

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO	
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DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 378 FASE NACIONAL INCLUIDO CAPITULO I
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 10
ACT: 430 REQUERSOLICITA	

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
OSCAR CORREA BARBOSA

Asunto: Radicación: 12-2109- -11
 Trámite: 11
 Evento: 378
 Actuación: 430
 Oficio: 4009
 Folios: 10

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/EP2010/059557				
Fecha de Presentación Internacional	05/07/2010				

1. Documentos estudiados

Descripción:	Radicado N°12-002109-00000-00000	06/enero/2012
Reivindicaciones:	Radicado N°12-002109-00000-00000	06/enero/2012
	Radicado N°12-002109-00004-00000	27/diciembre/2013
	Radicado N°12-002109-00007-00000	01/abril/2014
Respuesta solicitante.	Radicado N°12-002109-00004-00000	27/diciembre/2013
Negación:	Resolución N°9938	21/febrero/2014
Recurso:	Radicado N°12-002109-00007-00000	01/abril/2014
Prioridad:	EP09165019.2	9/julio/2009

	EP09167790.6	13/agosto/2009
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2. Análisis de la Prioridad

Se acepta la reivindicación de Prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del capítulo primero del Título 10 de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por el solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada.

Para el caso de la presente solicitud, al verificar la tasa pagada, todo el ultimo capitulo reivindicatorio presentado se tendrá en cuenta para realizar el examen de patentabilidad.

Título de la solicitud: “Estructura tipo lámina insecticida para proteger seres humanos y animales domésticos”

El problema planteado en esta solicitud se refiere a la necesidad de diseñar una estructura tipo lámina que tenga buena actividad insecticida contra insectos resistentes a piretroides, incluso después de procesos de lavado repetidos.

Para solucionar ese problema técnico la presente solicitud plantea una estructura tipo lámina terminada con una mezcla insecticida que comprende clorfenapir y uno o más piretroides seleccionados del grupo que comprende alfa-cipermetrina, deltametrina, permetrina y lambda-cihalotrina, debidamente definida (radicado N°12-002109-00000-00000, Págs. 2 a 3).

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A 01 N 053/008.

4. De la Solicitud de Patente

Titulo

El título no corresponde con el objeto de la solicitud porque es muy general y solo se menciona el resultado a alcanzar y no los componentes principales de la lámina, por lo cual se sugiere al solicitante hacer la corrección necesaria.

Reivindicaciones (Art 30 D 486, Art 22 PCT)

El capítulo reivindicatorio no se encuentra debidamente soportadas en la descripción puesto que en las realizaciones preferidas hace referencia a la preparación y actividad de la mezclas cuyas características que se mencionan en la tabla 1.

Se sugiere restringir la solicitud a una razonable cantidad de acuerdo al tipo de mezclas probadas, es decir, que se encuentran debidamente soportados en el capítulo descriptivo. Teniendo en cuenta la descripción en los ejemplos se encuentra:

Tabla 1. Ejemplos de la mezcla

Componentes de la mezcla	Soporte de los ejemplos presentados (proporciones)	
Alfa-cipermentrina	0 y 100(mg/m2)	0 y 3,2 (Concentración del baño g/L)
Clorfenapir	0 y 100(mg/m2)	0 y 3,2 (Concentración del baño g/L)
Aglutinante de acrilato (acrilonitrilo, acrilato de n-butilo, N-metilolmetacrilamida)	1 y 0.55% (% peso en la tela)	5 y 10(Concentración del baño g/L)

El solicitante debe definir específicamente la materia y el alcance de la invención a través de las características técnicas esenciales que se encuentren enteramente soportadas en la descripción.

La reivindicación 1 solo hace referencia a una mezcla de insecticidas por lo tanto debería cambiarse el preámbulo si desea reivindicar la mezcla, pero si lo que desea reivindicar es la tela compuesta por la mezcla pesticida y el aglutinante debe mencionar en la reivindicación 1 el tipo de aglutinante y su proporción.

La reivindicación 7 menciona el término “aglutinante”, se está haciendo alusión a un término muy amplio, puesto que para una persona de nivel medio versada en la materia estos evocan su función y no se está indicando la denominación común de los compuestos. Se sugiere definir el anterior término a qué tipo de aglutinantes se está haciendo referencia o compuesto por que componentes, pues no se puede definir sólo por su función. De acuerdo a lo sustentado en la descripción.

La reivindicación 8 y 9 describen características del proceso de elaboración de la tela, lo cual no define la lámina o la mezcla, puesto que no se define en base a su forma de elaboración sino en base a sus componentes y proporciones.

5. Determinación del Estado de la Técnica

El Estado de la Técnica se determinó con base en la Fecha de Presentación de la Solicitud de Prioridad, julio 9 de 2009 y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D2	Raphael N'guessan et al. Tropical medicine and international health, bd. 14, Nr. 4, Seiten 389-395.	"Control of pyrethroid-resistant Anophele gambiae and Culex quinquefasciatus mosquitoes with chlorfenapyr in Benin"	abril/2009
D3	F. W. MOSHA ET AL. Tropical Medicine And International Health, Bd. 13, Nr. 5, Seiten 644-652.	"Experimental hut evaluation for the pyrrole insecticide chlorfenapyr on bed nets for the control of Anopheles arabiensis and Culex	mayo/2008

		quinquefasciatus",	
D4	N'guessan R et al. Acta Tropica, Elsevier Science BV., Amsterdam, NL, Bd. 102, NR. 1, 1., seiten 69-78, , ISSN: 0001-706X, doi: 10.1016/j.actatropica.2007.03.003	"Chlorfenapyr: A pyrrole insecticide for the control of pyrethroid or DDT resistant Anopheles gambiae (Diptera: Culicidae) mosquitoes",	Abril/2007
D5	EP0771526	Pesticidal compositions containing 4-bromo-2-(4-chlorophenyl)-1-ethoxymethyl-4-trifluoromethylpyrrole-3-carbonitrile and pyrethroids	4/mayo/1997
D6	US2005132500	Composition for impregnation of fibers, fabrics and nettings imparting a protective activity against pests	23/junio/2005

El documento D2 ^[1] divulga un estudio para comparar la eficacia de chlorfenapyr aplicado sobre mosquiteros y como una pulverización residual en interiores contra las poblaciones de *Anopheles gambiae* y *Culex quinquefasciatus* en un área de Benin que muestra los niveles problemáticos de la resistencia a los piretroides. Se observa que en mosquiteros podría combinarse con un piretroide de contacto irritante para dar una mejor protección contra de picadura de mosquitos, mientras que matar mosquitos resistentes a los piretroides que entran en contacto con la red (abstract, Tabla 1 – 2).

El documento D3 ^[2] divulga un estudio para determinar la eficacia de la clorfenapir contra *Anopheles arabiensis* y *Culex quinquefasciatus* en el este de África y para identificar las dosis eficaces para el tratamiento neto en comparación con el piretroide deltametrina comúnmente utilizado. Se observó que combinando clorfenapyr con un piretroide más potente repelente en los mosquiteros para mejorar la protección personal, el control de los mosquitos resistentes a los piretroides y manejo de la resistencia a los piretroides sería ventajoso (abstract, Pág. 650, Col. 2; Tabla 1 – 3).

El documento D4 ^[3] divulga que debido al desarrollo y propagación de la resistencia a los piretroides en *Anopheles gambiae* en África hay una necesidad urgente de desarrollar insecticidas alternativos para complementar los piretroides. Clorfenapir tiene potencial para el control de la malaria en mosquiteros tratados o residuales de fumigación en las zonas donde los mosquitos son resistentes a los piretroides. Para aplicaciones mosquiteros tratados con clorfenapir podría combinarse con piretroide como una mezcla para proporcionar una protección personal, así como para dar el control de mosquitos resistentes (abstract)

El documento D5 ^[4] divulga proporciona una composición pesticida que comprende como ingredientes activos 4-bromo-2- (4-clorofenil) -1-etoximetil-5-trifluoro metilpirrol-3-carbonitrile (clorfenapir) y al menos un compuesto piretroide y un vehículo inerte.

El documento D6 ^[5] divulga un material textil o plástico impregnado con una composición insecticida que comprende al menos un insecticida y/o un repelente y al menos un aglutinante acrílico

6. Examen de Patentabilidad (Art 14 D486)

Novedad (Art 16 D486)

La **reivindicación 1** consiste en una estructura tipo lámina, en forma de una tela, que

¹anexo 1

²anexo 2

³anexo 3

⁴URL:[http://worldwide.espacenet.com/publicationDetails/biblio?](http://worldwide.espacenet.com/publicationDetails/biblio?DB=worldwide.espacenet.com&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=19970507&CC=E&P&NR=0771526A2&KC=A2)

[http://worldwide.espacenet.com/publicationDetails/biblio?](http://worldwide.espacenet.com/publicationDetails/biblio?DB=worldwide.espacenet.com&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=19970507&CC=E&P&NR=0771526A2&KC=A2)

⁵URL:[http://worldwide.espacenet.com/publicationDetails/biblio?](http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=13&ND=4&adjacent=true&locale=en_EP&FT=D&date=20050623&CC=US&NR=2005132500A1&KC=A1)

[http://worldwide.espacenet.com/publicationDetails/biblio?](http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=13&ND=4&adjacent=true&locale=en_EP&FT=D&date=20050623&CC=US&NR=2005132500A1&KC=A1)

comprende una mezcla insecticida debidamente definida (Rad. N°12002109-00007-0000, Pág. 15)

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D2	D3	D5
Estructura tipo lamina que comprende:	Mosquitero que comprende:	Mosquitero que comprende:	Composición pesticida que comprende
Clorfenapir 50 a 150 mg/m²	Clorfenapir 150 mg/m²	Clorfenapir 100, 250, 500 mg/m²	como ingredientes activos 4-bromo-2- (4-clorofenil) -1-etoximetil-5-trifluoro metilpirrol-3-carbonitrile (clorfenapir)
Uno o más piretroides: alfa-cipermetrina (50 a 150 mg/m²), deltametrina (15 a 45 mg/m²), permetrina (50 a 750 mg/m²), lambda-cihalotrina(5 a 30 mg/m²)	Piretroides (permentrina 500 mg/m², lambda-cihalotrina)	deltametrina 25 mg/m²	y al menos un compuesto piretroide (cipermetrina, deltametrina y permetrina) en una proporción 90,10 a 10:90

Es posible concluir que las características **esenciales** de la lámina de la reivindicación 1, habían sido divulgadas previamente en D2 o D3 o D5 porque de acuerdo con la tabla anterior las características técnicas de la invención descrita en la reivindicación 1 de la presente solicitud ya se enseñaban de manera independiente en cada anterioridad D2 o D3 o D5, de tal forma que se concluye que la composición descrita en la reivindicación 1, ya era conocido en el estado de la técnica.

En consecuencia, la reivindicación 1 carece de novedad, según lo divulgado de manera independiente en D2 o D3 o D5.

Las reivindicaciones dependientes 2 – 6 no mencionan alguna característica que haga nueva a la lámina de la reivindicación 1, por lo cual se considera que no son nuevas.

Nivel Inventivo (Art18 D486)

La **reivindicación 3** consiste en una estructura tipo lámina, en forma de una tela, que comprende una mezcla insecticida debidamente definida (Rad. N°12002109-00007-0000, Pág. 16)

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D2	D3	D4	D5
Estructura tipo lamina que comprende:	Mosquitero que comprende:	Mosquitero que comprende:	Mosquitero que comprende:	Composición pesticida que comprende
Clorfenapir 50 a 150 mg/m²	Clorfenapir 150 mg/m²	Clorfenapir 100, 250, 500 mg/m²	Clorfenapir 5-1000 mg/m²	como ingredientes activos 4-bromo-2-

				(4-clorofenil) -1-etoximetil-5-trifluoro metilpirrol-3-carbonitrile (clorfenapir)
Uno o más piretroides: alfa-cipermetrina (50 a 150 mg/m²), deltametrina (15 a 45 mg/m²), permetrina (50 a 750 mg/m²), lambda-cihalotrina(5 a 30 mg/m²)	Piretroides (permentrina 500 mg/m², lambda-cihalotrina)	deltametrina 25 mg/m²	Piretroides	y al menos un compuesto piretroide (cipermetrina, deltametrina y permetrina) en una proporción 90,10 a 10:90

Los documentos D2, D3, D4 y D5 son considerados el estado de la técnica cercano a la invención definida en la reivindicación 3 porque

D2 divulga un estudio donde se observa que en mosquiteros podría combinarse con un piretroide de contacto irritante para dar una mejor protección contra de picadura de mosquitos, mientras que matar mosquitos resistentes a los piretroides que entran en contacto con la red. Clorfenapir 150 mg/m2 y piretroides (permentrina 500 mg/m2, lambda-cihalotrina) (abstract, Tabla 1 – 2).

D3 divulga un estudio se observó que combinando clorfenapir con un piretroide más potente repelente en los mosquiteros para mejorar la protección personal, el control de los mosquitos resistentes a los piretroides y manejo de la resistencia a los piretroides sería ventajoso. Clorfenapir 100, 250, 500 mg/m2, deltametrina 25 mg/m2 (abstract, Pág. 650, Col. 2; Tabla 1 – 3).

D4 divulga Clorfenapir tiene potencial para el control de la malaria en mosquiteros tratados o residuales de fumigación en las zonas donde los mosquitos son resistentes a los piretroides. Para aplicaciones mosquiteros tratados con clorfenapir podría combinarse con piretroide como una mezcla para proporcionar una protección personal, así como para dar el control de mosquitos resistentes. Clorfenapir 5-1000 mg/m2 (abstract)

D5 divulga proporciona una composición pesticida que comprende como ingredientes activos 4-bromo-2- (4-clorofenil) -1-etoximetil-5-trifluoro metilpirrol-3-carbonitrile (clorfenapir) y al menos un compuesto piretroide en una proporción 90,10 a 10:90 y un vehículo inerte (Pág. 3, Líneas 37 – 40; reivindicaciones).

La diferencia entre la invención, definida en la reivindicación 3 y el mosquitero de D2 y D3 consiste en que solo se especifica permentrina y deltametrina, respectivamente, mientras que en la reivindicación 3 selecciona alfa-cipermetrina.

La diferencia entre la invención, definida en la reivindicación 3 y el mosquitero de D4 y composición de D5 consiste en que no se especifica la proporción de todos los componentes, mientras que en la reivindicación 3 se mencionan cada uno en una proporción.

El efecto que lograría las diferencias antes mencionadas en la lámina de la reivindicación 3, en cuanto a su acción insecticida incluso después de procesos de lavado, no puede ser determinado para todos los componentes abarcados, puesto que no hay nada que indique que dichas diferencias de cantidades y selección de piretroides, otorgan a la lámina ahora reivindicado una ventaja técnica sobre los mosquiteros ya conocidos en la anterioridad D2 o D3 o D4 o D5.

Por lo tanto, el problema técnico objetivo a resolver consiste en plantear una alternativa a los componentes de la lámina conocidos según D2 o D3 o D4 o D5 para que sean insecticidas más eficaces y con resistencia al lavado.

Teniendo en cuenta lo expuesto anteriormente, la personas de nivel medio versada en la materia derivara de manera obvia los componentes de la lámina descritos en la reivindicación 3 de la presente invención a partir de las enseñanzas de los componentes de los mosquiteros del documento D2 o D3 o D4 o D5 puesto que las diferencias se limitan en seleccionar una cantidad o un piretroide, buscando seguramente una mejor actividad y resistencia al lavado, sin que ese hecho confiera una mayor acción insecticida, por lo cual se considera obvia.

Puesto que las reivindicaciones dependientes 4 – 6 no mencionan características inventivas adicionales, tampoco pueden ser consideradas inventivas.

Conclusión:
Las reivindicaciones 3 – 6, cumplen el requisito de novedad (Art. 16), pero no cumplen con el requisito de nivel inventivo (Art. 18).

La **reivindicación 6** consiste en una estructura tipo lámina, en forma de una tela, que comprende una mezcla insecticida debidamente definida (Rad. N°12002109-00007-0000, Pág. 17)

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D2	D3	D4	D5	D6
Estructura tipo lamina que comprende:	Mosquitero que comprende:	Mosquitero que comprende:	Mosquitero que comprende:	Composición pesticida que comprende	Composición para material textil que comprende
Clorfenapir 50 a 150 mg/m ²	Clorfenapir 150 mg/m ²	Clorfenapir 100, 250, 500 mg/m ²	Clorfenapir 5-1000 mg/m ²	como ingredientes activos 4-bromo-2- (4-clorofenil) -1-etoximetil-5-trifluoro metilpirrol-3-carbonitrile (clorfenapir)	No se menciona
Uno o más piretroides: alfa-cipermetrina (50	Piretroides (permentrina 500 mg/m ² ,	deltametrina 25 mg/m ²	Piretroides	y al menos un compuesto piretroide	Piretroides (alfa-cipermetrina, deltametrina,

a 150 mg/m ²), deltametrina (15 a 45 mg/m ²), permetrina (50 a 750 mg/m ²), lambda- cihalotrina(5 a 30 mg/m ²)	lambda- cihalotrina)			(cipermetrina, deltametrina y permetrina) en una proporción 90,10 a 10:90	permetrina, lambda- cihalotrina)
Un aglutinante	No menciona	No menciona	No menciona	Un aglutinante	Un aglutinante acrílico

Los documentos D2, D3, D4 y D5 son considerados el estado de la técnica cercano a la invención definida en la reivindicación 6 porque

D2 divulga un estudio donde se observa que en mosquiteros podría combinarse con un piretroide de contacto irritante para dar una mejor protección contra de picadura de mosquitos, mientras que matar mosquitos resistentes a los piretroides que entran en contacto con la red. Clorfenapir 150 mg/m² y piretroides (permentrina 500 mg/m², lambda-cihalotrina) (abstract, Tabla 1 – 2).

D3 divulga un estudio se observó que combinando clorfenapir con un piretroide más potente repelente en los mosquiteros para mejorar la protección personal, el control de los mosquitos resistentes a los piretroides y manejo de la resistencia a los piretroides sería ventajoso. Clorfenapir 100, 250, 500 mg/m², deltametrina 25 mg/m² (abstract, Pág. 650, Col. 2; Tabla 1 – 3).

D4 divulga Clorfenapir tiene potencial para el control de la malaria en mosquiteros tratados o residuales de fumigación en las zonas donde los mosquitos son resistentes a los piretroides. Para aplicaciones mosquiteros tratados con clorfenapir podría combinarse con piretroide como una mezcla para proporcionar una protección personal, así como para dar el control de mosquitos resistentes. Clorfenapir 5-1000 mg/m² (abstract)

D5 divulga proporciona una composición pesticida que comprende como ingredientes activos 4-bromo-2- (4-clorofenil) -1-etoximetil-5-trifluoro metilpirrol-3-carbonitrile (clorfenapir) y al menos un compuesto piretroide en una proporción 90,10 a 10:90 y un vehículo inerte (Pág. 3, Lineas 37 – 40; reivindicaciones).

La diferencia entre la invención, definida en la reivindicación 6 y el mosquitero de D2 a D4 y la composición de D5 consiste en que no se menciona la presencia de un aglutinante.

El efecto que lograría las diferencias antes mencionadas en la lámina de la reivindicación 6, en cuanto a su acción insecticida incluso después de procesos de lavado, no puede ser determinado para todos los componentes abarcados, puesto que no hay nada que indique que dichas diferencias de cantidades y selección de piretroides, otorgan a la lámina ahora reivindicado una ventaja técnica sobre los mosquiteros ya conocidos en la anterioridad D2 o D3 o D4 o D5.

Por lo tanto, el problema técnico objetivo a resolver consiste en plantear una alternativa a los componentes de la lámina conocidos según D2 o D3 o D4 o D5 para que sean

insecticidas más eficaces y con resistencia al lavado.

Sin embargo el la presencia de un aglutinante con piretroides en textiles ya es conocida en el documento D6, donde D6 divulga un material textil o plástico de preferencia una tela o malla que ha sido tratada o impregnada con una formulación que comprende un ingrediente activo pesticida, se consideran formulaciones que contienen agentes insecticidas como por ejemplo clorfenapir, alfa-cipermetrina, deltametrina, permetrina ó lambda-cihalotrina, entre otros. Específicamente se divulga una tela o malla para matar o repeler insectos que comprende a) al menos un insecticida y/o al menos un repelente y b) un aglutinante acrílico. Según los ejemplos del documento D1, las telas así tratadas (ingrediente activo: alfa-cipermetrina o deltametrina) tienen una buena actividad insecticida frente a mosquitos incluso después de haber sido sometidas a procesos de lavado. (abstract, Pág. 4 a 6, reivindicaciones)

Como consecuencia de los hechos mencionados en los párrafos anteriores la persona medianamente versada en la materia habría derivado de manera obvia y sin mucho esfuerzo de su parte la lámina descrita en la reivindicación 6 a partir de incluir las enseñanzas de D6 en cuanto la presencia de un aglutinante en presencia de piretroides, junto con las enseñanzas de las composiciones del documento D2 o D3 o D4 o D5 toda vez que las diferencias se limitan en seleccionar compuestos y combinar buscando una aparente mejora que no se evidencia. Por lo cual al combinar D2 con D6 o D3 con D6 o D4 con D6 o D5 con D6 la reivindicación se considera obvia

Puesto que las reivindicaciones dependientes 7 – 9 no mencionan características inventivas adicionales, tampoco pueden ser consideradas inventivas.

Conclusión:

Las reivindicaciones 6 – 9, cumplen el requisito de novedad (Art. 16), pero no cumplen con el requisito de nivel inventivo (Art. 18).

7. Conclusión

Los requisitos de Patentabilidad de la presente Solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D2	Raphael N'guessan et al. Tropical medicine and international health, bd. 14, Nr. 4, Seiten 389-395.	1 – 6 /3 – 9	Novedad/ Nivel inventivo
D3	F. W. MOSHA ET AL. Tropical Medicine And International Health, Bd. 13, Nr. 5, Seiten 644-652.	1 – 6 /3 – 9	Novedad/ Nivel inventivo
D4	N'guessan R et al. Acta Tropica, Elsevier Science BV., Amsterdam, NL, Bd. 102, NR. 1, 1., seiten 69-78, , ISSN: 0001-706X, doi: 10.1016/j.actatropica.2007.03.003	1 – 6 /3 – 9	Novedad/ Nivel inventivo
D5	EP0771526	1 – 6 /3 – 9	Novedad/ Nivel inventivo
D6	US2005132500	7 – 9	Nivel inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir del día siguiente de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2015-04-21

Elaboró: Mayra Alejandra Quintero

Revisó: Gloria Jacqueline Alfonso Roza

Control of pyrethroid-resistant *Anopheles gambiae* and *Culex quinquefasciatus* mosquitoes with chlorfenapyr in Benin

Raphael N'Guessan^{1,2}, Pelagie Boko², Abiba Odjo², Bart Knols³, Martin Akogbeto² and Mark Rowland¹

¹ London School of Hygiene and Tropical Medicine, London, UK

² Centre de Recherches Entomologiques de Cotonou, Cotonou, Benin

³ Laboratory of Entomology, Wageningen University, The Netherlands

Summary

OBJECTIVE To compare the efficacy of chlorfenapyr applied on mosquito nets and as an indoor residual spray against populations of *Anopheles gambiae* and *Culex quinquefasciatus* in an area of Benin that shows problematic levels of pyrethroid resistance.

METHOD Eight-week trial conducted in experimental huts.

RESULTS Indoor residual spraying killed 82.9% of *An. gambiae* overall (mean mortality: 79.5%) compared to 53.5% overall (mean mortality: 61.7%) in the hut containing the lower dosed ITN. Analysis of data on a fortnightly basis showed high levels of mosquito mortality and blood-feeding inhibition during the first few weeks after treatment. Control of *C. quinquefasciatus* by the IRS and ITN interventions showed a similar trend to that of *An. gambiae* and though the average level of mortality was lower it was still much higher than with pyrethroid treatments against this population. Chlorfenapyr's reputation for being rather slow acting was evident particularly at lower dosages. The treatments showed no evidence of excito-repellent activity in this trial.

CONCLUSION Chlorfenapyr has the potential to control pyrethroid resistant populations of *A. gambiae*. There is a need to develop long-lasting formulations of chlorfenapyr to prolong its residual life on nets and sprayed surfaces. On nets it could be combined with a contact irritant pyrethroid to give improved protection against mosquito biting while killing pyrethroid-resistant mosquitoes that come into contact with the net.

keywords pyrethroid resistance, chlorfenapyr, indoor residual spraying, bednets, *Anopheles gambiae*, *Culex quinquefasciatus*, Benin

Introduction

Malaria ranks amongst the world's most prevalent tropical infectious diseases. Worldwide it causes over a million deaths annually, the majority among African children (WHO 2008a). The most effective way to control transmission is through measures that reduce vector longevity or provide protection against mosquito biting. The most widely used vector control interventions rely upon the use of insecticides either on pyrethroid-treated nets or applied during indoor residual spray campaigns (WHO 2008a).

During the last decade pyrethroid resistance has become widespread in *Anopheles gambiae* in sub-Saharan Africa (Chandre *et al.* 1999; Dabiré *et al.* 2008; Santolamazza *et al.* 2008), probably as a consequence of use of pyrethroids in agriculture (Chandre *et al.* 2001; Akogbeto *et al.* 2005) but also increasingly through exposure to long lasting insecticidal nets (LLINs), as coverage of treated nets is scaled up (Czeher *et al.* 2008). LLINs are being distributed

on a massive scale in many African countries with support from the Global Fund or President's Malaria Initiative, and this is having a demonstrable impact on malaria (Okiro *et al.* 2007). There is an air of optimism, and organizations such as the Bill and Melinda Gates Foundation are calling for more ambitious targets and ultimately the elimination of malaria. Central to their agenda is vector control (Anon 2007). But as we are now seeing, successful vector control through use of chemical killing agents comes at a price, and the price is resistance (Czeher *et al.* 2008).

The form of pyrethroid resistance now spreading through West African populations of the M molecular form of *An. gambiae* appears to have major operational significance (N'Guessan *et al.* 2007a; Sharp *et al.* 2007). Alternative insecticides to control pyrethroid-resistant mosquitoes and prevent the further spread of resistance genes need to be investigated urgently and thoroughly. New alternatives such as the pyrole chlorfenapyr, the oxadiazine indoxacarb and the neonicotinoid dinotefuron

were developed primarily for crop or household pests but also show contact activity against mosquitoes (Corbel *et al.* 2004a; Paul *et al.* 2006; N'Guessan *et al.* 2007b). Chlorfenapyr has a novel mode of action, targeting oxidative pathways in insect mitochondria (Black *et al.* 1994), and shows no cross resistance to older classes of insecticide mediated by target site insensitivity (pyrethroid/DDT resistance *kdr*, dieldrin resistant *Rdl* or insensitive acetylcholinesterase *Ace-1*) in *An. gambiae* (N'Guessan *et al.* 2007c). Application rates between 200 and 500 mg/m² on netting gave full control of *kdr* and *Ace-1* strains in laboratory tunnel tests (N'Guessan *et al.* 2007c). A field trial of chlorfenapyr-treated nets using the same 200–500 mg/m² dosage range in experimental huts in Tanzania worked significantly better against *An. arabiensis* and pyrethroid-resistant *Culex quinquefasciatus* than deltamethrin applied at its recommended dosage of 25 mg/m² (Mosha *et al.* 2008).

This paper reports on further investigations with chlorfenapyr as a possible alternative to pyrethroids for use on nets or indoor residual spray treatment against pyrethroid-resistant populations of *An. gambiae* M molecular form and *C. quinquefasciatus*.

Methods

Study site and experimental huts

The evaluation was carried out in experimental huts situated in Ladji, a village on the periphery of Cotonou, the capital of Benin. The village floods during the rainy season, which creates breeding sites for *An. gambiae*. The local population of *An. gambiae* is comprised entirely of the M taxon, and is resistant to pyrethroids and DDT, with frequent *kdr* presence and metabolic resistance (Weill *et al.* 2000; Corbel *et al.* 2007; N'Guessan *et al.* 2007a). The nuisance mosquito *Cx. quinquefasciatus* is present year round and is resistant to pyrethroid, carbamate and organophosphate insecticides (Corbel *et al.* 2007). Several experimental huts are situated at the edge of the village along the lakeside, and four of these were selected for this study. The design of the huts was similar to those used in Cote d'Ivoire by Darriet *et al.* (2000) and N'Guessan *et al.* (2001). Each hut is made from concrete bricks and has a roof of corrugated iron and ceiling of thick polyethylene sheeting covered with palm thatch on the interior surface. Each hut stands on a concrete base surrounded by a water-filled moat to exclude ants and other scavengers that would otherwise carry off any dead mosquito. Entry of mosquitoes occurs via four slits, 1 cm wide, located on three sides of the hut. Mosquitoes are able to exit into a verandah trap projecting from the fourth side.

The test ITN was a rectangular polyester net treated with an aqueous solution of chlorfenapyr (BASF, 'Phantom' 15% SC) to give a target dosage of 100 mg/m². Test and control nets had 80 holes, each of 4 cm² area, 25 along each side and 15 on each end to simulate a worn net. Chlorfenapyr SC solution was sprayed onto interior walls and ceiling at 1000 mg/m² using a Hudson compression sprayer equipped with a flat fan nozzle. The evaluation started 3 days after treatment.

Procedure

The trial took place over 8 weeks, 6 days per week, between 15 April and 10 June 2006. The chlorfenapyr-treated net and the untreated net control were rotated between two huts every week. The hut for IRS and the unsprayed control hut were fixed throughout the 8 weeks.

Sleepers were rotated between huts on successive nights to adjust for any differences in individual attractiveness to mosquitoes. Mosquitoes were collected each morning at 5:00 a.m. from floors, walls, ceilings and verandahs, and transported to the central laboratory for identification to species, mortality counts and determination of gonotrophic condition. Live mosquitoes were held in plastic cups and delayed mortality was recorded at 24-h intervals up to 72 h.

Analysis

Data was double entered in Excel and transferred to STATA 8.0 software for further analysis. The number of mosquitoes collected each night was analysed per treatment using the Wilcoxon rank sum test. Proportional data (% blood-feeding, % mortality after 24 h or 72 h, and % exiting to the verandahs) were analysed using logistic regression after adjusting for the effect of sleeper and hut. Insecticide-induced mortality, exiting rate and blood feeding inhibition were estimated by comparing summary data for each treatment against its control.

Results

An average of 2.1 and 5.3 *An. gambiae* females were collected each morning from inside the huts and verandahs of the ITN and IRS huts, respectively (Table 1). Mosquito population densities steadily increased during the course of the trial. There was no significant difference in the numbers collected between untreated control and ITN huts. A significantly higher number of *An. gambiae* was found in the IRS hut than in the unsprayed hut. This unexpected result was possibly due to differences in site attractiveness, with the IRS hut being closer to breeding sites or upwind of the control hut. The trend in overall numbers of

Table 1 Experimental hut results of chlorfenapyr treated net and indoor residual spray treatments against *Anopheles gambiae* in Ladjé field station

Treatments	Total females caught	Females Caught per night	% blood fed (95% CI)	% feeding inhibition	% in verandah trap (95% CI)	% 24 h mortality (95% CI)	% 72 h mortality (95% CI)	% 72h mortality (95% CI) corrected for control
ITN								
Untreated net	84 ^a	1.8	91.7 (85.8–97.6) ^a	–	32.1 (22.2–42.1) ^a	2.4 (0–5.7) ^a	9.5 (3.3–15.8) ^a	–
Chlorfenapyr 0.1g/m ²	116 ^a	2.4	87.1 (81.0–93.2) ^a	5.0	31.9 (23.4–40.4) ^a	27.5 (19.3–34.6) ^b	53.5 (44.4–62.5) ^b	48.6 (38.6–58.6)
IRS								
Unsprayed hut	199 ^a	4.1	98.0 (96.0–99.9) ^a	–	52.3 (45.3–59.2) ^a	1.0 (0–2.4) ^a	8.5 (4.7–12.4) ^a	–
Chlorfenapyr 1g/m ²	310 ^b	6.5	80.7 (76.2–85.0) ^b	17.6	48.1 (42.5–53.6) ^a	71.2 (67.7–84.1) ^b	82.9 (78.7–87.1) ^b	81.3 (76.7–85.9)

For each untreated-treated pair values not sharing the same superscript are significantly different at the 5% level.

C. quinquefasciatus collected was similar to that of *An. gambiae* (Table 2).

Blood feeding rates of *An. gambiae* and *C. quinquefasciatus* were high in both IRS and ITN treatments, with the highly holed nets presenting little or no barrier to host seeking mosquitoes. Any inhibition of blood feeding associated with the insecticide treatment was not statistically significant.

The proportions of *An. gambiae* and *C. quinquefasciatus* exiting into the verandahs of treated huts by dawn were similar to the proportions in the control verandahs.

We saw a trend of delayed mortality due to chlorfenapyr in the treated huts for both species and for both ITN and IRS. The percentage mortality recorded among *An. gambiae* in the ITN hut was 27.5% after 24 h increasing to 53.5% after 72 h ($P < 0.01$). The mortality rate of *An. gambiae* in the IRS hut was initially much higher than in the hut with the ITN, with 71.2% killed after 24 h and 82.9% after 72 h ($P = 0.019$). Mortality rates for *C. quinquefasciatus* were lower than for *A. gambiae*, but even so, after 72 h the percentage killed was as high as 34.4% in the ITN hut and 45.6% in the IRS hut.

Further analysis of mortality data revealed that the proportions killed by the ITN and IRS treatments were high during the first few weeks – for example, 97–100% of *An. gambiae* and 69% of *C. quinquefasciatus* died in the ITN and IRS huts during weeks 0–2 – but both species showed progressive decline in mortality over the following 5 weeks ($P < 0.001$) (Figures 1 and 2). The trend in mortality with the IRS was similar to that of ITN, but the rate of decline in mortality was faster with the ITN than with the IRS treatments. Owing to mosquitoes being more abundant during the second half of the trial, the values for overall mortality were skewed. Percentage mortality based on the mean of the four fortnightly mortality rates gives a more realistic picture; these values were 61.7% mortality for ITN and 79.5% mortality for IRS treatments. Blood feeding rates with the ITN during the initial period were approximately 40% compared to approximately 80% in the control. For the IRS treatment the difference in blood feeding rates between treatment and control was initially less than with the ITN. However blood feeding rates for IRS versus ITN were similar in subsequent observation periods.

The chlorfenapyr treatments were well tolerated. Neither the spraymen nor the sleepers complained or reported any side effects on record forms during daily interviews.

Discussion

In southern Benin there is a major loss of pyrethroid efficacy associated with resistance in the M taxon of *An. gambiae*, and the problem appears to be spreading to

Table 2 Experimental hut results of chlorfenapyr treated net and indoor residual spray treatments against *Culex quinquefasciatus* in Ladiji field station

Treatments	Total females caught	Females caught per night	% blood fed (95% CI)	% feeding inhibition	% in verandah trap (95% CI)	% 24 h mortality (95% CI)	% 72 h mortality (95% CI)	% 72h mortality corrected for control (95% CI)
ITN								
Untreated net	317 ^a	6.6	80.4 (76.1–84.8) ^a	–	36.3 (31.0–41.5) ^a	1.3 (0.05–2.6) ^a	6.9 (4.1–9.7) ^a	–
Chlorfenapyr 0.1 g/m ²	355 ^a	7.4	73.2 (68.6–77.8) ^a	8.9	37.2 (32.2–42.2) ^a	21.1 (19.6–27.3) ^b	34.4 (29.4–39.3) ^b	29.5 (24.2–34.8)
IRS								
Unsprayed hut	300 ^a	6.3	90.0 (86.6–93.4) ^a	–	48.7 (43.0–54.3) ^a	0.7 (0–1.6) ^a	4.7 (2.3–7.0) ^a	–
Chlorfenapyr 1 g/m ²	439 ^a	9.1	82.7 (79.1–86.2) ^a	8.1	50.3 (45.7–55.0) ^a	38.9 (32.6–47.1) ^b	45.6 (40.9–50.2) ^b	42.9 (38.0–47.7)

For each untreated-treated pair values not sharing the same superscript are significantly different at the 5% level.

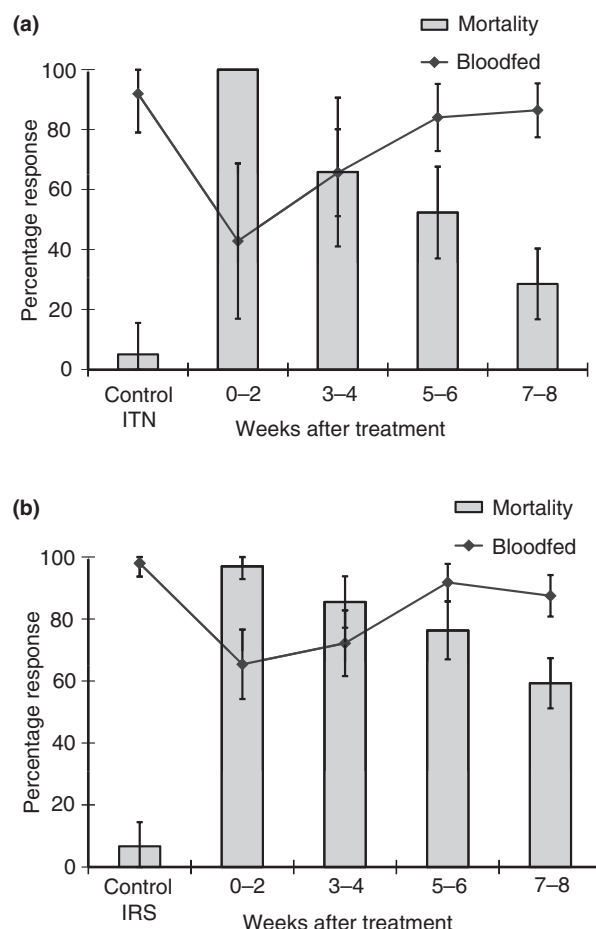


Figure 1 *Anopheles gambiae* mortality and feeding rates over fortnightly intervals during the hut trials with chlorfenapyr. Percentages based on all mosquitoes collected from the rooms and veranda traps. Control value is an average for all weeks. (a) ITN and (b) IRS.

Burkina Faso and Niger, probably as a result of increased LLIN coverage (N'Guessan *et al.* 2007c; WHO 2008b, Czeher *et al.* 2008). The experimental hut trial reported here demonstrates that chlorfenapyr has potential as a residual insecticide for control of pyrethroid-resistant mosquitoes of the M taxon. Whereas only 16.7% mortality and 29.8% mortality was recorded with pyrethroid treated nets using 500mg/m² permethrin and 18 mg/m² lambda-cyhalothrin respectively (Corbel *et al.* 2004b; N'Guessan *et al.* 2007a), the 100 mg/m² chlorfenapyr ITN achieved 53.5% mortality against the same population in Ladiji village. During the first 4 weeks of the trial, before chlorfenapyr started to decay or wear off, the average level of mortality was 83% against *An. gambiae* and 54% against *C. quinquefasciatus*. This suggests that if a newly

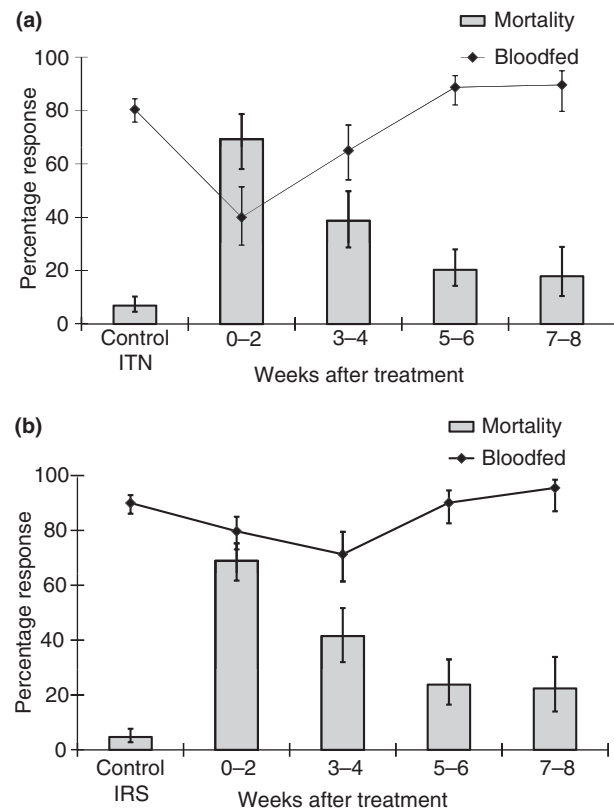


Figure 2 *Culex quinquefasciatus* mortality and feeding rates over fortnightly intervals during the hut trials with chlorfenapyr. Percentages based on all mosquitoes collected from the rooms and veranda traps. Control value is an average for all weeks. (a) ITN and (b) IRS.

developed long-lasting insecticidal net based on chlorfenapyr was to sustain this initial level of control against pyrethroid-resistant mosquitoes, it would help continue the progress against malaria currently being achieved in several parts of Africa. Without such alternative insecticides, pyrethroid resistance seems bound to spread and ultimately undermine the impact LLINs are currently having. The 100 mg/m² ITN tested in Ladji was no less effective than higher dosages (250–500 mg/m²) tested against *An. arabiensis* and *C. quinquefasciatus* in experimental huts in Tanzania (Mosha *et al.* 2008) and is a good starting point for development of an LLIN.

There was limited blood-feeding inhibition with the chlorfenapyr ITN. The present study was done at a time when all experimental hut trials in francophone West Africa were done with 80 holes per net. This was later considered excessive and trials are now done with six holes per net (WHO 2006). A recent trial comparing 80 with 6 holes per ITN against *C. quinquefasciatus* in Benin showed

a small but significant reduction in blood-feeding with the 6-hole nets. Had the current trial with chlorfenapyr used six holes per net, the level of personal protection might have been higher. However, a recent chlorfenapyr ITN trial in Tanzania used 6 holes per net but still showed only limited blood-feeding inhibition (Oxborough *et al.* 2008). We conclude that chlorfenapyr by itself can provide only limited personal protection on nets.

The IRS treatment was equally effective against pyrethroid-resistant mosquitoes. Over the 8 weeks it killed 82.9% of *An. gambiae* as compared to 30.8% killed by lambda-cyhalothrin IRS in the same locality (N'Guessan *et al.* 2007c). To maintain a high level of control, a longer-lasting IRS formulation of chlorfenapyr would need to be developed – perhaps based on microencapsulation or other advanced formulation technology. Microencapsulation technology can, for example, prolong for several months the residual activity of DEET repellent on nets or of pyrethroids in IRS formulations (N'Guessan *et al.* 2008; WHO 2007).

In our study, chlorfenapyr IRS or ITN seemed more toxic than excito-repellent against *An. gambiae* and *C. quinquefasciatus*. This contrasts with the action of pyrethroids which are both toxic and excito-repellent. The reduction in blood-feeding during the first few weeks of treatment was encouraging, suggesting an ephemeral personal protective effect, which again indicates the need for long lasting formulation technology to sustain the effect.

If chlorfenapyr treated nets were to be distributed on a large scale they should reduce malaria transmission rates thorough an effect on mosquito survivorship. This in turn would reduce the frequency of mosquito biting and hence provide personal protection indirectly. If distributed on an incremental scale, the delayed action of chlorfenapyr together with its lack of excito-repellency would provide limited personal protection initially. With the incremental approach, the chlorfenapyr treated nets would have limited impact. The best approach to ensure popularity and to provide personal protection is to combine chlorfenapyr with pyrethroid in a mixture or 2-in-1 treatment to encourage both reduced biting and increased mortality of pyrethroid-resistant mosquitoes (Guillet *et al.* 2001; Oxborough *et al.* 2008). Certain pyrethroids, more than others, retain excito-repellent activity and provide some personal protection against pyrethroid resistant *An. gambiae* (Corbel *et al.* 2004b; N'Guessan *et al.* 2007) and hence are better candidates to deploy in a chlorfenapyr combination treatment. This approach might also serve as a resistance management tactic to delay selection of pyrethroid resistance in areas where the vector is still susceptible. In fact there is a strong argument to deploy only combination nets in Africa from now on in order to prevent or slow down the spread of pyrethroid resistance to

areas that are currently susceptible and to control pyrethroid-resistant mosquitoes in areas where some mosquitoes or some species are still susceptible. One or other of these situations applies to all areas in malaria endemic sub-Saharan Africa. The argument for combination insecticides is analogous to that of combination therapy to preserve the effectiveness of antimalarial drugs (Nosten & White 2007).

The delayed mortality associated with chlorfenapyr is largely attributed to its unique mode of action involving disruption of oxidative phosphorylation in the mitochondria (Lovell *et al.* 1990). Slow action should be no obstacle to the deployment of chlorfenapyr as an IRS treatment. Provided the mosquito dies before any malaria parasite develops through to sporozoites or before an infected mosquito re-feeds after developing and laying its eggs, there will be interruption of transmission.

Culex quinquefasciatus is notoriously difficult to control with pyrethroids or organophosphates owing to the presence of multiple resistance mechanisms involving *kdr*, oxidases and esterases (Chandre *et al.* 1997; Corbel *et al.* 2007). Malaria control programmes deploy ITNs and IRS primarily for anopheline control but it is *C. quinquefasciatus*, a pyrethroid-resistant species often living sympatrically with *Anopheles*, that may determine acceptability and compliance of end-users towards these tools (Stephens *et al.* 1995). Once ITNs become holed, which they invariably do, a pyrethroid treatment offers little or no protection against pyrethroid-resistant *C. quinquefasciatus* (Irish *et al.* 2008). While *C. quinquefasciatus* is merely a nuisance mosquito in West Africa, it is an important filariasis vector in urban east Africa and India (Curtis *et al.* 1981; Bøgh *et al.* 1998). Chlorfenapyr achieved a high level of mortality against *C. quinquefasciatus* in the present trial, particularly in the initial weeks, compared to pyrethroid treatments. The prospect of providing additional protection against *Culex* biting or controlling filariasis transmission by means of a suitable combination pyrethroid-chlorfenapyr net deserves greater attention and further investigation.

As a novel residual insecticide chlorfenapyr shows a number of attributes. It shows potential as a substitute for pyrethroids to control pyrethroid resistance mosquitoes and as a supplement to pyrethroids to delay the selection of resistance. Ingenuity is required to improve the residual activity of the existing formulation and to identify the optimal strategy to deploy this product on ITNs/LLINs and IRS to achieve maximum effect and to preserve the limited arsenal of insecticides at our disposal. Attention needs to be given to this *now* to circumvent the problem being generated by pyrethroid resistance and, ironically, by the scale-up of pyrethroid-based LLINs.

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Corresponding Author Mark Rowland, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK.
E-mail: mark.rowland@lshtm.ac.uk

Experimental hut evaluation of the pyrrole insecticide chlorfenapyr on bed nets for the control of *Anopheles arabiensis* and *Culex quinquefasciatus*

F. W. Mosha¹, I. N. Lyimo¹, R. M. Oxborough², R. Malima³, F. Tenu³, J. Matowo¹, E. Feston¹, R. Mndeme¹, S. M. Magesa³ and M. Rowland²

¹ Kilimanjaro Christian Medical Centre, Moshi, Tanzania

² London School of Hygiene and Tropical Medicine, London, UK

³ Amani Medical Research Centre, National Institute of Medical Research, Muheza, Tanzania

Summary

OBJECTIVE To determine the efficacy of chlorfenapyr against *Anopheles arabiensis* and *Culex quinquefasciatus* in East Africa and to identify effective dosages for net treatment in comparison with the commonly used pyrethroid deltamethrin.

METHODS Chlorfenapyr was evaluated on bed nets in experimental huts against *A. arabiensis* and *C. quinquefasciatus* in Northern Tanzania, at application rates of 100–500 mg/m².

RESULTS In experimental huts, mortality rates in *A. arabiensis* were high (46.0–63.9%) for all dosages of chlorfenapyr and were similar to that of deltamethrin-treated nets. Mortality rates in *C. quinquefasciatus* were higher for chlorfenapyr than for deltamethrin. Despite a reputation for being slow acting, >90% of insecticide-induced mortality in laboratory tunnel tests and experimental huts occurred within 24 h, and the speed of killing was no slower than for deltamethrin-treated nets.

CONCLUSIONS Chlorfenapyr induced low irritability and knockdown, which explains the relatively small reduction in blood-feeding rate. Combining chlorfenapyr with a more excito-repellent pyrethroid on bed nets for improved personal protection, control of pyrethroid-resistant mosquitoes and pyrethroid resistance management would be advantageous.

keywords chlorfenapyr, mosquito nets, *Anopheles*, *Culex*

Introduction

Pyrethroid-treated nets are the primary tool for preventing malaria in Africa (Armstrong-Schellenberg *et al.* 2000; Curtis *et al.* 2003). This technology is threatened by the development and rapid spread of pyrethroid resistance in the *Anopheles gambiae* and *A. funestus* species complexes in several parts of Africa (Vulule *et al.* 1994, 1999; Chandre *et al.* 1999; Hargreaves *et al.* 2000; N'Guessan *et al.* 2007a). There is an urgent need to identify alternative insecticides, which meet criteria for vector control and show no cross-resistance to pyrethroids (Zaim & Guillet 2002). Such insecticides have been developed by pesticide manufacturers for the agricultural sector, where market size provides greater potential rewards and profitability than the public health sector.

A typical example is fipronil, a phenylpyrazole insecticide, which is effective against veterinary pests (Postal *et al.* 1995) and which has some activity against mosquitoes (Ali *et al.* 1998) but shows cross-resistance to dieldrin in

A. stephensi and hence was not developed further (Kolaczinski & Curtis 2001).

Some novel insecticides that have shown encouraging results against mosquito larvae are chlorfenapyr, hydamethylnon, indoxacarb and imidacloprid. Diafenthiuron and chlorfenapyr also have potential for adult mosquito control (Paul *et al.* 2006). Chlorfenapyr, a pyrrole, is a relatively new pro-insecticide which is now widely used for control of veterinary and agricultural pests (Lovell *et al.* 1990; Sheppard & Joyce 1998). This insecticide, developed in 1988 (Tracy *et al.* 1994) and commercialised by BASF Corporation for agricultural pest control, has a unique mechanism of action involving the uncoupling of oxidative phosphorylation in the mitochondria as the primary target site (Black *et al.* 1994). Owing to this, chlorfenapyr seems unlikely to show cross-resistance to conventional neurotoxic insecticides. Laboratory tests with a discriminating concentration of chlorfenapyr resulted in full mortality in the susceptible, *kdr* and *Ace-1^R* strains of *A. gambiae sensu stricto*, indicating no cross-resistance to

these pyrethroid and organophosphate resistance mechanisms (N'Guessan *et al.* 2007b). N'Guessan observed that 100 and 250 mg/m² dosages of chlorfenapyr had significantly better impact on the *kdr* strain than field rates of permethrin (500 mg/m²), deltamethrin (25 mg/m²) or lambdacyalothrin (18 mg/m²) under similar conditions in tunnel tests (WHO 2006). Thus, we conducted further investigations towards the development of chlorfenapyr as an alternative to pyrethroid insecticides for net treatment. This study focused on determining efficacy against *A. arabiensis* and *Culex quinquefasciatus* in East Africa and identifying effective dosages for net treatment in comparison with the commonly used pyrethroid deltamethrin.

Materials and methods

Study area

Evaluation of chlorfenapyr-treated nets was carried out under laboratory and experimental hut conditions at the Kilimanjaro Christian Medical Centre, Moshi, in Northern Tanzania. The laboratory studies involved contact bioassays and tunnel tests (WHO 2006). Experimental hut studies took place in an area of rice irrigation at Mabogini field station in Lower Moshi (Kulkarni *et al.* 2007). The only man-biting mosquitoes found in significant numbers in Lower Moshi are *A. arabiensis* and *C. quinquefasciatus* (Ijumba *et al.* 2002).

Test materials were rectangular nets made of polyester and impregnated with chlorfenapyr at various dosages (100, 250, 500 mg/m²) or with deltamethrin at a standard dosage (25 mg/m²).

Evaluation techniques

Mosquito net was subjected to WHO cone bioassay before proceeding with tunnel tests or experimental hut trials. Sugar-fed, 5-day-old laboratory-reared *A. arabiensis* (Dondotha) were tested on each net according to standard procedures (WHO 2006). A total of 30 mosquitoes in three replicates of 10 mosquitoes were exposed on each treatment for 3 min before transfer to paper cups with sugar solution for 24- and 72-h mortality counts.

Standard WHO resistance tests were carried out by exposing wild caught *C. quinquefasciatus* and *A. arabiensis* mosquitoes to insecticide test papers in WHO test kits. Exposure was for 1 h and mortality was scored after 24 h holding (WHO 2006).

All treatments plus control were subjected to a tunnel test (WHO 2006). The equipment consists of a square glass cylinder (60 × 25 × 25 cm) with three chambers: bait

chamber, middle chamber and releasing chamber. The release and middle chambers were separated by a paper divide with a 16 cm² window. The middle and bait chambers were separated by test netting material with nine 1 cm diameter holes supported by a wooden frame. Mosquitoes released into the tunnel are attracted by host odour into the middle chamber, and have the opportunity to enter the bait chamber through holes in the netting. The system represents a miniaturised room with access to a host occupying a torn-treated net. Three replicates of approximately 50 mosquitoes were tested as precursor to experimental hut trials. Non-blood-fed 5- to 8-days old *A. arabiensis* (Dondotha) were released into each tunnel at 18:00 and recovered at 8:00. Mosquitoes were then removed and scored as live or dead and unfed or blood fed. Live mosquitoes were held and delayed mortality scored after 24 and 72 h.

Experimental hut evaluation was carried out at Mabogini field station, Moshi, Northern Tanzania in three experimental huts constructed according to a design described by Smith (1965) and Smith and Webley (1969). Some slight modifications were made involving reduction of eave space, addition of cardboard and hessian cloth ceiling, concrete floor surrounded by a water filled moat, and improved screening of the veranda. The working principle of these huts has been described by Curtis *et al.* (1996) and Mosha *et al.* (2008).

Two separate trials, each lasting for 16 nights, were conducted:

- *Chlorfenapyr dosage rate evaluation: 12 February–3 March 2006.* Treatments of 100, 250, 500 mg/m² chlorfenapyr plus untreated control were rotated between three huts every 4 days, with one treatment being excluded during each rotation. During this time, *A. arabiensis* were abundant while *C. quinquefasciatus* were very infrequently captured in the experimental huts.
- *Comparison of chlorfenapyr with deltamethrin: 7 March–26 March 2006.* The high dosage of 500 mg/m² was dropped and the remaining dosages of 100 and 250 mg/m² were compared with the pyrethroid deltamethrin (25 mg/m²). *Anopheles arabiensis* numbers were still high and the number of *C. quinquefasciatus* had increased to a reportable level.

During each trial, three recently treated nets (2–3 days before) plus an untreated net were rotated through each of the three huts. Each net had six 4 cm diameter holes, two on the long side and one on the short side of the net, to simulate the condition of a worn net. Sleepers were rotated between huts on successive nights to reduce any bias

because of differences in individual attractiveness to mosquitoes. The direction of two open verandas was routinely changed from East–West to North–South orientation with each treatment rotation in order to minimise the potential confounding factor of preferential escape route through the eaves towards external light at sunrise.

Mosquitoes were collected in the morning at 06:00 from inside the net, window (exit) traps, and ceiling, walls and floors of the verandas and room. The collected mosquitoes were kept for species identification, determination of abdominal condition and mortality counts. All members of the *A. gambiae* complex identified by morphological characteristics were assumed to be *A. arabiensis* based on previous cytotoxic and PCR identification results (Ijumba *et al.* 2002; Kulkarni *et al.* 2006) and our own cytotoxic examination results of some mosquito samples.

All live mosquitoes were held in paper cups and provided with 10% glucose solution. Mortality counts were carried out after 24- and 72-h holding periods for calculation of delayed mortality rates.

Data processing and analysis

The number of mosquitoes in the two veranda traps was multiplied by two to adjust for the unrecorded escapes through the two open verandas, which are left unscreened to allow routes for entry of wild mosquitoes via the gaps under the eaves. The data was double entered and analysed to show the effect of each treatment in terms of: (1) insecticide-induced exiting rates – percentage of total mosquitoes collected from veranda and exit traps; (2) blood-feeding inhibition – percentage of unfed mosquitoes from treated hut relative to percentage from control; and (3) overall mortality – total number of mosquitoes found dead immediately and after holding for 24 or 72 h. Assessment of these outcome variables between treatments relative to the control was analysed by logistic regression using STATA 8.0 Statistical software.

Toxicology

Chlorfenapyr has a WHO toxicological classification III: an LD₅₀ oral toxicity in rats of >400 mg/kg body weight, acute dermal toxicity >2000 mg/kg and inhalation toxicity of 1.9 mg/l. Chlorfenapyr is placed in the category of 'slightly hazardous' to humans (Tomlin 2000), similar to many insecticides used in public health.

A risk assessment of the use of chlorfenapyr on nets was undertaken by BASF toxicologists [BASF unpublished observation: *Exposure and health risks associated to the treatment and subsequent use of long-lasting impregnated*

mosquito nets (LLIN) treated with chlorfenapyr] using the WHO generic risk assessment model (WHO 2004). Potential exposure to chlorfenapyr was evaluated using assumptions, parameters and default values defined in the WHO model, which refers to user-treated bed nets. The calculated exposure levels to chlorfenapyr for the relevant age groups (adult, child and newborn) are all below the corresponding relevant dermal and systemic acute reference doses or acceptable exposure levels for repeated exposure. Exposure was deemed acceptable based on the conservative scenarios from the WHO model, indicating safe use of the chlorfenapyr-treated nets for the intended use.

Ethical clearance

Informed consent was obtained from all volunteers recruited to the experimental hut studies. The study was approved by ethics committees of LSHTM and NIMR (Ref: NIMR/HQ/R.8a/Vol. X/86).

Results

Contact bioassays and resistance tests

Nets treated with dosages of chlorfenapyr of 100–500 mg/m² induced mortality rates in *A. arabiensis* that ranged from 54.2% with the lowest to 68.5% with the highest concentration (Figure 1). There was little increase in final mortality with dosages above 250 mg/m². There was delayed mortality of 15–25% between 24 and 74 h. Deltamethrin (25 mg/m²) caused higher mortality (100%) than chlorfenapyr.

Anopheles arabiensis showed 100% mortality on deltamethrin test papers in resistance tests. *Culex quinquefasciatus* showed 80% mortality on permethrin and 96% mortality on deltamethrin test papers (*n* = 100 mosquitoes per test).

In the tunnel tests, there was a non-linear relationship between dosage and passage rates through the chlorfenapyr-treated netting, with proportionately more mosquitoes penetrating the 250 mg/m² treatment than the lower or higher dosage treatments. Blood-feeding inhibition showed the same trend, as only mosquitoes that penetrated the netting could feed (Figure 2). Mortality rates with all treatments ranged between 96% and 100% after 72 h (Figure 3). Almost all mortality occurred during the first 24 h.

Experimental hut trials

A summary of the results of chlorfenapyr treatments ranging from 100 to 500 mg/m² for *A. arabiensis* is shown

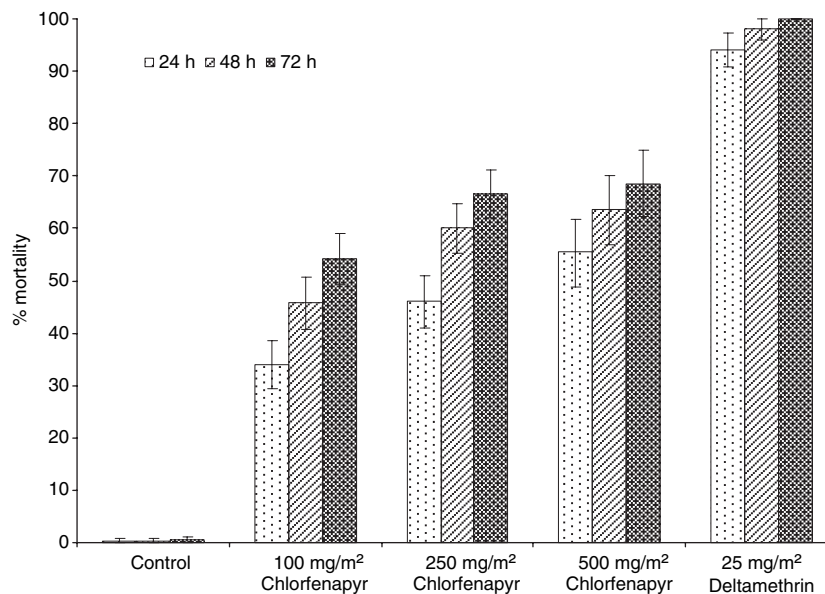


Figure 1 Mortality of *Anopheles arabiensis* 24, 48 and 72 h after exposure to chlorfenapyr or deltamethrin in cone bioassay tests.

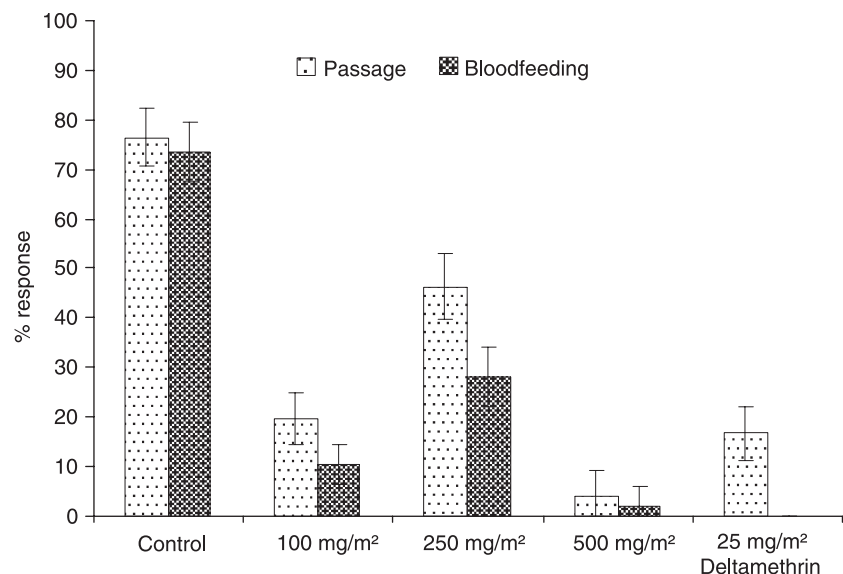


Figure 2 Behavioural responses of *Anopheles arabiensis* (Dondotha) in tunnel tests to chlorfenapyr- or deltamethrin-treated netting.

in Table 1. An average of 41.8 females per hut were collected from the huts and verandahs each morning. Numbers collected were similar in untreated control and insecticide-treated huts. Significantly fewer mosquitoes were found inside the holed chlorfenapyr 100 mg- and 500 mg-treated nets than inside the holed untreated nets. Significantly higher exiting rates of *A. arabiensis* (80.2%) were recorded with the 500 mg/m² dosage, while exiting rates with all other dosages were not significantly different

from the control. Significant blood-feeding inhibition was not observed for any dosage. High mortality of *A. arabiensis*, ranging between 58.5% and 63.9%, was recorded 72 h after exposure to chlorfenapyr. Most mortality occurred within the first 24 h. Delayed mortality between 24 and 72 h was about 5%.

For the comparison of the efficacy of chlorfenapyr and deltamethrin against *A. arabiensis* and *C. quinquefasciatus*, refer to Tables 2 and 3. An average of 26.0 *A. arabiensis*

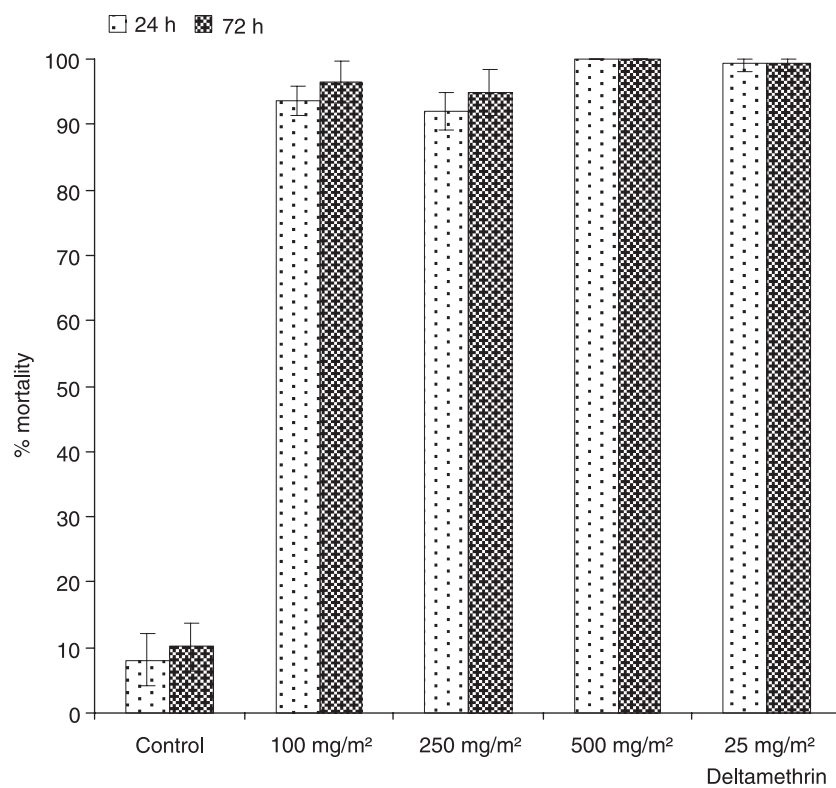


Figure 3 Mortality of *Anopheles arabiensis* (Dondotha) in tunnel tests to chlorfenapyr- or deltamethrin-treated netting.

and 3.5 *C. quinquefasciatus* females were collected from the room and verandahs of each hut per day. Fewer mosquitoes were collected from inside chlorfenapyr 250 mg and deltamethrin-treated nets than inside untreated nets. As in the previous trial, nets treated with 100 or 250 mg/m² chlorfenapyr showed no significant difference in exiting rate compared with the untreated control. Exiting rates between deltamethrin and chlorfenapyr did not differ significantly. A similar trend is observed for *C. quinquefasciatus*. There were higher

exiting rates with the deltamethrin treatment but, possibly as a result of the low numbers collected, no treatment showed an exiting rate significantly different from the control. In contrast to the previous trial, blood-feeding rates of *A. arabiensis* were significantly lower in the huts with chlorfenapyr-treated nets than in huts with untreated nets. There were no significant differences in feeding rate between the different concentrations of chlorfenapyr. The level of blood-feeding inhibition associated with deltamethrin treatment was no different from that of

Table 1 Summary of results obtained for *Anopheles arabiensis* in experimental huts with three different doses of chlorfenapyr

	Untreated net	Chlorfenapyr 100 mg/m ²	Chlorfenapyr 250 mg/m ²	Chlorfenapyr 500 mg/m ²
Total females caught	511	468	487	540
Females caught/night	42.6	39.0	40.6	45.0
24 h Mortality (%)	5.3 ^a (3.6–7.6)	58.3 ^b (53.8–62.7)	56.1 ^b (51.6–60.4)	54.3 ^b (50.0–58.4)
Corrected for control	–	56.0	53.6	51.7
72 h Mortality (%)	5.3 ^a (3.6–7.6)	63.9 ^b (59.4–68.1)	61.6 ^{bc} (57.2–65.8)	58.5 ^c (54.3–62.6)
Corrected for control	–	61.9	59.5	56.2
Blood feeding (%)	26.8 ^a (23.1–30.8)	26.1 ^a (22.3–30.2)	26.3 ^a (22.6–30.4)	26.5 ^a (22.9–30.4)
Blood-feeding inhibition (%)	–	2.6	1.9	1.1
Exiting rates (%)	73.6 ^a (69.6–77.2)	75.6 ^a (71.5–79.3)	72.5 ^a (68.3–76.3)	80.2 ^b (76.6–83.3)
% Caught in net	11.2 ^a (8.7–14.2)	4.5 ^b (2.9–6.8)	8.4 ^a (6.3–11.2)	3.1 ^b (2.0–5.0)

Numbers in the same row sharing a letter superscript do not differ significantly.

Table 2 Summary of results obtained for *Anopheles arabiensis* in experimental huts comparing two doses of chlorfenapyr and deltamethrin

	Untreated net	Chlorfenapyr 100 mg/m ²	Chlorfenapyr 250 mg/m ²	Deltamethrin 25 mg/m ²
Total females caught	321	422	265	238
Females caught/night	26.8	35.2	22.1	19.8
24 h Mortality (%)	3.4 ^a (1.9–6.1)	51.2 ^b (46.4–55.9)	42.3 ^c (36.5–48.3)	45.4 ^{bc} (39.2–51.7)
Corrected for control	–	49.5	40.3	43.5
72 h Mortality (%)	4.7 ^a (2.8–7.6)	56.9 ^b (52.1–61.5)	46.0 ^c (40.1–52.1)	49.6 ^{bc} (43.3–55.9)
Corrected for control	–	54.8	43.3	47.1
Blood feeding (%)	39.6 ^a (34.4–45.0)	25.1 ^b (21.2–29.5)	21.9 ^b (17.3–27.3)	22.3 ^b (17.4–28.0)
Blood-feeding inhibition (%)	–	36.6	44.7	43.7
Exiting rates (%)	81.9 ^a (77.3–85.8)	81.0 ^a (77.0–84.5)	85.7 ^a (80.9–89.4)	88.2 ^a (83.5–91.8)
% Caught in net	10.9 ^a (7.9–14.8)	7.8 ^{ab} (5.6–10.8)	4.5 ^b (2.6–7.8)	3.8 ^b (2.0–7.1)
Immediate mortality (% of total mortality)	0.0 ^a (0.0–0.0)	30.4 ^b (24.9–36.5)	35.2 ^b (27.3–44.1)	34.7 ^b (26.7–43.8)

Numbers in the same row sharing a letter superscript do not differ significantly.

Table 3 Summary of results obtained for *Culex quinquefasciatus* in experimental huts comparing two doses of chlorfenapyr and deltamethrin

	Untreated net	Chlorfenapyr 100 mg/m ²	Chlorfenapyr 250 mg/m ²	Deltamethrin 25 mg/m ²
Total females caught	61	47	28	32
Females caught/night	5.1	3.9	2.3	2.7
24 h Mortality (%)	1.6 ^a (0.2–10.7)	31.9 ^b (20.2–46.4)	17.9 ^{ab} (7.6–36.4)	12.5 ^a (4.8–28.9)
Corrected for control	–	30.8	16.6	11.1
72 h Mortality (%)	1.6 ^a (0.2–10.7)	31.9 ^b (20.2–46.4)	17.9 ^{ab} (7.6–36.4)	12.5 ^a (4.8–28.9)
Corrected for control	–	30.8	16.6	11.1
Blood feeding (%)	49.2 ^a (36.9–61.5)	23.4 ^b (13.5–37.5)	28.6 ^{ab} (15.0–47.6)	6.3 ^c (1.6–21.8)
Blood-feeding inhibition (%)	–	52.4	41.9	87.2
Exiting rates (%)	80.3 ^a (68.5–88.5)	78.7 ^a (64.8–88.2)	78.6 ^a (59.8–90.0)	96.9 ^a (80.9–99.6)

Numbers in the same row sharing a letter superscript do not differ significantly.

chlorfenapyr. Chlorfenapyr and deltamethrin treatments were associated with lower rates of blood feeding in *C. quinquefasciatus*, with deltamethrin producing significantly lower blood-feeding rates than any chlorfenapyr dosage.

Mortality rates in *A. arabiensis* were high across all dosages of chlorfenapyr relative to the control and there was no clear dosage effect. Mortality rates were similar to the previous trial. Mortality rates with the deltamethrin treatment were similar to the chlorfenapyr treatment and there was no evidence of delayed mortality being any less with deltamethrin. Immediate mortality rates (at the time of the morning collection) also did not differ between chlorfenapyr and deltamethrin. *Culex quinquefasciatus* mortality rates in the chlorfenapyr-treated huts were lower than for *A. arabiensis*, with the highest mortality (31.9%) occurring in the huts with the 100 mg/m²-treated net. Chlorfenapyr-induced mortality was significantly higher

than with the deltamethrin-treated net which killed only 12.5%. All chlorfenapyr-induced mortality in *C. quinquefasciatus* occurred within the first 24 h.

Discussion

The experimental hut trials demonstrate that chlorfenapyr has potential as a residual insecticide against mosquitoes on bed nets. Both hut trials, supported by tunnel tests, indicate that the lower chlorfenapyr dosages (100–250 mg/m²) are no less effective than higher dosages against *A. arabiensis* and *C. quinquefasciatus*. Against termites, the effectiveness of chlorfenapyr as a barrier treatment is largely attributed to its non-repellent toxic activity and to its long half-life which is about one year in soil (Rust & Saran 2006). Against mosquitoes, N'Guessan *et al.* (2007b) observed that chlorfenapyr is non-irritant at low dosages but stimulates take-offs at higher dosages.

The significantly increased exiting rates from huts containing nets treated with 500 mg/m² chlorfenapyr suggests that some excito-repellency may occur with higher dosages under natural conditions. At lower dosages, mosquitoes express little or no irritability and presumably spend more time on the treated surface, thereby picking up a more toxic dose. The reduced passage of *A. arabiensis* through the net in the tunnel test at higher dosages is possibly an expression of irritability to chlorfenapyr. With its low volatility of 1.3×10^{-5} Pa, chlorfenapyr does not have the characteristics of a spatial repellent. We could not detect or test for of spatial repellency expressed as deterred entry into huts owing to fluctuations in mosquito abundance during the course of the trial and of our need to systematically leave out treatments in order to test four treatments in the three available huts.

The mortality of *A. arabiensis* occurring between 24 and 72 h after exposure in all tests (contact, tunnel and hut) can be explained by the slow toxic action of chlorfenapyr, which in turn is attributed to its unique mode of action involving disruption of oxidative phosphorylation in the mitochondria (Lovell *et al.* 1990). Despite this, over 90% of mortality in huts and tunnels occurred within the first 24 h. There was no significant difference between deltamethrin and the chlorfenapyr treatments in terms of immediate mortality in the huts. This is an encouraging result from the perspective of users who might prefer to see an effect of treatment on numbers resting in the huts or lying dead on the nets or floors. High mortality during the first 24 h may be attributed to the more prolonged contact with treated nets in huts as opposed to contact bioassay exposure times.

A combination of low irritancy, low knockdown and the relatively slow-killing effect of chlorfenapyr explains why the rate of blood-feeding inhibition with chlorfenapyr-treated nets is less than what is commonly observed with pyrethroid-treated nets against susceptible populations. N'Guessan *et al.* (2007a), for example, recorded 96% reduction in blood-feeding with lambdacyhalothrin-treated nets against susceptible *A. gambiae* in northern Benin whereas we observed little or only partial reduction with chlorfenapyr. Blood-feeding inhibition with pyrethroids against other susceptible populations, for example in Ivory Coast, is not as pronounced as in N. Benin (Hougard *et al.* 2003). Species specific differences in response to pyrethroid may operate. In our trial, deltamethrin was only equivalent to chlorfenapyr in terms of feeding inhibition in *A. arabiensis*, a species known to differ in behaviour and geographic distribution to *A. gambiae* s.s. Resistance in *A. arabiensis* is not a factor affecting feeding inhibition as this species is fully suscep-

tible to deltamethrin in Lower Moshi (Kulkarni MA, Mosha FW, Temu E, Rowland M, Rau ME & Drakely C, unpublished observation).

The second hut trial indicated that chlorfenapyr produces a killing effect comparable with that of deltamethrin against *A. arabiensis* and superior to that of deltamethrin against *C. quinquefasciatus* (*Culex* is partially resistant to deltamethrin in this population). This is encouraging and warrants further evaluation of the lower dosage (100 mg/m²) of chlorfenapyr against *A. arabiensis* and *C. quinquefasciatus*. To optimise the personal protective effect of this insecticide it may be beneficial to produce a mixture with an insecticide having high excito-repellent action such as a pyrethroid (Lindsay *et al.* 1991, 1992; Miller *et al.* 1991; Curtis *et al.* 1992). This approach will not only contribute to increased overall efficacy but may also guard against development of chlorfenapyr resistance – as may be developing in some agricultural pests in Australia – by the pyrethroid component killing any chloroquine-resistant individual and *vice versa* (Gunning & Moores 2002; Herron *et al.* 2004). This is classic resistance management strategy through use of mixtures (Denholm & Rowland 1992). It is important to emphasise there is no reported resistance to chlorfenapyr in mosquito populations. Thus, the lower mortality rates observed with chlorfenapyr in *C. quinquefasciatus* compared with *A. arabiensis* may be due to behavioural differences that affect contact time with treated surfaces.

The reported negative cross-resistance action of chlorfenapyr to pyrethroids in some species of insect (Pimprale *et al.* 1997; Sheppard & Joyce 1998) places it as a good candidate for malaria vector control in areas with pyrethroid-resistant *A. gambiae* (Vulule *et al.* 1994; Chandre *et al.* 1999; N'Guessan *et al.* (2007a)) and *A. funestus* (Hargreaves *et al.* 2000). However, it is necessary to extend the residual activity of chlorfenapyr by developing a long-lasting formulation comparable with pyrethroid-based long-lasting insecticidal nets. Being a solid at ambient temperature (melting point of 100–101 °C) with low vapour pressure (5×10^{-3} mPa), and practically insoluble in water (0.12 mg/l), chlorfenapyr would appear to have the characteristics suitable for inclusion in long-lasting formulations (Rand 2004). A bi-treated net would have utility not only in areas of resistance but also in areas which are currently pyrethroid susceptible in order to delay the selection of pyrethroid resistance and to extend the useful life of pyrethroids. The characteristics of low excito-repellency and toxicity may combine to make chlorfenapyr a strong candidate for indoor residual spraying, provided adequate residual activity on interior cement and mud walls could be ensured.

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Corresponding Author Mark Rowland, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK. Tel.: +44 20 7299 4719; Fax: +44 20 7299 4720; E-mail: mark.rowland@lshtm.ac.uk

Chlorfenapyr: A pyrrole insecticide for the control of pyrethroid or DDT resistant *Anopheles gambiae* (Diptera: Culicidae) mosquitoes

R. N'Guessan^{a,b}, P. Boko^b, A. Odjo^b, M. Akogbéto^b, A. Yates^a, M. Rowland^{a,*}

^a London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

^b Centre de Recherche Entomologique de Cotonou, 06 BP 2604 Cotonou, Benin

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Abstract

Owing to the development and spread of pyrethroid resistance in *Anopheles gambiae* in Africa there is an urgent need to develop alternative insecticides to supplement the pyrethroids. Chlorfenapyr is a pyrrole insecticide first commercialized for the control of agricultural pests and termites. Performance against *An. gambiae* bearing *kdr* (pyrethroid and DDT resistance) or *Ace-1^R* insensitive acetylcholinesterase (organophosphate and carbamate resistance) mechanisms was studied using a variety of adult bioassay tests including a simulated-experimental hut system (tunnel tests) that allows uninhibited mosquito behaviour/insecticide interactions. Strains resistant to pyrethroids and organophosphates showed no cross resistance to chlorfenapyr. In cone bioassays on treated netting the mortality of adult mosquitoes showed an unexpected curvilinear response, with highest mortality occurring at intermediate dosages. Adults expressed irritability to chlorfenapyr at higher dosages, which might explain the dosage–mortality trend. Toxic activity of chlorfenapyr was slow compared to conventional neurotoxic insecticides and additional mortality occurred between 24 h and 72 h. In tunnel tests, the dosage–mortality trend showed a more typical sigmoid response and most mortality occurred during the first 24 h. Mosquito penetration through the holed, treated netting showed only limited inhibition and blood-feeding was not inhibited. Mortality rates in the *kdr* strain exposed to chlorfenapyr treated netting in tunnel tests were much higher than with permethrin treated netting over the same 100–500 mg/m² dosage range. Chlorfenapyr has potential for malaria control in treated-net or residual spraying applications in areas where mosquitoes are pyrethroid resistant. For treated-net applications chlorfenapyr might be combined with pyrethroid as a mixture to provide personal protection as well as to give control of resistant mosquitoes.

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1. Introduction

Malaria is a major cause of morbidity and mortality in Africa and Asia but is preventable through interventions

of insecticide treated nets (ITN) or indoor residual spraying (IRS) (Curtis et al., 1998; Rowland, 1999; Rowland et al., 2000). Pyrethroids are widely used for control of anophelines and are the only insecticides currently recommended for use on mosquito nets (Zaim et al., 2000). In the last decade, resistance to pyrethroids has become widespread in *Anopheles gambiae* in western and eastern Africa and in *An. funestus* in southern Africa

* Corresponding author. Tel.: +44 20 7299 4719; fax: +44 20 7299 4720.

E-mail address: mark.rowland@lshtm.ac.uk (M. Rowland).

(Chandre et al., 1999a; Etang et al., 2003; Hargreaves et al., 2000; Nikou et al., 2003; Vulule et al., 1999). A number of resistance mechanisms appear to be involved: *kdr* site insensitivity is prevalent in *An. gambiae* and enhanced oxidase mechanisms occur in both *An. gambiae* and *An. funestus*. Resistance is a threat to the future of ITNs and IRS but the distribution of resistance is patchy and the seriousness of the problem differs from region to region. For example, *kdr* resistance is present at high frequency in parts of the Ivory Coast but trials of pyrethroid-treated nets in that country continue to show effectiveness against malaria (Darriet et al., 2000; Asidi et al., 2004; Henry et al., 2005; Hougard et al., 2003a). Elsewhere in Ivory Coast or in neighbouring countries (Burkina Faso and Benin) there are instances of *kdr* homozygotes showing survival in the presence of ITN (Kolaczinski et al., 2000; Diabate et al., 2006) or of ITN or IRS failing to control resistant *An. gambiae* (N'Guessan et al., 2007). In South Africa IRS with deltamethrin failed to prevent the reappearance of *An. funestus* in resistant form or to stem the rise in malaria cases (Hargreaves et al., 2000). There is an urgent need to develop alternative insecticides to replace or supplement the pyrethroids before the situation grows worse (Zaim and Guillet, 2002). Whereas a number of insect growth regulators and bacterial insecticides have been registered for larval control in recent years, no new class of chemistry has emerged for adult control since the development of the pyrethroids more than 20 years ago. Malaria control is a small, rather risky market and the main pesticide manufacturers concentrate their efforts on developing pesticide for the agricultural sector where revenues and profits are larger and more reliable. Several new classes of agro-pesticide chemistry have emerged in recent years, but the majority seem unsuitable for adult mosquito control. The spinosads have potential as larvicides rather than adulticides (Romi et al., 2006), some tested neonicotinoids seem insufficiently active (Corbel et al., 2004a) and the fiproles show cross resistance to older classes of insecticide (Kolaczinski and Curtis, 2001). The primary characteristics required in a good adulticide are contact activity, long residual activity and low mammalian toxicity. These criteria are less essential in agro-insecticides. One exception that may meet the vector control criteria is chlorfenapyr, a member of the pyrrole class, used commercially for termite control and crop protection against a variety of insect and mite pests (Lovell et al., 1990; Pimprale et al., 1997; Sheppard and Joyce, 1998). Chlorfenapyr is a pro-insecticide that is activated by cytochrome P450 monooxygenases to its more active metabolite (Black et al., 1994). Unlike pyrethroids that affect nerve function chlorfenapyr is a

mitochondrial electron transport inhibitor (METI) whose mode of action is to disrupt the conversion of ADP to ATP (oxidative phosphorylation) in mitochondria of cells (Anon., 1995). Chlorfenapyr facilitates proton loss from inside to outside the mitochondria. When uncoupled from a proton energy source the mitochondria are unable to generate ATP and the cells cease to function (Anon., 1995). Because chlorfenapyr's mode of action is novel it is unlikely to show cross resistance to standard neurotoxic insecticides. It has a WHO toxicological classification III: an LD50 oral toxicity in rats of >400 mg/kg body weight, acute dermal toxicity of >2000 mg/kg, and inhalation toxicity of 1.9 mg/l. Chlorfenapyr is placed in the category of "slightly hazardous" to humans (Tomlin, 2000), the same category as many insecticides recommended by WHO for public health use. In recent laboratory tests against the mosquito *Aedes aegypti* it showed efficacy against larvae and adults (Paul et al., 2006).

The aim of the present project was to examine the efficacy and personal protection potential of chlorfenapyr against insecticide-resistant and susceptible *An. gambiae* and *An. stephensi* to assess its suitability for use in Africa and Asia as a residual insecticide on netting or other indoor surfaces.

2. Material and methods

2.1. Mosquito strains

Five laboratory colonies were used:

- *An. gambiae* Kisumu; a susceptible reference strain, from Kenya.
- *An. gambiae* Vkpr; pyrethroid resistant, fixed for *kdr*, from the Kou valley in Burkina Faso.
- *An. gambiae* Yao; resistant to organophosphates and carbamates conferred by insensitive acetylcholinesterase, and to pyrethroids conferred by *kdr*, from Yaokoffikro, Bouake, Ivory Coast. The frequency of the *Ace-1^R* allele was 100%.
- *An. stephensi* Beech; a susceptible reference strain.
- *An. stephensi* Dub234; resistant to pyrethroids and OPs, conferred by cytochrome P450 oxygenases, esterases and *kdr*-type target insensitivity, from Dubai.

2.2. Residual activity and cross resistance

The residual activity of chlorfenapyr and potential cross resistance in *An. gambiae* Kisumu, Vkpr and Yao strains was assessed by exposing adult females, 3–5 days of age, in WHO cylinder kits to filter paper impregnated

with 2% chlorfenapyr in silicon oil for periods upto 3 h; mortality was scored 24–72 h later. Test papers were initially treated with chlorfenapyr (BASF Corp, USA) dissolved in acetone (1.3 ml) and silicon oil (0.7 ml) (WHO, 1998). Approximately 100 females were tested at each exposure period. LT50s, LT95s and resistance ratios were estimated using probit analysis (Polo-PC, LeOra Software, Berkeley, CA).

Supplementary assays were performed using papers treated with 0.125–4% chlorfenapyr in silicon oil. Approximately 200 females were exposed to each concentration for 1 h and mortality scored 24–72 h later.

Activity of chlorfenapyr on *An. stephensi* Beech and Dub234 strains was assessed by exposing females to a range of chlorfenapyr concentrations for 1 h. Approximately 100 mosquitoes were tested per concentration.

2.3. Cone and wire ball bioassays

The WHO cone test, in which mosquitoes are exposed to pyrethroid-treated netting for 3 min within a plastic cone is the standard way to measure insecticide efficacy (WHO, 1998). An alternative approach is the wire ball bioassay (WHO, 2006), a metal frame completely covered with netting which has the advantage of presenting no non-insecticidal surfaces for mosquitoes to alight upon or avoid insecticide contact. Formulated chlorfenapyr ('Phantom' 15% SC) from BASF Corp was tested on 100-denier netting in cone and wire ball bioassays against *An. gambiae* Kisumu over a range of concentrations (5–1000 mg/m²) and exposure times (3–24 min). Mortality was scored after 24 h, 48 h and 72 h, and data analysed using logistic regression (STATA 6 software) or probit analysis (Polo-PC, LeOra Software, Berkeley, CA).

2.4. Irritability test

Two chlorfenapyr concentrations (100 mg/m² and 1000 mg/m²) were tested on netting for their irritancy to individual non-blood-fed Vkpr *An. gambiae* females, aged 2–5 days, in WHO plastic cones. After a settling period of 60 s the time elapsing until the next take-off was recorded as the "time to first take-off" (Mouchet and Cavalié, 1961). For each concentration and control, 50 mosquitoes were tested. Mosquitoes were grouped into geometric classes of 'time to first take-off' (0–1 s, >1–2 s, >2–4 s, >4–8 s, ..., >128–256 s). Probit analysis was used to calculate the time for 50% of mosquitoes to take-off (FT₅₀).

2.5. Tunnel tests

The same dosages of chlorfenapyr used in cone bioassays were evaluated in tunnel tests against pyrethroid-resistant *An. gambiae* (Vkpr). The tunnel test is a laboratory system designed to allow mosquitoes to freely express behavioural responses to insecticide that would occur when encountering ITNs under field conditions. Tunnel tests are used as a forerunner to experimental hut trials, and provide useful information on repellency, blood-feeding inhibition and mortality (WHO, 2006). The equipment consists of a 4-sided glass cylinder (25 cm high, 25 cm wide, 60 cm long) which is divided into two chambers by a netting insert which slots across the tunnel. In one of the chambers, a guinea pig is housed unconstrained in an open meshed cage and in the other chamber ca. 100 unfed female mosquitoes aged 2–5 days are released at dusk and left overnight. The netting is deliberately holed with nine 1 cm holes to give mosquitoes opportunity to penetrate into the baited chamber. The following morning, the number of mosquitoes alive or dead, fed or unfed in each chamber are scored. Live mosquitoes are given access to sugar solution and kept up to 72 h to record delayed mortality. Tunnel test data was analysed using analysis of variance of arcsine transformed proportions (STATA 6 software).

3. Results

3.1. Cross resistance

Log-time probit analyses of the mortality of susceptible and resistant strains of *An. gambiae* to 2% chlorfenapyr papers in WHO kits are presented in Table 1. A significant 2.5-fold tolerance to chlorfenapyr was expressed in the Vkpr strain but not in the Yao strain. A tolerance of this magnitude could be due to inter-strain variation, and was not apparent in the Yao strain which also carries *kdr*. With *An. stephensi* the toxicity of chlorfenapyr was similar in pyrethroid susceptible and resistant strains (Table 2).

Mortality of *An. gambiae* Kisumu induced by chlorfenapyr over a 0.125–4% range of concentrations showed a log-linear response. Owing to the delayed activity of chlorfenapyr, the LC₅₀ decreased 3-fold between 24 h and 48 h and 14-fold between 48 h and 72 h (Tables 3A and 3B). The LD₅₀ of 0.04% at 72 h represents the value computed when all insecticide-induced mortality had taken effect. The confidence interval is wide at this point because even the lowest dosage tested was killing more than 50% at 72 h.

Table 1

Log-time mortality probit analysis of 2% chlorfenapyr treated papers against *An. gambiae* susceptible and pyrethroid/OP strains in WHO cylinder kits after 24 h holding period

Strain	Slope (S.E.)	LT50 (mn) (95% CI)	LT95 (mn) (95% CI)	RR50 (95% CI)
Kisumu (S)	3.4 (0.35)	17.4 (11.4–23.7)	53.6 (36.4–126.8)	–
Vkpr (<i>kdr</i>)	2.6 (0.20)	43.7 (29.5–58.4)	184.0 (123.9–400.5)	2.5 (2.2–2.9)
Yao (<i>Ace-I</i>)	2.7 (0.17)	18.9 (5.6–34.9)	137.0 (64.4–1729)	1.1 (0.9–1.3)

RR50 represents the resistance factor for *kdr* or *Ace-I* strains relative to the susceptible strain at LT50 level.

Table 2

Log-dosage–mortality probit analysis of chlorfenapyr against *An. stephensi* pyrethroid susceptible (S) and resistant (R) strains in WHO cylinder kits after 24 h holding periods

Strain	Slope	LD50	LD95	RR50
Beech (S)	2.08 (0.19)	0.87 (0.70–1.08)	5.3 (3.5–10.5)	1.14 (0.85–1.51)
Dub234 (R)	2.30 (0.40)	0.99 (0.77–1.25)	5.1 (2.9–12.2)	

RR50 represents the resistance ratio between the two strains at the LD50 level. LD50 and LD95 are expressed as the % concentration of chlorfenapyr in silicon oil.

Table 3A

Summary of log-dosage–mortality probit analysis of chlorfenapyr against *An. gambiae* Kisumu in WHO cylinder kits after 24 h, 48 h and 72 h holding periods

Holding period (h)	Slope (S.E.)	LD50	LD95	TR50
24	1.9 (0.1)	0.6 (0.5–0.7)	4.6 (3.5–6.7)	–
48	1.6 (0.14)	0.2 (0.1–0.3)	2.3 (1.3–6.6)	2.6 (2.2–3.2)
72	1.2 (0.1)	0.04 (0.01–0.08)	1.0 (0.6–2.3)	14.3 (8.5–24.1)

LD50 and LD95 are expressed as % concentration of chlorfenapyr in silicon oil. TR50 represents the toxicity ratio at LD50 level at 24 h relative to that at 48 h and 72 h.

Table 3B

Mortality (%) of *An. gambiae* females (Kisumu, susceptible strain) after 1 h exposure to chlorfenapyr impregnated papers in WHO cylinder kits

	Chlorfenapyr concentration (no. of tested)						
	0% (205)	0.125% (180)	0.25% (213)	0.5% (200)	1% (206)	2% (167)	4% (227)
24 h	2.4	12.2	22.5	47.0	68.9	85.6	91.6
48 h	3.4	38.3	57.7	66.0	80.6	96.4	100
72 h	3.4	73.3	84.0	89.5	92.2	98.8	100

Mortality was recorded after 24 h, 48 h and 72 h holding periods.

Exposure to 4% chlorfenapyr for 1 h induced *ca.* 90% mortality after 24 h and 100% mortality after 48 h in the Kisumu susceptible, Vkpr and Yao strains, indicating no cross resistance between chlorfenapyr and *kdr* and *Ace-I^R* resistance mechanisms (Table 4).

3.2. Cone and wire ball tests

Mortality of mosquitoes to chlorfenapyr showed delayed expression in cone tests on treated netting; chlorfenapyr took up to 72 h to show full effect ($P < 0.001$).

Table 4

Mortality rates (%) of pyrethroid/OP resistant and susceptible *An. gambiae* on exposure to papers impregnated with 4% chlorfenapyr for 1 h

Post-exposure period (h)	Kisumu susceptible (N=99)	Vkpr <i>kdr</i> (N=102)	Yao <i>Ace-I^R</i> (N=122)
24	91.6	89.2	92.6
48	100	100	100

Mortality was recorded after 24 h and 48 h. Control mortality was <3% after 48 h.

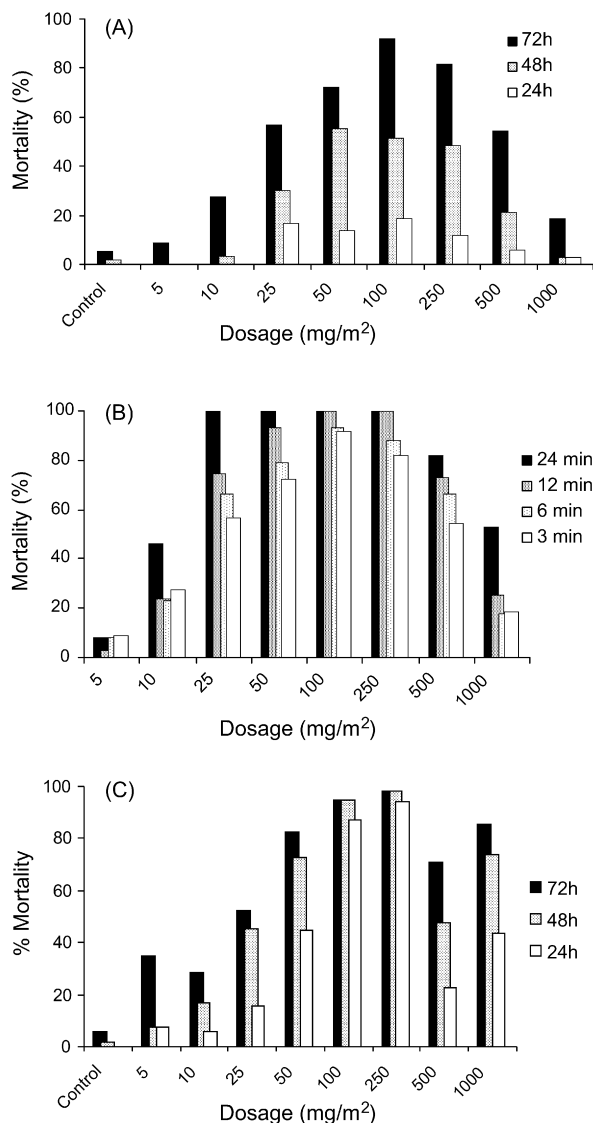


Fig. 1. Efficacy of chlorfenapyr treated netting over a range of concentrations against *An. gambiae* Kisumu. (A) Delayed mortality over 24–72 h holding periods after an initial 3 min exposure in WHO cone assays. (B) Mortality after exposure to a range of concentrations and exposure periods in cones (mortality scored after 72 h holding period). (C) Delayed mortality in wire ball assays after exposure to a range of concentrations for 3 min.

The dosage–mortality response was regular between 5 mg/m² and 100 mg/m² but showed a negative mortality trend at higher dosages (Fig. 1A). Extending the duration of exposure in the cones from 3 min to 24 min had a significant effect on mortality rates ($P < 0.001$) but the curvilinear dosage–mortality response observed with the shorter exposure period was still evident after longer exposures (Fig. 1B).

The value of the wire ball test over cone tests is that it provides only insecticidal surfaces for mosquitoes to rest upon. The ‘ball’ method produced a curvilinear trend but showed higher mortality rates at the higher dosages than did the cone tests (Fig. 1C).

3.3. Irritability tests

The proportion not taking off in the observation period was significantly lower among those exposed to 500 mg/m² treated netting than among those exposed to untreated netting ($\chi^2 = 5.9$, $P = 0.01$) or 100 mg/m² treated netting ($\chi^2 = 12.1$, $P < 0.001$). The proportion not taking off did not differ between those exposed to 100 mg/m² and untreated netting ($\chi^2 = 1.1$, $P = 0.29$) (Table 5). The time to first take-off as indicated by FT₅₀ was faster among those exposed to 500 mg/m² netting than among those exposed to untreated or 100 mg/m² netting; however, the difference was not significant at FT₅₀. The irritability expressed at the higher concentration of chlorfenapyr would be sufficient to explain the negative mortality trend shown in cone tests if contact time with insecticide was indeed reduced at higher concentrations.

3.4. Tunnel tests

The tunnel test results are summarised in Fig. 2A–G. Overall there was 21% reduced penetration of mosquitoes through the chlorfenapyr treated nets compared to the untreated control ($P = 0.007$) (Fig. 2A). The insecticide-induced inhibition did not show a clear dosage-dependent trend ($P = 0.75$). The effect on penetration was more apparent in Kisumu and Yao than in Vkpr strains. Since Yao and Vkpr both carry the *kdr* gene, any between-strain differences seem unlikely to be attributable to *kdr*. The pattern of % blood-feeding (Fig. 2B) showed the same trend as % penetration; this was because a regular proportion (ca. 75%) of individuals that penetrated the chlorfenapyr treated netting went on to feed (Fig. 2C). There were no between-strain differences in blood-feeding rate among those that penetrated the treated netting ($P = 0.755$). Unlike in cone tests, the dosage–mortality trend in tunnel tests was a typical sigmoid curve (Fig. 2D). Dosages of 250 mg/m² or higher induced >80% mortality within 24 h. Delayed mortality was more evident at lower dosages (50–100 mg/m²) than at higher. No cross resistance to chlorfenapyr between Kisumu and *kdr* or *Ace-1^R* bearing strains was evident in the tunnel tests ($P = 0.31$) (Fig. 2E). There was high mortality among all three strains even at the low dosage of 50 mg/m². Dosages of

Table 5

Time to first take-off (FT₅₀) of *An. gambiae* Kisumu exposed to chlorfenapyr treated netting in WHO irritability tests

	Dosage (mg/m ²)	N	% not taking off (95% CI)	FT ₅₀ # (s) (95% CI)	Induced irritancy at FT ₅₀ level relative to control
Control	0	100	77 (68.7–85.2)	181 (74–2019)	–
Chlorfenapyr	100	100	83 (75.6–90.4)	203 (86–1822)	0
Chlorfenapyr	500	100	61 (51.4–70.6)	140 (71–977)	23%

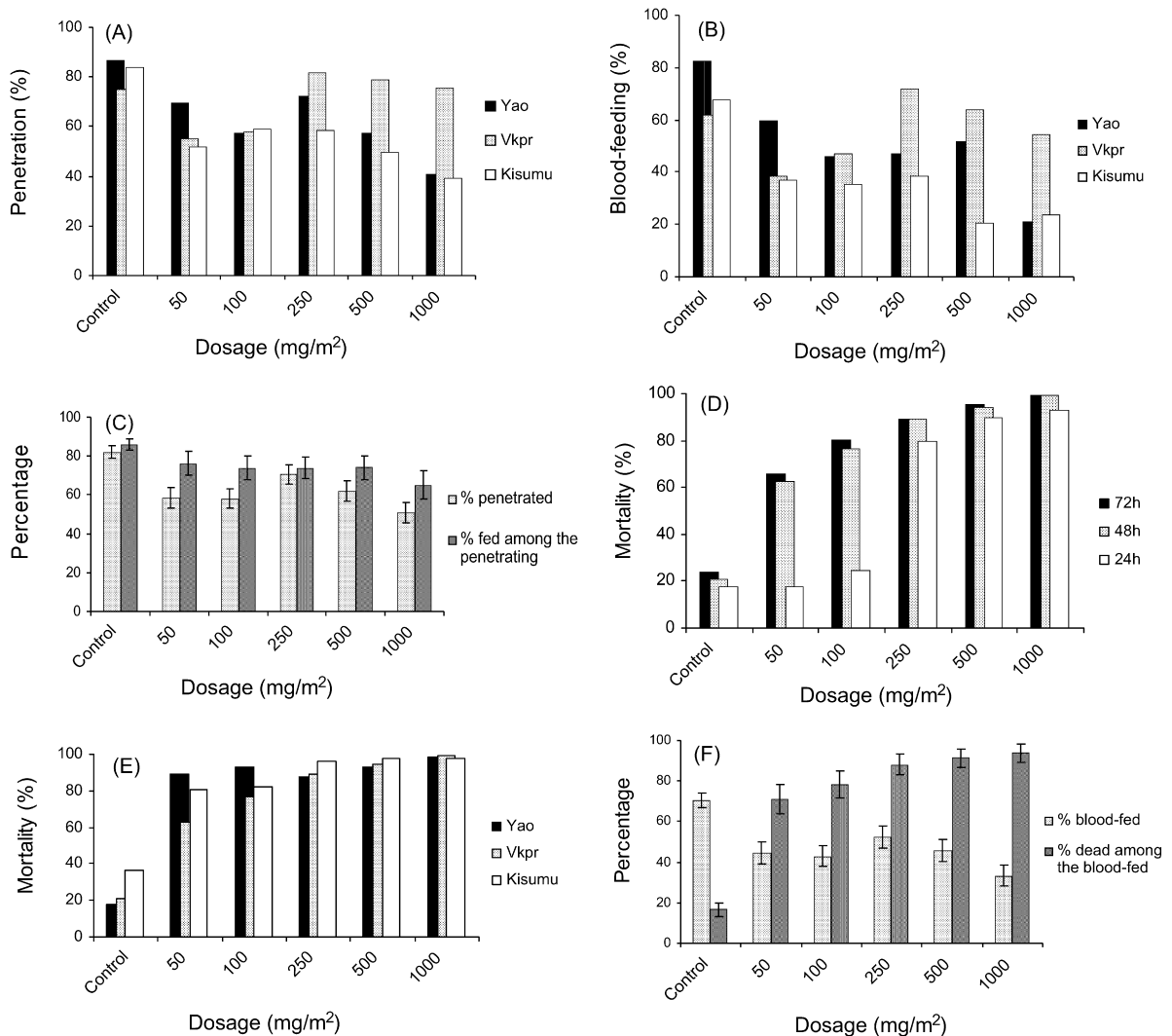


Fig. 2. Efficacy of chlorfenapyr treated netting over a range of concentrations against *An. gambiae* in tunnel tests. (A) Proportion of mosquitoes of Kisumu, Vkpr and Yao strains penetrating the holed netting. (B) Proportion blood-feeding among the three strains. (C) Overall proportion penetrating and the proportion blood-feeding among those that penetrated. (D) Delayed mortality of susceptible *An. gambiae* Kisumu over 24–72 h. (E) Mortality of Kisumu, Vkpr and Yao strains over 48 h. (F) Overall proportion blood-feeding and proportion dead after 48 h among those that blood-fed. (G) Comparison of response of Vkpr to chlorfenapyr and permethrin over a similar range of concentrations (permethrin tunnel data taken from Corbel et al., 2004b). Chlorfenapyr: stippled bars, permethrin: solid black bars.

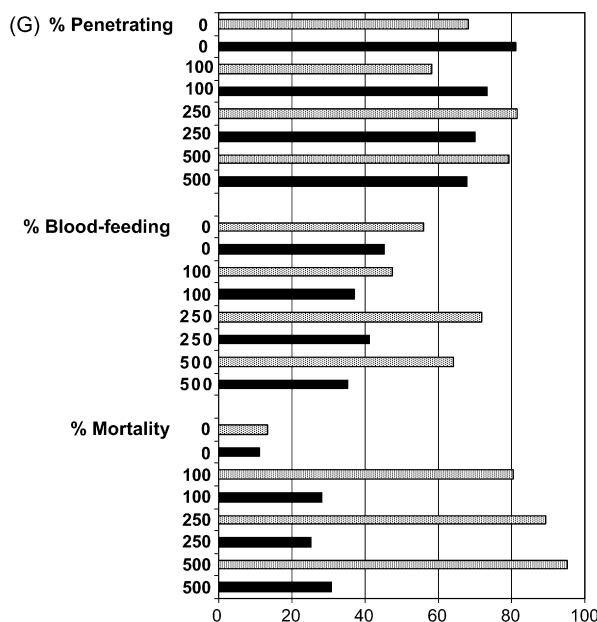


Fig. 2. (Continued).

100 mg/m² or more killed between 77% and 96% of *kdr* and *Ace-1^R* resistant *An. gambiae* within 48 h (Fig. 2E). In the chlorfenapyr tests, the majority (70–90%) of mosquitoes that blood-fed subsequently died (Fig. 2F). There was no significant differences between control and treatments in the proportions that had blood-fed among the dead mosquitoes ($P=0.88$). This indicates that mosquito capacity to blood-feed on the guinea pigs was not reduced in the period immediately after coming into contact with treated netting.

Fig. 2G compares the response of pyrethroid-resistant *Vkpr* in tunnels to chlorfenapyr and permethrin treated netting (cf. Corbel et al., 2004b) across a similar range of concentrations. Mortality is presented at the time at which any insecticide-induced mortality had taken full effect –15 h in the case of permethrin (Corbel et al., 2004b) and 48 h in the case of chlorfenapyr (see Fig. 2D). There was greater blood-feeding inhibition in response to the permethrin treatments but much greater mortality in response to the chlorfenapyr treatments.

4. Discussion

The various bioassay techniques that were used to measure intrinsic toxicity or behavioural responses to insecticide were consistent in showing no cross resistance between chlorfenapyr and the two common mechanisms, *kdr* and *Ace-1^R*, that confer resistance to pyrethroids, organophosphates and carbamates in *An.*

gambiae. Because chlorfenapyr's mode of action is to disrupt oxidative phosphorylation in the mitochondria rather than to target neural receptors, an absence of cross resistance to these mechanisms was anticipated (Anon., 1995; Lovell et al., 1990). Being a pro-insecticide which is metabolised to its active form by mixed function oxidases, chlorfenapyr has shown in some species the property of negative cross resistance. For example, chlorfenapyr is 2–5 times more toxic to pyrethroid resistant pests such as the cattle horn fly, *Haematobia irritans* or the tobacco budworm, *Heliothis virescens*, in which resistance is based on elevation of mixed function oxidase activity, than it is to pyrethroid susceptible strains (Pimprale et al., 1997; Sheppard and Joyce, 1998). In the present series of studies we tested chlorfenapyr against a strain of *An. stephensi* that once had multiple pyrethroid resistance mechanisms (Vatandoost, 1996) but it was never clear whether MFO mediated resistance was still present—certainly there was no evidence of negative cross resistance to chlorfenapyr. Strains of *An. gambiae* or *Culex quinquefasciatus* whose pyrethroid resistance is based on elevated oxidases do exist (Chandre et al., 1999a; Etang et al., 2003; Nikou et al., 2003; Vulule et al., 1999). The prospect of exploiting negative cross resistance to chlorfenapyr as a management strategy to control pyrethroid resistant mosquitoes is an attractive one. Evaluation against pyrethroid resistant *An. funestus* in southern Africa where pyrethroid failure was associated with selection of an MFO resistance mechanism (Hargreaves et al., 2000) is a priority.

The peculiar curvilinear dosage–mortality response expressed in the cone tests appeared to be due to irritability and reduced contact time at higher dosages. Such trends were not apparent in the overnight tunnel tests where contact time of host-seeking mosquitoes was potentially much longer. Some inhibition of penetration was evident with treated netting but the effect was not great (21% inhibition) and those that succeeded in penetrating the holed netting were not affected in their capacity to blood-feed, though many died later on.

The 2–3-fold tolerance to chlorfenapyr in the Vkpr strain was probably due to strain variation since the Yao strain which also carried *kdr* showed no such tolerance. Recently chlorfenapyr resistance has emerged in some agricultural pests (Van Leeuwen et al., 2006; Gunning and Moores, 2002). In the two-spotted spider mite, *Tetranychus urticae*, resistance to chlorfenapyr was associated with an increase in P450 mono-oxygenase activity and to a decrease in TMBZ peroxidation activity that is allegedly responsible for activation of chlorfenapyr (Van Leeuwen et al., 2004). In the housefly, some but not all strains that expressed enhanced mono-oxygenase mediated resistance showed negative cross resistance to chlorfenapyr (Scott et al., 2004; Van Leeuwen et al., 2006). In the cotton bollworm, *Helicoverpa armigera*, the emergence of chlorfenapyr resistance appears to be mediated by an esterase that is also responsible for pyrethroid resistance (Gunning and Moores, 2002). Drawing examples from other pests may provide some insight as to what might happen in mosquitoes but which mechanism of chlorfenapyr resistance will eventually evolve remains unpredictable.

Our 100 mg/m² and 250 mg/m² dosages of chlorfenapyr induced significantly greater mortality in the *kdr* strain than did field rates of permethrin (500 mg/m²), deltamethrin (25 mg/m²) or lambda-cyhalothrin (18 mg/m²) tested in tunnels (Hougard et al., 2003b). Dosages of chlorfenapyr within this range might be appropriate to test on nets in experimental huts against field populations of *kdr* resistant *Anopheles* and *Culex*. A key difference between chlorfenapyr and pyrethroids is the comparatively low personal protection to be gained when the chlorfenapyr-treated netting becomes holed, as shown by high penetration rates through holed netting and by the majority of penetrating mosquitoes then feeding before dying. Because chlorfenapyr treated nets seem more toxic than repellent to pyrethroid resistant mosquitoes, it would be predicted that at high coverage levels (for example, through roll-out programmes of free or subsidized nets), chlorfenapyr treated nets would collectively kill through a mass population effect a substantial proportion of

mosquitoes before they became infective, thereby reducing the force of infection and providing community protection. For individual protection against pyrethroid resistant mosquitoes the best approach might be to combine chlorfenapyr with pyrethroids in a mixture as together they would induce feeding inhibition and mortality of pyrethroid resistant mosquitoes. A mixture might also act to reduce the selection pressure for pyrethroid resistance in areas where mosquitoes are still largely susceptible, as predicted by theoretical models (Curtis, 1985). Chlorfenapyr has a safety profile similar to that of permethrin/deltamethrin and the hazard to users or occupational workers is within acceptable limits (BASF unpublished risk assessment). With its novel mode of action distinct from that of pyrethroids, a mixture of chlorfenapyr with pyrethroids is unlikely to present an enhanced toxicity risk to humans.

The twin characteristics of limited repellency and high toxicity might make chlorfenapyr a strong candidate for indoor residual spraying (IRS) provided good residual activity on interior walls can be achieved. Delayed toxicity is not a major limitation to IRS (DDT and once-effective organochlorines such as dieldrin and γ HCH are slow acting) because the interval between mosquitoes acquiring a malaria infection and being able to transmit it takes several days.

ITNs are used not only as protection against malaria mosquitoes but also as protection against *Cx. quinquefasciatus*, a species which often occurs alongside *Anopheles*. *Culex* is difficult to control with conventional insecticides owing to the presence of multiple resistance mechanisms involving *kdr* and oxidases against pyrethroids and elevated esterases and insensitive acetylcholinesterases against OPs (Khairandish and Wood, 1993; Chandre et al., 1997, 1998) at levels higher than what is observed in *Anopheles* (Curtis et al., 1996; Chandre et al., 1999b; N'Guessan et al., 2003). There is a good prospect for effective control of *Cx. quinquefasciatus* using chlorfenapyr, and this should reduce nuisance biting among net users and might even protect against filariasis transmission.

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concerning protection of animals used for experimental purposes.

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