journal*, *14*(1), 59.

Zollner, C., De Allegri, M., Louis, V.R., Ye, M., Sie, A., Tiendrebeogo, J., Jahn, A. & Muller, O. (2015). Insecticide-treated mosquito nets in rural Burkina Faso: assessment of coverage and equity in the wake of a universal distribution campaign. *Health Policy and Planning*, 30, 171–180.

ANNEX: PUBLICATIONS

MALARIA JOURNAL

RESEARCH Open Access

Efficacy of an insecticide paint against insecticide-susceptible and resistant mosquitoes - Part 1: Laboratory evaluation

Beatriz Mosqueira[1*], Stéphane Duchon[2], Fabrice Chandre[2], Jean-Marc Hougard[2], Pierre Carnevale[2], Santiago Mas-Coma[1]

Abstract

Background: The main malaria vector *Anopheles gambiae* and the urban pest nuisance *Culex quinquefasciatus* are increasingly resistant to pyrethroids in many African countries. There is a need for new products and strategies. Insecticide paint Inesfly 5A IGR™, containing two organophosphates (OPs), chlorpyrifos and diazinon, and insect growth regulator (IGR), pyriproxyfen, was tested under laboratory conditions for 12 months following WHOPES Phase I procedures.

Methods: Mosquitoes used were laboratory strains of *Cx. quinquefasciatus* susceptible and resistant to OPs. The paint was applied at two different doses (1 kg/6 m^2 and 1 kg/12 m^2) on different commonly used surfaces: porous (cement and stucco) and non-porous (softwood and hard plastic). Insecticide efficacy was studied in terms of delayed mortality using 30-minute WHO bioassay cones. IGR efficacy on fecundity, fertility and larval development was studied on OP-resistant females exposed for 30 minutes to cement treated and control surfaces.

Results: After treatment, delayed mortality was high (87-100%) even against OP-resistant females on all surfaces except cement treated at 1 kg/12 m^2. Remarkably, one year after treatment delayed mortality was 93-100% against OP-resistant females on non-porous surfaces at both doses. On cement, death rates were low 12 months after treatment regardless of the dose and the resistance status. Fecundity, fertility and adult emergence were reduced after treatment even at the lower dose ($p < 10^{-3}$). A reduction in fecundity was still observed nine months after treatment at both doses ($p < 10^{-3}$) and adult emergence was reduced at the higher dose ($p < 10^{-3}$).

Conclusions: High mortality rates were observed against laboratory strains of the pest mosquito *Cx. quinquefasciatus* susceptible and resistant to insecticides. Long-term killing remained equally important on non-porous surfaces regardless the resistance status for over 12 months. The paint's effect on fecundity, fertility and adult emergence may continue to provide an additional angle of attack in reducing overall population densities when the lethal effect of OPs diminishes over time. Some options on how to deal with porous materials are given. Implications in vector control are discussed.

Background

Every year, 300-500 million clinical episodes of malaria occur, resulting in about one million deaths [1]. A vast majority of these deaths involve children less than 5 years old in sub-Saharan Africa [2,3]. The fighting of malaria in sub-Saharan Africa is mainly focused on vector control through the use of insecticide-treated nets (ITNs) and indoor residual insecticide spraying (IRS) [4,5]. At present, pyrethroids are the only insecticides recommended for treatment of mosquito nets [6]. Despite the great value of pyrethroid-treated nets in malaria vector control, their efficacy may be threatened by resistance of major malaria vectors to this class of insecticides [7]. IRS is the main method of attacking adult mosquitoes in houses, but the technique requires

* Correspondence: bmosqueira@yahoo.com
[1]Departamento de Parasitología, Facultad de Farmacia, Universidad de Valencia, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain
Full list of author information is available at the end of the article

teams of trained personnel and special equipment to be transported to where they are needed [8].

A present recommendation towards resistance management is alternating or using in combination different insecticides or novel strategies in the framework of an integrated vector management [9] while respecting the Stockholm Convention on Persistent Organic Pollutants (POPs) of finding sustainable alternatives to POPs in integrated pest management practices where possible [10].

Insecticide paint Inesfly 5A IGR™ is composed of two organophosphates (OPs), chlorpyriphos (1.5%) and diazinon (1.5%) and an insect growth regulator (IGR), pyriproxyfen (0,063%). The product is a white vinyl paint with an aqueous base. Active ingredients reside within Ca CO^3 + resin microcapsules. The formulation allows a gradual release of active ingredients, increasing its durability. Microcapsules range from one to several hundred micrometers in size. Toxicology studies performed so far support the paint's safety in terms of irritancy (ocular, dermal and systemic), cytotoxicity and mutagenicity [11] and allergenicity [12]. Acute inhalation toxicity studies classified this paint as Category III (according to WHO) and category IV (according to EPA) - no warning label required in either case [12]. Analysis on cholinesterase levels showed no variations before/after treatment. Values were within reference values for all subjects [13].

The efficacy of Inesfly 5A IGR™ was studied under laboratory conditions for over 12 months at the *Laboratoire de Lutte contre les Insectes Nuisibles/Institut de Recherche pour le Développement* (LIN/IRD), the WHO reference laboratory for insecticide testing, in Montpellier, France. These highly-controlled evaluations against mosquitoes specifically resistant to the paint's insecticides were triggered by the encouraging results obtained during preliminary testing in malaria-endemic areas in Benin and Côte d'Ivoire, West Africa, against local populations of *Anopheles gambiae* the main malaria vector in sub-Saharan Africa. Resistance to OPs has been described in vector and pest mosquitoes in various parts of the world, including West Africa [14,15]. The paint's efficacy was tested against laboratory strains of the urban pest *Culex quinquefasciatus* susceptible and resistant to OPs. At the time of the study, there was no laboratory strain of *An. gambiae* specifically resistant to OPs. Efficacy was studied in two ways: delayed mortality and effect of the IGR on fecundity, fertility and larval development.

Methods

Delayed mortality

30 minute-WHO bioassay cones [16] were performed against two laboratory strains of *Cx. quinquefasciatus*: *Cx. quinquefasciatus* S-Lab is an insecticide susceptible reference strain [17]. *Culex.quinquefasciatus* SR is homozygote for the ace-1^R resistant gene involved in the resistance to OPs and carbamates, but has the same genetic background as S-Lab [18]. Unfed, 3-5 day old females bred at the LIN insectarium were placed in forced contact with four different surfaces: softwood and hard plastic (non-porous materials) and ready-mixed cement and ready-mixed stucco (porous materials). There were two treated and two controls for each kind of surface. Treated surfaces were painted at two doses, 1 kg/6 m^2 (manufacturer's recommended dose to leave surfaces completely white) and 1 kg/12 m^2. For each kind of surface, one control was left untreated and the other one was painted at 1 kg/6 m^2 with the same paint but without the insecticides and the IGR. Paint was applied undiluted with a regular brush and left to dry for 48 hours. After a 30-minute exposure, mosquitoes were introduced in 150-ml plastic cups provided with honey-juice. Tests were done in four repeats using 15 females per cone. Females were left at a temperature of 27 ± 1°C and a relative humidity of 80%, for 24-hour delayed mortality assessments. Tests were done at intervals of six months for one year. When not tested, surfaces were stored in aluminium foil at a temperature of 27 ± 1°C and a relative humidity of 80%. Delayed mortality was analysed using Epi-Info 6. Where values were <5, Fisher exact tests were used. Because bioassay tests are subject to variations, a 99% confidence interval was applied.

IGR efficacy on fecundity, fertility and larval development

Females were 4-5 day-old to increase the probability of having had females fertilised by male mosquitoes. LIN-reared *Cx. quinquefasciatus* OP-resistant females were exposed to treated and control surfaces for 30 minutes. Females alive 24 hours after a 30-minute exposition, were put in cages and allowed to blood-feed for one night. Females that had been well blood-fed were put in a new cage and given honey-juice every two days. At T0, 50 blood fed females were tested per surface. At T9, 30 blood-fed females were tested per surface. At T0 and T9, blood feeding took place about 36 hours after previous exposition to treated or control surfaces. Efficacy was measured in terms of fecundity (number of eggs laid), fertility (% hatching) and larval development (% pupation and % emergence). Tests were not done using susceptible *Cx. quinquefasciatus* S-Lab, because they all died during the 30-minute exposition. Tests were carried out on the most porous surface, cement, because not enough females survived exposition to other surfaces. Eggs were counted with a dissecting microscope and placed in plastic measuring containers with 2L of water for hatching. Water loss due to evaporation was replaced daily. Larvae were fed every two days.

The mean number of eggs was compared between treated and non-treated surfaces using a student T test. Differences in % hatching, % pupation, and % emergence were analysed using Epi-Info 6. Where values were <5, Fisher exact tests were used.

Results

Delayed mortality

Delayed 24-hour mortality at T0 was 98-100% (compared to control, $p < 10^{-3}$) for both, susceptible S-Lab and OP-resistant *Cx. quinquefasciatus* on non-porous surfaces and porous surfaces treated at 1 kg/6 m^2. While non-porous surfaces performed equally well regardless the dose and the resistance status, porous surfaces, cement and stucco, treated at the lower dose 1 kg/12 m^2 performed less optimally against OP-resistant mosquitoes yielding mortalities of 87% ($p < 10^{-3}$) and 15% ($p < 10^{-2}$) respectively. Efficacy had dropped by six months on cement surfaces treated at both doses on resistant and susceptible mosquitoes while, on stucco, only OP-resistant *Cx. quinquefasciatus* experienced a drop. Mortality at 24 hours was of 90-100% (compared to control, $p < 10^{-3}$) 12 months after treatment even against resistant mosquitoes at the lower dose on all but porous surfaces (Table 1).

IGR efficacy on fecundity, fertility and larval development

A significant reduction in the number of eggs laid was shown at 0 and 9 months after treatment at either dose ($p < 10^{-3}$) (Tables 2 and 3). A reduction in egg hatching was observed at T0 ($p < 10^{-3}$), but not at T9. An increased mortality from the nymph to the adult stage was shown 0 months after treatment at the lower dose ($p < 10^{-3}$), and 9 months after treatment only at the higher dose ($p < 10^{-3}$) No differences were found on the duration of the larval development cycle. No IGR effect was observed 12 months after treatment.

Discussion

After treatment with insecticide paint Inesfly 5A IGR™, 100% of OP-susceptible females died after 24-hours on all surfaces, porous and non-porous at both doses, 1 kg/6 m^2 and 1 kg/12 m^2. Killing was significant (87-100%) even against OP-resistant females on all surfaces except cement treated at the lower dose, 1 kg/12 m^2.

One year after initial treatment, mortality rates were still quite high, 93-100%, on non-porous surfaces (softwood and hard plastic) at both doses and against both, OP-resistant and susceptible females. On the other hand, the lethal effect on porous surfaces like cement had disappeared by six months after treatment against resistant and susceptible mosquitoes.

Long-term efficacy was an issue of porosity of materials rather than the pH of materials or the dose applied: active principles are kept in an acid pH within its microcapsule, making it more resistant to alkalinity than other conventional paints. To study whether efficacy hinged more on porosity than dose, a parallel study was performed. Cement-made surfaces painted with a control layer and an insecticide paint layer at 1 kg/6 m^2, performed as well as two insecticide paint layers at 1 kg/6 m^2, even though the latter had twice the dose (Mosqueira *et al.*, unpublished data). Hence, the first layer acted as a screen (even if it did not have insecticide) that allowed the bioavailability of the insecticide on the second layer. Porosity is also an issue for IRS. DDT may last for six months on cement surfaces, though it usually leaves walls stained [19].

A Phase II field study on this same paint, Inesfly 5A IGR™, was performed in Benin, West Africa for one year against local *An. gambiae* and *Cx. quinquefasciatus* populations resistant to pyrethroids. Experimental houses were built with locally-made cement. Long-term efficacy tests included 30 minute-WHO bioassay cones using the insecticide susceptible reference strain *Cx.*

Table 1 Delayed 24-hour mortality rates of susceptible *Cx. quinquefasciatus* S-Lab and OP-resistant *Cx. quinquefasciatus* after a 30-minute exposure to control and Inesfly(r) treated surfaces using WHO bioassay cones.

Culex % Delayed mortality 24 h (N = 60)	Control 1 No paint	Control 2 Control Paint	Cement 1 Kg/6 m2	Cement 1 Kg/12 m2	Stucco 1 Kg/6 m2	Stucco 1 Kg/12 m2	Softwood 1 Kg/6 m2	Softwood 1 Kg/12 m2	Plastic 1 Kg/6 m2	Plastic 1 Kg/12 m2
			Porous Surfaces				Non-Porous Surfaces			
T0 OP-Susceptible	0.5	0.4	100†	100†	100†	100†	100†	100†	100†	100†
T0 OP-Resistant	**2**	**2.2**	**100†**	**15.7†**	**100†**	**87.3†**	**100†**	**100†**	**100†**	**100†**
T6 OP-Susceptible	2.2	2.9	3.1	1.7	100†	96.7†	100†	100†	100†	100†
T6 OP-Resistant	**1.6**	**3.3**	**0**	**0**	**31.7†**	**3.3**	**100†**	**100†**	**100†**	**100†**
T12 OP-Susceptible	0	2.1	2	0	91.4†	20.3†	100†	100†	100†	100†
T12 OP-Resistant	**1.5**	**1**	**4.1**	**5.3**	**20.3†**	**5.3**	**100†**	**93.2†**	**100†**	**100†**

Control 1 = No paint; Control 2 = Paint without insecticide at 1 Kg/6 m^2. Culex = *Cx. quinquefasciatus*; T0 = 0 months after treatment, T6 = 6 months after treatment, T12 = 12 months after treatment, N = sample size per surface tested; (-) females had already died during the first hour. † = significant differences from control ($P < 0.05$).

Table 2 IGR effect on fecundity, fertility and larval development of females exposed to treated surfaces for 30 minutes

T0 - Cement (N = 50) OP-resistant *Culex*	Egg number	% Egg-hatching	% Pupation	% Emergence
C1/NO Paint	2104	51.8	39.6	79.5
C2/Paint NO insecticide	2473	48.8	40.0	85.9
Insecticide at 1 Kg/6 m^2	No survivors			
Insecticide at 1 Kg/12 m^2	800†	41.3†	45.5	52.7†

Control 1 = No paint; Control 2 = Paint without insecticide at 1 Kg/6 m^2; Culex = *Cx. quinquefasciatus*; T0 = 0 months after treatment; N = sample size per surface tested. † = significant differences from control ($P < 0.05$).

quinquefasciatus S-Lab. Six months after treatment, mortality rates in the Phase II study on cement-made surfaces treated with one layer at 1 Kg/6 m^2 were still very high, 98-100% [20] compared to the 3% observed in the Phase I study. The difference observed in the long-term efficacy may be due to the type of cement used in Phase I and II, ready-mixed cement *versus* traditionally made cement, respectively. The greater the proportion of water to cement, the more porous the hardened cement will be. To test this hypothesis, Phase I surfaces with locally made cement were made in Benin. Surfaces were kept away from light when not tested. Temperature and humidity were the same to Phase II experimental houses. Mortality rates were lower on the Phase I Benin surfaces but differences were not significant compared to Phase II cement houses (Mosqueira *et al*., unpublished data) as opposed to the mortality rates obtained on Phase I mixed-cement surfaces.

Another recent study has tested the efficacy and the residual effect of Inesfly 5A IGR™ insecticide paint against the main vector of Chagas disease in South America, *Triatoma infestans*, on different surfaces (wood, cement block and adobe bricks). Insecticide paint yielded longer and higher mortality rates in triatomines than other conventional products [21], and porosity also seemed to be an issue - cement surfaces performed worse than wood and even adobe-made surfaces. Insecticide paints have been used for some time concomitantly with home improvement as a control method for Chagas disease with good results [22,23].

Pyriproxyfen is toxic to a broad spectrum of insects during their developmental stages. Research on the dengue vector, *Aedes aegypti*, shows that contaminated adults can render oviposition sites unproductive by horizontal dissemination of pyriproxyfen even at small concentrations [24-26]. A study performed by Itoh *et al* [24] showed pyriproxyfen had a larger impact on fecundity when females were exposed to pyriproxyfen before blood feeding. Inversely, pyriproxyfen's effect on egg-hatching [24,27,28] and adult emergence [24,28] seems to be higher when females have blood fed before being exposed to treatment.

In the present study, the effect of pyriproxyfen was studied on OP-resistant *Cx quinquefasciatus* females that survived a 30-minute exposition to cement-treated surfaces. Cement surfaces were chosen because, being the most porous, they were the only ones that left enough females alive to follow their offspring. Females were exposed to treated surfaces about 36 hours before blood feeding, a timing that would favour a reduction in fecundity over fertility and adult emergence. This is in fact the observation made. For the first nine months, a reduction in fecundity was observed at both doses. A reduction in adult emergence was observed also for nine months but only at the higher dose. An effect on fertility was only observed after treatment and not after nine months. In a recent Phase I evaluation on adult *Anopheles stephensi* females exposed to pyriproxyfen 2% one day after blood feeding, results were the opposite. A reduction in fertility in treatment groups compared to control, whereas fecundity was also reduced but differences failed to be significant [29]. The potential application of horizontal dissemination in malaria vector control needs to be studied [30]. Could the pyriproxyfen that was picked up by females that have survived a

Table 3 IGR effect on fecundity, fertility and larval development of females exposed to treated surfaces for 30 minutes

T9 - Cement (N = 30) OP-resistant *Culex*	Egg number	% Egg-hatching	% Pupation	% Emergence
C1/NO Paint	1908	75.8	56.3	87.8
C2/Paint NO Insecticide	2002	73.1	60.0	84.4
Insecticide at 1 Kg/6 m^2	1216†	77.5	64.6	65.9†
Insecticide at 1 Kg/12 m^2	1156†	70.9	59.9	86.6

Control 1 = No paint; Control 2 = Paint without insecticide at 1 Kg/6 m^2; Culex = *Cx. quinquefasciatus*; T9 = 9 months after treatment; N = sample size per surface tested. † = significant differences from control ($P < 0.05$)

prolonged contact with painted walls be then transferred to the oviposition sites of malaria vectors? A project is in progress on different surfaces and blood feeding timing.

Results on non-porous surfaces are satisfying. There is a need to look for ways to deal with the porosity of surfaces like cement. Possible options may include two layers of paint, as discussed above, applying a coating resin, or even natural oil sealers first. The way surfaces are made also makes a difference: cement surfaces can be made less porous depending on the proportion of substances used. Hardwood is more porous than softwood. What would seem clear is that solutions need to be "user-friendly" and appealing in keeping with one of the paint's operational advantages.

There may be a reason to be optimistic about the potential that the insecticide paint may have as an additional tool in malaria and pest control: 1) High long-term killing rates against OP-resistant mosquitoes, 2) IGR's effect on fecundity, fertility and adult emergence and, 3) operational advantages: users can apply the paint themselves and take responsibility for their home improvement.

Conclusions

Laboratory assays against OP-resistant *Cx. quinquefasciatus* point at the paint's potential in attaining high mortality rates for up to 12 months despite resistance status. Ways to deal with the porosity of certain materials need to be explored. Pyriproxyfen's effect on the fecundity, fertility and adult emergence of exposed adult females affords an added tool in reducing overall pest and malaria vector population densities when the lethal effect of OPs diminishes over time. The paint is easily applied and improves communities' homes. A semi-field study performed following WHOPES Phase II procedures in Benin, West Africa against local populations of pyrethroid-resistant *An. gambiae* and *Cx. quinquefasciatus* populations has confirmed the product's promising profile. Future goals include performing a large-scale entomological, epidemiological and community acceptability study in West Africa.

Acknowledgements

This study was funded by Project Number 07-CAP3-1513 of the *Agencia Española de Cooperación Internacional para el Desarrollo* (AECID), Ministry of Foreign Affaires, Madrid, Spain, and by the *Red de Investigación de Centros de Enfermedades Tropicales* (RICET), Project Number RD06/0021/0017 "*Redes Temáticas de Investigación Cooperativa*", *Fondo de Investigación Sanitaria* (FIS), Ministry of Health, Madrid, Spain. We thank the University of Valencia and the IRD for their support, particularly Luc Finot for his help on some of the evaluations. We thank Dr. Thierry Baldet for his advice on the manuscript.

Author details

[1]Departamento de Parasitología, Facultad de Farmacia, Universidad de Valencia, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain. [2]UR016, Institut de Recherche pour le Développement (IRD), BP 64501, 34394 Montpellier Cedex 5, France.

Authors' contributions

PC and SMC conceived the protocol. PC, SMC, SD and BM designed the study. SD, FC and JMH critically contributed to the implementation of the study. BM conducted evaluations. The manuscript has been drafted by BM and has been revised by SMC and SD. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 1 October 2010 Accepted: 25 November 2010
Published: 25 November 2010

References

1. WHO: **The world malaria report 2005.** *World Health Organization* Geneva; 2005 [http://rbm.who.int/wmr2005/].
2. Breman JG, Egan A, Keusch GT: **The intolerable burden of malaria: a new look at numbers.** *Am J Trop Med Hyg* 2001, **64(1-2)**:iv-vii.
3. Snow RW, Craig MH, Newton C, Steketee RW: **The public health burden of *Plasmodium falciparum* malaria in Africa: deriving the numbers. Disease Control Priorities Project Working Paper No 11.** *Fogarty International Center, National Institutes of Health* Bethesda, Maryland; 2003.
4. Lengeler C, Sharp B: **Indoor residual spraying and insecticide-treated nets. In: Reducing malaria's burden: evidence of effectiveness for decision makers.** *Global Health Council* Washington D.C; 2003, 17-24.
5. Lengeler C: **Insecticide-treated bed nets and curtains for preventing malaria.** *Cochrane Database of Syst Rev* 2008 [http://www.cochrane.org].
6. Zaim M, Aitio A, Nakashima N: **Safety of pyrethroid-treated mosquito nets.** *Med Vet Entomol* 2000, **14**:1-5.
7. Baleta A: **Insecticide resistance threatens malaria control in Africa.** *Lancet* 2009, **374**:1581-1582.
8. Najera JA, Zaim M: **Malaria Vector Control. Insecticides for Indoor Residual Spraying. WHO/CDS/WHOPES/2001.3.** *World Health Organization* Geneva; 2001.
9. Beier JC, Keating J, Githure JI, Macdonald MB, Impoinvil DE, Novak RJ: **Integrated vector management for malaria control.** *Malar J* 2008, **7(Suppl 1)**:S4.
10. United Nations Environment Programme: **Stockholm Convention on Persistant Organic Pollutants (POPs).** *UNEP/Convention/The POPs* 2010.
11. Spanish Ministry of Health and Consumer Affairs: **Report on the study of the toxicity and irritability of Inesfly 5A.** *Health Institute Carlos III* Madrid; 1996.
12. International center of training and medical investigations (CIDEIM): **Toxicity studies on Inesfly 5A IGR.** CIDEIM, Cali (Colombia); 2003 [http://www.cideim.org.co].
13. **National Center of Tropical Diseases (CENETROP), Santa Cruz de la Sierra (Bolivia).** 2004 [http://www.cenetrop.org.bo].
14. Chandre F, Darriet F, Doannio JMC, Riviere F, Pasteur N, Guillet P: **Distribution of organophosphate and carbamate resistance in *Culex pipiens quinquefasciatus* (Diptera: Culicidae) from West Africa.** *J Med Entomol* 1997, **34**:664-671.
15. N'Guessan R, Darriet F, Guillet P, Carnevale P, Lamizana MT, Corbel V, Koffi AA, Chandre F: **Resistance to carbosulfan in field populations of *Anopheles gambiae* from Côte d'Ivoire based on reduced sensitivity of acetylcholinesterase.** *Med Vet Entomol* 2003, **17**:19-25.
16. WHO: **Test Procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces. Report of the WHO Informal Consultation. Document WHO/CDS/CPC/MAL/1998.12.** *World Health Organization* Geneva; 1998, 1-43.
17. Georghiou GP, Metcalf RL, Gidden FE: **Carbamate-resistance in mosquitoes: selection of *Culex pipiens fatigans* Wied (= *Culex quinquefasciatus*) for resistance to Baygon.** *Bull World Health Organ* 1966, **35**:691-708.
18. Berticat C, Boquien G, Raymond M, Chevillon C: **Insecticide resistance genes induce a mating competition cost in *Culex pipiens* mosquitoes.** *Genetics Research* 2002, **79**:41-47.

19. Najera JA, Zaim M: **Malaria Vector Control. Insecticides for Indoor Residual Spraying. (WHO/CDS/WHOPES/2001.3).** *World Health Organization* Geneva; 2001.
20. Mosqueira B, Chabi J, Chandre F, Akogbeto M, Hougard JM, Carnevale P, Mas-Coma S: **Efficacy of an insecticide paint against malaria vectors and nuisance in West Africa. Part II: Field evaluations.** *Malar J* 2010.
21. Amelotti I, Catalá SS, Gorla DE: **Experimental evaluation of insecticidal paints against *Triatoma infestans* (Hemiptera: Reduviidae), under natural climatic conditions.** *Parasites & Vectors* 2009, **2**:30-36.
22. Rozendaal JA: *Vector control methods for use by individuals and communities* Geneva: World Health Organization; 1997.
23. Oliveira Filho AM: **Use of new tools for controlling triatomines in different entomologic situations in the American continent.** *Rev Soc Bras Méd Trop* 1996, **30**:41-46.
24. Itoh T, Kawada H, Abe A, Eshita Y, Rongsriyam Y, Igarashi A: **Utilization of bloodfed females of *Aedes aegypti* as a vehicle for the transfer of the insect growth regulator pyriproxyfen to larval habitats.** *J Am Mosq Control Assoc* 1994, **10**:344-347.
25. Morrison AC, Zielinski-Gutierrez E, Scott TW, Rosenberg R: **Defining challenges and proposing solutions for control of the virus vector *Aedes aegypti*.** *PLoS Med* 2008, **5**:e68.
26. Devine GJ, Zamora Perea E, Killeen GF, Stancil JD, Clark SJ, Morrison AC: **Using adult mosquitoes to transfer insecticides to *Aedes aegypti* larval habitats.** *Proc Natl Acad Sci USA* 2009, **106**:11530-11534.
27. Dell Chism B, Apperson CS: **Horizontal transfer of the insect growth regulator pyriproxyfen to larval microcosms by gravid *Aedes albopictus* and *Ochlerotatus triseriatus* mosquitoes in the laboratory.** *Med Vet Entomol* 2003, **17**:211-220.
28. Sihuincha M, Zamora-Perea E, Orellana-Rios W, Stancil JD, Lopez-Sifuentes V, Vidal-Ortiz C, Devine GJ: **Potential use of pyriproxyfen for control of *Aedes aegypti* (Diptera: Culicidae) in Iquitos, Peru.** *J Med Entomol* 2005, **42**:620-630.
29. Aiku AO, Yates A, Rowland M: **Laboratory evaluation of pyriproxifen treated bednets on mosquito fertility and fecundity. A preliminary study.** *West Afr J Med* 2006, **25**:22-26.
30. Devine GJ, Killeen GF: **The potential of a new larviciding method for the control of malaria vectors.** *Malar J* 2010, **9**:142.

doi:10.1186/1475-2875-9-340
Cite this article as: Mosqueira *et al.*: **Efficacy of an insecticide paint against insecticide-susceptible and resistant mosquitoes - Part 1: Laboratory evaluation.** *Malaria Journal* 2010 **9**:340.

MALARIA JOURNAL

RESEARCH Open Access

Efficacy of an insecticide paint against malaria vectors and nuisance in West Africa - Part 2: Field evaluation

Beatriz Mosqueira[1*], Joseph Chabi[2], Fabrice Chandre[2,3], Martin Akogbeto[2], Jean-Marc Hougard[3], Pierre Carnevale[3], Santiago Mas-Coma[1]

Abstract

Background: Widespread resistance of the main malaria vector *Anopheles gambiae* to pyrethroids reported in many African countries and operational drawbacks to current IRS methods suggest the convenience of exploring new products and approaches for vector control. Insecticide paint Inesfly 5A IGR™, containing two organophosphates (OPs), chlorpyrifos and diazinon, and one insect growth regulator (IGR), pyriproxyfen, was tested in Benin, West Africa, for 12 months.

Methods: Field trials were conducted in six experimental huts that were randomly allocated to one or two layers of insecticide at 1 Kg/6 m^2 or control. Evaluations included: (i) early mosquito collection, (ii) mosquito release experiments, (iii) residual efficacy tests and (iv) distance tests. Early mosquito collections were performed on local populations of pyrethroid-resistant *An. gambiae* and *Culex quinquefasciatus*. As per WHOPES phase II procedures, four entomological criteria were evaluated: deterrence, excito-repellence, blood-feeding inhibition and mortality. Mosquito release experiments were done using local malaria-free *An. gambiae* females reared at the CREC insectarium. Residual efficacy tests and distance tests were performed using reference susceptible strains of *An. gambiae* and *Cx. quinquefasciatus*.

Results: Six months after treatment, mortality rates were still 90-100% against pyrethroid-resistant mosquito populations in experimental huts. At nine months, mortality rates in huts treated with two layers was still about 90-93% against *An. gambiae* and 55% against *Cx. quinquefasciatus*. Malaria-free local mosquito release experiments yielded a 90% blood-feeding inhibition in the absence of a physical barrier. A long-term residual efficacy of 12 months was observed by WHO-bioassays in huts treated with two layers (60-80%). Mortality after an overnight exposition at distances of 1 meter was 96-100% for up to 12 months.

Conclusion: The encouraging results obtained on the insecticide paint Inesfly 5A IGR™ in terms of mortality, be it in direct contact or at a distance, and its new operational approach could constitute an additional option in malaria control efforts in areas of pyrethroid resistance. Phase III studies will be performed to assess the product's epidemiological impact and sociological acceptance.

Background

Primary prevention of malaria on a large scale is essentially achieved through vector control. Currently, the two main vector control methods: 1) indoor residual insecticide spraying (IRS), and 2) insecticide-treated nets (ITNs), aim at the primary protection of individuals and populations against the bite of infected *Anopheles* mosquitoes [1,2]. Pyrethroids are presently the only insecticides recommended for treatment of mosquito nets because of their rapid knockdown, high insecticidal potency at low dosages, and relative safety for mammals [3]. While both, IRS and ITNs have been found to be efficient and cost-effective across a large number of settings [1] it is not clear whether these interventions

* Correspondence: bmosqueira@yahoo.com
[1]Departamento de Parasitologa, Facultad de Farmacia, Universidad de Valencia, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain
Full list of author information is available at the end of the article

alone will achieve those critical low levels of transmission that result in successful malaria vector control. Moreover, because of i) the expanding resistance of main malaria vectors to pyrethroids [4], and ii) operational drawbacks to IRS [5], there is need for novel strategies in the framework of an integrated vector management [6]. Insecticide paint Inesfly 5A IGR™ is a "cocktail" consisting of two organophosphates, chlorpyriphos and diazinon and an insect growth regulator (IGR), pyriproxyfen. The same paint has been evaluated under experimental conditions against *Triatoma infestans*, a main vector of Chagas disease in Argentina [7] and Bolivia [8]. Results showed high mortalities and long residual activity in both cases. The paint was well accepted and tolerated by populations exposed to it [8]. Studies performed at the Instituto de Salud Carlos III in Spain have shown the paint's safety in terms of irritancy (ocular, dermal and systemic), cytotoxicity and mutagenicity [9].

The efficacy and residual effect of Inesfly 5A IGR™ insecticide paint has been tested in the laboratory at LIN (Laboratoire de Lutte contre les Insectes Nuisibles) of the Institut de Recherche pour le Développement (IRD) in Montpellier, France, on different kinds of surfaces using laboratory strains of 100% OP-resistant and 100% OP-susceptible *Culex quinquefasciatus*. A residual efficacy of over 12 months was observed on most surfaces even against resistant mosquitoes (Mosqueira *et al.*, submitted). Community adherence to malaria control measures is higher if strategies are also effective against nuisance [10-12] which may be further complicated since the pest mosquito *Cx. quinquefasciatus* has become resistant to the most common insecticides used for bed net impregnation [13].

The objective of the present study was to evaluate the entomological efficacy and the residual effect of Inesfly 5A IGR™ insecticide paint in experimental huts in Benin, West Africa, against local wild pyrethroid-resistant populations of the major malaria vector, *Anopheles gambiae*, and pest mosquito, *Cx. quinquefasciatus*, for one year.

Methods

Study site

Ladji (6◦23N-2◦25) is a large village located by the Nokoué Lake that floods during the rainy season creating breeding sites for *An. gambiae*. The local population of *An. gambiae* is comprised entirely of the M molecular form and shows resistance to pyrethroids and DDT, *kdr* is present at a high frequency, but is susceptible to organophosphates and carbamates, the *ace-1*^{R} mutation was absent [14]. Pest mosquito *Cx. quinquefasciatus* is also present all year round and shows high resistance to DDT, pyrethroids and carbosulfan with high kdr frequency and elevated levels of esterases and GST activity [14]. The *ace-1*^{R} mutation was absent [14].

Insecticide paint

Inesfly 5A IGR™ contains two organophosphates, chlorpyriphos (1.5%) and diazinon (1.5%) and an insect growth regulator (IGR), pyriproxyfen (0.063%), as active ingredients. The formulation is vinyl paint with an aqueous base, with the active ingredients residing within Ca CO3 and resin microcapsules, allowing a gradual release of active ingredients. Microcapsules range from one to several hundred micrometers in size. The paint was applied with a regular brush.

Early morning collection (EMC)

Inesfly 5A IGR™ was evaluated in 6 experimental huts for over 12 months from September 2003 to September 2004 at the Ladji station. Mosquito collections were performed following WHO testing procedures [15]. Experimental huts were built similarly to those used in Cote d'Ivoire by Darriet *et al* [16]. Huts were treated with one or two layers of insecticide paint at 1 kg commercial product/6 m^2. Huts treated with two layers had the first layer diluted in 20% water following manufacturer's recommendations. The overall random disposition of huts was: H1: Control 1 (no paint); H2: one layer of insecticide paint on walls; H3: one layer of insecticide paint on walls and ceiling; H4: two layers of insecticide paint on walls; H5: Control 2 (Inesfly paint with no insecticide); and H6: two layers of insecticide paint on walls and ceiling. Team members working in mosquito collection were informed in writing and orally (though they were all literate) about the study and were given the time to think before giving Informed Consent. All team members were provided with intact non-treated bed nets to protect them. Ethical authorization for this research was obtained from the Ministry of Health. Confirmed *Plasmodium falciparum* parasitaemia would be treated as per Benin's Ministry of Health's recommendations. Before treating, mosquitoes were collected for several nights to check that there was no difference between huts in attractiveness to mosquitoes. Though generally done, in this study it was even more important since treatments could not be rotated. To reduce the effect of variation in individual attractiveness to mosquitoes, sleepers rotated between huts on successive study nights. Mosquito collections were performed for thirteen weeks during the first three months; and for six weeks minus/plus three weeks on time points 6, 9 and 12 months after treatment. Following WHO Phase II procedures, four entomological criteria were evaluated: (i) deterrent effect, (ii) excito-repellent effect, (iii) blood feeding inhibition, (iv) mortality rate.

Operationally speaking, the greatest advantage was found if and when females have not had an opportunity to blood-feed before they die. Blood-feeding inhibition rates leave the question open as to whether females would blood-feed the next day on some other individual. The product's impact on blood-feeding has been interpreted in terms of unfed mortality in treated *vs* control huts.

Mosquito release experiments

On two occasions, mosquito bed nets were removed to assess blood-feeding in the absence of a physical barrier. Mosquitoes used were malaria-free five-day old unfed *An. gambiae* females bred at CREC's insectarium from wild larvae caught at Ladji. Females were released in batches of 100 females per hut at 21:00, just after volunteers entered huts. The next morning, females were collected as per Early Morning Collections. Two replicates were performed at the start of the evaluation (T0).

Residual efficacy tests

Thirty-minute standard WHO cone bioassays [17] were carried out using 3-5 day old unfed females of *Cx. quinquefasciatus* S-Lab and *An. gambiae* Kisumu, both reference strains susceptible to all insecticides reared at the CREC insectarium. Tests were performed every three months after treatment.

Distance tests

Unfed females of *An. gambiae* Kisumu and *Cx. quinquefasciatus* S-Lab, 3-5 day old, reared at the CREC insectarium, and susceptible to all insecticides, were introduced into four 150-ml cups, with 15 females per cup per hut. Mosquito netting was placed at both ends to allow air to go through. Honey-soaked cotton was introduced to ensure that females did not die from starvation. Tubes containing females were placed horizontally inside huts from 19:00 to 7:00 h, at a distance of 1 m from two perpendicular walls. The following morning, females were taken to the insectarium for mortality assessment after 24 hours at 80 ± 10% relative humidity and 27 ± 2°C temperature. Tests were performed every three months after treatment.

Statistical analysis

χ^2 analyses were run to test whether differences were statistically significant. EMC and Mosquito release experiments: The Statcalc application of Epi-Info 6 (USD, Inc., Snellville, U.S.A.) was used to analyse differences in exophily, blood-feeding and mortality rates among huts; to analyse differences in entry rates, ANOVA was used. When mortality rates in control huts were between 5 and 20% Abbott's mortality correction formula was applied. Residual efficacy and distance tests: Immediate and delayed mortality were analysed using Epi-Info 6. Where values were <5, Fisher exact tests were used. Because bioassay tests are subject to variations, a 99% confidence interval was applied.

Results

Early morning collection (EMC)

As is common for OPs, no deterrent or excito-repellent effect was observed neither against *An. gambiae* nor *Cx. quinquefasciatus*. For the first three months, 100% of *An. gambiae* females in huts treated with two layers, and 76% in huts treated with one layer, died before blood feeding (Table 1) while only 12% died without blood feeding, in control huts. In the case of *Cx. quinquefasciatus*, 88% of females died unfed in huts with two layers and 80% in huts with one layer, while only about 3% died unfed in control huts (Table 2). Nine months after treatment, 83% of *An. gambiae* died unfed in huts treated with two layers on walls, and 59% on huts treated with two layers on walls and ceiling - this difference is due to the fact that the bed net was not fixed correctly in the hut treated with two layers on walls and ceiling for a week during the short period when we had most *An. gambiae* coming in. On huts treated with one layer on walls, 33% of *An. gambiae* died unfed (the only rate not significantly different from control), while a rate of 72% was observed in huts treated with one layer on walls and ceiling. Mortality of unfed females in control huts was 12-14%. In the case of *Cx. quinquefasciatus*, 6% of females died unfed in control huts, while 51-54% died unfed in both huts treated with two layers. On huts treated with one layer on walls, 22% of *Cx. quinquefasciatus* died unfed and 40% in huts treated with one layer on walls and ceiling. By 12 months after treatment, mortality rates of unfed females fell to near control levels for both species.

Mortality was 100% up to three months against both, local populations of *An. gambiae* and *Cx. quinquefasciatus* for all treated huts, differences being significant compared to control. Six months after treatment, mortality rates against *Cx. quinquefasciatus* were of 90-100% for all treated huts (Table 2). Due to seasonal factors, there is no data on *An. gambiae* for that time point. By nine months after treatment, mortality rates in huts treated with two layers were still 90-93% against *An. gambiae* and 54-57% against *Cx. quinquefasciatus* (Tables 1 and 2, respectively). By twelve months, mortality was still higher compared to control in huts treated with two layers ($p < 10^{-3}$) and one layer ($p < 0.05$) (Table 2).

Mosquito release experiments

Blood-feeding in treated huts went from 2 to 13%, whereas control huts yielded blood-feeding rates of 68.5

Table 1 Overall mortality and unfed mortality of *Anopheles gambiae* females collected from experimental huts during EMCs.

EMC *Anopheles gambiae*		Untreated bed net (Control)	Untreated bed net + 2 layers Control Paint on walls and ceiling	Untreated bed net +1 layer IP on walls	Untreated bed net +1 layer IP on walls and ceiling	Untreated bed net +2 layers IP on walls	Untreated bed net +2 layers IP on walls and ceiling
T0-T3	% Overall Mortality	0^{a}	0^{a}	100^{b}	100^{b}	100^{b}	100^{b}
	% Unfed Mortality	12.5^{a}	11.1^{a}	75^{b}	77.8^{b}	100^{c}	100^{c}
T9	% Overall Mortality	0^{a}	0.9^{a}	34.6^{a}	79.7^{b}	90.2^{b}	93.1^{b}
	% Unfed Mortality	15^{a}	14^{a}	$33.3^{a,c}$	72.4^{b}	83.3^{b}	58.8^{c}

IP = Insecticide Paint. T0-T3 and T9 = 0-3 and 9 months after treatment

Values in the same row sharing a letter superscript do not differ significantly ($P \geq 0.05$)

and 76.1% (Figure 1). Differences between treated and control huts were significantly different ($p < 10^{-3}$).

Residual efficacy tests

In huts treated with one layer, mortality rates of 98-100% were observed against both *An. gambiae* Kisumu and *Cx. quinquefasciatus* S-Lab for up to three months (Tables 3 and 4). *Anopheles gambiae*, mortality rates started dropping six months after treatment to values of 79.4 and 59.7%. *Culex quinquefasciatus* values of 98-100% continued to be observed 6 and 9 months after treatment. At nine months after treatment, mortality rates dropped to 14.7% against *An. gambiae* (Table 3). In huts treated with two layers, mortality rates of 98-100% were observed for both *An. gambiae* and *Cx. quinquefasciatus* for up to nine months (Tables 3 and 4). Twelve months after treatment mortality rates were of 70-80% against *An. gambiae* and *Cx. quinquefasciatus*.

Distance tests

Huts treated with one layer yielded mortalities of 90-100% against *An. gambiae* Kisumu (Table 5) and *Cx. quinquefasciatus* S-Lab (Table 6) for up to six months. By 12 months, a volume effect was observed in the hut treated with one layer just on walls (35.6% for *An. gambiae* and 60% *Cx. quinquefasciatus*) versus that treated on walls and ceiling (98.4% for *An. gambiae* and 96.2% *Cx. quinquefasciatus*), but differences were still significant with respect to control ($p < 10^{-6}$) for both. Huts

Table 2 Overall mortality and unfed mortality of *Culex quinquefasciatus* females collected from experimental huts during EMCs.

EMC *Culex quinquefasciatus*		Untreated bed net (Control)	Untreated bed net + 2 layers Control Paint on walls and ceiling	Untreated bed net +1 layer IP on walls	Untreated bed net +1 layer IP on walls and ceiling	Untreated bed net +2 layers IP on walls	Untreated bed net +2 layers IP on walls and ceiling
T0-T3	% Overall Mortality	0^{a}	0^{a}	100^{b}	100^{b}	100^{b}	100^{b}
	% Unfed Mortality	3.4^{a}	2.1^{a}	81.2^{b}	79.4^{b}	87.8^{c}	88^{c}
T6	% Overall Mortality	0^{a}	2.2^{a}	92.9^{b}	95.7^{c}	100^{d}	99.5^{d}
	% Unfed Mortality	5.6^{a}	7.6^{a}	78.3^{b}	$70.1^{b,c}$	$69.4^{b,c}$	$84.5^{b,d}$
T9	% Overall Mortality	0^{a}	2.1^{a}	20.8^{b}	40.1^{c}	56.7^{d}	54.5^{d}
	% Unfed Mortality	2.7^{a}	4.3^{a}	22^{b}	39.5^{c}	53.7^{d}	50.7^{d}
T12	% Overall Mortality	0^{a}	1.2^{a}	5.7^{b}	5.3^{b}	15.6^{c}	21.6^{d}
	% Unfed Mortality	5^{a}	$7^{a,b}$	9.7^{b}	$7.9^{a,b}$	17^{c}	23.9^{d}

IP = Insecticide Paint. T0-T3, T6, T9 and T12 = 0-3, 6, 9 and 12 months after treatment

Values in the same row sharing a letter superscript do not differ significantly ($P \geq 0.05$)

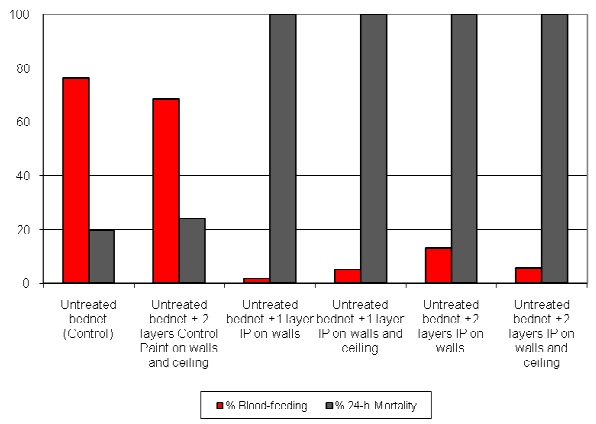

Figure 1 Delayed 24-hour mortality and blood-feeding rates during mosquito release experiments of local *Anopheles gambiae*. Malaria-free females reared at the CREC insectarium were released into each hut at 21:00 hours and collected between 5 and 7 hours the next day. Bed nets had been withdrawn and mosquito entry into the huts was blocked. Averages from two repeats of N > 30 each.

treated with two layers yielded mortalities 100% against *An. gambiae* and *Cx. quinquefasciatus* for 12 entire months (Tables 5 and 6).

Discussion

The efficacy of Inesfly 5A IGR™ was tested against pyrethroid-resistant *An. gambiae* and *Cx. quinquefasciatus*. Contrary to the results obtained by N'Guessan *et al* [18] and Assidi *et al* [19] in experimental huts, when testing OPs, neither a deterrent nor an exito-repellent effect was observed throughout the trial.

The product's best profile was found to be its capacity to kill mosquitoes. Mortality rates as high as 100% were obtained up to three months against both species. A nine-month residual efficacy was observed through bioassay testing as well as through Early Mosquito Collection, analogous to the nine-month residual activity obtained with chlorpyrifos-methyl applied by IRS in the same study area against the same mosquito populations of Ladji in Cotonou [18]. Mosquito killing was quick enough to prevent blood feeding: during mosquito release experiments, in the absence of the physical barrier provided by bed nets, only 2 to 13% of females blood fed in treated huts, whereas blood feeding in control huts was 72%, similar to the 83% obtained by Darriet *et al* [16] in Ivory Coast in huts with no bed nets. These findings were supported by Early Morning Collection data, where the number of females that died in treated huts without having blood-fed was significantly different compared to control.

Mortality rates observed in distance experiments were most striking. Females placed overnight at distances of one metre from treated walls died even twelve months after treatment. Because even highly endophilic pest or vector mosquitoes are not always in contact with an insecticide-treated surface before contacting a human or animal host, especially on pyrethroid-treated surfaces due to its excito-repellent effect, it is desirable to have a distance effect. The lethal effect at a distance observed in the insecticide paint goes in this direction. A possible mass protective effect as a result of mass house-treatment needs to be studied. On a product safety note, Acute Inhalation Toxicity studies classified this paint as Category III (according to WHO) and category IV (according to EPA) - no warning label required in either case [20].

As results show, a "layer effect"and a "volume effect" was observed by all three tests, EMC experiments, bioassays and distance tests. The "layer and volume effect" became more evident with time. Porous surfaces like cement benefited from treatment with two layers. Similarly, huts treated with only one layer benefited particularly from the treatment of a larger volume. Whether subsequent layers prolong the product's long lasting efficacy needs to be explored.

To test whether efficacy hinged more on porosity than dose, a parallel study was performed. Cement-made surfaces painted with a control layer and an insecticide

Table 3 Delayed 24-hour mortality of *Anopheles gambiae* Kisumu after a 30-minute exposure to treated and control walls.

WHO Bioassays% Mortality *Anopheles gambiae* Kisumu	Untreated bed net (Control)	Untreated bed net + 2 layers Control Paint on walls and ceiling	Untreated bed net +1 layer IP on walls	Untreated bed net +1 layer IP on walls and ceiling	Untreated bed net +2 layers IP on walls	Untreated bed net +2 layers IP on walls and ceiling
T0	12.5^{a}	14.1^{a}	100^{b}	100^{b}	100^{b}	100^{b}
T3	0^{a}	3.3^{a}	100^{b}	100^{b}	100^{b}	100^{b}
T6	0^{a}	1.8^{a}	79.4^{b}	59.7^{c}	100^{d}	100^{d}
T9	0^{a}	$3.4^{a, b}$	14.7^{b}	44.6^{c}	100^{d}	98.5^{d}
T12	1.7^{a}	$6.1^{a, b}$	0^{a}	12.9^{b}	80.6^{c}	71.9^{c}

IP = Insecticide Paint. T0, T3, T6, T9 and T12 = 0, 3, 6, 9 and 12 months after treatment

Values in the same row sharing a letter superscript do not differ significantly ($P \geq 0.05$)

Table 4 Delayed 24-hour mortality of *Culex quinquefasciatus* S-Lab after a 30-minute exposure to treated and control walls.

WHO Bioassays% Mortality *Culex quinquefasciatus* S-lab	Untreated bed net (Control)	Untreated bed net + 2 layers Control Paint on walls and ceiling	Untreated bed net +1 layer IP on walls	Untreated bed net +1 layer IP on walls and ceiling	Untreated bed net +2 layers IP on walls	Untreated bed net +2 layers IP on walls and ceiling
T3	5.5[a]	6.2[a]	100[b]	100[b]	100[b]	100[b]
T6	13.8[a]	10.3[a]	100[b]	98.3[b]	100[b]	100[b]
T9	1.6[a]	3.3[a]	72.6[b]	49.2[c]	100[d]	98.4[d]
T12	1.6[a]	0[a]	5[a]	8.1[a]	70[b]	72.4[b]

IP = Insecticide Paint. T0, T3, T6, T9 and T12 = 0, 3, 6, 9 and 12 months after treatment

Values in the same row sharing a letter superscript do not differ significantly (P ≥ 0.05)

Table 5 Delayed 24-hour mortality of *Anopheles gambiae* Kisumu after an overnight exposure at a distance of one meter from two perpendicular walls.

Distance tests% Mortality *An. gambiae* Kisumu	Untreated bed net (Control)	Untreated bed net + 2 layers Control Paint on walls and ceiling	Untreated bed net +1 layer IP on walls	Untreated bed net +1 layer IP on walls and ceiling	Untreated bed net +2 layers IP on walls	Untreated bed net +2 layers IP on walls and ceiling
T0	0[a]	3.4[a]	100[b]	100[b]	100[b]	100[b]
T6	0[a]	0[a]	91.8[b]	100[b]	100[b]	100[b]
T12	1.5[a]	3[a]	35.6[b]	98.4[c]	100[c]	100[c]

IP = Insecticide Paint. T0, T6 and T12 = 0, 6 and 12 months after treatment

Values in the same row sharing a letter superscript do not differ significantly (P ≥ 0.05)

paint layer at 1 kg/6 m^2, performed as well as two insecticide paint layers at 1 kg/6 m^2, even though the latter had twice the dose (Mosqueira *et al.*, unpublished data).

The paint offers a different operational approach that could be of value. Unlike IRS, people are able to apply the paint themselves, no need of trained personnel or special equipment. Homes' appearance would also improve leading, potentially, to changes in behaviour of public health significance [21].

Findings suggest the potential value of the insecticide paint as a vector control tool in areas of pyrethroid resistance and in urban settings. While it is clear that urban malaria represents a major challenge for public health in Africa [22], several factors make urban environments suitable for the insecticide paint: 1) superior resources, 2) the paint's effectiveness against nuisance; 3) population densities would facilitate coverage and a potential mass effect; 4) the vast majority of houses and public spaces, such as hospitals, schools, prisons, churches and mosques, are made of surfaces suitable for painting.

Conclusions

The lethal effect of the insecticide paint observed in the field against local populations of *An. gambiae* and *Cx. quinquefasciatus* resistant to pyrethroids was encouraging. Killing was not only high but quick enough to prevent blood feeding. A residual efficacy of nine months was observed as per mosquito collections and 30-minute bioassays. Females left overnight at distances of one meter continued dying significantly even after 12 months. The possible existence of a mass-effect needs to be studied in a large-scale epidemiological setting. Future endeavours will be directed towards the study of

Table 6 Delayed 24-hour mortality of Culex quinquefasciatus S-Lab after an overnight exposure at a distance of one meter from two perpendicular walls.

Distance tests% Mortality *Culex quinquefasciatus* S-Lab	Untreated bed net (Control)	Untreated bed net + 2 layers Control Paint on walls and ceiling	Untreated bed net +1 layer IP on walls	Untreated bed net +1 layer IP on walls and ceiling	Untreated bed net +2 layers IP on walls	Untreated bed net +2 layers IP on walls and ceiling
T0	8.3[a]	0[a]	100[b]	100[b]	100[b]	100[b]
T6	13.8[a]	10.3[a]	100[b]	98.3[b]	100[b]	100[b]
T12	1.8[a]	3[a]	60[b]	96.2[c]	100[c]	100[c]

IP = Insecticide Paint. T0, T6 and T12 = 0, 6 and 12 months after treatment

Values in the same row sharing a letter superscript do not differ significantly (P ≥ 0.05)

the insecticide paint's efficacy on the incidence of malaria as well as its acceptability.

Acknowledgements
We are grateful to volunteers at the Ladji Experimental Station for their continued effort for the entire year. This study was funded by Project Number 07-CAP3-1513 of the *Agencia Española de Cooperación Internacional para el Desarrollo* (AECID), Ministry of Foreign Affaires, Madrid, Spain, and by the *Red de Investigación de Centros de Enfermedades Tropicales* (RICET), Project Number RD06/0021/0017 "*Redes Temáticas de Investigación Cooperativa*", *Fondo de Investigación Sanitaria* (FIS), Ministry of Health, Madrid, Spain. We thank the University of Valencia in Spain, the Institut de Recherche pour le Développement (IRD) in France and the Centre de Recherche Entomologique de Cotonou (CREC) in Benin for their support. Special thanks to Dr. Thierry Baldet for his key input on the manuscript.

Author details
[1]Departamento de Parasitologa, Facultad de Farmacia, Universidad de Valencia, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain. [2]Centre de Recherches Entomologique de Cotonou, 06 BP 2604 Cotonou, Benin. [3]UR016, Institut de Recherche pour le Développement (IRD), BP 64501, 34394 Montpellier Cedex 5, France.

Authors' contributions
PC and SMC conceived the protocol. PC, SMC, FC and BM contributed to the design of the study. FC and JMH critically contributed to the implementation of the study. JC and BM conducted evaluations. MA was the director of the Centre de Recherche Entomologique de Cotonou (CREC). The manuscript has been drafted by BM and has been revised by PC. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Received: 15 August 2010 Accepted: 25 November 2010
Published: 25 November 2010

References

1. Lengeler C, Sharp B: **Indoor residual spraying and insecticide-treated nets. In: Reducing malaria's burden: evidence of effectiveness for decision makers.** *Global Health Council* Washington D.C; 2003, 17-24.
2. Lengeler C: **Insecticide-treated bed nets and curtains for preventing malaria.** *Cochrane Database of Syst Rev* 2008 [http://www.cochrane.org].
3. Zaim M, Aitio A, Nakashima N: **Safety of pyrethroid-treated mosquito nets.** *Med Vet Entomol* 2000, **14**:1-5.
4. Baleta A: **Insecticide resistance threatens malaria control in Africa.** *Lancet* 2009, **374**:1581-1582.
5. Najera JA, Zaim M: **Malaria vector control. Insecticides for indoor residual spraying.** *World Health Organ* Geneva; 2001, WHO/CDS/WHOPES/2001.3.
6. Beier JC, Keating J, Githure JI, Macdonald MB, Impoinvil DE, Novak RJ: **Integrated vector management for malaria control.** *Malar J* 2008, **7(Suppl 1)**:S4.
7. Amelotti I, Catalá SS, Gorla DE: **Experimental evaluation of insecticidal paints against *Triatoma infestans* (Hemiptera: Reduviidae), under natural climatic conditions.** *Parasit Vectors* 2009, **2**:30-6.
8. Dias JC, Jemmio A: **About an insecticidal paint for controlling *Triatoma infestans*, in Bolivia.** *Rev Soc Bras Med Trop* 2008, **41**:79-81.
9. Spanish Ministry of Health and Consumer Affairs: **Report on the study of the toxicity and irritability of Inesfly 5A.** *Health Institute Carlos III* Madrid; 1996.
10. Aikins MK, Pickering H, Greenwood BM: **Attitudes to malaria, traditional practices and bednets (mosquito nets) as vector control measures: a comparative study in five West African countries.** *Am J Trop Med Hyg* 1994, **97**:81-86.
11. Van Bortel W, Barutwanayo M, Delacollette C, Coosemans M: **Motivation à l'acquisition et à l'utilisation des moustiquaires imprégnées dans une zone à paludisme stable au Burundi.** *Trop Med Inter Health* 1996, **1**:71-80.
12. Samuelsen H, Paré-Toe L, Baldet T, Skovmand O: **Prevention of mosquito nuisance among urban populations in Burkina Faso.** *Social Sci Med* 2004, **59**:2361-2371.
13. Chandre F, Darriet F, Darder M, Cuany A, Doannio JMC, Pasteur N, Guillet P: **Pyrethroid resistance in *Culex quinquefasciatus* from West Africa.** *Med Vet Entomol* 1998, **12**:359-366.
14. Corbel V, N'Guessan R, Brengues C, Chandre F, Djogbénou L, Martin T, Akogbéto M, Hougard JM, Rowland M: **Multiple insecticide resistance mechanisms in *Anopheles gambiae* and *Culex quinquefasciatus* from Benin, West Africa.** *Acta Trop* 2007, **101**:207-16.
15. WHO: *Evaluation and testing of insecticides* World Health Organization, Geneva;, Report of the WHO Informal Consultation WHO/HQ, Geneva, 7-11 October 1996, (document CTD/WHOPES/IC/96.1).
16. Darriet F, N'Guessan R, Carnevale P: **Evaluation in test huts of the protective effects of untreated mosquito nets against *Anopheles gambiae* s.s. bites.** *Cahiers Santé* 2000, **10**:413-417.
17. WHO: *Test Procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces* World Health Organization, Geneva;1-43, Report of the WHO Informal Consultation. Document WHO/CDS/CPC/MAL/1998.12.
18. N'Guessan R, Boko P, Odjo A, Chabi J, Akogbeto M, Rowland M: **Control of pyrethroid and DDTresistant *Anopheles gambiae* by application of indoor residual spraying or mosquito nets treated with a long-lasting organophosphate insecticide, chlorpyrifos-methyl.** *Malar J* 2010, **9**:44.
19. Asidi A, N'Guessan R, Koffi AA, Curtis CF, Hougard JM, Chandre F, Corbel V, Darriet F, Zaim M, Rowland MW: **Experimental hut evaluation of bednets treated with an organophosphate (chlorpyrifos-methyl) or a pyrethroid (lambdacyhalothrin) alone and in combination against insecticide-resistant *Anopheles gambiae* and *Culex quinquefasciatus* mosquitoes.** *Malar J* 2005, **4**:25.
20. Internacional Centre of Medical Training and Research: **Acute Inhalatory Toxicity of Inesfly Saint 5 A IGR.** *CIDEIM* 2004, Reference Number: Inesfly Pintura 5A IGR-105-0903.
21. Rozendaal JA: *Vector control methods for use by individuals and communities* Geneva: World Health Organization; 1997.
22. Donnelly MJ, McCall PJ, Lengeler C, Bates I, D'Alessandro U, Barnish G, Konradsen F, Klinkenberg E, Townson H, Trape JF, Hastings IM, Mutero C: **Malaria and urbanization in sub-Saharan Africa.** *Malar J* 2005, **4**:12.

doi:10.1186/1475-2875-9-341
Cite this article as: Mosqueira *et al.*: Efficacy of an insecticide paint against malaria vectors and nuisance in West Africa - Part 2: Field evaluation. *Malaria Journal* 2010 **9**:341.

MALARIA JOURNAL

RESEARCH Open Access

Proposed use of spatial mortality assessments as part of the pesticide evaluation scheme for vector control

Beatriz Mosqueira[1*], Joseph Chabi[2], Fabrice Chandre[3], Martin Akogbeto[2], Jean-Marc Hougard[3], Pierre Carnevale[4] and Santiago Mas-Coma[1]

Abstract

Background: The WHO Pesticide Evaluation Scheme to evaluate the efficacy of insecticides does not include the testing of a lethal effect at a distance. A tool was developed to evaluate the spatial mortality of an insecticide product against adult mosquitoes at a distance under laboratory and field conditions. Operational implications are discussed.

Methods: Insecticide paint, Inesfly 5A IGR™, containing two organophosphates (OPs): chlorpyrifos and diazinon, and one insect growth regulator (IGR): pyriproxyfen, was the product tested. Laboratory tests were performed using "distance boxes" with surfaces treated with one layer of control or insecticide paint at a dose of 1 kg/6 sq m. Field tests were conducted up to 12 months in six experimental huts randomly allocated to control or one or two layers of insecticide paint at 1 kg/6 sq m. All distance tests were performed using reference-susceptible strains of *Anopheles gambiae* and *Culex quinquefasciatus* left overnight at a distance of 1 m from control or treated surfaces.

Results: After an overnight exposition at distances of 1 m, field and laboratory evaluations at 0 months after treatment (T0) yielded 100% mortality rates on surfaces treated with one layer at 1 kg/6 sq m against susceptible strains of *An. gambiae* and *Cx. quinquefasciatus*. Testing for long-term efficacy in the field gave mortality rates of 96-100% after an overnight exposition at a distance of 1 m for up to 12 months in huts where a larger volume was treated (walls and ceilings) with one or two layers of insecticide paint.

Conclusion: A comprehensive evaluation of the full profile of insecticide products, both upon contact and spatially, may help rationalize vector control efforts more efficiently. Treating a large enough volume may extend a product's mortality efficacy in the long-term, which contact tests would fail to assess. It is hereby proposed to explore the development of cost effective methods to assess spatial mortality and to include them as one additional measurement of insecticide efficacy against mosquitoes and other arthropod vectors in WHOPES Phase I and Phase II studies.

Keywords: Vector control, WHOPES, Insecticide-treated nets (ITNs), Long-lasting insecticidal nets (LLINs), Indoor residual spraying (IRS), Insecticide paint, Mass effect

Background

Vector-borne diseases, such as malaria and dengue, are among the major causes of morbidity and mortality and significantly impede the economic and social development of many countries, predominantly in tropical areas, although not only. In temperate regions, West Nile virus, dengue, leishmaniasis and chikungunya, among other vector-borne diseases, are also causing an increasing burden.

Control strategies rely mostly on vector control using insecticides, treatment using drugs, improving people's dwellings/modifying the environment, education, and the creation of new vaccines. A promising intervention strategy involves genetic control of the vectors [1]. The strategies chosen will depend on several factors, such as resistance to insecticides, availability of treatment and/or resistance to available drugs, difficulties in developing a vaccine, existence of operational genetic control programmes, and long-

* Correspondence: bmosqueira@yahoo.com
[1]Departamento de Parasitologia, Facultad de Farmacia, Universidad de Valencia, Av Vicent Andrés Estellés s/n, Burjassot 46100, Valencia, Spain
Full list of author information is available at the end of the article

term sustainability. A combination of the above disease control strategies will increase chances to succeed.

Vector control is one of these strategies and remains a key player in the control of major endemic and epidemic vector-borne diseases such as malaria [2,3]. The official World Health Organization Pesticide Evaluation Scheme (WHOPES) guidelines for the evaluation of the efficacy of insecticides [4,5] take into consideration products' impact on mortality, blood feeding, deterrence and repellence. Tests currently used include classical WHO contact bioassays [6,7], tunnel tests [8,9] and early morning collections in experimental huts [10,11]. These tests provide key information on the impact of insecticide products, such as long-lasting insecticidal nets (LLINs) or indoor residual spraying (IRS), upon contact both in the laboratory and the field, but does not provide information on the possible lethal effect at a distance.

Since even highly endophilic mosquitoes or other arthropod vectors are not always in contact with an insecticide-treated surface before biting a human or animal host, especially on pyrethroid-treated surfaces due to its irritant effect, it is desirable to evaluate the lethal effect spatially, that is, at a distance, without the mosquitoes ever entering into contact with an insecticide-treated surface.

Several studies on the community effect of ITNs on malaria indicate the presence of a beneficial mass effect [12-20]. A mass effect of IRS has also been documented in a number of trials [21].

In this study, distance tests were performed in the laboratory using "distance boxes", and in the field. In the field, evaluations were done in addition to WHO bioassays and early morning collections in experimental huts. The product evaluated consisted of an insecticide paint, Inesfly 5A IGR™, composed of two organophosphates (OPs): chlorpyriphos (1.5%) and diazinon (1.5%), and an insect growth regulator (IGR): pyriproxyfen (0.063%). The product was a white vinyl paint with an aqueous base. Active ingredients resided within Ca CO^3 + resin microcapsules ranging from one to several hundred micrometres in size. The formulation allows a gradual release of active ingredients, increasing its durability. Toxicology studies performed so far support the product's safety [22-24]. Inesfly 5A IGR™ had been evaluated previously under experimental conditions against the Chagas disease vector *Triatoma infestans* [25,26], Classical WHOPES tests were also performed on Inesfly 5A IGR™ in the laboratory (Phase I) against 100% OP-resistant *Culex quinquefasciatus* [27] and in the field (Phase II) against local wild pyrethroid-resistant populations of the major malaria vector, *Anopheles gambiae*, and pest mosquito, *Cx. quinquefasciatus* [28]. In parallel to the standard Phase I evaluations [27], it was decided to explore the idea of a possible efficacy at a distance by exposing mosquitoes to metal-treated surfaces at distances of 3 cm, 40 cm and 100 cm. Mortalities at shorter distance were almost the same as the ones upon contact (unpublished results). It was thus decided to test spatial mortality at distances of 100 cm from cement-treated surfaces so as to reproduce the same test on experimental huts during Phase II evaluations. The objective of this paper is to propose the use of spatial mortality tests as part of the WHOPES in the light of results obtained in the laboratory (Phase I) using "distance boxes" and in the field (Phase II) in experimental huts.

Methods

Phase I - laboratory tests using distance boxes

Two identical wooden boxes were built, one for control and one for treatment. The size of each wooden box was 50 cm wide × 50 cm high, length 100 cm with two horizontal slits of 4 cm × 50 cm on each side. The two horizontal slits were placed in the middle of each side of the box to allow air to flow. Wood was chosen as a material readily available and easy to work with. One end was left open and is where mosquitoes were placed inside 150 ml tubes. The other end was closed by a cement surface 50 cm × 50 cm – cement was chosen to reproduce the material experimental huts were made of. The box used as control had a cement surface with no paint. The box used for treatment had a cement surface with of one layer of Inesfly 5A IGR™ insecticide-paint at 1 kg/6 sq m. Boxes were placed in a closed room at 80 ± 10% relative humidity and 27 ± 2°C temperature.

Unfed females of *An. gambiae* Kisumu and *Cx. quinquefasciatus* S-Lab, three to five days old, reared at the Centre de Recherche Entomologique de Cotonou (CREC) insectarium, and susceptible to all insecticides, were used. Mosquitoes were introduced in four 150 ml tubes with mosquito netting at both ends to protect them from scavengers but allow air through. Honey juice-soaked cotton was introduced in each tube to prevent females from starvation. Four replicates were made with 15 females each, giving a total of 60 females per surface per test. Tubes were placed horizontally at the edge of the box at 1 m from the cement surface from 19:00 to 07:00. The following morning, females were taken to the insectarium for delayed mortality assessments after 24 hours at 80 ± 10% relative humidity and 27 ± 2°C temperature. Distance testing was done only at 0 months after treatment (T0) under laboratory conditions.

Phase II - field tests in experimental huts in Benin

Inesfly 5A IGR™ was evaluated in six experimental huts at the Ladji station in Cotonou (south of Benin) [28]. Experimental huts were built following the West African-style hut model [29]. Huts were treated with one or two layers of insecticide paint at 1 kg commercial product/6 sq m, that is 0,51 g a.i. per sq m. Based on huts' dimensions, 3.4 kg of paint were applied on walls per layer, and 1.0 kg

on ceilings. Huts treated with two layers had the first layer diluted in 20% water following recommendations of the manufacturer. The overall random disposition of huts was: H1: Control 1 - no paint; H2: Control 2 - two layers of control paint on walls and ceiling; H3: one layer of insecticide paint on walls; H4: one layer of insecticide paint on walls and ceiling; H5: two layers of insecticide paint on walls; and H6: two layers of insecticide paint on walls and ceiling.

Unfed females of *An. gambiae* Kisumu and *Cx. quinquefasciatus* S-Lab, three to five days old, reared at the CREC insectarium, and susceptible to all insecticides, were used. A total of 60 females were introduced into four tubes of 150 ml, with 15 females per tube. Mosquito netting was placed at both ends to allow air through. Honey-soaked cotton was introduced to ensure that females did not die from starvation. Tubes containing females were placed inside the hut, on the floor, horizontally from 19:00 to 07:00, at a distance of 1 m from two perpendicular walls inside the hut and 1.90 m from the ceiling. The following morning, females were taken to the insectarium for delayed mortality assessment after 24 hours at 80 ± 10% relative humidity and 27 ± 2°C temperature. Tests were performed again 12 months after treatment.

Results from laboratory and field distance tests were analysed using Epi-Info 6. When values were <5, Fisher exact tests were used.

Results

Phase I - laboratory tests using distance boxes

Distance boxes yielded 100% mortality at 0 months after treatment against both *An. gambiae* Kisumu and *Cx. quinquefasciatus* S-Lab (Table 1). Compared to control, mortality rates were significantly different for the treated surface ($p < 10^{-6}$).

Phase II - field tests in experimental huts in Benin

Under field conditions at T0, all huts, regardless of the surface treated and the number of layers, yielded 100% mortality against both *An. gambiae* Kisumu and *Cx. quinquefasciatus* S-Lab (Table 1). Twelve months after treatment, mortality rates observed at the huts where a larger volume was treated with one or two layers of paint were 98.4% for *An. gambiae* Kisumu and 96.2% for *Cx. quinquefasciatus* S-Lab (Table 2). Mortality rates in the hut treated on only walls with one layer of insecticide paint was lower than in the other three huts: 36% mortality against susceptible *An. gambiae* Kisumu and 60% against susceptible *Cx. quinquefasciatus* S-Lab ($p < 10^{-6}$), though still higher than control ($p < 10^{-6}$).

Discussion

The results obtained in the laboratory and the field were similar at T0: 100% mortality rates were observed on surfaces treated with one layer of insecticide paint at the recommended dose of 1 kg/6 sq m against susceptible *An. gambiae* Kisumu and *Cx. quinquefasciatus* S-Lab. Female mosquitoes were never in contact with the treated or control surfaces. A 1-m distance was respected in all cases for all repeats. The uniformity of results suggests distance boxes could be a useful and simple approach for testing the lethal efficacy of insecticide products at a distance in the laboratory during Phase I evaluations, but more data is needed. The distance of one metre was chosen because of the small size of experimental huts and of West African homes in general. The distance of one metre is thus proposed as an initial step, nevertheless the distance may be adapted depending on the nature of the insecticide (ie. vapour pressure) and the support used (ie. LLINs, IRS, DL, paint). The size of the dwellings to be treated may also play a role in deciding the distance to be tested – if large halls in schools, airports or hospitals are treated, it may be of interest to test for spatial mortality efficacy at greater distances.

To test for long-term spatial mortality efficacy, distance tests were performed again 12 months after treatment during Phase II studies in the field: spatial mortality after an overnight exposition at distances of 1 m remained high, 96-98%, against susceptible *An. gambiae* Kisumu and *Cx. quinquefasciatus* S-Lab in huts with one layer of insecticide paint if both, walls and ceiling were treated. On the other hand, huts treated with one layer but on walls only (not ceilings) performed less well, 36% mortality against susceptible *An. gambiae* Kisumu and 60% against susceptible *Cx. quinquefasciatus* S-Lab after 12 months. That is, provided that a large enough volume was treated, huts with one layer performed as well as huts with two layers despite the difference in dose – this finding was referred to as the "volume effect". This notion of volume effect seemed to be supported by results obtained during Phase II early morning collections in the field: A volume effect

Table 1 Comparison of phase I and phase II spatial mortality rates in control and treated surfaces

Cement tested at a distance of 1 m at T0	Control	One layer IP at 1 kg/6 sq m in distance box – phase I	One layer IP at 1 kg/6 sq m in experimental huts - phase II
An. gambiae Kisumu	0^a	100^b	100^b
Cx. quinquefasciatus S-Lab	0^a	100^b	100^b

Delayed 24-hour mortality of *Anopheles gambiae* Kisumu and *Culex quinquefasciatus* S-Lab after overnight exposure at a distance of 1 m in distance boxes in laboratory, and in experimental huts in the field.
IP, Insecticide paint, *T0*, 0 months after treatment. Values in the same row sharing a letter superscript do not differ significantly ($P \geq 0.05$).

Table 2 Phase II spatial long-term mortality rates in control and treated experimental huts

Phase II - cement tested at a distance of 1 m at T0 and T12	Timepoint	Control 1	Control 2 two layers of control paint on walls and ceiling	One layer IP on walls at 1 kg/6 sq m	One layer IP on walls and ceiling at 1 kg/6 sq m	Two layers IP on walls at 1 kg/6 sq m	Two layers IP on walls and ceiling at 1 kg/6 sq m
An. gambiae	T0	0^a	3.4^a	100^b	100^b	100^b	100^b
Kisumu	T12	1.5^a	3^a	35.6^b	98.4^c	100^c	100^c
Cx. quinquefasciatus S-Lab	T0	8.3^a	0^a	100^b	100^b	100^b	100^b
	T12	1.8^a	3^a	60^b	96.2^c	100^c	100^c

Delayed 24-hour mortality of *Anopheles gambiae* Kisumu and *Culex quinquefasciatus* S-Lab after overnight exposure at a distance of 1 m from two perpendicular walls in experimental huts in the field.
IP, Insecticide paint, *T0* and *T12*, 0 and 12 months after treatment.
Values in the same row sharing a letter superscript do not differ significantly ($P \geq 0.05$).

was observed during early morning collections performed in experimental huts in Ladji, south of Benin, with the same insecticide paint [28]. Likewise, a volume effect was observed when testing the efficacy of a pyrethroid-based insecticide paint against pyrethroid-susceptible populations of *An. gambiae* and *Cx. quinquefasciatus* during a Phase II study in experimental huts in the north of Benin: female mosquitoes exposed at a distance of 1 m from treated surfaces had a significantly higher mortality rate 12 months after treatment in huts where both walls and ceiling were treated even if with just one layer of paint, but not in huts with one layer on walls only [Mosqueira B, Chabi J, Soukou KB, Akogbeto M, Carnevale P, Corbel V, Mas-Coma S: Laboratory and field efficacy of a pyrethroid-based insecticide paint against insecticide-susceptible and resistant malaria-transmitting mosquitoes, in preparation]. Curiously, an irritant and deterrent effect was observed when comparing treated huts to control. Predictably, WHO contact bioassays failed to detect a volume effect: as far as WHO contact bioassays went, only the dose applied counted – two layers performing consistently better than one layer. Hence, Phase I and Phase II efficacy assessments based on contact may be overlooking important questions on the coverage and dose needed to achieve long-term efficacy such as: would reducing the total dose be possible if a larger surface was treated? Phase II early morning collections do offer an insight on the question, as not all wild mosquitoes entering the hut are in direct contact with treated surfaces, but the exact distances from treated surfaces would not be known, numbers would vary between huts and whether the insecticide is on the walls or bed nets might make a difference. Likewise, current Phase I and Phase II assessments may not fully explore the potential of high vapour pressure insecticides by evaluating efficacy chiefly upon contact as opposed to both, contact and distance.

The tested insecticide product was effective in killing mosquitoes that had not come closer than 1 m to the treated surface after an overnight exposition. It could be envisaged that the same could happen in a natural setting to mosquitoes resting on non-treated surfaces before and/or after biting, although it is not known the minimum amount of exposure time needed to achieve this. A study performed by Gimnig *et al.* [17] determined how the abundance of malaria vectors changed as a function of distance from houses with ITNs. The study used a geographic information system (GIS) to test the hypothesis that a community effect reduces the overall vector population, and that persons lacking ITNs who live near the compounds of those using ITNs are afforded some protection from vector mosquitoes. Another study performed by Hawley *et al.* [30] showed ITNs had a protective effect on child mortality, moderate anaemia, high-density parasitaemia, and haemoglobin levels in compounds lacking ITNs but located within 300 m of compounds with ITNs. A mass community effect against malaria transmission has also been observed in areas where the only malaria vector is largely exophagic and zoophilic [31].

In the case of dengue, a spatial analysis performed by Lenhart *et al.* [32] indicated that the effect of the presence of ITNs had spread to control houses located 50–100 m away from bed net houses by five months post-intervention, although control houses located more than 100 m from bed net houses experienced no significant change in entomological indices. Findings were particularly surprising since there were no previous indications that ITNs could be useful in reducing dengue transmission. Although the nature of that effect was not characterized, the study suggested that it was of both repellent and lethal nature and not by the barrier provided by the ITN itself [32].

Findings increasingly suggest the mass community effect depends on high rates of coverage [33,34] as well as the distance from treated clusters [35]. Despite evidence pointing at the mass community effect of vector control strategies, little is known on the exact mechanism. Contact alone may not be the sole factor. Recent findings emphasize the need to study the spatial repellency of insecticides in addition to the contact irritancy tests classically done [36]. Furthermore, spatial repellency may represent an effective tool in the fight against vector-borne disease transmission [37]. It is proposed that the spatial *mortality* of insecticide products is evaluated in addition to the contact mortality tests currently done in order to better rationalize vector control efforts. Future endeavours will be directed towards the testing of the

lethal efficacy at a distance of Inesfly 5A IGR™ against OP-resistant *An. gambiae* in the laboratory and the field.

Conclusions

Spatial mortality assessments provide additional information overlooked by the contact efficacy tests recommended at present. It is therefore proposed that tests to evaluate the spatial mortality effect of insecticides are added to the battery of Phase I and Phase II WHOPES tests in the laboratory and the field. A tool to evaluate an insecticide product's lethal effect at a distance in the laboratory may be distance boxes although further studies are needed before this method is standardised. In the field, exposing mosquitoes at a fixed distance from treated surfaces may provide valuable information with little added effort. In order to better rationalize integrated vector control strategies, it may be important to assess the full profile of an insecticide by doing both contact and spatial lethal efficacy tests.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SMC had the idea of performing distance tests as part of the protocol to test for insecticide efficacy. SMC, PC and BM conceived the protocol. PC, SMC and BM contributed to the design of the study. FC and JMH contributed to the implementation of the study. JC and BM conducted evaluations. MA was the director of the Centre de Recherche Entomologique de Cotonou (CREC). The manuscript has been written by BM and has been revised by SMC and JC. All authors read and approved the final manuscript.

Acknowledgements

This study was funded by Project No. 11-CAP2-1558 of the Agencia Española de Cooperación Internacional para el Desarrollo (AECID), Ministry of Foreign Affaires, Madrid, Spain, by project ISCIII-RETIC RD12/0018/0013, Red de Investigación de Centros de Enfermedades Tropicales – RICET, of the Program of "Redes Temáticas de Investigación Cooperativa" RETICS/FEDER, Fondo de Investigación Sanitaria (FIS), Ministry of Health and Consumption, Madrid, Spain; and by project PROMETEO/2012/042 of the programme of Ayudas para Grupos de Investigación de Excelencia, Generalitat Valenciana, Valencia, Spain. We are grateful to volunteers at the Ladji Experimental Station for their good work. We thank Lazare Hounkarin for his dedication and technical support at the Ladji Station and CREC. We thank the University of Valencia in Spain, the Institut de Recherche pour le Développement (IRD) in France, and the Centre de Recherche Entomologique de Cotonou (CREC) in Benin for their support.

Author details

[1]Departamento de Parasitologia, Facultad de Farmacia, Universidad de Valencia, Av Vicent Andrés Estellés s/n, Burjassot 46100, Valencia, Spain. [2]Centre de Recherches Entomologique de Cotonou, 06 BP 2604, Cotonou, Benin. [3]Institut de Recherche pour le Développement (IRD), Laboratoire de Lutte contre les Insectes Nuisibles, BP 64501, Montpellier Cedex 5 34394, France. [4]Immeuble Le Majoral, Avenue de la Tramontane, Portiragnes Plage 34420, France.

Received: 11 June 2013 Accepted: 22 September 2013
Published: 18 October 2013

References

1. McGraw EA, O'Neill SL: **Beyond insecticides: new thinking on an ancient problem.** *Nat Rev Microbiol* 2013, **11:**181–193.
2. Lengeler C, Sharp B: *Indoor residual spraying and insecticide-treated nets*, Reducing malaria's burden: evidence of effectiveness for decision makers. Washington D.C: Global Health Council; 2003:17–24.
3. Lengeler C: **Insecticide-treated bed nets and curtains for preventing malaria.** *Cochrane Database of Syst Rev* 2008. http://www.cochrane.org.
4. WHO: *Guidelines for laboratory and field testing of long-lasting insecticidal mosquito nets: document WHO/HTM/NTD/WHOPES/2013.1.* Geneva: World Health Organization; 2013.
5. WHO: *Guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets: document WHO/CDS/NTD/WHOPES/GCDPP/2006.3.* Geneva: World Health Organization; 2006.
6. WHO: *Test procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces: report of the WHO informal consultation: document WHO/CDS/CPC/MAL/1998.12 pp1-43.* Geneva: World Health Organization; 1998.
7. WHO: *Report of the WHO informal consultation on the evaluation and testing of insecticides: document WHO/CTD/WHOPES/IC/1996.1.* Geneva: World Health Organization; 1996.
8. Chandre F, Darriet F, Duchon S, Finot L, Manguin S, Carnevale P, Guillet P: **Modifications of pyrethroid effects associated with *Kdr* mutation in *Anopheles gambiae*.** *Med Vet Entomol* 2000, **14:**81–88.
9. World Health Organization: *Guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets: WHO/CDS/NTD/WHOPES/GCDPP/2006.3.* Geneva: World Health Organization; 2006:1–60.
10. Darriet F, Guillet P, N'Guessan R, Doannio JMC, Koffi AA, Konan L, Carnevale P: **[Impact of resistance of *Anopheles gambiae* s.s. to permethrin and deltamethrin on the efficacy of impregnated mosquito nets](in French).** *Med Trop (Mars)* 1998, **58:**349–354.
11. Darriet F, N'Guessan R, Hougard JM, Traoré-Lamizana M, Carnevale P: **Un outil expérimental indispensable à l'évaluation des insecticides: les cases-pièges.** *Bull Soc Path Exot* 2002, **95:**299–303.
12. Beach RF, Ruebush TK, Sexton JD, Bright PL, Hightower AW, Breman JG, Mount DL, Oloo AJ: **Effectiveness of permethrin impregnated bednets and curtains for malaria control in a holoendemic area of western Kenya.** *Am J Trop Med Hyg* 1993, **49:**290–300.
13. Lines J, Myamba J, Curtis CF: **Experimental hut trials of permethrin-impregnated mosquito nets and eave curtains against malaria vectors in Tanzania.** *Med Vet Entomol* 1987, **1:**37–51.
14. Magesa SM, Wilkes TJ, Mnzava AEP, Njunwa KJ, Myamba J, Kivuyo MVP, Hill N, Lines JD, Curtis CF: **Trial of pyrethroid impregnated bednets in an area of Tanzania holoendemic for malaria: part 2: effects on the malaria vector population.** *Acta Trop* 1991, **49:**97–108.
15. Maxwell C, Myamba J, Njunwa KJ, Greenwood BM, Curtis CF: **Comparison of bednets impregnated with different pyrethroids for their impact on mosquitoes and on re-infection with malaria after clearance of pre-existing infections with chloroproguanil- dapsone.** *Trans R Soc Trop Med Hyg* 1999, **93:**4–11.
16. Maxwell CA, Msuya E, Sudi M, Njunwa KJ, Carneiro IA, Curtis CF: **Effect of community-wide use of insecticide-treated nets for 3–4 years on malarial morbidity in Tanzania.** *Trop Med Int Health* 2002, **7:**1003–1008.
17. Gimnig JE, Kolczak MS, Hightower AW, Vulule JM, Schoute E, Kamau L, Phillips-Howard PA, Kuile FO, Nahlen BL, Hawley WA: **Effect of permethrin-treated bed nets on the spatial distribution of malaria vectors in western Kenya.** *Am J Trop Med Hyg* 2003, **68**(Suppl 4):115–120.
18. Smith T, Hii JLK, Genton B, Muller I, Booth M, Gibson N, Narara A, Alpers A: **Associations of peak shifts in age prevalence for human malarias with bednet coverage.** *Trans R Soc Trop Med Hyg* 2001, **95:**1–6.
19. Howard SC, Omumbo J, Nevill C, Some ES, Donnelly CA, Snow RW: **Evidence for a mass community effect of insecticide-treated bednets on the incidence of malaria on the Kenyan coast.** *Trans R Soc Trop Med Hyg* 2000, **94:**357–360.
20. Binka FN, Indome F, Smith T: **Impact of spatial distribution of permethrin-impregnated bed nets on child mortality in rural northern Ghana.** *Am J Trop Med Hyg* 1998, **59:**80–85.
21. Curtis CF, Mnzava AEP: **Comparison of house spraying and insecticide-treated nets for malaria control.** *Bull World Health Organ* 2000, **78:**1389–1400.
22. Spanish Ministry of Health and Consumer Affairs: *Report on the study of the toxicity and irritability of Inesfly 5A.* Madrid: Health Institute Carlos III; 1996.
23. International center of training and medical investigations (CIDEIM): *Toxicity studies on Inesfly 5A IGR.* Cali (Colombia): CIDEIM; 2003. http://www.cideim.org.co.
24. *National center of tropical diseases (CENETROP), santa cruz de la sierra (Bolivia).* http://www.cenetrop.org.bo.
25. Amelotti I, Catalá SS, Gorla DE: **Experimental evaluation of insecticidal paints against *Triatoma infestans* (Hemiptera: Reduviidae), under natural climatic conditions.** *Parasit Vectors* 2009, **2:**30.

26. Dias JC, Jemmio A: **About an insecticidal paint for controlling *Triatoma infestans*, in Bolivia.** *Rev Soc Bras Med Trop* 2008, **41:**79–81.
27. Mosqueira B, Duchon S, Chandre F, Hougard JM, Carnevale P, Mas-Coma S: **Efficacy of an insecticide paint against insecticide-susceptible and resistant mosquitoes - part 1: laboratory evaluation.** *Malar J* 2010, **9:**340.
28. Mosqueira B, Chabi J, Chandre F, Akogbeto M, Hougard JM, Carnevale P, Mas-Coma S: **Efficacy of an insecticide paint against malaria vectors and nuisance in West Africa: part II: field evaluations.** *Malar J* 2010, **9:**341.
29. Darriet F, N'Guessan R, Carnevale P: **Evaluation in test huts of the protective effects of untreated mosquito nets against *Anopheles gambiae* s.s. bites.** *Cahiers Santé* 2000, **10:**413–417.
30. Hawley WA, Phillips-Howard PA, Feiko O, Kuile T, Terlouw DJ, Vulule JM, Ombok M, Nahlen BL, Gimnig JE, Kariuki SK, Kolczac MS, Hightower AW: **Community-wide effects of permethrin-treated bed nets on child mortality and malaria morbidity in Western Kenya.** *Am J Trop Med Hyg* 2003, **68**(suppl 4):121–127.
31. Charlwood JD, Alcantara J, Pinto J, Sousa CA, Rompao H, Gil V, Rosario VE: **Do bednets reduce malaria transmission by exophagic mosquitoes?** *Trans R Soc Trop Med Hyg* 2005, **99:**901–904.
32. Lenhart A, Orelus N, Maskill R, Alexander N, Streit T, McCall PJ: **Insecticide-treated bednets to control dengue vectors: preliminary evidence from a controlled trial in Haiti.** *Trop Med Int Health* 2008, **13:**56–67.
33. Teklehaimanot A, Sachs JD, Curtis C: **Malaria control needs mass distribution of insecticidal bed nets.** *Lancet* 2007, **369:**2143–2146.
34. Vanlerberghe V, Trongtokit Y, Jirarojwatana S, Jirarojwatana R, Lenhart A, Apiwathnasorn C, McCall PJ, Van der Stuyft P: **Coverage-dependent effect of insecticide-treated curtains for dengue control in Thailand.** *Am J Trop Med Hyg* 2013, **89:**93–98.
35. Kroeger A, Lenhart A, Ochoa M, Villegas E, Levy M, Alexandrer N, McCall PJ: **Effective control of dengue vectors with curtains and water container covers treated with insecticide in Mexico and Venezuela: cluster randomized trials.** *BMJ* 2006, **332:**1247–1252.
36. Grieco JP, Achee NL, Chareonviriyaphap T, Suwonkerd W, Chauhan K, Sardelis MR, Roberts DR: **A new classification system for the actions of IRS chemicals traditionally used for malaria control.** *PLoS ONE* 2007, **2:**e716. doi:10.1371/journal.pone.0000716.
37. Achee NL, Bangs MJ, Farlow R, Killeen GF, Lindsay S, Logan JG, Moore SJ, Rowland M, Sweeney K, Torr SJ, Zwiebel L, Grieco JP: **Spatial repellents: from discovery and development to evidence-based validation.** *Malar J* 2012, **11:**164.

doi:10.1186/1475-2875-12-366
Cite this article as: Mosqueira *et al.*: Proposed use of spatial mortality assessments as part of the pesticide evaluation scheme for vector control. *Malaria Journal* 2013 **12**:366.

Acta Tropica 148 (2015) 162–169

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Pilot study on the combination of an organophosphate-based insecticide paint and pyrethroid-treated long lasting nets against pyrethroid resistant malaria vectors in Burkina Faso

Beatriz Mosqueira[a,*], Dieudonné D. Soma[b], Moussa Namountougou[b], Serge Poda[b], Abdoulaye Diabaté[b], Ouari Ali[b], Florence Fournet[c], Thierry Baldet[d], Pierre Carnevale[e], Roch K. Dabiré[b], Santiago Mas-Coma[a]

[a] Departamento de Parasitologia, Facultad de Farmacia, Universidad de Valencia, Av Vicent Andrés Estellés s/n, Burjassot, 46100 Valencia, Spain
[b] Institut de Recherche en Sciences de la Santé (IRSS)/Centre Muraz, Bobo-Dioulasso 01 BP 545, Burkina Faso
[c] Institut de Recherche pour le Développement (IRD), BP 64501, 34394 Montpellier Cedex 5, France
[d] Cirad, UMR15 CMAEE; INRA, UMR1309 CMAEE, Montpellier, France
[e] Immeuble Le Majoral, Avenue de la Tramontane, 34420 Portiragnes Plage, France

ARTICLE INFO

Article history:
Received 15 January 2015
Received in revised form 18 March 2015
Accepted 11 April 2015
Available online 7 May 2015

Keywords:
Inesfly 5A IGR™
LLINs
Pyrethroid resistance
Anopheles coluzzii
Malaria control

ABSTRACT

A pilot study to test the efficacy of combining an organophosphate-based insecticide paint and pyrethroid-treated Long Lasting Insecticide Treated Nets (LLINs) against pyrethroid-resistant malaria vector mosquitoes was performed in a real village setting in Burkina Faso. Paint Inesfly 5A IGR™, comprised of two organophosphates (OPs) and an Insect Growth Regulator (IGR), was tested in combination with pyrethroid-treated LLINs. Efficacy was assessed in terms of mortality for 12 months using Early Morning Collections of malaria vectors and 30-minute WHO bioassays. Resistance to pyrethroids and OPs was assessed by detecting the frequency of L1014F and L1014S *kdr* mutations and *Ace-1*^{R}G119S mutation, respectively. Blood meal origin was identified using a direct enzyme-linked immunosorbent assay (ELISA). The combination of Inesfly 5A IGR™ and LLINs was effective in killing 99.9–100% of malaria vector populations for 6 months regardless of the dose and volume treated. After 12 months, mortality rates decreased to 69.5–82.2%. The highest mortality rates observed in houses treated with 2 layers of insecticide paint and a larger volume. WHO bioassays supported these results: mortalities were 98.8–100% for 6 months and decreased after 12 months to 81.7–97.0%. Mortality rates in control houses with LLINs were low. Collected malaria vectors consisted exclusively of *Anopheles coluzzii* and were resistant to pyrethroids, with a L1014 kdr mutation frequency ranging from 60 to 98% through the study. About 58% of *An. coluzzii* collected inside houses had bloodfed on non-human animals. Combining Inesfly 5A IGR™ and LLINs yielded a one year killing efficacy against *An. coluzzii* highly resistant to pyrethroids but susceptible to OPs that exhibited an anthropo-zoophilic behaviour in the study area. The results obtained in a real setting supported previous work performed in experimental huts and underscore the need to study the impact that this novel strategy may have on clinical malaria and malaria exposure in children in a similar area of high pyrethroid resistance in South-Western Burkina Faso.

1. Background

Malaria transmission occurs in 97 countries, putting about 3.4 billion people at risk (WHO, 2013). In Africa, it is estimated that in 2012 alone, about 165 million people suffered from malaria and about 562,000 people died from causes attributed to malaria. About 86% of those deaths were among children under 5 years of age (WHO, 2013). In addition to the devastating impact on human health, malaria also imposes an enormous economic burden, estimated at 1.3% of economic growth per year in sub Saharan Africa (WHO, 2013). Primary prevention of malaria on a large scale is essentially achieved through vector control using mostly Long Lasting Insecticide Treated Nets (LLINs) and, to a lesser extent, Indoor Residual Spraying (IRS) (WHO, 2013). Between 2008 and 2010, 254 million LLINs were supplied to countries in sub-Saharan Africa (WHO, 2013). All currently recommended LLINs are treated

* Corresponding author. Tel.: +34 96 354 4298.
E-mail addresses: bmosqueira@yahoo.com (B. Mosqueira), dieusoma@yahoo.fr (D.D. Soma), namountougou_d@yahoo.fr (M. Namountougou), sergepoda71@yahoo.fr (S. Poda), a_diabate@hotmail.com (A. Diabaté), ouari_ali@yahoo.fr (O. Ali), florence.fournet@ird.fr (F. Fournet), thierry.baldet@cirad.fr (T. Baldet), pjcarnevale2001@yahoo.fr (P. Carnevale), dabire_roch@hotmail.com (R.K. Dabiré), s.mas.coma@uv.es (S. Mas-Coma).

http://dx.doi.org/10.1016/j.actatropica.2015.04.010
0001-706X/

with pyrethroids. Protection using IRS reached 58 million people in Africa – representing 8% of the global population at risk – in 2012 as reported by National Malaria Control Programmes (WHO, 2013). In 2009, pyrethroids were estimated to account for about 75% of IRS coverage, while DDT was the second most widely used insecticide; carbamates and organophosphates (OPs) represented only small percentages of global usage (WHO, 2012). LLINS and IRS remain efficient and cost-effective tools for malaria control across a large number of settings (Lengeler and Sharp, 2003). The raised levels of pyrethroid resistance among malaria mosquito vectors (Chandre et al., 1999; Diabaté et al., 2004; Dabiré et al., 2012) and subsequent reports on reduced efficacy of pyrethroid-based vector control tools (Toé et al., 2014) are a source of concern. However, there is yet no final consensus on whether *kdr* based modifications reduce significantly the efficacy of insecticides operationally speaking (Briët et al., 2013; Hemingway, 2014). Furthermore, the use of LLINs is advocated because, when well used and intact, it will help reduce bloodfeeding thus increasing individual protection (Trape et al., 2014). Apart from the issue of pyrethroid resistance, there are operational obstacles surrounding LLINs (Toé et al., 2009; MCHIP/USAID/PNLP, 2013) and IRS (Najera and Zaim, 2001) potentially rendering these tools less operationally effective in protecting againt malaria. To summarize, LLINs and IRS remain the cornerstone in malaria vector control but there is a growing need to find alternative malaria vector control strategies that can be added to the list of tools to choose from (Beier et al., 2008). The National Malaria Control Programme ("Programme National de Lutte contre le Paludisme"—PNLP) of the Ministry of Health in Burkina Faso, distributed more than 8 million nets were to a population of around 16 million targeted to the population at risk, children under 5 years old and pregnant women (MCHIP/USAID/PNLP, 2013). Thus, rather than departing from LLINs, the strategy implemented in this study enforced their use, in line with the PLNP efforts.

The LLINs in this study were PermaNet® 2.0 that had been distributed in the area by the PNLP in 2013 (MCHIP/USAID/PNLP, 2013) and were confirmed by the team to be well used by the population and intact. Insecticide paint Inesfly 5A IGR™ is a "cocktail" consisting of two OPs, chlorpyriphos and diazinon, and an insect growth regulator (IGR), pyriproxyfen. The paint was applied on plastic sheetings with no need of special equipment and placed in real houses, in a village in the Kou Valley, South-Western Burkina Faso, where there is high pyrethroid resistance among malaria mosquito vectors per the high frequency of the L1014F *kdr* mutation (Dabiré et al., 2008, 2009). Toxicology studies performed so far support the paint's safety (Spanish Ministry of Health and Consumer Affairs (SMHCA), 1996; International Center of Training and Medical Investigations (ICTM), 2003; National Center of Tropical Diseases, 2004). Inesfly 5A IGR™ has been evaluated previously under experimental conditions in South America against the Chagas disease vector *Triatoma infestans* (Dias and Jemmio, 2008; Amelotti et al., 2009; Maloney et al., 2013).

Tests were also performed following the WHO Pesticide Evaluation Scheme (WHOPES) procedures (WHO, 1996) on Inesfly 5A IGR™ in the laboratory (Phase I), against 100% OP-resistant *Culex quinquefasciatus* (Mosqueira et al., 2010a), and in experimental houses in the field (Phase II), against wild pyrethroid-resistant populations of the main malaria vector, *Anopheles gambiae*, and pest mosquito, *Cx. quinquefasciatus* in Benin (Mosqueira et al., 2010b). In the laboratory, one year after treatment delayed mortality was 93–100% even against OP-resistant females on non-porous surfaces like hard plastic or softwood (Mosqueira et al., 2010a). Pyriproxifen was added to the paint to confer and additional angle of attack against mosquito females once the OP effect diminishes over time. The effect of pyriproxyfen has been studied in the lab, where it was shown that pyriproxyfen had an effect on the fecundity, fertility and adult emergence of exposed adult females once the lethal effect of OPs diminished over time even against OP-resistant mosquitoes (Mosqueira et al., 2010a). In the field, on porous surfaces made of cement, mortality rates were 90–100% against pyrethroid-resistant mosquito populations six months after treatment. Nine months after treatment, mortality rates in huts treated with two layers was still about 90–93% against *An. gambiae* and 55% against *Cx. quinquefasciatus*, both resistant to pyrethroids (Mosqueira et al., 2010b). In addition, a high spatial long term mortality (96–100%) was obtained for 12 months in the field on mosquitoes that were kept at distances of one meter overnight, never entering in direct contact with treated surfaces (Mosqueira et al., 2010b, 2013).

The objective of the present study was to assess the efficacy of Paint Inesfly 5A IGR™ in combination with pyrethroid-treated LLINs in real-life houses in a village setting. This pilot study supported the previous Phase II studies performed in experimental huts (Mosqueira et al., 2010b) and provided useful information on the method to apply the paint, perform the mosquito collections and mosquito populations, in preparation for the forthcoming large scale Phase III cluster randomized controlled evaluation to assess the impact of this combination strategy on the incidence of clinical malaria and malaria exposure in children aged from 6 months to 14 years old in a similar area of high pyrethroid resistance in South-Western Burkina Faso.

2. Methods

2.1. Study site and mosquitoes

The study was conducted in the Kou Valley, a rice growing area in South-Western Burkina Faso, West Africa. It is located at 30 km in the North of Bobo-Dioulasso (lat. 11°23′14″N and long. 4°24′42″W) and is composed of 7 villages with a total of 4470 habitants in 2013. The study was conducted specifically at the VK1 village (Fig. 1). Irrigation has existed in this area since 1972, and is now semi-permanent with two crops grown per year: from February to June during the dry season and from July to November during the rainy season. The study area was chosen because of its high malaria transmission, its high frequency of the L1014F *kdr* mutation, rendering local malaria vector populations highly resistant to pyrethroids and DDT (Dabiré et al., 2008, 2009). Both *An. gambiae* (former *An. gambiae* S form) and *Anopheles coluzzii* (former *An. gambiae* M form) coexist in sympatry in the study area, but *An. coluzzii* is preponderant within the rice field habitats. As part of the necessary background information, the exact species were determined molecularly (Santolamazza et al., 2008). The study was performed continuously for six months, from June to December 2013, and then again in June 2014, 12 months after treatment.

2.2. Insecticide paint and LLINs

Inesfly 5A IGR™ contains two organophosphates, chlorpyriphos (1.5%) and diazinon (1.5%), and an insect growth regulator (IGR), pyriproxyfen (0.063%), as active ingredients. The formulation is vinyl white-coloured paint with an aqueous base, with the active ingredients residing within CaCO3 and resin microcapsules, allowing a gradual release of active ingredients. Microcapsules range from one to several hundred micrometers in size. The paint was applied on plastic sheetings with no need of special equipment, just a regular brush and gloves. Polypropylene plastic sheeting was bought at the local market and consisted of big plastic rolls cut and fit into the study houses. The plastic sheeting was used to homogenize test surfaces as some houses were made of adobe and some of cement. The plastic sheeting was then placed on the superior two thirds of interior house walls and ceilings. The lower part of all walls was left untreated for up to 1 m for all houses to reduce direct

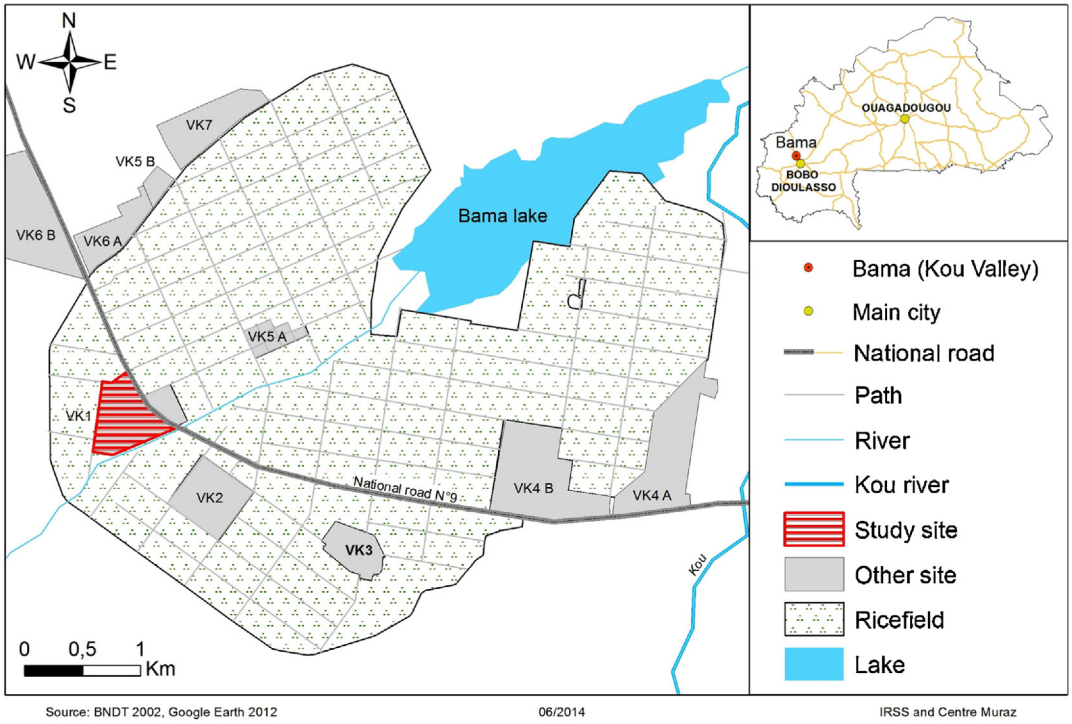

Fig. 1. Location of VK1 at Kou Valley in South-Western Burkina Faso.

exposure to both, babies and young toddlers. The LLINs in this study were PermaNet® 2.0, made of multifilament polyester netting (100 denier) factory impregnated with deltamethrin at 55 mg/m2 in a wash-resistant binder system that had been distributed locally by the PNLP in 2013. All nets were checked prior to the study and were found to be intact and correctly used by the owners.

2.3. Early morning collections (EMCs)

Inesfly 5A IGR^{TM} was evaluated in 14 real-life village houses at VK1. The 14 houses at VK1 were chosen based on owners' wish to participate and equivalence in dimensions. The control houses consisted on plastic sheetings with no paint, but with intact LLINs. For the treated houses, paint was applied on plastic sheetings with one or two layers of insecticide paint at 1 kg commercial product/6 m^2, that is 0.51 g a.i. per m^2. Huts treated with two layers had the first layer diluted in 20% water following recommendations of the manufacturer. The ceilings of certain houses were also covered with painted plastic sheeting per the configuration below. The different configurations were treated in duplicate, in two houses each. Experimental hut studies commonly use one single hut per configuration (WHO, 2006). However, because this study was done using real houses that were similar but not identical to each other, we used two houses per configuration, collected for several nights in a row and performed the week of blank collections prior to treatment and rotated the volunteers. Configurations were designed to allow the evaluation of a potential volume effect and dose effect.

(1) 2 houses = control sheeting with no paint + LLIN.
(2) 2 houses = regular paint 1 layer + insecticide paint 1 layer on walls only + LLIN.
(3) 2 houses = regular paint 1 layer + insecticide paint 1 layer on walls and ceiling + LLIN.
(4) 2 houses = insecticide paint: 1 layer on walls only + LLIN.
(5) 2 houses = insecticide paint: 1 layer on walls and ceiling + LLIN.
(6) 2 houses = insecticide paint: 2 layers on walls only + LLIN.
(7) 2 houses = insecticide paint: 2 layers on walls and ceiling + LLIN.

Mortality was the entomological indicator evaluated during this pilot study under real conditions. As there was no verandah, the exito-repellent effect generally assessed in Phase II WHOPES protocols, could not be implemented. Similarly, although house dimensions were similar, the number and size of openings (windows and doors) were too different to reliably evaluate the deterrent effect and bloodfeeding inhibition.

Before any treated sheetings were applied, mosquito collections took place for 1 full week just with LLINs to ensure there that there was no difference between houses in attractiveness to mosquitoes. Between June and December 2013 and again in June 2014, mosquito collections were performed nightly at VK1. The study was approved by the Ethics Committee of "Institut de Recherche en Sciences de la Santé" (IRSS) at Centre Muraz. Sixteen volunteers 18 years old or older were recruited from the population at VK1–2 volunteers served as back ups in case it was needed. After being informed about the study and discussing it, these volunteers provided an informed consent in writing or with a finger print if illiterate. The volunteers received training on mosquito collection procedures. At the first suspicion of malaria, volunteers were provided with the curative treatment recommended by the PNLP in Burkina Faso. Furthermore, all houses were checked and had intact well used LLINs. Volunteers rotated houses each night to avoid bias while avoiding contamination between houses. The lower part of doors were covered with cloth to reduce the number of scavengers from entering houses. Houses were broomed every morning and every evening to remove scavengers that made it in through other openings. There was one volunteer sleeping per house. Mosquito collections were performed to assess mortality rates. Volunteers would enter their houses at 18:00 h, one volunteer per house, and sleep under LLINs until 5:30 h, when they would be awaken to close the windows (that had been left open during the night as it is commonly done in the area). Once windows were closed at 5:30 h, the volunteer

proceeded to collect mosquitoes within the house. After classifying mosquito females as dead or alive, they were put in observation for delayed mortality assessments after 24 h. All mosquitoes were then conserved in silica gel at −20 °C to identify the species, resistance status and source of blood meal.

2.4. Residual efficacy tests

Thirty-minute standard WHO cone bioassays (WHO, 1998) were carried out using 2–4 days old unfed females of *An. gambiae* Kisumu, a reference strain susceptible to all insecticides reared at the IRSS/Centre Muraz insectarium. The local population identified molecularly as *An. coluzzii* and resistant to pyrethroids was reared at the insectarium from field caught larvae to the adult stage and was also tested in parallel to *An. gambiae* Kisumu. For each house, 10 females were introduced in 5 cones placed on five sides of the house (4 walls and ceiling) for 30 min. Cones were not placed on LLINs. Delayed mortality was observed 24 h later. Tests were performed monthly at T0, T1, T3, T6 and T12 after treatment.

2.5. Molecular analysis on resistance

The detection of *kdr* resistance genes was performed following protocols developed for the L1014F *kdr* mutation (Martinez-Torres et al., 1998), for the L1014S *kdr* mutation (Ranson et al., 2000), as well as the detection of the *Ace-1*^{R}G119S mutation (Weill et al., 2004). Testing took place each month for 5 months after treatment on *An. coluzzii* females collected in control houses and houses treated with 1 or 2 layers of insecticide paint on walls and ceiling.

2.6. Determination of blood meal source

Blood meal identification was performed using a direct enzyme-linked immunosorbent assay (ELISA) (Beier et al., 1988). The choice of antibodies tested was based on the animals that are more frequent in the study area. Six antibodies were tested: human, dog, sheep, donkey, cattle and pig. These antibodies, marked with peroxidase, were kept at +4 °C. Bloodfed *Anopheles* females collected during EMCs from June to December 2013 in control houses and houses treated with 1 or 2 layers of insecticide paint on walls and ceiling were tested. A total of 425 females identified molecularly as *An. coluzzii* were tested from each of those 3 configurations (>140 per configuration) to determine the source of the blood meal.

2.7. Statistical analysis

Results on mortality were compiled and analyzed using Epi-Info Version 6 to test for any significant difference in mortality rates between the different configurations via Chi square tests. A 95% confidence interval was applied. When mortality rates in control huts were between 5 and 20% Abbott's mortality correction formula was applied. Because bioassay tests are subject to variations, a 99% confidence interval was applied. The allelic frequency of each mutation (kdr and ace-1R) was calculated using the formula $F(R) = (2RR + RS)/2n$ where n is the total sample size, using GenePop version 4.

3. Results

3.1. Early morning collections (EMC)

No difference in house attractiveness was found prior to treatment. *An. coluzzii* (former *An. gambiae* form M) was the only *An. gambiae s.l.* species present in the study area as established from the molecular analysis performed during the study. Between June and December 2013 and June 2014, a total of 3903 females belonging to the *An. gambiae* complex identified molecularly as *An. coluzzii*, were collected in all houses combined. Full collections started one month after treatment (Table 1). For the first 6 months, the mortality rates observed in houses treated with the insecticide paint were 97–100%. Globally, 6 months after treatment, all houses treated with the insecticide paint, with 1 or 2 layers, on walls or on walls and ceiling, presented 100% mortality rates against wild populations of *An. coluzzii* whether they were bloodfed or not and were statistically significantly different from control ($p < 0.001$). By T12, mortalities were still high and significantly different from control ($p < 0.001$), but rates had slightly decreased to 69.5–82.2%. The highest mortality rates 12 months after treatment were observed in houses treated with 2 layers of insecticide paint and a larger volume (82.2%). No statistically significant differences were found between treated houses at T12. Mortality rates observed in control houses with no insecticide paint but with LLINs ranged from 5.2 to 9.5%, throughout the study (Table 1).

Table 1
Mortality rates on wild populations of *Anopheles coluzzii* at VK1 using EMCs. Averages taken for each configuration, 2 houses per configuration. C = control with LLINs only; RP = regular Paint; IP = insecticide paint; *T* = time in months since treatment. EMCs = early morning collections. Numbers in the same column sharing a letter superscript do not differ significantly ($p > 0.05$).

% Mortality in *Anopheles coluzzii* collected via EMCs	T1	T3	T6	T12
C (LLINs)	9.5^{a}	5.2^{a}	8.9^{a}	7.6^{a}
RP/1 layer + IP/1 layer walls + LLINs	100^{b}	100^{b}	100^{b}	78.6^{b}
RP/1 layer + IP/1 layer walls + ceiling + LLINs	100^{b}	100^{b}	100^{b}	69.5^{b}
IP/1 layer walls + LLINs	100^{b}	100^{b}	100^{b}	78.9^{b}
IP/1 layer walls + ceiling + LLINs	100^{b}	100^{b}	100^{b}	79.9^{b}
IP/2 layers walls + LLINs	100^{b}	99.9^{b}	100^{b}	78.5^{b}
IP/2 layers walls + ceiling + LLINs	100^{b}	100^{b}	100^{b}	82.2^{b}

3.2. Residual efficacy tests

Thirty-minute standard WHO cone bioassays on *An. gambiae* "Kisumu" and local populations of *An. coluzzii* from VK1, yielded mortality rates of 98–100% in all houses treated with insecticide paint (Table 2) regardless of the configuration. Mortality in control houses was lower and significantly different from treated houses, but because mortality was over 5% (but always less than 20%), the Abbott formula was applied. Mortality rates were 100% at T6 against both *An. gambiae* "Kisumu" and local populations of *An. coluzzii* from VK1, in all treated houses. Mortality rates at T12 were still 98–100% in all houses against *An. gambiae* "Kisumu". In the case of the local *An. coluzzii* from VK1, 12 months after treatment mortality rates were 97% in houses treated with 2 layers of insecticide paint on walls and ceiling, but slightly lower mortalities were observed in the other configurations. These differences were not statistically significant ($p > 0.05$). Mortality rates observed in control houses with LLINs only ranged from 1.7% to 10.9% (Table 2). Again, cones were only placed on walls and ceiling, not on LLINs.

3.3. Molecular Analysis on resistance

3.3.1. Allelic frequency of the L1014F and L1014S kdr mutations

All houses contained pyrethroid treated LLINs. Also, because the *Anopheles* females collected in treated houses were dead and around 89% to 94% of the females were alive in control houses, no comparisons could be done between dead and alive mosquitos within each given configuration. Thus, comparisons were done overtime between control houses with LLINs and treated houses with LLINs and 1 or 2 layers of insecticide paint. Overall, *An. coluzzii* females at VK1 were pyrethroid resistant: the allelic frequency of the L1014F *kdr* mutation was high, ranging from 60 to 98% (Table 3)

Table 2
Residual efficacy tests on (A) *Anopheles gambiae* "Kisumu" and (B) *Anopheles coluzzii* VK1 using WHO test cones. Averages taken for each configuration, 2 houses per configuration. C = control with LLINs only; RP = regular paint; IP = insecticide paint; *T* = time in months since treatment. Numbers in the same column sharing a letter superscript do not differ significantly ($p > 0.05$). Molecular analysis on resistance Allelic frequency of the L1014F and L1014S kdr mutations *Anopheles coluzzii* VK1 (B).

% Mortality in *Anopheles coluzzii* using WHO test cones	*Anopheles gambiae* Kisumu (A)					*Anopheles coluzzii* VK1 (B)				
	T0	T1	T3	T6	T12	T0	T1	T3	T6	T12
C (LLINs)	10.9[a]	7.9[a]	6.1[a]	5.6[a]	6.9[a]	1.7[a]	2.6[a]	2.9[a]	2.1[a]	2.1[a]
RP + IP/1 layer walls + LLINs	100[b]	100[b]	100[b]	100[b]	99.0[b]	100[b]	100[b]	100[b]	98.9[b]	90.9[b]
RP + IP/1 layer walls + ceiling + LLINs	100[b]	100[b]	98.1[b]	100[b]	99.0[b]	100[b]	100[b]	100[b]	99.0[b]	91.3[b]
IP/1 layer walls + LLINs	100[b]	100[b]	98.0[b]	100[b]	99.0[b]	100[b]	100[b]	100[b]	100[b]	85.0[b]
IP/1 layer walls + ceiling + LLINs	100[b]	100[b]	100[b]	100[b]	98.1[b]	100[b]	100[b]	100[b]	100[b]	81.8[b]
IP/2 layers walls + LLINs	100[b]	100[b]	100[b]	100[b]	100	100[b]	100[b]	100[b]	98.8[b]	88.9[b]
IP/2 layers walls + ceiling + LLINs	100[b]	100[b]	100[b]	100[b]	100	100[b]	100[b]	100[b]	100[b]	97.0[b]

Table 3
Distribution of the frequency of L1014F and L1014S kdr mutations in *Anopheles coluzzii* in VK1. C = control with LLINs only; IP = insecticide paint; *n* = number of mosquitoes tested; *T* = time in months since treatment; *F*(*kdr*) = frequency of the mutation *kdr*; *p* (HW) = value for Hardy–Weinberg equilibrium hypothesis; "–" = non determinable.

Treatments	Month	*n*	SS	RS	RR	*F*(*L1014F kdr*)	*p* (HW)	SS	RS	RR	*F*(L1014S *kdr*)	*p* (HW)
C (LLINs)	T0	30	7	3	20	0.717	0.0001	30	0	0	0	–
	T1	30	0	0	30	0.98	–	30	0	0	0	–
	T2	30	11	2	17	0.6	0	30	0	0	0	–
	T3	31	8	3	20	0.694	0	31	0	0	0	–
	T4	30	7	0	23	0.767	0	30	0	0	0	–
	T5	25	5	0	20	0.8	0	25	0	0	0	–
IP/1 layer walls + ceiling + LLINs	T0	30	3	0	27	0.9	0.0001	30	0	0	0	–
	T1	28	1	0	27	0.964	–	30	0	0	0	–
	T2	30	4	3	23	0.817	0.002	27	3	0	0.05	1
	T3	31	6	1	24	0.79	0	30	1	0	0.016	–
	T4	29	3	6	20	0.793	0.066	24	5	0	0.086	1
	T5	30	4	7	19	0.75	0.048	23	7	0	0.117	1
IP/2 layers walls + ceiling + LLINs	T0	30	4	0	26	0.867	0	30	0	0	0	–
	T1	31	0	0	31	0.98	–	30	0	0	0.001	–
	T2	30	4	0	26	0.867	0	30	0	0	0	–
	T3	31	9	0	22	0.71	0	31	0	0	0	–
	T4	29	3	0	26	0.897	0.0001	29	0	0	0	–
	T5	30	4	10	16	0.7	0.378	20	10	0	0.167	0.563

with no significant difference between alive specimens collected from the control and dead specimens collected from the treated houses during the period tested, up to 5 months after treatment. Similarly, no increasing or decreasing trends were identified on the allelic frequency overtime. The L1014S *kdr* was not found in the samples collected in control houses with LLINs and was weakly detected in the heterozygous form in houses treated with 1 layer starting at T2, T4 and T5, and in houses treated with 2 layers, at T5, though only in the heterozygote form (Table 3).

3.3.2. *Allelic frequency of the mutation Ace-1R*

The *Ace1*^{R} mutation was detected at low allelic frequencies and was heterozygous. It was only randomly found at T0 and T5 in the control houses at frequencies of 8.3 and 4.0%, respectively (Table 4) and at no point in the treated houses.

3.3.3. *Determination of the bloodfeeding origin*

There were no statistical differences between control houses, houses treated with 1 insecticide paint layer, and houses with 2 insectide paint layers (Table 5). The averages of all houses combined from T0 to T6, showed about 27% of females had fed on humans, about 58% on other animals and about 16% on both. All in all, the rate of zoophily was high (58%). Of the females having bloodfed on other animals (non human), about 45% of them had not blood fed on any of the domestic animals chosen as the most typical blood meal sources in the area. Of the identified domestic animals, cattle remained the most common blood meal source (Table 5).

4. Discussion

The study area was chosen based on parameters such as insecticide resistance and malaria transmission levels (Dabiré et al., 2008, 2009). In addition, the team was drawn by the population's interest on the paint and the efforts that home owners had previously undergone to try to paint the interior of their homes and the edges of windows and doors when their economic level allowed it. From that standpoint, the study area presented an optimal profile to perform a pilot study on the efficacy of combining an OP-based paint and LLINs. The fact that classical WHOPES Phase II experimental huts were not used posed some liminations on the measurement of certain entomological parameters (discussed throughout the text), but allowed the assessment of how the Phase III trial may be implemented. Furthermore, the results obtained in this pilot study supported previous findings observed during the WHOPES Phase I in the laboratory and Phase II study in experimental huts in the South of Benin using the same paint, Inesfly 5A IGR^{TM}, in terms of entomological mortality rates, the porosity of materials and the notion of volume effect discussed below. In this pilot study, the combination of the insecticide paint Inesfly 5A IGR^{TM} consisting of two different OPs with an IGR, and pyrethroid-treated LLINs was able to control *An. coluzzii* (former *An. gambiae* form M) populations yielding mortality rates of 100% for 6 months after treatment regardless of the treatment configuration in terms of volume (walls or walls + ceiling), dose of insecticide paint and number of layers. With time, however, houses with two layers of insecticide paint and a larger volume benefited with a higher long term efficacy. The mortality rates observed during mosquito collections on the pilot

Table 4
Allelic frequency and genotype of the *Ace-1*^{R} mutation in *Anopheles coluzzii* at VK1. C = control with LLINs only; IP = insecticide paint; *n* = number of mosquitoes tested; *T* = time in months since treatment; *f*(119S) = allelic frequency of the mutation ace-1 119S; *p* (HW) = value for Hardy–Weinberg equilibrium hypothesis; "–" = non determinable.

Treatment	Month	*n*	Genotypes			*f*(119S)	[95%CI]	*p* (HW)
			119G 119G	119G 119S	119S 119S			
C (LLINs)	T0	30	25	5	0	0.083	[0.00–0.18]	1
	T1	30	30	0	0	0	–	–
	T2	30	30	0	0	0	–	–
	T3	30	30	0	0	0	–	–
	T4	30	30	0	0	0	–	–
	T5	23	21	2	0	0.04	[0.00–0.12]	1
IP/1 layer walls + ceiling + LLINs	T0	30	30	0	0	0	–	–
	T1	30	30	0	0	0	–	–
	T2	30	30	0	0	0	–	–
	T3	30	30	0	0	0	–	–
	T4	30	30	0	0	0	–	–
	T5	30	30	0	0	0	–	–
IP/2 layers walls + ceiling + LLINs	T0	30	30	0	0	0	–	–
	T1	30	30	0	0	0	–	–
	T2	30	30	0	0	0	–	–
	T3	30	30	0	0	0	–	–
	T4	30	30	0	0	0	–	–
	T5	24	24	0	0	0	–	–

Table 5
Analysis of the blood source of bloodfed *Anopheles coluzzii* collected using EMCs at VK1. C = control with LLINs only; IP = insecticide paint; *n* = numbers of mosquitoes tested; T0–T6 = period from June to December 2013 when collected *Anopheles coluzzii* were pooled and randomly tested for bloodfeeding source. Numbers in the same column sharing a letter superscript do not differ significantly ($p > 0.05$).

Treatment	*Anopheles coluzzii* females tested (T0–T6)	Humans		Other animals								Mixed	
		n	%	Cattle	Sheep	Donkey	Pig	Dog	Other	*n*	%	*n*	%
C (LLINs)	141	35	24.8[a]	16	8	18	7	5	39	93	66.0 [a]	13	9.2[a]
IP/1 layer walls + ceiling + LLINs	143	51	35.7[a]	21	4	3	5	4	33	70	49.0[a]	22	15.4[a]
IP/2 layers walls + ceiling + LLINs	141	28	19.9 [a]	30	3	9	0	2	38	82	58.2[a]	31	22.0[a]
Total	425	114	26.8	67	15	30	12	11	110	245	57.6	66	15.5

study in VK1, in real houses, were also supported by the long-term residual tests using WHO cone tests. Mortality rates in all treated houses remained 98.9–100% for 6 months against both *An. gambiae* "Kisumu" (the insecticide-susceptible laboratory reference strain) and the pyrethroid-resistant *An. coluzzii* populations in VK1. Results obtained 12 months after treatment using WHO cones confirm that, in the long term, houses with two layers and a larger volume performed best. The results obtained using EMCs and WHO cones are in consistence with previous studies performed in an experimental field setting in Benin with the same paint (Mosqueira et al., 2010b), where huts treated with two layers of insecticide paint and, particularly, a larger volume had a longer lasting efficacy. The observed volume effect was in line with previous observations during the Phase II trial in the South of Benin (Mosqueira et al., 2010b) and a study performed in experimental huts on carbamate-treated plastic sheeting used concomitantly with nets treated with deltamethrin at 25 mg/m^2 (Djènontin et al., 2009). Overtime, starting mildly at T6 but becoming more evident by T12, the mortality rates observed in treated houses were higher on this study than those observed in experimental huts made of cement in Ladji, South of Benin (Mosqueira et al., 2010b). This was probably linked to the high porosity of cement compared to plastic sheeting used in VK1 as supported by Phase I studies exploring the effect that the porosity of materials have on the long term efficacy of insecticide treated surfaces (Mosqueira et al., 2010a). The treatment of plastic sheeting was seeked as an interim decision to test the efficacy of the paint under optimal conditions while the manufacturer improves the sealing qualities of the paint so the paint is applied directly on walls.

Several studies have assessed vector mortality rates when combining sheetings or IRS with pyrethroid-treated nets: a study carried in experimental huts in the Kou Valley in Burkina Faso showed mortality rates of carbamate-treated plastic sheeting and LLINs were superior to sprayed carbamates via IRS and control using just LLINs (Djènontin et al., 2010). A study performed in experimental huts in Tanzania that tested several IRS compounds used concomitantly with LLINs showed IRS with DDT or pyrethroids did not confer additional value to LLINs alone, but showed IRS with OPs could be effective in preventing blood feeding and increasing vector mortality when combined with LLINs (Okumu et al., 2013). These studies suggest there may be value in adding a non-pyrethroid insecticide paint, insecticide treated plastic sheetings or IRS to LLINs.

Understanding the bio-ecology and spatio-temporal distribution of the malaria vector in the study area is important (Ferguson et al., 2010; The malERA Consultative Group on Vector Control, 2011; Sinka et al., 2012). During the study period, local wild populations were genomically identified as *An. coluzzii* (former *An. gambiae* form M) exclusively. The two reproductive units formerly referred to 'M' and 'S' molecular forms, are now officially recognised as *An. coluzzii* Coetzee & Wilkerson 2013 and *An. gambiae s.s.* Giles 1902 based on population genomic evidence (Coetzee et al., 2013). Whilst implementing vector control strategies, old or new, monitoring insecticide resistance is increasingly central (Enayati & Hemingway, 2010). *Anopheles coluzzii* in the study area showed high frequencies (ranging from 60 to 98%) of the target site L1014F *kdr* mutation that confers cross-resistance to pyrethroids and DDT (Martinez-Torres et al., 1998). There is a concern that concomitant

use of pyrethroids for IRS and LLINs could increase the pressure for resistance development in vector populations (WHO, 2011, 2012). The potential of this novel strategy for resistance development was assessed briefly during five months. Tests performed during the testing period showed the allelic mutation *kdr* L1014F did not vary significantly during the testing period. This was not the case for the mutation *kdr* L104S revealed in Burkina Faso in recent years (Dabiré et al., 2009). The distribution of the allelic frequencies of *kdr* L104S were low and heterozygous, but appeared 3 months after treatment in houses treated with insecticide paint and LLINs but not in control houses with LLINs alone. The above results provide only some indication that the combination of LLINs and the insecticide paint Inesfly does not select for this mutation. In order to properly assess this risk, a longer term full protocol will be developed and carried during the phase III study. With regard to the *ace-1*^{R} mutation, *An. coluzzii* in VK1 are considered to be susceptible to OPs as the distribution of the *ace-1*^{R} mutation is still low thus far (less than 10% overall) and in the heterozygous form.

The mortality rates observed in control houses with LLINs (no insecticide paint) were low. While it is acknowledged that the study design may have allowed for some limitations such as increasing the chances of having unwanted scavengers eat the dead mosquitoes thus underestimating the mortality, the low mortality rates observed in control houses with LLINs in the VK1 area in this study are supported by recent findings in the nearby VK7 village, also in the Bama area (Toé et al., 2014). Toé et al. (2014) study measured the efficacy of several pyrethroid-treated LLINs, including PermaNet 2.0 distributed by the PNLP (such as the ones in VK1) against local populations of pyrethroid-resistant *An. gambiae* s.l. using WHO bioassays among other tests. PermaNet 2.0 used yielded mortality rates of about 20% against pyrethroid-resistant *An. gambiae* s.l. from VK7 in forced contact (Toé et al., 2014).

Assessing the impact that vector control tools have on blood feeding inhibition may yield misleading information as it cannot distinguish females entering houses to feed on humans, from females that have bloodfed outside (on either humans or animals, or both) and then enter the houses to complete their bloodfeeding and/or to rest. Analysis on the source of blood meals showed that an average of 58% of the *An. coluzzii* collected had bloodfed on other animals (non human) versus about 27% on humans, and about 16% had bloodfed on both other animals and humans. There were no differences between control and treated houses with regard to the rate of zoophily or anthropophily. It is worth noting that out of the 58% of females having blood fed on other animals (non human), about 45% obtained their blood meals on animals not identified as neither human nor any of the five chosen domestic animal antibodies. The surprisingly relatively low rate of anthropophily of *An. coluzzii* in this particular rice-field area had already been highlighted in previous studies and may be explained by the large mosquito densities and extensive livestocking activities (Robert, 1989; Baldet et al., 2003). In this anthropo-zoophilic context, the insecticide paint consisting on OPs may have provided a more optimal coverage by decreasing the longevity of both, malaria vectors having bloodfed outside on humans or other animals and entering houses to rest, as well as malaria vectors entering houses to bloodfeed (Killeen et al., 2014).

To summarize: the advantages of combining Inesfly 5A IGR^{TM} and LLINs could be many-fold in terms of the insecticides' mode of action as well as operational coverage: (a) combining different insecticides may help reduce the pressure for resistance development in vector populations (WHO, 2011, 2012); (b) the lethal effect of OPs coupled with pyrethroids' excito repellent effect may broaden the efficacy spectrum and thus increase protection to users; (c) the paint may provide protection before and after regular sleeping hours, when users are not yet under the net; (d) the paint may kill indoor resting as well as indoor bloodfeeding mosquitoes; (e) whilst, IRS provides similar benefits, the application of the paint may lead to a perceived improvement of people's homes and requires no special equipment. IRS leaves a residue on walls and needs special equipment leading to some operational obstacles (Najera and Zaim, 2001). In fact, in the area where the study was performed, most owners had seeked painting their homes and volunteers saw the study's paint as an added benefit.

Results obtained during this pilot study on the combination of Inesfly 5A IGR^{TM} and LLINs in a real village in an area of high pyrethroid resistance were positive: the average mortality rates were well above the 80% threshold recommended by WHOPES as a criteria for an effective vector control tool for over 6 months in all six configurations of insecticide paint and LLINs. Houses with LLINs and where a larger volume had been treated still met the criteria after 12 months. The next phase is to test if clinical malaria incidence and malaria exposure are reduced when combining Inesfly 5A IGR^{TM} and LLINs in children aged from 6 months to 14 years. The Phase III cluster randomized controlled study on the combination of Inesfly 5A IGR^{TM} and LLINs will be conducted in South-Western Burkina Faso, where villages are being currently identified in an area similar to VK1, with pyrethroid-resistant malaria vectors and holoendemic malaria.

5. Conclusions

The combination of Inesfly 5A IGR^{TM} and LLINs yielded a long-term mortality of 80% against *An. coluzzii* highly resistant to pyrethroids for about 12 months in houses where a larger volume was treated. The encouraging results obtained during this pilot study in a real village on malaria vector mortality sets the basis for the upcoming Phase III to study the impact of combining Inesfly 5A IGR^{TM} and LLINs on clinical malaria incidence and malaria exposure in children aged 6 months to 14 years in a pyrethroid-resistant and holoendemic malaria area in South-Western Burkina Faso.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SMC, RKD, PC, TB, FF, AD and BM contributed to the design of the study. TB and RKD critically contributed to the implementation of the study. DDS, SP, MN conducted evaluations. The manuscript has been written by BM and has been revised by RKD, TB, FF and DDS. All authors read and approved the final manuscript.

Acknowledgements

We are grateful to the house owners and volunteers at VK1 for their continued high value work and to the technicians at IRSS/Centre Muraz for their key assistance. This study was funded by Project No. 11-CAP2-1558 of the Agencia Española de Cooperación Internacional para el Desarrollo (AECID), Ministry of Foreign Affaires, Madrid, Spain, by Project No. ISCIII-RETIC RD12/0018/0013, Red de Investigación Colaborativa en Enfermedades Tropicales—RICET, of the Program of "Redes Temáticas de Investigación Cooperativa" RETICS/FEDER, Fondo de Investigación Sanitaria (FIS), Ministry of Health and Consumption, Madrid, Spain; and by project PROMETEO/2012/042 of the programme of Ayudas para Grupos de Investigación de Excelencia, Generalitat Valenciana, Valencia, Spain. We thank the University of Valencia in Spain, the Institut de Recherche pour le Développement (IRD) in France and the IRSS/Centre Muraz in Bobo-Dioulasso in Burkina Faso for their support.

References

Amelotti, I., Catalá, S.S., Gorla, D.E., 2009. Experimental evaluation of insecticidal paints against *Triatoma infestans* (Hemiptera: Reduviidae), under natural climatic conditions. Parasit. Vectors 2 (1), 30, http://dx.doi.org/10.1186/1756-3305-2-30

Baldet, T., Diabaté, A., Guiguemdé, T.R., 2003. Malaria transmission in 1999 in the rice field area of the Kou Valley (Bama), (Burkina Faso). Santé (Montrouge, France) 13 (1), 55–60.

Beier, J.C., Perkins, P.V., Wirtz, R.A., Koros, J., Diggs, D., Gargan, T.P., Koech, D.K., 1988. Bloodmeal identification by direct enzyme-linked immunosorbent assay (ELISA), tested on Anopheles (Diptera: Culicidae) in Kenya. J. Med. Entomol. 25 (1), 9–16.

Beier, J.C., Keating, J., Githure, J.I., Macdonald, M.B., Impoinvil, D.E., Novak, R.J., 2008. Integrated vector management for malaria control. Malar. J. 7 (Suppl. 1), S4, http://dx.doi.org/10.1186/1475-2875-7-S1-S4

Briët, O.J.T., Penny, M.A., Hardy, D., Awolola, T.S., Van Bortel, W., Corbel, V., Dabiré, K.R., Koudou, B.G., Tingu, P.K., Chitnis, N., 2013. Effects of pyrethroid resistance on the cost effectiveness of a mass distribution of long-lasting insecticidal nets: a modelling study. Malar. J. 12, 77, http://dx.doi.org/10.1186/1475-2875-12-77

Chandre, F., Darrier, F., Manga, L., Akogbeto, M., Faye, O., Mouchet, J., Guillet, P., 1999. Status of pyrethroid resistance in Anopheles gambiae sensu lato. Bull. World Health Organiz. 77 (3), 230–234.

Coetzee, M., Hunt, R.H., Wilkerson, R., Torre, A.D., Coulibaly, M.B., Besansky, N.J., 2013. *Anopheles coluzzii* and Anopheles amharicus, new members of the *Anopheles gambiae* complex. Zootaxa 3619 (3.), http://dx.doi.org/10.11646/zootaxa.3619.3.2

Dabiré, K.R., Diabaté, A., Djogbenou, L., Ouari, A., N'Guessan, R., Ouédraogo, J.-B., Hougard, J.M., Chandre, F., Baldet, T., 2008. Dynamics of multiple insecticide resistance in the malaria vector Anopheles gambiae in a rice growing area in South-Western Burkina Faso. Malar. J. 7, 188, http://dx.doi.org/10.1186/1475-2875-7-188

Dabiré, K.R., Diabaté, A., Namountougou, M., Toé, K.H., Ouari, A., Kengne, P., Bass, C., Baldet, T., 2009. Distribution of pyrethroid and DDT resistance and the L1014F kdr mutation in Anopheles gambiae s.l. from Burkina Faso (West Africa). Trans. R. Soc. Trop. Med. Hyg. 103 (11), 1113–1120, http://dx.doi.org/10.1016/j.trstmh.2009.01.008

Dabiré, K.R., Diabaté, A., Namountougou, M., Djogbenou, L., Wondji, C., Chandre, F., Simard, F., Ouédraogo, J.-B., Martin, T., Weill, M., Baldet, T., 2012. Trends in insecticide resistance in natural populations of malaria vectors in Burkina Faso, West Africa: 10 Years' surveys. In: Perveen, F. (Ed.), Insecticides—Pest Engineering. InTech, Retrieved from ⟨http://www.intechopen.com/books/insecticides-pest-engineering/trends-in-insecticide-resistance-in-natural-populations-of-malaria-vectors-in-burkina-faso-west-africa⟩.

Diabaté, A., Brengues, C., Baldet, T., Dabiré, K.R., Hougard, J.M., Akogbeto, M., Kengne, P., Simard, F., Guillet, P., Hemingway, J., Chandre, F., 2004. The spread of the Leu-Phe kdr mutation through *Anopheles gambiae* complex in Burkina Faso: genetic introgression and de novo phenomena. Trop. Med. Int. Health: TM IH 9 (12), 1267–1273, http://dx.doi.org/10.1111/j.1365-3156.2004.01336.x

Dias, J.C.P., Jemmio, A., 2008. [About an insecticidal paint for controlling *Triatoma infestans*, in Bolivia]. Rev. Soc. Bras. Med. Trop. 41 (1), 79–81.

Djènontin, A., Chabi, J., Baldet, T., Irish, S., Pennetier, C., Hougard, J.-M., Corbel, V., Akogbéto, M., Chandre, F., 2009. Managing insecticide resistance in malaria vectors by combining carbamate-treated plastic wall sheeting and pyrethroid-treated bed nets. Malar. J. 8, 233, http://dx.doi.org/10.1186/1475-2875-8-233

Djènontin, A., Chandre, F., Dabiré, K.R., Chabi, J., N'guessan, R., Baldet, T., Akogbéto, M., Corbel, V., 2010. Indoor use of plastic sheeting impregnated with carbamate combined with long-lasting insecticidal mosquito nets for the control of pyrethroid-resistant malaria vectors. Am. J. Trop. Med. Hyg. 83 (2), 266–270, http://dx.doi.org/10.4269/ajtmh.2010.10-0012

Enayati, A., Hemingway, J., 2010. Malaria management: past, present, and future. Annu. Rev. Entomol. 55, 569–591 (2010).

Ferguson, H.M., Dornhaus, A., Beeche, A., Borgemeister, C., Gottlieb, M., Mulla, M.S., Gimnig, J.E., Fish, D., Killeen, G.F., 2010. Ecology: a prerequisite for malaria elimination and eradication. PLoS Med. 7 (8), e1000303, http://dx.doi.org/10.1371/journal.pmed.1000303

Hemingway, J., 2014. The role of vector control in stopping the transmission of malaria: threats and opportunities. Philos. Trans. R. Soc. Lond., Ser. B: Biol. Sci. 369 (1645), 20130431, http://dx.doi.org/10.1098/rstb.2013.0431

International Center of Training Medical and Investigations, 2003. Toxicity Studies on Inesfly 5A IGR. CIDEIM, Cali, Colombia, ⟨http://www.cideim.org.co⟩ (2003).

Killeen, G.F., Seyoum, A., Gimnig, J.E., Stevenson, J.C., Drakeley, C.J., Chitnis, N., 2014. Made-to-measure malaria vector control strategies: rational design based on insecticide properties and coverage of blood resources for mosquitoes. Malar. J. 13, 146, http://dx.doi.org/10.1186/1475-2875-13-146

Lengeler, C., Sharp, B., 2003. Indoor residual spraying and insecticide-treated nets. In: Reducing Malaria's Burden: Evidence of Effectiveness for Decision Makers, Global Health Council, Washington, DC, pp. 17–24, 2003.

Maloney, K.M., Ancca-Juarez, J., Salazar, R., Borrini-Mayori, K., Niemierko, M., Yukich, J.O., Naquira, C., Keating, J.A., Levy, M.Z., 2013. Comparison of insecticidal paint and deltamethrin against *Triatoma infestans* (Hemiptera: Reduviidae) feeding and mortality in simulated natural conditions. J. Vector Ecol.: J. Soc. Vector Ecol. 38 (1), 6–11, http://dx.doi.org/10.1111/j.1948-7134.2013.12003.x

Martinez-Torres, D., Chandre, F., Williamson, M.S., Darriet, F., Bergé, J.B., Devonshire, A.L., Guillet, P., Pasteur, N., Pauron, D., 1998. Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector *Anopheles gambiae* s.s. Insect Mol. Biol. 7 (2), 179–184.

MCHIP/USAID/PNLP, 2013. Rapport sur la mise en œuvre du programme de lutte contre le paludisme au Burkina Faso. MCHIP/USAID.

Mosqueira, B., Duchon, S., Chandre, F., Hougard, J.-M., Carnevale, P., Mas-Coma, S., 2010a. Efficacy of an insecticide paint against insecticide-susceptible and resistant mosquitoes—Part 1: Laboratory evaluation. Malar. J. 9 (1), 340, http://dx.doi.org/10.1186/1475-2875-9-340

Mosqueira, B., Chabi, J., Chandre, F., Akogbeto, M., Hougard, J.-M., Carnevale, P., Mas-Coma, S., 2010b. Efficacy of an insecticide paint against malaria vectors and nuisance in West Africa—Part 2: Field evaluation. Malar. J. 9, 341, http://dx.doi.org/10.1186/1475-2875-9-341

Mosqueira, B., Chabi, J., Chandre, F., Akogbeto, M., Hougard, J.-M., Carnevale, P., Mas-Coma, S., 2013. Proposed use of spatial mortality assessments as part of the pesticide evaluation scheme for vector control. Malar. J. 12, 366, http://dx.doi.org/10.1186/1475-2875-12-366

Najera, J.A., Zaim, M., 2001. Malaria vector control Insecticides for indoor residual spraying. In: WHO/CDS/WHOPES/2001.3. World Health Organization, Geneva (2001).

National Center of Tropical Diseases, 2004. CENETROP. Santa Cruz de la Sierra, Santa Cruz de la Sierra, Bolivia, ⟨http://www.cenetrop.org.bo⟩ (2004).

Okumu, F.O., Mbeyela, E., Lingamba, G., Moore, J., Ntamatungiro, A.J., Kavishe, D.R., Kenward, M.G., Turner, E., Lorenz, L.M., Moore, S.J., 2013. Comparative field evaluation of combinations of long-lasting insecticide treated nets and indoor residual spraying, relative to either method alone, for malaria prevention in an area where the main vector is *Anopheles arabiensis*. Parasit. Vectors 6, 46, http://dx.doi.org/10.1186/1756-3305-6-46

Ranson, H., Jensen, B., Vulule, J.M., Wang, X., Hemingway, J., Collins, F.H., 2000. Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan Anopheles gambiae associated with resistance to DDT and pyrethroids. Insect Mol. Biol. 9 (5), 491–497.

Robert, V., 1989. La transmission du paludisme humain: la zone des savanes d'Afrique de l'Ouest. Université de Paris 6, pp. 325 (Ph.D. Thesis, 1989).

Santolamazza, F., Mancini, E., Simard, F., Qi, Y., Tu, Z., della Torre, A., 2008. Insertion polymorphisms of SINE200 retrotransposons within speciation islands of *Anopheles gambiae* molecular forms. Malar. J. 7, 163, http://dx.doi.org/10.1186/1475-2875-7-163

Sinka, M.E., Bangs, M.J., Manguin, S., Rubio-Palis, Y., Chareonviriyaphap, T., Coetzee, M., Mbogo, C.M., Hemingway, J., Patil, A.P., Temperley, W.H., Gething, P.W., Kabaria, C.W., Burkot, T.R., Harbach, R.E., Hay, S.I., 2012. A global map of dominant malaria vectors. Parasit. Vectors 5, 69, http://dx.doi.org/10.1186/1756-3305-5-69

Spanish Ministry of Health and Consumer Affairs, 1996. Report on the study of the toxicity and irritability of Inesfly 5A. Health Institute Carlos III Madrid.

The malERA Consultative Group on Vector Control, 2011. A research agenda for malaria eradication: vector control. PLoS Med. 8 (1), e1000401, http://dx.doi.org/10.1371/journal.pmed.1000401

Toé, L.P., Skovmand, O., Dabiré, K.R., Diabaté, A., Diallo, Y., Guiguemdé, T.R., Doannio, J.M., Akogbeto, M., Baldet, T., Gruénais, M.-E., 2009. Decreased motivation in the use of insecticide-treated nets in a malaria endemic area in Burkina Faso. Malar. J. 8, 175, http://dx.doi.org/10.1186/1475-2875-8-175

Toé, K.H., Jones, C.M., N'Fale, S., Ismail, H.M., Dabiré, R.K., Ranson, H., 2014. Increased pyrethroid resistance in malaria vectors and decreased bed net effectiveness, Burkina Faso. Emerg. Infect. Dis. 20 (10), http://dx.doi.org/10.3201/eid2010.140619

Trape, J.-F., Tall, A., Sokhna, C., Ly, A.B., Diagne, N., Ndiath, O., Mazenot, C., Richard, V., Badiane, A., Dieye-Ba, F., Faye, J., Ndiaye, G., Diene Sarr, F., Roucher, C., Bouganali, C., Bassène, H., Touré-Baldé, A., Roussilhon, C., Perraut, R., Spiegel, A., Sarthou, J.-L., Pereira da Silva, L., Mercereau-Puijalon, O., Druilhe, P., Rogier, C., 2014. The rise and fall of malaria in a West African rural community, Dielmo Senegal, from 1990 to 2012: a 22 year longitudinal study. Lancet Infect. Dis. 14 (6), 476–488, http://dx.doi.org/10.1016/S1473-3099(14)70712-1

Weill, M., Malcolm, C., Chandre, F., Mogensen, K., Berthomieu, A., Marquine, M., Raymond, M., 2004. The unique mutation in ace-1 giving high insecticide resistance is easily detectable in mosquito vectors. Insect Mol. Biol. 13 (1), 1–7.

WHO, 1996. Evaluation and testing of insecticides. In: Report of the WHO Informal Consultation WHO/HQ, Geneva, 7–11 October 1996, (document CTD/WHOPES/IC/96.1). World Health Organization, Geneva.

WHO, 1998. Test procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces. In: Report of the WHO Informal Consultation. Document WHO/CDS/CPC/MAL/1998.12. World Health Organization, Geneva, pp. 1–43.

WHO, 2006. Evaluation and testing of insecticides Guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets. In: WHO/CDS/NTD/WHOPES/GCDPP/2006.3. World Health Organization.

WHO., 2011. The Technical Basis for Coordinated Action against Insecticide Resistance: Preserving the Effectiveness of Modern Malaria Vector Contro. World Health Organization, Geneva, ⟨http://whqlibdoc.who.int/publications/2011/9789241501095_eng.pdf⟩.

WHO, 2012. Global Plan for Insecticide Resistance Management in Malaria Vectors. World Health Organization, Geneva, ⟨http://www.who.int/malaria/publications/atoz/gpirm/en/index.html⟩.

WHO, 2013. World Malaria Report 2013. World Health Organization, Geneva, ⟨http://www.who.int/malaria/publications/world_malaria_report_2013/report/en/⟩.