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Duration of Fipronil WHO Glass Jar Method Toxicity Against Susceptible and Feral German Cockroach Strains

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Abstract: The duration of fipronil WHO glass jar method toxicity against twelve strains of feral German cockroaches, *Blattella germanica* (L.), was determined. In the WHO glass jar bioassay, the average LT_{50} of susceptible strain was 16.4, 14.3, 12.4 and 11.3 exposure minutes after 24, 48, 72 and 96 h, respectively and the average LT_{95} was 20.3, 19.9, 19.5 and 19.1 exposure minutes after 24, 48, 72 and 96 h, respectively. As with the susceptible reference strain, where LT_{50} was halved from 24 to 96 h, the LT_{50} of fipronil decreased with time in the feral German cockroach strains. LT_{50} varied > 8-folds from 16.2 to 24.7 exposure min at 24 h, 8.4 folds from 14.4 to 22.8 min at 48 h and almost 8.8 folds from 12.5 to 21.3 exposure minutes at 72 h. At the end of the bioassay at 96 h, LT_{50} varied from 11.6 to 19.7 exposure minutes, which is 1.0 and 1.7 folds exposure min higher than the standard susceptible value of fipronil. All German cockroach strains showed a similar susceptibility or very low tolerance (1.5 to 1.7 folds) to fipronil compared with the susceptible laboratory strain and the steep slopes of time exposure-mortality curves indicated that the feral German cockroach strains was homogenous in time exposures to fipronil. These results indicate that the fipronil was relatively slow-acting in WHO glass jar method bioassay, with LT_{50} values decreasing until 96 h and becoming stable thereafter.

Key words: Feral German cockroach strains, fipronil, time exposure-mortality, WHO glass jar method, duration

INTRODUCTION

Chemical insecticides play a fundamental role in integrated pest management due to their effect on controlling insects. The continuous or periodic use of insecticides against pest populations may result in the development of insecticide resistance. Insecticide resistance continues to be an important problem in insect pest management. Geoghiou (1990) reported resistance development in 447 species of insect and mites. Presently, at least 536 species have developed resistance to pesticides and > 300 active ingredients have been subject to resistance by at least one insect or mite species (Whalon *et al*., 2004; Cutler *et al*., 2005).

The search for new insecticides and new methods of insecticide delivery to control the German cockroach, *Blattella germanica* (L.), 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. One of the newest insecticides is fipronil, a fast-acting phenylpyrazole that blocks the transmission of signals by the inhibitory neurotransmitter g-aminobutyric acid (Colliot *et al*., 1992; Cole *et al*., 1993; Moffat, 1993). Fipronil (Colliot *et al*., 1992) is a relatively new insecticide that is beginning to see widespread use against an array of arthropod pests of agricultural, medical and veterinary importance. Fipronil kills insects by interacting agonistically with gamma-aminobutyric acid (GABA)-gated chloride channels (Gant *et al*., 1998), a mode of action that Colliot *et al*. (1992) called unique.

Monitoring insects' susceptibility to insecticides is required for effective use of insecticides. This requirement prompted this study to evaluate the susceptibility of the most abundant species in urban areas of worldwide, *B. germanica*, to commonly used insecticides. Fipronil was relatively slow-acting in topical application bioassay, with LD_{50} values decreasing until 72 h and becoming

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stable thereafter (Scott and Wen, 1997; Nasirian *et al*., 2006), thus no studies focused on the duration of fipronil WHO glass jar method toxicity. The objective of this study is to determine the duration of fipronil WHO glass jar method toxicity against susceptible and feral German cockroach, *B. germanica*, strains.

MATERIALS AND METHODS

Cockroach strains: A total of 12 strains of *B. germanica* were evaluated for duration of fipronil WHO glass jar method toxicity at the School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences, Iran during 2002 and 2003. 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 M strain was collected from infested Habitable Convened, the eight strains (D_1 to D_8) were collected from different infested student dormitories and the two strains (H_1 to H_2) were collected from two infested hospitals in Tehran, Iran (Nasirian, 2004).

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 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 as a 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.

WHO Glass Jar method bioassay: Technical grade fipronil (95% [AI]; Rhone-Poulenc, Research Triangle Park, NC), was prepared in acetone and 2.5 mL was pipetted into a 0.4 liter glass jar (inner surface area = 302.6 cm^2), with its rim painted with Fluon AD-1. The jars were rolled horizontally over a flat surface until all of the acetone had evaporated, so that the insecticide was deposited evenly over the inner surface of the jar. In a series of contact experiments, a concentration of 0.0625 μg m^{-2} fipronil (technical grade) at a 20 min exposure time was found to be a discriminating dose for adults to distinguish SS from RR strains. Adult male cockroaches (1-3 week old) were treated with 5-6 time exposures and each time exposures was replicated 3-6 times (10 cockroaches for each replicate). Control groups received acetone alone. A 5-6 time exposure giving >0 and<100% mortality at 24, 48, 72 and 96 h after insecticide time exposures was used for each experiment. In addition, it should be mentioned that mortality after fipronil exposure was recorded for 6 days but mortality after 96 h becoming stable. Fipronil exposure males were placed in 150 by 25 mm plastic Petri dishes, provided with food and water and monitored for mortality for 24, 48, 72 and 96 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 time exposure mortality was assessed by probit analysis (Finney, 1972), with a SPSS package on an IBM computer. Resistance ratios were calculated as the 50% response value (LT_{50}) of RR strain divided by the 50% response value of the SS strain. For comparison, bioassays are visualized in Fig. 1 by plotting time exposures of insecticide (log_{10}) versus percentage mortality (probit) at LT_5, LT_{50} and LT_{95}.

RESULTS AND DISCUSSION

In the WHO glass jar bioassay, the average LT_{50} of the susceptible strain was 16.4, 14.3, 12.4 and 11.3 exposure minutes after 24, 48, 72 and 96 h, respectively and the average LT_{95} was 20.3, 19.9, 19.5 and 19.1 exposure minutes after 24, 48, 72 and 96 h, respectively (Table 1-4).

The toxicity of fipronil was examined in feral German cockroach strains that collected from 11 different infested sites during 2002 and 2003 (Nasirian, 2004). As with the susceptible reference strain, where LT_{50} was halved from 24 to 96 h, the LT_{50} of fipronil decreased with time in the feral German cockroach strains (Table 1 and Fig. 1). LT_{50} varied > 8 folds from 16.2 to 24.7 exposure min at 24 h, 8.4 folds from 14.4 to 22.8 min at 48 h and almost 8.8 folds from 12.5 to 21.3 exposure min at 72 h. At the end of the bioassay at 96 h, LT_{50} varied from 11.6 to 19.7 exposure

Table 1: Toxicity of fipronil to a susceptible and feral-reared German cockroach strains by WHO glass jar method

	24 h after exposure times						
Strains	n	Slope±SE	χ^2	LT_{50}[a] (CI)	LT_{90}[a] (CI)	LT_{95}[a] (CI)	RR[b]
S	168	0.42±0.06	0.24	16.4 (15.5-17.2)	19.4 (18.4-21.0)	20.3 (19.2-22.1)	-
D_1	160	0.39±0.06	2.70	16.3 (15.4-17.2)	19.6 (18.5-21.3)	20.5 (19.3-22.6)	0.99
D_2	196	0.24±0.03	13.30	19.5 (13.3-30.6)	24.9 (20.8-74.4)	26.4 (21.8-41.5)	1.20
D_3	248	0.21±0.03	3.90	24.7 (23.6-26.0)	30.8 (29.0-33.4)	32.5 (30.5-35.6)	1.50
D_4	160	0.41±0.06	0.25	16.4 (15.5-17.2)	19.5 (18.5-21.2)	20.4 (19.2-22.4)	1.00
D_5	248	0.16±0.02	6.50	20.6 (19.2-22.0)	28.8 (26.8-31.5)	31.1 (28.8-34.4)	1.20
D_6	284	0.13±0.01	3.50	25.6 (24.1-27.1)	35.3 (32.9-38.6)	38.0 (35.3-41.9)	1.60
D_7	160	0.46±0.07	0.04	16.9 (16.1-17.7)	19.6 (18.7-21.2)	20.4 (19.3-22.3)	1.00
D_8	160	0.41±0.06	0.25	16.4 (15.5-17.2)	19.5 (18.5-21.2)	20.4 (19.2-22.4)	1.00
M	284	0.13±0.01	4.70	23.3 (21.9-24.8)	33.1 (30.9-36.1)	35.8 (33.3-39.4)	1.40
H_1	160	0.45±0.07	0.14	16.2 (15.4-17.0)	19.1 (18.1-20.7)	19.9 (18.8-21.9)	0.99
H_2	200	0.27±0.03	2.10	18.2 (17.2-19.3)	23.1 (21.7-25.1)	24.4 (22.9-26.8)	1.10

[a]LT_{50} ,LT_{90}, LT_{95} values in time exposure minutes (95% CI). [b]Resistance ratio: LT_{50} of feral strain/LT_{50} of susceptible strain

Table 2: Toxicity of fipronil to a susceptible and feral-reared German cockroach strains by WHO glass jar method

	48 h after exposure times						
Strains	n	Slope±SE	χ^2	LT_{50}[a] (CI)	LT_{90}[a] (CI)	LT_{95}[a] (CI)	RR[b]
S	168	0.24±0.03	6.4	14.3 (3.6-41.7)	18.9 (15.2-25.4)	19.9 (16.3-29.9)	-
D_1	160	0.33±0.05	5.1	15.5 (7.2-32.5)	19.4 (16.1-35.3)	20.5 (16.9-25.4)	1.1
D_2	196	0.18±0.02	12.7	17.3 (11.6-26.1)	24.6 (19.6-56.9)	26.6 (21.0-35.4)	1.2
D_3	248	0.19±0.02	9.3	22.8 (20.1-26.2)	29.6 (26.2-37.8)	31.6 (27.7-41.4)	1.6
D_4	160	0.24±0.03	5.8	14.4 (5.3-34.9)	19.7 (15.4-31.4)	21.2 (16.5-42.5)	1.0
D_5	248	0.14±0.02	3.4	17.6 (16.3-19.1)	26.5 (24.5-29.3)	29.0 (26.7-32.4)	1.2
D_6	284	0.12±0.01	9.6	22.1 (19.2-25.4)	33.3 (29.2-40.8)	36.5 (31.7-45.6)	1.6
D_7	160	0.26±0.03	13.3	14.8 (9.6-24.4)	19.8 (15.9-56.8)	21.2 (16.9-66.8)	1.0
D_8	160	0.30±0.04	7.0	14.5 (11.6-17.9)	18.7 (16.1-28.7)	20.0 (17.0-32.1)	1.0
M	284	0.12±0.01	3.9	19.4 (17.9-21.0)	30.4 (28.1-33.5)	33.5 (30.8-37.3)	1.4
H_1	160	0.27±0.03	13.8	14.9 (9.3-25.6)	19.7 (15.9-63.9)	21.1 (16.9-75.6)	1.0
H_2	200	0.22±0.02	2.2	15.8 (14.6-16.9)	21.7 (20.2-23.9)	23.4 (21.6-26.0)	1.1

[a]LT_{50} ,LT_{90}, LT_{95} values in time exposure minutes (95% CI). [b]Resistance ratio: LT_{50} of feral strain/LT_{50} of susceptible strain

Table 3: Toxicity of fipronil to a susceptible and feral-reared German cockroach strains by WHO glass jar method

	72 h after exposure times						
Strains	n	Slope±SE	χ^2	LT_{50}[a] (CI)	LT_{90}[a] (CI)	LT_{95}[a] (CI)	RR[b]
S	168	0.23±0.03	4.0	12.4 (7.6-17.6)	18.0 (14.3-36.6)	19.5 (15.4-42.8)	-
D_1	160	0.24±0.03	5.8	14.4 (5.3-34.9)	19.7 (15.4-33.2)	21.2 (16.5-37.5)	1.2
D_2	196	0.16±0.02	7.6	15.7 (11.7-20.2)	23.5 (19.3-37.1)	25.7 (20.8-42.4)	1.3
D_3	248	0.16±0.2	9.3	21.3 (18.4-24.9)	29.3 (25.6-38.1)	31.6 (27.2-42.1)	1.7
D_4	160	0.23±0.38	3.9	12.5 (7.6-18.1)	18.1 (14.3-38.5)	19.6 (15.5-45.0)	1.0
D_5	248	0.13±0.01	4.7	15.0 (13.5-16.5)	24.9 (22.8-28.0)	27.7 (25.2-31.4)	1.2
D_6	284	0.11±0.01	8.9	20.7 (17.7-23.7)	32.4 (28.4-39.8)	35.7 (31.0-44.6)	1.7
D_7	160	0.22±0.03	5.0	12.7 (5.8-21.5)	18.5 (14.3-60.0)	20.1 (15.5-72.2)	1.0
D_8	160	0.24±0.03	3.5	12.8 (11.7-13.9)	18.1 (16.6-20.3)	19.6 (17.9-22.2)	1.0
M	284	0.11±0.01	3.0	17.5 (15.8-19.1)	29.1 (26.8-32.4)	32.4 (29.6-36.4)	1.4
H_1	160	0.24±0.03	5.2	12.7 (5.7-21.8)	18.1 (14.1-60.2)	19.7 (15.2-72.4)	1.0
H_2	200	0.20±0.02	1.9	14.5 (13.4-15.7)	20.9 (19.3-23.1)	22.7 (20.8-25.4)	1.2

[a]LT_{50} ,LT_{90}, LT_{95} values in time exposure minutes (95% CI). [b]Resistance ratio: LT_{50} of feral strain/LT_{50} of susceptible strain

Table 4: Toxicity of fipronil to a susceptible and feral-reared German cockroach strains by WHO glass jar method

	96 h after exposure times						
Strains	n	Slope±SE	χ^2	LT_{50}[a] (CI)	LT_{90}[a] (CI)	LT_{95}[a] (CI)	RR[b]
S	168	0.21±0.03	3.2	11.3 (10.2-12.5)	17.4 (15.8-19.7)	19.1 (17.2-22.0)	-
D_1	160	0.22±0.03	4.1	12.4 (6.9-18.4)	18.2 (14.3-42.1)	19.8 (15.5-49.7)	1.1
D_2	196	0.18±0.2	4.6	14.0 (12.7-15.2)	21.1 (19.4-23.6)	23.1 (21.2-26.2)	1.2
D_3	248	0.17±0.02	5.3	19.7 (18.5-21.0)	27.2 (25.4-29.8)	29.4 (27.3-32.4)	1.7
D_4	160	0.21±0.03	4.1	11.8 (5.4-17.9)	18.0 (14.0-45.2)	19.8 (15.2-54.1)	1.0
D_5	248	0.12±0.01	5.3	12.4 (10.6-14.0)	23.6 (21.3-27.0)	26.8 (24.0-31.0)	1.1
D_6	284	0.11±0.01	9.8	18.8 (15.5-21.9)	30.7 (36.6-38.3)	34.1 (29.3-43.5)	1.7
D_7	160	0.22±0.03	2.8	11.6 (10.5-12.8)	17.6 (16.0-20.0)	19.3 (17.4-22.1)	1.0
D_8	160	0.20±0.03	4.5	11.7 (4.2-19.0)	18.2 (13.9-55.3)	20.0 (15.2-67.1)	1.0
M	284	0.08±0.01	5.8	14.1 (11.6-16.1)	29.6 (26.6-34.1)	34.1 (30.4-39.7)	1.2
H_1	160	0.23±0.03	3.3	11.8 (10.6-12.9)	17.3 (15.8-19.5)	18.8 (17.1-21.5)	1.0
H_2	200	0.18±0.02	2.7	13.5 (12.2-14.8)	20.5 (18.8-22.9)	22.5 (20.5-25.4)	1.2

[a]LT_{50} ,LT_{90}, LT_{95} values in time exposure minutes (95% CI). [b]Resistance ratio: LT_{50} of feral strain/LT_{50} of susceptible strain

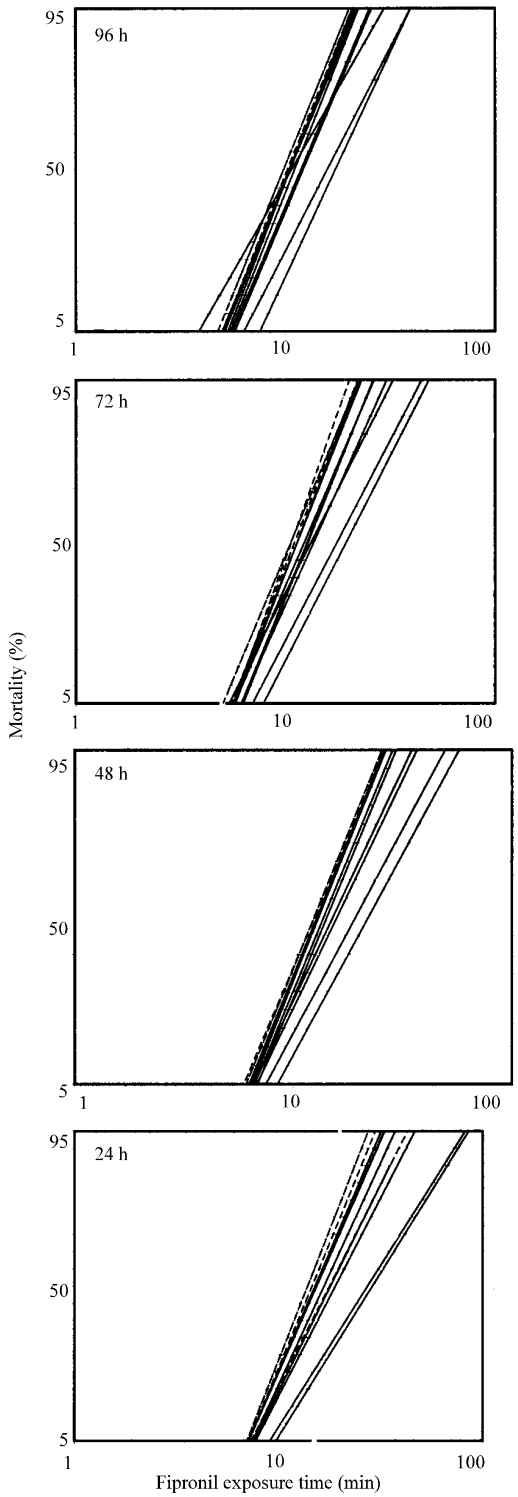

Fig. 1: Duration of WHO glass jar fipronil toxicity to German cockroaches. Solid lines indicate feral strains and broken lines show replicates with the susceptible laboratory strain

minutes (Table 1), which is 1.0 and 1.7 folds exposure minutes higher than the standard susceptible value of fipronil. All German cockroach strains showed a similar susceptibility or very low tolerance (1.5 to 1.7 folds) to fipronil compared with the susceptible laboratory strain and the steep slopes of time exposure-mortality curves indicated that the field population of these German cockroach strains was homogenous in time exposures to fipronil.

Several factors, such as the application rate, formulation, surface and most importantly, the length of time a cockroach contacts the treatment, affect the performance of residual insecticides (Cornwell, 1976; Chadwick, 1985; Wickham, 1995). Male mortality was affected by the duration of exposure to fipronil on a glass surface. A 19.9-38.0 min exposures to 0.0625 ug m^{-2} fipronil (technical grade) killed all males within 24 h (Table 1). Shorter exposures the same residues, however, required 48, 72 and 96 h, respectively, to kill all cockroaches (Table 2-4). With less exposure of adult male German cockroaches to a concentration of fipronil (0.0625 μg m^{-2}), significantly more time needed to kill the all cockroaches. In contrast, males that walked on a fipronil-treated surface in the WHO glass jar method probably picked up insecticide on their tarsi and transferred it to other parts of the body but in the topical application bioassay method the insecticide to be imported directly in the insect body.

The toxicity of insecticides against insects usually terminated for 24 h after exposure times but in fipronil last for more 24 h after exposure times. No previous reports have been found for insecticide susceptibility of *B. germanica* to fipronil in WHO glass jar method in Iran; however, the result of this study revealed a similar trend to that of *B. germanica*, being nearly susceptible to fipronil in this method. In a study by Scott and Wen (1997) and Nasirian *et al.* (2006) fipronil was relatively slow-acting in topical application bioassay, with LD_{50} values decreasing until 72 h and becoming stable thereafter. In conclusion, these results support the hypothesis that fipronil was relatively slow-acting in WHO glass jar method bioassay, with LT_{50} values decreasing until 96 h and becoming stable thereafter.

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