

## Original Article

# Duration of Fipronil and Imidacloprid Gel Baits Toxicity against *Blattella germanica* Strains of Iran

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(Received 2 Dec 2006; accepted 13 Oct 2007)

## Abstract

The current study was conducted to investigate the duration of fipronil and imidacloprid gel baits toxicity against German cockroach strains in Iran during 2003-2004. In order to conduct this study, nine German cockroach strains were used. Newly emerged adult male German cockroaches starved for one scotophase (12 h), and ingested fipronil and imidacloprid gel baits for 2 h. After the given time was over, the bait was removed and replaced with mouse pellet. Mortality was recorded at 12 intervals for 144 h (6 days). Mortality data of the replicates were pooled and was tested using probit analysis. Both gel baits were toxic to adult male German cockroaches. In the ingested bait method, the susceptible strain showed  $LT_{50}$  of 47.1 and 11.3 h for fipronil and imidacloprid gel baits, respectively, and the average  $LT_{90}$  was 74.2 and 19.3 h, respectively.  $LT_{50}$  of the feral German cockroach strains varied 14.9 h from 30.5 to 45.4 h and 4.4 h from 12.4 to 16.8 h for fipronil and imidacloprid gel baits, respectively. All German cockroach strains showed a similar susceptibility to fipronil and imidacloprid gel baits, compared with the susceptible laboratory strain. The steep slopes of ingested bait mortality curves indicated that the feral German cockroach strains were homogenous to fipronil and imidacloprid ingested gel baits. These results suggest that fipronil and imidacloprid gel baits appear to have considerable potential as a bait for insecticide-resistant strains of German cockroach.

**Keywords:** German cockroach, Gel bait, Fipronil, Imidacloprid, Iran

## Introduction

The German cockroach, *Blattella germanica* (L.), is a common indoor pest in low income housing. Cockroaches not only spoil food but also transfer pathogens and cause allergic reactions and psychological distress (Brenner 1995).

Management of cockroach populations in urban environments has recently shifted from the predominant use of insecticide sprays to the inclusion of baits in management programs (Reiersen 1995). This change has been facilitated by the general perception that baits are safer and therefore they appeal to the public's concern about risks associated with pesticides in the domestic environment. Moreover, baits fit well into the integrated pest management

(IPM) objectives of reducing pesticide usage while maintaining effective suppression of cockroach populations (Schal and Hamilton 1990, Rust et al. 1995).

Application of insecticidal baits is one of the most common and effective strategies for controlling the *B. germanica* (Reiersen 1995, Bennett et al. 1997). Toxic baits are commonly used in urban pest and management programs and provide several advantages over other insecticide application methods. Baits greatly reduce problems encountered with run off and drift from liquid and dust insecticide formulations (Jech et al. 1993). Baits are useful when control programs are conducted near water, or in areas where threatened and endangered species occur, and where preservation of beneficial

species of arthropods is important. Baits offer the advantage of low odor, stability, and ease of application compared with aerosol and spray formulations (Appel 2004). Unlike liquid or dust forms, baits have little dispersion. In addition, using baits substantially reduces the overall amount of active ingredient needed and therefore reduces the amount of insecticide exposed to the environment. Baits are often specific to the target organism, or at least provide greater selectivity than liquids and dusts.

Fipronil ( $C_{12}H_4C_{12}F_6N_4OS$ ), a phenylpyrazole insecticide, was discovered by Rhone-Poulenc Agro in 1987, introduced in 1993, and registered as a pesticide in the United States in 1996. Fipronil (5-amino-1-[2, 6-dichloro-4-(trifluoromethyl) phenyl]-4-[(trifluoromethyl) sulfinyl]-1H-pyrazole), is a relatively new insecticide that is at the beginning of a widespread use against an array of arthropod pests of agricultural, medical, and veterinary importance (Colliot et al. 1992). Application rates vary between 0.6 and 200 g a.i./ha, depending on the target pest and formulation. Fipronil slowly degrades on vegetation and relatively slowly in soil and water, with a half-life ranging between 36 hour and 7.3 month. This very different time span depends on substrate and conditions. It is relatively immobile in soil and has low potential to leach into underground water. It is moderately toxic to laboratory mammals by oral exposure ( $LD_{50}$ = 97 mg/kg for rats,  $LD_{50}$ = 91 mg/kg for mice). Technical fipronil is in toxicity categories II and III, depending on the route of administration, and is classed as a non-sensitizer (Tingle et al. 2003). Baits containing fipronil have already, in the 5 year use since their introduction, become popular among consumers and professionals alike for control of domestic cockroaches and ants. The great appeal of fipronil can be attributed, in large part, to its considerable lethality (Kaakeh et al. 1997), but equally attractive is its distinctly greater toxicity to insects than mammals (Gant et al. 1998, Hainzl et al. 1998). 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. Recently, a toxic gel bait containing fipronil became available commercially (Colliot et al. 1992).

Neonicotinoid insecticides exhibit high selective toxicity to insects over vertebrates. It has been shown that the selective toxicity of neonicotinoids is, at least in part, due to their selectivity to insect nicotinic acetylcholine receptors (nAChRs) (Shimomura 2005). Imidacloprid ( $C_9H_{10}ClN_5O_2$ ), 1-(6-chloro-3-pyridylmethyl)-N-nitro-imidazolidin-2-ylideneamine, is a member of the class of neonicotinic insecticides. Produced commercially by Bayer CropScience, it is active both topically and by ingestion (Lagadic et al. 1993) and is currently registered for crop, ornamental, and turf plant protection as well as seed treatments, companion animal parasite control, and as a soil termiticide. The oral  $LD_{50}$  of imidacloprid is > 4000 mg/kg body weight in rats and 131 mg/kg in mice, the 24 h dermal  $LD_{50}$  in rats is greater than > 2000 mg/kg. It is not irritating to eyes or skin in rabbits and guinea pigs. Imidacloprid is rated as "moderately toxic" acutely by the WHO and the EPA (class II or III). Imidacloprid has low vapor pressure. The chemical breaks down to inorganic molecules by both photolysis and microbial action, in the air and with a half-life of 30 days in water and 27 days in soil anaerobically. Because of its activity against a wide range of insects and its relatively low mammalian toxicity, imidacloprid is an excellent candidate for use against cockroaches (Appel and Tanley 2000).

Toxic gel baits are used more and more frequently to control urban cockroach populations (Appel 1990, Koehler et al. 1995). Gel baits have been the main method for German cockroach control in the United States for at least 5-8 year (Harbison et al. 2003). Gel baits are proven to be convenient to use and highly effective (Appel 1992, Ross 1993, Appel and Benson 1995, Kaakeh et al. 1997, Appel and Tanley, 2000). They are also safer and more

environmentally friendly than insecticide sprays due to their targeted application. As a result, gel baits have become a very popular cockroach management tool for pest management professionals. When the active ingredient is incorporated into palatable bait, cockroaches readily consume a lethal dose from a single meal. Therefore, baits were considered less likely to select for high-level cockroach resistance than insecticide sprays and other formulations (Wang et al. 2004).

The current study was designed to investigate duration of fipronil and imidacloprid gel baits toxicity against German cockroach strains in Iran.

## Materials and Methods

### Cockroach Strains

Nine strains of *B. germanica* were evaluated for duration of fipronil and imidacloprid gel baits toxicity at the School of Public Health, Medical Sciences/University of Tehran, Iran during 2003 and 2004. A standard susceptible strain (SS) which has been maintained since 1975 without exposure to any insecticide in the insectary (Ladonni 2001), was used as reference strain.

Eight strains of German cockroach (D<sub>1</sub> to D<sub>7</sub> and M) were collected from different locations in Tehran (51° 22'-51° 24' E, 35° 42'-35° 44' N) including one infested habitable convened, and the seven different infested student dormitories of different Universities (Medical Sciences/University of Tehran, Tehran, Sanatee Sharif and Tarbiat Modares), after insecticide spraying control failure with pyrethroid insecticides (Nasirian 2004).

### Cockroach 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 was 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 (Nasirian 2004).

All cockroach collected strains 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 aforementioned address. 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 mouse pellet, water ad libitum and a cardboard as a shelter.

### Insecticides

Chemicals used were imidacloprid gel bait 2.15%, Bayer AG Leverkusen, Germany, fipronil gel bait 0.05%, commercialized as Goliath, Rhone-poulenc Rhodic, Lyon, France, and CO<sub>2</sub> as an anesthetizer.

### Toxic bait method

Only adult males (1-3 wk old) were used in bait ingested because their weight and physiology were more uniform than those of adult female cockroaches were (Appel et al. 1983). Moreover, female cockroaches were needed for further reproduction. Adult male German cockroaches starved for one scotophase (12 h) with 5-6 bait ingested, and each bait ingested was replicated 3-6 times (10 cockroaches for each replicate), and then ingested fipronil and imidacloprid gel baits (fipronil gel 0.05%, commercialized as Goliath, and imidacloprid gel bait 2.15%, for 2 h. After the given time was over, the gel baits were removed and replaced with mouse pellet. Control groups (10 cockroaches for each replicate) ingested mouse pellet alone. A 1-6 day monitored giving > 0% and < 100% mortality at 144 h after ingested fipronil and imidacloprid gel baits were used for cockroaches. Ingested fipronil gel bait males were placed in 150 by 25-mm plastic Petri dishes, provided with mouse pellet food and water, and monitored for mortality at 12 intervals (12 h) for 144 h under the same temperature and photoperiod as the colony. In addition, it should be mentioned that mortality after ingested fipronil and imidacloprid gel baits was recorded for 12 days but mortality became stable after 6 days. 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. Resistance ratios were calculated as the 50% response value ( $LT_{50}$ ) of RR strain divided by the 50% response value of the SS strain. Significant differences ( $\alpha=0.05$ ) between  $LT_{50}$  and  $LT_{90}$  of each strain to fipronil and imidacloprid gel baits were verified by One-Way ANOVA. Distribution of variables was analyzed using One-Sample Kolmogorov-Smirnov Test.

### Results

Both gel baits were consumed and toxic to adult male German cockroaches. There was negligible (< 1%) control groups (ingested mouse pellet alone) mortality. After probit analysis (Finney 1972) in the ingested bait method, the susceptible strain showed  $LT_{50}$  of 47.1 and 11.3 h for fipronil and imidacloprid gel baits (Fig. 1), respectively, and the average  $LT_{90}$  was 74.2 and 19.3 h, respectively (Table 1 and 2).  $LT_{50}$  of the feral

German cockroach strains varied 14.9 h from 30.5 to 45.4 h and 4.4 h from 12.4 to 16.8 h for fipronil and imidacloprid gel baits, respectively (Table 1 and 2). After calculation of the 50% response value ( $LT_{50}$ ) of RR strain divided by the 50% response value of the SS strain, all German cockroach strains showed a similar susceptibility to fipronil and imidacloprid gel baits compared with the susceptible laboratory strain and the steep slopes of ingested bait-mortality curves indicated that the feral German cockroach strains were homogenous to fipronil and imidacloprid ingested gel baits. Table 1 shows that the field collected strains are more susceptible to fipronil gel bait than the susceptible strain. It is probably due to the field collected strains which were more attractant to fipronil gel bait than the susceptible strain.

No significant differences ( $P < 0.05$ ) were observed between  $LT_{50}$  and  $LT_{90}$  of each strain to fipronil and imidacloprid gel baits using One-Way ANOVA. One-Sample Kolmogorov-Smirnov Test was showed that the distribution of variables were normal (Fig 1.).

**Table 1.** Toxicity of fipronil toxic gel bait to a susceptible and feral-reared German cockroach strains

Strains	n	Slope±SE	X <sup>2</sup>	LT <sub>50</sub> <sup>a</sup> (CI)	LT <sub>90</sub> <sup>a</sup> (CI)	RR <sup>b</sup>
S	160	0.05±0.01	2.13	47.1 (42.0-53.2)	74.2 (65.7-88.3)	-
D <sub>1</sub>	160	0.08±0.01	10.13	36.7 (27.2-48.1)	53.5 (43.8-66.3)	0.8
D <sub>2</sub>	196	0.07±0.01	8.9	32.9 (23.1-43.0)	51.8 (42.0-81.5)	0.7
D <sub>3</sub>	248	0.1±0.02	3.4	30.5 (27.5-33.6)	53.0 (39.1-69.3)	0.7
D <sub>4</sub>	160	0.05±0.01	6.1	30.5 (25.2-35.3)	56.5 (49.6-68.0)	0.6
D <sub>5</sub>	240	0.17±0.03	0.4	32.9 (29.2-35.1)	50.6 (37.9-65.6)	0.7
D <sub>6</sub>	284	0.04±0.01	3.1	45.4 (39.3-52.2)	76.2 (66.2-85.1)	1.0
D <sub>7</sub>	160	0.03±0.01	1.4	39.1 (25.3-47.4)	77.3 (65.2-88.5)	0.8
M	220	0.05±0.01	2.5	37.5 (25.9-43.7)	63.1 (55.6-81.2)	0.8

<sup>a</sup>  $LT_{50}$  and  $LT_{90}$  values in hours (95% CI)

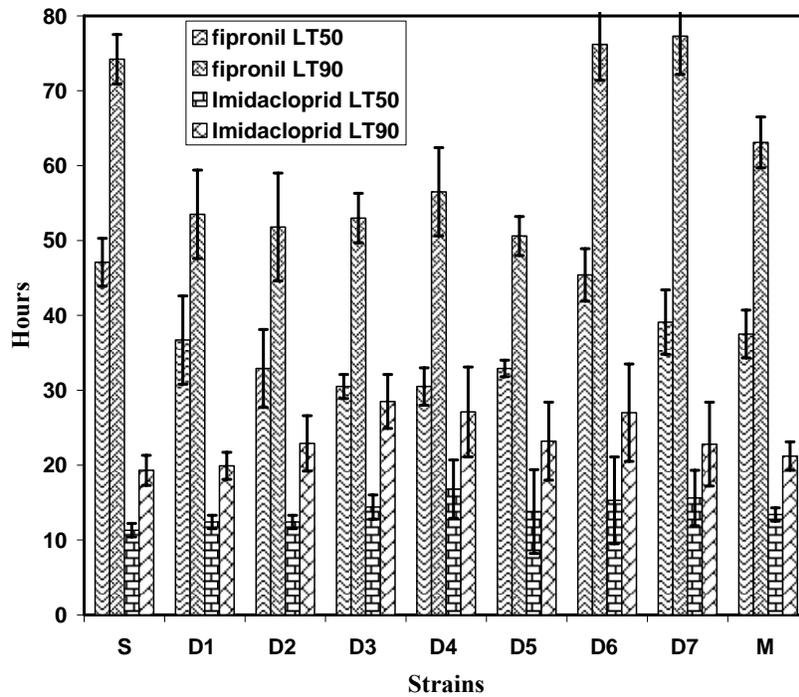
<sup>b</sup> Resistance ratio:  $LT_{50}$  of resistant strain/  $LT_{50}$  of susceptible strain

**Table 2.** Toxicity of imidacloprid toxic gel bait to a susceptible and feral-reared German cockroach strains

Strains	n	Slope±SE	X <sup>2</sup>	LT <sub>50</sub> <sup>a</sup> (CI)	LT <sub>90</sub> <sup>a</sup> (CI)	RR <sup>b</sup>
S	160	0.16±0.03	1.3	11.3 (9.29-13.05)	19.3 (17.07-23.19)	-
D <sub>1</sub>	160	0.17±0.03	1.4	12.4 (10.6-14.09)	19.9 (17.72-23.47)	1.1
D <sub>2</sub>	186	0.12±0.03	0.8	12.4 (7.6-14.09)	22.9 (20.12-30.0)	1.1
D <sub>3</sub>	228	0.09±0.02	4.8	14.4 (9.72-17.4)	28.5 (24.86-35.39)	1.3
D <sub>4</sub>	160	0.14±0.02	6.2	16.8 (8.52-24.33)	27.1 (21.73-38.75)	1.4
D <sub>5</sub>	240	0.02±0.01	0.3	13.8 (7.54-31.22)	23.2 (15.42-33.23)	1.2
D <sub>6</sub>	264	0.03±0.01	6.7	15.29 (6.0-26.52)	27.0 (14.12-39.61)	1.4
D <sub>7</sub>	160	0.18±0.02	8.8	15.6 (9.44-20.64)	22.8 (18.60-33.59)	1.4
M	180	0.16±0.02	3.2	13.4 (11.62-15.11)	21.2 (18.88-24.93)	1.2

<sup>a</sup>LT<sub>50</sub> and LT<sub>90</sub> values in hours (95% CI).

<sup>b</sup>Resistance ratio: LT<sub>50</sub> of resistant strain/ LT<sub>50</sub> of susceptible strain.



**Fig. 1.** Comparison of fipronil and imidacloprid gel baits toxicity to a susceptible and feral-reared German cockroach strains

**Discussion**

These results indicated that the fipronil and imidacloprid gel baits relatively killed the German cockroaches in ingested bait method, with time until 144 h and becoming stable thereafter.

Although both fipronil and imidacloprid gel baits tested had similar high efficacy levels after direct ingestion, important differences in death rates were observed under laboratory conditions. Cockroaches died more rapidly after ingestion imidacloprid gel bait than after ingestion

of fipronil gel bait. In comparison the fipronil gel bait  $LT_{50}$  (or  $LT_{90}$ ) of susceptible strain and the average  $LT_{50}$  (or  $LT_{90}$ ) of the feral German cockroach strains was more than to imidacloprid gel bait. The fipronil gel bait was more slowly affect the German cockroach than the imidacloprid gel bait. The observed differences between the two baits might be inherent to their different modes of actions.

Appel and Tanley (2000) reported that the imidacloprid gel bait was toxic to all stages of the German cockroach (Appel and Tanley, 2000). Baits affected cockroaches differently in relation to their developmental stage, small nymphs died more rapidly than large nymphs and adults. This may be related to differences in size, action on metabolism, or physiology (Cornwell 1976, Rust et al. 1995).

Fipronil is active at very low concentration so that cockroaches are killed after eating only a very small amount of bait (Kaakeh et al. 1997, Valles et al. 1997, Durier and Rivault 2000). Furthermore, the fact that the presence of fipronil in gel did not influence its level of attractiveness indicates that fipronil is not repellent to cockroaches (Durier and Rivault 2000).

Extensive use of insecticides has led to the development of resistance in German cockroach to a wide range of insecticides including organochlorines, organophosphates, carbamates and pyrethroids (Lee et al. 1996, Cochran 1997, Ladonni 2001, Nasirian et al. 2006) and consequent control failures in some field populations have been reported (Cochran 1989, Scott et al. 1990, Atkinson et al. 1991, Valles and Yu 1996, Dong et al. 1998, Valles 1999, Valles et al. 2000, Wei et al. 2001). Fipronil and imidacloprid are relatively new and acting at new target sites, which are currently not affected by resistance than other previous insecticides that will be used for pest control especially against German cockroach in Iran. However, Nasirian et al. (2006) investigated fipronil toxicity against these strains of German cockroach. The RRs of these strains to fipronil ranged from 1.5- to 2.6-fold (Nasirian et al. 2006). Since fipronil has not

been used previously in the area, this relatively low resistance could be due to German cockroaches collected from the field exhibited higher susceptibility level to fipronil than the susceptible strain (Kaakeh et al. 1997). Therefore, based on the data from Nasirian et al. (2006), fipronil could be recommended as a valuable tool in resistance management of German cockroach in this study area.

It is concluded that fipronil and imidacloprid toxic gel baits appear to have considerable potential as bait insecticide for insecticide resistant German cockroach strains in Iran.

### Acknowledgments

The author would like to thank Dean of School of Public Health, and Head of Medical Entomology and Vector Control Department of Medical Sciences/University of Tehran for their support to carry out this research. This investigation received financial support from the School of Public Health (Medical Sciences/University of Tehran), via MSPH thesis.

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