Insecticidal Effects of the Insect Growth Regulators Methoprene and Pyriproxyfen on the Cat Flea (Siphonaptera: Pulicidae)

HITOSHI KAWADA AND MASACHIKI HIRANO
Agricultural Chemicals Research Laboratory, Sumitomo Chemical Company Limited, Takazakura, Hyogo 665, Japan


ABSTRACT Residual effectiveness of the insect growth regulators pyriproxyfen and methoprene against cat flea, *Ctenocephalides felis* (Bouché), larvae were evaluated under simulated household conditions. Pyriproxyfen provided control of larvae for >12 mo when applied at 1 mg and 0.2 mg/m² and ≥3 mo at the rate of 0.04 mg/m². Methoprene applied at 1 mg/m² provided control for >12 mo and 6 mo at 0.2 mg/m². No significant mortality was observed when methoprene was applied at 0.04 mg/m².

KEY WORDS cat flea, pyriproxyfen, methoprene, juvenile hormone mimic, insect growth regulator

The cat flea, *Ctenocephalides felis* (Bouché), is a major pest of humans and companion animals because they are more synanthropic and have a wider host range than other flea species. The larvae are found in carpets and other areas of the house and high populations of larvae are also found in home yards, which provide a constant source of flea infestation (Palma and Meola 1990). The larvae appear to be the best life stage to target for control because they are often distributed in the pets bedding area (Ösbrink et al. 1986).

Insect growth regulators (IGRs), such as juvenile hormone mimics or chitin synthesis inhibitors, have been reported to be effective control agents for flea larvae (El-Gazzar et al. 1986, Marchiondo et al. 1990, Palma and Meola 1990, Henderson and Foil 1993, Palma et al. 1993, Hinkle et al. 1995). Pyriproxyfen (Nylar or Sumilarv) acts as a juvenile hormone mimic and interrupts nymphal–adult or pupal–adult metamorphosis (Hatakoshi et al. 1987, 1988). Its potential as a biorational insecticide covers a wide range of insect species of public health or household importance, such as synanthropic flies (Kawada et al. 1987, Langley et al. 1988, Miller 1989, Bull and Meola 1993), mosquitoes (Kawada et al. 1988, Muller and Darwazeh 1988, Schaefer et al. 1988, Suzuki et al. 1989, Kamimura and Arakawa 1991, Okazawa et al. 1991), chironomid midges (Ali et al. 1993, Trayler et al. 1994), cockroaches (Kawada et al. 1989, Koehler and Patterson 1991), termites (Su and Scheffrahn 1989), and fleas (Palma and Meola 1996, Hinkle et al. 1995). Palma and Meola (1990) reported that Nylar, an emulsifiable concentrate formulation of pyriproxyfen, used at the rate of 32 mg/m² prevented development of >80% cat flea larvae for 3 wk in home yard conditions. Total-release aerosol formulation of pyriproxyfen used at the rate of >0.39 mg/m² (0.01% [AI] wt:wt for 0.18-lit aeronsol can) significantly reduced adult flea emergence by >80% for a >7-mo duration (Hinkle et al. 1995). In similar experiments using total-release aerosols, we reported that 0.91 mg/m² of pyriproxyfen (0.0375% [AI] wt:wt for a 0.18-lit aerosol can) prevented the emergence of the cat flea for >7 mo in the laboratory, and that 2.4 mg/m² of pyriproxyfen controlled a cat flea population >1 mo in private houses infested with the cat flea (Senbo et al. 1993). The objective of this study was to compare the residual effectiveness of spray formulation of pyriproxyfen and methoprene under simulated household conditions.

Materials and Methods

Test Compounds. A 5% emulsifiable concentrate formulation of pyriproxyfen (4-phenoxypyphenyl (RS)-2-(2-pyridyloxy) propyl ether [Sumitomo, Takarazuka, Hyogo, Japan]) and methoprene (isopropyl-(2E,4E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate [Sandoz, Des Plaines, IL]) was used to prepare the dilution of test sprays.

Test Insects. Second-instar cat flea larvae were used as test insects. The fleas were collected in Sakai, Osaka, Japan, in 1991. reared with cats as a host for several generations at Osaka Seiyaku (Higashi Osaka, Japan) and introduced to our laboratory in 1993. The larvae were reared in a medium that contained a mixture of 80 g sand, 15 g powdered animal food (CE-2, Clea Japan, Tokyo), 3 g dried yeast (Ebis, Tanabe Pharmaceutical, Tokyo), and 2 g dried bovine blood (Wako, Tokyo). The strain showed normal susceptibility to pyre-
Table 1. Residual effectiveness of IGRs on carpet under simulated household conditions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dosage, mg/m²</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>9</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>93.3a</td>
<td>90.0a</td>
<td>93.3a</td>
<td>76.7a</td>
<td>93.3a</td>
<td>76.7a</td>
<td>96.7a</td>
<td>85.0a</td>
<td>83.3a</td>
</tr>
<tr>
<td>Pyriproxyfen</td>
<td>1</td>
<td>0b</td>
<td>3.33b</td>
<td>0b</td>
<td>3.33bc</td>
<td>0b</td>
<td>0b</td>
<td>0b</td>
<td>3.33b</td>
<td>0b</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>0c</td>
<td>10.0b</td>
<td>0b</td>
<td>0c</td>
<td>0b</td>
<td>0b</td>
<td>0b</td>
<td>3.33b</td>
<td>0b</td>
</tr>
<tr>
<td>Methoprene</td>
<td>1</td>
<td>0.04</td>
<td>10.0c</td>
<td>6.67b</td>
<td>0b</td>
<td>0c</td>
<td>6.67b</td>
<td>0b</td>
<td>10.0b</td>
<td>6.67a</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>0.04</td>
<td>36.7c</td>
<td>16.7c</td>
<td>3.33b</td>
<td>6.67bc</td>
<td>13.3b</td>
<td>16.7b</td>
<td>23.3b</td>
<td>50.0a</td>
</tr>
</tbody>
</table>

Numbers followed by the same letter are not significantly different (P = 0.01; Tukey HSD test).

Evaluations Methods. Polyester carpet (0.4 cm deep) was cut into square pieces (15 by 15 cm) and glued to plywood backing. Twelve carpet samples were prepared for each IGR concentration. A 5% emulsifiable concentrate formulation of the IGR was diluted with water to the appropriate concentration and the carpet was treated at 50 ml/m². Each sample was vacuumed with 4 strokes by a vacuum cleaner (MC-104H, Matsushita Electric, Tokyo) and then 1 g of dried animal food (CE-2, Clea Japan) was distributed uniformly onto the surface of the carpet. The carpets were maintained at 25°C and 60% RH. A series of each concentration of the test samples was prepared each week. Three pieces of round plugs (5.5 cm diameter) were cut from each carpet test sample and then placed in an aluminum cup (5.5 cm diameter, 5.5 cm high). Ten 2nd-instar fleas were released onto each plug and the cup was covered with a plastic lid that contained 4 small ventilation holes. A piece of adhesive paper (3 by 3 cm) was stapled on the inside wall of the aluminum cup to trap adults that emerged. The cups were stored in a desiccator that provided 80–90% RH until adult emergence was completed. Adult emergence was observed, and inhibition of emergence was determined by comparison with untreated test samples. Bioassays were conducted every 4 wk for 12 mo.

Results and Discussion

The untreated control survival was 76.7–96.7% during the 12-mo period. Larval survival in the pyriproxyfen treatment was significantly lower than that in the control for >12 mo at concentrations of 1 and 0.2 mg/m², and for ≥3 mo at 0.04 mg/m² (Table 1). The effective duration of the methoprene treatments was for >12 mo at 1 mg/m² and for 6 mo at 0.2 mg/m². No significant difference in larval survival was obtained even immediately after treatment when fleas were exposed to the carpet samples treated with methoprene at 0.04 mg/m².

The LD₅₀ values of pyriproxyfen and methoprene during the 12-mo period are shown in Table 2. The LD₅₀s were consistently <0.04 mg/m² by 4 mo and 0.04–0.2 mg/m² by 12 mo after exposure of the flea larvae to pyriproxyfen. The LD₅₀ of methoprene tended to increase gradually monthly. Hinkle et al. (1994) reported that the survival of cat flea larvae exposed to commercial total-release aerosol formulation of methoprene (mixture of 0.075% [S]-methoprene, 1.0% propoxur, 0.47% dichlorvos) became insignificant when compared with the control by the 3rd mo and that 0.025% pyriproxyfen showed a significant reduction in the survival by the 7th mo. In their experiment the concentrations of 0.075% [S]-methoprene and 0.025% pyriproxyfen were calculated to be ~2.41 mg/m². This amount is equivalent to ~4.82 mg/m² of methoprene because [S]-isomer is thought to be twice as active as racemic methoprene (Chamberlain et al. 1998) and 0.975 mg/m², respectively. Our results indicate a significant difference in the effective dosages for pyriproxyfen and methoprene between the treatment with total-release aerosol and that of emulsifiable concentrate. Actual amounts of IGR in the carpet appear to be much higher using the emulsifiable concentrate spray treatment than the total-release aerosol treatment. Moreover the formulation factors, such as inner pressure, liquid/gas ratio, particle size, and discharge rate, may affect the actual amount of IGR on the floor when the
total release aerosol treatment is used. The relative activity ratio of pyriproxyfen to methoprene was approximately >5 times in the different treatments. These results indicate the higher persistence of pyriproxyfen on the carpet under household conditions than methoprene, although the relative activity of pyriproxyfen had been reported to be less than twice the activity of methoprene (Kobayashi et al. 1994b). Hinkle et al. (1995) attributed the lack of residual effectiveness in the methoprene treatment to its high volatility, because the structure of methoprene is similar to hydroprene and is reported to translocate or move from the point of application (Donahue and Young 1992). Their conclusions, however, are not consistent with the vapor pressure of the 2 compounds within the same range of 2.37 x 10^-5 mmHg at 25°C for methoprene (Farm Chemical Handbook 1995) versus 2.8 x 10^-3 mmHg at 20°C for pyriproxyfen (Preiss and Untiedt 1994). Volatility, therefore, is not a factor by which the difference in residual activities of 2 chemicals can be explained. High stability of chemicals in the carpet matrix or in the house dust is more likely to be the reason for the long-term residual effectiveness in the carpet treatments. Diflubenzuron (Uniroyal, Amsterdam), a chitin synthesis inhibitor with a relatively high stability like pyriproxyfen, was reported to have a long residual effectiveness under simulated household conditions (Henderson and Foil 1993). The dosage of diflubenzuron, however, was much higher (53.82 mg/m²) than the dose of pyriproxyfen used in the current study. Such a high dosage of diflubenzuron was required because of its lower inhibitory activity than the other IGRs such as methoprene (Moser et al. 1992).

Carpets seem to be one of the more difficult substrates to be treated effectively with an insecticide because of their increased surface area (Hinkle et al. 1995). Osbink et al. (1986) reported the lack of residual effectiveness of pressurized aerosols containing pyrethrins and tetramethrin against cat flea larvae. They also found that the combination of IGRs with the chemicals noted above reinforced their residual effectiveness. The use of IGRs with high stability and high inhibitory activity, such as pyriproxyfen, would minimize the total amount of chemicals used in houses and reduce the operational cost in the household flea control program.

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