Effect of Metofluthrin-Impregnated Spatial Repellent Devices Combined with New Long-Lasting Insecticidal Nets (Olyset® Plus) on Pyrethroid-Resistant Malaria Vectors and Malaria Prevalence: Field Trial in South-Eastern Malawi

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SUMMARY: Long-lasting insecticidal nets (LLINs) experience some operational problems that reduce their effectiveness, such as limited spaces for hanging, biting of mosquitoes outdoors, a shift of key biting time from midnight to dawn or dusk, and development of pyrethroid resistance in mosquitoes. The concept of spatial repellency may be a countermeasure to overcome the above issues. The effect of the combined use of metofluthrin-impregnated spatial repellent devices (MSRDs) and LLINs (Olyset® Plus) on malaria prevalence and vector mosquitoes were examined in malaria endemic villages in south-eastern Malawi. The intervention reduced the infection rate in children as well as the number of pyrethroid-resistant vector mosquitoes. To achieve effective malaria control, continued intervention using MSRDs with 2 strips per 10 m² at 3-month intervals to reduce the density of malaria mosquitoes is recommended.

INTRODUCTION

Several studies have shown the effectiveness of insecticide-treated nets (ITNs) for malaria control (1, 2). However, ITNs and Long-lasting insecticidal nets (LLINs) have several operational limitations (3). ITNs or LLINs effectively protect people from mosquito blood sucking only while they sleep in their bed nets; however, outside of the nets, they are not protected (3-6). Sleeping arrangements and availability of locations for hanging the nets affect bed net use (4). The use of bed nets is sometimes limited to parents and babies, and remaining family members have to sleep without bed nets (6). A major obstacle to successful malaria vector control program is pyrethroid resistance of mosquitoes. Pyrethroid resistance is high in Anopheles gambiae s.l. in West and Central Africa (7). Pyrethroid resistance in An. funestus has also been reported in South Africa, Mozambique (8), and Malawi (9). New, convenient, and sustainable self-protection measures substituting or complementing LLINs are required for more effective malaria control (3).

Metofluthrin (SumiOne®) belongs to a new pyrethroid group that has unique characteristics (10). Metofluthrin has a high vapor pressure (1.96 × 10⁻³ Pa at 25°C) and can be vaporized without heating. Another unique characteristic is its high insecticidal and spatial repellent activity against mosquitoes. The spatial repellency of metofluthrin-impregnated spatial repellent devices (MSRDs) has been evaluated against An. gambiae in Tanzania (11) and western Kenya (Kawada et al. unpublished) and the possibility of its effective use against pyrethroid-resistant mosquitoes was demonstrated. Airborne metofluthrin vapor repelled mosquitoes through two main modes of action, namely, knockdown and excito-repellency (11-15).

In the present study, we demonstrated the effectiveness of MSRDs as a possible, novel, self-protection measure. The effects of the devices in combination with LLINs on the density of pyrethroid-resistant mosquitoes and malaria prevalence in children are discussed based on the results of field trials in south-eastern Malawi.

MATERIALS AND METHODS

Study site: The study was performed in three adjacent villages, Chiliko, Chilore, and Lamusi, in Likangala, an eastern area of Zomba district, in the south-eastern region of Malawi (Fig. 1). Zomba district belongs to a malaria endemic area with high malaria prevalence (16). Malaria is one of the leading causes of morbidity in the district, accounting for 66.8% of the total deaths (Zomba District Health office, 2009).

Insecticide susceptibility tests for mosquitoes found at the study sites: An. arabiensis larvae were collected from paddy fields and water pools between February and May 2015. Larvae were reared until the emergence of adults for susceptibility testing. An. funestus s.s. adult mosquitoes were collected by aspiration with a battery-powered aspirator (BioQuip Products, Rancho Dominguez, CA) from February to March 2018.
Insecticide susceptibility tests were performed using World Health Organization test tube kits (WHO/CDS/CPC/MAL/98.12). Papers impregnated with 0.75% permethrin, 0.05% deltamethrin, 0.5% etofenprox, 4% DDT, 0.1% propoxur, and 1.0% fenitrothion were used. Cytochrome P450 monooxygenase inhibitor, piperonyl butoxide (PBO; 98%, WAKO Pure Chemical Industries, Osaka, Japan), glutathione S-transferase inhibitor, DEM (diethyl maleate; 96%, Sigma-Aldrich, Saint Louis, MO, USA), and esterase inhibitor, DEF (Tribuphos; 97%, WAKO Pure Chemical Industries, Ltd, Japan) were used as synergists. Papers impregnated with permethrin/PBO (0.75/0.75 and 0.25/0.25), permethrin/DEM (0.75/0.75 and 0.25/0.25), and permethrin/DEF (0.75/0.75 and 0.25/0.25) were used. One to three-day-old blood-unfed females of An. arabiensis and field-collected, blood-fed and blood-unfed females of An. funestus s.s. were used for the tests. Knockdown was assessed during 60 min and mortality was determined after 24 h. Knockdown of An. funestus was observed in a mixed group consisting of blood-fed and unfed mosquitoes and mortality was recorded separately. Three to six replications using approximately 10 mosquitoes were performed for each insecticide.

Species identification: Mosquitoes were identified microscopically as An. gambiae s.l. and An. funestus s.l. based on the identification keys (17). DNA was extracted using the RedExtract-N-Amp Tissue PCR kit (Sigma-Aldrich Japan, Tokyo, Japan) and species identification was performed using multiplex PCR (18, 19).

Detection of point mutations in the voltage-gated sodium channels of An. arabiensis and An. funestus s.s.: To verify the presence of point mutations at L1014 in An. arabiensis and An. funestus s.s., PCR and direct DNA sequencing was conducted according to the procedures described by Kawada et al. (20).

Preliminary small-scale field survey to determine the number of MSRDs required for each house: A preliminary small-scale trial comprising 40 houses of Chiliko village was performed between January and April 2015.

Intervention using Olyset® Plus LLINs and MSRDs: Olyset® Plus (2% permethrin and 1% w/w PBO, Sumitomo Chemical Co., Ltd., Tokyo, Japan) nets were delivered to 29 houses after removing the old bed nets and 11 houses were left unchanged as controls (The control houses used Olyset® Net with 2% w/w permethrin). Olyset® Plus nets were supplied in sufficient numbers to cover all the residents. Of the 29 houses, 20 were treated with MSRDs (polyethylene net materials of 8 m x 15 cm impregnated with 10% w/w metofluthrin, Sumitomo Chemical Co., Ltd., Tokyo, Japan) at 2 different dose regimens; 10 houses were treated with 2 strips per 10 m² and the other 10 houses with 3 strips per 10 m². The house residents were informed about the study and their written consent was obtained before conducting the interventions. The intervention was performed on January 22 and 23, 2015. Four months after hanging the MSRDs, all strips were collected on May 18, 2015, and the remaining
quantities of active ingredients were analyzed using gas chromatography.

**Mosquito collection:** Mosquitoes were collected from the 40 houses after pyrethrum was sprayed. Pre-intervention collections were performed on January 10 and 15, 2015, and post-intervention collections were conducted after 1 week (January 27, 31, and February 1, 2015), 1 month (February 24 and 25, 2015), 2 months (March 28 and 31, 2015), and 3 months (April 24 and 27, 2015).

**Large-scale field trial involving all residential houses in 2 villages:** Large-scale trial was performed to investigate the effect of the MSRD intervention combined with Olyset® Plus against the malaria infection rate in children, between May 2015 and June 2016.

**Intervention using Olyset® Plus nets and MSRDs:** All houses in Chilore (215 houses) and Lamusi (408 houses) were part of the study. Sufficient numbers of Olyset® Plus nets to cover all residents were delivered to all houses after removing the old bed nets. MSRDs were used in two different intervention regimens; a single treatment at the rate of 2 strips per 10 m² and a two-time treatment at the same dosage with a 3-month interval.

The house residents were informed about the study and their written consents were obtained before conducting the treatment at the same dosage with a 3-month interval. The house residents were informed about the study and their written consents were obtained before conducting the interventions. Olyset® Plus nets were delivered on December 9 and 10, 2015. The first intervention of MSRDs in the 2 villages was implemented from January 6 to 12, 2016, and the 2nd MSRD intervention in Chilore was conducted on April 22 and 23, 2016.

**Mosquito collection:** Twenty houses each in Chilore and Lamusi were randomly selected for mosquito collections. Pyrethrum spray collection for the 40 houses were performed. Pre-intervention collections were performed on December 3 and 7, 2015, and post-intervention collections were conducted after 1 month (February 12 and 21, 2016), 2 months (March 7 and 8, 2016), 3 months (April 12, 13, 16 and 17, 2016), and 4 months (May 10 and 11, 2016). The collected mosquitoes were identified according to the procedures described above.

**Blood collection and detection of malaria parasites:** Pre-intervention blood collections for children (under 10 years old) were conducted from May 5 to 8, 2015 with 74 children in Chilore and 112 children in Lamusi. Post-intervention blood collections were conducted on May 2 and 3, 2016 with 50 children in Chilore and 105 children in Lamusi. A finger-prick blood sample (5 μL) was obtained from each child. The presence of malaria parasites was determined using a rapid diagnostic test (RDT) for detecting histidine-rich protein II antigen parasites was determined using a rapid diagnostic test (RDT) for detecting histidine-rich protein II antigen. High resistance was also observed for 4% DDT. In contrast, 0.1% propoxur and 1.0% fenitrothion showed high mortality (both 100%) (Fig. 2-A). Among the synergists examined in combination with permethrin, PBO was the most active, enhancing both mortality and knockdown in An. arabiensis (Fig. 2-B). An. funestus s.s. showed resistance to permethrin, DDT, and propoxur, but showed a high mortality rate (100%) with 1.0% fenitrothion. As observed in An. arabiensis, PBO was also an effective synergist, enhancing both the mortality and knockdown (Fig. 3).

**Ethics:** The protocol for the study was reviewed and approved by the Chancellor College Research and Ethics Committee, University of Malawi, and the Ethical Committee of Institute of Tropical Medicine, Nagasaki University (Approval No. 140005123-2).

**RESULTS**

**Insecticidal susceptibility of An. arabiensis, and An. funestus s.s.:** An. arabiensis collected from the study site showed high resistance to the following diagnostic levels of pyrethroids: 0.75% permethrin, 0.05% deltamethrin, and 0.5% etofenprox. The mortality rate was 60.5%, 82.8%, and 87.2%, respectively. High resistance was also observed for 4% DDT. In contrast, 0.1% propoxur and 1.0% fenitrothion showed high mortality (both 100%) (Fig. 2-A). Among the synergists examined in combination with permethrin, PBO was the most active, enhancing both mortality and knockdown in An. arabiensis (Fig. 2-B). An. funestus s.s. showed resistance to permethrin, DDT, and propoxur, but showed a high mortality rate (100%) with 1.0% fenitrothion. As observed in An. arabiensis, PBO was also an effective synergist, enhancing both the mortality and knockdown (Fig. 3). Not a single point mutation at the voltage-gated sodium channel (L1014F or L1014S) was detected among 62 female mosquitoes, and nested-PCR was performed in the same manner as described above.

**Data analysis:** A generalized linear mixed model (GLMM) with Poisson distribution was used to analyze the variation in the number of mosquitoes collected in the different types of interventions. The date of collection was considered a random factor. GLMM with binomial distribution was used to analyze the variation in P. falciparum parasitemia in children belonging to houses wherein different types of interventions were implemented. The family (house) of the children was considered a random factor. GLMMs were conducted using the lme4 package in R software (http://www.R-project.org). Tukey’s multiple comparison tests were performed using the multcomp package for R software.

**Ethics:** The protocol for the study was reviewed and approved by the Chancellor College Research and Ethics Committee, University of Malawi, and the Ethical Committee of Institute of Tropical Medicine, Nagasaki University (Approval No. 140005123-2).

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**Preliminary small-scale field survey to determine the number of MSRDs required per house:** A total of 232 An. arabiensis and 82 An. funestus s.s. female mosquitoes were collected from the 40 houses in Chiliko. More An. arabiensis (157 females) were collected in the beginning and middle of the rainy
season (January to February 2015) than that of *An. funestus* s.s. (17 females). In contrast, the number of *An. funestus* s.s. (65 females) increased to as many as that of *An. arabiensis* (75 females) at the end of the rainy season (March and April 2015).

The differences in the number of mosquitoes collected among the 4 intervention types were significant at the day before the intervention, 1 week, 1 month, 2 months, and 3 months after intervention ($P < 0.001$). The reduction in the number of mosquitoes was highest in the houses treated with Olyset® Plus + MSRDs (both, at 2 strips per 10 m$^2$ and 3 strips per 10 m$^2$). Significant reduction in the number of mosquitoes compared to the control houses was observed at 3 months after the abovementioned intervention regimes were implemented, although the mosquito numbers slightly resurged after 3 months of intervention (Fig. 4). The residual amount of active ingredient in the MSRDs (total 124 strips from 20 houses, initially impregnated with 10% w/w) 4 months after intervention was $2.75 \pm 0.34\%$.

![Fig 2.](Color online) Insecticide susceptibility of *Anopheles arabiensis* females (A) and synergistic activity of PBO, DEF, and DEM on permethrin (B) by WHO tube test. Figures in parenthesis are the number of insects used.
**Fig 3.** (Color online) Insecticide susceptibility of *Anopheles funestus* females by WHO tube test. Figures in parenthesis are the number of insects used in the bioassay (F, Blood-fed female; UF, Blood-unfed female).

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Mortality (%) - Unfed</th>
<th>Mortality (%) - Fed</th>
<th>KD (%) at 60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenitrothion 1.0% (22F+25UF)</td>
<td></td>
<td></td>
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<tr>
<td>Propoxur 0.1% (22F+24UF)</td>
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<tr>
<td>DDT 4.0% (27F+22UF)</td>
<td></td>
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<td></td>
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<tr>
<td>Permethrin / PBO 0.75 / 0.75% (27F+31UF)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Permethrin 0.75% (26F+26UF)</td>
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</table>

**Fig 4.** (Color online) Changes in the number of female *An. gambiae* s.l. and *An. funestus* s.s. before and after the intervention. The different letters in the same color bar indicate significant difference (*P* < 0.01) by GLMM using the Poisson distribution with dates of collection as random effect.

Large-scale field trial including all residential houses in two villages: The total number of female mosquitoes collected from the randomly selected 40 houses included 187 *An gambiae* s.l. (185 *An. arabiensis* and 2 *An. gambiae* s.s.) and 77 *An. funestus* s.l. (71 *An. funestus* s.s., 2 *An. rivulorum* Leeson, and 4 *An. parensis* Gillies). The number of mosquitoes collected in the pre-intervention survey (December 2015, at the beginning of the rainy season) was small for both
Field Trial of Metofluthrin Devices in Malawi

An. gambiae s.l. (20 females) and An. funestus s.l. (4 females). A greater number of An. gambiae s.l. (134 females) mosquitoes were collected in the middle of the rainy season (February and March 2016) than that of An. funestus s.l. (23 females). In contrast, the dominance was reversed at the end of the rainy season (April and May 2016), during which the number of An. funestus s.l. (50 females) was greater than that of An. gambiae s.l. (33 females). Average *P. falciparum* sporozoite positive rates during the trial in *An. arabiensis* and *An. funestus* s.s.

Table 1. Changes in the number of female Anopheline mosquitoes per house (*Anopheles gambiae* s.l., and *Anopheles funestus* s.l.) before the intervention, and 1, 2, 3, and 4 months after the intervention of MSRD (2 strips/10 m²) + Olyset® Plus

<table>
<thead>
<tr>
<th>Village</th>
<th>Pre-intervention</th>
<th>1 month after first intervention</th>
<th>2 months after first intervention</th>
<th>3 months after first intervention</th>
<th>1 month after 2nd intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chilore village (MSRD 2 times treatment)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Anopheles gambiae</em> s.l.</td>
<td>0.75</td>
<td>1.8</td>
<td>0.17</td>
<td>0.33</td>
<td>0</td>
</tr>
<tr>
<td><em>Anopheles funestus</em> s.l.</td>
<td>0</td>
<td>0.67</td>
<td>0.44</td>
<td>0.94</td>
<td>0.35</td>
</tr>
<tr>
<td>Total</td>
<td>0.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.61&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.35&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Lamusi village (MSRD single treatment)</td>
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<td></td>
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<td></td>
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<tr>
<td><em>Anopheles gambiae</em> s.l.</td>
<td>0.25</td>
<td>2.0</td>
<td>0.41</td>
<td>0.21</td>
<td>0.94</td>
</tr>
<tr>
<td><em>Anopheles funestus</em> s.l.</td>
<td>0</td>
<td>0</td>
<td>0.12</td>
<td>0.58</td>
<td>0.56</td>
</tr>
<tr>
<td>Total</td>
<td>0.45&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.95&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.53&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.79&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1)</sup> The first intervention of the strips in Chilore and Lamusi villages was performed from January 6–12, 2016, and the 2nd intervention in Chilore was performed on April 22–23, 2016. Olyset® Plus nets were delivered to all houses in Chilore and Lamusi on December 9 and 10, 2015.

<sup>2)</sup> Contains 185 *Anopheles arabiensis* and 2 *Anopheles gambiae* s.s.

<sup>3)</sup> Contains 77 *Anopheles funestus* s.s., 2 *Anopheles rivulorum,* and 4 *Anopheles parensis.*

<sup>4)</sup> $\chi^2 = 1.52, df = 1, P = 0.22.$

<sup>5)</sup> $\chi^2 = 1.27, df = 1, P = 0.26.$

<sup>6)</sup> $\chi^2 = 0.10, df = 1, P = 0.75.$

<sup>7)</sup> $\chi^2 = 1.63, df = 1, P = 0.20.$

<sup>8)</sup> $\chi^2 = 10.0, df = 1, P = 0.0014.$

Table 2. Number of children infected with parasites before and after the intervention of MSRD with combination of Olyset® Plus

<table>
<thead>
<tr>
<th>Village</th>
<th>RDT</th>
<th>PCR</th>
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<tr>
<td></td>
<td>Total</td>
<td>Positive %</td>
</tr>
<tr>
<td>Chilore Village (MSRD 2 times intervention)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>74</td>
<td>51.4&lt;sup&gt;1)&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. examined</td>
<td>38</td>
<td>28.6&lt;sup&gt;2)&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasmodium falciparum positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After</td>
<td>50</td>
<td>34.0&lt;sup&gt;1)&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. examined</td>
<td>17</td>
<td>24.8&lt;sup&gt;3)&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasmodium falciparum positive</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>1)</sup> $\chi^2 = 3.68, df = 1, P = 0.055.$

<sup>2)</sup> $\chi^2 = 1.30, df = 1, P = 0.25.$

<sup>3)</sup> $\chi^2 = 0.40, df = 1, P = 0.53.$

<sup>4)</sup> $\chi^2 = 2.33, df = 1, P = 0.13.$

An. gambiae s.l. (20 females) and An. funestus s.l. (4 females). A greater number of An. gambiae s.l. (134 females) mosquitoes were collected in the middle of the rainy season (February and March 2016) than that of An. funestus s.l. (23 females). In contrast, the dominance was reversed at the end of the rainy season (April and May 2016), during which the number of An. funestus s.l. (50 females) was greater than that of An. gambiae s.l. (33 females). Average *P. falciparum* sporozoite positive rates during the trial in *An. arabiensis* and *An. funestus* s.s.
were 16.4% (Chilore) and 5.7% (Lamusi), and 20.5% (Chilore) and 11.1% (Lamusi), respectively.

The differences in the number of mosquitoes between the 2 intervention types were not significant before intervention and 1, 2, and 3 months after intervention (P = 0.22, 0.26, 0.75, and 0.86, respectively), while the difference after 4 months (1 month after 2nd intervention of the strips was implemented in Chilore) was significant (P = 0.016), indicating decreasing spatial repellency with the single treatment of MSRD after 4 months but continued high spatial repellency by the 2nd intervention of MSRD (Table 1).

Parasitemia in blood samples collected from children: *P. falciparum* infection rates were reduced after intervention in both the villages as compared to those before the intervention, although these differences were not statistically significant (Table 2). No significant difference in body temperature after the intervention was observed between parasite positive and parasite negative children [35.9 ± 0.8°C and 35.4 ± 0.9°C in Chilore (P = 0.40), and 36.3 ± 1.0°C and 36.0 ± 1.0°C in Lamusi (P = 0.60), respectively].

**DISCUSSION**

Two major malaria vectors, *An. arabiensis* and *An. funestus* s.s., obtained from the study site, showed high resistance to pyrethroids. Some mixed function oxidase (MFO)-related oxidative mechanisms were thought to be the major factors responsible for metabolic resistance.

In the preliminary field study, significant reduction in the number of mosquitoes was observed in the houses treated with MSRDs in combination with Olyset® Plus compared to that of the control houses. The effective duration of the single treatment of MSRD is thought to be less than 4 months with 2 strips per 10 m², since the mosquito numbers slightly resurgened after 3 months of intervention. Given the results described above and in view of reducing the practical cost, 2 strips per 10 m² of MSRD is recommended as the intervention dosage for a house. In the study site, the rainy and mosquito breeding season normally starts in November and lasts until early May. Therefore, two-time interventions of MSRDs would be required to cover this season (6 months) for effective reduction of mosquitoes in the study sites.

In the large-scale trial, a reduction in *P. falciparum* infection rates was observed in children in Chilore and Lamusi, 4 months after the first intervention of MSRD (1 month after the second intervention in Chilore). As the body temperature of parasite positive children was within the normal range, these children were thought to be asymptomatic carriers of the parasite along with most of the adults in the villages. The infection rate did not decrease sharply possibly because of the presence of these asymptomatic carriers who harbored parasites that might not be directly affected by MSRD and LLIN interventions and were supplying sources of gametocytes to mosquitoes (22, 23). A decrease in mosquito blood feeding frequency does not only indicate a reduction in the frequency of sporozoite inoculation but also a reduction in the rate of gametocyte transfer from humans to mosquitoes, resulting in a gradual decrease in infection rates. Although parasites infecting the inhabitants may not disappear immediately, prevention of mosquito blood feeding by the continued intervention using MSRDs will be a convenient and effective measure to maintain the contact frequency with vector mosquitoes at low levels.

Although the mechanism underlying the effectiveness of metofluthrin against pyrethroid-resistant mosquitoes has not yet been elucidated, it might be explained by the difference in chemical structure between the phenoxybenzyl alcohol-based pyrethroids, such as permethrin and deltamethrin, and tetrafluorobenzyl alcohol-based pyrethroids, such as transfluthrin and metofluthrin. Transfluthrin has the potential to control pyrethroid-resistant mosquitoes with P450-mediated enhanced metabolic factor, since the P450-detoxifying enzymes preferably bind at the phenoxybenzyl alcohol moiety, but not at the tetrafluorobenzyl moiety (24). Similarly, the resistance ratio of transfluthrin and metofluthrin was much smaller than that of permethrin against permethrin-resistant wild colonies of *Culex quinquefasciatus* Say (25).

The mode of action of airborne metofluthrin against mosquitoes is also of interest. In a previous study, *Aedes aegypti* (L.) exposed to metofluthrin vapor were inhibited from biting humans mainly due to quick knockdown and high killing activity of metofluthrin but not due to repellency (26). Most of the *Ae. aegypti* adult mosquitoes present in the metofluthrin-treated room, died, or were knocked down without escaping (27). Contrastingly, significant excito-repellency of *An. gambiae* s.s. was reported in the artificial huts treated with metofluthrin (28). The above inconsistency may be caused by the difference in mosquito species with different host preferences. Therefore, the spatial repellency of metofluthrin is the combined result of multiple functions, such as, killing and knock down effects, excito-repellency, and the declining effect of host-seeking eagerness. Studies on the mode of action of metofluthrin in pyrethroid-resistant mosquitoes and further verification of the spatial repellency in field with MSRDs might provide breakthroughs in possible measures for malaria control in endemic areas.

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**Conflict of interest** None to declare.

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