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REVIEW ARTICLE OPEN ACCESS

Effects of Permethrin and Chlorfenapyr Susceptibility Patterns in the *Anopheles gambiae* Complex: Molecular Mechanisms and Implications for Malaria Vector Control with IG2 Bed Net

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Abstract

The rising threat of insecticide resistance in malaria vectors restricts the effectiveness of global malaria control initiatives. The *Anopheles gambiae* complex is a group of efficient vectors that has demonstrated extensive resistance to copious insecticides, particularly pyrethroids (permethrin). Consequently, alternative insecticides with different mechanisms of action, such as chlorfenapyr, are being applied frequently. Target site mutations, such as knockdown resistance (kdr) mutations (L1014F/S) in the voltage-gated sodium channel gene, along with increased metabolic detoxification mediated by cytochrome P450 monooxygenases, glutathione S-transferases, and esterases, are associated with permethrin resistance in *An. gambiae* complex. Conversely, chlorfenapyr, a novel insecticide involving metabolic activation, has been introduced as an alternative. Understanding these differential and overlapping resistance mechanisms is vital for strategic deployment of insecticides and designing effective resistance management programs. This review evaluates the susceptibility patterns of *An. gambiae* complex to both permethrin and chlorfenapyr, an alternative insecticide with a novel mode of action, while emphasizing their molecular resistance mechanisms and implications for malaria vector control. More focus is given to the Interceptor® G2 (IG2) long-lasting insecticidal net, which combines alpha-cypermethrin and chlorfenapyr to enhance the control of resistant mosquito populations. Compared to previous reviews, this paper provides an integrated analysis of the synergetic mechanism of IG2 that circumvents resistance, delay its spread, and revamps the efficacy of malaria interventions. By highlighting recent findings from field trials and molecular studies, this review underscores the need for strategic deployment, resistance surveillance, and policy support to sustain the effectiveness of dual-insecticide tools in endemic regions.

Keywords: *Anopheles gambiae* Complex, Insecticide Resistance, Permethrin, Chlorfenapyr, Interceptor G2, Malaria Control, Molecular Mechanisms

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INTRODUCTION

Malaria remains a significant health concern in sub-Saharan Africa, where *Anopheles* mosquitoes serve as key vectors of *Plasmodium* parasites. It accounts for high rates of morbidity and mortality, contributing to around 94% of global malaria cases and 95% of malaria-associated fatalities worldwide in 2023. Unlike other countries, Nigeria bears approximately 30.9% of the total global malaria mortalities, highlighting the severity of the disease in this region.[1] Control of disease-transmitting parasites has improved considerably through the use of vector control and synthetic insecticides such as pyrethroids, coupled with insecticide-treated sprays utilized in IRS and LLINs, owing to their low toxicity to humans and high effectiveness against mosquitoes.[2] The main malaria prevention strategy in sub-Saharan Africa involves the use of pyrethroid-treated long-lasting insecticidal nets (LLINs), which have significantly prevented an estimated 1.5 billion cases of malaria and 7.3 million deaths attributed to the disease over the last two decades. However, the decline in the number of cases stagnated after 2015, and certain regions in sub-Saharan Africa experienced increased malaria transmission from 2019 to 2020.[3] This resurgence is attributed to sparse coverage of efforts due to the spreading resistance to pyrethroid insecticides among malaria-carrying mosquitoes, coupled with stagnation in malaria control efforts.[4] The resurgence that sub-Saharan Africa experienced from the 1980s onward necessitated immediate mitigations.

The *Anopheles gambiae* complex susceptibility to insecticides affects the control of malaria vectors and determines the effectiveness of LLIN and IRS interventions.[5] Susceptibility to pyrethroid permethrin and the newly introduced member of the pyrrole insecticide known as chlorfenapyr can significantly affect malaria transmission, the dynamics of the vector population, and the strategies employed for managing resistance. The effects of these susceptibility patterns extend across multiple domains, including vector control efficacy, resistance evolution, behavioral adaptations, and epidemiological consequences.[6]

Although pyrethroid resistance in general is a growing concern in malaria vector control, this review specifically focuses on permethrin resistance in the Anopheles gambiae complex. Permethrin, a Type I pyrethroids that act as neurotoxins to mosquitoes has been one of the most exploited insecticide in conventional LLINs across the endemic regions, especially in sub-Saharan Africa. However, commercial use and gross application have led to increased selection pressure and emergence resistance in populations of the *Anopheles gambiae* complex mosquitoes.[7] This focus will facilitate more targeted understanding of these mechanisms, significant for assessing cross-resistance potential with newer tools such as Interceptor® G2 nets and PermaNet® Dual, which incorporate a combination of alpha-cypermethrin and chlorfenapyr as well as chlorfenapyr and deltamethrin. The use of pyrethroids, particularly permethrin insecticides, has been instrumental in the control of malaria vectors because they act as neurotoxins to mosquitoes. However, commercial use and overapplication have led to increased resistance in populations of the *Anopheles gambiae complex* mosquitoes.[7] There is growing interest in promising but unstudied insecticides such as chlorfenapyr due to its different mechanism of action that could address this challenge. Its application in the field, however, also poses potential problems, such as developing resistance if not used judiciously.[8]

A specific type of pyrethroid, permethrin, induces paralysis and death in mosquitoes through the voltage-gated sodium channel' (VGSC) in the sodium channel pathway of the nervous system.[9] However, target-site mutations and metabolic detoxification mechanisms cause widespread resistance to permethrin, which has been observed in *An. gambiae* as a result of its extensive use.[10] An identified resistance mechanism is the knockdown resistance, which occurs at the VGSC gene. Several single-nucleotide polymorphism mutations, such as L1014F and L1014S decrease the binding affinity of pyrethroids, rendering them ineffective.[11]

Chlorfenapyr has promise in fighting malaria vectors; a halogenated pyrrole pro-insecticide, it induces energy loss and death in species of concern by inhibiting oxidative phosphorylation through mitochondria.[12] As an integrated vector management tool, it is valuable as it has a novel mode of action that minimizes the

risk of cross-resistance compared to permethrin. There are also concern of the effect of permethrin resistance mechanisms such as target site mutation or overexpression of detoxifying enzymes in chlorfenapyr.[13] Chlorfenapyr was effective against permethrin-resistant mosquitoes when combined with other insecticides.[3] Chlorfenapyr-pyrethroid long-lasting insecticidal bed nets (LLINs), including Interceptor® G2 (IG2), were promising in more recent studies, especially in regions where vectors had high resistance to pyrethroids, particularly permethrin.[14]

These dual-active ingredient nets resulted in a substantial reduction in malaria prevalence compared to traditional pyrethroid-only LLINs.[15] The synergistic effect of combining two insecticides with distinct modes of action alpha-cypermethrin (a pyrethroid that targets the mosquito's nervous system), and chlorfenapyr (a pyrrole compound that inhibits mitochondrial oxidative phosphorylation), enhances efficacy against resistant strains and improves control over insecticide resistance.[16] The effectiveness of IG2 bednets is significantly diminished by reducing the potential for spreading resistance to both active components, one of which, similar the other, can develop while simultaneously being used for vector control due to selective pressure from several biological mechanisms. More importantly, these mosquitoes may demonstrate a remarkable capacity to convert chlorfenapyr into its active form, tralopyril, thus inducing resistance, as certain cytochrome P450 enzymes, commonly overexpressed in resistant populations, activate chlorfenapyr.[17]

Insecticide resistance generally arises from biological mechanisms beyond systematic adaptation, such as metabolic adaptation, behavioural adaptation, or genetic mutation.[18] The goal of chlorfenapyr is to enhance its insecticidal effectiveness against resistant populations of *Anopheles gambiae*. While previous reviews have evaluated insecticide resistance in *Anopheles gambiae* or studied the individual effects of permethrin and chlorfenapyr, few have integrated their combined implications in the context of dual-treated, long-lasting insecticidal nets including Interceptor® G2. Understanding these resistance pathways is essential not only for evaluating the limitations of current interventions but also for assessing the potential of dual-active IG2 bed nets, which incorporate distinct insecticidal actions to overcome these challenges. This review examines the molecular mechanisms driving the effects of both permethrin and chlorfenapyr against the *An. gambiae* complex, including the interactions of both insecticides' metabolites and physiological modulators within the IG2 net, and offers recommendations on optimizing the anti-malaria vector control policy and managing insecticide resistance in Africa.

By addressing these knowledge gaps, this review aims to contribute to creating sustainable, efficient vector control methods and revamps the ongoing reduction of the malaria burden worldwide.

The *Anopheles gambiae* complex and its role in the spread of malaria

An. gambiae (*sensu stricto*)*, An. coluzzii, An. arabiensis, An. merus, An. melas*, and *An. bwambae* are members of the *An. gambiae* complex. They are physically identical but genetically different.[19] Animals from these sibling species exhibit distinctive ecological and behavioural traits, and these differences determine their ability to spread disease and insecticide resistance. *An. gambiae s.s.*, *An. coluzzii*, and *An. arabiensis* are the primary malaria vectors due to their anthropophilic and endophilic behaviours.[20] *An. arabiensis* shows remarkable ecological plasticity, feeding on both humans and animals and exhibiting higher exophilic and exophagic tendencies, which reduce its exposure to IRS and LLINs. *An. melas* and *An. merus* are saltwater-tolerant species with localized distributions. According to a recent study, *Anopheles* mosquitoes are expanding their habitats to include contaminated populations, while *Anopheles* larvae prefer transparent, stagnant water sources that are remote from human regions.[21] To implement targeted control strategies, understanding the insecticide resistance of these sibling species is required.

Permethrin resistance in malaria vector control

Permethrin, a type of synthetic pyrethroid insecticide, has historically been among the most effective traditional pyrethroid insecticides due to its long-lasting residual efficacy, low toxicity

Table 1. Application in Vector Control Programs

Application	Description
Insecticide-treated nets (ITNs)	Permethrin is extensively used to impregnate bed nets to provide dual action: killing mosquitoes upon contact and acting as a physical barrier, significantly reducing mosquito biting rates and disease transmission[26]
Indoor Residual Spraying (IRS)	It is applied to walls and other indoor surfaces where vector insects rest, effectively controlling endophilic species such as *Anopheles gambiae*, a primary malaria vector[1]
Clothing and Equipment Treatment	Permethrin-treated clothing offers protection against ticks, mosquitoes, and other arthropods, which is important for military personnel, travelers, and outdoor workers exposed to vector-borne diseases[27]
Public Health Infrastructures	Permethrin is employed in aerial and ground-level applications for vector control during disease outbreaks including dengue and Zika[24]

to mammals, and affordability.[22] However, many *Anopheles* populations have developed resistance because of the extensive and prolonged use of the chemical in large quantities, which threatens the effectiveness of malaria control efforts.[23] Permethrin is a commonly used insecticide and repellent that effectively targets numerous arthropod vectors, such as mosquitoes, ticks, flies, and lice (Table 1). It particularly impacts the nervous system by causing sodium ion channels to open, leading to paralysis and death in these insects.[24] It demonstrates high efficacy against primary disease vectors, including *Anopheles* mosquitoes (malaria), *Aedes aegypti* (dengue, Zika, yellow fever), and *Culex* mosquitoes (filariasis and West Nile virus).[25]

Pattern of permethrin resistance

The pattern of permethrin resistance is a growing concern in the fight against malaria. For decades, traditional insecticide-treated nets (ITNs), especially those impregnated with permethrin, have been a key part of malaria control efforts. Permethrin, a widely used synthetic pyrethroid insecticide, is paramount for global initiatives aimed at controlling mosquitoes causing diseases, including malaria, dengue, Zika, and yellow fever.[26] It is a principal component in long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), and agricultural pest management, helping to significantly reduce malaria rates in many regions. However, the frequent and widespread use of permethrin, without proper resistance management, has inadvertently led to the emergence of resistance in mosquito populations, posing a serious challenge to the effectiveness of these traditional control methods.[27]

The rise of permethrin resistance is particularly noticeable in areas that have experienced repeated IRS campaigns and long-term distribution of permethrin-treated bed nets.[28] Over time, the constant pressure from permethrin exposure has driven evolutionary changes in mosquito populations, increasing the prevalence of resistance alleles and enabling resistant mosquitoes to survive and reproduce. This issue is further exacerbated by the use of agricultural insecticides, particularly in rural areas where crops are treated with insecticides that work similarly to permethrin, as other pyrethroids.[29]

As these resistance alleles accumulate in mosquito populations, the effectiveness of permethrin-based ITNs begins to decline. The associated mechanisms of this resistance primarily involve mutations in target sites, such as the voltage-gated sodium channels that pyrethroids target, as well as metabolic resistance driven by enzymes, including cytochrome P450 monooxygenases.[30] This cross-resistance between permethrin and other pyrethroids, such as deltamethrin and lambda-cyhalothrin, further complicates the situation, limiting the efficacy of these crucial control measures in managing resistant vector populations.[31]

An additional complication arises from the spread of resistance alleles through gene flow and vector migration. In regions where resistant mutations, such as knockdown resistance, were initially identified in West Africa, these mutations have expanded to East Africa via human and

Table 2. Diverse genetic and biochemical mechanisms contribute to permethrin resistance

Mechanism	Description	Key Genes/Proteins
Target Site Insensitivity	Mutations in voltage-gated sodium channels (VGSC) reduce insecticide binding.	Knock down resistance (L1014F, L1014S)[10]
Metabolic Resistance	Overexpression of detoxifying enzymes metabolizes permethrin.	P450s, GSTs, esterases[37]
Behavioral Resistance	Altered behaviors minimize contact with insecticides	Behavioural adaptations[10]
Cuticular Resistance	A thickened cuticle reduces insecticide penetration	Cuticle protein genes[37]

mosquito migrations.[32] This geographic spread of resistance complicates efforts to control malaria and other mosquito-borne diseases. The failure of traditional ITNs due to resistance underscores the urgent need for new, innovative solutions to maintain effective malaria control.

Mechanisms of permethrin resistance

Permethrin resistance in malaria vectors arises from various mechanisms. A primary factor is metabolic resistance, where mosquitoes increase the production of detoxifying enzymes such as cytochrome P450 monooxygenases, glutathione S-transferases (GSTs), and carboxylesterases. These enzymes break down or sequester pyrethroids, diminishing their toxicity.[33] In resistant populations of *Anopheles* mosquitoes, cytochrome P450 enzymes, particularly CYP6P3, play a principal role in detoxifying permethrin.[34] Another important aspect involves mutations in the voltage-gated sodium channel gene, commonly known as knockdown resistance. These mutations, such as L1014F and L1014S, reduce the binding affinity of permethrin, rendering it less effective.[35] The prevalence of knockdown resistance in Africa presents a significant challenge for pyrethroid-based treatments.[36] Additionally, there is behavioural resistance, where mosquitoes adapt by avoiding treated areas or resting outdoors to minimize contact with insecticides. Cuticular resistance also contributes, as it involves alterations in the composition of the mosquito cuticle that minimizes the absorption of insecticides. Table 2 consists of various genetic and biochemical mechanisms that contributes to permethrin resistance.

Chlorfenapyr: Mode of action and efficacy

Chlorfenapyr is a differential pyrrole insecticide, distinguishing itself from neurotoxic pyrethroids of permethrin. While pyrethroids such as permethrin target the voltage-gated sodium channels in mosquito nerve cells, leading to paralysis and death, chlorfenapyr exhibits a different mechanism as a pro-insecticide.[38] It must be activated within the insect, where it is converted into its active form of CL 303268. This active form inhibits mitochondrial respiration by obstructing the cytochrome bc1 complex in the electron transport chain, which halts ATP production, the primary energy source for cells.[16] The resulting energy depletion causes paralysis and ultimately leads to the mosquito's destruction.[39]

Chlorfenapyr targets cellular respiration rather than neural pathways, making it significantly less vulnerable to the cross-resistance mechanisms that is frequently associated with the application of pyrethroids. For instance, resistance mechanisms, including knockdown resistance, which reduce the effectiveness of pyrethroids by altering sodium channel proteins, along with metabolic resistance that enhances detoxifying enzymes including P450 monooxygenases do not impact chlorfenapyr's ability to inhibit mosquitoes' vector capacity.[40] Consequently, chlorfenapyr remains highly effective, even against populations of *Anopheles gambiae s.l.* and *Aedes aegypti* mosquitoes that have developed resistance to pyrethroids.

One of the standout properties of chlorfenapyr, especially when combined with IG2 nets, is its delayed mortality effect. Unlike permethrin, which kills mosquitoes rapidly upon contact, chlorfenapyr causes delayed mortality, typically occurring within 24 to 72 hours after exposure. This delay enables mosquitoes to continue interacting with their surroundings, including other mosquitoes, for a limited time before they succumb.[41] Consequently contributing to a secondary kill effect in which the already exposed mosquitoes can transfer this insecticide

to other surfaces or mosquitoes through their interaction.[42]

This delayed mortality also reduces the chances of behavioural changes avoidance by mosquitoes, an approach with fast-acting insecticides such as permethrin. Mosquitoes are less likely to alter their behaviour in response to exposure to chlorfenapyr, allowing for continued mosquito interactions with treated nets and surfaces before they are destroyed by the insecticide.[42]

Another prominent benefit of chlorfenapyr is its long-lasting effectiveness. Studies have demonstrated that chlorfenapyr maintains its insecticidal efficacy on treated surfaces for several months, even in the challenging environmental conditions found in malaria-prone regions.[41] This extended efficacy ensures that IG2 bed nets can provide ongoing protection against malaria vectors throughout the entire transmission season. The durability of chlorfenapyr not only supports its use in bed nets but also makes it a key player in indoor residual spraying (IRS) programs, where consistent vector control is principal for keeping mosquito populations in check between spray applications.[43]

Additionally, chlorfenapyr has a low risk of developing resistance, which enhances the long-term effectiveness of IG2 nets. While no insecticide is completely immune to the possibility of resistance development, chlorfenapyr's potential to target cellular respiration instead of the typical neurotoxic pathways as represented by pyrethroids and other insecticides, makes it significantly less susceptible to the rapid emergence of resistance.[44] This low resistance risk positions chlorfenapyr as a component of Integrated Vector Management (IVM) programs, where multiple control methods are integrated to minimize resistance and prolong the effectiveness of interventions.[16]

Synergistic Potential of Chlorfenapyr in Combination Nets

A promising ally in the battle against insecticide resistance in malaria vector control is chlorfenapyr.[45] This compound shows impressive synergistic potential when combined with insecticide-treated nets, increasing the effectiveness of treatments by targeting resistant mosquito populations.[46] It is used to address several challenges in malaria vector control. First, it helps fight resistance, particularly since pyrethroid resistance is widespread in *Anopheles gambiae* and other malaria vectors due to mutations at target sites, such as knockdown resistance, and enhanced detoxification processes.[17] Chlorfenapyr's discrete mode of action sets it apart from pyrethroids, allowing it to work effectively even against resistant mosquito strains.[8] Moreover, these synergistic nets are designed to effectively target both resistant and susceptible mosquitoes by combining chlorfenapyr with pyrethroids, enhancing overall vector control. Another advantage is that chlorfenapyr, when used with nets, helps delay the development of resistance. Its distinct mechanisms of action minimizes the selection pressure on pyrethroid resistance alleles, which could slow the emergence of resistance and prolong the effectiveness of pyrethroid-based strategies.[14]

Furthermore, these combination nets offer extended residual efficacy as chlorfenapyr-treated nets maintain their lethal potency for a longer duration compared to standard pyrethroid-only nets, even after multiple washes. This durability makes them more practical and reliable in malaria-endemic areas, where it is essential to maintain net efficacy for ongoing mosquito control.[47]

Integration into IG2 net

The Interceptor G2 (IG2) net is specially designed to address the growing problem of insecticide resistance in malaria control.[44] Unlike the standard bed nets that are impregnated with only pyrethroids, IG2 nets use a blend of two active ingredients chlorfenapyr and pyrethroid insecticide that disrupts the mosquito's nervous system, leading to rapid knockdown and death.[48] Compared to permethrin another type of pyrethroid, alpha-cypermethrin has shown effectiveness against resistant mosquito populations due to some slight chemical modifications.[49] The combination of alpha-cypermethrin and chlorfenapyr in IG2 bednet aims to enhance control over malaria vectors, especially in regions where resistance to pyrethroids, including permethrin, is prevalent.[50] Each chemical targets different physiological pathways, creating a powerful synergy. Alpha-cypermethrin focuses on neurotoxic pathways, delivering immediate knockdown and repellent

effects against mosquitoes, while chlorfenapyr, acts more slowly by disrupting mitochondrial function, ensures that mosquitoes that survive the initial exposure to alpha-cypermethrin will eventually face mortality, particularly those resistant to pyrethroids.[16] This dual-action strategy offers a significant edge in areas with high pyrethroid resistance, providing effective control over both resistant and susceptible mosquito populations.[17]

In addition to Interceptor® G2, which combines alpha-cypermethrin with chlorfenapyr, studies have shown another dual-active net, PermaNet® Dual, incorporates deltamethrin (a type II pyrethroid) alongside chlorfenapyr.[43] This combination similarly targets both the nervous system and mitochondrial pathways of mosquitoes, enhancing efficacy against pyrethroid-resistant *Anopheles gambiae* populations.

Field studies have demonstrated that IG2 bed nets are highly effective in reducing malaria transmission, even in areas with extensive insecticide resistance. In countries such as Burkina Faso, Cameroon, and Uganda, IG2 nets have been shown to provide long-lasting protection, with research confirming their combined effectiveness in combating resistant mosquito populations.[50] The studies highlight that IG2's dual-action formula is particularly adept at addressing the high levels of resistance found in mosquito populations, especially those that have developed resistance to pyrethroids such as permethrin.[44] Remarkably, these bednets remain effective for up to three years, offering extended protection in regions with high malaria transmission rates. These combination approaches significantly reduce mosquito biting rates, which in turn lowers the incidence of malaria.[14] The development and deployment of IG2 nets represent a strategic approach to managing resistance, decreasing reliance on single insecticides.[44] The dual action of chlorfenapyr and alpha-cypermethrin helps combat this challenge by minimizing dependence on any one class of insecticide.[50] This combination not only extends the effectiveness of insecticide interventions but also reduces the risk of resistance developing in malaria vector populations.[44]

By diversifying the insecticides used, IG2 nets provide resilience against evolving resistance, ensuring that they remain a prominent tool in the fight against malaria. Despite their effectiveness, there are associated challenges that include the costly production of IG2 bednets compared to standard pyrethroid-only nets among others.[44] To ensure widespread distribution, especially in resource-limited areas, cost-effective strategies will be essential. Furthermore, while chlorfenapyr has not yet faced significant resistance, its ongoing use could eventually lead to resistance issues.[44]

Effects of permetehrin and chlorfenapyr susceptibility patterns

Effective vector control and an understanding of insecticide resistance depend on the effects of susceptibility patterns to permethrin and chlorfenapyr in insect populations, particularly in *Anopheles gambiae*, the primary malaria vector. Table 3 shows the categories and the assocate effects.

Molecular interactions between permethrin-resistance and chlorfenapyr

Chlorfenapyr exhibit a different mechanism compared to permethrin with regards to circumventing mutations and resistance related to metabolic detoxification. While permethrin is a neurotoxic pyrethroid that targets sodium channels, chlorfenapyr operates as a pro-insecticide that disrupts mitochondrial oxidative phosphorylation. Research has demonstrated its impressive effectiveness against *Anopheles* populations that show significant resistance to permethrin, particularly in studies using insecticidal nets such as the Interceptor® G2.[14] Additionally, in permethrin regions, recent field tests in Tanzania revealed a marked reduction in malaria transmission after the introduction of chlorfenapyr-based nets.[57] By targeting the overexpressed P450 enzymes associated with pyrethroid resistance, chlorfenapyr interestingly leverages on the vector's resistance adaptations for its activation. The molecular interactions between chlorfenapyr and permethrin resistance involve various metabolic and functional pathways. One key factors is cytochrome P450 which is an enzyme that not only contributes to pyrethroid resistance through metabolic detoxification but also activates chlorfenapyr.[16] In the presence of P450s, including CYP6M2, chlorfenapyr is converted to its active form more efficiently

Table 3. Category and the effects of susceptibility patterns to permethrin and chlorfenapyr in insect population

Category	Effect
Impact on Malaria Vector Control Interventions	• Reduced efficacy of LLINs and IRS: Pyrethroid resistance has made LLINs less effective, which raises the risk of malaria transmission and mosquito survival rates. Although introducing nets treated with chlorfenapyr (Interceptor® G2) provides an alternative. However, its long-term effectiveness can be challenged if resistance develops.[51] • Dual-insecticide strategies are required: Although vector control strategies have been improved by combining chlorfenapyr with pyrethroids, thus monitoring is necessary to identify early indications of resistance development.[52]
Evolution of Resistance Mechanisms	• Genetic adaptations in the Anopheles gambiae complex: kdr mutations and upregulation of metabolic enzymes are well resistance. Although chlorfenapyr resistance mechanisms are still being studied, metabolic alterations and mitochondrial mutations might be involved.[53] • Cross-resistance Concerns: Permethrin-resistant mosquitoes may display metabolic pathways that influence their susceptibility to chlorfenapyrs, making vector control initiatives more difficult.[54]
Behavioral and Ecological Changes	• Altered feeding and resting behaviour: Resistant mosquitoes avoid treated surfaces by shifting to outdoor resting (exophily) or modifying feeding times, reducing insecticide contact.[55] • Shifts in species composition: Selective pressure from insecticide use may prefer species with lower susceptibility, altering vector population structures and potentially increasing malaria transmission.[56]

to effectively target resistance populations thereby turning their resistance mechanisms into vulnerable ones than in susceptible mosquitoes.[58] Since chlorfenapyr directly impacts energy metabolism and bypasses the nervous system, it remains effective even in populations with high rates of kdr mutations. This independence ensures that chlorfenapyr maintains its efficacy in these resistant groups.[8]

Another concern is the risk of adaptive resistance; chlorfenapyr can exploit the overexpression of P450 enzymes, and overuse could lead to resistant strains. Its activation or effectiveness could be compromised by adaptive changes, mutations in mitochondrial target sites, or the downregulation of specific P450 enzymes.[16] Due to its differential mechanism of action, chlorfenapyr continues to combat populations that have developed resistance. However, it is crucial to identify and address any emerging resistance to chlorfenapyr.[14] Even though chlorfenapyr has proven effective, some mosquito populations are beginning to show reduced susceptibility.[8] This could be due to adaptive changes in P450 enzyme activity or mutations in mitochondrial target sites, which might diminish chlorfenapyr's activation or effectiveness.[16] Research indicates that *Anopheles gambiae* populations across southern Africa exhibit varying levels of susceptibility to chlorfenapyr, highlighting the importance of continuous monitoring.

The interaction between permethrin resistance mechanisms and chlorfenapyr are is crucial for developing effective vector control strategies aimed at reducing malaria transmission.[17]

Efficacy in permethrin resistant populations

Chlorfenapyr has proven to be highly effective against malaria vector populations that have developed resistance to pyrethroids, combating one of the biggest hurdles in malaria vector control.[59] The mutations that cause target-site and knockdown resistance, which prevent permethrin from binding to voltage-gated sodium channels, do not affect chlorfenapyr, since chlorfenapyr works by disrupting mitochondrial function, a process that operates independently of these resistance mechanisms.[57] Additionally, in permethrin-resistant mosquitoes that overproduce cytochrome P450 enzymes which converts the bio-activated chlorfenapyr into its toxic form, making it particularly potent in these resistant populations.[60]

As a result, chlorfenapyr can effectively manage mosquito populations compared to traditional pyrethroid-based methods.

Potential for combination strategies

The complementary mechanisms of action of permethrin and chlorfenapyr support laudable possibilities of combination strategies consisting of dual-action bed net that combine both permethrin and chlorfenapyr insecticides (Interceptor® G2). This approach effectively targets both permethrin-susceptible and resistant mosquito populations, facilitating a reduction in their survival rates and slowing down the development of resistance.[57] Pyrethroids may work synergistically with chlorfenapyr to maximize vector mortality rates in mixed mosquito groups, which could enhance the potential of vector control programs.[46] Although chlorfenapyr has shown effectiveness against pyrethroid-resistant mosquitoes, however its effectiveness needs to be managed to sustain its efficacy. Selection pressure could help mosquitoes with P450 variants that can deactivate both pyrethroids and chlorfenapyr. Although there's limited evidence of this cross-resistance, it highlights the need for resistance surveillance by rotating chlorfenapyr with other insecticides this can minimize the selection pressure and the emergence of new resistance mechanisms.[8]

Chlorfenapyr's distinct mode of action presents opportunities and challenges for malaria control efforts. Its efficacy in managing pyrethroid-resistant mosquitoes can restore the effectiveness of long-lasting insecticidal nets (LLINs) and indoor residual sprays (IRS) in areas with significant levels of resistance.[14] However, chlorfenapyr formulation, especially those that combine multiple actions, tend to be more expensive than traditional pyrethroid based ones, which can strain budgets in malaria-endemic countries.[61]

Incorporating chlorfenapyr formulation into the malaria elimination strategies offers several long-term advantages including the significant reduction of mosquito survival, which directly helps limit the spread of *Plasmodium* parasites.[62] By widely using chlorfenapyr combined with existing insecticides, reduces the sole dependence on pyrethroids aiding in the slow the spread of pyrethroid resistance.[58]

CONCLUSION

The increasing prevalence of permethrin resistance in malaria vectors, driven by target site mutations and enhanced metabolic detoxification, presents a significant challenge to malaria control and elimination efforts. However, introducing chlorfenapyr as a complementary or alternative insecticide offers a promising solution. Unlike pyrethroids, chlorfenapyr acts through a distinct mechanism involving the disruption of mitochondrial oxidative phosphorylation, making it effective against permethrin-susceptible and resistant mosquito populations.

Notably, the Interceptor® G2 (IG2) bed net, which combines chlorfenapyr and alpha-cypermethrin, has demonstrated substantial efficacy in field studies by addressing both susceptible and resistant vector populations. Its dual-action design leverages the metabolic resistance traits of *An. gambiae* complex, turning them into vulnerabilities through chlorfenapyr activation.

The molecular interactions between permethrin resistance and chlorfenapyr reveal that the overexpression of P450 enzymes, a hallmark of metabolic resistance, paradoxically enhances chlorfenapyr's activation and efficacy. This unique relationship allows chlorfenapyr to target resistant populations otherwise unaffected by pyrethroid-based interventions. Furthermore, its lack of dependence on voltage-gated sodium channel function ensures that chlorfenapyr remains effective in mosquitoes harbouring knockdown resistance mutations

The implications for malaria vector control are profound with chlorfenapyr-based interventions, such as dual-action insecticidal nets and indoor residual sprays, which have demonstrated significant potential in reducing malaria transmission in areas burdened by pyrethroid resistance. Nonetheless, the successful integration of chlorfenapyr into vector control strategies requires careful resistance management, routine surveillance, and the adoption of rotational or combination approaches to prevent the emergence of new resistance mechanisms. While chlorfenapyr represents a powerful tool for combating pyrethroid resistance, its long-term efficacy relies on sustainable implementation

strategies and continuous investment in developing new insecticides with novel modes of action. Through proactive efforts in resistance monitoring and management, chlorfenapyr could play a pivotal role in maintaining the effectiveness of malaria vector control programs and advancing global malaria elimination goals.

Recommendations

To effectively manage malaria vectors and respond to the growing threat of insecticide resistance in Africa, the African malaria control programs should adopt an Integrated Vector Management (IVM) strategy that combines the use of dual active ingredient long-lasting insecticidal nets (LLINs), such as Interceptor® G2, with indoor residual spraying (IRS) with non-pyrethroid insecticides and larval source management in high-risk breeding locations. This integrated approach reduces the over-reliance on any single intervention and mitigates the emergence of resistance. Additionally, it is important to rotate insecticides and use combinations with different modes of action such as alternations between chlorfenapyr and other insecticide classes (neonicotinoids or organophosphates) across transmission seasons can decrease selection pressure. Deploying combination products of IG2 bed nets that combine chlorfenapyr with alpha-cypermethrin targets multiple resistance mechanisms and enhances intervention efficiency.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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