Original Article

Insecticide-impregnated net originally invented to control malaria vectors can be used to control other arthropod pests

Kazumasa Ogino*, 1), 2), Hiroyuki Hara2), Osamu Araki3), Kenji Takai4) and Tamotsu Kanazawa1)

* Corresponding author: kzogino@med.uoeh-u.ac.jp

1) Department of immunology and Parasitology, University of Occupational and Environmental Health, Japan, 1–1 Iseigaoka, Yahatanishi-ku, Kitakyushu, Fukuoka 807–8555, Japan
2) Sanix Incorporated, 2–1–23 Hakataekihigashi, Hakata-ku, Fukuoka 812–0013, Japan
3) Sunaim Incorporated, 1–9–19 Kiyokawa, Chuo-ku, Fukuoka 810–0005, Japan
4) Department of Immunology and Medical Zoology, St. Marianna University School of Medicine, 2–16–1 Sugao, Miyamae-ku, Kawasaki, Kanagawa 216–8511, Japan

(Received: 14 May 2019; Accepted: 1 August 2019)

Abstract: We evaluated an insecticide-impregnated net, originally invented for malaria vector control, as a tool to control vectors and stored product insects in a laboratory trial. First, we performed the standard continuous contact test on German cockroach, American cockroach, house fly, cigarette beetle, rice weevil, and red flour beetle; lethal effects were found on all of these insects, except red flour beetle. The house fly and cigarette beetle were affected very rapidly in the contact tests. Second, we disclosed that seating the net proved to act as a barrier against American cockroach. From the third day of the experiment, the roaches did not enter the shelter in the treated zone, and the quantity of feces left on the control shelters was greater than that on the treated shelters. Thus, the net was shown to repel the roaches from the treated shelters. Third, suspending a strip of the net in a cage had knockdown and lethal effect on house flies and flesh flies. This study has shown the applicability and the utility of an insecticide-impregnated net to control pests other than malaria vectors.

Key words: insecticide-impregnated net, vectors, stored product insects, knockdown effects, barrier effects

Introduction

In recent years, insecticide-impregnated or repellent-impregnated nets have been used as one of the main tools to control vectors of infectious diseases, especially mosquitoes. Moreover, insecticide-impregnated fiber products, nets, clothes, and mats, were developed and used to control vectors in fields and in households (Sholdt et al., 1989; Hossain and Curtis, 1989; Hill, 1996; Wood et al., 1999; Kroeger et al., 1999, 2003, 2004; Cameron and Hill, 2002; Herber and Kroeger, 2003; Das et al., 2007; Courtenay et al., 2007; Levy et al., 2008; Schwarz et al., 2011; Banks et al., 2014). During the development of appropriate insecticide-incorporated nets, different materials, methods of insecticide incorporation, weaving procedures, and other parameters, have been tested. In particular, an insecticide-impregnated polyethylene net was developed with long-lasting effectiveness of insecticide (Itoh and Okuno, 1996; Alaii et al., 2003a, 2003b; Dagne and Deressa, 2008; Dutta et al., 2011; Woyessa et al., 2014).

For the control of agricultural pests, the concept of integrated pest management (IPM), which avoids the usage of insecticides, agrichemicals, and other harmful chemicals as far as possible, has been refined for more than two decades. This aims to control pests without causing environmental pollution. The concept of IPM was accepted in the fields of medicine and economic entomology (Shiff, 2002). IPM was later applied to the field of vector control (WHO, 2004; Ehler, 2006; Zha et al., 2018) and to the control of other arthropod pests, including nuisances (Mosqueira et al., 2010; Durel et al., 2015). As insecticide replacements, harmless or negligibly weakly harmful substances that act on the pests were proposed. These substances were obtained from plants or natural sources (Shaaya et al., 1997; Rajendran and Sriranjini, 2008; Müller et al., 2008; Odaka et al., 2019).

The use of insecticide-impregnated nets for the control of pests requires the consideration of insecticide usage. However, we believe that it results in far lower levels of environmental pollution than simple spraying and drifting methods of insecticides in the field. Moreover, the setting of the insecticide-impregnated nets is safer for operators than spraying...
the insecticide, which may result in drifting, and the control effect of this method is higher; thus, it is more economical. Besides, the net has advantages to be used in various ways as just putting on, spreading over, suspending at, and sticking the target place for control. However, there are not ample studies that have evaluated the beneficial effects of insecticide-impregnated net on insects in the laboratory.

Therefore, in this study, we performed a laboratory evaluation of the effect of insecticide-impregnated nets on vectors other than malaria vectors, as well as on stored product insect pests.

We investigated two cockroach species and one fly species as representative creeping arthropod vectors and a flying vector, respectively. We aimed to obtain basic efficacy of the insecticide-impregnated net on these pests for comparison with other studies. And we examined three beetle species as typical stored product insect pests, which bred in facilities that recused insecticides to be sprayed and drifted even in the preliminary trial. One of the beetles also played a role as a mechanical carrier of pathogenic fungal spores (Nakagawa et al., 2008) and the others of which served as intermediate hosts of helminth.

Next, we evaluated the insecticide-impregnated net as having a repelling effect on American cockroach when combined with a shelter. This roach invaded kitchen and food storages from sewage and outfield, and often bit foodstuffs containers which evaded insecticides to be sprayed and drifted even in the preliminary trial. One of the beetles also played a role as a mechanical carrier of pathogenic fungal spores (Nakagawa et al., 2008) and the others of which served as intermediate hosts of helminth.

In addition, we studied the effect of the insecticide-impregnated net on flies in a way different from the way mentioned above. It was suspending the net for caged flies. Two fly species tested were typical flying vectors in livestock and recently became problems in facilities around livestock.

The net we tested was the “Toughguard® net” (SC Environmental Science Co., Ltd., Osaka, Japan). To evaluate the effect of the net on various pests and also its useful application in the field, the apparatus equipped with the net was selected in each experiment.

Materials and Methods

1. Insecticide-impregnated net

The net used in this study was made of polyethylene and incorporated 2% permethrin. The mesh size was a 1 mm × 1 mm square. It was produced and purchased under the commercial name of Toughguard®, net, which was made from the same materials of Olyset®, net, by SC Environmental Science Co., Ltd.

2. Insects

1) German cockroach Blattella germanica Linnaeus

The colony of German cockroach belonged to an insecticide-sensitive strain, donated from Japan Environmental Sanitation Center, and routinely maintained in our laboratories with solid mouse food (commercial rodent MF pellets: Oriental Yeast, Kyoto, Japan) exposed to long (16L: 8D) photoperiods at 25°C ± 2°C.

2) American cockroach Periplaneta americana (Linnaeus)

American cockroaches were donated from Fukuoka University and were reared in our laboratories. The rearing conditions were the same as those for German cockroach.

3) House fly Musca domestica Linnaeus

House flies were of an insecticide-sensitive strain and their pupae were donated from Japan Environmental Sanitation Center. After pupal-adult ecdisys, they were reared on sugar and cream powder in our laboratories exposed to long (16L: 8D) photoperiods at 25°C ± 2°C. At 3 days after pupal-adult ecdisys, the flies were used in this experiment.

4) Flesh fly Sarcophaga peregrina (Robineau-Desvoidy)

The colony of flesh flies was routinely maintained on sugar and cream powder in our laboratories exposed to long (16L: 8D) photoperiods at 23°C. The larvae were bred with chicken liver. Mature larvae were kept and developed to adults in accordance with the method of Ohtaki et al. (1968). At day three after pupal-adult ecdisys, the flies were used in this experiment.

5) Cigarette beetle Lasioderma serricorne (Fabricius)

Cigarette beetles were collected from a rice milling plant in Fukuoka Prefecture, Japan, and were reared in our laboratories. The rearing conditions were the same as those for German cockroach.

6) Red flour beetle Tribolium castaneum (Herbst)

Red flour beetles were collected from a rice milling plant in Fukuoka Prefecture, Japan, and reared in our laboratories. The rearing conditions were the same as those for German cockroach.

7) Rice weevil Sitophilus oryzae (Linnaeus)

Rice weevils were collected from a polished rice warehouse in Oita Prefecture, Japan, and were reared in our laboratories. The rearing conditions were the same as those for German cockroach.

3. Experimental Methods

3-1. Continuous exposure tests

Continuous exposure tests were performed to obtain the basic profile of the insecticide-impregnated net against pests. We were able to compare it with the profile of other products of insecticides in accordance with general bioassay methods (Busvine and Barnes 1947; Yasutomi et al., 1966; Wadleigh et al., 1989; Hemingway et al., 1993a, 1993b; Lee et al., 1996, 1997). Briefly, a container was prepared and the pest to be tested was boxed in. The inside bottom surface was
occupied by the insecticide-treated net, in a manner to ensure that the pest was always in contact with the net. The other surfaces of the container were covered with a lubricating substance, such as petrolatum, or the container was glass on the inside upper and side surfaces, so the pests were unable to perch. The pests to be tested were vectors (German and American cockroaches and house fly) and stored product insects (cigarette beetle, red flour beetle, and rice weevil).

1) German cockroach
We prepared a plastic box (W150 mm × L300 mm × H200 mm) with the inner surface coated with petrolatum. The test net (150 mm × 300 mm) was attached with double-sided tape. Cotton wool immersed in sugar solution in a small plastic container was placed in the box to allow the inside tested insects' minimal food. For the experiment, 10 adult males were released in the box with the treated net. After 60 minutes of exposure, the roaches were transferred to a plastic box of the same size without the treated net and supplied with water and food (Fig. 1). The number of knocked down roaches was recorded to determine the 50% knockdown value (KT50) and 24-hour mortality. This experiment was performed at 25°C ± 2°C with humidity deregulation. The transferred roaches were kept in the standard rearing condition and checked until 24 hours after the exposure test began. The experiment was repeated three times. For the control experiment, we used a commercial plastic net as a control net, which had the same mesh size as the test net without insecticide treatment.

2) American cockroach
The experiment was performed in the same manner as for the German cockroach, and 10 adult males were used. The individuals not knocked down were also transferred to the untreated box and dealt with in the same way as the other roaches transferred to the box.

3) House fly
We prepared a glass petri dish (diameter 90 mm × H90 mm), and a square cardboard of a larger size than the dish. The test net was attached to the upper surface of the cardboard (we called it net-cardboard) and inserted between the dish and the lid. The upper and lower closed spaces were created with the height of the lid and the dish, respectively. Ten adult males were anesthetized by carbon dioxide for a short time and released in the upper space of the lid, to be exposed to the insecticide on the net. The exposure time was set at 60 minutes. The flies on the net-cardboard were fallen down in the dish by pulling the net-cardboard outside entirely. In the lower space of the dish, cotton wool immersed with sugar solution was placed as a minimum food source (Fig. 2). The number of knocked down flies was recorded to determine KT50 and 24-hour mortality, as for the experiment on cockroaches. The temperature conditions and humidity deregulation were the same as for the cockroach experiment. The flies in the dish were kept in the standard rearing condition and checked until 24 hours after the exposure test began. The experiment was repeated three times. For the control experiment, we used a commercial plastic net with the same mesh size.

4) Cigarette beetle
The experiment was performed in the same manner as for the house fly, without anesthesia, and tested 10 adults.

Fig. 1. Schematic of continuous exposure tests of German cockroach and American cockroach. Test net impregnated with or without (for control) permethrin was laid on the bottom in the box. Insects were transferred to another clean box after 60 min exposure to the net.

Fig. 2. Schematic of continuous exposure tests of house fly and cigarette beetle. The test net was put on cardboard between cover and dish and insects were put in the cover initially. After 60 min exposure to the net, the cardboard with the net was removed and the insects dropped to the bottom of the clean dish.
5) Red flour beetle

We prepared a glass petri dish (diameter 90 mm × H20 mm). A filter paper was placed over the entire bottom surface of the dish and the test net of the same size was attached on. Ten adults were released inside the dish, with a lid placed on top, and allowed to contact the net for 60 minutes. Subsequently, the insects were transferred to a fresh dish with cotton wool immersed in sugar solution, and the number of beetles knocked down was recorded over 24 hours, to obtain KT50 and 24-hour mortality (Fig. 3). This experiment was performed at 25°C ± 2°C with humidity deregulation and repeated three times. For the control experiment, we used a commercial plastic net with the same mesh size.

6) Rice weevil

The experiment was performed in the same manner and the same test number as for red flour beetle.

7) Analysis

To obtain KT50, probit analysis was made in accordance with the method of Finney (1971). We calculated slope, confidence limits, chi-squared and p-value by KTS&C program using Excel® VBA.

3-2. Inhibition test of the insecticide impregnated net for habitation on American cockroach

We evaluated the inhibition effect of the insecticide-impregnated net on American cockroach by underlaying the shelter. We prepared American cockroaches (five males and five middle-instar nymphs) in this experiment. We excluded female adults in this test, because their behavior changed depending on their reproductive condition. The experimental equipment consisted of a plastic box and inside structures as shelters of cockroaches. The box (W320 mm × L650 mm × H200 mm) was coated on its inside surface with petrolatum. The shelter was made of three wooden plates (100 mm × 100 mm) and had a two-story structure with a ceiling height of 10 mm. The shelter made of the same material with the same sizes was also used in rearing. Two unused shelters were set in the box at its diagonal corners. Solid mouse food and water in plastic dishes of 35 mm in diameter were placed in the center of the box. Under a shelter, the test net (150 mm × 150 mm) was placed, and the corners were adjusted. Owing to the size difference between the shelter and net, a 50 mm wide net with an L-shaped zone adjacent to two sides of the shelter was created. We placed a control net on the control shelter (Fig. 4).

We released roaches in the box and recorded the number of roaches alive and dead inside and outside shelters for 7 days. The types of the response were alive, remained under the treatment shelter, and remained under the control shelter. It was well characterized that the excreted quantity of feces in an area was proportional to the sojourn time of the cockroaches in that area (Stejskal, 1996, 1997). As the feces of the cockroach were granular and countable, the quantity of feces was roughly proportional to the number of droppings. The feces of American cockroaches were so viscous that they usually adhered to the structure of the apparatus; it was difficult to quantify precisely the feces excreted at the back of the shelter. Observation of the rearing box showed that the cockroach excreted feces in all parts of the shelter, with no apparent preference for a particular area of the shelter. Based on this observation, we tried to count the feces on and around the shelter.

This experiment was performed at 25°C ± 2°C under long (16L: 8D) photoperiods with humidity deregulation and repeated three times.

3-3. Suspended insecticide-impregnated net test for freely flying flies

We evaluated effects of insecticide-impregnated net on flies, which were vectors in livestock barns and facilities near them by suspending from a ceiling.

We prepared a metal wired cage (W200 mm × L300 mm × H200 mm) and a rectangular test net (150 mm × 100 mm), which was suspended inside the center of the cage (Fig. 5). Cotton moistened with sugar was supplied daily to prevent the caged insects from drying out and to serve as the minimum food source. The experimental procedure was as follows: a control net was suspended in the cage at the start of the experiment. Subsequently, for the treatment
experiment, 10 flies were released inside the cage. After 24 hours, the net was exchanged for the insecticide-impregnated net. Then after additional 60 minutes, the net was changed back to the control net. For the control experiment, the control net in the cage was unchanged during the experimental period (Fig. 5).

We counted the knocked down individuals over the treatment period of 60 minutes and determined KT₅₀. Afterwards, the insecticide-impregnated net was removed and the number of dead flies over 24 hours was recorded.

Experiments were conducted at 25°C±2°C with humidity deregulation, and repeated three times. KT₅₀ and the 24-hour mortality were determined.

**Results**

1. Continuous exposure test

The KT₅₀ and 24-hour mortality determined from this test are shown in Table 1. KT₅₀ in vectors, presented in the top three lines of the table, was lowest in the house fly. Two species of cockroaches had KT₅₀ values 2.5–8.5 times larger than for the house fly. In the stored product insects, listed in the bottom three lines of the table, the cigarette beetle had the lowest KT₅₀ and the red flour beetle and rice weevil had KT₅₀ values 5.7 and 10.4 times larger, respectively, than for cigarette beetle. For all insects except the red flour beetle, 100% mortality occurred in 24 hours. In stored product insects, the cigarette beetle had the most sensitive KT₅₀ of 5.4 minutes. Red flour beetle had the second most sensitive KT₅₀ of 30.9 minutes, and rice weevil had the least sensitive KT₅₀ of 56.2 minutes. In contrast, for vectors, the most sensitive insect was the house fly, with a KT₅₀ of 6.7 minutes. The KT₅₀ values for German cockroach and American cockroach were 17.0 and 57.2 minutes, respectively.

Over 24 hours, 100% mortality occurred in all insects except the red flour beetle. Although the red flour beetles were knocked down in a short time, they were roused within at least 24 hours. The insecticide-impregnated net had caused all the other insects in this experiment to be knocked down after they were exposed to the insecticide for 60 minutes.

2. Inhibition test of the insecticide-impregnated net for habitation for American cockroach

The repellency of the insecticide-impregnated net for American cockroaches was shown in the experiment. The responses of cockroaches in this experiment are shown in Table 2. Initially, cockroaches entered both shelters, and some cockroaches made contact with the insecticide-impregnated net. Dead individuals appeared until the second day, but no dead insects occurred from the third day onwards. The cockroaches appeared to avoid entering the shelter with the impregnated net from the third day onwards. This result provided evidence of the repellent effect of the insecticide-impregnated net on the cockroaches, when the net was underlaid.

This behavior of the cockroaches inside the cage was assessed by the distribution of feces. But we were able to count accumulated feces only on the roof of the shelter, because of contamination of the feces at the bottom of the box by their activated behavior. On average, the number of droppings on day 7 on the control shelter was 12, and the number on the treated shelter was 0.6.

3. Suspended insecticide-impregnated net test for freely flying flies

The KT₅₀ and 24-hour mortality values in the flies exposed to the suspended net for 60 minutes are shown in Table 3. The KT₅₀ values for house fly and flesh fly were 31.9 and 31.3 minutes, respectively.

Table 1. Efficacy of the insecticide-impregnated net for the vectors (cockroaches and a fly) and the stored product insects (beetles and a weevil) by the continuous exposure test.

<table>
<thead>
<tr>
<th>Insect (Species)</th>
<th>n</th>
<th>Slope</th>
<th>KT₅₀ (min.)</th>
<th>Limits of 95% confidence interval</th>
<th>χ²</th>
<th>p</th>
<th>Mortality in 24 hr (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>German cockroach (Blattella germanica)</td>
<td>30</td>
<td>1.2</td>
<td>17.0</td>
<td>(14.8, 19.5)</td>
<td>0.45</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>American cockroach (Periplaneta americana)</td>
<td>30</td>
<td>2.2</td>
<td>57.2</td>
<td>(50.2, 71.2)</td>
<td>2.71</td>
<td>0.75</td>
<td>100</td>
</tr>
<tr>
<td>House fly (Musca domestica)</td>
<td>30</td>
<td>3.2</td>
<td>6.7</td>
<td>(6.1, 7.4)</td>
<td>7.13</td>
<td>0.52</td>
<td>100</td>
</tr>
<tr>
<td>Cigarette beetle (Lasioderma serricorne)</td>
<td>30</td>
<td>3.2</td>
<td>5.4</td>
<td>(4.9, 6.0)</td>
<td>7.43</td>
<td>0.49</td>
<td>100</td>
</tr>
<tr>
<td>Red flour beetle (Trichilium castaneum)</td>
<td>30</td>
<td>3.3</td>
<td>30.9</td>
<td>(27.9, 34.3)</td>
<td>3.6</td>
<td>0.89</td>
<td>0</td>
</tr>
<tr>
<td>Rice weevil (Sitophilus oryzae)</td>
<td>30</td>
<td>3.7</td>
<td>56.2</td>
<td>(49.6, 64.1)</td>
<td>5.34</td>
<td>0.72</td>
<td>100</td>
</tr>
</tbody>
</table>

χ² indicates goodness of fit of data points to the theoretical line. 50% knockdown time (KT₅₀) and mortality in 24 hours after treatment were shown.
An insecticide-impregnated net was originally developed to control malaria vectors, and its efficacy has been confirmed worldwide (WHO, 2014). This method contributes to the suppression of the malaria, and has been recommended as a primary control method (Lengeler, 2004). The method was proven to be useful not only to control malaria vector mosquitoes, but also to control other arthropod pests, such as flies, bugs, and mites, some of which were disease vectors in the field (Goodyer et al., 2010; Banks et al., 2014).

In this study, we characterized the effects of the net. In continuous exposure tests, we believe that a significant amount of the insecticide oozed out from the surface of the net. Landonni (2001) performed a continuous contact test for a sensitive strain of German cockroach and recorded a KT50 of 4.4 minutes for 20 mg/m² permethrin. Compared with the results of Landonni (2001), the order of magnitude of the effective volume of the insecticide on the surface of the insecticide-impregnated net was presumed to be almost the same as residual spraying of 20 mg/m² permethrin.

The effect of the insecticide-impregnated net on flies was investigated by Komagata et al. (2012). In their spontaneous contact tests, a KT50 was recorded 341 seconds in blow fly and 338 seconds in house fly. In our data, a KT50 was 6.7 minutes (402 seconds) in house fly, and the difference seemed to be caused by the method.

Control of the stored product insects was made by the insecticide-impregnated net, as shown by the results of the continuous exposure tests in Table 1. The KT50 values were 5.4 minutes (cigarette beetle) and 56.2 minutes (rice weevil). Although the 24-hour mortality for these two insects was 100%, 100% mortality did not occur in the red flour beetle, despite a KT50 of 30.9 minutes. As the red flour beetle was known to be resistant to pyrethroids (Watters et al., 1983), they did not die after exposure to permethrin in our experiment. The difference of red flour beetle from the other two insects implied that there was a possibility that this insect was inherently resistance to pyrethroid insecticides.

It was indicated that the insecticide-impregnated net would be successful for the control of creeping insects when it was attached on a wall, placed on over an air vent or window, or covered over the furniture. For example, Cameron and Hill (2002) reported the lethal effects of permethrin-impregnated nets on house dust mites in the laboratory and the reduction of house dust mites and their allergens through the use of permethrin-impregnated mattress liners. Therefore, permethrin-impregnated nets and cloths may be used to control insects and mites within households. Curtis (1993) suggested an effective method for the use of insecticide-impregnated nets against bedbugs and Goodyer et al. (2010) reviewed a method for control of body lice. Insecticide-impregnated nets and curtains were tested for the control of assassin bugs, vectors of Chagas disease, in South America and were shown to serve as protective tools against these vectors (Kroeger et al., 1999, 2003; Herber and Kroeger, 2003).

Therefore, in the field, the net would protect men and animals from invading, contacting, and damaging arthropods. Household and industrial materials, particularly stored products that were the target of these arthropods, would be also freed from contamination of those arthropods.

To explore the effects of the insecticide-impregnated

### Table 2. Repellent effect of underlaying the insecticide-impregnated net in the shelter on the number of cockroaches staying in there.

<table>
<thead>
<tr>
<th>Time in days</th>
<th>No. alive*</th>
<th>No. in control shelter</th>
<th>No. in treated shelter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>

*: Dead individuals were removed from the experimental boxes at the day when they were dead. I, II, and III are three replications.

### Table 3. Efficacy of the suspended insecticide-impregnated net in a test cage of flies.

<table>
<thead>
<tr>
<th>Insect (Species)</th>
<th>n</th>
<th>Slope</th>
<th>KT50 (min.)</th>
<th>Limits of 95% confidence interval</th>
<th>χ²</th>
<th>p</th>
<th>Mortality in 24 hr (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>House fly (Musca domestica)</td>
<td>30</td>
<td>1.9</td>
<td>31.9</td>
<td>(29.7, 34.4)</td>
<td>6.92</td>
<td>0.33</td>
<td>100</td>
</tr>
<tr>
<td>Flesh fly (Sarcophaga peregrina)</td>
<td>30</td>
<td>2.2</td>
<td>31.3</td>
<td>(29.0, 34.0)</td>
<td>7.58</td>
<td>0.37</td>
<td>100</td>
</tr>
</tbody>
</table>

χ² indicates goodness of fit of data points to the theoretical line. 50% knockdown time (KT50) and mortality in 24 hours after treatment were shown.
net on flying insects, we have compared our data with the tests on flies.

In comparison with Kitagaki (1973), our results indicated that the effect of the insecticide-impregnated net was the same as the effect of residual spraying of 160 mg/m² fenitrothion. This dose of 160 mg/m² was not excessive for residual application in the field against flies. Therefore, suspending a net in the field may be an effective substitute for the residual spray of insecticides, because the insecticide impregnated into the net can be removed immediately and easily from the environment; hence, the insecticide exposure period was short.

According to Itoh and Okuno (1996), a suspended insecticide-impregnated net had an effect on *Anopheles* mosquitoes, and suspending a piece of insecticide-impregnated net also showed effect on flies in this study. Komagata et al. (2012) demonstrated spontaneous fly contact assay of the insecticide-impregnated net attached on all the six rectangular sides of the cage and time-limiting spontaneous fly contact assay in well-regulated methodology. In this study, we allowed flies to contact suspended insecticide-impregnated net incidentally and deregulated the contact time.

Our preliminary investigation showed that flies had more chances to be exposed to the insecticide when they encountered a suspended insecticide-impregnated net than creeping insects. The flying insects such as flies collided with a vertical net and would often cling on to the net. This behavior of the insect would increase their contact time with the insecticide and increase the amount of insecticide absorbed into their body.

The results of this study emphasize that the use of a suspended insecticide-impregnated net was a simple and effective means to control fly. The method proved to be effective in the control of malaria vector mosquitoes (Binka et al., 1996; Nevell et al., 1996; Sriehari et al., 2007), and may be applicable to the control of mosquitoes breeding in sewage and to the control of various flying insect pests breeding in areas of human dwellings, business buildings, and farms. In addition, a deltamethrin-impregnated net often resulted in the death of sand flies inside and outside the bednet (Courtenay et al., 2007). Insecticide appeared to act on the insect after a short time in contact with the bednet. This situation was similar to the experiments with the suspended net in this study.

It seemed that beetles of stored products insects were affected by a suspended net, because the beetles often fly and cling on the wall and the screen door net. We considered that suspending insecticide-impregnated net was an easy task and it was effective when used in the house, in the duct, in the place of tableware storage, and so on.

Recently, as an example of insecticide-impregnated net-based technology, Kawada et al. (2012) set the permethrin-impregnated net on the ceiling as a more effective methodology against malaria. Tsunoda et al. (2013) reported a novel control method of using permethrin-impregnated nets as the lids over jars to control *Aedes aegypti* (L.). The combination of these methods proved to be effective for the control of malaria and dengue vector mosquitoes. To increase the controlling efficiency of the method by using an insecticide-impregnated net, the net has been combined with other methods, including UV light, attractant chemicals, and a suction trap. An attractant increased the efficacy of the Olyset® net in controlling against house flies and stable flies in a cattle shed (Ogino and Kanazawa, 2011).

As insecticide pollution in the environment causes a harmful effect on living organisms and accelerates the resistance of the arthropod pests to the insecticide, replacements for their use have been explored for the control of arthropod pests; these include electronic frames, adhesive traps, suction traps, UV light traps, attractants, and natural or synthesized repellents. This study, which utilized a permethrin-impregnated net for the control of pests was limited as it only tested an insecticide. However, permethrin belonged to a type of insecticide with limited toxicity to the majority of mammals, including humans (Brent, 1999). The permethrin-impregnated net offered the advantages that the minimum and effective amount of insecticide was released from the net over an extended period. Thus, the environmental pollution by the insecticide was made in a relatively limited fashion. The insecticide-impregnated net was originally invented for global use in controlling malaria vectors and is industrially produced. The insecticide-impregnated net can be applied to control other arthropod pests.

**Acknowledgements**

The authors gratefully acknowledge Dr. Fumio Yokohari, Department of Earth System Science, Faculty of Science, University of Fukuoka for the donation of experimental animals and expert advice on the methodology of the study. We also thank Japan Environmental Sanitation Center, Kawasaki, Kanagawa, Japan for the donation of experimental animals and expert advice on the methodology of the study. We also thank Japan Environmental Sanitation Center, Kawasaki, Kanagawa, Japan for the donation of experimental animals. We would like to thank Editage (Cactus Communications, www.editage.jp) for English language editing.

**Conflicts of Interest**

Author Kazumasa Ogino is an employee of Sanix Incorporated that works for pest control. No other author has reported a potential conflict of interest relevant to this article.

**References**

Alaie, J. A., Van den Borne, H. W., Kachur, S. P., Shelley, K.,


