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Duration of Fipronil Topical Application Toxicity in *Blattella germanica* Field Population Strains

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Abstract: The duration of fipronil topical application toxicity of twelve strains of German cockroaches, *Blattella germanica* (L.), was determined. The Saman strain was collected in 2002, from infested Habitable Convened and the Bustan-7, Bustan-8, Bustan-10, Kouye-Pezeshky, Shahmorady, Zanjan and Kouye-Tehran strains were collected in 2001, from different infested student dormitories and the Fayyazbaghesh, Mogtameh and Kouye-Dokhteran strains were collected in 1997 from two infested hospitals and one student dormitory, respectively in Tehran, Iran. In the topical application bioassay, the average LD_{50} of susceptible strain was 1.8, 1.3 and 0.96 ng per insect after 24, 48 and 72 h, respectively and the average LD_{95} was 3.4, 2.6 and 2.2 ng per insect after 24, 48 and 72 h, respectively. As with the susceptible reference strain, where LD_{50} was halved from 24 to 72 h, the LD_{50} of fipronil decreased with time in the field populations. LD_{50} varied > 3-fold from 1.2 to 3.6 ng per insect at 24 h and almost 2.5-fold from 1.2 to 3.0 ng per insect at 48 h. At the end of the bioassay at 72 h, LD_{50} varied from 0.94 to 2.5 ng per insect, which is 1.6- and 2.6-fold higher than the standard susceptible value of fipronil and the steep slopes of dose-response curves indicated that the field population of these German cockroach strains was homogenous in response to fipronil. All German cockroach strains showed a similar susceptibility or lower tolerance (1.5 to 2.6-folds) for fipronil compared with the susceptible laboratory strain and the steep slopes of dose-response curves indicated that the field population of these German cockroach strains was homogenous in response to fipronil. These results indicate that the fipronil was relatively slow-acting in topical application bioassay, with LD_{50} values decreasing until 72 h and becoming stable thereafter.

Key words: *Blattella germanica*, fipronil, insecticide resistance, topical application, duration

INTRODUCTION

Control of the German cockroach, *Blattella germanica* (L.), by using insecticides has a history going back to the 1950s (Heal *et al*., 1953). However, extensive application of a variety of formulated insecticides like organophosphates, carbamates and pyrethroids for control of German cockroaches has led to the development of insecticide resistance in German cockroaches in many countries including the United States (Lionel *et al*., 2000; Ladonni, 2001). Pyrethroids have been used extensively for control of insects because of their rapid action, low odor and safety (Cornwell 1976, Zhai and Robinson 1996). Frequent use has resulted in the development of resistance to pyrethroids in some field populations of German cockroaches (Atkinson *et al*., 1991; Horwood *et al*., 1991; Vagn Jensen, 1993).

The search for new insecticides and new methods of insecticide delivery to control the German cockroach continues because this insect remains one of the most economically and medically important pests of the urban environment (Brenner, 1995). In this ongoing process, older chemicals are displaced because of insecticide resistance, increasingly strict regulations and public demand for safer and more effective products. New active ingredients and innovative delivery tools emerge to provide effective means of dealing with infestations (Buczkowski and Schal, 2001). One of the newest insecticides is fipronil, a relatively fast-acting phenylpyrazole that blocks the transmission of signals by the inhibitory neurotransmitter Gama-aminobutyric acid (Colliot *et al*., 1992; Cole *et al*., 1993; Moffat, 1993). Fipronil is a highly effective, broad-spectrum insecticide with potential value for the control of a wide range of crop, public hygiene, amenity and veterinary pests. It can generally be applied at low to very low dose rates to achieve effective pest control. It is higher toxicity of fipronil in insects than in mammals is due partially to the higher sensitivity of GABA receptors (Zhao *et al*., 2003)

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and highly toxic to the German cockroach (Kaakeh *et al*., 1997) and other pests of public and medical importance (Scott and Wen, 1997).

The development of resistance and cross-resistance are potential problems resulting from the over-use of any pesticides and fipronil is relatively new and thus currently free from this problem. One of the principal justifications for continued research on insecticide efficacy, longevity and application is to prevent the development of resistance in the target insect population. Although attempts to monitor resistance of field-collected strains at the adult stage have been conducted (Ladonni, 2001), in Iran very little information is currently available on the toxicity of fipronil to development of resistance German cockroach or no studies conducted.

In this study, we address the question of the duration of fipronil topical application toxicity against susceptible and field populations of German cockroach, *B. germanica*, strains.

MATERIALS AND METHODS

Cockroach strains: A total of 12 strains of *B. germanica* were evaluated for duration of fipronil topical application toxicity at the School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences, Iran during 2001 and 2002. A Standard Susceptible (SS) strain was maintained since 1975 in the insectary at the School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences, without exposure to any insecticide (Ladonni, 2001). The Saman strain was collected in 2002, from infested Habitable Convened and the Bustan-7, Bustan-8, Bustan-10, Kouye-Pezeshky, Shahmorady, Zanjan and Kouye-Tehran strains were collected in 2001, from different infested student dormitories (Nasirian, 2004) and the Fayyazbaghesh, Mogtameh and Kouye-Dokhteran strains were collected in 1997 from two infested hospitals and one student dormitory, respectively, in Tehran, Iran (Farzinnia,1998; Moosavi, 1999).

Cockroaches collecting and rearing: Cockroaches were collected with a piece of a radiology film (10 × 10 cm) and transferred to an apparatus by hand catch in the last hours of the night. The apparatus manufactured from two parts, the upper inside surface of the upper part (5 cm) was lightly greased with petroleum jelly to prevent cockroaches from escaping, after collecting cockroaches and in the insectary the lower part separated from the upper part and cockroaches transferred to glass rearing jars to prevent cockroaches from greasing.

All cockroaches were maintained and colonized at 27±2°C, 60±10% RH and a photoperiod of 12:12 (L:D) h in the insectary at the School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences. Each strain was kept in separate labeled glass rearing jars of the same size (500 mL). The upper inside surface of the jars was lightly greased with petroleum jelly to prevent escape. Cockroaches were provided with cat food, water ad libitum and a cardboard shelter.

Insecticide: Chemicals used were technical grade fipronil (95% [AI]; Rhone-Poulenc, Research Triangle Park, NC), CO_2 as an anesthetic and acetone as a solvent.

Topical application bioassays: Technical grade fipronil (95% [AI]; Rhone-Poulenc, Research Triangle Park, NC), was delivered in 0.5 μL acetone to the first abdominal sternum of briefly CO_2-anesthetize adult male cockroaches by topical application with a micro applicator (Hamilton, Reno, NV) equipped with a 1.0 mL hypodermic glass syringe fitted with a 27-gauge needle (Scott*et al*., 1990) Adult male cockroaches (1-3 wk old) were treated with 5-6 concentration of insecticide and each concentration was replicated 3-6 times (10 cockroaches for each replicate). Control groups received acetone alone. A 5-6 dose giving > 0% and < 100% mortality at 24, 48 and 72 h for fipronil after insecticide treatment was used for each experiment. In addition, it should be mentioned that mortality after fipronil treated was recorded for 6 days but mortality after 72 h becoming stable. Treated males were placed in 150 by 25-mm plastic petri dishes, provided with food and water and monitored for mortality for 24, 48 and 72 h under the same temperature and photoperiod as the colony. If insects on their backs were unable to right themselves when prodded, they were considered dead.

Statistical analysis: Mortality data from the replicates were pooled and the dose-response was assessed by probit analysis (Finney, 1972), with a SPSS package on an IBM computer. Resistance ratios were calculated as the 50% response value (LD_{50}) of RR strain divided by the 50% response value of the SS strain. For comparison, bioassays are visualized in Fig. 1 by plotting concentration of insecticide (log_{10}) versus percentage mortality (probit) at LD_5, LD_{50} and LD_{95}.

RESULTS AND DISCUSSION

Toxicity of fipronil against susceptible strain: In the topical application bioassay, the average LD_{50} (n = 6) was 1.8, 1 .3 and 0.96 ng per insect after 24 h (not shown),

Table 1: Toxicity of topically applied fipronil to laboratory-reared German cockroach strains

	Fipronil				
Strains	n	Slope±SE	x^2	LD_{50}[a] (CI)	RR[b]
Susceptible	285	1.3±0.13	3.0	0.96(0.81–1.1)	_
Bustan-7	280	1.1±0.10	4.4	1.4(1.2–1.6)	1.5
Bustan-8	314	1.0±0.1	5.0	1.4(1.2–1.6)	1.5
Bustan-10	402	0.9±0.1	15.2	2.5(2.2–2.8)	2.6
Kouye-Pezeshky	463	1.3±0.1	4.4	0.94(0.82–1.1)	0.96
Shahmorady	279	1.3±0.13	2.6	1.0(0.85–1.2)	1.0
Zanjan	400	1.0±0.1	4.6	2.5(2.4–2.7)	2.6
Kouye-Tehran	362	1.2±0.2	4.6	1.5(1.3–1.6)	1.6
Saman	406	0.9±0.1	9.8	2.3(2.1–2.5)	2.4
Mogtameh	360	1.2±0.11	8.5	1.0(0.96–1.2)	1.0
Kouye-Dokhteran	399	1.0±0.1	9.3	1.9(1.8–2.1)	2.0
Fayyazbaghesh	355	1.4±0.13	4.2	1.0(0.90–1.2)	1.0

[a] LD_{50} values in nanograms per insect (95% CI). [b] Resistance ratio: LD_{50} of resistant strain/ LD_{50} of susceptible strain

48 h (not shown) and 72 h (Table 1), respectively and the average LD_{95} (n = 6) was 3.4, 2.6 and 2.2 ng per insect after 24 h (not shown), 48 h (not shown) and 72 h (not shown), respectively. The values are similar to the insecticide-susceptible strains CSMA and Chochran-S with LD_{50} values 2.6 and 3.0 ng per insect, respectively (Scott and Wen 1997). Fipronil was highly toxic to German cockroaches (LD_{50} to the susceptible CSMA strain was 2.6 ng per cockroach) that indicated the fipronil was 100-fold more toxic than commonly used carbamate and organophosphate insecticides (i.e., bendiocarb and chlorpyrifos LD_{50} values are 360 and 260 ng per cockroach, respectively). The toxicity of fipronil to susceptible cockroaches is similar to, or slightly higher than, common pyrethroids (pyrethroid values generally range from 4 to 110 ng per cockroach (Scott *et al*., 1990), whereas in our study the susceptible strain showed that the fipronil was 238-fold more toxic than permethrin (fipronil and permethrin LD_{50} values are 1.8 and 430 ng per cockroach after 24 h, respectively) (Nasirian, 2004).

Toxicity of fipronil against field populations German cockroach strains: The toxicity of fipronil was examined in German cockroach field population collected from 11 different infested sites during 2001 and 2002. As with the susceptible reference strain, where LD_{50} was halved from 24 to 72 h, the LD_{50} of fipronil decreased with time in the field populations (Fig. 1). LD_{50} varied > 3-fold from 1.2 to 3.6 ng per insect at 24 h and almost 2.5-fold from 1.2 to 3.0 ng per insect at 48 h (Fig. 1). At the end of the bioassay at 72 h, LD_{50} varied from 0.94 to 2.5 ng per insect (Fig. 1; Table 1), which is 1.6- and 2.6-fold higher than the standard susceptible value of fipronil and the steep slopes of dose-response curves indicated that the field population of these German cockroach strains was homogenous in response to fipronil. Toxicity of insecticides varies between susceptible field populations

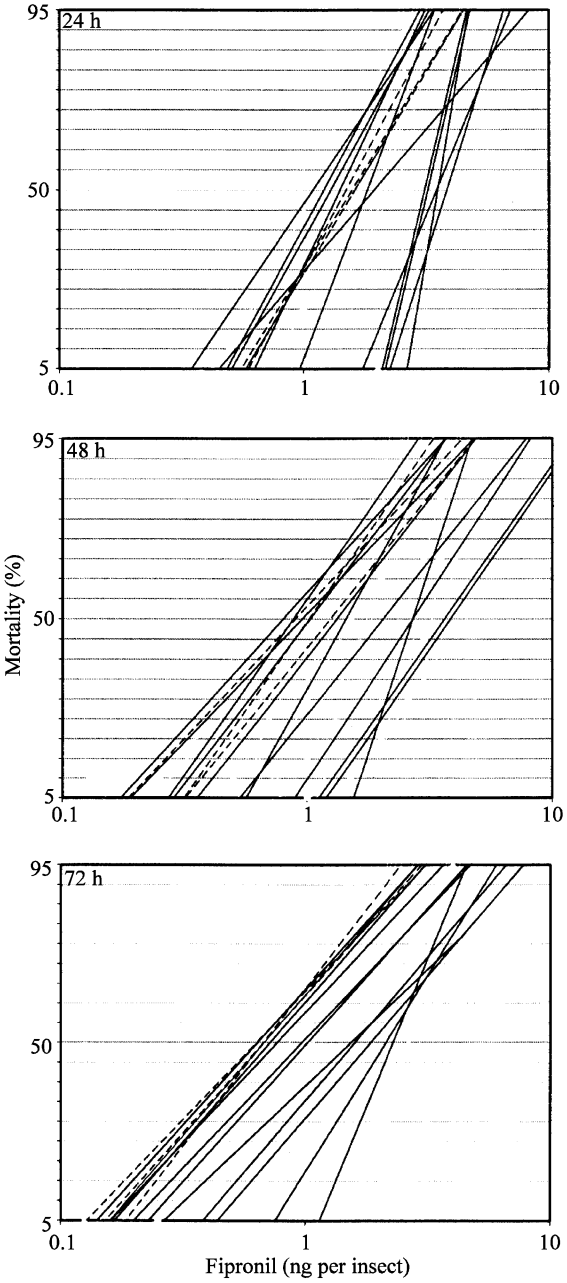

Fig. 1: Toxicity of fipronil to German cockroaches. Solid lines indicate field population strains, and broken lines show replicates with the susceptible laboratory strain

and susceptible laboratory strains, as well as between field populations, where large unexplained variations of toxicity of unexposed field populations occur (Sawicki, 1987). These differences or natural variation could be referred to as differences in tolerance or sensitivity, whereas the term resistance is best defined as a reduction in susceptibility beyond natural variation

(Schaub *et al*., 2002). In a study by Scott and Wen (1997), fipronil was highly toxic to German cockroaches. Valles *et al.* (1997) reported that fipronil effectively killed German cockroaches when applied in nanogram quantities; insecticide-susceptible, carbamate-and organophosphorus-resistant, pyrethroid-resistant strains were equally susceptible to fipronil with LD_{50} values between 4.6 and 5.4 ng per insect. A pyrethroid-organophosphorus and carbamate-resistant was 1.6-fold tolerant to fipronil. German cockroaches strains collected from field were considerably tolerant to fipronil (Valles *et al*., 1997).

All German cockroach strains showed a similar susceptibility or lower tolerance (1.5 to 2.6-folds) for fipronil compared with the susceptible laboratory strain (Table 1) and the steep slopes of dose-response curves indicated that the field population of these German cockroach strains was homogenous in response to fipronil (Fig. 1). Present results confirm those of Kaakeh *et al.* (1997) who reported that fipronil was very effective against laboratory-reared German cockroaches in nanogram quantities. Furthermore, German cockroaches collected from the field exhibited higher susceptibility level toward fipronil than the susceptible strains (Kaakeh *et al*., 1997).

In conclusion, fipronil was relatively slow-acting in topical application bioassay, with LD_{50} values decreasing until 72 h and becoming stable thereafter. These data confirm the reported study by Scott and Wen (1997) that determined the toxicity of fipronil in German cockroach and housefly (Scott and Wen, 1997).

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