



Behavioural Effects of Pesticides in Bees—Their Potential for Use in Risk Assessment

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Abstract. This paper reviews a wide variety of behavioural effects that have been reported in bees following exposure to pesticides, primarily insecticides. These range from effects on odour discrimination in the individual to the loss of foraging bees due to disruption of their homing behaviour. Some of these effects have the potential to have a significant impact on the development and survival of colonies. However, there is currently little guidance available on the types of behavioural data which should be collected during laboratory, semi-field or field regulatory studies or how they should be included and interpreted in risk assessment. Further work is required to allow risk assessment to include significant behavioural effects and their longer term consequences on colony survival and development. Such an approach will require a larger base set of data to predict the longer-term consequences on colonies of short-term effects on individuals, e.g. through population modelling.

Keywords: pesticides; sublethal; honey bees; risk assessment

Introduction

Kevan (1999) considered pollinators, such as honeybees and bumble bees as crucial to the functioning of almost all terrestrial ecosystems including those dominated by agriculture. Honeybees are excellent samplers of environmental pollution (Bromenshenk et al., 1991, 1996; Kevan, 1999). Haynes (1988), in considering the sublethal effects of neurotoxic insecticides, concluded that the rationale for studying such effects in pest insects, e.g. to increase the efficacy of insecticidal materials, is fundamentally different from that in beneficial insects such as the honeybee which is a more immediate problem. “The assumption that a colony of honeybees is healthy simply because no increase in mortality is noted

immediately after exposure to an insecticide may not be valid”.

There is increasing concern amongst beekeepers that sub-lethal behavioural effects may have significant impacts on honeybee colonies (Pajot, 2001). Both Kevan (1999) and a Canadian government publication on Pesticide Pollinator Interactions (NRCC, 1981) considered that sublethal effects, particularly from long term, low level exposure to pesticides, are less understood and probably overlooked. However, these data have not been summarised and the potential for use of sublethal effects in risk assessment addressed. From the European perspective Annex VI to EU Directive 91/414 section 2.5.3 requires that “Where there is a possibility of honeybees being exposed no authorisation will be granted if the hazard quotients for oral or contact exposure of honeybees are greater than 50, unless it is clearly established through appropriate risk assessment that under field

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conditions there are no unacceptable effects on honeybee larvae, honeybee behaviour, or colony survival and development after use of the plant protection product according to the proposed conditions of use". The risk assessment is undertaken using data generated from laboratory data (LD_{50}) and semi-field and field data for chemicals with a hazard quotient (application rate (g as/ha): LD_{50} ($\mu\text{g as/bee}$)) over 50, with a specific mode of action (e.g. IGRs) or where there are indications of indirect effects such as delayed action or modification of bee behaviour (Annex II 91/414/EC, EPPO, 1993). This approach requires that laboratory studies are capable of highlighting concerns that need to be addressed in semi-field and field tests for pesticides which have a hazard quotient less than 50. It also requires that higher tier tests are capable of detecting and interpreting the consequences of behavioural effects caused by pesticides. The design of these higher tier studies, semi-field or field tests, is the subject of EPPO guidelines (EPPO, 1992) but the emphasis is on data collection for assessment of mortality of adult bees with confirmatory data that bees are foraging on the crop. With the introduction of a wider range of pesticides which, although applied at low rates, may be extremely toxic to honeybees and therefore result in behavioural effects at very low concentrations, it is important to provide a framework within which this data can be collected and interpreted. This paper represents the first stage of this process by summarising the effects reported to date in published literature to identify the types of behaviour which could be assessed in laboratory and higher tier studies.

There have been a small number of reviews of the sublethal effects of pesticides on insects but these have been limited in the classes of chemicals included to neurotoxic insecticides (Moriarty, 1969; Haynes, 1988). The reported effects on insect behaviour included impacts on reproduction, host-finding and feeding, dispersal and general locomotion and perception of insecticides. Only one review deals specifically with the sublethal effects of pesticides on bee species (NRCC, 1981) but is limited in its consideration of these effects within a wider discussion of the impact of pesticides on bees, although it does highlight their potential importance. The review presented here is restricted to sublethal behavioural effects in bee species, primarily honeybees (*Apis mellifera* L.) and bumble bees (*Bombus* sp.). The differences in sensitivity between species is not

normally assessed in studies but there are few data, e.g. for cyhalothrin and fipronil the toxicity is greatest to the alfalfa leafcutter bee (*Megachile rotundata*) less to the honeybee and least to the alkali bee (*Nomia melanderi*) (Mayer et al., 1998; Mayer and Lunden, 1999). Only honeybees are routinely included in the risk assessment process in Europe under Directive 91/414/EC. Although, because of the amount of data available, this review is biased towards insecticides it is not limited to their effects.

Table 1 gives a summary of the reported effects and also includes a summary of the dose levels at which effects were observed. Risk assessment requires both toxicity and exposure to the pesticide to be taken into account. For honeybees the application rate is used as the indirect measure of exposure. However, many of the studies summarised in Table 1 did not involve field applications and doses were applied as a direct contact dose per bee or by feeding. There are very few data available on the exposure levels of bees to pesticides to allow comparison of laboratory and field dose levels. Koch and Weiber (1997) reported the results of a field study using a fluorescent tracer at 20 g/ha to assess the exposure of honey bees to pesticides applied to crops. The only route which could be assessed in this manner is contact exposure which may be an underestimate of total exposure but this can be counteracted by using a maximum rather than mean exposure level. The mean deposit per bee over 20–30 min post-application at 20 g/ha to *Phacelia* was 6.34–35.77 ng (maximum 48 ng). These data allowed calculation that 1 kg/ha would give a maximum residue of 2.4 $\mu\text{g/bee}$. To allow comparison of the tested doses with the likely exposure after field applications, and therefore likelihood of effects, Table 1 includes an estimate of the exposure level reported as a percentage of the likely maximum exposure after application to *Phacelia* (based on maximum application rates for the pesticides in the UK (or nearest equivalent approved pesticide)). In the following discussion greater emphasis is placed on the effects which were detected at doses at or below those likely to be observed in the field.

Division of labour

Honeybees workers perform an ontogenic sequence of tasks in the nest during their first 3 weeks of adulthood and then shift to foraging in the final 2–3 weeks of

Table 1. Summary of reported sublethal behavioural effects of pesticides

Species	Chemical	Dose	Estimated dose ($\mu\text{g}/\text{bee}$) ²	Approx. percentage maximum exposure ¹	Stage	Effect	Reference
<i>Division of labour</i>							
<i>Apis mellifera</i>	Diazinon	25% LD ₅₀	0.18	19	Adult	Precocious foraging	Mackenzie and Winston, 1989
<i>Apis mellifera</i>	Juvenile hormone mimic (Law-Williams mixture)	50–200 $\mu\text{g}/\text{bee}$	50–200	140–550	Adult	No hypopharangeal gland development	Jaycox et al., 1974
<i>Apis mellifera</i>	Range of OPs, carbamates, OCs	NK			Adult	Decreased house-cleaning	Nation et al., 1986
<i>Apis mellifera</i>	Kinoprene	NK			Adult	Inhibited hypopharangeal gland development	Gerig cited by Tasei (2001)
<i>Apis mellifera</i>	Juvenile hormone	1 $\mu\text{g}/\text{bee}$	1	300	Adult	Regression hypopharangeal glands	Rutz (1974) cited by Tasei (2001)
<i>Apis cerana indica</i> , <i>Apis mellifera</i>	Diflubenzuron, penfluron	50 $\mu\text{g}/\text{bee}$	50	140	Adult	Significantly suppressed hypopharangeal gland development	Gupta and Chandel, 1995
<i>Apis mellifera</i>	Methoprene	250 $\mu\text{g}/\text{bee}$	250	700	Adult	Early degeneration of hypopharangeal gland, precocious foraging	Robinson, 1985
<i>Conditioned responses and learning</i>							
<i>Apis mellifera</i>	Dicofof	NK		NK	Adults	Decreased acquisition and persistence in conditioned response test	Stone et al., 1997
<i>Apis mellifera</i>	Permethrin	25% LC ₅₀	0.013	22	Adults	Decreased learning ability, abnormal behaviour (antennae cleaning, rubbing together of hind legs)	Mamood and Waller, 1990
<i>Apis mellifera</i>	Imidacloprid	50 ppb	0.01	3	Semi-field	Decreased flight activity and olfactory discrimination performance	Decourtye et al., 1999
<i>Apis mellifera</i>	Imidacloprid	4–40 ppb	0.008–0.08	3–30	Adults	Decreased olfactory learning performance	Decourtye et al., 1999
<i>Apis mellifera</i>	Imidacloprid	0.1–10 ng/bee	0.0001–0.01	0.03–3	7–8 day old adults	Increase in number of trials to abolish habituation at 7 days, increase in number of trials to achieve habituation at 8 days	Guez et al., 2001
<i>Apis mellifera</i>	Flucythrinate, cyfluthrin, permethrin, fenvalerate, cypermethrin, fluralinate	LC ₅₀	0.05	80	Adults	Slower learning of odour mediated response flucythrinate = cyfluthrin > permethrin = fenvalerate = cypermethrin > fluralinate	Taylor et al., 1987

Table 1. (Contd)

Species	Chemical	Dose	Estimated dose ($\mu\text{g}/\text{bee}$) ²	Approx. percentage maximum exposure ¹	Stage	Effect	Reference
<i>Apis mellifera</i>	Serine proteinase inhibitors	0.01–0.1 mg/ml		NK	Adults	Slower learning of odour mediated response	Pham-Delegue et al., 2000
<i>Foraging Apis mellifera</i>	Parathion	0.3 $\mu\text{g}/\text{bee}$	0.3	30	Adult	Incorrect direction (angle of dance) on vertical surface, incorrect distance on horizontal surface	Schricker and Stephen, 1970; Stephen and Schricker, 1970
<i>Apis mellifera</i>	Permethrin	6% LD ₅₀	0.017	30	Adults	Failure to return to colony, increased self-cleaning, trembling dance, abdomen tucking, rotating and cleaning of abdomen while rubbing hind legs together, decreased walking, body insertion in cells, food giving and foraging	Cox and Wilson, 1984
<i>Apis mellifera</i>	Deltamethrin	4% LD ₅₀	0.002	3	Adult	Failure to return to colony	Vandame et al., 1995
<i>Apis mellifera</i>	Methoxychlor	5 ppm		NK	Semi-field trial	Decreased brood, pollen patty consumption and foraging, decreased house-cleaning	Nation et al., 1986
<i>Apis mellifera</i>	Imidacloprid	20–100 ppb	0.004–0.02	1–7	Semi-field	Decreased foraging	Schmuck, 1999
<i>Apis mellifera</i>	Deltamethrin, cypermethrin, alpha-methrin, lambda-cyhalothrin	10 pmol/bee (=5 ng/bee deltamethrin)	0.005	8	Adults	Hypothermia	Belzunces et al., 2001
<i>Apis mellifera</i>	Cypermethrin	10–30 g ai/ha	0.02–0.07	30–120	Semi-field trial	Reduced foraging	Le Blanc, 1985
<i>Colony development Apis mellifera</i>	Imidacloprid	0.02 mg/kg	0.004	1	Chronic feeding of colonies	Slight differences in queen egg laying cycle, and numbers of larvae and pupae	Schmuck et al., 2001
<i>Bombus terrestris</i>	Imidacloprid	10–25 $\mu\text{g}/\text{kg}$	0.002–0.005	0.6–2	Chronic feeding small colonies	Lower numbers of larvae ejected and decreased brood production	Tasei et al., 2000
<i>Apis mellifera</i>	Acephate	1 ppm	0.2	20	Field colonies	Significant reduction in surviving brood (10 ppm toxic to queens)	Stoner et al., 1985
<i>Megachile rotundata</i>	Deltamethrin	20% LD ₅₀	0.01	17	Adult females	Less eggs laid	Tasei et al., 1988

<i>Apis mellifera</i>	Diflubenzuron	10 ppm	2	555	Field colonies	Eliminated brood production, dec pollen and water intake, dec production comb, eggs and workers	Barker and Taber, 1977
<i>Bombus terrestris</i>	Teflubenzuron	150 ppm	30	>1000	Small colonies	Decreased sucrose intake, arrested egg development, larval mortality	De Wael et al., 1995
<i>Apis mellifera</i>	Dimethoate	10 ppm	2	200	Field colonies	Queens ceased laying	Waller et al., 1979
<i>Apis mellifera</i>	Dimethoate	1 ppm	0.2	20	Field colonies	Decreased comb production, egg laying	Waller et al., 1979
<i>Apis mellifera</i>	Cypermethrin	12.5 ppb	0.0025	4	Field colonies	Significantly increased queen superscedure	Bendahou et al., 1999
<i>Larval behaviour</i>							
<i>Apis mellifera</i>	Dimethoate	0.313 µg/g royal jelly	NK		96h larvae	Stimulated of growth and maturation, failure to spin cocoon, hypersensitivity to stimulation	Davis et al., 1988
<i>Apis mellifera</i>	Dimethoate	LD ₁₀	0.04	4	1-2, 3-4, 5-6 day old larvae	Adults emerge with wing malformation, stunted bodies, crippled legs and wings	Atkins and Kellum, 1986
<i>Apis mellifera</i>	Malathion	LD ₁₀	0.029	3	1-2, 3-4, 5-6 day old larvae	Adults emerge with wingless, crumpled or shortened wings	Atkins and Kellum, 1986
<i>Apis mellifera</i>	Carbaryl	LD ₁₀	0.13	13	1-2, 3-4, 5-6 day old larvae	Emergence of very small adults and in some cases wingless	Atkins and Kellum, 1986
<i>Apis mellifera</i>	Captan	1-10 µg/larva	NK	7.2	1-2, 3-4, 5-6 day old larvae	Adults emerge with malformed wings, small size and light pigmentation	Atkins and Kellum, 1986
<i>Repellency</i>							
<i>Apis mellifera</i>	Aldicarb sulfoxide	3 ppm	0.6	63	Semi-field	Inhibited foraging	Nigg et al., 1991
<i>Apis mellifera</i>	Permethrin, cypermethrin	4% LD ₅₀	NK		Treated float	Transient inhibition of activity	Rieth and Levin, 1988
<i>Apis mellifera</i>	Fenvalerate, flucythrinate	1 mg/disc	NK		Treated float	Transient inhibition of activity	Rieth and Levin, 1988
<i>Apis mellifera</i>	Cyhalothrin	2 ppm	0.4	666	Syrup	Reduced honeybee visitation	Mayer et al., 1998
<i>Apis mellifera</i>	Cypermethrin	10-20 g ai/ha	0.02-0.05	33-83	Field application	Decreased foraging	Shires et al., 1984
<i>Apis mellifera</i>	Formulated cypermethrin (Cymbush)	1-160 µl/l	0.02-3.2	33->1000	Syrup	Reduced honeybee visitation	Delabie et al., 1985
<i>Apis mellifera</i>	Formulated cypermethrin (Cymbush)	50 g ai/ha	0.12	200	Glasshouse and field	Reduction in foraging	Delabie et al., 1985

Table 1. (Contd)

Species	Chemical	Dose	Estimated dose ($\mu\text{g}/\text{bee}$) ²	Approx. percentage maximum exposure ¹	Stage	Effect	Reference
<i>Nomia melanderi</i>	Cyhalothrin	0.028 kg ai/ha	0.07	117	Field	Decreased populations at nesting blocks	Mayer et al., 1998
<i>Apis mellifera</i>	Fipronil	100–500 ppm	20–100	> 1000	Syrup	Reduced honeybee visitation	Mayer and Lunden, 1999
<i>Apis mellifera</i>	Neem	0.1 ppm	NK	NK	Syrup	Reduced honeybee visitation	Naumann et al., 1994
<i>Apis mellifera</i>	Pirimicarb	NK			Field application	Reduced honeybee visitation	Le Blanc, 1985
<i>Apis mellifera</i>	Cypermethrin	10–30 g ai/ha	0.02–0.07	33–117	Field application	Reduced honeybee visitation	Le Blanc, 1985
<i>Apis mellifera</i>	Range of fungicides	NK			Syrup	Reduced honeybee visitation	Solomon and Hooker, 1989

NK: not known.

¹Maximum estimated exposure based on maximum application rate for pesticide (or nearest approved in the UK) and maximum exposure rate of 2.4 $\mu\text{g}/\text{bee}$ at 1 kg ai/ha.

²Dose for feed intake (ppm, ppb) based on 200 $\mu\text{l}/\text{bee}$.

life. Juvenile hormone is thought to be involved in the regulation of the age dependent activities, although demographic changes within the colony, e.g. shortage of nurse bees, also have a significant effect (Winston and Punnett, 1982). Treatment of honeybees with juvenile hormone analogues (e.g. methoprene) results in a shift in the activity of worker bee activity from the brood nest to food handling early, early degeneration of the hypopharyngeal glands and precocious foraging ability (Jaycox et al., 1974; Rutz, 1974 cited by Tasei, 2001; NRCC, 1981; Robinson, 1985). However, this precocious foraging ability does not appear to affect the foraging performance of these honeybee workers (Robinson, 1985; Deng and Waddington, 1997). The effect of diazinon exposure on the division of labour suggests greatest effects occur when newly emerged bees were exposed and effects were usually related to the onset and duration of foraging and handling of nectar (MacKenzie and Winston, 1989). The age dependence of the effects was presumably due to the lower levels of detoxifying enzymes (Smirle, 1993). This change in sequence of tasks may also affect longevity with a reduction in lifespan of up to 20% in bees exposed the same dose of diazinon (MacKenzie and Winston, 1989).

The effects of pesticide exposure on the division of labour, and on their subsequent lifespan, in bees may have a serious impact on the survival of a colony but many studies have been undertaken at unrealistically high exposure levels. Impacts on the distribution of tasks amongst the bees may have effects at the colony level from brood rearing through to the collection of stores. For example, the level of house-cleaning by colonies treated with a range of pesticides was reduced compared with the controls (Nation et al., 1986). Many of the treated colonies sustained extensive wax moth (*Galleria mellonella* L.) damage such that in some of the colonies there was little remaining comb for brood rearing.

Foraging

Foraging honeybees associate odour, among other cues, with resources, and on return to the colony communicate the direction and distance of the food resource. The process involves memory, learning, communication, navigation, an internal clock and a number of other flexible responses, e.g. the ability to integrate local landmarks (Menzel, 1993; Menzel

et al., 1998). Similar cues are used by bumble bees to locate their nest site (Goulson and Stout, 2001). Two types of learning are associated with foraging—latent (observatory) and associative (reward) (Menzel, 1993). Latent learning is important in spatial orientation and dance communication. Associative learning is associated with stimuli experienced immediately before rewards. Spatial orientation and dance communication is discussed below and associative learning through classical conditioning of olfactory stimuli is reviewed in a discussion of conditioned responses.

Although not directly related to the effects of pesticides on honeybee foraging behaviour, the effects of the dosing method must be taken into account in undertaking these studies as it may confound the observed effects. The effects of carbon dioxide and low temperature narcotisation on the behaviour of honeybee workers and queens have been reviewed (Ebadi et al., 1980). Carbon dioxide treatment of greater than 2 min affected the ability of the bees to return to the hive and treatment for 30 s or greater resulted in reduced survival and pollen gathering ability. Therefore it is important to understand the impact of handling and dosing procedures on foraging behaviour.

Organophosphorus and carbamate insecticides affect the ability of the bees to communicate the source of food through the wagtail dance. The dance of the honeybee requires integration of information on the location of food sources and a key in this is the angle of the dance. Sublethal oral exposure of honeybees to parathion (at levels below those likely to be encountered following field applications) prevented the bees from communicating the direction of an artificial food source to other bees (Schricker and Stephen, 1970). Bees performed the dance below the appropriate angle immediately after dosing and then overcompensated, they also made no compensation for the changes in the angle of the sun usual in untreated bees. This was followed by a second error below the angle and then a further overcompensation. Therefore, the treated bees performed their dance in a stepwise fashion rather than the continuous compensation for the sun's angle made by untreated bees. The dance angles of the poisoned bees returned to normal (similar to control bees) by 23 h after treatment. The deviations suggested that parathion affected the ability of the bee to orientate relative to gravity (Stephen and Schricker, 1970). The exposure to parathion had no adverse effects on the direction of flights of the poisoned bees on foraging trips suggesting it had no effect on their ability to

navigate and parathion treated bees flew approx 10% faster than untreated bees. However, the errors in the dance pattern of the foragers resulted in significant misdirection of novice foragers showing the importance of the dance in directing foraging bees to nectar sources. Similarly the bees made errors in distance for up to 6 h which affected not only the unpoisoned recruits, which foraged too close to the hive, but also the poisoned bees which stopped short of the resource. The variation in recovery time from specific sublethal effects suggests recovery from one effect does not indicate complete recovery.

Pyrethroids, at realistic exposure levels, appear to affect the ability of the foraging honeybee to return to the hive. Permethrin treatment resulted in 43% of foragers returning once to the colony and only 4% returned twice with none of the treated bees present the following morning (89% of the control bees were present) (Cox and Wilson, 1984). Most of the treated bees became so disorientated they could not return to the colony. These bees also showed serious disturbance of behaviour, e.g. spending more time in self-cleaning, trembling dance, abdomen tucking, rotating and abdomen cleaning and less time than controls foraging. Deltamethrin altered the homing-flight ability of bees in cages at levels below those that affect flight muscles and coordination (Vandame et al., 1995). After treatment 54% of the foragers flew towards the sun and 81% did not return to the colony within 30 s after release (the mean return time of control bees was 10 s). Vandame et al. (1995) concluded that this disorientation resulted from incorrect spatial perception or increased phototropism or information retrieval problems (comparison of actual and memorised patterns). They suggested that the bees failed to include, or integrate, the visual pattern of local landmarks with that of the direction of the sun. In cooler climates the ability of foragers to return to the colony after exposure to pyrethroids may also be affected by inhibition of temperature regulation by blocking the flight muscle involved in thermogenesis (Belzunces et al., 2001).

Treatment of foragers with a neonicotinoid insecticide, imidacloprid, was observed to have a slight effect on the preciseness of the communicated direction and a significant effect on the communicated distance of the food source on return to the colony (Kirchner, 1998; cited by Schmuck, 1999). There was a lower motivation to perform wagging dances and an increase in trembling dance. Given the distance is

communicated by dance rate the effect was suggested to be due to the effects of imidacloprid on motor neuron signal transmission. These changes result in a decreased foraging activity in honeybee colonies at approx. 20 ppb imidacloprid and resulted in inhibition of foraging at levels above 100 ppb after 30–60 min (Schmuck, 1999). Larger colonies of bees have been reported to show transitory disruptions of foraging activity at doses above 50 ppb which was observed to be time dependent and persisted overnight at doses of 100 ppb (Schmuck, 1999). The delay in the inhibition of foraging was suggested by Schmuck (1999) to be due to the reaction of the bees in the hive to the acceptability of the nectar rather than effects on the foragers, this may be related to effects on conditioned responses (see below).

The ability of the foragers to return may greatly affect colony survival, as recruitment of nurse bees to forage may reduce brood production. Effects on the communication of the source of forage may be less significant as bees are likely to seek out forage rather than return without nectar or pollen.

Conditioned responses

Odour perception and responses to pheromones are important to the survival of honeybee colonies. Nicotinic cholinergic systems are integral in attention, learning and memory in animals and have been reported to be responsible for the proboscis extension reflex pathway, habituation and memory formation in the honeybee (Guez et al., 2001). Several studies have investigated the effects of insecticides on learning (Decourtye et al., 1999) and on the habituation (Guez et al., 2001) of the proboscis extension reflex (PER), an appetitive component of honeybee feeding behaviour, to sucrose stimulation in honeybees. Learning responses are based on training the bees to associate an odour (conditioned stimulus) with sucrose stimulation of the antenna with proboscis extension rewarded by a food reward. The test is then conducted in the presence of the odour alone which induces a conditioned proboscis extension. Habituation is the decline in a behavioural response to a repeated stimulus of touching one antenna with a droplet of sucrose solution.

The neonicotinoid insecticides are nicotinic agonists. At doses as low as 0.1 ng/bee imidacloprid (well below those likely to occur following field

application, Table 1) increased the number of trials 15 min after treatment required to achieve habituation in 7-day-old bees and an increase in 8-day-old bees (Guez et al., 2001). A large change in this habituation behaviour occurs around days 7–8 in control bees suggesting changes in brain function occur at this stage of adult development with an increase in the mushroom body neurophil around the time that orientation flights begin (Guez et al., 2001).

Pyrethroid treated bees learned odour-mediated learned responses more slowly and achieved fewer positive responses after several training periods compared with control bees (Taylor et al., 1987; Mamood and Waller, 1990). Training response was least affected by fluvalinate and most affected by flucythrinate and cyfluthrin; permethrin, fenvalerate and cypermethrin were intermediate in their effect. Effects were observed for up to 3 days after treatment and at doses below those likely to be encountered following field application (assuming no repellency). Those authors suggested that decreased foraging on pyrethroid treated crops may therefore not be the effect of repellency but sublethal toxic dysfunction. Bees trained prior to exposure to permethrin showed no effects on their response suggesting that the pyrethroid affects learning (association of two inputs) rather than recall of memory (Mamood and Waller, 1990).

The effects in the ability of bees to learn and become habituated to odour based signals may have an impact on the colony by reducing the ability of the foragers to detect floral odours and thus nectar sources. However, olfactory learning performance in the individual under laboratory conditions has not been robustly correlated with effects in treated honeybee colonies. Imidacloprid reduced olfactory learning performance in individual bees (4–40 ppb, 3–33% LD₅₀) and affected flight activity and olfactory discrimination performance in a colony (50 ppb in sucrose) (Decourtye et al., 1999) but has not been correlated with effects following field applications. Therefore more studies are required before laboratory studies can be routinely used to predict effects in the field.

Effects on colony development

Haynes (1988) reviewed the effects of neurotoxic insecticides on the reproductive behaviour of insects and concluded that every class of insecticide, including

insect growth regulators, has been shown to decrease production of offspring. In honeybees reductions in brood and new bees may be more damaging to colony survival than the loss of foragers; foragers would be quickly replaced if there are sufficient brood and nurse bees. The following discussion relates only to effects on behaviour which may affect colony development and shown in Table 1 to be observed at levels of exposure likely to occur following field applications and not with effects related to brood mortality, e.g. by IGRs (Chandel and Gupta, 1992).

The effects of pesticide exposure on colony survival appear to vary depending on the level of alternative forage, i.e. nectar flow into the colony, with more severe effects shown during low nectar flows when there was less dilution within the colony. With little alternative forage, large effects were observed on comb production, egg laying and foraging at 1 ppm dimethoate (Waller et al., 1979). However, when alternative forage was available these effects were not observed (Stoner et al., 1983). Many studies have investigated short-term effects on colony development and survival but longer-term survival, e.g. over winter, is important but rarely addressed. Colonies fed 1 ppm carbofuran survived the summer, producing significantly fewer adult bees, but died over the winter (Stoner et al., 1982).

Pesticides may affect both the queen status (presence of a queen and her ability, through pheromone release, to prevent further queens being raised) of colonies and the ability of the queen to lay eggs. Queens were usually among the last individuals in the population to die following exposure to carbofuran, diflubenuron and methyl parathion pesticide exposure suggesting these are not systemic within the colony. The queens died because of reduction in the numbers of attendant workers in the treated colonies (Stoner et al., 1985). Acephate, dimethoate and fenthion exposure resulted in failure of the colonies to requeen themselves (Stoner et al., 1982, 1983, 1985). The queen supersedure rate in colonies treated with very low doses of cypermethrin (80%) was statistically significantly greater than in controls (30%) (Bendahou et al., 1999).

Pesticides may also affect the fecundity of the queens. Treatment of female leafcutter bees (*Megachile rotundata*) with a pyrethroid (20% of the LD₅₀) resulted in 20% fewer eggs laid throughout a 6-week period after dosing (Tasei et al., 1988). Honey bee colonies fed with the neonicotinoid insecticide imidacloprid showed

no effects on the development of the colonies (brood area) but there were some differences apparent in the egg-laying cycle and the abundance of larval and pupal stages (Schmuck et al., 2001). A laboratory feeding study with small, queenless, bumble bee colonies showed that imidacloprid reduced brood production and the ejection of larvae by the workers although there was no effect on larval development time (Tasei et al., 2000). The reduced ejection of larvae could have been a consequence of the reduced brood area.

The impacts of pesticides on the colony may be severe, including effects on comb production (which reduces the available area for brood rearing and honey storage), egg laying, overwintering survival, queen supersedure and ability of the colony to requeen. All these effects have the potential to have a large impact on colony survival.

Larval behaviour

The honey bee larvae cannot defecate, as they have a closed gut, and therefore food and metabolic waste is retained until pupation. Laboratory tests have been devised to determine the effects of pesticides on larvae by direct application and semi-field brood tests are well established for determining colony level effects (Oomen et al., 1992). Many of the effects on larvae reported relate to development and there are few which relate to behaviour.

Larvae dosed with low levels of dimethoate (0.313 µg/g royal jelly) showed stimulation of growth and maturation compared to controls, although some of the larvae lost their typical C shape and were stretched out dorsally or dorsolaterally (Davis et al., 1988). Some of these larvae also failed to spin cocoons and were hypersensitive to stimulation. These results were similar to those reported in larvae fed on lipid free diets suggesting effects were related to juvenile hormone and ecdysone levels. Davis et al. (1988) suggested that dimethoate may interfere with the normal metabolism of sterols or 10-hydroxy-2-decanoic acid which may regulate the secretory activity of the corpora allata and therefore the ratio of juvenile hormone to ecdysone in the larva.

Developmental effects in larvae may have impacts on the behaviour of adults and therefore affect colony survival. Dimethoate, malathion, carbaryl and the fungicide captan have been reported to result morphogenic effects in adults exposed as larvae, e.g. very small

adults, wing malformations, in some cases wingless, stunted bodies, crippled legs and wings (Jay, 1964; Atkins and Kellum, 1986). All these effects are likely to severely affect the ability of the adults to perform duties within the colony and forage effectively and were observed at realistic levels of exposure.

Nestmate recognition

Honeybee workers discriminate between nestmates and non-nestmates using both self-produced and environmentally acquired cues. However, self-produced cues are only important for inter-colony recognition when environmental cues are missing (Downs et al., 2000). Environmental cues include odour from food, comb wax or the queen, e.g. queen fecal pheromones (Breed et al., 1992). There is anecdotal evidence from beekeepers to the National Bee Unit that forager bees are ejected from the colony after foraging on some herbicide and fungicide treated crops. The rejection of bees returning from treated fields may be the result of chemicals within the formulation masking the environmental cues on which the bees rely for recognition. Mortality of large numbers of workers may occur because of the aggression of the guard bees which would not be identified in laboratory studies and therefore no further testing would be undertaken.

Repellency

Some insects change their behaviour in response to their sensory perception of insecticides, e.g. by repellency, excitation, irritation, reduced feeding or feeding stimulation (Haynes, 1988). Pyrethroids are probably the best known repellent pesticides in use. Both the phenylacetate-ester and cyclopropanecarboxylate pyrethroids exhibit repellency through similar modes of action (Delabie et al., 1985; Rieth and Levin, 1988, 1989), i.e. sublethal effects after contact exposure of the tarsi and ventral abdomen (Mamood and Waller, 1990). The pyrethroids are highly irritating to the bees which then transfer the pesticide from the tarsi to the proboscis and antennae during grooming. The bees return to the colony to recover, before they receive a lethal dose, in a similar manner to the knockdown effects of pyrethroids in other insects. Given that the effects of pyrethroids are temperature dependent the

ability of the bee to return to the hive may be affected by the temperature—i.e. the lower the temperature the less likely that the bee will return to the hive before knockdown, i.e. it is not true repellency but a sublethal effect.

The presentation of the test chemical is important in evaluating the repellency of the compound to honeybees. In most cases repellency has been tested in semi-field conditions, e.g. in polytunnels, by dissolving the chemical in sucrose and assessing the level of foraging to a feed (Solomon and Hooker, 1989). The problems in extrapolating from laboratory or semi-field experiments using treated feed to the field was reported for 12 chemicals (Atkins et al., 1975a,b) and are highlighted by two more recent studies. Neem (azadirachtin) treated sucrose was repellent to bees in a semi-field study but not when it was applied to flowering oilseed rape (Naumann et al., 1994). The neonicotinoid insecticide fipronil reduced the visitation rate of honeybees to sucrose feeders, although the mechanism for repellency could not be ascertained, and application at field rates to flowering oilseed rape showed no repellency (Mayer and Lunden, 1999). These differences between treated feed and field applications may result from the attractiveness of the crop overriding any repellency.

Under field conditions the repellency of pyrethroids may be lower than suggested by semi-field experiments. The more affected bees may not return to the colony, as discussed above, and therefore low mortality is observed at the hive but loss of large numbers of foraging bees occurs. Cypermethrin sprayed on oilseed rape during periods of peak foraging activity resulted in a slight decline in the level of foraging and the levels of pollen collected (Shires et al., 1984). The number of bees foraging was only affected by 60% for 3–4 h in the field sprayed at the lower rate (10 g ai/ha). In the field sprayed at a higher rate (20 g ai/ha) the number of bees foraging was reduced by 85% immediately after spray application but had recovered by the following day. Evidence for repellency was also questioned by the detection of relatively high residues of cypermethrin in honey and wax (0.01–0.04 mg/kg) from colonies in the 20 g ai/ha treated site (pollen levels in the treated crop were 0.1 mg/kg immediately after application and declined rapidly). The numbers of bees in the colonies at the beginning and end of the study were not determined so assessment could not be made as to whether affected bees returned to the hive or were lost.

It is important to determine whether decreases in foraging levels result from repellency or mortality of foragers. There is some evidence for repellency of anticholinesterase insecticides, foraging was inhibited by treating sucrose solution with aldicarb sulfoxide at levels below those that caused mortality (Nigg et al., 1991). However, reductions in foraging following application of methyl parathion was associated with high levels of mortality at the hive entrance (Shires et al., 1984). A number of fungicides have also been shown to be repellent, e.g. Captan, when dissolved in sucrose (Solomon and Hooker, 1989).

Behavioural effects in regulatory studies

Data for use in pesticide risk assessment for honeybees concentrates primarily on mortality. For laboratory studies the standard guidelines are those produced by the OECD (1998a,b), EPPO (1992) and EPA (draft 1996). The OECD and EPPO guidelines require all abnormal behavioural effects to be recorded and numbers of bees with adverse effects to be reported but give no guidance on the types of effects to be recorded. The draft EPA Honey Bee Acute Contact Toxicity guideline (Ecological Effects Guideline OPPTS 850.3020) is more prescriptive “any signs of intoxication, other abnormal behaviours, including ataxia, lethargy and hypersensitivity that may or may not be attributed to the test substance should be recorded throughout the test period and reported by time of onset, duration, severity and number affected at each dosage level”.

The primary endpoint of the laboratory study is mortality and the dose levels are selected to determine an LD₅₀ as precisely as possible. However, there is increasing evidence that mortality curves, and possibly sublethal effects, may not be simple functions of dose. Imidacloprid, a neonicotinoid insecticide, shows biphasic mortality at lower doses, particularly following contact exposure, and at higher doses mortality was delayed (Suchail et al., 2000). Therefore, sublethal effects may occur at lower doses than those used to generate mortality curves in the laboratory. There are additional observations that could be routinely included in laboratory toxicity studies, e.g. trembling, abdomen tucking or excessive cleaning, knockdown, aggressiveness and lack of coordination (stumbling) (NRCC, 1981). Observation of these types

of behaviour may suggest further investigation of behaviour is required in semi-field and field studies. Knockdown may be particularly important as trophylaxis (sharing of food) occurs between non-affected and affected/recovering bees in laboratory studies. In the field trophylaxis is unlikely to occur outside the colony and extended periods of knock-down may result in starvation and death. However, the primary concern in routine collection of further sublethal data in laboratory studies is that there are currently few data available at realistic exposure levels (following field applications) on the consequences for colonies of sublethal effects observed in the laboratory. For example, the effects of pesticide exposure on conditioned responses can only be readily assessed under laboratory conditions. Further work is also required to determine the importance of sublethal effects of pesticides with low acute toxicity. Until there is a wider database on the types of effects which occur and their relevance to the field their incorporation in risk assessment will be limited.

Although EU Directive 91/414/EC requires assessments of effects on behaviour, colony development and survival there is no guidance in the types of effects to be investigated and few data on which to base interpretation. At the higher tier, the EPPO (1992) cage test guidelines refer to recording foraging activity and behaviour of bees on the crop and around the hive, other assessments refer to "those appropriate to the type of test product". EPPO (1992) field test guidelines refer to recording foraging activity and behaviour of bees on the crop and around the hive, and brood status at the test initiation and test termination (usually 2–3 weeks). The review presented here has shown that there are a number of behavioural effects that could have a severe effect on colony development and survival but the longer-term impact on the colony is rarely reported. Semi-field and field studies should always include assessments of behaviour at the hive entrance, e.g. excessive fanning, aggressiveness to returning foragers (or observers), number of flights into and out of the colony. In this way effects observed in laboratory studies may also be correlated over time with effects in semi-field and field tests to provide a larger database on which to base correlations and allow interpretation. Some tests are too complex to be undertaken routinely, e.g. the effects of pesticide exposure on conditioned responses (responses to odours) in the field requires the training of foragers to respond to odour cues associated with

food sources, and interpretation of the effects at colony level is unclear. Therefore, until further data are available on the implications for the colony odour discrimination should not be routinely assessed.

Direct assessments of foraging on the crop described in the EPPO guidelines primarily provide information on the exposure of the bees and an assessment of any repellency. The repellency of treated sucrose in semi-field trials cannot be used to predict the repellency of treated surfaces, e.g. leaves, following field applications. In many cases the basis of the repellency is unclear and reductions in foraging resulting from mortality must be clearly identified in semi-field and field studies.

Conclusions

A wide variety of sublethal behavioural effects have been reported in bees following their exposure to pesticides, primarily insecticides. These range from effects on odour discrimination to loss of foraging bees from disruption of their homing behaviour. Many of the reported effects occurred at levels at or below those estimated as likely to occur, in the short term, following field applications. Particular attention should be paid to the significance of sublethal effects in the laboratory for compounds which may not otherwise undergo higher tier testing because of their low acute toxicity or low application rates but may result in effects at the colony level, e.g. rejection of foragers returning to the colony. Semi-field and field studies should routinely include observations of excessive fanning, aggressive behaviour and activity levels at the hive entrance, full colony assessments both at the termination of the trial and delayed effects as well as the behaviour of foraging bees as all of these may impact on colony development and survival. The longer term consequences of sublethal changes in colonies, e.g. over-wintering survival, should also be assessed. Such an approach requires a larger base set of data to predict longer-term consequences of short-term effects on colonies, e.g. through population modelling.

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