# ASSESSMENT OF TOXICITY OF FIPRONIL AND ITS RESIDUES TO HONEY BEES

## FİPRONİL VE REZİDÜLERİNİN BAL ARILARI ÜZERİNDEKİ TOKSİSİTESİNİN BELİRLENMESİ

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**Summary:** *Laboratory bioassays were conducted to assess the toxicity of fipronil to seven-day-old worker honey bees, using topical and oral applications duplicating likely field exposure. In addition, residual effects of fipronil were assessed after potted cotton plants were sprayed with full and half recommended field rates, exposed to field conditions, then bees were exposed to different age residues. The acute dermal $LD_{50}$ was 1.9 ng / bee, and acute oral $LC_{50}$ was 0.4 ng / bee. The residual toxicity of fipronil on cotton leaves remained high for an extended period of 25 d and 20 d for full and half recommended rates of fipronil, respectively. These studies show that fipronil is highly toxic to honey bees via direct spray contact, ingestion, and contact with residues. The application of fipronil in flowering cotton is, therefore, unlikely to be compatible with use of managed honey bees.*

**Keywords:** *Apis mellifera, fipronil, toxicity, residual activity, cotton*

**Özet:** *Arazi ortamında iki tekrarlı olarak topikal ve oral uygulamalar ile fipronilin 7-günlük bal arıları üzerindeki toksik etkileri saptanmıştır. Buna ek olarak, fipronilin rezidüel etkileri; arazi ortamında uygulanan dozun tamamı ve yarısının pamuk bitkileri üzerinde uygulamak üzere, farklı yaş aralıklarındaki arılara rezidüler uygulanmıştır. Akut dermal LD50 değeri 1.9 ng/arı ve akut oral LC50 değeri ise 0.4 ng/arı olarak tespit edilmiştir. Fipronilin pamuk bitkisi üzerindeki toksisitesi 25 günlük bir sürede, dozun tamamı ve yarısının uygulanması ise 20 günlük bir sürede yüksek seviyelerde devam etmiştir. Bu çalışmalar, fipronilin direkt spray ile sıkılması, yenmesi ve rezidüler ile temas edilmesi suretiyle bal arıları üzerinde yüksek derecede toksik etkileri saptanmıştır. Çiçek veren pamuk üzerinde fipronil uygulamasının ise bu nedenle üretimi yapılan bal arıları ile uyumlu olması beklenmemelidir.*
**Anahtar kelimeler:** *Apis mellifera, fipronil, toksisite, reziduel aktivite, pamuk*

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## Introduction

Cotton, *Gossypium hirsutum* L., a major agricultural crop grown world-wide, including in Australia, historically received multiple treatments of pesticides during the growing season to manage a range of pests attacking the cotton buds and bolls. Although cotton is primarily self-pollinated, cotton flowers visited by the European honey bee, *Apis mellifera* L., have been reported to produce heavier bolls (Moffett *et al*., 1980; Vaissiere, 1991; Free, 1993; Keshlaf, 2009) with improved lint quality (Rhodes, 2002) and seed-oil content (El-Sarrag *et al*., 1993). Unfortunately, insecticide applications during the flowering period often kill honey bees, *Apis mellifera* L., and other pollinators, which contact the insecticide-treated flowers or foliage causing high bee mortality (Waller 1982; Estesen et al. 1992; Robertson and Rhodes 1992). Therefore, many beekeepers avoid providing bees for the pollination of cotton crops (Bennett 1993; Stace 1994). With the introduction of transgenic cotton expressing Cry genes for *Bacillus thuringiensis* (Bt) toxins, it was anticipated the level of pesticide use would be significantly reduced (Perlak et al. 2001; Qaim 2003; Fitt 2004), possibly to the extent that it might be safe to honey bees. However, infestations of some non-lepidopterous pests unaffected by the toxin in the plants have increased, probably due to reduced use of broad spectrum pesticides to control *Helicoverpa* spp. (Fitt 2000). In particular, sucking insects have become an increasing problem in the USA (Greenplate et al. 2001) and Australia (Ward 2005). In Australia the green mirid, *Creontiades dilutus*, feeds on small squares, flowers and young bolls, causing bolls to drop (Pyke and Brown 1996; Ward 2002; Whitehouse et al. 2007).

The most commonly used insecticide against green mirid is fipronil, 5-amino-1-[2,6 dichloro-4-(trifluoromethyl) phenyl]-4-[(1-R,S) (trifluoromethyl) sulfinyl]-1H-pyrazole-3-carbonitrile, a member of the phenylpyrazole insecticide class (Cox 2005), with broad-spectrum activity and both contact and stomach action. Since 2003, fipronil has been used more than twice as frequently in Australian transgenic cotton than in conventional cotton (Doyle et al. 2005).

Fipronil disrupts the nerves in the brain and central nerve cord of insects by interfering with the ability of these nerve cells to transmit nerve impulses, resulting in uncontrolled activity, leading to death (Tingle et al. 2003; Kadar and Faucon 2006; Gunasekara et al. 2007). When fipronil is exposed to light it degrades to produce a number of metabolites, one of which, fipronil-desulfinyl, is extremely stable and more toxic than fipronil itself (Tingle et al. 2003; Gunasekara et al. 2007).

The purpose of this study was to examine in the laboratory and in potted plant trials, the toxic effects of fipronil (viz. topical, oral, and contact with fresh and aged fipronil residues on cotton foliage) to honey bees.

## Materials and methods

### *Honey bee samples*

Honey bees, *A. mellifera ligustica*, were obtained from the University of Western Sydney (UWS) apiary. In this study all bees used in the bioassays were the same age. A frame of sealed worker brood from the bee hive was placed into an incubator (33 ± 0.25 ˚C, 40 ± 10 % RH). The following day all emerged bees (viz. 1-day-old) were marked with non-toxic pilot-paint on the dorsal side of their prothoraces. For topical application bioassays, where the insecticide was applied to the thorax, bees were marked on their abdomen. After marking, bees (400 - 600) were collected singly by forceps and transferred into small (10 × 5 × 3 cm) wire-mesh cages, which had an exit hole (1 cm diam.) sealed with bee candy. The cages were set over the top of brood nest frames of their original hive. The bee candy provided sufficient time to enable the marked bees to be accepted into the hive when they left the cage visa the exit hole. After 6 d, all marked bees were re-collected from the hive using forceps, placed into the same wire-mesh cages and transferred to the laboratory for the bioassay investigations.

### *Bioassay techniques*

#### *Topical acute toxicity of fipronil to honey bees*

Fipronil (Regent®200SC, Nufarm Australia Limited, Laverton North, Victoria 3026; containing 200 g/L active ingredient [a.i]) was dissolved in absolute ethanol. Preliminary range-finding tests were conducted to determine the approximate $LC_{50}$ for honey bees. The doses tested were 3.2, 2.6, 2.4, 2.0, 1.6 and 1.0 ppm a.i. This procedure was repeated three times in order

to obtain three true treatment replicates. A wire-mesh cage containing seven-day-old worker bees was placed into a plastic bag then anaesthetized using medical grade carbon dioxide for 1 min, to facilitate handling and treatment. Seventeen bees were treated with each insecticide solution.

For each bee, 1.0 µL of solution was drawn into the tip and gently dispensed on the middle of the dorsal side of its pronotum with a precision hand micro-applicator PAX 100-3 (Burkard Scientific, PO BOX 55 Uxbridge Middx,UB8 2RT, UK). The control group was treated with 1.0 µL of absolute alcohol only. After treatment, the sub-batches of (n=17) bees were kept separately in 50 mL Schott glass vials and placed on the laboratory bench. Vials were covered with muslin netting supported with a rubber band. Food was provided as a bee-candy (a small block of hardened sugar fondant), placed outside the vial on the muslin. All treated vials were retained under laboratory conditions (25 ± 2 °C, 50 - 70 % RH). Observations of bee mortality were recorded 24 h after treatment. Obviously healthy, active bees were counted as alive; all others were deemed dead.

***Oral acute toxicity of fipronil to honey bees***

To evaluate the oral toxicity, fipronil was suspended in 50 % honey syrup. This stock solution was used to prepare further serial dilutions 1.0, 0.8, 0.6, 0.4, 0.1 a.i. using the same solution. This procedure was repeated four times in order to obtain four true treatment replicates. Preliminary range-finding tests were conducted to determine the approximate $LC_{50}$ for honey bees, and also to ensure bees fed freely on fipronil-treated honey syrup. In addition, several preliminary experiments were conducted to develop the most suitable method for feeding bees with this syrup, using honey and honey solution in small plastic Petri dishes (30 mm diam), and honey solution in a Parafilm™ (Pechiney Plastic Packaging Chicago II) cell. The most successful feeding station was the Parafilm cell, which was subsequently used in all oral toxicity bioassays. The cell was set up as follows: 1 mL of the appropriate concentration syrup was poured onto a 3.5 × 3.5 cm sheet of Parafilm; which was then used as the lid of a small plastic 50 mm diameter Petri dish, with the syrup located on the inside of the Petri dish. The remaining edges of the Parafilm were stretched to produce a seal on the base of the dish, thereby forming a single transportable unit. The Parafilm cell was perforated with an entomological pin to produce five holes around the location of the honey solution, to enable the bees to reach the honey solution with their mouth parts without contaminating their bodies. The cell was then gently placed, with forceps, on the base of a 200 mL Pyrex beaker.

Bees were starved for 4 h prior to commencement of the bioassay. The control group was provided with honey syrup only. A group of 15 bees was used as a replicate. These were randomly selected and anaesthetized as described previously, then transferred to each concentration treatment Pyrex beaker. The beaker was then covered with muslin netting, supported with a rubber band. Observations on bee mortality were recorded 24 h after release, as previously described.

***Toxicity to honey bees of fipronil residues on cotton foliage exposed to normal weather conditions***

At the farm of university of western Sydney, fifty transgenic Bt (Bollgard II® Sicot 71BR) cotton plants were sown and grown on 5 November 2007in 8-L plastic pots containing composted sawdust-based general open potting mix (Debeco Pty Ltd, Vineyard 2765, NSW). Plants were maintained in a pesticide-free area in a greenhouse. After two months, plants were approximately 1 m high with their first floral buds (squares). Eighteen plants of uniform size and development were selected, and randomly allocated to the insecticide treatments. After labelling, they were transferred outside the greenhouse for application of the insecticide treatments. Plants were thoroughly and uniformly sprayed with a 450 mL pressurized Plaspak sprayer (Plaspak Co., Jannali 2226, NSW) at the rate 4.2 mL/plant. Six replicate plants were used for each of the following treatments; Full recommended rate, Half of the recommended rate, and Control (water only)

Calculations of rates were based on the recommended field full rate (125 mL/ha) (Farrell 2007) and normal field plant density (10 plants/m); thus at this rate, each cotton plant would received $125\times10^{-5}$ g of Regent®200SC. Accordingly, a stock solution of 0.03% was prepared by dissolving 0.3 g of Regent®200SC in 1.0 L of distilled water. Each plant was sprayed with 4.2 mL of the insecticide solution. Half rate solutions

were similarly prepared and applied. After treatment, the cotton plants were left outside in separate treatment batches for 2 h to ensure foliage dryness, before being transferred to a mesh house (2mm).

Samples of cotton foliage with exposed fipronil residues were collected from each treated plant 1, 2, 6, 12 and 24 d after initial application. Two mature leaves from each plant, approximately 200 $cm^2$, were randomly selected, cut and placed singly into plastic bags and taken for laboratory bioassay. Plastic Petri dishes (90 mm diam.) were modified to provide ventilation and to facilitate bee feeding for the bioassays, by cutting two circular holes (30 mm diam.) in the middle of the lid and covering them with nylon mesh which was glued to the lid. Each excised cotton leaf was directly placed onto the base of a Petri dish, to avoid hand contamination which may have reduced the deposited fipronil, then cut exactly to fit in the dish on top of a filter paper that was located on the base of the dish.

One of the pair of discs from each plant was allocated to each of two bee exposure times. The leaf discs were placed so their upper surface was exposed to the bees. Batches of ten anaesthetized bees were randomly allocated to each of the treatment concentrations, and were placed into their respective Petri dish. They were exposed continuously to the treated leaves for two different time periods, 3 h or 24 h. All treatments were replicated six times. Thus, for each bioassay 12 Petri dishes were used. Bees in the 3 h exposure treatment were immediately transferred after this time had elapsed to similarly modified, but clean, Petri dishes with a filter paper on their base. They were then provided with 1 g bee candy which was placed on the mesh. At the same time, bees in the 24 h exposure treatment were also similarly fed. Food was not provided to bees earlier, in order to maximize their movement over the treated leaf surface rather than remaining static and eating candy. Mortality was assessed 24 h after initial exposure.

***Statistical Analysis***

Probit analysis was carried out for dose-mortality (topical and oral), and heterogeneity of regressions was determined by the Pearson $\chi^2$-test statistics (Busvine 1971). Abbott's (1925) formula was used to correct for the natural mortality prior to Probit analysis.

Data on toxicity of aged residues were analysed using mixed general linear model of analysis of variance (ANOVA) SPSS® for Windows™ Version 14 (SPSS Inc. 2007). The age of residue, rate of fipronil and time of bee exposure to the residues were fixed factors and replicate was a random factor. The assumption of normal distribution was checked using P-P plot and homogeneity of variance using Levene's test of equality of error variances. When the assumption of homogeneity of variance was met Ryan's Q test was used to separate treatment means and when the assumption was not met Dunnett's T3 test was used. Data for all analyses were arcsin (sqrt(X)) transformed. The value X was the proportion of dead bees.

## Results

***Topical acute toxicity of fipronil to honey bees***

The results from the analysis of the acute topical toxicity investigation are presented in Table 1. The calculated $LD_{50}$ and $LD_{90}$ values were 1.9 ng/bee (15.1 ng/g bee weight) and 2.8 ng/bee (22.2 ng/g), respectively. The estimated regression line has $r^2 = 0.876$.

***Oral acute toxicity of fipronil to honey bees***

The results from the analysis of the acute oral toxicity investigation are presented in Table 2. The estimated $LC_{50}$ and $LC_{90}$ values were 0.4 ng/bee and 1.3 ng/bee, respectively. The estimated regression line has $r^2 = 0.710$.

***Bioassay of fipronil residues on cotton foliage collected from the field***

Bee mortality was consistently higher in the full rate fipronil treatments, and also for bees exposed to similar age residues for 24 h compared to 3 h. For the full rate treatment, mortality < 100% occurred with 1-day-old residues when bee exposure was 3 h, but did not reach this point for 24 h exposure until residues were 12 days old. With 24-day-old residues, mortality in all treatments was negligible.

Statistical analysis showed significant rate × age of residue interaction ($F_{8,155} = 11.837$, $P < 0.001$), and age of residue × time of exposure interaction ($F_{4,155} = 2.385$, $P = 0.05$); hence, analyses were performed for each exposure time of each rate separately. There were significant differences between effects of age of

***Table 1.*** *Statistical summary of results for acute toxicity of topically applied fipronil (1 µL) to seven-day old A. mellifera workers, after 24 h.*

| Treatment | n[a] | Slope ± s.e. | $LD_{50}$ [b] (ng/bee) (95% CL[e]) | $LD_{90}$ [b] (ng/bee) (95% CL[e]) | $\chi^2$ | df | *P* |
|---|---|---|---|---|---|---|---|
| Topical | 270 | 7.61 ± 1.34 | 1.9 (1.6 - 2.2) | 2.8 (2.5 - 7.0) [c] | 20.97 | 16 | 0.180 |
| Oral | 360 | 3.63 ± 0.44 | 0.4 (0.4 - 0.8) | 1.3 (0.9 - 2.0) [d] | 24.24 | 15 | 0.061 |

***[a] number of insects tested.***
***[b] $LD_{50}$ and $LD_{90}$ data were determined by probit analysis (SPSS Version 15, 2008); concentration (ppm) in alcohol[c] and honey solution [d]***
***[e] CL confidence limits.***

***Table 2.*** *Statistical summary of results for orally toxicity of orally applied fipronil to seven-day old A. mellifera workers, after 24 h.*

| n[a] | Slope ± s.e. | $LC_{50}$ [b] (ng/bee) (95% CL[c]) | $LC_{90}$ [b] (ng/bee) (95% CL) | $\chi^2$ | df | *P* |
|---|---|---|---|---|---|---|
| 360 | 3.63 ± 0.44 | 0.4 (0.4 - 0.8) | 1.3 (0.9 - 2.0) | 24.24 | 15 | 0.061 |

***[a] number of insects tested.***
***[b] $LD_{50}$ and $LD_{90}$ data were determined by probit analysis (SPSS Version 15, 2008); concentration (ppm) in honey solution.***
***[c] CL confidence limits.***

fipronil residues in the half rate treatment, when bees were exposed to treated leaves for 3 h ($F_{4,20} = 9.779$, $P < 0.001$) and 24 h ($F_{4,20} = 4.052$, $P = 0.014$). When bees were exposed to the residues for 3 h, mortality was significantly higher in 1- and 2-day-old residues compared to 12- and 24-day-old ones. There were no significant differences between other aged residues. When bees were exposed for 24 h to treated leaves of the same treatment rate, 2-day-old residues were significantly different to 24-day-old residues. There were no significant differences between other differently aged residues.

***Table 3.*** *The percentage of bee mortality ± SD after 24 h of confinement in a Petri dish with treated cotton leave, at field rates of fipronil, for periodically (3 h) or continuously (24 h).*

| Age of residues (day) | Mean Percentage Mortality (n = 6) ± s.e. | | | |
|---|---|---|---|---|
| | Half rate fipronil | | Full rate fipronil | |
| | 3 of exposure (h) | 24 of exposure (h) | 3 of exposure (h) | 24 of exposure (h)[c] |
| 1 | 75[b] ± 13.3 a[a] | 60.0 ± 20.0 ab | 100.0 ± 0.0 a | 100.0 ± 0.0 a |
| 2 | 60 ± 13.1 a | 86.7 ± 4.9 b | 70.0 ± 17.5 a | 100.0 ± 0.0 a |
| 6 | 40 ± 17.8 ab | 61.7 ± 19.5 ab | 66.7 ± 16.8 ab | 100.0 ± 0.0 a |
| 12 | 8.3 ± 4.7 b | 35.0 ± 20.6 ab | 31.7 ± 14.4 b | 33.3 ± 13.8 b |
| 24 | 6.6 ± 4.9 b | 0.0 ± 0.0 c | 0.0 ± 0.0 c | 0.0 ± 0.0 c |

***[a] Values in a column followed by the same letter are not significantly different ($P < 0.05$).***
***[b] Mean percentage mortality (n=6) ± SE***
***[c] Data could not meet assumption of normal distribution even after transformation so data analysis was not performed***

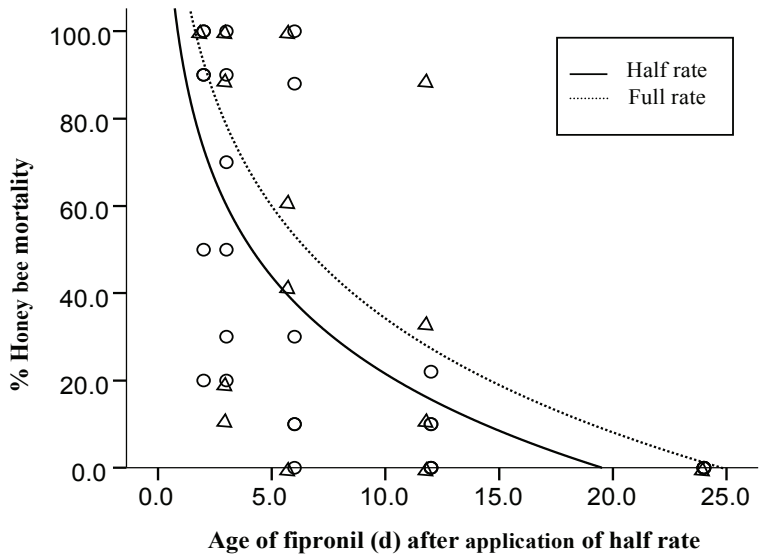


**Figure 1** ***Mortality of honey bees, after 3 h exposure to aged residues of half field rate (  ) and full field rate (  ) of fipronil, on cotton leaves (mortality recorded 24 h after commencement of exposure).***

$r^2 = 0.527$, $Y = 95.615 - 32.176 \times \log_e$
$r^2 = 0.591$ , $Y = 123.196 - 38.300 \times \log_e$

There were significant differences between the different age residues with full rate fipronil, when bees were exposed to treated leaves for 3 h ($F_{4,20} = 10.073$, $P < 0.001$). Mortality of bees exposed for 3 h to 1-day-old residues was significantly higher than for 12- and 24-day-old residues. Two-day-old residues were significantly different from 24-day-old residues. There were no significant differences between other differently aged residues. When bees were exposed to 1-day-old residues, data analysis could not be performed because there were no variations between replicates in four out of five tested age residues and so the assumptions of analysis of variance could not be met.

## Discussion

The combination of residual and oral toxicity studies provided valuable information on the highly hazardous nature of fipronil to honey bees. The data show high mortality, via acute toxicity, of worker bees when they were exposed to direct contact (e.g. via topical application; viz., equivalent to spray application in the field), oral (e.g., ingestion of nectar, contaminated pollen and honey), and to fresh and aged residues of fipronil.

In the current study, the $LD_{50}$ of fipronil, when 1 µL was applied topically to the dorsal prothorax of 7-day-old *A. mellifera* workers, was 0.002 µg / bee. This figure is similar to, but slightly lower than, that reported by Anon (2000, cited in Cox 2005) of 0.004-0.005 µg/bee, 0.006 µg/bee (Decourtye 2002, cited in Hassan et al. 2005; Roper 2002, cited in Chauzat et al. 2006), 0.0037-0.006 µg/bee (Anon 2002, cited in Colin et al. 2004), 0.004 µg/bee (Anon 1995, cited in Tingle et al.) and < 0.005 µg/bee (Hassan et al. 2005). However, it differs greatly from the first reported figure of 0.013 µg/bee by Mayer and Lunden (1999), which is much higher than all subsequent reports.

Differences between our results and those of others may be a result of the amount applied (for instance, Mayer and Lunden [1999] applied 2 µL), the carrier

(Mayer and Lunden [1999] used acetone, and we used ethanol), or the age of bees tested (Chauzat et al. 2006). Both life stage and age impact on insect response to intoxicants (Busvine 1971). We used verified 7-day-old workers which were maintained in hives, so that we could use uniform organisms for the bioassays. Mayer and Lunden (1999) used presumably 4-5-week-old bees, and Hassan et al. (2005) used bees of unknown age because they "caught worker bees through a hole in the top of the hive". Decourtye et al. (2005), on the other hand, used 14-day-old bees which were reared under artificial conditions in an incubator. The youngest stages of larvae are generally the most susceptible, and newly emerged adult bees are more susceptible to DDT and carbaryl (Davis 1989), and aldicarb, sulfone and methomyl (Atkins and Kellum 1986). In contrast, older bees are more susceptible to endosulfan and malathion (Atkins and Kellum 1986). With foraging adult bees, it is also difficult to distinguish between mortality due to natural ageing or exhaustion due to intense foraging activity and mild poisoning. While older foraging workers would be more likely to be directly exposed to fipronil, contaminated bees, nectar and/or pollen would potentially expose younger bees and larvae in the hive to the pesticide. Acute toxicity of fipronil for honey bees was < 2.0 ppm and, according to the basis of pesticide classification (Johansen 1977; Anon 2005), it is highly toxic product.

Fipronil was also highly toxic to honey bees when ingested. In the current study, the $LC_{50}$ of fipronil, when provided in contaminated honey syrup to 7-day-old *A. mellifera* workers after 24 h, was 0.0004 % a.i. It was not possible to assess the $LD_{50}$, as we did not measure the intake of individual bees. However, the $LD_{50}$ has been reported to be 0.006 µg/bee after 48 h (Decourtye 2002), and 0.004 µg/bee after 24 h (Roper 2002). Fipronil has been detected in some pollen samples (Chauzat et al. 2006; Jimenez et al. 2007), and can thus pose a risk to larvae and young, non-foraging bees.

The above figures relate to direct mortality of honey bees. The effects on bee foraging have occurred at even lower exposures (Colin et al. 2004). Decourtye et al. (2005) compared the sub-lethal effects of nine pesticides on olfactory learning performance (proboscis extension response) in *A. mellifera*, and concluded that even doses lower than 5% of the $LD_{50}$ could impact negatively. Hassan et al. (2005), however, reported that concentrations of fipronil approximately an order of magnitude below the $LD_{50}$ did not interfere with the locomotive activity of honey bees regardless of the route of administration (topical at 0.1, 0.5 ng/bee, and oral at 1 ng/bee). They also recorded that while these concentrations impaired olfactory learning they did not impair learning or memory retention (Hassan et al. 2005).

The potted plant bioassays to assess activity of fipronil residues, in the current study, indicate that fipronil is highly toxic to honey bees for an extended period after its initial application. Fipronil applied at the full recommended rate (0.025 kg $a.i.ha^{-1}$) was still toxic to bees between 12 and 25 d after application, and at half recommended rate for more than 12 d. This residual activity could be shorter under full field conditions, where treated plants would be exposed to rain, irrigation and other agricultural practices. Considering normal honey bee foraging behaviour, 3 h exposure to fipronil residues is a realistic timeframe to assess likely toxicity in the field.

There are very limited published data with which to compare our results. Mayer and Lunden (1999) reported that mortality of honey bees, when exposed for 24 h to 2-h- and 8-h-old fipronil field residues applied at a rate of 0.22 $kg/ha^{-1}$ to canola leaves, was 76% and 46%, respectively. They concluded that fipronil at rates of 0.11 $kg/ha^{-1}$could be considered as non-hazardous to honey bees, and could be applied only in the evening to flowering crops where bees are foraging. Based on our results, this conclusion is incorrect. Our data are supported by Mulrooney (1999) who recorded 100 % mortality in cotton weevil after exposure to 3-day-old residues of fipronil on field cotton, initially applied at 0.056 kg $a.i.\ ha^{-1}$. It is also consistent with the current label recommendations for a 28 d period between use of fipronil and likely managed honey bee foraging (Nufarm Australia Limited 2006). While it was not obvious in our investigations, it is reported that fipronil residues increase their toxicity several days after their initial application (Cox 2005; Anon 2006).

A number of insecticides can be repellent over a range of concentrations (Mamood and Waller 1990), reducing likely field toxicity, especially to non-target species. This is not the case with fipronil in canola (Mayer and Lunden 1999), which suggests that its residues in cotton are unlikely to be detectable by honey bees. These studies show that fipronil is highly toxic to honey bees via direct spray contact, ingestion, and contact with residues, and toxic also to young adult bees and maybe larvae. The use of fipronil in flowering cotton is, therefore, unlikely to be compatible with use of managed honey bees.

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