EXTERNAL SCIENTIFIC REPORT

Interaction between pesticides and other factors in effects on bees[1]

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ABSTRACT

Bees are important pollinators of both managed crops and wild flora. An overview of the interactions between pesticides and other factors in effects on bees considered: 1) The importance of the different exposure routes in relation to the overall exposure of bees to pesticides; 2) Multiple exposure to pesticides (including substances used in bee medication) and potential additive and cumulative effects; and 3) Interactions between diseases and susceptibility of bees to pesticides. Nectar foraging bees are likely to experienced highest exposure to both sprayed and systemic seed and soil treatments compounds followed by nurse and brood-attending bees. In both cases the major contribution to exposure was contaminated nectar with direct overspray playing a significant role in exposure. However, there are a variety of other routes (and other bee species) where there is currently insufficient data to fully total exposure: There are a large number of studies that have investigated the interactions between pesticides in bees. By far the majority have related to the interactions involving EBI fungicides and can be related to their inhibition of P450. The scale of the synergy is shown to be dose and season-dependent in acute exposures but there are few data relating to the effect of time between exposures, the effect of route of exposure or on chronic exposure effects at realistic exposure levels. There are a wide range of factors which affect the immunocompetence of bees including diet quality, pest and diseases. Although there are a limited number of laboratory based studies which suggest effects of a pesticide on disease susceptibility there is no clear evidence from field-based studies that exposure of colonies to pesticides results in increased susceptibility to disease or that there is a link between colony loss due to disease and pesticide residues in monitoring studies.

KEY WORDS

Honeybees, bumble bees, pesticides, disease, mixtures, synergism

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SUMMARY

1. Honeybees are important pollinators of both managed crops and wild flora. Changes in the use of agricultural land are thought to play a major role in reported pollinator declines, e.g. the nutritional value of pollen affects the physiology of developing bees. However, parasites and diseases are likely to produce significant additional pressures on the remaining populations. This review considered state-of-the-knowledge through search of information from scientific literature, study reports and other documents. To provide an overview of the interactions between pesticides and other factors in effects on bees considering:

    - The importance of the different exposure routes in relation to the overall exposure of bees to pesticides.

    - Multiple exposure to pesticides (including substances used in bee medication) and potential additive and cumulative effects.

    - Interactions between diseases and susceptibility of bees to pesticides.

2. The database searches, after clearly unrelated references and duplicate references were removed, yielded

    - 148 references containing data directly relevant to routes of exposure in bees

    - 103 references for mixtures of which 84 were specific to honeybees, and 19 related to other insects

    - 112 references for pesticide interactions with disease of which 71 were specific to honeybees, 7 to bumble bees and 34 other insects.

3. Residues per unit dose (RUD) were identified for directly over-sprayed honeybees, pollen and nectar, and stored pollen and nectar. These were combined with intake levels for honeybees to determine the relative importance of different routes of exposure for different ages of bees. These showed that nectar foraging bees are likely to experienced highest exposure to both sprayed and systemic seed and soil treatments compounds followed by nurse and brood-attending bees. In both cases the major contribution to exposure was contaminated nectar with direct overspray playing a significant role in exposure.

4. However, there are a variety of other routes where there is currently insufficient data to fully quantify their contribution to total exposure:

    - If dusts are produced during sowing of treated seeds this may be a significant source of exposure and may result in residues in pollen and nectar of nearby flowering weeds or crops.

    - Contact exposure to newly sprayed crops is likely to be integrated in the RUD for over-sprayed bees.

    - Inhalation may be a significant route of exposure for compounds with high vapour pressure and present in stored pollen or collected in water and further data are required.

    - Beeswax may be a significant route of exposure for highly lipophilic chemicals and more information is required to evaluate transfer to brood.

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- Propolis probably has a low contribution to overall exposure except where applications are made to trees producing resin, e.g. trunk injection, and the pesticides are systemic and therefore may be exuded in resin.

- Water may be sourced from puddles or guttation droplets which may contain high residues for periods of days-weeks and further data is required on the relative importance of these routes.

5. The same approach was assessed for bumble bee workers and larvae. However, the intake of foragers is not reported and therefore the data only relate to intake for metabolic requirements. Overspray can be related to the surface area of the bee but bumble bees are also far more variable in size than honeybees making any predictions unreliable.

6. There was insufficient data available to assess the exposure of solitary bee species. More data are required to fully evaluate the importance of differing routes of exposure for bumble bees and other non-Apis species.

7. Honeybees and other bees may be exposed to mixtures of pesticides through multiple applications, overspray of residues already present, e.g. systemic pesticides, collection of pollen and nectar from a variety of sources and stored within the colony and, in addition, the use of treatments within hives by beekeepers. As previously there is very limited data on bees other than honeybees.

8. There is evidence in the literature of multiple residues of pesticides detected in honeybees, honey and pollen and wax within the hive but this is limited by the direction of the analysis to chemicals of interest to the researchers and rarely are levels of individual components reported. More data are required on realistic levels and combinations of pesticides at the individual colony level within the EU to more fully evaluate the effects of multiple pesticide exposure..

9. Additive toxicity is an appropriate approach for most mixtures where synergy can be excluded and can be applied to residues in pollen and nectar to assess the total exposure of adult and larval bees to pesticides.

10. There are a large number of studies that have investigated the interactions between pesticides in bees. By far the majority have related to the interactions involving EBI fungicides and can be related to their inhibition of P450. The scale of the synergy is shown to be dose and season-dependent in acute exposures but there are few data relating to the effect of time between exposures or on chronic exposure effects at realistic exposure levels.

11. The vast majority of the studies have concentrated on the contact toxicity of the combinations. However the exposure section shows that a significant proportion of the exposure may be through ingestion of contaminated nectar. It appears that pesticides which induce P450s in other insects do not induce these enzymes in honeybees but natural chemicals, such as quercetin present in honey and propolis do induce P450s and reduce the toxicity of some pesticides. Given the role of the midgut enzymes in the metabolism of xenobiotics the shortage of data foollowing oral exposure of mixtures is a major gap in our understanding of the potential interactions between chemicals, particularly those present in pollen and nectar, and the effects of diet quality in maintaining xenobiotic metabolising capacity within the gut.

12. Significant synergy has been reported between EBI fungicides and both neonicotinoids and pyrethroid insecticides but in some cases where high levels of synergy are reported the doses

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of fungicides have been well in excess of those identified in the exposure section of this report. At lower, more realistic ratios of synergist to insecticide generally lower levels of synergy are identified with field application ratios of pyrethroids and neonicotinoids although the data for the latter are very limited.

13. Greater synergy is observed in the laboratory between EBI fungicides at field rates application rates and pyrethroids used as varroacides (flumethrin and fluvalinate) and between coumaphos and fluvalinate varroacides. Given the persistence of residues of varroacides detected in monitoring studies further evaluation of the combined effects of these with agricultural pesticides is warranted.

14. As effects are dose-dependent synergism may be an area where modelling is applicable both from toxicokinetic/toxicodynamic and QSAR approaches but also needs to take into account formulation differences in affecting rate of uptake.

15. More recently data has shown that antibiotics used in hives may increase the susceptibility of bees to organophosphorus, pyrethroid and neonicotinoid insecticides through interaction with the membrane bound transporter proteins and further work is required to more fully understand the implications of these findings. It is therefore important that all treatments used on colonies used in studies are reported.

16. In all studies the interactions between two chemicals has been reported. However, the exposure data demonstrate that bees are often exposed directly through applications of multiple active ingredients or indirectly through consumption of stored pollen and nectar to several pesticides over a period of time. Data are required to determine the effects of such long term low level exposure to multiple pesticides on the health and functioning of honeybee colonies foraging in agricultural environments.

17. The only reported studies for interactions between pesticides and bees were for honeybees.

18. There are a small number of studies in honeybees which suggests that infection by *Nosema* or viruses may increase the susceptibility to pesticides. The reported levels of increase in toxicity are less than 3 fold to date (but the number of reported studies are small and the reported levels of apparent infection are high).

19. There are data that may demonstrate increased spore counts of *N.ceranae* in bees previously chronically exposed to pesticides but there are also reports that spore count decreased following exposure to some pesticides. However, spore count may not be a reliable indicator of the impact of *N ceranae* infection in bees. There is a need for improved methods of assessment for some pathogens, e.g. *N cerenae* which more clearly link to the impact of the disease on the individual and the colony.

20. There are a wide range of factors which affect the immunocompetence of bees including the quality of the pollen diet, the presence of other diseases, such as *N ceranae*, or pests, e.g. Varroa, and in-hive treatments such as antibiotics. In addition, the confinement of colonies or individuals may result in stress leading to immunosuppression. It is important that these factors are taken into account in studies determining the effects of pesticides on both individual and social immunity.

21. The effect of the diet on both the immunocompetence and the xenobiotic metabolising enzymes within the gut are important and impact on both the effects on the toxicity of other pesticides and the impacts on disease susceptibility. Pathogens may also impact on some

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measures of sublethal effects of pesticides. It is therefore important that the realistic routes of exposure are used in mixture studies, i.e. oral for contaminated pollen and nectar, and that the disease status of bees used in pesticide studies is fully understood.

22. Currently there is no clear evidence from field based studies that exposure of colonies to pesticides results in increased susceptibility to disease or that there is a link between colony loss due to disease and pesticide residues in monitoring studies.

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TERMS OF REFERENCE AS PROVIDED BY EFSA

To compile all available scientific information for Lot 5 Interaction between pesticides and other factors in effects on bees considering:

Multiple exposure to pesticides (including substances used in bee medication) and potential additive and cumulative effects.

Interactions between diseases and susceptibility of bees to pesticides.

The importance of the different exposure routes in relation to the overall exposure of bees to pesticides.

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INTRODUCTION AND OBJECTIVES

Honeybees are important pollinators of both managed crops and wild flora. Changes in the use of agricultural land are thought to play a major role in reported pollinator declines, e.g. the nutritional value of pollen affects the physiology of developing bees. However, parasites and diseases are likely to produce significant additional pressures on the remaining populations. The review considered state-of-the-knowledge through search of information from scientific literature, study reports and other documents

MATERIALS AND METHODS

The set of search terms selected and databases searched for the study are shown in Appendix 1. All search results are fully documented in Appendix 1. The database was searched for duplicates which were removed and the cleaned database transferred to EndNote. The literature was evaluated systematically and the criteria for including or excluding the references stated (see Appendix 1).

Any reports of studies that were identified as useful were evaluated to assess their reliability. Reliability covers the inherent quality of the test relating to the test methodology and the way the performance and results of the test are described. The criteria used for assessing reliability of the identified literature were based on that identified in "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009" which provides a definition of scientific peer-reviewed open literature and instructions on how to minimise bias in the identification, selection and inclusion of peer-reviewed open literature in dossiers, according to the principles of systematic review (i.e. methodological rigour, transparency, reproducibility). For each identified data source the reason for inclusion or exclusion is clearly stated in the main report for those included and in Appendix 1 for those excluded.

The information from previous reports and the review of 'new' literature was combined to provide an overview of the interactions between pesticides and other factors in effects on bees considering:

- The importance of the different exposure routes in relation to the overall exposure of bees to pesticides.
- Multiple exposure to pesticides (including substances used in bee medication) and potential additive and cumulative effects.
- Interactions between diseases and susceptibility of bees to pesticides.

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RESULTS

1. EXPOSURE OF BEES TO PESTICIDES

The database searches yielded 386 exposure related papers of which 148 were relevant to evaluating honeybee routes of exposure, i.e. they related to the residues present in bees or matrices collected by bees. The papers and reports identified as not relevant did not contain detailed information on pesticide or in-hive treatment residues and are separated in the database.

Bees are exposed to pesticides via a number of routes and the relative importance of each depends on the life stage of the insect and the mode of application of the pesticide. Adults may be exposed directly to pesticides through direct overspray or flying through spray drift, by consumption of pollen and nectar (which may contain directly over-sprayed or systemic residues), by contact with treated surfaces (such as resting on recently treated leaves or flowers), by contact with dusts generated during drilling of treated seeds, or by exposure to guttation fluid potentially as a source of water or as dried residues on the surface of leaves. The exposure of larvae is primarily via processed pollen and nectar in brood food. Data available in the literature includes residues in pollen, wax and nectar within colonies, pollen and nectar residues from plants, in pollen loads on bees returning to the hives and in adult workers. Such data also includes the residues of veterinary medicines detected and the distribution of chemicals around the hive.

The routes of exposure of bees to pesticides has been assessed, e.g. (Babendrier et al., 2004; Rortais et al., 2005) and recently reviewed in an EFSA Scientific Opinion particularly in relation to quantifying uptake and extended to include other non-Apis species where data were available. The exposure of bumble bees to pesticides has also been reviewed and showed there are key times in the year when exposure of queens may be particularly important in determining the fate of a colony (Thompson, 2001). Therefore this review was directed at identifying the levels of pesticides identified with specific routes of exposure associated with spray and seed/soil treatments. Data from directed field studies outside the EU have been included as these relate residues to application rates but monitoring data from outside the EU (e.g. Mullin et al., 2010) have been excluded due to the wide range of active ingredients, modes of application and application rates outside the EU for both in-hive and pesticide applications, which makes their applicability limited. In the following discussion where possible a residue (mg/Kg) per unit dose approach has been estimated for spray applications of 1Kg/Ha and seed treatments of 1mg/seed. This, together with intake data from Rortais et al. (2005) and similar estimates for bumble bees from the literature allows comparison of the relative importance of the different routes of exposure.

At the outset it must be stated that there is very limited data on the residues of pesticides in matrices specific to non-Apis bees and therefore much of the following is directed at honeybees but where non-Apis bee data were identified these are included.

1.1. Residues in bees

There are a number of studies available which report the residues in honeybees following in-hive treatments or pesticide applications. These are summarised in Table 1.1. The in-hive treatments provide information on the range of active ingredients and level of exposure of bees to such treatments within the hive (not all are authorised in the EU) and the residues of acaracides (tau-fluvalinate, coumaphos, amitraz) detected in live and dead bees collected from hives (Table 1.2). These two tables show that residues of in-hive treatments are detected in bees in some cases, e.g. antibiotics and coumaphos, at high levels after application.

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The residues after pesticide applications (Table 1.1) provide evidence of the exposure of bees to applications aggregated through all routes of exposure, i.e. through direct overspray, foraging on treated crops and consumption of treated food and water as samples were collected over time after exposure. This shows peak residues in the first sample after application with declines for spray applications over the following week. No data from systemic seed or soil application field studies were available but residues of imidacloprid and its metabolite 6-chloronitoctinic acid and fipronil and its metabolites were detected at low levels in monitoring studies (Table 1.2). Table 1.2 provides information on the residues detected in live and dead bees during the conduct of monitoring studies which provides a realistic scenario of honeybee exposure where pesticides are in widespread use in the agricultural environment. The monitoring data clearly show the presence of residues higher than the published data for the LD_{50} in many cases where dead bees were analysed (multiple residues in dead bees are discussed in Chapter 2); particularly when considering that residues may be 80-90% lower in dead bees than the LD_{50} when samples are collected over a period of time after poisoning (Greig-Smith et al., 1994). However, the data from the live bees and the range of residues detected in dead bees demonstrates exposure of bees to a wide range of pesticides at sub-lethal levels, including seed treatments and varroacides. In summary, residues collected from live foraging bees from field studies are likely to be more reliable in their use in estimating actual aggregate exposure than field collected samples of dead bees which are subject to a wide range of variables before analysis.

1.2. Forager bees

There are three main routes of exposure for foragers bees identified in the literature:

- Direct overspray
- Contact with treated surfaces including from dusts and spray drift
- Intake of contaminated pollen, nectar and water

1.2.1. Aggregate exposure of foragers following spray applications

Studies have been undertaken in which bees were allowed to fly during tracer applications to apple trees and Phacelia ((Koch and Weisser, 1997) and in wind tunnel experiments with both dead and tethered flying bees (Ucar et al., 2003). Initial deposits measured on dead bees in laboratory studies has shown that the residue is not affected by the volume of the application but by the product rate per hectare (Koch and Spieles, 1993). Therefore, extrapolation is appropriate for data generated on exposure of bees by the use of tracers.

The wind tunnel studies ensured bees were present in the direct spray from the application (76 mg on a target of 5625 $cm^{2;}$ 13.5 µg/cm^2) and showed that at low wind speed deposits on flying bees were 4.6 ± 1.9 µg/bee and on dead bees 4.1 ± 0.9 µg/bee and at higher wind speeds 8.0 ± 2.1 µg/bee on flying bees and 7.9 ± 0.5 µg/bee on dead bees (Ucar et al., 2003). Given the application was only to the upper surface of the dead bees the surface area of a honeybee is 0.93 cm^2 (Johansen et al., 1983) this suggests that the maximum residue equated to 63% efficiency. At lower wind speeds the efficiency was approximately 38%.

The data from the wind tunnel study in which bees were directly placed in the spray application zone can be compared with free-flying bees to determine a more realistic exposure than direct overspray. Use of a fluorescent tracer at 20 g/ha (200 ng/cm^2) in apple orchards resulted in a maximum exposure of 20.8 ng/bee (mean 6.33 ng/bee) and in Phacelia 35.77 ng/bee (mean 4.52 ng/bee) or maximum of 11% (mean 3.4%) of the available dose in apples and a maximum of 19% (mean 2.4%) in Phacelia.

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This suggests that the actual exposure is likely to be lower than direct overspray. This more realistic exposure equates to an application rate of 1Kg/ha resulting in a mean residue (RUD) of 0.32 µg/bee in apples and 0.23 µg/bee in Phacelia (overall mean 0.28 µg/bee; 2.80 mg/kg) and maximum residues of 1.04 µg/bee in apples and 1.79 µg/bee in Phacelia (overall maximum 1.79 µg/bee; 17.9 mg/kg).

As the major contribution to the residues on at the early sampling points is likely to be direct overspray. The RUD (residue per unit dose - mg/kg in dead bees for a 1Kg/ha application) was calculated for the data in Table 1.1 This showed that the RUD for the applications ranged from mean of 4.43 to a 90th percentile of 15.28 which is similar to the those identified in the study using tracers (2.8 mean 17.9 maximum) and suggests such a value may reflect exposure of foraging honeybees to spray applications during and immediately after application. Such an RUD may be extrapolated to other bee species based on their surface area (Johansen et al., 1983).

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Table 1.: Summary of honeybee residue data from published studies

Active ingredient	Application rate	Method	Potential route of exposure	Mean Residue µg/Kg (range)	Reference
In-hive treatments					
ampicillin	30 mg in 1 litre sucrose	5 colonies	oral	Two day old larvae 1 day 60 3 day <LOD (10) Five day old larvae 1 day 3000 3 day 600 7 day 80 14 day <LOD (10)	(Nakajima et al., 1997)
Cymiazole as Apitol	Acaracide 2g/hive	10 colonies treated samples collected up to 115 days after treatment	oral	Mean 84000 ± 20800 1 day after treatment to 70 ± 40 15 days after treatment	(Cabras et al., 1994)
Fluvalinate	Acaracide 1600mg/treatment for 1 month	2 treated hives, samples taken before to 180 days after treatment	contact	Day 0 <10 Day 0.8 24-82 Day 1 79-160 Day 2 68-100 Day 4 130-160 Day 7 14-58 Day 10 25-64 Day 15 62-66 Day 30 46-66	(Bonzini et al., 2011)
fluvalinate	Acaracide 100 µl fluvalinate on wooden strips	Direct application into hive	contact	Adult bee heads 1 day 228 2 days 258 4 days 204 8 days 105 Larvae 1 day <LOD (10) 2 days 1038-1170 4 days 98-110	(Skerl et al., 2010)

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Active ingredient	Application rate	Method	Potential route of exposure	Mean Residue µg/Kg (range)	Reference
				8 days <LOD (10)	
Mirosamicin	200mg in pollen paste	6 colonies 1 week of dosing	oral	Adult bees 0 day 7000 3 day 1000 7 day 200 14 day 100 Two day old larvae 0 day 1000 3 day <LOD (10) Five day old larvae 0 day 8000 3 day 80 7 day <LOD (10)	(Nakajima et al., 1998)
Oxtetracycline	0.3 g/colony spring	5 colonies 48 hrs after last dosing	oral	Adult bees 28000 ± 42700 Larvae 22400 ± 17100 Honey 19500 ± 9900	(Lodesani et al., 1994)
Oxtetracycline	0.75 g/colony spring	5 colonies 48 hrs after last dosing	oral	Adult bees 10100 ± 5700 Larvae 17400 ± 7000 Honey 24300 ± 7800	(Lodesani et al., 1994)
Oxtetracycline	1.5 g/colony spring	5 colonies 48 hrs after last dosing	oral	Adult bees 97600 ± 11800 Larvae 68400 ± 43900 Honey 44900 ± 13100	(Lodesani et al., 1994)
Oxtetracycline	0.3 g/colony autumn	5 colonies 48 hrs after last dosing	oral	Adult bees 5500 ± 7400 Larvae 2500 ± 4300 Honey 18700 ± 5900	(Lodesani et al., 1994)
Oxtetracycline	0.75 g/colony autumn	5 colonies 48 hrs after last dosing	oral	Adult bees 10900 ± 11800 Larvae 5200 ± 5600 Honey 40100 ± 20400	(Lodesani et al., 1994)
Oxtetracycline	1.5 g/colony autumn	5 colonies 48 hrs after last dosing	oral	Adult bees 10900 ± 12800 Larvae 8500 ± 13900 Honey 31600 ± 13300	(Lodesani et al., 1994)

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Pesticides

Active ingredient/LD50	Application rate	Method	Potential route of exposure	Residue µg/Kg	RUD	Reference
Acephate (LD50 0.7 µg/bee; 7000 µg/kg)	500 mg/L bait syrup 30 ±11 mg consumed/colony	35 colonies; dead bees	oral	1 day 10000 µg/kg acephate/3000 µg/kg methamidophos (metabolite)		(Danka et al., 1991)
Carbofuran (0.16 µg/bee; 1600 µg/kg)	1.1 Kg ai/ha, 8 ha field alfalfa Furadan	6 Colonies moved adjacent to treated field before application; dead bees	all	1 day 3317-6367 2 days 1300-1900 3 days 367-1217 4 days 467-483 5 days 33-83 6 days 33	5788	(Moffett et al., 1986)
Carbofuran (0.16 µg/bee; 1600 µg/kg)	1.1 Kg ai/ha, 9 ha field alfalfa Furadan + sticker	6 Colonies moved adjacent to treated field before application; dead bees	all	1 day 3333 2 days 2500 3 days 1417 4 days 100 5 days 467 6 days 250	3030	(Moffett et al., 1986)
Cypermethrin (0.37 µg/bee; 3700 µg/kg)	25 Ha 25 g ai/ha	Dead bees	all	54 ng/bee – 540 µg/kg	21600	(Andreescu et al., 2008) abstract
ethyl parathion (0.175 µg/bee; 1750 µg/kg)	Aerial application on sunflowers, 1.1 kg/ha	Dead bees, 6 colonies	all	Day 1 3600 µg/kg	3272	(Cox et al., 1986)
Fluvalinate (1.9 µg/bee ; 19000 µg/kg)	144 g/ha	applied to apple trees in flower; live bees	all	1 day 75 2 day 72 3 day 52 4 day 32 5 day 20 6 day 7 7 day <LOD	520	(Haouar et al., 1990)
Malathion 0.27 µg/bee; 2700 µg/kg	Aerially applied bait spray 159 ml malathion /ha	Collection of pollen samples and dead bees after weekly applications	all	Dead bees 960-5280		(Gary and Mussen, 1984)
Methyl parathion	Flowering cotton sprayed	5 hives dead bees	all	200		(Robertson and

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Active ingredient/LD50	Application rate	Method	Potential route of exposure	Residue µg/Kg	RUD	Reference
(0.165 µg/bee; 1650 µg/kg)	rate not known					Rhodes, 1992)
Methyl parathion (microencapsulated) (0.165 µg/bee; 1650 µg/kg)	1.1 Kg ai/ha, 8 ha field alfalfa Penncap M	4 Colonies moved adjacent to treated field 5 hrs after application dead bees	all	1 day 2500 2 days 810 3 days 696 4 days 358 5 days 280 6 days 52 7 days 2	2272	(Moffett et al., 1986)
Methyl parathion (microencapsulated) (0.165 µg/bee; 1650 µg/kg)	1.1 Kg ai/ha, 9 ha field alfalfa Penncap M = sticker	4 Colonies moved adjacent to treated field 5 hrs after application dead bees	all	1 day 762 2 days 368 3 days 890 4 days 224 5 days 80 6 days 134 7 days 62	809	(Moffett et al., 1986)
Methyl parathion (microencapsulated) (0.165 µg/bee; 1650 µg/kg)	1.1 Kg ai/ha, 8 ha field alfalfa Penncap M	6 Colonies moved adjacent to treated field before application dead bees	all	1 day 883-1582 2 days 117-177 3 days 48-122 4 days 50-72 5 days 12-45 6 days 2	1438	(Moffett et al., 1986)
Methyl parathion (microencapsulated) (0.165 µg/bee; 1650 µg/kg)	1.1 Kg ai/ha, 9 ha field alfalfa Penncap M = sticker	6 Colonies moved adjacent to treated field before application dead bees	all	1 day 202-1230 2 days 42-458 3 days 28-85 4 days 18 5 days 15-140 6 days ND-30	1118	(Moffett et al., 1986)
Methyl parathion microencapsulated (0.165 µg/bee; 1650	Birdsfoot trefoil treated at 1 kg ai/ha	2 nucleus colonies placed on edge of 0.5ha treated area	all	Max 87% contamination on day 1 decreased to 10% on day 9		(Burgett and Fisher, 1980)

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Active ingredient/LD50	Application rate	Method	Potential route of exposure	Residue µg/Kg	RUD	Reference
µg/kg)		every 48 hrs until 8 nuclei present, Identification of microcapsules in stored pollen				
Zineb	Aerial application on vineyards rate not known	Dead bees	all	1.1-1.82 µg/bee – 11820 µg/Kg		(Belliardo and Nano, 1975)
Tracers						
Fluorescent tracer	4g/l, 114 ml/min, spray time 10 secs = 76 mg (76g/0.0563 ha or 1.35 kg/ha) wind speeds 1.5, 2.5, 3.5 m/s	Wind tunnel 75 x 75 cm 3m long (0.563 m^2), target at 1.5m live bees on end of string, dead bees to simulate bees at rest,	contact	Flying bees 1.5 m/s 4.6 ± 1.9 µg/bee – 46000 µg /kg 2.5 m/s 6.6 ± 2.2 µg./bee – 66000 µg/kg 3.5 m/s 8.0 ± 2.1 µg/bee -80000 µg/kg Dead bees 1.5 m/s 4.1 ± 0.9 µg/bee -41000 µg/kg 2.5 m/s 6.7 ± 0.2 µg./bee – 67000 µg/kg 3.5 m/s 7.9 ± 0.5 µg/bee – 79000 µg/kg		(Ucar et al., 2003)
Sodium fluorescein (fluorescent tracer)	20g/ha	Flowering apple orchards (0.4 -1.6 Ha) bees collected at hive entrance every 20-30min	contact	Mean initial deposit 6.33 ng/bee – 63 µg/kg (1.62-20.84 ng/bee 16.2 - 208.4 µg/kg) decreased to below <5 ng/bee 30mins after end of application	Mean 3165 Max 10,420	(Koch and Weisser, 1997)
Sodium fluorescein (fluorescent tracer)	20g/ha	Flowering Phacelia (0.1 - 1.0 Ha) collected at hive entrance every 20-30min	contact	Mean initial deposit 4.52 -45.2 µg/kg (6.34-35.77 ng/bee, 63.4-357.7 µg/kg) <10 ng/bee 30mins	Mean 2260 Max	(Koch and Weisser, 1997)

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Active ingredient/LD50	Application rate	Method	Potential route of exposure	Residue µg/Kg	RUD	Reference
				after end of application	17885	

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Table 1.2: Data honeybee residue data from monitoring studies honey bees (µg/kg)

pesticide	LD50 µg/kg	Range detected in bees in field studies	France 2002-2005 (Chauzat et al., 2011) (Chauzat et al., 2009) live bees; mean (range)	France 2008-2009 (Wiest et al., 2011) live bees; max values only	Poland (Walorczyk and Gnusowski, 2009) dead bees; mean (range)	Italy 2000 (Ghini et al., 2004) dead bees; mean (range)	UK 1981-1991 (Greig-Smith et al., 1994) dead bees; range
aldicarb	2300-15640		13.1 (10.9-15.3)				
aldicarb sulfoxide			12.6 (>LOD-19.2)				
aldicarb sulfone			16.0 (11.0-21.0)				
aldrin	125500						850
alphacypermethrin	330-500						49-280
amitraz (varroacide)	*20-550*			*30*			
azinphos ethyl	630		ND			56 (20-94)	600-1300
azinphos methyl	550-4200		<LOD			69 (47-91)	
azoxstrobin							
benalaxyl	>1,000,000			<LOQ			
bendiocarb	1000						630-13000
boscalid	>1,660,000				33		
bromopropylate							333000
bupirimate	>50000			nd			
carbaryl	13000		214.3 (single sample)	<LOQ		21 (12-31)	60-21500
carbendazim	>500,000			66			
carbofuran	1600	3317-6367 (dead)	13.0 (11.0-14.9)	nd		148 (9-669)	
chlorpyrifos ethyl	59-590		ND	180	33 (10-56)	43(30-57)	2-13800
chlorpyrifos methyl	3800			nd		9 (1-36)	1300
coumaphos (varroacide)	*29000-203000*		*1545.6 (>LOD-24840)*	*47*		*208 (2-2777)*	
cyfluthrin	50-370		ND				

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pesticide	LD50 µg/kg	Range detected in bees in field studies	France 2002-2005 (Chauzat et al., 2011) (Chauzat et al., 2009) live bees; mean (range)	France 2008-2009 (Wiest et al., 2011) live bees; max values only	Poland (Walorczyk and Gnusowski, 2009) dead bees; mean (range)	Italy 2000 (Ghini et al., 2004) dead bees; mean (range)	UK 1981-1991 (Greig-Smith et al., 1994) dead bees; range
cypermethrin	3700	54-540 (dead)	ND	49			24-43
cyproconazole	>1,000,000		ND	nd			
2,4-D	607000-961000						1300
DDT	3900-96000						50
deltamethrin	510-6770		16.9 (>LOD-43.0)				40-230
demeton-S-methyl	26700						50-700
diazinon	0.129-3720			<LOQ		3 (1-7)	
dieldrin	1320-3210			nd			380-9000
diethofencarb	200,000			nd			
dimethoate	800-6610		ND	nd	1676 (258-4864)	19 (1-237)	20-21800
dinoseb							50
diquat	470,000-1,000,000						300
endosulfan	217,500-2,750,000		8.3 (>LOD-17.0)	nd			
epoxyconazole	>1,000,000		10.4 (>LOD-13.7)				
fenitrothion	180-4200		ND		718 (473-963)	544 (1-10330)	50-14000
fenoxycarb	>1,000,000			20		38 (30-157)	
fenthion	3080		ND			16 (2-38)	
fenvalerate	2110-63000						1-30
fipronil	41.7-59.3		0.5 (>LOD-0.7)		25 (10-64)		

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pesticide	LD50 µg/kg	Range detected in bees in field studies	France 2002-2005 (Chauzat et al., 2011) (Chauzat et al., 2009) live bees; mean (range)	France 2008-2009 (Wiest et al., 2011) live bees; max values only	Poland (Walorczyk and Gnusowski, 2009) dead bees; mean (range)	Italy 2000 (Ghini et al., 2004) dead bees; mean (range)	UK 1981-1991 (Greig-Smith et al., 1994) dead bees; range
Fipronil desulfinyl			1.2 (>LOD-2.5)				
Fipronil sulfone	64		0.4 (>LOD-0.6)				
Flusilazole	337500-1650000		11 (>LOD-18.0)	<LOQ			
Gamma HCH	2300-5600						2-40000
heptenophos						104 (68-162)	
hexaconazole			15.8 (>LOD-22.7)				
hexathiazox	>2,000,000			<LOQ			
imazalil	351,000-390,000			nd			
Imidacloprid	90-400		1.2 (>LOD-11.1)	nd			
6-chloronicotinic acid			1.0 (>LOD-1.7)				
Iprodione	>250,000->2,000,000			nd			
Lambda cyhalothrin	380-9600		18.6 (>LOD-47.0)				
Lindane	2000-5600		10.5 (3.0-17.4)				
malathion	2200-410000	960-5280 (dead)	ND			360 (1-3780)	120000
mercaptodimethur	3750		14.8 (>LOD-27.0)				
Mercaptodimethur sulfon			9.5 (>LOD-11.5)				
Mercaptodimethur sulfoxide			ND				
methidamiphos						23 (1-38)	
methidathion	2200-4200		ND			8 (1-18)	
methiocarb	3750					231 (184-346)	

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pesticide	LD50 µg/kg	Range detected in bees in field studies	France 2002-2005 (Chauzat et al., 2011) (Chauzat et al., 2009) live bees; mean (range)	France 2008-2009 (Wiest et al., 2011) live bees; max values only	Poland (Walorczyk and Gnusowski, 2009) dead bees; mean (range)	Italy 2000 (Ghini et al., 2004) dead bees; mean (range)	UK 1981-1991 (Greig-Smith et al., 1994) dead bees; range
methomyl	680-12900		ND				
mevinphos	700-5510		ND				
myclobutanil	>3,620,000		9.5 (>LOD-29.2)				
omethoate	830				411 (93-1156)	9 (4-14)	100-1100
oxamyl	3800-4700		ND				
Oxydemeton methyl	5400						250
paraquat	48000-64000						2000-43000
Parathion ethyl	1750	3600 (dead)	ND			45 (1-5)	
Parathion methyl	1650-3200	ND-2500	ND			139 (1-694)	
Penconazole	>50,000		7.5 (>LOD-<LOQ)				
permethrin	230-2100						500
Phentoate						1 (1)	
phosalone	89000			<LOQ	66	4 (4)	650-1000
phosmet	1400-4200			62		96 (96)	
phosphamidon	14600					30 (1-50)	
phoxim				nd		198 (19-355)	
Piperonyl butoxide				<LOQ			
pirimicarb	27900->540000						210
Pirimiphos ethyl	660					6 (1-30)	
Pirimiphos methyl	660-3900					6 (1-62)	300-103000
prochloraz	50000-6850000			nd	412		550
Procymidone			NA				

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pesticide	LD50 µg/kg	Range detected in bees in field studies	France 2002-2005 (Chauzat et al., 2011) (Chauzat et al., 2009) live bees; mean (range)	France 2008-2009 (Wiest et al., 2011) live bees; max values only	Poland (Walorczyk and Gnusowski, 2009) dead bees; mean (range)	Italy 2000 (Ghini et al., 2004) dead bees; mean (range)	UK 1981-1991 (Greig-Smith et al., 1994) dead bees; range
profenophos	40-34600					14 (7-17)	
Propiconazole	570,000-610,000		ND	<LOQ			
pyrazophos						22 (4-53)	
pyriproxyfen	1,000,000			<LOQ			
quinalphos	11600					23 (1-70)	92
Tau-fluvalinate (varroacide)	*184000*	*130-258*	*65.5 (>LOD-326)*	*53*			
Tebuconazole	830,000-2,000,000		18.2 (>LOD - 31.1)	nd	172(10-1146)		
temephos						235 (2-689)	
Tetraconazole	>1,000,000		17.3 >LOD-31.3)				
Thiophanate methyl	>1,000,000			5			
triazophos	550					7 (5-9)	
trifloxystrobin	>2,000,000						
triphenylphosphate				62			
vamidothion						15 (6-24)	
vinclozolin	>1,000,000		NA	nd	352 (185-657)		

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LD50 µg/kg based on bee weighing 0.1g –data from EPA Ecotox database, ANSES Agritox database, Greig-Smith et al (1994)
(Faucon et al., 2002) 14.6-29 µg/kg tau fluvalinate
(Ghini et al., 2004) 14 monitoring stations of 2 hives observed weekly, analysis of when threshold of 250 dead bees/week reached
(Wiest et al., 2011)16 apiaries in Pay de Loire 145 samples
(Chauzat et al., 2009) 24 apiaries at 5 sites across France to give a total of 120 hives.

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Country -region	Overview	Method	Results	Reference
Italy- Emilia-Romagna	21 monitoring stations of 2 hives 1995/96; observed weekly, analysis of dead bees when threshold of 350 dead bees/week reached	Multi-residue analysis + palynology; Use of index of pesticide toxicity based on toxicity and persistence of residues	1995 Threshold exceeded on 16 occasions (3.4%)of weekly observations. Only in 5 cases could the deaths be ascribed to pesticides (parathion, methyl parathion, lindane, endosulfan, dimethoate detected) associated with orchards and home/garden uses. 1996 Threshold exceeded on 10 occasions (2.2%)of weekly observations. Only in 9 cases could the deaths be ascribed to pesticides (acephate, azinphos methyl, chlorothalonil, dimethoate, formothion, methamidaphos, omethoate, parathion, vinclozolin, deltamethrin, diazinon detected) associated with orchards and vegetable crops	(Porrini et al., 1998)
Italy - Forli	24 monitoring stations of 2 hives 1983; observed weekly, analysis of dead bees when threshold of 500-700 dead bees/week reached	Multi-residue analysis	Threshold exceeded on 19 occasions: (dithiocarbamate, parathion (paraoxon) endosulfan, captafol, chlorbenside, methidathion detected)	(Celli et al., 1985)
Italy - Ferrara	1987 20 monitoring stations, 1988 28 monitoring stations, of 2 hives observed weekly, analysis of dead bees when threshold of 500-700 dead bees/week reached	Multi-residue analysis	1987 Threshold exceeded on 46 occasions, (dithiocarbamates, azinphos methyl, dimethoate detected) 1988 Threshold exceeded on 30 occasions, (dithiocarbamates, azinphos methyl, dimethoate detected)	(Celli et al., 1991)

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1.2.2. Spray drift and dust

Studies on residues deposited as dust are summarised in Table 1.3. Drift during agricultural treatment determines the deposition of pesticides within a small distance from the field edge (Ganzelmeier et al. 1995) and these standardised scenarios have been used to determine the drift of sprays onto field margins. Tremolada et al., (2010) used a similar scenario to identify dust drift in the air and on to vegetation. Based on stable atmospheric conditions they inferred that pesticide residues dispersing to air from the seed drill would be dispersed up to a height of 5 m over the sown fields with concentrations greater in close proximity to the seed drill and much lower further away from the point source due to both dispersion and deposition processes. Therefore they considered that the volume of air for consideration should correspond to the surface of the sowing area and a uniform height of 5 m. Based on a 7 Ha field this corresponded to 350,000 m^3 of air. If a bee crossed the treated site during sowing operations, exposure can be calculated by using the estimated contact air volume (ignoring the wing surface area) and the concentration of the insecticide in the air. Tremolada et al. (2010) calculated that the volume of air that a bee would come into contact with during foraging activity can be calculated by multiplying the flight distance over the treated fields from the hive (500 m) by the front surface area of the bee (12.5 mm^2 derived from the approximation of the bee body to a cylinder with the dimensions of 12–13 mm of length and 4 mm of height) equating to an air exposure volume of 0.00625 m^3. They recognised that a bee's anatomy is characterised by thick hairs on the body that will efficiently trap airborne particulates. Based on a drilling rate of 7.35 g ai/ha on treated seed, and if 1% of the active ingredient is present in exhaust dust, if there is uniform dispersion in this air package the concentration of the active substance in the air is 1.47 µg ai/ m^3. Using this theoretical air concentration an exposure level for bees flying to and from their foraging areas can be calculated by multiplying the air exposure volume by the insecticide air concentration, resulting in a dose of 9.2 ng/bee, a deposition rate of 6.25 ng/bee per 1µg ai/ m^3 . The far higher levels reported when bees were held in cages (Tapparo et al., 2012) reflects an artificial situation in which bees could not move through or away from the dust plume. This is demonstrated by the lower residues in live bees sampled at the hives following application of clothiandin treated seed in which residues of 1.8 ng/bee were detected (Chauzat, Martel et al. 2010) which suggests that the use of the concentration in air over the treated field may be protective but not unrealistic.

Obviously if the 1% dust is emitted and deposited directly on the ground immediately under the generated dust cloud then the mean insecticide deposition is 0.735 ng cm^{-2} (1% of the drilled rate).This does not take into account recent EU requirements to limit dust emissions through the use of professionally treated seeds and deflectors. Tremolada et al. (2010) also demonstrated that a theoretical deposition could be calculated for the front of the hive if dust was directly deposited onto the front of the hive. What is less obvious is how to calculate the drift onto flowering weeds in the field margin as dust drift is unlikely to behave in the same way as spray drift due to the wide variations in particle size. Unfortunately the deposition data generated to date for grasses and flowering weeds in flower margins have limitations in terms of the reporting of sowing rates and seed treatment rates. However, for the purpose of this discussion it has been assumed that rates of 0.5 mg ai/seed were used and 75,000 seeds/ha were sown on a 1Ha plot for both studies i.e. an application rate of 37.5 g ai/ha (Greatti et al., 2003; Greatti et al., 2006). Based on a 1% of the active ingredient emitted as dust deposited directly on the ground would be 37.5 µg/m^2. Measured residues of 21-58 and 32-124 µg/kg, equal to µg/m^2 for these studies, resulted in grasses and wild flowers respectively. Therefore, in the absence of dust reduction measures 1% emission may be a realistic assessment and deposition on weeds depends on density.

If the dust deposition on flowering weeds in used in the same way as a spray application (section 1.3.1) the residues in pollen/bee bread the residues from a 0.375g/ha dust deposition would be 0.015

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mg/kg (15 µg/kg) which is similar to reported values of 6-40 µg/kg in the absence of dust concentration information (Chauzat et al., 2010b; Kruype et al., 2012)).

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Table 1.3: Residue data from studies with dust

Active ingredient	Application rate	Method	Residue	Reference
Clothianidin	1.25 mg/seed	Maize dusts <3g/100Kg Drill modified but no deflectors	Dusts 140 µg/m^2 after 1 hr of drilling, air 10m residues 0.8-1.5 µg/m^3PM10 0.2-1.2 µg/m^3 Residues in caged bees downwind 1m 1394 ± 0.6 ng/bee 2.25m 808 ±0.2 4.5m 64 ±4 6.75m 164 ±4 9m 101 ± 0.7 Calculated 0.55-1.84% of insecticide released	(Tapparo et al., 2012)
clothianidin	Bees held in cages alongside drilled maize field	10 bees /cage and 36 bees/cage upwind and downwind of drilling	10 of 10 downwind died within 5-10h but no others died	(Marzaro et al., 2011)
clothianidin	Bees held in cages alongside drilled maize field	30 bees /cage downwind of drilling	5 of 30 at low humidity (unknown) 22 of 30 died at high humidity (73%) Residue in bees 279 ± 142 ng/bee at high humidity, 514 ±175 ng/bee at lab humidity	(Marzaro et al., 2011)
Clothianidin	Dust generated during maize drilling Info on levels of Nosema spores and virus in dead bees	Field monitoring following colony losses; large amounts of osr pollen in bee bread (82%)	1.8 ng/bee 25 and 40 µg/kg bee bread	(Chauzat et al., 2010b)
clothianidin	Dust generated from pneumatic drill during maize drilling; area treated and treatment rate not stated	Dust on planter following planting Links with levels in pollen returned to hive	4900-15030 ppm In hive pollen 2.9 ±1.3 – 10.7±2.3 µg/kg	(Kruype et al., 2012)
fipronil	0.5 mg/seed	Maize dusts <3g/100Kg Drill modified but no deflectors	Calculated 1.37% insecticide released air 10m residues 0.8-1.5 µg/m^3PM10 0.2-1.2 µg/m^3	(Tapparo et al., 2012)
Imidacloprid	0.5 mg/seed	Maize dusts <3g/100Kg Drill	air 10m residues 0.8-1.5	(Tapparo et al., 2012)

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		modified but no deflectors	µg/m^{3}PM10 0.2-1.2 µg/m^3	
Imidacloprid	Dust generated from pneumatic drill during maize drilling 1Ha; 75000 seeds/ha; rate per seed not stated	Residues on filter papers at fan outlet for 240 seconds and grass and flowers from border of treated field	0.5 -1 µg/min output onto filter paper Grass mean 58.2 µg/kg (1m^2) Flowers mean 123.7 µg/kg (1m^2) Heavy rain reduced residues to below LOD	(Greatti et al., 2006)
Imidacloprid	Dust generated from pneumatic drill during maize drilling; area treated and treatment rate not stated	Residues on filter papers at fan outlet for 120 seconds and grass and flowers from border of treated field	Grass 21 ±5 µg/kg (1m^2) Flowers 32 ±15 µg/kg (1m^2)	(Greatti et al., 2003)
metalaxyl	Dust generated from pneumatic drill during maize drilling; area treated and treatment rate not stated	Dust on planter following planting	92-263 ppm	(Kruype et al., 2012)
thiamethoxam	0.6 mg/seed	Maize dusts <3g/100Kg Drill modified but no deflectors	Calculated 1.85% insecticide released air 10m residues 2.8 µg/m^{3}PM10 1.6 µg/m^3	(Tapparo et al., 2012)
thiamethoxam	Bees held in cages alongside drilled maize field	30 bees /cage downwind of drilling	15 of 30 at low humidity (unknown) 28 of 30 died at high humidity (73%)	(Marzaro et al., 2011)
thiamethoxam	Dust generated from pneumatic drill during maize drilling; area treated and treatment rate not stated	Dust on planter following planting	70-13240 ppm In hive pollen 6.2 ±4.9 – 20.4±2.7 µg/kg	(Kruype et al., 2012)
trifloxystrobin	Dust generated from pneumatic drill during maize drilling; area treated and treatment rate not stated	Dust on planter following planting	3.8-503 ppm	(Kruype et al., 2012)

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1.2.3. Contact residues

Contact of bees with treated surfaces may occur through resting on treated leaves or during foraging on treated flowers. The major issue is that bees do not come into contact with the treated plant over the entire surface of their body but primarily through their feet; however during resting and cleaning they may transfer residues from their feet to other parts of their bodies. Therefore a worst case scenario would be to assume that contact exposure is directly related to surface area of the bee in the same way a direct overspray, i.e. a mean of 3.4% based on the treated surface area as identified above (section 1.2.1).

Unfortunately, there are few studies (Table 1.4) which assess the dislodgeable residue on the surface of leaves and the toxicity of the leaf surface to honeybees. However, the single study providing this information (Chukwudebe et al., 1997) showed high mortality when bees were in contact with emamectin benzoate treated leaves. The contact LD_{50} of emamectin benzoate is 0.0027 µg/bee (2.7 ng/bee) and a surface containing 3.6 ng/cm^2 resulted in 46% mortality. Therefore, to result in the LD_{50} the bee needs to come into contact with 0.75 cm^2 which is close to the 0.93 cm^2 surface area of the body (Johansen et al., 1983). Such a measure may be useful in extrapolating to other bee species with differing surface areas, e.g. leafcutter bee (0.47cm^2 and alkali bee (1.65 cm^2) (Johansen et al., 1983). This suggests that dislodgeable foliar residue data with the information on surface area of the bee may be more useful than only direct overspray data, in assessing the additional risk to bees from applications to foliage in flowering crops, e.g. over time.

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Table 1.4: Residues related to exposure through contact with treated surfaces

Active ingredient	Application rate	Method	Residue	Reference
Emamectin benzoate	0.0168 kg ai/ha	Contact with treated alfalfa leaves sampled over time; dissipation half life 10 hours samples taken at 3, 8 and 24 hrs	9.1 ng/cm^2 =100% mortality; 3.6 mg/cm^2 = 46% mortality, 1.3 mg/cm^2= 3% mortality	(Chukwudebe et al., 1997)
ethyl parathion	Aerial application on sunflowers, 1.1 kg/ha	leaves	Day 1 41000-107000 µg/kg Day 5 12000-20000	(Cox et al., 1986)

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1.2.4. Pesticides in plant matrices

1.2.4.1. Pesticides in pollen

The exposure of bees to pesticides in pollen depends on both the residues present and the amounts of pollen collected by the bees.

The amount of pollen collected by a colony per day is highly variable and depends on both the pollen availability and the needs of the colony. For example in snap beans annual collections ranged from 57-96 g/ day/ hive to 10-17 g /day/hive (Erickson et al., 1994). On oilseed rape the amount of pollen collected varied with the stage of flowering with most collected in the latter stages (Figure 1.1) (Free and Ferguson, 1980).

The collection of pollen also varies according to crop species, (Wallner, 1997) reported that vine flowers produce no nectar and only approximately 1mg of pollen is yielded by a flower cluster. At optimal foraging approximately 2000 bees can be expected to forage per ha and 250g of vine pollen will be returned to the hive per day. The structure of the vine flowers differs from many others in that closed buds are closely associated with open flowers and thus pesticides applied pre-flowering may be transferred by contact to bees foraging on neighbouring open flowers.

Three methods have been used for collection of pollen for residue analysis:

Collection directly at the flower (Table 1.5).

Collection in pollen traps at the hive (Table 1.6).

Collection of processed pollen (bee bread) from within the hive (Table 1.10 discussed later in relation to stores within the hive).

Obviously residues in pollen differ considerably depending on the route of exposure with residues following spray applications exceeding those following systemic treatment via seed coatings or soil applications.

Collection of pollen at the flower can be very time consuming and there are a limited number of reported data (Table 1.5). Only 2 studies which provide sufficient data to enable calculation of the residue per unit dose (RUD) from directly collected pollen which showed a range of 40-43. Collection of pollen at the hive in traps in field studies (Table 1.6) has the complexity of multiple sources of such pollen not just from the treated field and this is demonstrated by the generally lower peak RUDs calculated for the field studies than in the tunnel studies reported (RUDs15-43) and the field studies in which known oilseed rape foragers were collected (RUDs 4.1-28). However, such pollen may more representative of the pollen entering the hive as shown by the monitoring studies in which pollen was collected at the hive (Table 1.7). Therefore for spray applications, with the limitations of the reporting of data in the literature, the mean RUD for all methods of pollen collection (RUD - mg/Kg in pollen per 1Kg/ha pesticide applied) is 7.9 and the 90th percentile is 21.1, with a 95th percentile of 24.8. More studies should be undertaken to determine residues in semi-field studies in which bees can only forage on the treated crop to ensure a robust RUD value is generated.

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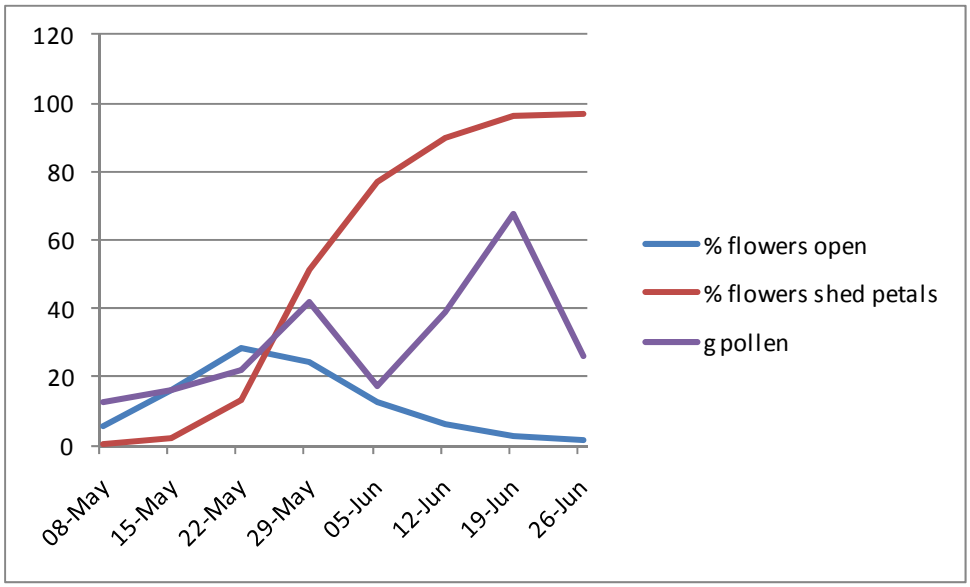

Figure 1.1 Mean % oilseed rape flowering and amount of oilseed rape pollen (per week) returned to colonies situated in UK oilseed rape fields with a mean area of 27Ha (Free and Ferguson, 1980).

For seed and soil treatments the data are summarised separately from spray applications in Table 1.6. A similar approach has been taken in assessing the residue in pollen as a function of the treatment rate (in some cases the treatment rate has been assumed due to the lack of information provided in the reports).

The data are limited and would be improved by consideration of the application rate per hectare rather than the rate per seed as drilling rate may affect the residue present and the data may also then be applicable to granules and potentially follow-on crops. However based on the seed application rate it can be determined that the concentration in pollen (mg/kg) can be calculated from the seed treatment rate using a RUD of between 0.002 and 0.086.

Monitoring data in Table 1.7 shows that residues are lower than those reported in field studies suggesting field studies are realistic but worst-case. Varroacide residues (coumaphos, tau-fluvalinate) are present even in pollen collected at the hive entrance, presumably transferred from the external surface of the bees.

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Table 1.5: Residues in pollen collected from flowers

Active ingredient	Application rate	Method	Residue µg/kg	Peak mg/kg based on application rate of 1 Kg/ Ha	Reference
cypermethrin	Spray application 44 g ai/ha	Oilseed rape field study	0 day 1900 1 day 110 2 day 70	43.1	(Fries and Wibran, 1987)
Dimethoate	304 ppm applied to run off	Spray application to potted alfalfa (*Medicago sativa*)	1 day 500± 400 traces at 8, 10, 12 and 14 days		(Barker et al., 1980)
malathion	Aerial application 210 ml/ha 32 ha oilseed rape in full flower	Oilseed rape field study	1 hr after spraying 5700 ± 1600 2hrs 2000 4 hrs 500		(Pankiw and Jay, 1992)
Metalaxyl	Dust generated at sowing	Maize pollen field study	3.1		(Kruype, Hunt et al. 2012)
Methyl parathion microencapsulated	Birdsfoot trefoil treated at 1 kg ai/ha	2 nucleus colonies placed on edge of 0.5ha treated area every 48 hrs until 8 nuclei present, Identification of microcapsules in stored pollen	100% of pollen loads on bees contaminated on first 2 days post-spray decreasing to 32% on 9th day post-spray; 79% of all foragers had contaminated pollen loads over 10 day period		(Burgett and Fisher, 1980)
PP321 (pyrethroid)	Spray application 5 g ai/ha	Oilseed rape field study	0 day 200 1 day 500 2 day 10	40	(Fries and Wibran, 1987)
trifloxystrobin	Dust generated at sowing	Maize pollen field study	1.7		(Kruype et al., 2012)

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Table 1.6: Residues in pollen collected at the hive entrance

Active ingredient	Application rate	Method	Residue µg/kg	Peak mg/kg based on application rate of 1 Kg/ Ha	Reference
Boscalid	500 g ai/ha 8 ha flowering oilseed rape	9 colonies 200m from field, bees collected directly at hive only rape pollen collected	0 day 13900 1 day 26200 2 day 4700 7 days 3000	27.8	(Wallner, 2009)
Captan	10 Ha Apples 2000 g ai/ha	10 colonies samples combined to single sample per day field study	3 day 18970 ± 2400 4 day 14660 ± 2800 5 day 5540 ± 200 6 day 290 ± 70 9 day 200 ± 30 10 day 160 ± 30 11 day 150 ± 30 12 day 40 ± 4	9.5	(Kubik et al., 2000)
Carbaryl	Aerial spray application 1.1 kg/ha 65Ha	Alfalfa field study	240	0.22	(Stanger and Winterlin, 1975)
carbofuran	1.1 Kg ai/ha, 8 ha field alfalfa Furadan	6 Colonies moved adjacent to treated field before application	1 day 10183-11517 2 days 5100-6600 3 days 1233-1500 4 days 316-717 5 days 233 6 days 33	10.5	(Moffett et al., 1986)
carbofuran	1.1 Kg ai/ha, 9 ha field alfalfa Furadan + sticker	6 Colonies moved adjacent to treated field before application	1 day 4550 2 days 4267 3 days 3317 4 days 1517 5 days 1000 6 days 67	4.1	(Moffett et al., 1986)

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Active ingredient	Application rate	Method	Residue µg/kg	Peak mg/kg based on application rate of 1 Kg/ Ha	Reference
chlorantraniprole	Spray application 60 g ai/ha	flowering Phacelia 2 tunnels	1 day 2601 3 days 763 7 days 264	43	(Dinter et al., 2009)
diazinon	Apples in flower 15L/ha	2 hives per site 2 sites- field study	1 day 1980 6 days 140-180 10 days 30-90		(Skerl et al., 2009)
dichlofluanid	4.5 Ha, 5kg euparen /ha spray application	Strawberry 4 colonies field study	2 days 256 ± 58 7 days 12.9 ± 5.1 13 days 0.9 ± 0.3		(Kubik et al., 1992)
difenoconazole	10 Ha Apples 200 g ai/ha	10 colonies, samples combined to single sample per day field study	3 day 166 ± 3 4 day 191 ± 3 5 day 20 ± 1 6 day 29 ± 1 9 day 19 ± 2 10 day 33 ± 2 11 day 23 ± 8 12 day 43 ± 9	0.83	(Kubik et al., 2000)
difenoconazole	Apples in flower 50 g/ha	2 hives per site 2 sites field study	1 day 10 6 days <LOD (10)	0.2	(Skerl et al., 2009)
Endosulfan	Spray application 525 g ai/ha	2003-2004 Mustard –bees collected at crop 3 replications	0hr 2224 ± 20 1hr 1553 ± 20 4hr 1235 ± 13 8hr 892 ± 3 24hr 745 ± 5 48hr 398 ± 6 72hr 125 ±1 120hr <LOD 240hr <LOD	4.2	(Choudhary and Sharma, 2008)
Endosulfan	Spray application 525 g ai/ha	2004-2005 Mustard –bees collected at crop 3	0hr 2127 ± 73 1hr 1412 ± 22	4.1	(Choudhary and Sharma, 2008)

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Active ingredient	Application rate	Method	Residue µg/kg	Peak mg/kg based on application rate of 1 Kg/ Ha	Reference
		replications	4hr 1102 ± 2 8hr 792 ± 3 24hr 592 ± 3 48hr 308 ± 0.1 72hr 97 ± 6 120hr <LOD 240hr <LOD		
ethyl parathion	Aerial application on sunflowers, 1.1 kg/ha	6 colonies field study	Day 1 3700 µg/kg	3.4	(Cox et al., 1986)
Flufenoxuron	Phacelia 40 g/ha	Tunnel -Germany	60-600	15	Flufenoxuron DAR
Flufenoxuron	Phacelia 40 g/ha	Tunnel -Germany	40-730	18.3	Flufenoxuron DAR
Flufenoxuron	Phacelia 40 g/ha	Field Spain	30-60	1.5	Flufenoxuron DAR
Flufenoxuron	Phacelia 40 g/ha	Field Italy	<10		Flufenoxuron DAR
Flufenoxuron	Phacelia 40 g/ha	Field France	10-690	17.3	Flufenoxuron DAR
Flufenoxuron	Phacelia 40 g/ha	Field France	20-73 (3620 noted as unrealistic)	1.8	Flufenoxuron DAR
Flufenoxuron	Phacelia 40 g/ha	Vineyard France	10-60	1.5	Flufenoxuron DAR
fluvalinate	144 g/ha	applied to apple trees in flower –field study	262 day 1 138 day 2 75 day 3 28 day 4 5 day 5 <LOD day 6	1.8	(Haouar et al., 1990)
Iprodione	4.5 ha in flower cherry plantation sprayed 0.75 kg ai/ha 6 days preflower and 0.188 kg ai/ha during flower	5 colonies pollen trap sampling time during flowering	Mean 33.2 ± 38.1 (max after prespray application 118 , max post spray application 57.2)	Prespray 0.16 Postspray 0.30	(Kubik et al., 1999)
Lambda-cyhalothin	Spray application 75 g ai/ha	2003-2004 Mustard –bees collected at crop 3 replications	0hr 1672 ± 4 1hr 1264 ± 125 4hr 1023 ± 33	22.3	(Choudhary and Sharma, 2008)

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Active ingredient	Application rate	Method	Residue µg/kg	Peak mg/kg based on application rate of 1 Kg/ Ha	Reference
			8hr 806 ± 47 24hr 437 ± 15 48hr 197 ± 3 72hr 43 ±3 120hr <LOD 240hr <LOD		
Lambda-cyhalothin	Spray application 75 g ai/ha	2004-2005 Mustard –bees collected at crop 3 replications	0hr 1612 ± 29 1hr 1161 ± 2 4hr 951 ± 19 8hr 771 ± 10 24hr 442 ± 10 48hr 163 ± 4 72hr 11 ±1 120hr <LOD 240hr <LOD	21.4	(Choudhary and Sharma, 2008)
Malathion	Aerially applied bait spray 159 ml malathion /ha	Collection of pollen samples and dead bees after weekly applications –field study	Pollen 240-7640		(Gary and Mussen, 1984)
Methyl parathion	Flowering cotton sprayed rate not known	5 hives –field study	600		(Robertson and Rhodes, 1992)
Methyl parathion (microencapsulated)	1.1 Kg ai/ha, 8 ha field alfalfa Penncap M	6 Colonies moved adjacent to treated field before application	1 day 2168-2177 2 days 263-942 3 days 63-275 4 days 33-42 5 days 2-43 6 days ND-8	2.0	(Moffett et al., 1986)
Methyl parathion (microencapsulated)	1.1 Kg ai/ha, 9 ha field alfalfa Penncap M + sticker	6 Colonies moved adjacent to treated field before application	1 day 1907-2342 2 days 860-1513 3 days 80 4 days 20 5 days 40-65	2.1	(Moffett et al., 1986)

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Active ingredient	Application rate	Method	Residue µg/kg	Peak mg/kg based on application rate of 1 Kg/ Ha	Reference
			6 days 25		
Methyl parathion microencapsulated	Spray application 0.5 lb ai/acre (0.56 kg/ha)	Alfalfa - field study	70-2330	4.2	(Johansen and Kious, 1978)
Methyl parathion microencapsulated	Spray application 0.5 lb ai/acre (0.56 kg/ha)	Alfalfa - field study	10-6630	11.8	(Johansen and Kious, 1978)
Methyl thiophanate	4.5 ha in flower cherry plantation sprayed 1 kg /ha 6 days preflower and 1 kg /ha during flower	5 colonies pollen trap sampling time during flowering –field study	Mean 319 ± 332 (max after prespray application 1195, max post spray application 1000)	Prespray 1.2 Post spray 1.0	(Kubik et al., 1999)
monocrotophos	Aerial spray application 0.6 kg/ha 65Ha	Alfalfa field study	280	0.47	(Stanger and Winterlin, 1975)
procymidone	4.5 Ha, 0.75 kg ai/ha spray application	Strawberry 4 colonies field study	3 days 31.1 ± 6.1 9 days 4.9 ± 0.5	0.04	(Kubik et al., 1992)
Spiromesifen	Spray application 225 g ai/ha	2003-2004 Mustard –bees collected at crop 3 replications	0hr 2101 ± 7 1hr 1437 ± 19 4hr 1151 ± 9 8hr 950 ± 7 24hr 719 ± 31 48hr 317 ± 67 72hr 2 ±0.1 120hr <LOD 240hr <LOD	9.3	(Choudhary and Sharma, 2008)
Spiromesifen	Spray application 225 g ai/ha	2004-2005 Mustard –bees collected at crop 3 replications	0hr 1827± 42 1hr 1242 ± 8 4hr 1037 ± 14 8hr 900 ± 48 24hr 592 ± 64 48hr 296 ± 31 72hr 4 ±2	8.1	(Choudhary and Sharma, 2008)

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Active ingredient	Application rate	Method	Residue µg/kg	Peak mg/kg based on application rate of 1 Kg/ Ha	Reference
			120hr <LOD 240hr <LOD		
thiacloprid	Apples in flower 96 g/ha	2 hives per site 2 sites - field study	1 day 90 6 days <LOD(10)- 30 10 days <LOD (10)	0.94	(Skerl et al., 2009)
Vinclozolin	4.5 ha in flower cherry plantation sprayed 375 g ai./ha	5 colonies pollen trap sampling time during flowering –field study	Mean 393 ± 422 (max 1518)	4.1	(Kubik et al., 1999)

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Seed and soil treatments

Active ingredient	Application rate	Method	Residue µg/kg	Peak mg/kg based on application rate of 1mg/ seed	Reference
Chlorantraniprole	Soil application 253.5 g ai/ha	flowering Phacelia 2 tunnels	3 days 10 7 day 18		(Dinter et al., 2009)
Clothianidin	0.03 mg/seed	Flowering oilseed rape	Max 2.59	0.086	(Cutler and Scott-Dupree, 2007)
Clothianidin	Rate not given assumed 0.6mg/seed	Maize pollen	3.9	0.0065	(Kruype, Hunt et al. 2012)
Fipronil + 3 derivatives	Rate not given	Sunflower, corn, buckwheat pollen collected at flowers	Mean 0.42 max 8.3		(Bonmatin et al., 2007)
Fipronil + 3 derivatives (77% fipronil)	Rate not given	Sunflower, corn, buckwheat pollen – pollen trap	Mean 0.29 max 4.3		(Bonmatin et al., 2007)
Imidacloprid	1 mg/seed	Radiolabelled in sunflower	<0.5-36 (mean 13) Residues in pollen similar to floret dish which was 33% that of the top stem	0.036 (mean 0.013)	(Laurent and Rathahao, 2003)
Imidacloprid	Commercial treated fields use rate not reported – assumed 1 mg/seed	Survey in France of treated maize fields	2.1 ± 2.7	0.002	(Bonmatin et al., 2005)
Imidacloprid	Rate not given- assumed 0.7 mg/seed	Review of sunflower data	3.0	0.004	(Bonmatin et al., 2005)
Imidacloprid	1mg/seed	Seed treatment on maize, samples of leaves over time and on pollen	Pollen <1 (panicles 2) at flowering (day 130) Leaves 253 ± 50 day 30 13 ± 3 day 45 5 ± 1 day 60 2 ± 1 day 80	<0.001	(Donnarumma et al., 2011)
imidacloprid	Not given –assumed 0.7	sunflower pollen (n=24)	Mean 3 (1-11)	0.015	(Bonmatin et al., 2003)

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Active ingredient	Application rate	Method	Residue µg/kg	Peak mg/kg based on application rate of 1mg/ seed	Reference
	mg/seed				(Bonmatin et al., 2007)
imidacloprid	Not given –assumed 1.0 mg/seed	Maize pollen (n=5)	Mean 2 (1-3)	0.003	(Bonmatin et al., 2003) (Bonmatin et al., 2007)
imidacloprid	0.7 mg/seed radiolabelled	Sunflower grown in greenhouse -22 plants	3.9 (no detectable residues in field grown plants LOD 10)	0.0056	(Schmuck et al., 2001)
imidacloprid	0.7 mg/seed	Sunflower field 54 samples; 9 sites	<LOQ (10)		(Schoning and Schmuck, 2003)
imidacloprid	1 mg/seed	Maize field 18 samples 9 sites	<LOQ (10)		(Schoning and Schmuck, 2003)
imidacloprid	0.05 mg/seed	Oilseed rape field 80 samples 9 sites	<LOQ (10)		(Schoning and Schmuck, 2003)
thiamethoxam	Rate not given – assumed 1.0	Maize pollen	1.7	0.0017	(Kruype et al., 2012)
thiamethoxam	315 g s.a./100 Kg = 1.2mg/seed @ 0.38g/seed	maize	<1-15	0.0125	AFSSA 2007

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Table 1.7: Monitoring data for residues in pollen collected at the hive entrance at hive (µg/kg)

pesticide	Range of residues collected in field studies	France 2006 (Chauzat et al., 2006) mean	France 2009 (Chauzat et al., 2009) mean	France 2002-2005 (Chauzat et al., 2011) mean (range)	France 2008-2009 (Wiest et al., 2011) 130 samples (max)
aldicarb		19.2		ND	
Aldicarb sulfon				ND	
Aldicarb sulfoxide				ND	
Amitraz (varroacide)					*115*
Azinphos methyl		ND (LOD 57)	<LOD	ND	
benalaxyl					nd
bupirimate					<LOQ
buprofezine					nd
Carbaryl	240	218.7	142.4	142.4 (93-276.9)	15
carbendazim					2595
Carbofuran	33-11517	14	32.7	32.7 (>LOD-137.5)	2
Chlorpyrifos ethyl		ND (LOD10)		35.0	140
Chlorpyrifos methyl					nd
Coumaphos(varroacide)		*925*	*423.5*	*423.5 (>LOD -1700)*	*40*
cyfluthrin		ND (LOD 7)		ND	
cypermethrin		ND(LOD 3.8)		ND	nd
cyproconazole		7.5		7.5 (>LOD -<LOQ)	22
Deltamethrin		ND(LOD 0.1)	39.0	39.0	
diazinon	30-1980				nd
dieldrin					<LOQ
diethofenocarb					3
dimethoate		ND(LOD 18)		ND	<LOQ
Endosulfan	97-2224	81.2	45.8	45.8(>LOD -340)	nd
epoxyconazole		ND(LOD 5)		ND	
fenitrothion		ND(LOD 19)		ND	
fenoxycarb					nd
Fenthion		ND(LOD 8)		ND	
Fipronil	Mean 0.42 max 8.3 (fipronil + 3 derivatives)	1.2	1.2	1.2 (>LOD -<LOQ)	

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pesticide	Range of residues collected in field studies	France 2006 (Chauzat et al., 2006) mean	France 2009 (Chauzat et al., 2009) mean	France 2002-2005 (Chauzat et al., 2011) mean (range)	France 2008-2009 (Wiest et al., 2011) 130 samples (max)
Fipronil desulfinyl		1.3	1.0	1.0 (>LOD-1.5)	
Fipronil sulfone		1.2	1.7	1.7 (>LOD-3.7)	
Flusilazole				16.2 (>LOD-71)	52
hexaconazole		18.0		54.7 (11-106)	
hexythiazox					nd
imazalil					nd
Imidacloprid	<0.5-36	1.2	0.7	0.9 (>LOD-5.7)	<LOQ
6-chloronicotinic acid		1.2	1.2	1.2 (>LOD-9.3)	
iprodione	33.2 ±38.1				<LOQ
Lindane		ND(LOD 0.1)	7.0	7.0 ((6.0-9.0)	
Malathion	240-7640	ND (LOD 9)		ND	
Mercaptodimethur		ND (LOD 5)		ND	
Mercaptodimethur sulfon				ND	
Mercaptodimethur sulfoxide				ND	
Methidathion		ND (LOD 13)		ND	
methomyl		ND (LOD 5)		ND	
mevinphos		ND (LOD 3.8)		ND	
myclobutanil		13.9		13.5 ((>LOD-20.3)	
Oxamyl		38.4		38.4	
Parathion ethyl	3700	19.2		19.2 (>LOD- <LOQ)	
Parathion methyl	8-6630	24.8		24.8 (>LOD-<LOQ)	
Penconazole		23.6	17.6	17.6 (>LOD-176)	
phosalone					nd
phosmet					78
phoxim					nd
Piperonyl butoxide					nd
prochloraz					nd
Propiconazole		ND (LOD 5)	<LOD	ND	nd
pyriproxyfen					<LOQ

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pesticide	Range of residues collected in field studies	France 2006 (Chauzat et al., 2006) mean	France 2009 (Chauzat et al., 2009) mean	France 2002-2005 (Chauzat et al., 2011) mean (range)	France 2008-2009 (Wiest et al., 2011) 130 samples (max)
Tau-fluvalinate (varroacide)	5-262	487.2	334.1	334.1 (>LOD-2020)	85
Tebuconazole		12.3	16.5	16.5 (>LOD-33.2)	nd
Tetraconazole		ND (LOD 5)	<LOD	ND	
Thiophanate methyl					3674
triphenylphosphate					<LOQ
vinclozolin	393 ± 422				70

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1.2.4.2. Residues in nectar

Flower morphology is an important factor in the pesticide content of nectar: flowers in which the nectar is deeper, such as clover, were less contaminated (0.005 µg parathion/flower) than shallower flowers such as cabbage (0.027 µg/flower) and nectar yield/flower was less important in determining pesticide content (Celli and Porrini, 1988)

The amount of nectar consumed by bees whilst foraging and thus their ability to return toxic nectar to the hive was evaluated by Waller et al. (1979) who assessed the effects on foraging by honeybees. The bees continued to make foraging trips to contaminated sucrose solutions until 2.9- 3.9 µg/bee had been consumed (20-25 times the oral LD_{50}). Therefore a mean of 45 trips to a 1ppm solution or 11 trips to a 5ppm solution were observed before foraging ceased (50% acetylcholinesterase inhibition). Therefore bees effectively consumed 5% of the dimethoate and transferred 95% of the nectar collected to the hive. This is confirmed by observations by Gary and Lorenzen (1976) (cited by Gary and Mussen, 1984) who reported that bees 5- 10% of nectar in the honey stomach may be involuntarily taken into the proventriculus during foraging. Thus for a bee carrying 60µl nectar in the honey stomach (Schmid-Hempel et al., 1985) the bee may consume up to 6 µl during the foraging flight.

The residues in nectar collected directly from plants or from the honey stomach of returning foragers is shown in Table 1.8. For nectar, again within the limitations of the reported data, the mean RUD for nectar collected directly from the plant or from the honey stomach of foragers returning to the hive (RUD - mg/Kg in nectar per 1Kg/ha pesticide applied) is 5.3 and the 90th percentile is 11.3, with a 95th percentile of 11.9. There are only two studies which permit the calculation of the RUD for nectar from use as seed and soil treatments and the RUD (mg/Kg pollen per 1 mg/seed) is 0.05-0.075. In addition, recent patent applications suggest there may be wide variations in systemicity based on formulation (e.g. Dieckmann, 2010). As with pollen there is a need to generate further data under semi-field conditions to develop robust RUD values for pollen and nectar from spray and seed/soil applications.

1.2.4.3. Aphid honeydew.

Honey bees are known to collect honeydew primarily from tree-feeding aphid species such as *Sternorrhyncha* but they they have also been regularly shown to exploit honeydew from aphid-infested cereal and other crops, e.g. potatoes (Maurizio, 1985). Other bee species have also been reported to collected honeydew such as Osmia (Konrad et al 2009) and bumble bees (Bishop 1994).

The sugar content of aphid honeydew is highly influenced by both the species of aphid and the host plant (Fischer and Shingelton 2001). Hogervorst et al (2007) and Wykes (1953) reviewed the sugar content of aphid honeydews from different aphid species and host plants. Aphids excrete not only the sugars taken up from the plant phloem but also synthesize specific sugars, melezitose, to attract ants. These species-specific differences are demonstrated with aphids on some plants excreting up to 30-70% melezitose whilst other species of aphid not tended by ants excreting no melezitose (Fischer and Shingelton 2001). Leroy et al (2011) reported plant derived phloem sµgars comprised 67-89% of the sµgar content. Due to the small volume of exudates available Fischer and Shingleton (2001) could not ascertain the actual content of the sugars in aphid honeydew but did report wide differences between the presence of ants on the sugar composition of excreta of 3 species of aphids feeding on two species of poplar.

Hogervorst et al (2007) identified that although honeydew may be a food source (e.g. for parasitoids) it can be far inferior to nectar based on sugar composition, amino acid composition and the presence

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of plant secondary chemicals. However, honeydew on crops may be a significant percentage of the sugar flow into colonies at times of limited, or less attractive, alternative forage.

Santas, (1985) reported honeydew excreted by the soft scale *Parthenolecanium corni* which is associated with filbert trees (*Corylus avellana*), Prunus, Rosaceae and numerous other plants is a major source of honeydew for honeybees in Greece. In Greece honeydew excretion is a significant source of bee forage in April to early June. In some cases changes can be made to the timing of insecticide application practices to control honeydew producing aphids according to their biology, e.g. by use of oils in the dormant season or by reducing populations juvenile populations of those species such as *Parthenolecanium corni* which have a single generation per year.

To date, there are no reports of pesticide residues in aphid honeydew after spray application but the intake by bees may be expected to be similar to that of nectar sources.

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Table 1.8: Residues in nectar collected at plant or honey stomach from foragers

Pesticide	Application rate	method	Peak residue µg/Kg	RUD	Reference
Acephate (+ metabolite methamidophos)	Spray application 750 g ai/ha 1 day before flowering	raspberry	1 day 14390 acephate/1130 methamidophos 6 days 2800 acephate/300 methamidophos 11 days 800 acephate/ 90 methamidophos 13 days 450 acephate/ 40 methamidophos	20.7	(Fiedler, 1987)
Acephate (+ metabolite methamidophos)	Spray application 750 g ai/ha 13 days before flowering	raspberry	1 day 800 acephate/ 150 methamidophos 6 days 150 acephate/ 40 methamidophos	1.2	(Fiedler, 1987)
Acephate (+ metabolite methamidophos)	Spray application 750 g ai/ha 2 days before flowering	raspberry	5 days 1990 acephate/ 330 methamidophos	3.09	(Fiedler, 1987)
Acephate (+ metabolite methamidophos)	Spray application 750 g ai/ha 2 days before flowering	cherry	2 days 2840 acephate/ 270 methamidophos 7 days 1690 acephate/ 140 methamidophos	4.1	(Fiedler, 1987)
Acephate (+ metabolite methamidophos)	Spray application 750 g ai/ha 20 and 5 days before flowering	raspberry	3 days 2150 acephate/ 200 methamidophos 6 days 450 acephate/ 40 methamidophos	3.1	(Fiedler, 1987)
Acephate (+ metabolite methamidophos)	Spray application 750 g ai/ha 3 days before flowering	cherry	7 days 1950 acephate/ 180 methamidophos 16 days 190 acephate/ 20 methamidophos	2.8	(Fiedler, 1987)

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Pesticide	Application rate	method	Peak residue µg/Kg	RUD	Reference
Acephate (+ metabolite methamidophos)	Spray application 750 g ai/ha 9 days before flowering	cherry	0 days 850 acephate/ 80 methamidophos 6 days 320 acephate/ 50 methamidophos 11 days 210 acephate/ 30 methamidophos 14 days 200 acephate/ 40 methamidophos	1.2	(Fiedler, 1987)
Acephate (+ metabolite methamidophos)	Spray application 750 g ai/ha 1 day before flowering collection by holding flowers in plastic bags	apple	7 days 1400-8440 acephate/ 150 -650 methamidophos 14 days 730 acephate/ 80 methamidophos	11.3	(Fiedler, 1987)
Acephate (+ metabolite methamidophos)	Spray application 750 g ai/ha 3 days before flowering collection by holding flowers in plastic bags	apple	9 days 2250 acephate/ 90 methamidophos	4.2	(Fiedler, 1987)
Acephate (+ metabolite methamidophos)	Spray application 750 g ai/ha 7 days before flowering collection by holding flowers in plastic bags	apple	7 days 2930 acephate/ 330 methamidophos 9 days 1670 acephate/ 70 methamidophos 14 days 270 acephate/ 30 methamidophos	4.3	(Fiedler, 1987)
Boscalid	500 g ai/ha 8 ha flowering oilseed rape	9 colonies 200m from field – honey stomachs of returning foragers	0 day 1430 1 day 130 2 day 17 7 days 25	2.9	(Wallner, 2009)
chlorantraniprole	Spray application 60 g ai/ha	flowering Phacelia 2 tunnels	1 day 33 3 days 9.6 7 days 3.6	0.55	(Dinter et al., 2009)

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Pesticide	Application rate	method	Peak residue µg/Kg	RUD	Reference
Dimethoate	1.12 Kg ai/ha 55 ha Lemons	Field grown flowering lemons	Max Day 1 600 Day 2 300 Day 3 220 Day 4 1400 Day 8 370 Day 9 80 Day 13 40 Day 15 30 Day 16 130 Day 20 10	1.4	(Waller et al., 1984)
Dimethoate	304 ppm	Spray application to alfalfa (Medicago sativa) to run off	1 day 16000 in uncovered florets, 5000 in covered florets with first order decay log ng/ul = 1.17 - 0.12 x days		(Barker et al., 1980)
dimethoate	Spray application 4.25 l/ha	Apples at green bud stage	15 day 310 16 day 480		(Belanger and Rivard, 1980)
dimethoate	Spray application 4.25 l/ha	Apples at pink tip stage	5 day 5200 6 day 3320		(Belanger and Rivard, 1980)
Endosulfan	Spray application 525 g ai/ha	2003-2004 Mustard – bees collected at crop 3 replications	0hr 1825 ± 43 1hr 1551 ± 28 4hr 1333 ± 13 8hr 1098 ± 101 24hr 919 ± 35 48hr 715 ± 41 72hr 313 ± 31 120hr 10 0.1 240hr <LOD	3.5	(Choudhary and Sharma, 2008)

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Pesticide	Application rate	method	Peak residue µg/Kg	RUD	Reference
Endosulfan	Spray application 525 g ai/ha	2004-2005 Mustard – bees collected at crop 3 replications	0hr 1614 ± 13 1hr 1493 ± 9 4hr 1214 ± 91 8hr 998 ± 39 24hr 865 ± 30 48hr 600 ± 5 72hr 286 ± 32 120hr 11 ± 1 240hr <LOD	3.1	(Choudhary and Sharma, 2008)
Lambda-cyhalothin	Spray application 75 g ai/ha	2003-2004 Mustard – bees collected at crop 3 replications	0hr 858 ± 38 1hr 655 ± 1 4hr 536 ± 2 8hr 428 ± 15 24hr 240 ± 4 48hr 139 ± 1 72hr 13 ±1 120hr <LOD 240hr <LOD	11.4	(Choudhary and Sharma, 2008)
Lambda-cyhalothin	Spray application 75 g ai/ha	2004-2005 Mustard – bees collected at crop 3 replications	0hr 836± 46 1hr 660 ± 16 4hr 540 ± 10 8hr 450 ± 29 24hr 442 ± 10 48hr 253 ± 54 72hr 134 ±29 120hr 4 ± 3 240hr <LOD	11.1	(Choudhary and Sharma, 2008)
malathion	Aerial application 210 ml/ha 32 ha oilseed rape in full flower	Contents of honey stomach	90 ± 30 µg/ml 1 hr after treatment		(Pankiw and Jay, 1992)

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Pesticide	Application rate	method	Peak residue µg/Kg	RUD	Reference
Prothioconazole	250 g ai/ha 8 ha flowering oilseed rape	9 colonies 200m from field – honey stomachs of returning foragers	0 day 690 1 day 60 2 day 17 7 days 9	2.8	(Wallner, 2009)
Spiromesifen	Spray application 225 g ai/ha	2003-2004 Mustard – bees collected at crop 3 replications	0hr 1452 ± 29 1hr 1121 ± 6 4hr 801 ± 49 8hr 705 ± 74 24hr 508 ± 9 48hr 257 ± 21 72hr 94 ± 3 120hr <LOD 240hr <LOD	6.5	(Choudhary and Sharma, 2008)
Spiromesifen	Spray application 225 g ai/ha	2004-2005 Mustard – bees collected at crop 3 replications	0hr 1413± 33 1hr 1011 ± 24 4hr 784 ± 2 8hr 594 ± 103 24hr 491 ± 31 48hr 224 ± 1 72hr 83 ± 9 120hr <LOD 240hr <LOD	6.3	(Choudhary and Sharma, 2008)

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Soil and seed treatments

Pesticide	Application rate	method	µg/Kg	RUD mg/kg per 1mg/seed	Reference
Carbofuran	Cut stems with open flowers and no leaves placed in vials containing radiolabelled ai, 5 or 50 µg/ml	Oilseed rape (nectaries supplied by phloem) nectar collected up to 120hrs	5 µg/ml max 3 µg/ml 50 µg/ml Max 160 µg/ml		(Davis et al., 1988)
chlorantraniprole	Soil application 253.5 g ai/ha	flowering Phacelia 2 tunnels	3 days 3.2 7 day <1		(Dinter et al., 2009)
clothianidin	Seed treatment 0.03 mg/seed	Flowering oilseed rape	Max nectar 2.24	0.074	(Cutler and Scott-Dupree, 2007)
clothianidin	Seed treatment –rate not given assumed 0.06mg/seed	9 colonies 200m from oilseed rape field – honey stomachs of returning foragers	1-3	0.05	(Wallner, 2009)
dimethoate	25 mg/pot	Nasturtium grown in treated soil in pots	Maximum identified at 6 days by biological assay, samples contained 2890 ± 550		(Lord et al., 1968)
dimethoate	25mg/pot	Fuschia grown in treated soil in pots	Maximum identified at 6 days by biological assay, samples contained 741 ± 259		(Lord et al., 1968)
dimethoate	50 mg/pot	Field bean grown in treated soil in pots	4 days 21000		(Lord et al., 1968)
Dimethoate	Cut stems with open flowers and leaves placed in vials	Field bean (nectaries supplied by phloem, extra floral nectarines	5 µg/ml max 2 µg/ml 50 µg/ml Max 60 µg/ml		(Davis et al., 1988)

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Pesticide	Application rate	method	µg/Kg	RUD mg/kg per 1mg/seed	Reference
	containing radiolabelled ai, 5 or 50 µg/ml	suppied by xylem and phloem) nectar collected up to 120hrs			
Dimethoate	Cut stems with open flowers and leaves placed in vials containing radiolabelled ai, 5 or 50 µg/ml	Ajµga repens (nectaries supplied by xylem and phloem) nectar collected up to 120hrs	Flowers 5 µg/ml max 5 µg/ml 50 µg/ml Max 20 µg/ml Extrafloral nectaries 5 µg/ml max 50 µg/ml 50 µg/ml Max 150 µg/ml		(Davis et al., 1988)
Dimethoate	Cut stems with open flowers and leaves placed in vials containing radiolabelled ai, 5 or 50 µg/ml	Field bean (nectaries supplied by phloem, extra floral nectarines suppied by xylem and phloem) nectar collected up to 120hrs	5 µg/ml max 12 µg/ml 50 µg/ml Max 120 µg/ml		(Davis et al., 1988)
Dimethoate	Cut stems with open flowers and leaves placed in vials containing radiolabelled ai, 5 or 50 µg/ml	Ajµga repens (nectaries supplied by xylem and phloem) nectar collected up to 120hrs	Flowers 5 µg/ml max 5 µg/ml 50 µg/ml Max 85 µg/ml Extrafloral nectaries 5 µg/ml max 50 µg/ml 50 µg/ml Max 900 µg/ml		(Davis et al., 1988)
Dimethoate	Cut stems with open flowers and no leaves placed in vials containing radiolabelled ai, 5 or 50 µg/ml	Oilseed rape (nectaries supplied by phloem) nectar collected up to 120hrs	5 µg/ml max 5 µg/ml 50 µg/ml Max 140 µg/ml		(Davis et al., 1988)
imidacloprid	0.05 mg/seed	Oilseed rape field 80 samples 9 sites	<LOQ (5)		(Schoning and Schmuck, 2003)

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Pesticide	Application rate	method	µg/Kg	RUD mg/kg per 1mg/seed	Reference
imidacloprid	0.7 mg/seed	Sunflower field 54 samples; 9 sites	<LOQ 5		(Schoning and Schmuck, 2003)
imidacloprid	0.7 mg/seed (radiolabelled)	Sunflower grown in greenhouse	1.9 (no detectable residues in field grown plants LOD 10)		(Schmuck et al., 2001)
imidacloprid	1 mg/seed	Maize field 18 samples 9 sites	<LOQ 5		(Schoning and Schmuck, 2003)
Phorate	100 mg/pot	Field bean grown in treated soil in pots	4 days 7		(Lord et al., 1968)
phorate	25 mg/pot	Nasturtium grown in treated soil in pots	Maximum identified at 6 days by biological assay, samples contained ≤100		(Lord et al., 1968)
phorate	25mg/pot	Fuschia grown in treated soil in pots	Maximum identified at 6 days by biological assay, samples contained ≤100		(Lord et al., 1968)

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1.2.5. Water collection

Water is collected by honeybees to dilute thickened honey, to produce brood food from stored pollen, to maintain humidity within the hive and to maintain temperature within the brood area. Water is not stored in combs by temperate bee colonies. The amount of water required depends on the outside air temperature and humidity, the strength of the colony and the amount of brood present. The production of water by evaporation of nectar to form honey may address at least some of this need. Water consumption by honeybee colonies has been assessed using confined of colonies provided with a source of water within the hive. Average water consumption per colony (7-13 combs of bees with pollen and nectar present)) when it is unconfined was 0.11-0.14 L in colonies over a 36 hr period and 0.175-0.273 L over a 72 hour period (Al-Fattah and El-Shemy, 1990) when outside air temperatures were 35-41^{o}C. Assuming a colony of 10 combs of bees represents 21,000 bees and of these approx 30% will be foragers (Harbo, 1986) then the consumption over a 24 hrs period equates to collection of 10-14 µl/forager.

Large bees require concentrated nectar at low to moderate ambient temperature because of their very high metabolic water production in flight and bumble bees have been observed to evaporate water on their tongues when provided with 30% rather than 50% sucrose whereas desert bees use dilute nectar for rehydration purposes (Nicolson, 2009).

Guttation is the appearance of drops of xylem sap on the tips or edges of leaves of some vascular plants, such as grasses, and should not be confused with dew, which condenses from the atmosphere onto the plant surface. A recent paper on "translocation of neonicotinoid insecticides from coated seeds to seedling guttation drops: a novel way of intoxication for bees" was published by (Girolami et al., 2009) and this has focussed significant interest on the possible risks posed to honeybees by such a method of exposure.

There are a wide variety of crops that guttate (Thompson, 2010) and with soil and seed treatment applications produce high levels of residues during the early developmental stages of the crop. The data published to date on migration from seeds and following spray applications is shown in Table 1.9 and conditions favouring guttation are shown in the lower section of Table 1.9. Figure 1.2 shows that high levels of residues are produced soon after emergence and residues decline over time. To date there have been no published studies that demonstrate exposure of bees to guttating crops in the field. Guttation fluid is unlikely to be identified by honeybees as a source of sugar due to the low levels present. Bees are less subject to dessication than most terrestrial insects due to their nectar diet and high metabolic water production ((Nicolson, 2009).

Sprayed residues on the surface of leaves may also be redissolved in dew formed on the surface after the spray application. Shawki et al (2006) investigated the potential impact on honeybees collecting water of a spray formulation containing chlorpyrifos and cypermethrin (both non-systemic) applied to oilseed rape plants at growth stage 21-51. Guttation was encouraged by irrigation before being covered by plastic covers overnight and samples were collected before or soon after sunrise. They collected samples of guttation fluid and dew up to 10 days after spray application. Analytical data showed that chlorpyrifos residues were below the limit of detection (0.8 µg/kg) and cypermethrin was also not detected in guttation fluid, chlorpyrifos residues in dew peaked at 3.7 µg/kg on day 4 and 1.5 µg/kg on the day 5 after spray application.

Water present on the soil surface may also become contaminated by overspray or migration of residues in the surrounding soil, e.g. granules. The concentration of pesticides in puddles has been

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reviewed in the EFSA Bird and Mammal Guidance Document (2010). There were no reports in the literature of residues associated with bees drinking at contaminated puddles.

Studies on the use of crops by bees suggest that the majority of bees are observed adjacent to the bee hives. They are mostly observed resting on plants or scanning the leaf area, presumably searching for water. Only single bees are observed taking up water from crop plants; these bees seemed not to distinguish between rain-, dew, or guttation droplets. Water collection has been observed up to a distance of 30m from the hive with less bees observed with increasing distance from the hive (Joachimsmeier et al 2011 (ICPBR poster)). This concurs with (Woyciechowski, 2007) who identified that water collecting bees were less likely to return to the hive than those fed sucrose when they were released 500-600m away. They also demonstrated that water collecting bees consume more nectar/honey in hive prior to their foraging trips than nectar foragers even though they covered the same distance outside the hive.

There is insufficient data currently to allow modelling of the residues in guttation fluid or to assess with certainity the actual water collection of bees from guttating crop plants; it appears this is an infrequent occurrence.

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Table 1.9: Residues in guttation fluid

Active ingredient	Application rate	Method	Residue	Reference
2,4,5-T	Water supply treated with 1000ppm	5 colonies	Max 150000 during exposure	(Morton et al., 1974)
Chlorpyrifos/cypermethrin	Chorpyrifos 500g/l; cypermethrin 50 g/L applied to 3 replicate plots of oilseed rape plants at 0.6 l/ha	Daily collection of guttation fluid	<LOD (0.8)	(Shawki et al., 2006)
Clothianidin	Dust from drilled fields	Guttation droplets from vegetation on field margins	17.5-27 at 1 hr, 6.5-12.5 at 24 hrs	(Marzaro et al., 2011)
Clothianidin	0.5 mg/seed	maize	7400 ng/ml on emergence decreasing to <1000 ng/ml after 1 week	(Reetz et al., 2011)
Clothianidin	0.5 mg /seed	maize	25-35 mg/L within 20 days after sowing	FOAG 2009
Clothianidin	1.25 mg/seed	Droplets collected from maize plants grown from treated seeds during first 3 weeks of growth	23.3 ± 4.2 mg/L	(Girolami et al., 2009)
Clothianidin	1.25 mg/seed	maize	8000 ng/ml on emergence decreasing to 1000 ng/ml after 1 week	(Reetz et al., 2011)
Clothianidin	1.25 mg/seed	Maize lab and field grown	Lab 1mg/seed 1 day 35.99 mg/L 8-10 day 8.82 mg/L, 11-20 days 31.64 mg/L Field 19-46 mg/L	(Tapparo et al., 2012)
Fipronil	0.5, 0.75, 1 mg/seed	maize	Not detected	(Tapparo et al., 2012)
Fipronil	0.17 nmol/g soil	Radiolabelled distribution from treated soil to sunflowers	Xylem fluid nmol/mL day 0- 6 0.06 6-13 0.10 13-18 0.25	(Aajoud et al., 2006)
Imidacloprid	1.75 mg/seed	triticale	13 ng/ml	(Reetz et al., 2011)
Imidacloprid	0.5, 1, 1.25 mg/seed	Maize lab and field grown	Lab1mg/seed 1 day 80.87 mg/L 8-10 day 17.30 mg/L, 11-20 days 60.13 mg/L Level on seed did not significantly	(Tapparo et al., 2012)

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Active ingredient	Application rate	Method	Residue	Reference
			affect residues Field 77-222 mg/L	
Imidacloprid	0.5 mg/seed	Droplets collected from maize plants grown from treated seeds during first 3 weeks of growth	47 ±9.96 mg/L max 110 mg/L	(Girolami et al., 2009)
Thiamethoxam	1mg/seed	Droplets collected from maize plants grown from treated seeds during first 3 weeks of growth	11.9 ± 3.32 mg/L	(Girolami et al., 2009)
Thiamethoxam	0.6, 1mg/seed	Maize lab and field grown	Lab 1mg/seed 1 day 24.29 mg/L 8-10 day 3.55 mg/L, 11-20 days 8.32 mg/L Field 1 day 79-227 mg/L	(Tapparo et al., 2012)

Conditions in laboratory studies which encourage guttation and subsequent residues in guttation fluid

Crop	Growth stage	Method	Volume collected (ul/leaf)	Residue ai	Ref
Wheat (cultivar Apollo)	8 day old seedling	Lab, 100% humidity 60mins	6.4	1.37ppm Triforine (36hrs after application of 300ppm ai to leaves)	Bickers et al 1996
Wheat (cultivar Avalon)	1-2 fully expanded leaves)	20C, leaf enclosed in vial	Not reported	0.15 ug (Exp) (36hrs after application of 1ug to leaf) <0.01 ug tebuconazole (48 hrs after application of 1ug to leaf)	Harris 1999
Barley (4 cultivars_ Alexis, Jana, Trixi, Corona)	8 day old seedling	Lab 100% humidity 60mins	4.8-5.6	1.36-3.95 Triforine (36hrs after application of 300ppm ai to leaves)	Bickers et al 1996
Tomato	14 day old seedling	Lab 100% humidity 60mins	74.5	8.70ppm Triforine (36hrs after application of 300ppm ai to leaves)	Bickers et al 1996
Grapevine	21 day old cutting (after 3 weeks in hydroculture)	Lab 100% humidity 60mins	56.5	2.95ppm Triforine (36hrs after application of 300ppm ai to leaves)	Bickers et al 1996
Grass (Yorkshire fog)	Mature	Field conditions	1.73	N/A	Hughes and Brimblecombe 1994
Rice	125-135 days	Field- rainy season (30-	62-110	N/A	Singh et al 2008

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Crop	Growth stage	Method	Volume collected (ul/leaf)	Residue ai	Ref
		32C, 80-85% RH)			
Field horsetail	4-6 shoots, 15cm high	20-25C, 50-7-%RH, 14/10 light cycle		Amitrole 2.88 ug Fosamine 0.004 ug, glyphosate 0.96 ug (36 hrs after application of 2ul 20g ai/L)	Coupland and Peabody (1981)
Tobacco	2 months	95% humidity 26C light, 18C dark, 14/10h light dark cycle	1-2ml /g dry leaf per day (5ul/cm2 leaf area)	N/A	Komarnytsky et al 2000
Field bean			6ul/cm2 leaf area	N/A	Yarwood 1952 cited by Komarnytsky et al 2000
Couch grass (Agropyron repens)	3 tillers	16C light, 6C dark, 77% RH light, 95% RH dark, 14/10hr light/dark		0.11 ug glyphosate (8days after application of 50ug)	Coupland and Caseley 1979
Rice	12-18 days	12/12 hr light/dark 20C dark, 35C light, closed chamber with air flow (1L/min)		50 ug/ml Carbaryl (1 day after root soak uptake 305 ug/g plant) 254 ug/ml Carbofuran (1 day after root soak uptake 297 ug/g plant) 2152 ug/ml Aldicarb (1 day after root soak uptake 328 ug/g plant)	Ferreira and Seiber 1981

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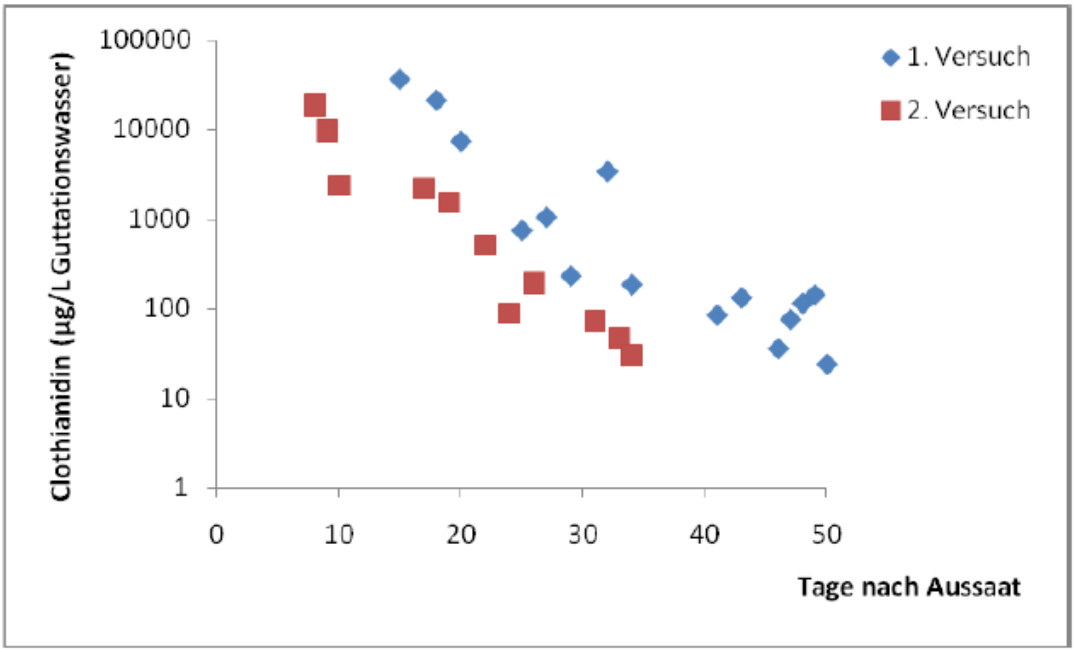

Figure 1.2 Clothianidin concentration in guttation fluid in maize in two trials in Switzerland

[Ordinate: Clothianidin (µg/L guttation fluid. Abscissa: Days after sowing. Versuch = Trial]

1.3. Exposure of bees within the hive

1.3.1. In-hive pollen (bee bread)

Bee bread is pollen processed from the pollen loads by bees for storage by combining with nectar or honey and addition of antimicrobial agents ((Nicolson, 2009). The reported residues in bee bread collected from studies are shown in Table 1.10 and those collected during monitoring studies are shown in Table 1.11. "Entombed pollen" which is visible as sunken capping on sealed bee bread within the colony appears to be related to high residues of chlorothalonil (40 times that in apparently normal pollen) is a phenomenon reported in the USA (vanEngelsdorp et al., 2009a). The cappings contained a high concentration of propolis and the cells appeared to contained empty pollen grain husks. Feeding pollen from these cells appeared to have no significant effect on the survivorship of adults or on larvae reared *in vitro*.

The RUD for bee bread in field studies (Table 1.10) are far higher than in the collected pollen from flowers or from pollen traps (section 1.2.4.1) with a mean RUD of 20.8 and a 90th percentile of 62.7. These higher values may relate to differences in availability for residue analysis following processing of the pollen by bees which has been reported (Kubik et al., 1999) where residues of fungicides in bee bread were up to 200-300 times those identified in pollen pellets. The monitoring studies (Table 1.11) also show the presence of acaracides in bee bread within the hives but the range of analyses conducted were not broad enough to detect the full range of pesticides which may have been present.

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Table 1.10: Residues in bee bread samples from colonies

Active ingredient	Application rate	Method	Residue µg/kg	RUD mg/kg	Reference
Captan	10 Ha Apples 2000 g ai/ha	10 colonies samples combined to single sample per day	4740 ± 1010 – 8090 ± 790	4.0	(Kubik et al., 2000)
carbaryl	Spray application to vines	Vines	0,5%		(Belliardo et al., 1975)
Carbaryl	Aerial spray application 1.1 kg/ha 65Ha	alfalfa	33-44	0.04	(Stanger and Winterlin, 1975)
chlorantraniprole	Soil application 253.5 g ai/ha	flowering Phacelia 2 tunnels	7 day <0.3		(Dinter et al., 2009)
chlorantraniprole	Spray application 60 g ai/ha	flowering Phacelia 2 tunnels	1 day 2863 7 days 108		(Dinter et al., 2009)
diazinon	Apples in flower 15L/ha	2 hives per site 2 sites	15- 16 days50- 90		(Skerl et al., 2009)
dichlofluanid	4.5 Ha, 5kg euparen /ha spray application	Strawberry 4 colonies	End of flowering 1.8 ± 0.5		(Kubik et al., 1992)
difenoconazole	10 Ha Apples 200 g ai/ha	10 colonies, samples combined to single sample per day	157 ± 67 – 411 ±75	2.1	(Kubik et al., 2000)
Flufenoxuron	Phacelia 40 g/ha	Tunnel -Germany	60	1.5	Flufenoxuron DAR
Flufenoxuron	Phacelia 40 g/ha	Field Spain	10-320	8.0	Flufenoxuron DAR
Flufenoxuron	Phacelia 40 g/ha	Field Italy	<0.01		Flufenoxuron DAR
Flufenoxuron	Phacelia 40 g/ha	Field France	10-70	1.8	Flufenoxuron DAR
Flufenoxuron	Phacelia 40 g/ha	Field France	20-120	3.0	Flufenoxuron DAR
Flufenoxuron	Phacelia 40 g/ha	Vineyard France	<0.01		Flufenoxuron DAR
Iprodione	4.5 ha in flower cherry plantation sprayed 0.75 kg ai/ha 6 days preflower and 0.188 kg ai/ha during flower	5 colonies sampling time during flowering	Mean 3055 ± 1436 (1795 ± 135 – 5511 ±2396)	29.3	(Kubik et al., 1999)
Methyl parathion microencapsulated	Birdsfoot trefoil treated at 1 kg ai/ha	2 nucleus colonies placed on edge of 0.5ha treated area every 48 hrs until 8 nuclei present, Identification of microcapsules in stored pollen	Mean 9.9% of pollen cells contaminated (max 15%)		(Burgett and Fisher, 1980)
Methyl parathion microencapsulated	Spray application 0.5 lb ai/acre (0,56 kg/ha)	alfalfa	9 days 530-1030 3.5 months 110-1030	2.1	(Johansen and Kious, 1978)

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Active ingredient	Application rate	Method	Residue µg/kg	RUD mg/kg	Reference
			7.3 months 340-1170		
Methyl tiophanate	4.5 ha in flower cherry plantation sprayed 1 kg /ha 6 days preflower and 1 kg /ha during flower	5 colonies sampling time during flowering	Mean 1929 ± 1034 (196 ± 6 – 2904 ±75)	2.9	(Kubik et al., 1999)
monocrotophos	Aerial spray application 0.6 kg/ha 65Ha	alfalfa	28	0.05	(Stanger and Winterlin, 1975)
procymidone	4.5 Ha, 0.75 kg ai/ha spray application	Strawberry 4 colonies	End of flowering 1.2 ± 0.5	0.002	(Kubik et al., 1992)
Teflubenzuron	Oilseed rape semi-field study 157.5 g ai/ha	One colony 1 frame	1 day 23600 7 days 150	149.8	Teflubenzuron DAR
teflubenzuron	Oilseed rape field study 78.75 g ai/ha	One colony 2 frames	1 day 1710 4-14 days 110-160	21.7	Teflubenzuron DAR
thiacloprid	Apples in flower 96 g/ha	2 hives per site 2 sites	<LOD (10)		(Skerl et al., 2009)
Vinclozolin	4.5 ha in flower cherry plantation sprayed 375 g ai./ha	5 colonies sampling time during flowering	Mean 23628 ± 7045 (13107 ± 3754 – 31909 ± 7543)	85	(Kubik et al., 1999)

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Table 1.11: Reported residue levels in bee bread collected during monitoring studies

varroacide	Bee bread µg/kg Spain 2006-2007 (Orantes-Bermejo et al., 2010)	Spain Salamanca 2008 (Orantes-Bermejo et al., 2010)
bromopropylate	1.31 ± 6.3	
chlorfenvinphos	35.9 ± 60.86	17.81 ± 17.15
Coumaphos	6.04 ± 25.3	
Tau-fluvalinate	200.68 ± 563.11	

Spain

Mean residues in samples of stored pollen collected in the spring and autumn of 2008 in Spain ((Bernal et al., 2010)

Apiary	Fipronil (parent + sulphide, sulfone and desulfinyl)	*Chlorfenvinphos µg/kg (varroacide)*	Endosulfan sulphate µg/kg	*Fluvalinate µg/kg (varroacide)*	*Bromopropylate µg/kg (varroacide)*	HCB µg/kg	trifluralin µg/kg
C1	ND	24-62	2-7	ND	ND	ND	1-5
C2	ND	20-34	3-12	ND	ND	ND	1-4
C3	ND	ND	ND	8-18	ND	ND	3-13
C4	ND	16-48	4-27	ND	2-12	ND	ND
C5	ND	ND	ND	ND	ND	ND	5-7
C6	ND	22-31	1-3	3-27	2-6	ND	ND
A1	ND	11-27	ND	ND	3-7	23-57	5-12
A2	ND	ND	22-78	3-5	8-20	ND	4-22
A3	ND	32-60	7-20	ND	ND	14-44	3
A4	ND	44-132	ND	4-12	ND	ND	1-5
A5	ND	ND	2-14	ND	ND	18-94	2-7
A6	ND	4-12	ND	15-24	7-17	3-21	ND

Pollen was collected from hives near treated sunflower fields (sunflowers treated with fipronil). Chlorfenvinphos is described as a varroacide. Only representative data are provided in the publication but are shown below (µg/kg). They also assessed the presence of disease in the colonies and detected N cerenae on most occasions (but not N apis which is less tolerant of the high temperatures 31-38^{o}C)

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1.3.2. Residues in stored honey and nectar

Residues in honey formed from contaminated nectar and stored within the hive will depend on two competing factors:

The concentration of nectar through evaporation of water to produce honey

Degradation of residues through biological and chemical factors in honey

Both factors are slow and counter each other to some extent and there are differences between honey contained in open and sealed cells (Winterlin et al., 1973).

Table 1.12 shows the residues of in-hive treatments and pesticides detected in stored nectar and honey in field studies and Table 1.13 summarises the available monitoring data for samples taken directly from colonies. Monitoring data for processed honey has been excluded as honey is combined from a large number of colonies and therefore residues may be diluted. For pesticides (not acaracides) the residues detected in the monitoring studies are lower than those reported in field studies.

Table 1.12 shows that nectar/ honey stored over a longer period of time in the hive the data are far more limited (many relate to in-hive treatments) and suggest the mean RUD is 0.4 with the 90th percentile 1.2 and the 95th percentile 1.6 but it should be recognised that the dataset are very limited and residues in the hive will be highly dependent on the persistence of the pesticide.

Table 1.13 shows that there are a number of pesticides detected in honey during monitoring studies including multiple pesticides within the same sample (this is discussed in more detail in the section on multiple pesticide exposure). The variability with pesticide probably precludes the development of generic data for honey stored within the hive but this may be possible using the residue data for nectar and first order kinetics as a precautionary approach: in practice the primary determinant of turnover of pesticides in honey within the hive is removal by the beekeeper and consumption by the colony.

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Table 1.12 Residues in stored nectar/honey in hive

Active ingredient	Application rate	Method	Residue µg/kg	Reference
Amitraz	acaracide	Review article	1992 Italy 0.061	(Menkissoglu-Spiroudi, Tsigouri et al. 2001)
Amitraz as Taktic	40 mg/colony aerosol autumn	16 samples March 11 samples May 9 Samples July	March <1 May <1 July <1	(Lodesani et al., 1992)
Ampicillin	30mg in sucrose	5 colonies	3 Day 4400 14 day 50 21 day <LOD 10	(Nakajima et al., 1997)
Bromopropylate	acaracide	Review article	1990 Switzerland 204 max 1995 Austria 63.3 max 1999 Germany 0.5-15 (54.9% n=226) 1999 EU 0.5-10 (20.9% n=158)	(Menkissoglu-Spiroudi, Tsigouri et al. 2001)
Bromopropylate as Folbex VA	Acaracide 1600 mg/treatment	3 hives treated samples taken in spring after Standard treatment in autumn/winter	10	(Bogdanov, 2006)
Bromopropylate as Folbex VA	1480 mg/colony fumigation autumn	46 samples March 12 samples May 8 Samples July	March 18 ±3 May 94 ± 18 July 26 ± 9	(Lodesani et al., 1992)
Coumaphos	acaracide	Review article	1988 Greece 0.08 -2.8 (100% n=10) 1995 Austria 63.5 (71% n=41) 1999 Germany 0.5-25 (61% n=221) 1999 EU 0.5-20 (19% n=158)	(Menkissoglu-Spiroudi, Tsigouri et al. 2001)
Coumaphos as Perizin	Acaracide 32 mg/treatment	3 hives treated samples taken in spring after Standard treatment in autumn/winter	15	(Bogdanov, 2006)

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Active ingredient	Application rate	Method	Residue µg/kg	Reference
Cymiazole as Apitol	Acaracide 2g/hive	10 colonies treated samples collected up to 115 days after treatment	Unsealed cells Mean 2450 ± 900 1 day after treatment to 140 ± 60 112 days after treatment Sealed cells 260 ± 200 112 days after treatment	(Cabras et al., 1994)
Flumethrin	acaracide	Review article	1995 Austria 15 max	(Menkissoglu-Spiroudi, Tsigouri et al. 2001)
Flumethrin as Bayvarol	Acaracide 14.4 mg/strip	3 hives treated samples taken in spring after Standard treatment in autumn/winter	<1	(Bogdanov, 2006)
Fluvalinate	acaracide	Review article	1989-1993 Belgium 1-100 (25-93% n=250) 1999 Germany 0.5-10 37.2% n=226) 1999 EU 0.5-15 (55.1% n=158) 2000 Greece 0.44- 30.1 (100% n=66)	(Menkissoglu-Spiroudi, Tsigouri et al. 2001)
Fluvalinate	Acaracide 1600mg/treatment for 1 month	2 treated hives, samples taken before to 180 days after treatment	<2.5	(Bonzini et al., 2011)
Fluvalinate as Apistan	Acaracide 1600 mg/strip	3 hives treated samples taken in spring after Standard treatment in autumn/winter	<1	(Bogdanov, 2006)
Fluvalinate as Apistan	1600 mg/colony contact (strips) autumn	25samples March 33 samples May 29 Samples July	March 2 ± 3 May <1 July 1 ± 1	(Lodesani et al., 1992)
fumagillin	240 mg/hive in winter	10 colonies	Brood chamber 4 months <LOQ (1) -172	(Nozal et al., 2008)
fumagillin	120 mg/hive in winter	10 colonies	Brood chamber 4 months <LOD (1)-57	(Nozal et al., 2008)

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Active ingredient	Application rate	Method	Residue µg/kg	Reference
Menthol	60g foam strip for evaporation	5 colonies	21 days 6200	(Nelson et al., 1993)
menthol	30g foam strip for evaporation	5 colonies	21 days 9700	(Nelson et al., 1993)
menthol	60g paste for evaporation	5 colonies	21 days 1730	(Nelson et al., 1993)
menthol	30g paste for evaporation	5 colonies	21 days 1250	(Nelson et al., 1993)
menthol	30g on cardboard for evaporation	5 colonies	21 days 800	(Nelson et al., 1993)
Miroamicin	30mg in sucrose	2 colonies 24hr dosing	2 day 9000 6 day 700 13 day 300 20 day 300	(Nakajima et al., 1998)
Mirosamicin	200mg in pollen paste	6 colonies 1 week of dosing	0 day 1000 3 day 200 7 day <LOD (10)	(Nakajima et al., 1998)
Oxalic acid	44 ml of 35.8 g/L /colony total over 5 applications	2000 50 colonies 2001 66 colonies	2000 17000 ± 10800 spring honey 2001 26000 ± 12600 spring honey	(Moosbeckhofer et al., 2003)
Oxytetracycline	0.3g/colony	5 colonies each on 2 sites	2 days 16100 ± 5400- 19400 ± 10500 22 days 700 ± 1000	(Lodesani et al., 1994)
Oxytetracycline	0.75g/colony	5 colonies each on 2 sites	2 days 21700 ± 8700- 28900 ± 10700 22 days 7600 ± 3800	(Lodesani et al., 1994)
Oxytetracycline	1.5g/colony	5 colonies each on 2 sites	2 days 37300 ± 8700- 46900 ± 14100 22 days 16400 ± 5310	(Lodesani et al., 1994)
oxytetracycline	1g/colony in 200-250ml sucrose	6 colonies	7 days 67000 (26000-150000) 8 weeks 3700 (600-11000) 12 weeks 440 (300-900)	(Thompson et al., 2005)

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Active ingredient	Application rate	Method	Residue µg/kg	Reference
oxytetracycline	1g/colony in icing sµgar powder	6 colonies	7 days 11500 (1600-22000) 8 weeks 450 (300-600) 12 weeks 260 (90-800)	(Thompson et al., 2005)
rotenone	In hive varroa treatment PVC high dose 1g ai	4 colonies	At end of treatment 60 ±30 4 months <LOQ	(Satta et al., 2008)
rotenone	In hive varroa treatment PVC low dose 0.5 g ai	4 colonies	At end of treatment 50 ±40 4 months <LOQ	(Satta et al., 2008)
rotenone	In hive varroa treatment cardboard treatment 1g ai	4 colonies	At end of treatment 30 ±20 4 months <LOQ	(Satta et al., 2008)
thymol	acaracide	Review article	<20-480	(Bogdanov, 2006)
Thymol	4500-6000 mg/colony sublimation autumn	39 samples March 11 samples May 11 Samples July	March 315 ± 315 May 47 ± 45 July <1	(Lodesani et al., 1992)
Thymol (Apilife Var)	20g containing 76% thymol, 16.4% eucalyptol, 3.8% methol, 3.8% camphor	Direct application in hive 4 colonies	2000 ±980- 2600 ± 2000 thymol after 8 weeks 30 ±10 – 100 ± 90 after 8 months (overwinter)	(Imdorf et al., 1994)
Tylosin	Antibiotic treatment, 1.1g/colony	Dosed directly into colony, 4 colonies, brood and super honey sampled separately each from 3 points.	Mean of brood chamber and super Day 3 17000 ± 15000 Day 7 12000 ± 6200 Day 14 9900 ± 6600 Day 21 7400 ± 6100 Day 28 6100 ± 4700 Day 56 3300 ±2300 Day 84 1700 ±1600 Day 140 920 ± 840 Day 238 (overwinter) 930 ± 770	(Adams et al., 2007)
Lincomysin	1.2g/colony	Dosed directly into colony, 4 colonies, brood and super	Mean of brood chamber and super	(Adams et al., 2009)

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Active ingredient	Application rate	Method	Residue µg/kg	Reference
		honey sampled separately each from 3 points.	Day 3 24000 ± 31000 Day 7 13000 ± 12000 Day 14 13000 ± 13000 Day 21 8700 ± 8100 Day 28 6600 ± 5000 Day 56 3800 ±3500 Day 84 2200 ±2200 Day 129 3500 ± 2800 Day 290 (overwinter) 3100 ± 3800	
Chloramphenicol	1.0g/colony	Dosed directly into colony, 4 colonies, brood and super honey sampled separately each from 3 points.	Mean of brood chamber and super Day 3 17000 ± 19000 Day 7 26000 ± 7200 Day 14 11000 ± 2700 Day 21 3500 ± 1600 Day 28 2000 ± 860 Day 56 2500 ±2000 Day 84 1400 ±650 Day 126 690 ± 400 Day 332 (overwinter) 1000± 1000	(Adams et al., 2008)

RUDs cannot be calculated due to the multiple modes of application for veterinary medicines used in-hive

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Pesticides

Active ingredient	Application rate	Method	Residue µg/kg	RUD	Reference
2,4,5-T	Water supply treated with 1000ppm	5 colonies	Max 50000 during exposure		(Morton et al., 1974)
Captan	10 Ha Apples 2000 g ai/ha	10 colonies samples combined to single sample per day	4 ± 3 – 19 ± 13	0.01	(Kubik et al., 2000)
Carbaryl	Aerial spray application 1.1 kg/ha 65Ha	alfalfa	2	0.002	(Stanger and Winterlin, 1975)
Carbendazim (slightly hydrophilic compared with other fungicides)	360 g/ha sprayed on flowering oilseed rape	3 colonies at edge of field sampled 7 days after treatment	Mean 145 (61-227)	0.76	(Buchler and Volkmann, 2003)
carbofuran	1.1 Kg ai/ha, 8 ha field alfalfa Furadan	6 Colonies moved adjacent to treated field before application	1 day 133-167 2 days 233 3 days 100 4 days 33 5 days 17 6 days 17	0.15	(Moffett et al., 1986)
carbofuran	1.1 Kg ai/ha, 9 ha field alfalfa Furadan + sticker	6 Colonies moved adjacent to treated field before application	1 day 217 2 days 267 3 days 183 4 days 83 5 days 17 6 days 50	0.20	(Moffett et al., 1986)
chlorantraniprole	Soil application 253.5 g ai/ha	flowering Phacelia 2 tunnels	7 day <0.3		(Dinter et al., 2009)
chlorantraniprole	Spray application 60 g ai/ha	flowering Phacelia 2 tunnels	1 day 47.2 7 days 1.3	0.79	(Dinter et al., 2009)
clothianidin	Seed treatment 0.03 mg/seed	Flowering oilseed rape	Max honey 0.93		(Cutler and Scott-Dupree, 2007)
dichlofluanid	4.5 Ha, 5kg euparen /ha spray application	Strawberry 4 colonies	32 ± 1		(Kubik et al., 1992)
difenoconazole	10 Ha Apples 200 g ai/ha	10 colonies, samples combined to single sample per day	3 ± 1 – 9 ±1	0.045	(Kubik et al., 2000)
Flufenoxuron	Phacelia 40 g/ha	Tunnel -Germany	<0.01		Flufenoxuron DAR

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Active ingredient	Application rate	Method	Residue µg/kg	RUD	Reference
Flufenoxuron	Phacelia 40 g/ha	Field Spain	<0.01		Flufenoxuron DAR
Flufenoxuron	Phacelia 40 g/ha	Field Italy	<0.01		Flufenoxuron DAR
Flufenoxuron	Phacelia 40 g/ha	Field France	<0.01		Flufenoxuron DAR
Flufenoxuron	Phacelia 40 g/ha	Field France	10-80	2	Flufenoxuron DAR
Flufenoxuron	Phacelia 40 g/ha	Vineyard France	<0.01		Flufenoxuron DAR
Iprodione	4.5 ha in flower cherry plantation sprayed 0.75 kg ai/ha 6 days preflower and 0.188 kg ai/ha during flower	5 colonies sampling time not stated (end of experiment)	Mean 23.1 ± 5.4 (13.6 ± 22.8 – 266 ±8.7)	1.4	(Kubik et al., 1999)
Methyl thiophanate	4.5 ha in flower cherry plantation sprayed 1 kg /ha 6 days preflower and 1 kg /ha during flower	5 colonies sampling time not stated (end of experiment)	Mean 58.9 ± 17 (58.9 ± 32.1 – 101 ±31.5)	0.10	(Kubik et al., 1999)
monocrotophos	Aerial spray application 0.6 kg/ha 65Ha	alfalfa	8	0.01	(Stanger and Winterlin, 1975)
procymidone	Two spray applications 7 days apart 1.25 kg ai/ha, 2 Ha	Raspberry 4 colonies	1 month storage after collection at end of exposure period 14 ± 12 – 56 ± 43	0.04	(Kubik et al., 1991)
procymidone	4.5 Ha, 0.75 kg ai/ha spray application	Strawberry 4 colonies	39 ± 2	0.052	(Kubik et al., 1992)
tebuconazole	377 g/ha sprayed on flowering oilseed rape	3 colonies at edge of field sampled 7 days after treatment	Mean 18 (<LD- 25)	0.07	(Buchler and Volkmann, 2003)
teflubenzuron	Oilseed rape field study 78.75 g ai/ha	One colony 2 frames	1 day 70	0.9	Teflubenzuron DAR
Vinclozolin	4.5 ha in flower cherry plantation sprayed 375 g ai./ha	5 colonies sampling time not stated (end of experiment)	Mean 107 ± 17 (66.4 ± 45.4 – 173 ± 53.8)	0.46	(Kubik et al., 1999)

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Table 1.13 Honey residue monitoring data (µg/Kg)
Marketed honeys are excluded as these are blended from several apiaries/ areas and are not therefore representative of exposure in the colonies.

Location	Method	Analytical method	Residue	Reference
Greece (north, central and south)	Collected randomly from 16 apiaries associated with citrus	Multiresidue methods for organophosphorus pesticides	*Chlorfenvinphos 0.15-0.20 µg/kg (n=4)* Chlorpyrifos methyl 0.12-0.22 µg/kg (n=10) Phorate 0.07-0.17 µg/kg (n=2) *Coumaphos 0.14 -3.1 µg/kg*	(Balayiannis and Balayiannis 2008
Greece (north, central and south)	Collected randomly from 17 apiaries associated with cotton	Multiresidue methods for organophosphorus pesticides	Chlorpyrifos methyl 0.10-0.24 µg/kg (n= Phorate 0.50-0.89 µg/kg *Coumaphos 0.10 -4.5 µg/kg*	(Balayiannis and Balayiannis 2008
Greece (north, central and south)	Collected randomly from 9 apiaries associated with sunflower	Multiresidue methods for organophosphorus pesticides	Phorate 0.09-0.68 µg/kg (n=4) *Coumaphos 1.4 -4.8 µg/kg*	(Balayiannis and Balayiannis 2008

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pesticide	Reported residues in field studies (range)	France 2002-2005 (Chauzat et al., 2011) Chauzat, Carpentier et al. 2009) mean	Belgium 2004-2005 (Nguyen et al., 2009) mean	France 2008-2009 (Wiest et al., 2011) 142 samples (max)	Spain 1988-1990 (Garcia et al., 1995) 177 samples (OP only) mean
aldicarb		ND			
Aldicarb sulfone		ND			
Aldicarb sulfoxide		ND			
Amitraz (varroacide)	*0.061*			*26*	
Azinphos methyl		21.8			5.7
benalaxyl				nd	
bitertanol			0.12		
bromopropylate	0.15 - 204		27.5		
bupirimate				<LOQ	
buprofezine				43	
Carbaryl		30.8		<LOQ	
carbendazim	61-227			88	
Carbofuran	17-267	16.1	0.6	<LOQ	
Chlorpyrifos ethyl		ND		nd	
Chlorpyrifos-methyl				<LOQ	
Coumaphos (varroacide)	*0.08-63.5*	*37.9*	*576*	*29*	*6*
cypermethrin				<LOQ	
cyproconazole		ND		4	
Deltamethrin		2.6			
diazinon				14	41.3
dieldrin				nd	
diethofencarb				<LOQ	
dimethoate		NA		nd	
Endosulfan		ND		<LOQ	
epoxyconazole		ND			
ethion					3
fenitrothion		ND			
fenoxycarb				<LOQ	
fenthion		ND			
Fipronil		ND			
Fipronil desulfinyl		ND			
Fipronil sulfone		ND			
flusilazole			0.0275	<LOQ	
hexaconazole		ND			
hexythiazox				<LOQ	
Imidacloprid		0.7	0.275	<LOQ	
6-chloronicotinic acid		1.2			
imazalil				<LOQ	
iprodione	13.6 ±22.8-266 ±8.7			nd	
Lindane		8.5			
malathion		ND			
mercaptodimethur		ND			
Mercaptodimethur sulfone		ND			
Mercaptodimethur		ND			

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pesticide	Reported residues in field studies (range)	France 2002-2005 (Chauzat et al., 2011) Chauzat, Carpentier et al. 2009) mean	Belgium 2004-2005 (Nguyen et al., 2009) mean	France 2008-2009 (Wiest et al., 2011) 142 samples (max)	Spain 1988-1990 (Garcia et al., 1995) 177 samples (OP only) mean
sulfoxide					
methidathion		ND			
methamidophos					8.6
methiocarb			2.75		
methomyl		ND			
mevinphos		ND			
myclobutanil		ND			
oxamyl		ND			
Parathion ethyl		7.5			
Parathion methyl		ND			
Penconazole		ND			
phosalone				nd	10.3
phosmet				42	
phoxim				<LOQ	
Piperonyl butoxide				<LOQ	
prochloraz				<LOQ	
Propiconazole		ND		nd	
Pyriproxyfen				<LOQ	
rotenone			15.2		
Tau-fluvalinate (varroacide)	*<1-100*	*44.7*		*30*	
Tebuconazole	<LOD-25	ND		<LOQ	
Tetraconazole		ND			
Thiophanate methyl	58.9 ±32.1 – 101 ± 31.5			5	
trifloxystrobin			0.275		
triphenylphosphate				<LOQ	
vinclozolin	66.4 ± 45.4 – 173 ± 53.8	109.4		nd	

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1.3.3. Residues in beeswax

Beeswax is produced by worker bees within the colony to house stores of nectar and pollen and for brood production. Production begins when the worker is slightly less than one week old, peaking at around two weeks and then reducing (Hepburn et al., 1991). It takes between 24 and 48 hours for any particular honeybee worker to produce a moderate-sized wax scale. If unchanged by a beekeeper wax within the colony may accumulate lipophilic residues over time both from contaminated pollen and nectar brought into the hive and from chemicals used within the hive, e.g. varroacides. Two different types of residue data for beeswax are available: data from monitoring studies where residues cannot be linked to a specific application and directed studies around the time of application. By far the majority of studies conducted have been in relation to varroacides used by beekeepers (coumaphos, fluvalinate, chlorfenvinphos) and there are concerns relating to residue levels of these chemicals in foundation introduced into beehives (Table 1.14).

The migration of pesticides into wax is directly related to their lipophilicity and therefore the calculation of RUDs is inappropriate. There is no data available directly on the uptake of pesticides into larvae from contaminated wax but reports suggest at high levels these may pose a risk (Pettis et al., 1991; Wu et al., 2011). Further work is required to identify the transfer of pesticide residues from wax into brood.

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Table 1.14 Residues in beeswax

Active ingredient	Application rate	Method	Residue µg/kg	Reference
In-hive treatments				
Amitraz	acaracide	Review article	1988 Italy 1-16 (10% n=20); 96-260 (n=4) 1988-1991 Spain 420-1820 (9% n=221) 1986-1990 East Germany <1 ->50 (68.5% n=330) 1992 Italy <1-34 (n=109)	(Menkissoglu-Spiroudi et al., 2001)
Amitraz as Taktic	40 mg/colony aerosol autumn	12samples March 6 samples May 5 Samples July	March <1 May <1 July <1	(Lodesani et al., 1992)
Bromopropylate	acaracide	Review article	1986 Germany 1-139 (30% n=112) 1987 Germany 50- 94 (44% n=50) 1988 Denmark; Italy 10-90; 125-715; 2-12 (22% n=64) 1989 Belgium 320-1612 1990 Switzerland 30-160 1990 Spain 5-60 (16% n=101) 1992 Italy <1-245 (=74) 1995 Austria >2-33 (13% n=105)	(Menkissoglu-Spiroudi, Tsigouri et al. 2001)
Bromopropylate as Folbex VA	Acaracide 1600 mg/treatment	3 hives treated samples taken in spring after Standard treatment in autumn/winter	Brood combs 47800 Honey combs 2400	(Bogdanov, 2006)
Bromopropylate as Folbex VA	1480 mg/colony fumigation autumn	46 samples March 12 samples May 8 Samples July	March 17 ±2 May 12 ± 7 July 6 ± 3	(Lodesani et al., 1992)
Chlorfenvinphos as Supona (unregistered treatement)	200 mg/colony	Colonies in which used for at least 5 years	Brood combs 730 ±110 Honey combs ND	(Lodesani et al., 2008)

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Active ingredient	Application rate	Method	Residue µg/kg	Reference
Coumaphos	acaracide	Review article	1988 Greece; 1988 Italy 2-5 (10% n=21); 23-50 (100% n=5); 10-180 (60% n=10); 10-30 (40% n=15); 23 (10% n=10) 1988-1991 Spain 1-53 (14% n=221) 1989 Germany 88-252 (100% n=4) 1990 Czechoslovakia 5-53; 235 (n=1) 1995 Austria >2-12 (12% n=84)	(Menkissoglu-Spiroudi, Tsigouri et al. 2001)
Coumaphos as Asuntol (unregistered treatement)	250 mg/colony	Colonies in which used for at least 5 years	Brood combs 4460 ± 470 Honey combs 2880 ± 260	(Lodesani et al., 2008)
Coumaphos as Perizin	Acaracide 32 mg/treatment	3 hives treated samples taken in spring after Standard treatment in autumn/winter	Brood combs 3800 Honey combs 700	(Bogdanov, 2006)
Coumaphos as Perizin	30 mg/colony	Colonies in which used for at least 5 years	Brood combs 240 ± 51 Honey combs 86 ±7	(Lodesani et al., 2008)
Cymiazole	acaracide	Review article	1995 Austria >15-217 (22% n=32)	(Menkissoglu-Spiroudi, Tsigouri et al. 2001)
Flumethrin as Bayvarol	Acaracide 14.4 mg/strip	3 hives treated samples taken in spring after Standard treatment in autumn/winter	Brood combs 50 Honey combs <1	(Bogdanov, 2006)
Fluvalinate	acaracide	Review article	1988-1991 Spain 1-15 (n=18% 221) 1990 Spain 10-100 (12% n=101) 1992 Italy, <1-100 (n=114) 1994 Belgium 4 (0.5% n=215) 1995 Austria >2-9 (3% n=94) 1999 Germany 2-7 (1%) 2000 Greece <1-39 (100% n=93); <1-4 (91% n=32)	(Menkissoglu-Spiroudi, Tsigouri et al. 2001)
Fluvalinate	Acaracide 1600mg/treatment for 1 month	2 treated hives, samples taken before to 180 days after treatment	Max residue 120-180 days after treatment 1130-1540	(Bonzini et al., 2011)

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Active ingredient	Application rate	Method	Residue µg/kg	Reference
Fluvalinate as Apistan	Acaracide 1600 mg/strip	3 hives treated samples taken in spring after Standard treatment in autumn/winter	Brood combs 2900 Honey combs 100	(Bogdanov, 2006)
Fluvalinate as Apistan	1600 mg/colony contact (strips) autumn	25samples March 33 samples May 29 Samples July	March 415 ± 194 May 842 ± 289 July 767 ± 142	(Lodesani et al., 1992)
Fluvalinate as Apistan	1600 mg/colony	Colonies in which used for at least 5 years	Brood combs 3570 ±740 Honey combs 965 ± 53	(Lodesani et al., 2008)
Malathion	Acaracide	Review article	1985 Greece 1-5 (11% n=6) 1988 Greece <1-4 (6% n=31)	(Menkissoglu-Spiroudi, Tsigouri et al. 2001)
rotenone	In hive varroa treatment PVC high dose 1g ai	4 colonies	At end of treatment 22200±13800 4 months 4800 ±2500	(Satta et al., 2008)
rotenone	In hive varroa treatment PVC low dose 0.5g ai	4 colonies	At end of treatment 144400 ± 137900 4 months 3300 ± 4700	(Satta et al., 2008)
rotenone	In hive varroa treatment cardboard treatment 1g ai	4 colonies	At end of treatment 1500 ±1500 4 months 1500 ±2000	(Satta et al., 2008)
thymol	acaracide	Review article	Brood combs 169000-1989000 Honey combs 400-20000	(Bogdanov, 2006)
Thymol	4500-6000 mg/colony sublimation autumn	39 samples March 11 samples May 11 Samples July	March 45370 ±5719 May 240 ± 189 July 150 ± 194	(Lodesani et al., 1992)
Pesticide				
chlorantraniprole	Soil application 253.5 g ai/ha	flowering Phacelia 2 tunnels	7 day <0.3	(Dinter et al., 2009)
chlorantraniprole	Spray application 60 g ai/ha	flowering Phacelia 2 tunnels	1 day 10.5 7 days 7.6	(Dinter et al., 2009)

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Table 1.15 Monitoring data for residues in beeswax µg/kg

pesticide	France (Chauzat et al., 2010a)	(Jimenez et al., 2005) Spain 21 beekeepers White beeswax (n=9)	(Jimenez et al., 2005) Spain 21 beekeepers yellow beeswax (n=12)	(Jimenez et al., 2005) Spain Foundation beeswax (n=31)	France (Faucon et al., 2002)	France (Chauzat et al., 2011) France (Chauzat et al., 2009)	Spain 2006-2007 (Orantes-Bermejo et al., 2010)	Spain Salamanca 2008 (Orantes-Bermejo et al., 2010)
acrinathrin		58-570		220-590				
Amitraz (varroacide)		*100-240*	*150-560*	*80-690*				
Azinphos methyl						228.2		
Bromopropylate		330	68-120	41-110			14.41 ± 1.03	
Chlorfenvinphos (varroacide?)		*340*	*160-4820*	*160-7620*			*449.28 ± 708.42*	*1983.52 ± 914.25*
Chlorpyrifos ethyl						14.9		
Coumaphos (varroacide)	*<LOD - 4700*			*270-380*		*647.5*	*3.98 ± 2.85*	
cyfluthrin						85.3		
cypermethrin						35.0		
Deltamethrin						14.7		
Endosulfan		240-360	120-140	210-370		51.0		
fenitrothion						511		
fenthion						ND		
Lindane				130		18.8		
malathion						15.1		
methidathion						ND		
mevinphos						138		
Parathion ethyl						99		
Parathion methyl						ND		
Procymidone						27.7		
Tau-fluvalinate (varroacide)	*500-2400*	*440-1860*	*850-5100*	*64-3440*	*35-3260*	*220*	*996.49 ±2384.37*	*445.56 ± 1394.31*

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pesticide	France (Chauzat et al., 2010a)	(Jimenez et al., 2005) Spain 21 beekeepers White beeswax (n=9)	(Jimenez et al., 2005) Spain 21 beekeepers yellow beeswax (n=12)	(Jimenez et al., 2005) Spain Foundation beeswax (n=31)	France (Faucon et al., 2002)	France (Chauzat et al., 2011) France (Chauzat et al., 2009)	Spain 2006-2007 (Orantes-Bermejo et al., 2010)	Spain Salamanca 2008 (Orantes-Bermejo et al., 2010)
tetradifon			54-76	32-580				
vinclozolin						21.5		

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1.3.4. Residues in propolis

Propolis is collected by bees as resin from trees, e.g. buds, primarily poplars and pine trees and is used within the hives to block small gaps and as a defense at the hive entrance against ants etc. and also as an anti-bacterial antifungal agent within the hive. The main propolis plants in Europe are poplar, birch, oak, alder, willow and hazel (Konig 1985). Foragers collect the resin in their pollen baskets to return it to the hive and can carry approximately 10 mg (Konig, 1985). The chemical composition of propolis varies between sources but is a mixture of resins, terpenes and volatiles. Due to the range of sources of propolis and storage within the hive propolis collected for human use, e.g. due to its antibacterial and antifungal properties,it can contain a range of contaminants.

There are only a small number of reports of trace residues of pesticides present in propolis collected from colonies and propolis tinctures prepared from this (Table 1.16). Contaminants include organophosphate pesticides (coumaphos, chlorpyrifos, ethion in Uruguay (Perez-Parada et al 2011); dichlorvos, diazinon, malathion, methyl parathion and coumaphos in Mexico (Acosta-Tejada et al 2011)); pyrethroid residues in Brazil (dosSantos et al 2008) and varroacide residues in Croatia (Cvek et al 2009). Bogdanov (2006) reviewed pesticide residues in bee products and concluded the major contaminants of concern in propolis are lead and persistent lipophilic acaracides, i.e. varroacides applied within the hive.

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Table 1.16 Residues of pesticides in propolis

Active ingredient	Application rate	Method	Residue	Reference
Bromopropylate	Acaracide 1600 mg/treatment	Samples from 27 beekeepers in Switzerland	600-1170	(Bogdanov et al., 1998)
fluvalinate	Acaracide 1600 mg/strip	Samples from 27 beekeepers in Switzerland	500-38700	(Bogdanov et al., 1998)
flumethrin	Acaracide 14.4 mg/strip	Samples from 27 beekeepers in Switzerland	1300-3700	(Bogdanov et al., 1998)

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1.3.5. Inhalation exposure

There are three possible sources of inhalation exposure of bees to pesticides (Table 1.14). During applications of pesticides (is a similar manner to flying through spray), through vapour generated from residues on the crop after application and from stored pollen and nectar within the hive (and potentially water evaporated within the hive). The harmfulness of 3 formulations of carbamate pesticides after spray applications has been reported to be more closely correlated with the vapour pressure of the chemical than the toxicity under laboratory conditions (Hurny and Zieba, 1980). Dichlorvos fog demonstrated the link between distance from source and exposure time to effects on bee colonies (Gaal and Herczeg, 1972). The only other pesticide which has been related to a poisoning incident was zineb applied as a powder which resulted in asphyxiation of foraging bees rather than as a result of the toxicity of the active ingredient.

There are a number of in-hive treatments, such as varroacides which act through vapour, e.g. menthol, thymol. (Tremolada et al., 2004) assessed the possible distribution of coumaphos applied as Perizin within a beehive following a varroacide treatment with 32mg/hive. Based on the vapour pressure of 0.013 mPa at 20C this equates to an air saturation of 1.9 µg/m3 and the volume of air within a hive of 0.2m3 gives a saturation in the air of 0.000001%. Even based on a temperature of the centre of the brood nest of 34-35C the vapour pressure will increase only approximately 5-fold. Analysis of residues in honeybees during monitoring studies (Chauzat et al., 2011) have shown mean residues of coumaphos of 1545 µg/kg although it is not known when this was direct intake/contact exposure or exposure to vapour following varroacide treatment.

Methyl parathion vapour generated from pollen dosed at 10mg/hive has been demonstrated to result in residues in bees of 200-600 µg/kg (Boelter and Wilson, 1984) which is similar to the residues generated after a spray application (Moffett et al., 1986). The vapour pressure of methyl parathion at 20oC is 1.3 mPa (Spencer et al., 1979). Therefore the vapour pressure of pesticides from residues within pollen may be used to determine whether residues within the hive pose a risk by inhalation.

Using n (moles)= P (pressure in atm) x V (volume in m3)/ R (universal gas constant (0.0821 L/atm) x T (temperature in Kelvin)

The above relationship suggests that 140 µg methyl parathion /m3 will occur within the hive which will increase proportionately with temperature (Spencer et al., 1979). (Sonnet, 1978) monitored the residues in air above methyl parathion treated fields and showed levels of up to 7.4 µg/m3 for EC formulations and 3.8 µg/m3 for an encapsulated formulation. Further assessments should be made of the actual concentrations of vapour within the hive from contaminated pollen and nectar, including mixtures of pesticides, to ensure this exposure is more fully understood.

The effects of non-Apis bees have only been reported in a single study on wintergreen oil and formic acid fumigants on Osmia showing the relationship between concentration in air and time to death (White et al., 2009).

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Table 1.14 Inhalation studies

Active ingredient	Application rate	Method	Residue µg/Kg	Comments	Reference
Dichlorvos	2%	Gas oil warm fog produced by fog generator as a function of distance and exposure time		Effects on bees	(Gaal and Herczeg, 1972)
Methyl parathion	Introduced in pollen 10 and 500mg/hive, as EC and microencapsulated (ME)	5 replicates, 50g pollen per hive packed into cells in brood comb and covered to inaccessible –only vapour effect	2.0 ng/L days 1-2 Residues in dead bees 10mg/hive EC 200 ± 200 500 mg/hive EC 700 ± 900 10 mg/hive ME 600 ± 500 500 mg/hive ME 3400 ± 1400		(Boelter and Wilson, 1984)
Thymol	4500-6000 mg/colony sublimation autumn	39 samples March 11 samples May 11 Samples July	Bees March 222 ± 45 May 1 ± 2 July <1		(Lodesani et al., 1992)
Thymol (Apilife Var)	20g containing 76% thymol, 16.4% eucalyptol, 3.8% methol, 3.8% camphor	Direct application in hive 4 colonies	1.1-21.3 µg/L thymol <0.02 -2.4 µg/L eucalyptol, menthol and camphor		(Imdorf et al., 1994)
Zineb and sulfur	Helicopter 30m above hives in vineyard application	650 hives killed following application	Not reported	Fungicides known to be non-toxic - thought to be asphyxiation, respiratory problems also reported in humans	(Vidano, 1975)

1.4. Distribution around hives

Nectar collected by foragers from plants is transferred to in-hive bees at the colony entrance which then to further bees for transport to storage or brood combs. During spring and summer large quantities of nectar are stored for use in periods of shortage, e.g. during breaks in nectar flow, periods of poor weather, or for over-wintering. Nectar is placed both in storage combs and also in brood

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combs close to larvae so it is readily available for brood rearing. The majority of published studies relate to in-hive treatments with varroacides and antibiotics and solely measured residues in honey intended for human consumption (e.g. (deGrandi-Hoffman and Hagler, 2000; Nakajima et al., 1997; Nakajima et al., 1998; Bonzini et al., 2011; Tremolada et al., 2004; Tremolada et al., 2011). However, there are a small number of studies which specifically address the distribution of incoming contaminated nectar within hives including Nixon and Ribbands (1952), who demonstrated that releasing just six foragers fed with radiolabelled sμgar into a colony resulted in about 20% of the workers in the brood area receiving some labelled food within 3.5 hours and this included nurse bees which demonstrated the potential exposure of brood. DeGrandi-Hoffman and Hagler (2000) used a marker (rabbit IgG) to assess the distribution of labelled sucrose and demonstrated rapid transport to both food storage and brood combs. Within 2 hours a similar percentage of worker bees from food combs, nurse bees and nectar samples from the combs tested positive for the marker showing workers deposit nectar loads into food storage or brood combs rapidly and with equal frequency. The nectar delivered to brood comb is used rapidly by nurse bees to feed larvae and this was demonstrated by detection of the marker in 31% of the larvae from 2h hours onwards with 70% testing positive from 6 hours onwards.

Contamination of royal jelly and brood food may occur when larvae, including queen larvae, are fed. The contamination does not appear to occur systemically through the hypopharangeal glands responsible for the glandular part of the food but from the honey sac during brood food production (Davis and Shuel, 1988). Higher levels of dimethoate than of carbofuran have been detected in royal jelly and queen larvae and it was considered that the relative amounts of sugar in the diets of differing age larvae and castes may directly impact on the level of contaminant received. Skerl et al. (2010) demonstrated that coumaphos and fluvalinate were also detected in royal jelly after in-hive treatments whereas diazinon, malathion and amitraz were below the levels of detection. Only fluvalinate was detected at significant levels in bee heads and in larvae suggesting high levels of trophyllaxis occur within the hive.

Tremolada et al. (2004) followed the distribution of the varroacide coumaphos in 2 hives. Although deliberately introduced into the hive as a varroacide, unlike other varroacides such as fluvalinate which is formulated in a plastic strip, the formulation was applied in water to the top bars of the frames as a liquid and can thus provide some information on the distribution around the hive if the "indirectly contaminated" compartments are considered. High residues were detected in bees (440-1000 μg/kg) soon after treatment and then this dropped rapidly to 2.9-9.1 μg/kg by day 44. Sampled honey contained 1-2.9 μg/kg and the coumaphos rapidly migrated into wax with residues up to 82,000 μg/kg present in brood comb by 15 days after treatment but this was thought to be a hot spot due to the treatment. Larvae sampled 7 days after treatment contained 14 μg/kg which continued to the final sample point 44 days after treatment (32 μg/kg). The solution also contaminated stored pollen with residues of 18-45 μg/kg throughout the sampling period. Calculation of the half life of the chemical within the colony showed that in abiotic matrices such as wax and honey the half life was approximately 100 days whereas in bees it was approximately 1 week. Therefore bees are the most effective method of dissipating such a chemical from within the colony.

1.5. Conclusions

1.5.1. Honeybees

Where RUDs can be calculated, these can be used to determine the relative amounts of pesticide available through each of the exposure routes:

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Route	Mean		90th percentile	
	mg/Kg	µg/mg	mg/Kg	µg/mg
In bees after overspray from 1 kg/ha	4.4		15.3	
Nectar (spray) from 1kg/ha Nectar (seed and soil treatments) from 1mg/seed	5.3 0.05	0.0053 0.00005	11.3	0.0113
Pollen (spray) from 1 kg/ha Pollen (seed and soil treatments) from 1 mg/seed	7.1 0.002-0.086	0.0071 0.000002-0.000086	21.1	0.0211
Stored nectar/honey (spray) from 1Kg/ha	0.4	0.0004	1.2	0.0012
Stored pollen (spray) from 1 Kg/ ha	20.8	0.0208	62.7	0.0627

These RUDs can be combined with the intake levels for honeybees summarised by (Rortais et al., 2005) to determine the relative importance of different routes of exposure for different ages of bees (Tables 1.15 - 1.17). These show that nectar foraging bees are likely to experienced highest exposure to both sprayed and systemic seed and soil treatments compounds followed by nurse and brood-attending bees.

However, there are a variety of other routes where there is currently insufficient data to fully evaluate their contribution to total exposure.

If dusts are produced during sowing of treated seeds this may be a significant source of exposure for a brief period of time (bees flying through clouds of dust or in contact with surfaces where dusts have been deposited) and may result in residues in pollen and nectar of nearby flowering weeds or crops.

Contact exposure to newly sprayed crops is likely to be integrated in the RUD for spray applications.

Inhalation may be a significant route of exposure for compounds with high vapour pressure and present in stored pollen or collected in water.

Beeswax may be a significant route of exposure for highly lipophilic chemicals.

Propolis probably has a low contribution to overall exposure except where applications are made to trees producing resin, e.g. trunk injection, and the pesticides are systemic and therefore may be exuded in resin.

Water may be sourced from puddles or guttation droplets which may contain high residues for periods of days-weeks and further data is required on the relative importance of these routes.

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Table 1.15 Total exposure based on mean RUDs for 1Kg/Ha spray application

Category of bee	days	µg/bee from overspray	Intake sugar mg/bee	µg/bee from nectar	µg/bee from stored nectar/honey	Intake pollen mg/bee	µg/bee from pollen	µg/bee from bee bread	max exposure µg/bee
Worker Larvae	5		59.4	0.79	0.06	5.4	0.04	0.11	0.90
Drone larvae	6.5		98.2	1.31	0.10				
Nurse bees/brood attending	10		272-400	5.32	0.40	6.5	0.05	0.14	5.46
Wax producing bees	6		108	1.44	0.11				1.44
Winter bees	90		792		0.79				0.792
Nectar foragers	7	0.44	224–898.8	11.95	0.90				12.39
Pollen foragers	7	0.44	72.8–109.2	1.45	0.11				1.89

µg/mg sugar in nectar (40% sugar) = 0.0053*(100/40)= 0.0133; µg/mg sugar in honey (80% sugar) = 0.0004 * (100/80)= 0.001

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Table 1.16 Total exposure based on maximum RUDs for 1Kg/Ha spray application

Category of bee	days	µg/bee from overspray	Intake sugar mg/bee	µg/bee from nectar	µg/bee from stored nectar/honey	Intake pollen mg/bee	µg/bee from pollen	µg/bee from bee bread	max exposure µg/bee
Worker Larvae	5		59.4	1.68	0.09	5.4	0.11	0.34	2.02
Drone larvae	6.5		98.2	2.78	0.15				
Nurse/ brood attending bees	10		272-400	11.32	0.60	6.5	0.14	0.41	11.73
Wax producing bees	6		108	3.06	0.16				3.06
Winter bees	90		792		1.19				1.19
Nectar foragers	7	1.53	224–898.8	25.44	1.35				26.97
Pollen foragers	7	1.53	72.8–109.2	3.09	0.16				4.62

µg/mg sugar in nectar (40% sugar) = 0.0113*(100/40)=0.0283; µg/mg sugar in honey (80% sugar) = 0.0012 * (100/80)= 0.0015

Table 1.17 Total exposure based on mean RUDs for 1mg/seed treatment for systemic pesticides

Category of bee	days	Intake sugar mg/bee	µg/bee from nectar	Intake pollen mg/bee	µg/bee from pollen	max exposure µg/bee
Worker Larvae	5	59.4	0.00743	5.4	0.00046	0.0012
Drone larvae	6.5	98.2	0.0122			
Nurse bees/brood attending	10	272-400	0.0500	6.5	0.00056	0.0506
Wax producing bees	6	108	0.0135			0.0135
Winter bees	90	792	0.099			0.099
Nectar foragers	7	224–898.8	0.112			0.112
Pollen foragers	7	72.8–109.2	0.0137			0.0137

µg/mg sugar in nectar = 0.00005*(100/40) = 0.000125

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1.5.2. Bumble bees

For bumble bees intake data are far more limited than for honeybees but data from Tasei and Aupinel (2008) suggest that in queenless microcolonies under laboratory conditions workers consume 30 mg pollen/day/worker (for nest construction) and larvae consume 22 mg pollen/day. For sucrose intake the values are 0.14-0.19g/day/bee (Tasei et al 1994, Tasei et al 2004). For larvae intake of sucrose is unclear but an approximation is provided by Pereboom (2000) where 34% of the 64 µl fed per day (0.88µl 3 times per hour for 24hrs) equates to 42 µl/day/larva. However, the intake of foragers is not reported and therefore the data only relate to intake for metabolic requirements. These data were used to assess RUDs in Table 1.18.

Overspray can be related to the surface area of the bee which suggests the RUD for honeybees should be increased from 4.4 and 15.3 but the surface area of bumble bees is likely to increase significantly due to their greater size but they are also far more variable in size making any predictions unreliable (Van der Steen 2001).

There was insufficient data available to assess the exposure of solitary bee species.

Table 1.18 Total exposure of bumble bees based on RUDs for spray and seed treatments

Category	**Intake nectar mg/bee/day**	**µg/bee/day from nectar**	**Intake pollen mg/bee/day**	**µg/bee/day from pollen**	**Max µg/bee/day**
Sprays per 1 Kg/Ha					
Worker	140-190	0.742-1.007 (mean) 1.58-2.15 (90^{th} percentile)	30	0.213 (mean) 0.633 (90^{th} percentile)	1.22 (mean) 2.78 (90^{th} percentile)
Larvae	42	0.30 (mean) 0.47 (90^{th} percentile)	22	0.156 (mean) 0.464 (90^{th} percentile)	0.456 (mean) 0.934 (90^{th} percentile)
Systemic seed treatments per 1mg/seed					
Worker	140-190	0.00700-0.00950	30	0.00258	0.0121
Larvae	42	0.0021	22	0.00189	0.00399

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2. Multiple exposure to pesticides (including substances used in bee medication) and potential additive and cumulative effects.

The database searches yielded 103 references of which 84 related to bees and 19 to other insects. Although the searches were not primarily directed at other insects (and therefore should not be regarded as comprehensive for other species) these references were used as a comparison where they provided additional data.

As shown in Chapter 1 pesticides widely used both in the agricultural and urban environment (pest control and home and garden uses) as well as by beekeepers to control pests, e.g. fluvalinate, amitraz, coumaphos to control varroa, are detectable in bees and hive matrices. Exposure of honeybees to any single pesticide application may occur over the short term or, unlike many organisms, over a longer period if residues are present in pollen and/or nectar stored within the colony or due to migration of lipophilic compounds into wax. These more persistent residues are likely to be available to the colony over a period of time depending on the active ingredient and the frequency of use, e.g. multiple applications. Pesticide residues in samples taken from colonies have been reported in France (e.g. Chauzat et al., 2006; Chauzat et al., 2011) and limited data are available for residues in pollen and nectar from treated crops (see Chapter 1 - Exposure). This part of the review assesses the likely combinations of pesticides used on flowering crops attractive to bees, varroacides and other chemicals used within the hive and the potential for additive and synergistic interactions as well as accumulation of residues within the hive. Cumulative effects of pesticides have been reviewed in the Honeybee Risk Assessment Scientific Opinion and this has demonstrated that there is very little data published in a form, e.g. half-life information, which can be used to quantitatively assess the cumulative effects even of individual pesticides.

2.1. Sources of multiple pesticide residues

Multiple chemical residues may occur in colonies due to

- Presence of multiple pesticide ingredients in a single application or application of a spray to a crop in which systemic residues are present in pollen/nectar/guttation fluid
- Collection of pollen/nectar from a variety of sources in space and/or time
- Treatments applied by beekeepers to colonies which also contain residues from agricultural applications

2.1.1. Presence of multiple pesticide ingredients in a single application

Bees may be exposed to mixtures of products applied to plants on which they forage. Recent data from a Defra report (PS2354) indicates the extent of mixing of formulations that occur on arable, vegetable orchards and soft fruit crops in the UK. For arable crops, vegetables and orchards over 50%

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of the treated area was treated with mixtures. For arable crops mixtures contained up to 9 products. For vegetables, orchards and soft-fruit mixtures contain up to 7, 8 or 6 products respectively. Comparing arable data from 1998 and 2008 there was a slight increase not only in the proportion of total area treated with mixtures over time (56% and 61% respectively), but also in the complexity of the mixtures (means of 2.99 and 3.25 products per mixture respectively). Due to an increase in total area treated of 8.5% with respect to 1998, the area treated with mixtures in 2008 was 17.5% greater. Tables 2.1-2.4 show the type of mixtures applied to arable crops, vegetables, orchards and soft fruit in the UK. This demonstrates the scale of tank mixing in the UK is not confined to mixtures with the same target but includes a wide range of modes of action. Tables 2.5-2.7 demonstrate the tank mixing of EBI fungicides with pyrethroids and neonicotinoid insecticides in the UK.

In addition to the application of products as tank mixes, the increasing use of seed treatments raises the possible scenario of nectar, pollen or guttation water containing active ingredients also being contaminated with sprays applied during the flowering period (Defra report PS2368). An example of this is winter oilseed rape which may be treated with a neonicotinoid seed treatment and during the flowering period spray applications of an EBI fungicide (Figure 2.1).

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Table 2.1: Classes of compounds used in mixtures of 2 to 9 products applied to arable crops in 2008 indicating the number of unique combinations, area treated and % of total area treated with mixtures (from Defra PS2354).

Mixture	N	Area (ha)	% area
Fungicide(s) + Herbicide(s) + PGR(s)	1117	2585761	19.32
Fungicide(s) + Herbicide(s)	991	1906643	14.24
Fungicides	723	1837802	13.73
Fungicide(s) + PGR(s)	850	1672300	12.49
Herbicide(s) + Insecticide(s)	721	1616471	12.07
Herbicides	618	1488168	11.12
Fungicide(s) + Insecticide(s)	610	1404836	10.49
Fungicide(s) + Herbicide(s) + Insecticide(s)	205	417120	3.12
Herbicide(s) + PGR(s)	78	161796	1.21
Fungicide(s) + Insecticide(s) + PGR(s)	24	121246	0.91
Fungicide(s) + Herbicide(s) + Insecticide(s) + PGR(s)	24	97756	0.73
PGRs	8	27764	0.21
Herbicide(s) + Insecticide(s) + PGR(s)	6	20739	0.15
Molluscicides	4	13559	0.10
Insecticides	10	8670	0.06
Insecticide(s) + PGR(s)	3	6427	0.05

Table 2.2. Classes of compounds used in mixtures of 2 to 7 products applied to vegetable crops in 2007 indicating the number of unique combinations, area treated and % of total area treated with mixtures (from Defra PS2354).

Mixture	N	Area (ha)	% area
Herbicides	279	105419	31.20
Fungicide(s) + Insecticide(s)	749	102753	30.41
Insecticides	91	57273	16.95
Fungicides	133	47136	13.95
Herbicide(s) + Insecticide(s)	124	15851	4.69
Fungicide(s) + Herbicide(s)	76	4378	1.30
Fungicide(s) + Herbicide(s) + Insecticide(s)	58	3778	1.12
Molluscicides	2	1044	0.31
Fungicide(s) + PGR(s)	5	176	0.05
Fungicide(s) + Insecticide(s) + PGR(s)	1	33	0.01

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Table 2.3. Classes of compounds used in mixtures of 2 to 8 products applied to orchards in 2008 indicating the number of unique combinations, area treated and % of total area treated with mixtures (from Defra PS2354).

Mixture	N	Area (ha)	% area
Fungicides	243	62758	46.02
Fungicide(s) + Insecticide(s)	384	33911	24.87
Fungicide(s) + PGR(s)	175	17224	12.63
Herbicides	54	13298	9.75
Fungicide(s) + Insecticide(s) + PGR(s)	181	6602	4.84
Acaricide(s) + Fungicide(s)	21	1370	1.00
Insecticides	13	614	0.45
Insecticide(s) + PGR(s)	13	254	0.19
PGRs	5	238	0.17
Acaricide(s) + Fungicide(s) + PGR(s)	6	92	0.07
Fungicide(s) + Herbicide(s)	2	7	0.01
Herbicide(s) + PGR(s)	2	4	0.003

Table 2.4: Classes of compounds used in mixtures of 2 to 6 products applied to soft fruit in 2006 indicating the number of unique combinations, area treated and % of total area treated with mixtures (from Defra PS2354).

Mixture	N	Area (ha)	% area
Fungicides	259	21832	50.12
Fungicide(s) + Insecticide(s)	361	13915	31.94
Herbicides	145	5710	13.11
Acaricide(s) + Fungicide(s)	58	1002	2.30
Acaricide(s) + Fungicide(s) + Insecticide(s)	35	379	0.87
Insecticides	16	330	0.76
Acaricide(s) + Insecticide(s)	7	311	0.71
Fungicide(s) + Herbicide(s)	5	30	0.07
Molluscicides	2	19	0.04
Herbicide(s) + Insecticide(s)	2	18	0.04
Acaricide(s)	1	16	0.04

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Table 2.5: Combinations of EBI fungicides with pyrethroid and neonicotinoid pesticides (X) found in mixtures applied to UK arable crops (2008) From Defra report PS2354.

		Insecticides												
EBI fungicides		**Pyrethroids**										**Neonicotinoids**		
Type	Compound	Alpha-cypermethrin	Bifenthrin	Cyfluthrin	Cypermethrin	Deltamethrin	Esfenvalerate	Fenpropathrin	Lambda-cyhalothrin	Tau-fluvalinate	Zeta-cypermethrin	Acetamiprid	Thiacloprid	Thiamethoxam
DMI	Cyproconazole	X	X	X	X		X		X	X	X		X*	
DMI	Difenoconazole	X			X	X			X					
DMI	Epoxiconazole	X*			X		X		X	X	X		X	
DMI	Fenarimol													
DMI	Fenbuconazole													
DMI	Fluquinconazole									X*				
DMI	Flusilazole	X	X	X	X	X			X	X	X			
DMI	Myclobutanil													
DMI	Penconazole													
DMI	Prochloraz	X*			X		X*		X	X				
DMI	Propiconazole				X*				X*	X	X*		X*	
DMI	Tebuconazole	X	X	X*	X	X	X		X	X	X			
DMI	Triadimenol		X*	X*			X*		X*	X*	X*			
DMI	Tetraconazole				X		X							
DMI	Metconazole	X	X		X	X	X		X	X	X		X	
DMI	Prothioconazole	X	X*		X	X	X		X	X	X		X	
Morpholine	Fenpropidin								X*					
Morpholine	Fenpropimorph	X*			X		X		X	X*	X*			
Morpholine	Spiroxamine				X*		X*		X*	X*	X*			

* indicates that combination only occurred where mixture also included other EBI fungicides.

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Table 2.6: Combinations of EBI fungicides with pyrethroid and neonicotinoid pesticides found in mixtures applied to UK orchards (2008) Defra PS2354.

EBI fungicides		**Insecticides: Pyrethroids**										**Neonicotinoids**		
Type	Compound	Alpha-cypermethrin	Bifenthrin	Cyfluthrin	Cypermethrin	Deltamethrin	Esfenvalerate	Fenpropathrin	Lambda-cyhalothrin	Tau-fluvalinate	Zeta-cypermethrin	Acetamiprid	Thiacloprid	Thiamethoxam
DMI	Cyproconazole													
DMI	Difenoconazole													
DMI	Epoxiconazole													
DMI	Fenarimol													
DMI	Fenbuconazole				X								X	
DMI	Fluquinconazole													
DMI	Flusilazole													
DMI	Myclobutanil				X							X	X	
DMI	Penconazole				X								X	X
DMI	Prochloraz													
DMI	Propiconazole													
DMI	Tebuconazole		X											
DMI	Triadimenol													
DMI	Tetraconazole													
DMI	Metconazole													
DMI	Prothioconazole													
Morpholine	Fenpropidin													
Morpholine	Fenpropimorph													
Morpholine	Spiroxamine													

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Table 2.7: Combinations of EBI fungicides with pyrethroid and neonicotinoid pesticides (X) found in mixtures applied to UK soft fruit (2006) from Defra report PS2354.

EBI fungicides		**Insecticides : Pyrethroids**										**Neonicotinoids**		
Type	Compound	alpha-cypermethrin	bifenthrin	cyfluthrin	cypermethrin	deltamethrin	esfenvalerate	fenpropathrin	lambda-cyhalothrin	tau-fluvalinate	zeta-cypermethrin	Acetamiprid	thiacloprid	thiamethoxam
DMI	Cyproconazole													
DMI	Difenoconazole													
DMI	Epoxiconazole													
DMI	Fenarimol							X						
DMI	Fenbuconazole													
DMI	Fluquinconazole													
DMI	Flusilazole													
DMI	Myclobutanil		X			X		X	X				X	
DMI	Penconazole													
DMI	Prochloraz													
DMI	Propiconazole													
DMI	Tebuconazole													
DMI	Triadimenol													
DMI	Tetraconazole													
DMI	Metconazole													
DMI	Prothioconazole													
Morpholine	Fenpropidin													
Morpholine	Fenpropimorph		X										X	
Morpholine	Spiroxamine													

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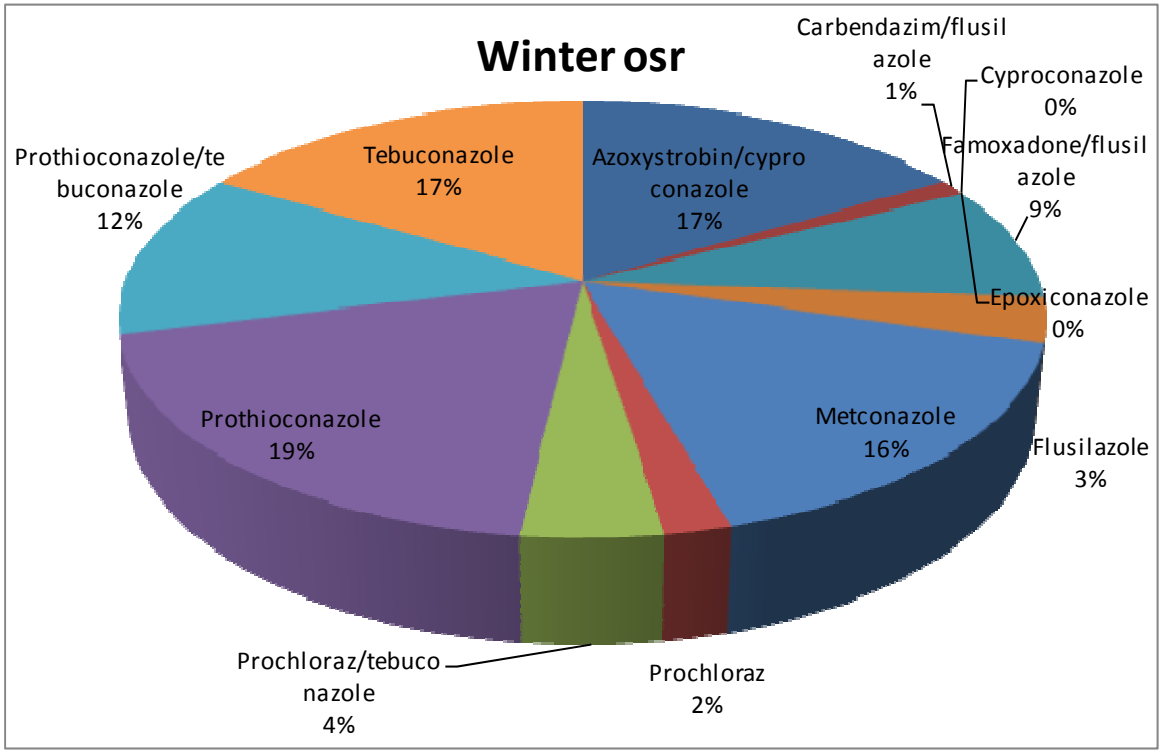

Figure 2.1: Applications of in the UK of EBI fungicides during the flowering of winter oilseed rape (April/May) (from Defra report PS2368)

2.1.2. Collection of pollen/nectar from a variety of sources in space and/or time

Honeybees forage over a large area; with a mean radius of 5km from their hive giving a potential foraging surface area of over 7500 Ha.

There are two main sources of information on the levels of pesticides being returned to the hive, pollen and nectar returned to and stored within the hive and honeybees collected from the hive. Although many reports of residues in pollen being returned to the hive by foragers are published the majority of these are based on individual pesticide residues rather than assessments of the total pesticide residue levels (see Chapter 1). The primary source of information identified was the data reported by (Chauzat et al., 2011) which showed 37.8% of all pollen samples collected from 120 hives from 24 apiaries at 5 sites across France (main types of honey were chestnut, oilseed rape, sunflower, and local mixed flower honey) contained at least two different pesticide residues with 22.2, 12.7, 2.4, and 0.5% containing two, three, four, or five different residues, respectively. 14.7% of all honeybee samples contained at least two pesticides with two (11.2%), three (2.3%), four (1.0%), or five (0.2%) active ingredients.

The UK Wildlife Incident Investigation Scheme which investigates reports of dead bees has been increasingly reporting multiple pesticide residues, due to changes in analytical approaches particularly since 2009 (http://www.pesticides.gov.uk/guidance/industries/pesticides/topics/ reducing-environmental-impact/wildlife/wildlife-incident-investigation-scheme.htm). The combination of

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pesticides reported in live and dead bees are shown in Table 2.8 and demonstrate the range of pesticides detected. In all cases the analyses has not included all pesticides, e.g. many herbicides, and therefore the number of residues which may be present is probably unreported.

2.1.3. Treatments applied by beekeepers to colonies which also contain residues from agricultural applications

Chapter 1 of this review shows that data reported for residues of chemicals applied by beekeepers in honeybee colonies have primarily been directed at single varroacides with some limited data after antibiotic dosing. The exceptions to this are the monitoring studies reported in France, Spain, Italy and the UK which report residues detected in pollen within the hive, honey, wax and bees (live or dead). This shows that very high levels of varroacides may be present within colonies and are regularly detected in live bees (up to 30 µg/Kg bromopropylate, 24840 µg/Kg coumaphos, 326 µg/Kg tau-fluvalinate), bee bread (bromopropylate max 20 µg/Kg, chlorfenvinphos max 132 µg/Kg, coumaphos 6.04 ± 25.3 µg/Kg, tau-fluvalinate 221 ±563 µg/Kg) wax (up to 7620 µg/Kg chlorfenviphos, 648 µg/Kg coumaphos, 5100 µg/Kg tau-fluvalinate) and in honey (up to 27.5 µg/Kg bromopropylate, 576 µg/Kg coumaphos, 44.7 µg/Kg tau-fluvalinate). Unfortunately such data are not available for other chemicals such as the *Nosema* control agent fumagillin or antibiotics, although in many EU countries their use is far more closely controlled than varroacides. During the monitoring study in France survey (Chauzat et al., 2011), observed home-made coumaphos preparations made from the canine medication Asuntol (Bayer) and applied in hives, often at quantities higher than the recommended doses.

2.2. Accumulation of residues within hives

Unfortunately although there are a number of reports of multiple residues detected in monitoring studies, the data are often not reported in sufficient detail to determine the residue levels of the individual components within these multiple detections (Table 2.8).

In France (Chauzat et al., 2009) and (Chauzat et al., 2011) reported the levels of a range of pesticides on honey and wax samples collected from 24 apiaries at 5 sites across France, a total of 120 hives. The main types of honey were chestnut, oilseed rape, sunflower, and local mixed flower honey and 14.2% of honey samples contained two or three active ingredients had frequencies of 12.1 and 2.1%, respectively but unfortunately these were not specificd. Multiple residues have also been reported in bee-bread and honey in samples from Spain and Greece where again the range of analyses were limited (Tables 1.11 and 1.13).

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Table 2.8: Binary and multiple combinations detected in honeybees in France (live bees -bold) and in the UK (dead bees- non-bold) (Chauzat et al., 2011; Greig-Smith et al., 1994) – varroacides are shown in italics

	EBI fungicides	**pyrethroids**	**organochlorines**	**organophosphorus**	**carbamates**
Neonicotinoids	Epoxyconazole + imidacloprid Imidacloprid + tebuconazole	Imidacloprid + *tau fluvalinate* Deltamethrin + 6 – chloronicotinic acid			Bendiocarb + imidacloprid
Pyrethroids	Myclobutanil + fluvalinate Permethrin + Propiconazole *Fluvalinate* + Tebuconazole *Fluvalinate* + propiconazole	*Fluvalinate* + Permethrin Bifenthrin + *fluvalinate*	Lindane + *tau fluvalinate*	Deltamethrin + coumaphos Triazophos + cypermethrin Dimethoate + fenvalerate Dimethoate + omethoate + fenvalerate	
organophosphorus			Gamma HCH + triazophos Gamma HCH + pirimiphos methyl Fenitrothion + DDT	Triazophos + dimethoate Triazophos + phosalone Triazophos + quinalphos Dimethoate+ omethoate	
EBI fungicides	Epoxyconazole + tebuconazole Tebuconazole +flusilazole				
carbamates	Bendiocarb + Propiconazole Bendiocarb + tebuconazole	Carbaryl + permethrin bendiocarb + *fluvalinate*	Gamma HCH + carbaryl Bendiocarb + dieldrin	Bendiocarb + pirimiphos-methyl	

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Combinations of pesticides detected in dead bees in the UK 1997-2011 (http://www.pesticides.gov.uk/guidance/industries/pesticides/topics/reducing-environmental-impact/wildlife/wildlife-incident-investigation-scheme.htm)

bumble bee	Boscalid; prothioconazole-desthio;tebuconazole
bumble bee	Chlorpyrifos; dieldrin
bumble bee	Azoxystrobin; boscalid; cypermethrin
honey bee	Myclobutanil; penconazole; pirimicarb; thiacloprid
honey bee	Carbendazim; dieldrin; HCH-gamma
honey bee	Bendiocarb; permethrin; propiconazole; tebuconazole
honey bee	Boscalid; carbendazim; *fluvalinate*; propiconazole; thiacloprid
honey bee	Chlorothalonil; cyproconazole; deltamethrin; *fluvalinate*
honey bee	Bendiocarb; deltamethrin; propiconazole
honey bee	Bendiocarb; DDE-pp; pirimiphos-methyl
honey bee	Dieldrin; HCH-gamma; permethrin; propiconazole; thiacloprid
honey bee	DDT-pp; fipronil; propiconazole
honey bee	DDT-pp; methomyl; propiconazole
honey bee	Chlorpyrifos; dimethoate; *fluvalinate*; thiacloprid
honey bee	Chlorpyrifos; cyhalothrin-lambda; difenoconazole; dimethoate; propiconazole; thiacloprid
honey bee	Chlorpyrifos; cyhalothrin-lambda; dimethoate; *fluvalinate*; thiacloprid
honey bee	Dieldrin; HCH-gamma; permethrin
honey bee	Chlorpyrifos; glyphosate; thiacloprid
honey bee	Permethrin; piperonyl butoxide
honey bee	MCPA; mecoprop-P
honey bee	Bendiocarb; imidacloprid; permethrin; tebuconazole

2.3. Additive toxicity

The risk from most mixtures can be assessed using the additive approaches of concentration addition (or dose addition) and independent action (IA) (Commission, 2009). Which approach to use is dependent on whether the chemicals do not interact (no synergism or antagonism) but either have the same site of action (simple similar joint action) or different sites of action (independent joint action).

Concentration addition (CA)

This approach is used where chemicals have the same site of action (simple similar joint action) but do not affect biological activity of each other (no interaction). For this method the endpoint must be the same for each chemical.

Total Toxicity = $(C_a/T_a + (C_b/T_b) + ... + (C_n/T_n)$ Concentration addition (CA)

Where C = concentration (or dose)

T = toxicity

While the endpoint (e.g. lethality, immobilisation, reproductive impairment) must be the same for each chemical, the measure of toxicity (e.g. LC50, EC50) does not.

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Independent action (IA)

This approach is used where chemicals have different sites of action (independent joint action) but do not affect the biological activity of each other (no interaction). Here each component of the mixture acts on a different physiological or biological system but contributes to a common response.

This requires biological response (BR) expressed as % toxic effect for the assessed concentration from dose response curve for each constituent.

Total toxicity = $BR_1 + BR_2 + ... + BR_n$ Response addition

The disadvantage of this method is that it requires dose response data for all of the mixture constituents and species being assessed.

CA is recognised as a conservative method for assessing the toxicity of mixtures (Commission, 2009; Verbruggen and Van den Brink, 2010; Wilkinson et al., 2000). In all cases the estimated toxicity using this approach is higher than that predicted by IA (Commission, 2009). When comparing estimates using CA it has been estimated that the majority of estimates do not deviate by more than a factor of 2 (Deneer, 2000), 2.5 (Warne, 2003) or 3(Commission, 2009). There is also some evidence that this deviation is greatest for mixtures containing small numbers of chemicals and decreases as the complexity of the mixture increases. Applying such a factor (of 2 to 3) to the values of toxicity should therefore make the estimate of toxicity protective for most mixtures, and the more complex the mixture, the more conservative this value is likely to be.

Ideally mixture toxicity would be assessed by using the method most suited to the type of chemicals under investigation. However, of these CA appears to be the most conservative and also the one that can be applied using existing toxicity data. For these reasons there is an emerging consensus that the CA approach is the most suitable for routine mixture assessments where synergism is not predicted.

The greatest impact of additive toxicity in bees will be in multiple insecticide residues and therefore these have been identified for assessment of the implications of the presence of these residues in live and dead bees. In undertaking this in the absence of the actual residue data for the reported combinations highlighted in the sections above the approach is illustrated using the residues detected in bees submitted to the UK Wildlife Incident Scheme in 2009 and 2010 when bees were screened for a wider range of residues (Table 2.9). Although this is a scheme based on analysis of dead bees it allows the contributions of the components to the overall toxic residue to be assessed. The limitation of this is that the residues are not the same as an applied dose in an LD_{50} study and in many cases the residues represents 10-20% of the applied dose (Greig-Smith et al., 1994). A similar approach could be adapted for live bee samples but it must be recognised that the residues relate to the half life within the bee rather than a toxic residue and therefore time after exposure will be a confounding factor.

In addition the limited data available on residues reported in bee bread from colonies in Spain and in honey from colonies sampled in Greece were used to assess the approach of using additive toxicity to determine the implications for honeybee exposure (Tables 2.10 and 2.11). These show the ability of the approach to provide an assessment of the total toxic unit contribution of the mixture from its components and how this can be used to assess the exposure of nurse bees and larvae (in the absence of larval toxicity data it has been assumed that the toxicity to larvae and adults are similar).

In the absence of parallel data for nectar and pollen from the same colonies the data were combined to give the maximum toxic units consumed by a nurse bee of 21.1×10^{-5} (pollen)+ 269×10^{-5} (honey) =

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0.0029, i.e. 0.29% of the combined LD_{50} of the individual components. For larvae the intake is 17.8 x 10^{-5} (pollen) + 40 x 10^{-5} (honey) = 0.00058, i.e. 0.058% of the adult LD_{50}.

Table 2.9: Residues detected in honeybee samples submitted to the UK in 2009 and 2010 in which multiple pesticides were detected, the calculated total toxic units for the pesticide residues and the major contributor to the toxicity.

Sample No.	Sample type	Pesticide	Major contributor to TU	Total toxic units (TU)	% LD50
80404	bumble bee	Azoxystrobin; boscalid; cypermethrin	cypermethrin 96%	0.106	10.6
81443	honey bee	Bendiocarb; deltamethrin; propiconazole	Bendiocarb 98%	0.172	17.2
81522	honey bee	Bendiocarb; fluvalinate (varroacide)	Bendiocarb 99%	0.073	7.3
82187	honey bee	Bendiocarb; DDE-pp; pirimiphos-methyl	Bendiocarb 86%	0.067	6.7
85780	honey bee	Fluvalinate (varroacide); tebuconazole	Fluvalinate 75%	3.68 x 10 $^{-5}$	0.0037
85781	honey bee	Imidacloprid; tebuconazole	Imidacloprid 96%	0.0060	0.6
85878	honey bee	Dieldrin; HCH-gamma; permethrin; Propiconazole; thiacloprid	Permethrin 93%	0.051	5.1
85943	honey bee	Chlorpyrifos; propiconazole	Chlorpyrifos 99%	0.0027	0.27
86418	honey bee	Fluvalinate (varroacide); propiconazole	Fluvalinate 76%	9.9 x 10 $^{-5}$	0.0099
86419	honey bee	DDT-pp; methomyl; propiconazole	Methomyl 96%	0.073	7.3
86420	honey bee	DDT-pp; fipronil; propiconazole	Fipronil 99% (veterinary use)	0.100	10
86781	honey bee	Chlorpyrifos; dimethoate; fluvalinate (varroacide) thiacloprid	Dimethoate 99%	0.220	22
86782	honey bee	Chlorpyrifos; cyhalothrin-lambda; difenoconazole; dimethoate; propiconazole; thiacloprid	Dimethoate 94%	0.285	28.5
86783	honey bee	Chlorpyrifos; cyhalothrin-lambda; dimethoate; fluvalinate (varroacide); thiacloprid	Dimethoate 92%	0.182	18.2
87110	honey bee	Bendiocarb; imidacloprid	Bendiocarb 95%	0.733	73.3
87543	honey bee	Bendiocarb; permethrin; propiconazole tebuconazole	Bendiocarb 96%	0.364	36.4
87729	honey bee	Dieldrin; HCH-gamma; permethrin	Permethrin 39%	0.0182	1.82
88040	honey bee	Chlorpyrifos; glyphosate; thiacloprid	Chlorpyrifos 97%	0.0174	1.74

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Table 2.10 Spain: Mean residues (µg/kg) in samples of stored pollen collected in the spring and autumn of 2008 in Spain ((Bernal et al., 2010)

Apiary	**Fipronil (parent + sulphide, sulfone and desulfinyl)**	***Chlorfenvinphos µg/kg (varroacide) LD50 4.1µg/bee***	**Endosulfan sulphate 7.1 *µg/bee***	***Fluvalinate (varroacide) 18.4 µg/bee***	***Bromopropylate (varroacide) LD50 not known***	**HCB *LD50 not known***	**Triflural in >100 µg/bee**	**Total toxic units/kg pollen**	**Toxic unit intake worker larvae 5.4 mg pollen /bee over 7 days**	**Toxic unit intake nurse bees 6.5 mg pollen /bee over 10 days**
C1	ND	24-62	2-7	ND	ND	ND	1-5	=(62/4.2)+(7/7.1)+(5/100) = 15.79	8.5×10^{-5}	10.2×10^{-5}
C2	ND	20-34	3-12	ND	ND	ND	1-4	=(34/4.1)+(12/7.1)+(4/100) =10.02	5.4×10^{-5}	6.4×10^{-5}
C3	ND	ND	ND	8-18	ND	ND	3-13	=(18/18.2)+(13/100) = 1.12	0.60×10^{-5}	0.72×10^{-5}
C4	ND	16-48	4-27	ND	2-12	ND	ND	=(48/4.1)+(27/7.1) = 15.5	8.4×10^{-5}	9.9×10^{-5}
C5	ND	ND	ND	ND	ND	ND	5-7	0.07	0.038×10^{-5}	$0.045x\ 10^{-5}$
C6	ND	22-31	1-3	3-27	2-6	ND	ND	=(31/4.1)+(3/7.1)+(27/18.4) =9.45	5.1×10^{-5}	6.0×10^{-5}
A1	ND	11-27	ND	ND	3-7	23-57	5-12	(27/4.1)+(12/100) = 6.71	3.6×10^{-5}	4.3×10^{-5}
A2	ND	ND	22-78	3-5	8-20	ND	4-22	(78/7.1)+(5/18.4)+(22/100)=11.49	6.2×10^{-5}	7.4×10^{-5}
A3	ND	32-60	7-20	ND	ND	14-44	3	=(60/4.1)+(20/7.1)+(3/100) = 17.47	9.4×10^{-5}	11.2×10^{-5}
A4	ND	44-132	ND	4-12	ND	ND	1-5	=(132/4.1)+(12/18.4)+(5/100)= 32.9	17.8×10^{-5}	21.1×10^{-5}
A5	ND	ND	2-14	ND	ND	18-94	2-7	=(14/7.1)+(7/100) = 2.04	1.1×10^{-5}	1.3×10^{-5}
A6	ND	4-12	ND	15-24	7-17	3-21	ND	=(12/4.1)+(24/18.1) = 4.26	2.3×10^{-5}	2.7×10^{-5}

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Table 2.11 Honey sampled from colonies sampled in Greece (Balayiannis and Balayiannis, 2008)

Location	**Method**	**Analytical method**	**Residue**	**Toxic units/kg honey**	**Toxic unit intake worker larvae 74.2 mg honey (80% sugar) /bee over 7 days**	**Toxic unit intake nurse bees 500 mg honey (80% sugar) /bee over 10 days**
Greece (north, central and south)	Collected randomly from 16 apiaries associated with citrus	Multiresidue methods for organophosphorus pesticides	*Chlorfenvinphos 0.15-0.20 µg/kg (n=4)* Chlorpyrifos methyl 0.12-0.22 µg/kg (n=10) Phorate 0.07-0.17 µg/kg (n=2) *Coumaphos 0.14 -3.1 µg/kg*	=(0.2/4.1)+(0.22/0.059)+(0.17/0.32)+(3.1/2.9) = 5.38	40×10^{-5}	269×10^{-5}
Greece (north, central and south)	Collected randomly from 17 apiaries associated with cotton	Multiresidue methods for organophosphorus pesticides	Chlorpyrifos methyl 0.10-0.24 µg/kg (n= Phorate 0.50-0.89 µg/kg *Coumaphos 0.10 -4.5 µg/kg*	=(0.24/0.38)+(0.89/0.32)+(4.5/2.9)= 4.96	37×10^{-5}	248×10^{-5}
Greece (north, central and south)	Collected randomly from 9 apiaries associated with sunflower	Multiresidue methods for organophosphorus pesticides	Phorate 0.09-0.68 µg/kg (n=4) *Coumaphos 1.4 -4.8 µg/kg*	=(0.68/0.32)+(4.8/2.9)= 3.79	28×10^{-5}	190×10^{-5}

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2.4. Synergy between chemicals

2.4.1. Detoxifying enzymes in honeybees as a basis of synergy

The honeybee genome has substantially fewer protein coding genes than *Drosophila melanogaster* and *Anopheles gambiae* with some of the most marked differences occurring in three superfamilies encoding xenobiotic detoxifying enzymes (Claudianos et al., 2006). This variation makes extrapolation of responses to both individual pesticides and pesticide mixtures between species less reliable as there are only about half as many of the three major xenobiotic metabolising enzymes glutathione-S-transferases (GSTs), cytochrome P450 monooxygenases (P450s) and carboxyl/cholinesterases (CCEs) in the honeybee. The glutathione-S-transferase group of enzymes catalyse the metabolism of pesticides by conjugation of reduced glutathione — via a sulfhydryl group — to electrophilic centers on a wide variety of substrates. The P450s catalyse a range of reactions including oxidation and demethylation which may result in decrease in activity or produce active metabolites, e.g. the conversion of the thion to oxon forms of organophosphorus pesticides or the conversion of the neonicotinoid thiamethoxam to clothianidin. Tau-fluvalinate and flumethrin used in varroa control differ from the more toxic pyrethroids in that they share an aromatic ring on the acid moiety. One of the numerous reactions catalyzed by P450s is the oxidation of aromatic rings (Ortiz de Motellano and De Voss, 2005). The additional aromatic ring on tau-fluvalinate and flumethrin may present an additional site for modification by P450s, or the additional aromatic ring may affect the interaction between the pyrethroid molecule and the P450 active site, resulting in enhanced activity on the aromatic rings of the alcohol moiety. In either case, the additional aromatic ring may allow more rapid detoxification and result in the lower toxicities exhibited by these two pyrethroids. Thus, inhibition of P450s may result in a greater increase in toxicity. The carboxyl/cholinesterase catalyse the cleavage of esters bonds to give carboxylate and alcohol, e.g. cholinesterases cleave acetylcholine at nerve junctions and inhibition of these esterases may also result in increased toxicity if this is a major route of metabolism.

The midgut in the honeybee is a major site of metabolism for ingested pesticides and interactions between chemicals at least in part may be influenced by effects on the detoxifying enzymes within the midgut, including microsomal oxidases, glutathione S transferases and esterases. Microsomal oxidase assay required intact midgut because an inhibitor of P450 is released when midguts are dissected and midgut microsomal preparations contained mainly cytochrome P-420, the inactive form of cytochrome P-450, which may explain the low microsomal oxidase activity in microsomes (Johnson etal 2009). The microsomal oxidase activities include aldrin epoxidase activity which is inhibited by malathion and permethrin, N-demethylase activity which is induced by diazinon and EPN and O-demethlase activity which is induced by diazinon. Of the glutathione S-transferases, aryltransferase activity is significantly induced by diazinon and moderately induced by permethrin. Carboxylesterase activity is moderately inhibited by malathion and permethrin (Suh and Shim, 1988; Yu et al., 1984).

The P450s are thought to play a central role in insects in the metabolism of phytochemicals (Li et al., 2007). Examples of such phytochemicals relevant to honeybees are the flavonoids (flavonoles e.g.quercetin, kaempherol, galangin, fisetin, flavanones e.g. pinocembrin, naringin, hesperidin and flavones e.g. apigenin, acacetin, chrysin, luteolin) which occur as glycosides in nectar and are hydrolysed to aglycones during the formation of honey and are also present in propolis and pollen (Viuda-Martos et al., 2008). However, when compared with other insects there are a significantly lower number of CYP3 clans (which include the CYP6s and CYP9s) associated with xenobiotic metabolism encoded in the honeybee genome (Johnson et al., 2010). Although this may be related to the reduced exposure to chemically-defended plant tissues there is some suggestion that others e.g. the CYP6AS subfamily, have undergone an expansion relative to other insects (Mao et al., 2011). CYP6

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enzymes are recognised as being involved in the metabolism of dietary constituents in herbivorous insects (Liu et al., 2006). Therefore this expansion may be due to the presence of specific phytochemicals in the diet, e.g. in pollen and nectar, which may be concentrated in honey and bee bread (Adler, 2000). The link to phytochemical exposure in honeybees is supported by the upregulation of three of the CYP6AS genes in response to consumption of honey (Johnson, 2008).

In identifying the relevance of synergy in determining the toxicity of mixtures it is important to understand the route of metabolism of pesticides in honeybees and the effects of age, season etc on this (Nielsen et al., 2000; Smirle, 1988; Smirle and Winston, 1987). The classic P450 synergist piperonyl butoxide has a temperature dependent effect in honeybees in concurrent exposure to carbamates which reflects the relationship between temperature and enzymatic rates of reaction (Table 2.14) (Georghiou and Atkins Jr, 1964).

Synergy is highly dependent on the enzymatic profile of the individual. The level of synergy shown between pyrethroid deltamethrin and the P450-inhibiting fungicide prochloraz is much reduced during winter months, suggesting a reduced role for P450-mediated detoxification in winter bees (Meled et al., 1998). During this review it was noted that few, if any studies identified the time of year that the study was conducted, whether studies were conducted concurrently or even whether any in-hive treatments had taken place prior to the study- in one case it was clearly stated that antibiotics had been used prior to the study ((Johnson et al., 2009). As identified later antibiotics and varroacides may interact with other chemicals within the hive.

Unlike a wide range of vertebrates and invertebrates synthetic pesticides do not appear to readily induce P450s in bees (Johnson, 2008). Although there is some evidence of induction by benzo(a) pyrene and by the pyrethroids fluvalinate and cymiazole hydrochloride (Kezic et al., 1992). Therefore the majority of synergistic effects observed have been ascribed to inhibition rather than induction of P450s involved in pesticide metabolism.

2.5. Non-pesticide synergists

A limited number of studies have been undertaken with classical enzyme inhibitors on the toxicity of pesticides to honeybees but even this limited number suggest significant differences between honeybees and other insects. Phenobarbital, a barbiturate drug, induces P450s in many organisms, and phenobarbital, xanthotoxin, salicylic acid and indole-3-carbinol are all effective inducers in other insects, but no induction has been observed in bees fed these P450 inducers and no change in P450gene expression has been observed in phenobarbital-treated bees (Johnson, 2008).

Table 2.12 summarises the laboratory studies reported in which the toxicity of pesticides applied as mixtures or sequentially with classic pharmacological synergists has been assessed. In all cases the synergists have been applied at a single dose so no dose-response or threshold effect can be identified.

Piperonyl butoxide (PBO) is a classic P450 inhibitor. Johnson et al. (2006) showed that PBO increased the toxicity of the pyrethroid insecticides cyfluthrin, lambda cyhalothrin and tau-fluvalinate by factors ranging from 30 to 980 fold (Table 2.12A). Iwasa et al. (2004a) investigated the effect of PBO on the neonicotinoid insecticides and showed that under the same conditions it had little effect on the toxicity of imidacloprid but increased the toxicity of acetamiprid 6 fold and thiacloprid 154 fold (Table 2.12B). These data show that even with known pesticide synergists the scale of the increase in toxicity differs widely between compounds and is related to the relative importance of the affected P450s on the metabolism of the pesticides.

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Quercetin, the main flavinoid component of pollen, nectar and honey, is a substrate shared by a number of CYP6AS enzymes (1, 3, 4 and 10) (Mao et al., 2011) and has been demonstrated to reduce the toxicity of tau-fluvalinate in honeybees whilst microarray analysis of the guts of bees fed extracts of honey, pollen and propolis also showed elevated expression of three CYP6AS subfamily P450 genes (Johnson et al., 2012). Johnson et al. (2012) identified that bees fed honey extracts had more robust guts than those fed sucrose, as evidenced by their larger dimensions, and concluded that prophylactic induction of P450s through consumption of pollen and honey flavonoids may enhance bee survival (Table 2.13). This work suggests that regulation of honey bee P450s is highly tuned to chemicals that occur naturally in the hive environment and that, in terms of toxicological capacity, a diet of sugar fed to a colony or in laboratory studies is not equivalent to a diet of honey (Johnson et al., 2006; Johnson et al., 2012). Feeding honey or propolis to bees increased their resistance to aflatoxin B1 compared with sucrose or high fructose corn syrup (Table 2.13) whereas the P450 inhibitor PBO decreased survival time (Niu et al., 2011). A wide range of plant alkaloids are present in honey but there is no information on their potential effects on metabolising enzymes in honeybees (Jullien, 2009; Reinhard et al., 2009).

The carboxylesterase inhibitor DEF has been shown to increase the toxicity of the pyrethroids cyfluthrin 2 fold and tau-fluvalinate 5 fold and of the neonicotinoid acetamiprid 3 fold demonstrating the role of esterases in the detoxification of these pesticides whereas the inhibition of glutathione-S-transferases by DEM resulted in 2.3 fold increase in the toxicity of lambda cyhalothrin but no effect on the toxicity of tau-fluvalinate or acetamiprid (Tables 2.12A and B) (Johnson et al., 2006; Iwasa et al., 2004a).

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Table 2.12A: Synergism of three pyrethroid insecticides administered 1 hr after treatment with acetone control or detoxification enzyme inhibitors (DEM, a glutathione-S-transferase inhibitor; DEF, a carboxylesterase inhibitor; or PBO, a cytochrome P450 monooxygenase inhibitor) (Johnson et al., 2006)

Insecticide	Inhibitor	n	LD50 ng/bee (95% CI)	Synergism		
				X_2	DF	Ratio LD50
Cyfluthrin	None	440	62.0(67.7-74.5)	2.9	4	-
	DEF	280	29.0(20.5-51.5)	9.5	3	**2.3***
	PBO	380	2.24(1.48-4.18)	13.8	4	**30***
Lambda-cyhalothrin	None	360	102(73.0-133)	6.0	3	-
	DEM	260	38.0(27.5-50.7)	3.2	3	2.7*
	PBO	360	1.28(1.12-1.46)	2.0	3	80*
Tau-fluvalinate	None	820	9450(7480-12000)	11.1	5	-
	DEM	620	8260 (7570-9030)	2.2	4	1.1ns
	DEF	480	1960(830-4170)	23.8	4	4.8*
	PBO	880	9.64(6.61-14.9)	11.6	4	980*

*Statistically significant, X_2: X_2 value; DF: degrees of freedom, Ratio LD50: ratio of LD50 control single compounds versus mixture.

Table 2.12B: Pretreatment effect of general insecticide synergists on honey bee mortality (LD50 after 24 h) (Iwasa et al., 2004a)

Insecticide synergist	n	LD50 (ug/bee)c	95% CI	Chi-square	Slope SE	SR	95% CI
Acetamiprid							
Alone	465	7.07	4.57–11.2	0.826	1.78	1	
PBO	202	1.17	0.342–3.79	1.18	1.56	6.04	4.29–8.51
DEF	124	2.39	0.278–12.4	5.85	2.97	2.96	1.83–4.76
DEM	123	6.94	4.10–13.2	0.278	1.47	1.02	0.783–1.33
Imidacloprid							
Alone	137	0.0179	0.0092–0.0315	0.303	1.71	1	
PBO	152	0.0105	0.0061–0.0172	0.0889	1.67	1.70	1.29–2.26
Thiacloprid							
Alone	158	14.6	9.53–25.4	0.480	2.74	1	
PBO	193	0.0948	0.0406–0.211	0.424	1.67	154	115–207

DEM, glutathione-S-transferase inhibitor; DEF, carboxylesterase inhibitor; PBO, cytochrome P450 monooxygenase inhibitor, SR; synergism ratio.

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2.6. Synergy between pesticides

There are a range of pesticides which are also varroacides (e.g. coumaphos, tau-fluvalinate) and where their primary use is as in-hive treatments they are discussed in section 2.6.

The synergy of pesticides may be predicted by their mode of action (Cedergreen et al., 2006; Jullien, 2009) and biomarkers have been proposed to determine the scale of any synergism (Walker, 1998). However, metabolomics is showing increasing applications to understand the interactions of pesticides with organisms including their bioactivation (Alferis and Jabaji, 2011; Morimoto et al. 2011). Table 2.14 summarises the laboratory studies conducted with honeybees and pesticide mixtures, Table 2.15 summarises semi-field studies with pesticide mixtures and honeybees and other bee species and Table 2.16 summarises laboratory studies with other bee species and pesticide mixtures. There were some reports identified where there were indications of interactions but the details of the studies were insufficient to fully evaluate the information, (Kuhn, 1985; de Batista et al., 1976; Jullien, 2009; Wang et al., 2006; van der Steen and Dinter, 2007; Ibrahim and Eshbah, 1989; Volckaert and van Laere, 1984; Anonymous, 1988; Anonymous, 1989; Bernard, 1990; Floch, 2003).

2.6.1. Carbamate insecticides

The toxicity of carbamate insecticides is affected by the P450 inhibitor piperonyl butoxide (Table 2.14) and therefore potentially by pesticides interacting by the same mechanism. Sulfenyl-propoxur is greater than 33 times more toxic to house flies (LD_{50} 24.5 µg/g) than honeybees (LD_{50} > 800 µg/g) and is enhanced more than 18.2 fold by the synergist piperonyl butoxide. However the metabolite propoxur, is more toxic to the honey bee (LD_{50} 4.5 µg/g) than to house flies (LD_{50} 24.5 µg/g). Two factors account for these differences in toxicity of sulfenyl-propoxur 1) reduced penetration in the honey bee and 2). the slower *in vivo* conversion of sulfenyl-propoxur to its active toxic parent carbamate, propoxur. The high susceptibility of bees to propoxur (and hence the effect of the PBO synergist in inhibiting metabolism of propoxur) was related to high internal amounts of unchanged propoxur found soon after treatment (Lagier et al., 1974; Mallipudi, 1978).

Sonnet et al. (1978) demonstrated that co-exposure of carbamates with 100ppm atrazine resulted in no increase in toxicity with carbaryl and up to a 5 fold decrease in toxicity of carbofuran (Table 2.14) in an inverted dose-response, i.e. greatest decreases at lower doses. This is in accordance with work that suggested other insect P450s are unaffected by atrazine (Anderson and Lydy, 2002).

The use of fungicides to enhance the toxicity of carbamates to the green leafhopper, *Nephotettix cincticeps* showed they were effective in the order carbendezim (EBI fungicide) > zineb > dinocap > maneb > sulfur > tridemorph but no mode of action for the enhancement was proposed (Prakash and Srivastava, 1997).

Topical applications of DEET and propoxur (carbamate), in mosquitos showed that synergism between DEET and propoxur disappeared in the presence of PBO but not with DEF (an esterase inhibitor) suggesting that DEET interacts with P450s (Bonnet et al., 2009).

2.6.2. Neonicotinoid insecticides

There was only one report of interactions between classic chemical synergists and neonicotinoid insecticides (Iwasa et al., 2004b) (Table 2.12B). Piperonyl butoxide at 10µg/bee increased the contact toxicity of acetamiprid 6 fold and imidacloprid 1.7 fold based on the LD_{50}. This suggests for the

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cyano-substituted neonicotinoids, acetamiprid and thiacloprid, oxidation is an important neonicotinoid detoxification pathway in the honey bee as the acetamiprid metabolites N-demethyl acetamiprid,6-chloro-3-pyridylmethanol and 6-chloro-nicotinic acid when applied topically, produced no mortality at 50 µg/bee. However, for imidacloprid the low increase in contact toxicity when PBO was applied suggests that P450s are less relevant and their importance may vary between species. The metabolites of imidacloprid in the honey bee are a hydroxy derivative at the 5' position and an olefin derivative in the imidazolin ring (Suchail et al., 2004). The olefin has higher not lower insecticidal activity than the parent (Nauen et al., 2001). However, species differences in metabolism exist as, in the house fly, PBO increased imidacloprid toxicity 10.7-fold (Liu et al., 1995) while O-propyl-O-(2-propynyl)phenylphosphate (PPP) increased both imidacloprid and acetamiprid toxicity (Yamamoto et al., 1988).

The carboxylesterase inhibitor DEF significantly increases the toxicity of acetamiprid 3 fold but the glutathione inhibitor DEM has no significant effects (Iwasa et al., 2004b) suggesting carboxylesterases also play a role in the metabolism of acetamiprid although a less important than the oxidative route.

The role of oxidative metabolism in detoxification of the cyano-substituted neonicotinoids in bees is highlighted by the increase in toxicity of acetamiprid and thiacloprid in combination with the EBI fungicides (Table 2.14). These fungicides act by inhibiting P450s involved in ergosterol biosynthesis in fungi and inhibit P450 in insects (Brattsten et al., 1994). The mortality following thiacloprid, acetamiprid or imidacloprid 1 hour after treatment with 10 µg/bee propiconazole shows similar effects to PBO (Table 2.12B) with the LD_{50} of imidacloprid effectively unchanged whereas that of acetamiprid decreased 105 fold and thiacloprid 559 fold (Figure 2.2) (Iwasa et al., 2004b). Similar effects were observed with epoxiconazole, triadimefon, triflumizole and uniconazole-P. Tebuconazole at 3 µg/bee also increases the toxicity of thiacloprid with mortality increasing from 3 to 70% (Schmuck et al., 2003a). Alptekin et al., (2011) has suggested that identification of the P450s over-expressed following exposure with thiacloprid may offer the opportunity to design of future synergists to protect these enzymes.

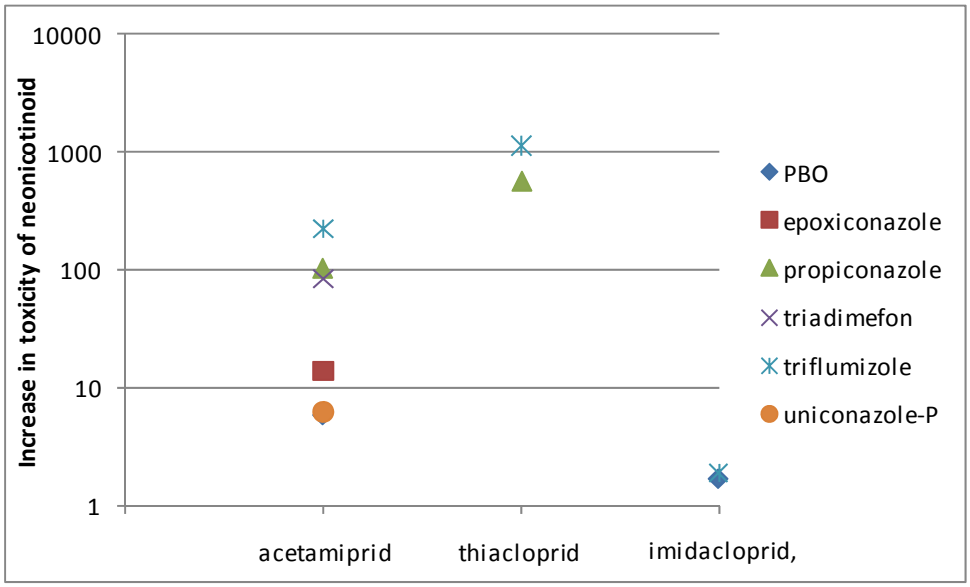

Figure 2.2 Increase in contact toxicity (decrease in LD_{50}) of acetamiprid, thiacloprid and imidacloprid in the presence of 10 µg/bee a range of P450 inhibitors (data from Iwasa et al., 2004b)

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The level of exposure to the synergist also affects the scale of the synergy. Prochloraz at 1µg/bee contact dose has no effect on the toxicity of thiacloprid but increases mortality from 10 to 87% when bees were doses with 10 µg prochloraz/bee (Schmuck, Stadler et al. 2003). At a contact dose of 10 µg/bee propiconazole increased the toxicity of thiacloprid 559 fold (Iwasa et al 2004). Similar data have been demonstrated for the relationship between the contact dose of propiconazole and thiamethoxam (Figure 2.3) (Defra report PS2368).

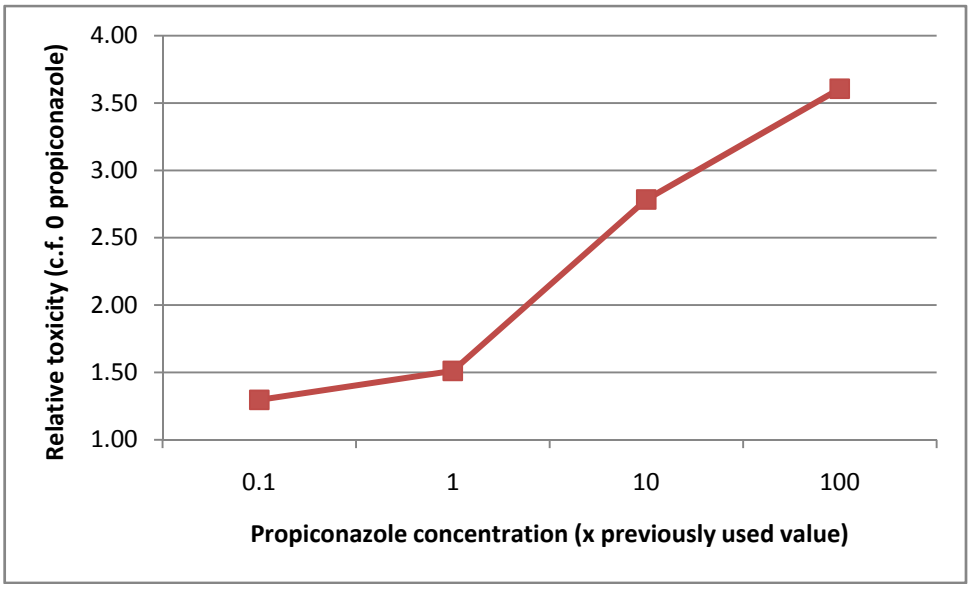

Figure 2.3: Effect of propiconazole exposure on the contact LD50 of thiamethoxam (Defra project PS2368).

The effect of exposure on the scale of synergy is important as many of the laboratory studies have been undertaken with high doses of synergists, e.g. 3-10 µg/bee and at more realistic exposure levels such high increases of toxicity have not been observed even under laboratory conditions (Defra report PS2368). This is confirmed by semi-field studies with field rates of thiacloprid and tebuconazole and of acetamiprid and triflumizole in which no increase in mortality was observed (Iwasa et al., 2004b; Schmuck, Stadler et al. 2003) (Table 2.14). Based on the exposure scenarios identified in section 1 the 90th percentile exposure for a forager bee from a spray application of 1 Kg/ha fungicide would be 1.53 µg/bee from spray application and 3.63 µg/bee/day from nectar therefore for many of the EBI fungicides the maximum EU application rate is 250 g ai/Ha resulting in a 90th percentile exposure of 0.38 µg/bee from spray and potentially a total including oral exposure of 1.3 µg/bee on the day of application.

Defra project PS2368 assessed the impact of joint contact:contact and oral:oral exposures to a range of neonicotinoid insecticides (clothianidin, thiamethoxam, imidacloprid and thiacloprid) and EBI fungicides (flusilazole, myclobutanil, propiconazole and tebuconazole) at realistic fungicide exposure rates (extrapolated from Koch and Weisser, 1997). This showed only lower order increases in the toxicity of the neonicotinoids (up to 3 fold) but differences between effects following contact and oral exposures.

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Four other non-EBI fungicides have been assessed for their effects on the toxicity of thiacloprid (Schmuck, Stadler et al. 2003). Cyprodinil (an anilinopyrimidine fungicide) at 8 µg/bee and tolyfluanid (a phenylsulfamide fungicide) at 11 µg/bee slightly increased the mortality assocated with a contact dose of 2 µg thiacloprid /bee from 3 to 20% and 3 to 13% respectively. Mancozeb (a dithiocarbamate fungicide) at 8 µg/bee and azoxystrobin (a methoxyacrylate stobilurin fungicide) at 3 µg/bee had no effect on the toxicity of a contact dose of 2 µg thiacloprid /bee.

2.6.3. Phenylpyrazole insecticides

Only one phenylpyrazole insecticide is in widespread use – fipronil and metabolism appears unaffected by the EBI fungicide and the LD_{50} for both contact and oral toxicity was additive (Table 2.14).

2.6.4. Organochlorine insecticides

The organochlorine insecticides are metabolised by the P450 system as shown by the effects of oral exposure to 5mg phenobarbital /g in candy on the toxicity of aldrin and dieldrin (Table 2.14). The LD_{50} of aldrin decreased from 60.5 to 38.5 ng/bee and the LD_{50} of dieldrin decreased from 37.2 to 20.7 ng/bee (Johnson et al., 2012).

2.6.5. Organophosphorus insecticides

(see also section 2.6 for information relating to coumaphos)

Organophosphorus pesticides are more toxic in their oxon form and the conversion of the thion to oxon form is P450 dependent. The metabolism of the oxon occurs through multiple pathways including P450s esterases and glutathione-S-transferases. Therefore P450 inhibitors would be expected to have little or no effect on the toxicity organophosphorus pesticides due to reduced conversion to the active oxon form. 2,4-D, atrazine, monuron and simazine had no effect or decreased the toxicity of a range of organophoshorus pesticides and with atrazine this effect was dose dependent (Sonnet et al., 1978) (Figure 2.4) and iprodione had no effect on the toxicity of phosalone (Table 2.14) (Twinn et al., 1984).

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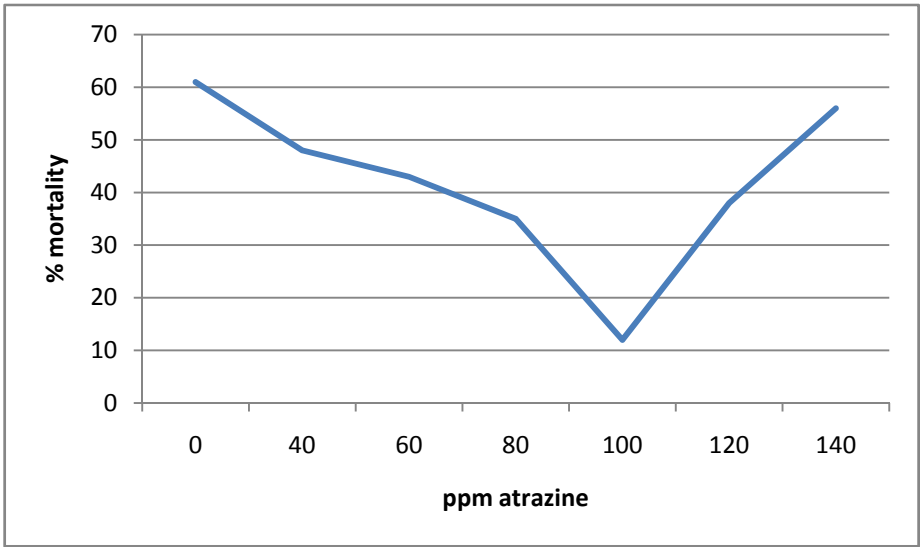

Figure 2.4 Effect of atrazine concentration on the mortality associated with oral exposure to 3ppm malathion

2.6.6. Pyrethroid insecticides

(see also section 2.6 for information relating to tau-fluvalinate and flumethrin)

A major pathway of metabolism for many of the pyrethroid insecticides involves P450s. This is demonstrated by the effects of the classic P450 inhibitors PBO and phenobarbital which have been shown to increase the toxicity of lambda-cyhalothrin and permethrin up to 9 fold with dose-dependence (Hagler et al., 1989; Pilling, 1992) (Table 2.14) and tau-fluvalinate (also a varroacide) up to 42 fold (Table 2.17) (Johnson et al., 2012). Xanthotoxin, salicyclic acid and indole-3-carbinol which are known to induce P450s in other insects, and thus decrease the toxicity of pesticides which are metabolised by these enzymes, only had no effect or increased the toxicity of tau-fluvalinate in honeybees with toxicity increased up to 230 fold with xanthotoxin (Table 2.17) (Johnson et al., 2012).

The effects of prior exposure to a range of natural synergists in the honeybee diet has shown the potential for these to induce P450s unlike phenobarbital. Quercetin which is naturally present in honey decreased the toxicity of tau-fluvalinate 1.4 fold (Table 2.16) (Johnson et al., 2012).

By far the greatest number of reports of synergy in honeybees involve EBI (ergosterol biosynthesis inhibitor) fungicides and pyrethoid insecticides (Table 2.14). EBI fungicides act by inhibiting the P450s involved in ergosterol biosynthesis in fungi and appear to be potent inhibitors of P450s involved in pyrethroid metabolism in honeybees e.g. (Colin and Belzunces, 1992b; Pilling, 1992; Pilling et al., 1995). The data shown in Table 2.14 primarily relate to realistic exposure ratios and show that the very high ratios identified in laboratory studies with classical synergists (Table 2.12) are usually at unrealistic ratios in relation to field uses and result in less than 15 fold synergy and more often 3 fold or less.

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There were no reported effects, other than a slight decrease in toxicity, in mixtures of carbendazim with alphacypermethrin or lambda cyhalothrin (Thompson and Wilkins, 2003). It should be noted that the treatment rates used were the maximum application rates for the fungicides are similar to the expected exposure rates as identified in Chapter 1. For example propiconazole is applied at 125g ai/ha which using the RUD calculated in Chapter 1 would relate to 90th percentile exposure of 0.19 µg/bee and the ratio used in the study was 0.3 µg/bee. Difenoconazole effects appear to vary depending on the treatment rate and whether the formulations or active ingredients are tested with effects varying from no change in toxicity to 3.7 fold increase in toxicity with a lambda-cyhalothrin in a 1:33 ratio. Belzunces and Colin (1993) showed a difference depending on whether dosing with the deltamethrin and difenoconazole and deltamethrin and prochloraz formulations occurred as a mixture or sequentially and this suggests that the rate of uptake of the two active ingredients may vary. This differing effect between active ingredients and formulations occurs for a number of pyrethroid fungicide mixtures including but effects are not consistent, e.g. lambda cyhalothrin and flusilazole, shows 2.2-2.5 fold synergy between active ingredients and between formulations but alphacypermethrin and flusilazole shows synergy between the active ingredients but not between the formulations (Figure 2.5).

This suggests it is important to assess the combination in the ratio and formulation to be used in the field to determine whether significant synergy is likely to occur. In tunnel studies with mixtures of EBI fungicides and pyrethroids (Table 2.16) no effects were observed in mixtures of tau-fluvalinate and difenoconazole + carbendazim (Lefebvre and Bassand, 2001; Rouas, 1987) whereas lambda cyhalothrin and flusilazole resulted in increased mortality which is in agreement with the synergy observed in the laboratory (Defra PN0945). The increased toxicity of pyrethroid/EBI fungicide tank mixes have resulted in incidents in normal use. For example incidents have been reported in Germany in 1996 and 1997 following tank mixing of pyrethroids and EBI fungicides (Brasse, 2001). In 1997 4 incidents occurred in flowering oilseed rape related to the tank mixing of alphacypermethrin and in one case fluvalinate with EBI fungicides. In 1998 there were 47 incideints with mixtures of EBI fungicides with alphacypermethrin (36), lambda-cyhalothrin (8) and tau-fluvalinate (3). As a result tank mixing of pyrethroids and EBI fungicides was prohibited in Germany. In the UK 17 incidents involving mixtures occurred in 11 years from 1988 to 1998 (Brobyn, 2001) and of these 6 potentially involved a pyrethroid mix with a fungicide. These involved tanks mixes of alphacypermethrin, vinclozolin and iprodione, alphacypermethrin and prochloraz, deltamethrin and prochloraz, alphacypermethrin, carbendazim and iprodione, alphacypermethin, iprodione and thiophanate methyl, lambda-cyhaolthrin and carbendazim. It was concluded that given some of the incidents involved direct overspray of foraging bees (which contravened the label) that the situation would be monitored to determine whether changes to labelling or user education was required.

Pyrethroid mixtures have also been assessed with the non-EBI fungicide chlorothalonil (a chloronitrile) and this shows a 1.4-2.1 fold increase in contact toxicity suggesting interaction with the metabolism of the pyrethoids alphacypermethrin and lambda-cyhalothrin (Thompson and Wilkins, 2003). Mixtures of the same two pyrethroid insecticides with the dithiocarbamate fungicide mancozeb suggested 2 to 4 fold decrease in contact toxicity (Thompson and Wilkins, 2003). Mixtures of deltamethrin with pirimicarb suggested the combined effect was less than additive as increased acetylcholinesterase activity following the pyrethroid exposure was not reduced by pirimicarb exposure (Badiou and Belzunces, 2008). Oral exposure to fluvalinate and permethrin with carbaryl, mancozeb and paraquat resulted in only additive toxicity suggesting no interaction (Chaney, 1988). Esterases are also involved in the detoxication of pyrethroids in other insects as shown by the synergy between pyreoids and organophoshates (which inhibit esterases) in mosquitos (Bonnet et al., 2004).

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As effects are dose-dependent synergism may be an area where modelling is applicable both from toxicokinetic/toxicodynamic and QSAR approaches. To date mathematical modelling has been limited and has used compartmental models based on degradation of toxic agents rather than structural approaches (Chalvet-Monfray et al., 1996; ChalvetMonfray et al., 1995). The formulation differences shown in Figure 2.5 suggest such approaches are currently limited in applicability.

There are very limited data reported on the combined effects of pesticides in bee species other than honeybees but these are shown in Table 2.15 for laboratory studies and Table 2.16 for semi-field studies. Three studies have been reported with N. melanderi the alkali bee with mixtures of the organophosphorus insecticides demeton and trichlorfon, the sulphite ester acaracide propargite and trichlrofon and propargite, demeton and trichlorfon. In all cases it appeared that the results were greater than additive but there was insufficient data in the report to clearly define the scale of the reported synergism (Johansen et al., 1983).

2.6.7. Other insect species

There are a small number of reports of combinations of other pesticides resulting in synergy in other insects. For example (Abdel-Hafez and Mohamed, 2009) reported spinetoram (Radiant 12 SC) and its mixtures with the chitin synthesis inhibitor (chlorfluazuron 5 EC) and pyrethroid insecticide (fenpropathrin 20 EC) against the second instar larvae of *Spodoptera littoralis*. The results indicated that spinetoram and chlorfluazuron or fenpropathrin revealed showed synergy in all treatments at different concentration of binary mixtures, except the: mixture of spinetoram at (LC_{50}), with fenpropathrin (LC_{10}) which produced an additive effect.

Formamidines (e.g. chlordimefon) have been identified as synergists for the synthetic pyrethroid insecticides in larvae of the boll worm, *Heliothis zea* and the tobacco budworm, *H. virescens* . Toxicity of permethrin and fenvalerate was increased 2-15 fold against both *Heliothis* spp. but lower levels of synergism were observed with decamethrin. (Plapp, 1979).

A rotenone-pyrethrum mixture showed increased toxicity to honeybees but no detailed data were available (Maccagnani et al., 2008).

Data from other insect species suggests not just fungicides but also plant growth regulators which interact with P450 are likely to affect the toxicity of other pesticides requiring this pathway of metabolism (Brattsten et al., 1994; Ramoutar et al., 2010).

Modes of action other than enzyme induction/inhibition have been reported in other species. For example the synergy between pyrethroid and carbamate insecticides has been ascribed to changes at the nerve synapse as no effects were observed when P450 and esterase inhibitors were present and a sublethal dose of nicotine increased the effect (Corbel et al., 2006). The pyrethroid - carbamate mixture increased ACh concentration within the synaptic cleft in American cockroaches, and thus stimulated a negative feedback of ACh release. Atropine, a muscarinic receptor antagonist, reversed the effect demonstrating the implication of the presynaptic muscarinic receptors in the negative feedback regulation process and in synergism. These type of effects have also be reported in mole crickets (Kostromytska et al., 2011) where combining a sodium channel toxin (bifenthrin) and a synaptic toxin (imidacloprid) led to greater than additive neurophysiological and toxic effects, evidence of synergistic neurological ‘‘potentiation’’.

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There are a number of patents involving pesticide mixtures to enhance efficacy against pests and pathogens including nicotinic acid agonists/antagonists and fipronil/ethiprole with azole fungicides (Colliot, 1999; Heuer, 1999; Jamet, 2011; Krohn, 2008). This highlights the importance of testing formulated products. The use of such products as seed treatments or granules also raises the possibility that they are distributed around the plant as shown by their enhanced efficacy. Such enhanced efficacy may be paralleled with residues with higher inherent toxicity as mixtures in guttation fluid, pollen and nectar.

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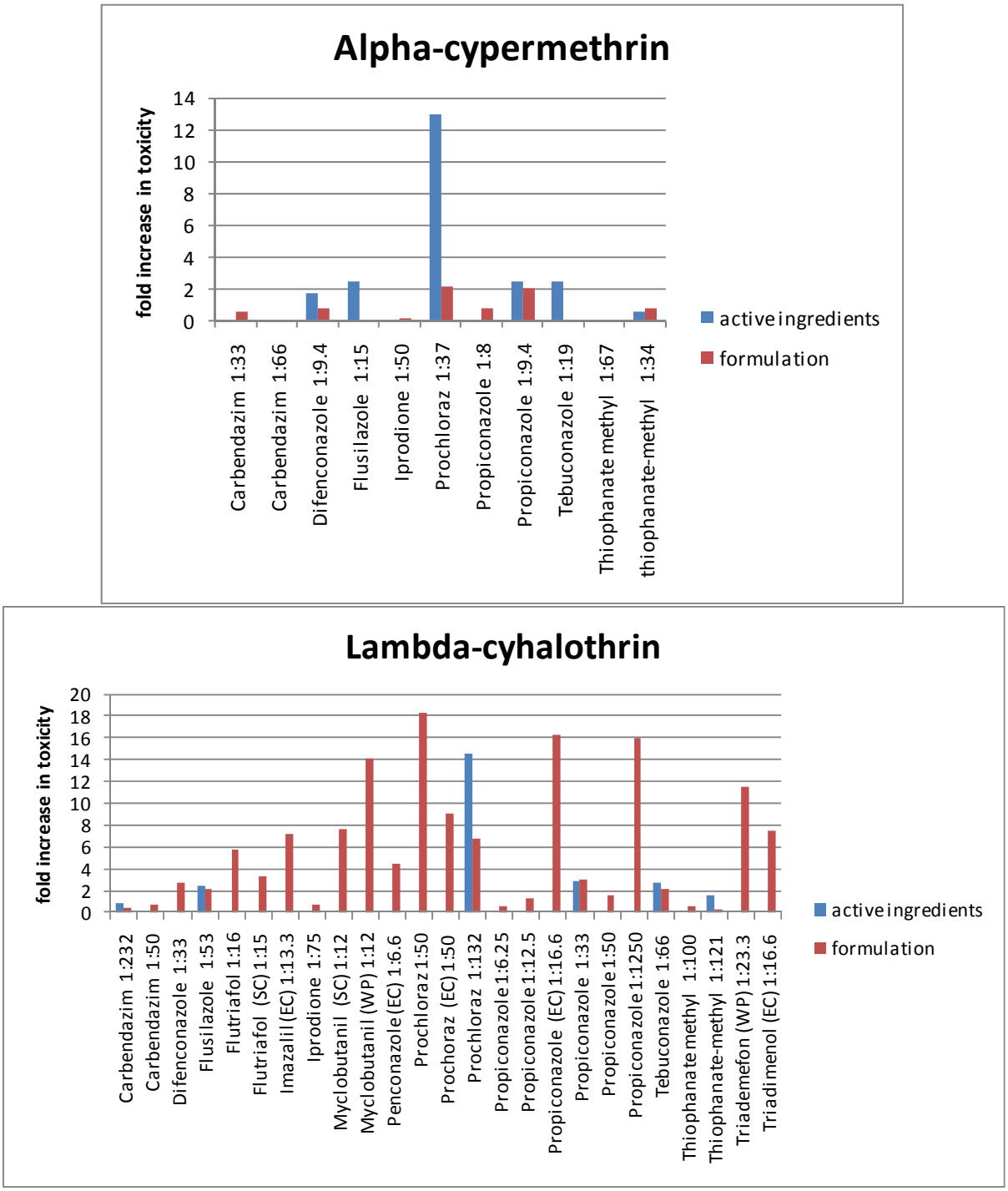

Figure 2.5 Synergy observed in combinations of active ingredients and formulations in contact toxicity studies (data from Defra report PN0945 and Pilling and Jepson (1993).

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2.7. Synergy between in-hive chemicals and pesticides

2.7.1. Varroacides

Chemicals are directly placed within the hive to control varroa and these include the organophosphorus pesticide coumaphos and two pyrethroids tau-fluvalinate and flumethrin. Chlorfenvinphos, amitraz and bromopropylate have also been used as acaracides to control varroa and acarine (tracheal mite) but there were no data reported on their interactions with other chemicals.

Table 2.17 summarises the available data for mixtures of pesticides and in-hive treatments.

The classic P450 inhibitors PBO and phenobarbital synergise the varroacides tau-fluvalinate 423 fold and coumaphos 4 fold (Table 2.17). Other P450 inhibitors/inducers cause lower levels of synergy with indole-3-carbinol resulting in no change and salicyclic acid 1.8 fold increase in toxicity. Exposure to quercetin, which is present in honey, resulted in a decrease in tau-fluvalinate toxicity by 1.4 fold due to induction of P450s (Johnson et al., 2012). Carboxylesterase also plays a role in the metabolism of coumaphos as demonstrated by the 2.7 fold increase in toxicity resulting from exposure to the carboxylesterase inhibitor DEF whereas glutathione-S-transferase plays little role as shown by the no change in toxicity following exposure to the inhibitor DEM (Johnson et al., 2012).

The effects of EBI fungicides on the contact toxicity of the active ingredients of the pyrethroid varroacides flumethrin and tau-fluvalinate shown they are synergised by the fungicides (Table 2.17 and Figure 2.6) with increases in toxicity up to 53 fold with prochloraz and tau-fluvalinate and 19 fold with flumethrin and flusilazole (Defra PN0945). It should be noted that the treatment rates used were the maximum application rates for the fungicides are similar to the expected exposure rates as identified in Chapter 1. For example propiconazole is applied at 125g ai/ha which using the RUD calculated in Chapter 1 would relate to 90th percentile exposure of 0.19 µg/bee and the ratio used in the study was 0.3 µg/bee. These effects were generally reflected when bees treated with the fungicides were co-exposed to the formulated varroacide in the laboratory with slight increases in the toxicity of formulated flumethrin (Bayvarol) and fluvalinate (Apistan) although there were some difference apparent, e.g. increased toxicity of formulated fluvalinate with co-exposure to thiophanate methyl which may be due to the effects of the formulation on uptake. This is supported by the observation in the study that there were difference between the scale of synergy between co-exposure of fungicides with the Apistan formulation of fluvalinate and the pesticide formulation Mavrik (Table 2.18).

Synergy is observed between the two varroacides coumaphos and tau-fluvalinate and is dose related (Figure 2.7) (Johnson, 2009; Johnson et al., 2009). Given the persistence of these varroacides within colonies and the high residues detected in the monitoring studies identified in the exposure section of this report interactions between varroacides may occur if both varroacides are used in a colony over a short period of time. For example in Chapter 1 monitoring studies identified mean residues of coumaphos up to 24840 µg/kg bee (2.48 µg/bee) and tau-fluvalinate up to 326 µg/kg bee (0.0326 µg/bee) (although care should be taken in interpreting these data as non-registered treatments were observed during the surveys (Chauzat et al., 2011).

Pre-exposure to formulated tau-fluvalinate (Apistan) for 48 hours prior to exposure to the pyrethroid insecticide bifenthrin resulted in additive toxicity as may be expected and had no effect on the toxicity of carbaryl or methyl parathion (Ellis et al., 1997).

2.7.2. Antibioticsand other medicines

More recently data have been reported that suggest that antibiotics used in colonies may affect susceptibility to both varroacides and other pesticides (Table 2.19) (Hawthorne and Dively, 2011).

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Such antibiotics may be used in colonies to treated brood diseases American foulbrood or European foulbrood. Exposure of newly emerged bees to 1.4mM oxytetracycine (the dose per bee calculated following treatment of a colony) or the L-type calcium channel blocker 1mM Verpamil (a classic MDR transporter inhibitor used as an anti-hypertension drug in humans) for 2 days prior to contact dosing with the varroacides coumaphos or fluvalinate resulted in mortality of bees increasing for <10% in the absence of the drugs to 90-100% following Verpamil treatment and 39-51% following oxytetracycline treatment. Pre-treatment of bees also increased the oral toxicity of neonicotinoid insecticides. Pretreatment with Verpamil increased the toxicity of imidacloprid, acetamiprid and thiacloprid (Figure 2.8). These effects were ascribed to the effects of the drugs on membrane bound transporter proteins (Buss and Callaghan, 2008) and suggested that the acaricides coumaphos and tau-fluvalinate, and 3 neonicotinoid insecticides are substrates of these transporters in insects as they are in mammals. These increases in sensitivity to the selected pesticides by inhibition of MDR (multi-drug reistance) transporters were suggested to indicate that these transporters may mediate adverse synergisms of diverse toxins in bees. This is the first report in honeybees of interactions between antibiotics used in the treatment of bacterial diseases in bee colonies and pesticides/varroacides.

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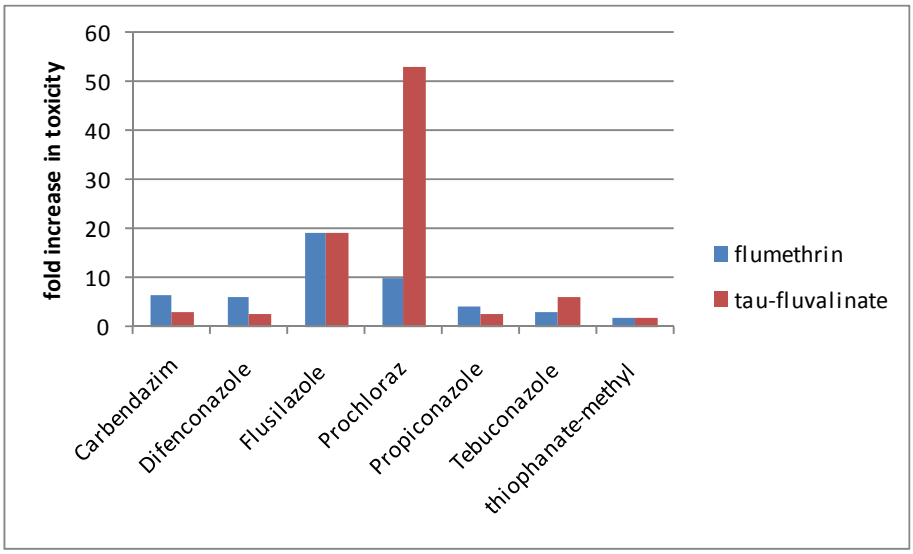

Figure 2.6 Effects on the contact toxicity of flumethrin and tau-fluvalinate active ingredients of co-exposure to fungicides at their maximum field application rate (Defra PN0945)

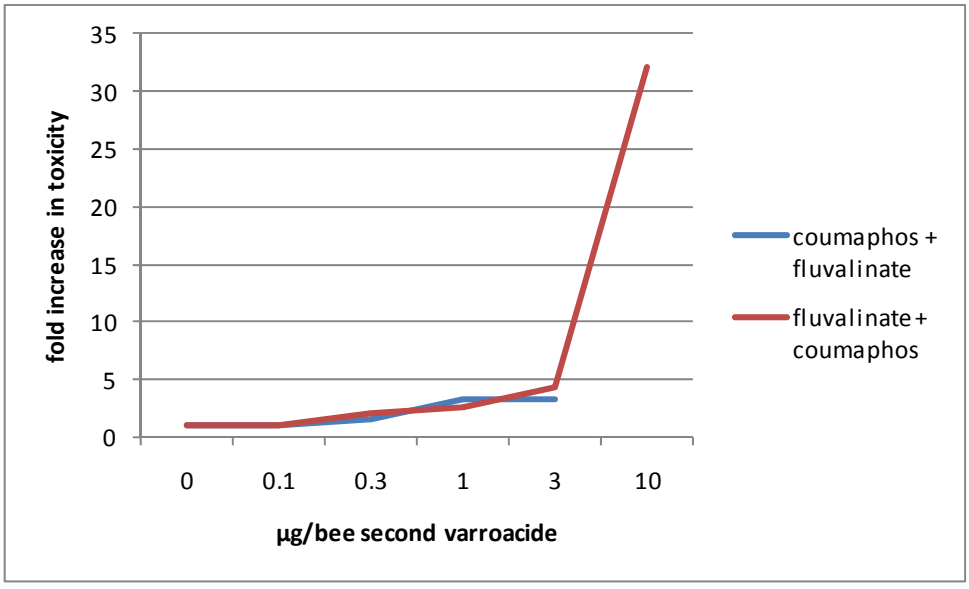

Figure 2.7 Effects of varying exposure to a second varroacide on the toxicity of coumaphos (contact LD_{50} 20 µg/bee) and tau-fluvalinate (contact LD_{50} 6.75 µg/bee) (data from Johnson et al., 2009)

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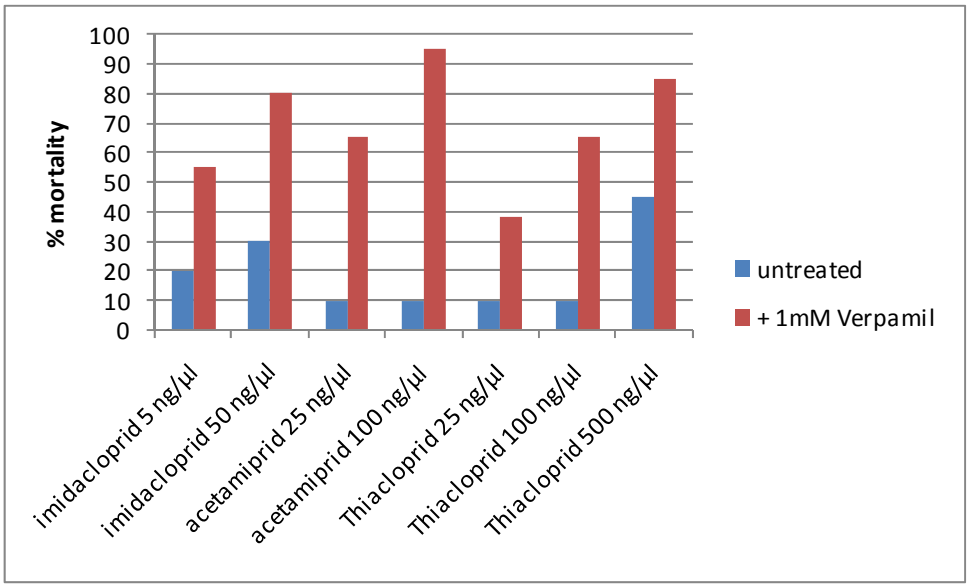

Figure 2.8 Effects of pretreatment with MDR transporter inhibitor Verpamil (1mM) on the oral toxicity of neonicotinoid insecticides (data from Hawthorne and Dively, 2011)

2.8. Conclusions

Honeybees and other bees may be exposed to mixtures of pesticides through multiple applications, overspray of residues already present, e.g. systemic pesticides, collection of pollen and nectar from a variety of sources and stored within the colony and, in addition, the use of treatments within hives by beekeepers. As previously there is very limited data on bees other than honeybees.

There is evidence in the literature of multiple residues of pesticides detected in bees, honey and pollen and wax within the hive but this is limited by the direction of the analysis to chemicals of interest to the researchers and rarely are levels of individual components reported.

Additive toxicity is an appropriate approach for most mixtures where synergy can be excluded and can be applied to residues in pollen and nectar to assess the total exposure of adult and larval bees to pesticides.

There are a large number of studies that have investigated the interactions between pesticides in bees. By far the majority have related to the interactions involving EBI fungicides and can be related to their inhibition of P450. The scale of the synergy is shown to be dose and season -dependent in acute exposures but there are few data relating to the effect of time between exposures or on chronic exposure effects at realistic exposure levels.

The vast majority of the studies have concentrated on the contact toxicity of the combinations. However the exposure section shows that a significant proportion of the exposure may be through ingestion of contaminated nectar. Given the role of the midgut enzymes in the metabolism of xenobiotics this is a major gap in our understanding of the potential interactions between chemicals, particularly those present in pollen and nectar, and the effects of diet in maintaining xenobiotic metabolising capacity within the gut.

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It appears that pesticides which induce P450s in other insects do not induce these enzymes in honeybees but natural chemicals, such as quercetin present in honey and propolis do induce P450s and reduce the toxicity of some pesticides.

Significant synergy has been reported between EBI fungicides and both neonicotinoids and pyrethroid insecticides but in some cases where high levels of synergy are reported the doses of fungicides have been well in excess of those identified in the exposure section of this report. At lower, more realistic ratios of synergist to insecticide generally lower levels of synergy are identified with field application ratios of pyrethroids and neonicotinoids although the data for the latter are very limited.

Greater synergy is observed between EBI fungicides at field rates application rates and pyrethroids used as varroacides (flumethrin and fluvalinate) and between coumaphos and fluvalinate varroacides.

As effects are dose-dependent synergism may be an area where modelling is applicable both from toxicokinetic/toxicodynamic and QSAR approaches but also needs to take into account formulation differences in affecting rate of uptake.

More recently data has shown that antibiotics used in hives may increase the susceptibility of bees to organophosphorus, pyrethroid and neonicotinoid insecticides through interaction with the membrane bound transporter proteins and further work is required to more fully understand the implications of these findings. It is therefore important that all treatments used on colonies used in studies are reported.

In all studies the interactions between two chemicals has been reported. However, the exposure data demonstrate that bees are often exposed directly through applications of multiple active ingredients or indirectly through consumption of stored pollen and nectar to several pesticides over a period of time. Data are required to determine the effects of such long term low level exposure to multiple pesticides on the health and functioning of honeybee colonies.

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Table 2.12: Summary of studies undertaken in the laboratory with honeybees and effects of diet

Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
Non-pesticides							
High fructose corn syrup	Aflatoxin B1 20 ug/g candy	mixture	Decreased longevity from 75.9 to 47.3 hrs	Apis mellifera	Not stated	Active ingredients, 3 day old bees, 20 bees/replicate 32-34C, 72h mortality, Probit analysis	(Johnson et al., 2012)
honey	Aflatoxin B1 20 ug/g candy	mixture	Decreased longevity from 76.5 to 55 hrs	Apis mellifera	Not stated	Active ingredients, 3 day old bees, 20 bees/replicate 32-34C, 72h mortality, Probit analysis	(Johnson et al., 2012)
Sucrose	Aflatoxin B1 20 ug/g candy	mixture	Decreased longevity from 67.9 to 40.9 hrs	Apis mellifera	Not stated	Active ingredients, 3 day old bees, 20 bees/replicate 32-34C, 72h mortality, Probit analysis	(Johnson et al., 2012)
Mycotoxin aflatoxin B1, 10ug/g candy	Piperonyl butoxide 0.05%, 0.1% in candy	mixture	Decreased survival time from 66hrs to 39hrs	Apis mellifera	Not stated	Newly emerged bees, dark, humid, 32–34^{o}C., 3g of bees (approx 30) Probit and ANOVA	(Niu et al., 2011)
Mycotoxin ochratoxin A, 10ug/g, 40 ug/g candy	Piperonyl butoxide 0.05%, 0.1% in candy	mixture	No effect on survival	Apis mellifera	Not stated	Newly emerged bees, dark, humid, 32–34 ^{o}C., 3g of bees (approx 30) Probit and ANOVA	(Niu et al., 2011)
Mycotoxin aflatoxin B1, 20ug/g candy	Propolis 50-300 mg/g candy	mixture	Increased survival time from 44 hrs to 126-154hrs	Apis mellifera	Not stated	Newly emerged bees, dark, humid, 32–34 ^{o}C., 3g of bees (approx 30) Probit and ANOVA	(Niu et al., 2011)

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Table 2.13 Summary of studies undertaken in the laboratory with honeybees and pesticide mixtures

Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
Carbamate insecticides							
+ Classical synergists							
Sevin (carbaryl) contact	PBO ratio 1:5 contact	Mixture	Inc mortality from 38 to 97% mortality at 16C, 8 to 97% at 27C and 8 to 87% at 32C	Apis mellifera	Not stated	Active ingredient, 20/replicate, 4 replicates, 16, 27, 32 C, 60%RH control treated with acetione mortality 4, 8, 16, 24hrs, < 5% mortality in controls	(Georghiou and Atkins Jr, 1964)
+ herbicides							
Carbaryl 1 ,3, 5 ppm	Atrazine 100ppm oral	mixture	mortality 1.0-1.1 fold	Apis mellifera	Not stated	Commercial grade (?) 25°C, 40-45% RH, amount consumed not measured, 4 trials 30-60bees/trial control mortality not stated, statistics binomial random variable	(Sonnet et al., 1978)
Carbofuran 0.3, 0.5, 1 ppm	Atrazine 100ppm oral	mixture	mortality 0.2-1.0 fold	Apis mellifera	Not stated	Commercial grade (?) 25°C, 40-45% RH, amount consumed not measured, 4 trials 30-60bees/trial control mortality not stated, statistics binomial random variable	(Sonnet et al., 1978)
Neonicotinoids							
+ Classical synergists							
Acetamiprid (LD50 0.007 mg/bee) contact	PBO 0.010 mg/bee contact	Synergist 1hr prior	Inc LD50 6.0 fold	Apis mellifera	No treatment prior to study	Active ingredients, 30 insects per replicate, 5-7 doses, 2-3 replicates, 27±1°C, 50% RH, 14:10 light :dark, mortality (incl knocked down) at 24hrs, 3.7% control mortality, Statistics: Abbotts correction, probit	(Iwasa et al., 2004a)

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
						analysis, Students t-test for means	
Imidacloprid (LD50 0.0000179 mg/bee) contact	PBO 0.010 mg/bee contact	Synergist 1hr prior	Inc LD50 1.7 fold	Apis mellifera	No treatment prior to study	Active ingredients, 30 insects per replicate, 5-7 doses, 2-3 replicates, 27±1°C, 50% RH, 14:10 light :dark, mortality (incl knocked down) at 24hrs, 3.7% control mortality Statistics: Abbotts correction, probit analysis, Students t-test for means	(Iwasa et al., 2004a)
Thiacloprid (LD50 0.0146 mg/bee) contact	PBO 0.010 mg/bee contact	Synergist 1hr prior	Inc LD50 154 fold	Apis mellifera	No treatment prior to study	Active ingredients, 30 insects per replicate, 5-7 doses, 2-3 replicates, 27±1°C, 50% RH, 14:10 light :dark, mortality (incl knocked down) at 24hrs, 3.7% control mortality Statistics: Abbotts correction, probit analysis, Students t-test for means	(Iwasa et al., 2004a)
Acetamiprid (LD50 0.007 mg/bee) contact	DEF 0.010 mg/bee contact	Synergist 1hr prior	Inc LD50 3.0 fold	Apis mellifera	No treatment prior to study	Active ingredients, 30 insects per replicate, 5-7 doses, 2-3 replicates, 27±1°C, 50% RH, 14:10 light :dark, mortality (incl knocked down) at 24hrs, 3.7% control mortality Statistics: Abbotts correction, probit analysis, Students t-test for means	(Iwasa et al., 2004a)
Acetamiprid (LD50 0.007 mg/bee) contact	DEM 0.010 mg/bee contact	Synergist 1hr prior	No effect on LD50	Apis mellifera	No treatment prior to study	Active ingredients, 30 insects per replicate, 5-7 doses, 2-3 replicates, 27±1°C, 50% RH, 14:10 light :dark, mortality (incl knocked down) at 24hrs, 3.7% control mortality Statistics: Abbotts correction, probit analysis, Students t-test for means	(Iwasa et al., 2004a)
+ EBI fungicides							
Acetamiprid (LD50	Epoxiconazole	Synergist	Inc LD50 14	Apis	No treatment	Active ingredients, 30 insects per	(Iwasa et al., 2004a)

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
0.007 mg/bee) contact	0.010 mg/bee contact	1hr prior	fold	mellifera	prior to study	replicate, 5-7 doses, 2-3 replicates, 27±1°C, 50% RH, 14:10 light :dark, mortality (incl knocked down) at 24hrs, 3.7% control mortality	
Thiacloprid 0.001, 0.010 mg/bee contact	Prochloraz 0.001, 0.010 mg/bee contact	mixture	0% mortality at 0.001mg/bee; inc mortality from 10 to 87% at 0.01 mg/bee,	A mellifera carnica	no antibiotics or varrocides within 4 weeks	Formulations 25±2 °C, 50-60% RH, 24h dark, 3 replicates of 10 bees per dose level, mortality and behaviour (discoordinated movements, staggering, apathy) up to 96hrs, 0% control mortality	(Schmuck et al., 2003b)
Acetamiprid (LD50 0.007 mg/bee) contact	Propiconazole 0.010 mg/bee contact	Synergist 1hr prior	Inc LD50 105 fold	Apis mellifera	No treatment prior to study	Active ingredients, 30 insects per replicate, 5-7 doses, 2-3 replicates, 27±1°C, 50% RH, 14:10 light :dark, mortality (incl knocked down) at 24hrs, 3.7% control mortality Statistics: Abbotts correction, probit analysis, Students t-test for means	(Iwasa et al., 2004a)
Imidacloprid (LD50 0.0000179 mg/bee) contact	Propiconazole 0.010 mg/bee contact	Synergist 1hr prior	Inc LD50 1.5 fold	Apis mellifera	No treatment prior to study	Active ingredients, 30 insects per replicate, 5-7 doses, 2-3 replicates, 27±1°C, 50% RH, 14:10 light :dark, mortality (incl knocked down) at 24hrs, 3.7% control mortality Statistics: Abbotts correction, probit analysis, Students t-test for means	(Iwasa et al., 2004a)
Thiacloprid (LD50 0.0146 mg/bee) contact	Propiconazole 0.010 mg/bee contact	Synergist 1hr prior	Inc LD50 559 fold	Apis mellifera	No treatment prior to study	Active ingredients, 30 insects per replicate, 5-7 doses, 2-3 replicates, 27±1°C, 50% RH, 14:10 light :dark, mortality (incl knocked down) at 24hrs, 3.7% control mortality Statistics: Abbotts correction, probit	(Iwasa et al., 2004a)

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
						analysis, Students t-test for means	
Thiacloprid 0.002 mg/bee contact	Tebuconazole 0.003 mg/bee contact	mixture	Inc mortality from 3 to 70%	A mellifera carnica	no antibiotics or varrocides within 4 weeks	Formulations 25±2 ^{o}C, 50-60% RH, 24hr dark, 3 replicates of 10 bees per dose level, mortality and behaviour (discoordinated movements, staggering, apathy) up to 96hrs, 0% control mortality	(Schmuck et al., 2003b)
Acetamiprid (LD50 0.007 mg/bee) contact	Triadimefon 0.010 mg/bee contact	Synergist 1hr prior	Inc LD50 84 fold	Apis mellifera	No treatment prior to study	Active ingredients, 30 insects per replicate, 5-7 doses, 2-3 replicates, 27±1^{o}C, 50% RH, 14:10 light :dark, mortality (incl knocked down) at 24hrs, 3.7% control mortality Statistics: Abbotts correction, probit analysis, Students t-test for means	(Iwasa et al., 2004a)
Acetamiprid (LD50 0.007 mg/bee) contact	Triflumizole 0.010 mg/bee contact	Synergist 1hr prior	Inc LD50 244 fold	Apis mellifera	No treatment prior to study	Active ingredients, 30 insects per replicate, 5-7 doses, 2-3 replicates, 27±1^{o}C, 50% RH, 14:10 light :dark, mortality (incl knocked down) at 24hrs, 3.7% control mortality Statistics: Abbotts correction, probit analysis, Students t-test for means	(Iwasa et al., 2004a)
Imidacloprid (LD50 0.0000179 mg/bee) contact	Triflumizole 0.010 mg/bee contact	Synergist 1hr prior	Inc LD50 1.9 fold	Apis mellifera	No treatment prior to study	Active ingredients, 30 insects per replicate, 5-7 doses, 2-3 replicates, 27±1^{o}C, 50% RH, 14:10 light :dark, mortality (incl knocked down) at 24hrs, 3.7% control mortality Statistics: Abbotts correction, probit analysis, Students t-test for means	(Iwasa et al., 2004a)
Thiacloprid (LD50	Triflumizole	Synergist	Inc LD50	Apis	No treatment	Active ingredients, 30 insects per	(Iwasa et al., 2004a)

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
0.0146 mg/bee) contact	0.010 mg/bee contact	1hr prior	1141 fold	mellifera	prior to study	replicate, 5-7 doses, 2-3 replicates, 27±1°C, 50% RH, 14:10 light :dark, mortality (incl knocked down) at 24hrs, 3.7% control mortality Statistics: Abbotts correction, probit analysis, Students t-test for means	
Acetamiprid (LD50 0.007 mg/bee) contact	Uniconazole-P 0.010 mg/bee contact	Synergist 1hr prior	Inc LD50 6.3 fold	Apis mellifera	No treatment prior to study	Active ingredients, 30 insects per replicate, 5-7 doses, 2-3 replicates, 27±1°C, 50% RH, 14:10 light :dark, mortality (incl knocked down) at 24hrs, 3.7% control mortality Statistics: Abbotts correction, probit analysis, Students t-test for means	(Iwasa et al., 2004a)
+ Other fungicides							
Thiacloprid 0.002 mg/bee contact	Cyprodinil (Anilino-pyrimidine) 0.008 mg/bee contact	mixture	Inc mortality from 3 to 20%	A mellifera carnica	no antibiotics or varrocides within 4 weeks	Formulations 25±2 °C, 50-60% RH, 24hr dark, 3 replicates of 10 bees per dose level, mortality and behaviour (discoordinated movements, staggering, apathy) up to 96hrs, 0% control mortality	(Schmuck et al., 2003b)
Thiacloprid 0.002 mg/bee contact	Azoxystrobin (methoxyacrylate strobilurin) 0.003 mg/bee contact	mixture	No effect on mortality	A mellifera carnica	no antibiotics or varrocides within 4 weeks	Formulations 25±2 °C, 50-60% RH, 24hr dark, 3 replicates of 10 bees per dose level, mortality and behaviour (discoordinated movements, staggering, apathy) up to 96hrs, 0% control mortality	(Schmuck et al., 2003b)
Thiacloprid 0.002 mg/bee contact	Tolyfluanid (phenylsulfamide) 0.011 mg/bee contact	mixture	Inc mortality from 3 to 13%	A mellifera carnica	no antibiotics or varrocides within 4 weeks	Formulations 25±2 °C, 50-60% RH, 24hr dark, 3 replicates of 10 bees per dose level, mortality and behaviour (uncoordinated movements, staggering, apathy) up to 96hrs, 0%	(Schmuck et al., 2003b)

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
						control mortality	
Thiacloprid 0.002 mg/bee contact	Mancozeb (dithiocarbamate) 0.008 mg/bee contact	mixture	No effect on mortality	A mellifera carnica	no antibiotics or varrocides within 4 weeks	Formulations 25±2 °C, 50-60% RH 24hr dark , 3 replicates of 10 bees per dose level, mortality and behaviour (discoordinated movements, staggering, apathy) up to 96hrs, 0% control mortality	(Schmuck et al., 2003b)
Phenylpyrazole insecticides + EBI fungicides							
Fipronil contact (LD50 0.005546 µg/bee) and oral (LD50 0.1015µg/bee)	Trichlorfon contact (LD50 4.065 µg/bee) and oral (LD50 13.89 mg/bee)	mixture	Additive toxicity	Apis mellifera (Italian)	Not stated	LD50, 48hr mortality	(Cang et al., 2008) (abstract only available)
Organochlorine insecticides + classic synergists							
aldrin	Phenobarbital 5 mg/g candy oral	3 days prior	Dec LD50 from 60.5 to 38.5 ng/bee	Apis mellifera	Not stated	Active ingredients, 3 day old bees, 20 bees/replicate 32-34C, 24h mortality, Probit analysis	(Johnson et al., 2012)
dieldrin	Phenobarbital 5 mg/g candy oral	3 days prior	Dec LD50 from 37.2 to 20.7 ng/bee	Apis mellifera	Not stated	Active ingredients, 3 day old bees, 20 bees/replicate 32-34C, 24h mortality, Probit analysis	(Johnson et al., 2012)
Organophosphorus insecticides							
+ herbicides							
Malathion 3ppm oral	2,4D oral	mixture	dec mortality from 61 to 24%	Apis mellifera	Not stated	Commercial grade (?) 25°C, 40-45% RH, amount consumed not measured, 4 trials 30-60bees/trial control mortality not stated, statistics binomial random variable	(Sonnet et al., 1978)
Methyl parathion 1ppm oral	2,4D oral	mixture	dec mortality from 50 to 28%	Apis mellifera	Not stated	Commercial grade (?) 25°C, 40-45% RH, amount consumed not measured, 4 trials 30-60bees/trial control mortality not stated, statistics binomial	(Sonnet et al., 1978)

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
						random variable	
Diazinon 3, 5 ppm	Atrazine 100ppm oral	mixture	dec mortality	Apis mellifera	Not stated	Commercial grade (?) 25°C, 40-45% RH, amount consumed not measured, 4 trials 30-60bees/trial control mortality not stated, statistics binomial random variable	(Sonnet et al., 1978)
Malathion 3, 5 pmm	Atrazine 100ppm oral	mixture	Dec mortality	Apis mellifera	Not stated	Commercial grade (?) 25°C, 40-45% RH, amount consumed not measured, 4 trials 30-60bees/trial control mortality not stated, statistics binomial random variable	(Sonnet et al., 1978)
Methyl parathion 1, 5 ppm	Atrazine 100ppm oral	mixture	Dec mortality	Apis mellifera	Not stated	Commercial grade (?) 25°C, 40-45% RH, amount consumed not measured, 4 trials 30-60bees/trial control mortality not stated, statistics binomial random variable	(Sonnet et al., 1978)
Mevinphos 0.3, 0.5 ppm	Atrazine 100ppm oral	mixture	dec mortality	Apis mellifera	Not stated	Commercial grade (?) 25°C, 40-45% RH, amount consumed not measured, 4 trials 30-60bees/trial control mortality not stated, statistics binomial random variable	(Sonnet et al., 1978)
Monocrotophos 0.5, 1 ppm	Atrazine 100ppm oral	mixture	Dec mortality	Apis mellifera	Not stated	Commercial grade (?) 25°C, 40-45% RH, amount consumed not measured, 4 trials 30-60bees/trial control mortality not stated, statistics binomial random variable	(Sonnet et al., 1978)
Parathion 7, 10 ppm	Atrazine 100ppm oral	mixture	Dec mortality	Apis mellifera	Not stated	Commercial grade (?) 25°C, 40-45% RH, amount consumed not measured, 4 trials 30-60bees/trial control mortality not stated, statistics binomial random variable	(Sonnet et al., 1978)

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
Malathion 3ppm oral	Monuron oral	mixture	dec mortality from 61 to 28%	Apis mellifera	Not stated	Commercial grade (?) 25°C, 40-45% RH, amount consumed not measured, 4 trials 30-60bees/trial control mortality not stated, statistics binomial random variable	(Sonnet et al., 1978)
Methyl parathion 1ppm oral	Monuron oral	mixture	dec mortality from 50-to 27%	Apis mellifera	Not stated	Commercial grade (?) 25°C, 40-45% RH, amount consumed not measured, 4 trials 30-60bees/trial control mortality not stated, statistics binomial random variable	(Sonnet et al., 1978)
Malathion 3ppm oral	Simazine oral	mixture	dec mortality 61-7%	Apis mellifera	Not stated	Commercial grade (?) 25°C, 40-45% RH, amount consumed not measured, 4 trials 30-60bees/trial control mortality not stated, statistics binomial random variable	(Sonnet et al., 1978)
Methyl parathion 1ppm oral	Simazine oral	mixture	dec mortality from 50-to 27%	Apis mellifera	Not stated	Commercial grade (?) 25°C, 40-45% RH, amount consumed not measured, 4 trials 30-60bees/trial control mortality not stated, statistics binomial random variable	(Sonnet et al., 1978)
+ organophosphorus insecticide							
Thio and dithiophosphoric ester pesticides – ethyl parathion, dimethoate, dialifos oral	Coumaphos varroacide	Pretreatment in artificial swarm four days prior	Inc toxicity	A mellifera carnica	Pretreatment with varroacide	Artificial swarm 34C, 70% RH 48hr mortality	(Lienau, 1990) (abstract only available)
Pyrethroids							
Lambda-cyhalothrin dose response LD50 contact	PBO contact (1:10 pyrethroid:PBO)	mixture	Dec LD50 from 0.15 ug ai/bee to 0.077 ug ai/bee (2.0	Apis mellifera	Not stated	Formulations, 25C, 75%RH mortality 24hr	(Pilling, 1992)

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
			fold)				
Permethrin 0-75 ug/ml	PBO ratio 1:4 and 1:9 (pyrethroid:PBO)	Mixture	5.4 fold inc toxicity 1:4 9 fold increase toxicity 1:9	Apis mellifera	Not stated	Active ingredient, 24 C, 30% RH dark, filter paper exposure, 10 bees per dose, 6 doses, replicated 7 times,48hr mortality Probit analysis	(Hagler et al., 1989)
Lambda-cyhalothrin	Phenobarbital 5 mg/g candy oral	3 days prior	Dec LD50 from 47.5 to 16.9 ng/bee	Apis mellifera	Not stated	Active ingredients, 3 day old bees, 20 bees/replicate 32-34C, 24h mortality, Probit analysis	(Johnson et al., 2012)
+ EBI fungicides							
Alphacypermethrin	Carbendazim (ratio related to application rate) 1:66	mixture	No change in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003)(Defra PN0945)
Alphacypermethrin	Carbendazim (ratio related to application rate) 1:66	mixture	No change in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Active ingredients,, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003)(Defra PN0945)
Alphacypermethrin	Carbendazim (ratio related to application rate) 1:33	mixture	1.6 fold decrease in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Folkard-Ward, 2001)
Lambda-cyhalothrin	Carbendazim (ratio related to application rate) 1:50	mixture	1.3 fold decrease in toxicity	Apis mellifera	No antibiotic no varroacide within 4	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t	(Thompson and Folkard-Ward, 2001)

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
					weeks of start of study	test vs additive toxicity	
Lambda-cyhalothrin	Carbendazim (ratio related to application rate) 1:232	mixture	2.6 fold decrease in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003)(Defra PN0945)
Lambda-cyhalothrin	Carbendazim (ratio related to application rate) 1:232	mixture	1.3 fold decrease in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Active ingredients, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003)(Defra PN0945)
Alphacypermethrin	Difenconazole (ratio related to application rate) 1:9.4	mixture	1.2 fold decrease in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003)(Defra PN0945)
Alphacypermethrin	Difenconazole (ratio related to application rate) 1:9.4	mixture	1.8 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Active ingredients,, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003)(Defra PN0945)
Deltamethrin 0.75 g ai/ha	Difenoconazole +carbendazim 125-250 g/ha (1:167-333)	mixture	Increased mortality	Apis mellifera	Not stated	Formulations sprayed in Potter Tower, 28±1^{o}C, 50-70%RH, 50 /replicate, 12 replicates, 24-48hrs mortality,	(Belzunces and Colin, 1993) (Colin and Belzunces, 1992a)
Deltamethrin 0.75 g	Difenoconazole	sequential	No effect	Apis	Not stated	Formulations sprayed in Potter Tower,	(Belzunces and Colin,

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
ai/ha	+carbendazim 125-250 g/ha (1:167-333)			mellifera		28±1°C, 50-70%RH, 50 /replicate, 12 replicates, 24-96hrs mortality,	1993) (Colin and Belzunces, 1992a)
Lambda-cyhalothrin	Difenconazole (ratio related to application rate) 1:33	mixture	2.7 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003)(Defra PN0945)
Lambda-cyhalothrin	Difenconazole (ratio related to application rate) 1:33	mixture	No change in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Active ingredients, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003)(Defra PN0945)
Alphacypermethrin	Flusilazole (ratio related to application rate) 1:15	mixture	No change in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003)(Defra PN0945)
Alphacypermethrin	Flusilazole (ratio related to application rate) 1:15	mixture	2.5 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Active ingredients, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003)(Defra PN0945)
Lambda-cyhalothrin	Flusilazole (ratio related to application rate) 1:53	mixture	2.2 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t	(Thompson and Wilkins, 2003)(Defra PN0945)

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
					weeks of start of study	test vs additive toxicity	
Lambda-cyhalothrin	Flusilazole (ratio related to application rate) 1:53	mixture	2.5 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Active ingredients, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003)(Defra PN0945)
Lambda-cyhalothrin dose response LD50 contact	Flutriafol (SC) ratio related to application rate (1:15)	mixture	Dec LD50 from 0.068 ug ai/bee to 0.0205 ug ai/bee (3.3 fold)	Apis mellifera	Not stated	Formulations, 25C, 75%RH, 10 bees per replicate, 3 replicates per dose, 6 doses, mortality 24hr	(Pilling and Jepson, 1993)
Lambda-cyhalothrin dose response LD50 contact	Flutriafol ratio related to application rate contact (1:16)	mixture	Dec LD50 from 0.15 ug ai/bee to 0.026 ug/bee (5.8 fold)	Apis mellifera	Not stated	Formulations, 25C, 75%RH mortality 24hr	(Pilling, 1992)
Lambda-cyhalothrin dose response LD50 contact	Imazalil (EC) ratio related to application rate (1:13.3)	mixture	Dec LD50 from 0.068 ug ai/bee to 0.0095 ug ai/bee (7.16 fold)	Apis mellifera	Not stated	Formulations, 25C, 75%RH, 10 bees per replicate, 3 replicates per dose, 6 doses, mortality 24hr	(Pilling and Jepson, 1993)

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
Alphacypermethrin	Iprodione (ratio related to application rate) 1:50	mixture	6 fold decrease in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Folkard-Ward, 2001)
Lambda-cyhalothrin	Iprodione (ratio related to application rate) 1:75	mixture	1.5 fold decrease in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Folkard-Ward, 2001)
Deltamethrin 0.75 g ai/ha	Iprodione + carbendazim 525-262.5 g/ha	mixture	No effect	Apis mellifera	Not stated	Formulations sprayed in Potter Tower, 28±1^{o}C, 50-70%RH, 50 /replicate, 12 replicates, 24-48hrs mortality,	(Belzunces and Colin, 1993)
Lambda-cyhalothrin dose response LD50 contact	Myclobutanil (SC) ratio related to application rate (1:12)	mixture	Dec LD50 from 0.068 ug ai/bee to 0.0089 ug ai/bee (7.64 fold)	Apis mellifera	Not stated	Formulations, 25C, 75%RH, 10 bees per replicate, 3 replicates per dose, 6 doses, mortality 24hr	(Pilling and Jepson, 1993)
Lambda-cyhalothrin dose response LD50 contact	Myclobutanil (WP) ratio related to application rate (1:12)	mixture	Dec LD50 from 0.068 ug ai/bee to 0.0048 ug ai/bee (14.1 fold)	Apis mellifera	Not stated	Formulations, 25C, 75%RH, 10 bees per replicate, 3 replicates per dose, 6 doses, mortality 24hr	(Pilling and Jepson, 1993)
Lambda-cyhalothrin	Penconazole (EC)	mixture	Dec LD50	Apis	Not stated	Formulations, 25C, 75%RH, 10 bees	(Pilling and Jepson, 1993)

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
dose response LD50 contact	ratio related to application rate (1:6.6)		from 0.068 ug ai/bee to 0.0154 ug ai/bee (4.42 fold)	mellifera		per replicate, 3 replicates per dose, 6 doses, mortality 24hr	
Alphacypermethrin	Prochloraz (ratio related to application rate) 1:37	mixture	2.2 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003) (Defra PN0945)
Alphacypermethrin	Prochloraz (ratio related to application rate) 1:37	mixture	13 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Active ingredients, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003) (Defra PN0945)
Deltamethrin	Prochloraz	mixture	Isolated heart 100 fold inc cardiotoxicity of deltamethrin, 10 fold inc cardiotoxicity prochloraz Suggested joint action at target site in heart – gap junctional intercellular	A mellifera macedonica	Not stated	Active ingredients No temp info N=6 3 concentrations deltamethrin and prochloraz, six studies ANOVA and Dunnetts test	(Papaefthimiou and Theophilidis, 2001)

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
			communication				
Deltamethrin 0.125 g ai/ha	Prochloraz 25 g ai/ha (1:200)	Sequential separated by 0.8 days	Inc mortality from 0 to 23.8-27.5% at 50h (depending on order of applications)	Apis mellifera	Not stated	Active ingredients, sprayed in Potter-Tower, 24±1°C, 50-70%RH, 50/replicate, 3 replicates per dose 4 doses, ANOVA	(Belzunces and Colin, 1993; Colin and Belzunces, 1992b)
Deltamethrin 125 mg ai/ha	Prochloraz 25 g ai/ha (1:200)	Mixture potter tower	Inc mortality from 0 to 67.5% at 24 hrs, 74.1% at 50h	Apis mellifera	Not stated	Active ingredients, sprayed in Potter-Tower, 24±1°C, 50-70%RH, 50/replicate, 3 replicates per dose 4 doses, ANOVA	(Belzunces and Colin, 1993; Colin and Belzunces, 1992b)
Deltamethrin 125 mg/ha	Prochloraz 25 g/ha (1:200)	Mixture potter tower	63 ±5% mortality	Summer A mellifera	Not stated	Active ingredient, 28 ± 1C, 60±10%RH, 3 replicates of 100 sprayed, repeated 10 times, 24hr mortality	(Meled et al., 1998)
Deltamethrin 500 mg/ha	Prochloraz 25 g/ha (1:50)	Mixture potter tower	47 ±11% mortality	Winter A mellifera	Not stated	Active ingredient, 28 ± 1C, 60±10%RH, 3 replicates of 100 sprayed, repeated 10 times, 24hr mortality	(Meled et al., 1998)
Deltamethrin 62.5 mg/ha	Prochloraz 25 g/ha (1:400)	Mixture potter tower	32.5 ±3.5% mortality	Summer A mellifera	Not stated	Active ingredient, 28 ± 1C, 60±10%RH, 3 replicates of 100 sprayed, repeated 10 times, 24hr mortality	(Meled et al., 1998)
Deltamethrin 0.5, 1 ng/bee, contact	Prochloraz, difenoconazole 850 ng/bee contact (1:850-1700)	mixture	Significant increased hypothermia	A mellifera	Not stated	22°C, no other info on study design	(Vandame and Belzunces, 1998b) (Vandame and Belzunces, 1998a)
Lambda-cyhalothrin	Prochloraz ratio	mixture	Decreased	Apis	Not stated	Formulations, 25C, 75%RH mortality	(Pilling, 1992)

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
dose response LD50 contact	related to application rate contact (1:50)		LD50 from 0.15 ug/bee to 0.0082 ug/bee (18.3 fold)	mellifera		24hr	
Lambda-cyhalothrin dose response LD50 contact	Prochoraz (EC) ratio related to application rate (1:50)	mixture	Dec LD50 from 0.068 ug ai/bee to 0.0075 ug ai/bee (9.1 fold)	Apis mellifera	Not stated	Formulations, 25C, 75%RH, 10 bees per replicate, 3 replicates per dose, 6 doses, mortality 24hr	(Pilling, 1992; Pilling and Jepson, 1993)
Lambda-cyhalothrin radiolabelled	Prochloraz (EC)ratio 1:50	mixture	Delayed metabolism and excretion of lambda cyhalothrin for 16hrs	Apis mellifera	Not stated	Active ingredient and formulation, 25C, 75%RH, 300 bees dosed per treatment, frass collected at 2,4,16 and 24 hrs	(Pilling et al., 1995)
Lambda-cyhalothrin	Prochloraz (ratio related to application rate) 1:132	mixture	6.7 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003) (Defra PN0945)
Lambda-cyhalothrin	Prochloraz (ratio related to application rate) 1:132	mixture	14.5 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Active ingredients, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003) (Defra PN0945)
Alphacypermethrin	Propiconazole (ratio related to application rate) 1:8	mixture	1.2 fold decrease in toxicity	Apis mellifera	No antibiotic no varroacide	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates.	(Thompson and Folkard-Ward, 2001)

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
					within 4 weeks of start of study	Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	
Alphacypermethrin	Propiconazole (ratio related to application rate) 1:9.4	mixture	2.1 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003) (Defra PN0945)
Alphacypermethrin	Propiconazole (ratio related to application rate) 1:9.4	mixture	2.5 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Active ingredients,, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003) (Defra PN0945)
Lambda-cyhalothrin	Propiconazole 1:12.5	mixture	1.3 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Folkard-Ward, 2001)
Lambda-cyhalothrin	Propiconazole 1:1250	mixture	16 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Folkard-Ward, 2001)
Lambda-cyhalothrin	Propiconazole 1:50	mixture	1.6 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t	(Thompson and Folkard-Ward, 2001)

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
					weeks of start of study	test vs additive toxicity	
Lambda-cyhalothrin	Propiconazole 1:6.25	mixture	1.6 fold decrease in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Folkard-Ward, 2001)
Lambda-cyhalothrin dose response LD50 contact	Propiconazole (EC) ratio related to application rate (1:16.6)	mixture	Dec LD50 from 0.068 ug ai/bee to 0.0042 ug ai/bee (16.2 fold)	Apis mellifera	Not stated	Formulations, 25C, 75%RH, 10 bees per replicate, 3 replicates per dose, 6 doses, mortality 24hr	(Pilling and Jepson, 1993)
Lambda-cyhalothrin	Propiconazole (ratio related to application rate) 1:33	mixture	3.0 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003) (Defra PN0945)
Lambda-cyhalothrin	Propiconazole (ratio related to application rate) 1:33	mixture	2.9 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Active ingredients, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003) (Defra PN0945)
Alphacypermethrin	Tebuconazole (ratio related to application rate) 1:19	mixture	No change in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003) (Defra PN0945)

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
					start of study		
Alphacypermethrin	Tebuconazole (ratio related to application rate) 1:19	mixture	2.5 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Active ingredients, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003) (Defra PN0945)
Lambda-cyhalothrin	Tebuconazole (ratio related to application rate) 1:66	mixture	2.2 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003) (Defra PN0945)
Lambda-cyhalothrin	Tebuconazole (ratio related to application rate) 1:66	mixture	2.7 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Active ingredients, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003) (Defra PN0945)
Lambda-cyhalothrin	Thiophanate methyl (ratio related to application rate) 1:100	mixture	1.9 fold decrease in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003) (Defra PN0945)
Alphacypermethrin	Thiophanate methyl (ratio related to application rate) 1:67	mixture	20 fold decrease in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Folkard-Ward, 2001)

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
Alphacypermethrin	thiophanate-methyl (ratio related to application rate) 1:34	mixture	1.2 fold decrease in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003) (Defra PN0945)
Alphacypermethrin	thiophanate-methyl (ratio related to application rate) 1:34	mixture	1.6 fold decrease in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Active ingredients, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003) (Defra PN0945)
Lambda-cyhalothrin	Thiophanate-methyl (ratio related to application rate) 1:121	mixture	3 fold decrease in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003) (Defra PN0945)
Lambda-cyhalothrin	thiophanate-methyl (ratio related to application rate) 1:121	mixture	1.6 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Active ingredients, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003) (Defra PN0945)
Lambda-cyhalothrin dose response LD50 contact	Triademefon (WP) ratio related to application rate (1:23.3)	mixture	Dec LD50 from 0.068 ug ai/bee to 0.0059 ug ai/bee (11.5 fold)	Apis mellifera	Not stated	Formulations, 25C, 75%RH, 10 bees per replicate, 3 replicates per dose, 6 doses, mortality 24hr	(Pilling and Jepson, 1993)
Lambda-cyhalothrin	Triadimenol (EC)	mixture	Dec LD50	Apis	Not stated	Formulations, 25C, 75%RH, 10 bees	(Pilling and Jepson, 1993)

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
dose response LD50 contact	(ratio related to application rate (1:16.6)		from 0.068 ug ai/bee to 0.0090 ug ai/bee (7.55 fold)	mellifera		per replicate, 3 replicates per dose, 6 doses, mortality 24hr	
+ other fungicides							
alphacypermethrin	Chlorothalonil (chloronitrile) (ratio related to application rate) 1:34	mixture	2.1 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003) (Defra PN0945)
Lambda-cyhalothrin	Chlorothalonil (chloronitrile) (ratio related to application rate) 1:121	mixture	1.4 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003) (Defra PN0945)
alphacypermethrin	Mancozeb (dithiocarbamate) (ratio related to application rate) 1:107	mixture	2 fold decrease in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Folkard-Ward, 2001)
Lambda-cyhalothrin	Mancozeb (dithiocarbamate) (ratio related to application rate) 1:160	mixture	4.3 fold decrease in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Folkard-Ward, 2001)
Other pesticides							

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
Fluvalinate, 1 and 10 ppm oral	Carbaryl, paraquat, mancozeb 1 and 10 ppm oral	mixture	Additive toxicity	Apis mellifera (Italian)	Not stated	Formulations, 25 bees/rep, 18 and 25C, 50-70% RH, 4 repeated studies, randomised block design, 12, 24 and every 24h to 5 days after treatment, ANOVA	(Chaney, 1988)
Permethrin, 1 and 10 ppm oral	Carbaryl, paraquat, mancozeb 1 and 10 ppm oral	mixture	Additive toxicity	Apis mellifera (Italian)	Not stated	Formulations, 25 bees/rep, 18 and 25C, 50-70% RH, 4 repeated studies, randomised block design, 12, 24 and every 24h to 5 days after treatment, ANOVA	(Chaney, 1988)
Deltamethrin 12.5 ng contact	Pirimicarb 2.5 µg contact	mixture	Inc in AChE activity (soluble form in dead bees, soluble and membrane forms in live bees) resulting from deltamethrin treatment not reduced by pirimicarb	Apis mellifera	Not stated	Active ingredients, dead bees collected every 30mins for 24hr, live bees collected at 24hr, AChE activity by electrophoresis, 10 experiments in triplicate, ANOVA, paired t-test	(Badiou and Belzunces, 2008)

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Table 2.14 Assessment of the toxicity of mixtures in non-Apis bees

Pesticide	Pesticide	Time between treatments	Effect	Species	Pre-exposure treatments	Method	Reference
Demeton 0.2 kg/ha	Trichlorfon 0.9 kg/ha	mixture	Inc mortality from 0 to 17%	Nomia melanderi Alkali bees	Not stated	2hr exposure to residues from treated crop	(Johansen et al., 1983)
Propargite + 1.8 kg/ha	Trichlorfon 0.9 kg/ha	mixture	Inc mortality from 0 to 22%	Nomia melanderi Alkali bees	Not stated	2hr exposure to residues from treated crop	(Johansen et al., 1983)
Propargite 1.8 kg/ha	Trichlorfon 0.9 kg/ha + demeton 0.2 kg/ha	mixture	Inc mortality from 0 to 50%	Nomia melanderi Alkali bees	Not stated	2hr exposure to residues from treated crop	(Johansen et al., 1983)

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Table 2.15 Semi-field studies with pesticide mixtures

Pesticide	Pesticide	Acute effect	Chronic effect	Test system	Reference
Honeybee					
Thiacloprid 96, 144 g ai/ha	Tebuconazole 375 g ai/ha	No increased adult mortality	No change in hive strength	Flowering phacelia or oilseed rape	(Schmuck et al., 2003b)
Acetamiprid 168.1 g/ha	Triflumizole 280 g/ha	No effect on adult mortality		alfalfa	(Iwasa et al., 2004a)
Tau fluvalinate 48 g ai/ha	Difenoconazole 126 g ai/ha + carbendazim 250 g ai/ha	No effect on adult mortality	No effect on colony development	Flowering phacelia or oilseed rape	(Lefebvre and Bassand, 2001)
Lambda cyhalothrin	Flusilazole	Increased mortality immediately post application and days 1and 2	Decreased foraging but increased mortality compared with fungicide alone	Flowering phacelia	Defra (PN0945, 2004)
Other bee species					
Iprodione 2.24 kg Rovral/Ha),	Surfactant Dyne-Amic (0.75% v/v) + inorganic foliar feed (5% vol/vol)	No effects	No effect	Osmia lignaria, 10-14 females, 15-18 males flowering phacelia time spent inside the nest depositing pollen-nectar loads, foraging time, cell production rate, and survival, control + toxic reference (dimethoate)	(Ladurner et al., 2008)

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Table 2.16 Laboratory studies with pesticide and varroacides

Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
+ classical synergists							
Tau-fluvalinate	Indole-3-carbinol 1 mg/g candy oral	3 days prior	No change in LD50	Apis mellifera	Not stated	Active ingredients, 3 day old bees, 20 bees/replicate 32-34C, 24h mortality, Probit analysis	(Johnson et al., 2012)
Tau-fluvalinate	Phenobarbital 5 mg/g candy oral	3 days prior	Dec LD50 from 8050 to 190 ng/bee	Apis mellifera	Not stated	Active ingredients, 3 day old bees, 20 bees/replicate 32-34C, 24h mortality, Probit analysis	(Johnson et al., 2012)
Tau-fluvalinate	Quercetin 10 mg/g candy oral	3 days prior	Inc LD50 from 8050 to 11400 ng/bee	Apis mellifera	Not stated	Active ingredients, 3 day old bees, 20 bees/replicate 32-34C, 24h mortality, Probit analysis	(Johnson et al., 2012)
Tau-fluvalinate	Salicylic acid 2.5 mg/g candy oral	3 days prior	Dec LD50 from 8050 to 4450 ng/bee	Apis mellifera	Not stated	Active ingredients, 3 day old bees, 20 bees/replicate 32-34C, 24h mortality, Probit analysis	(Johnson et al., 2012)
Coumaphos LD50 20 ug/bee contact	DEF (carboxylesterase inhibitor) 10ug/bee contact	Synergist 1hr prior	LD50 7.3 ug/bee	Apis mellifera	Terramycin and fumadil used in colonies	Active ingredients, no temp/humidity/lighting information, mortality (incl knocked down) at 24hrs statistics probit analysis	(Johnson et al., 2009)
Coumaphos LD50 20 ug/bee contact	DEM (glutathione transferase inhibitor) 100 ug/bee contact	Synergist 1hr prior	LD50 19.9 ug/bee	Apis mellifera	Terramycin and fumadil used in colonies	Active ingredients, no temp/humidity/lighting information, mortality (incl knocked down) at 24hrs statistics probit analysis	(Johnson et al., 2009)
Coumaphos LD50 20 ug/bee contact	PBO (P450 inhibitor) 10ug/bee contact	Synergist 1hr prior	LD50 5.0 ug/bee	Apis mellifera	Terramycin and fumadil used in colonies	Active ingredients, no temp/humidity/lighting information, mortality (incl knocked down) at 24hrs statistics probit analysis	(Johnson et al., 2009)
+ EBI synergists							
Flumethrin	Carbendazim max	mixture	6.4 fold	Apis	No antibiotic	Active ingredients, 25 ±1 C,	(Thompson

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
	field application rate 4.4 mg/ml		increase in toxicity	mellifera	no varroacide within 4 weeks of start of study	65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs,	and Wilkins, 2003) (Defra PN0945)
Flumethrin	Difenconazole max field application rate 0.63 mg/ml	mixture	6.0 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Active ingredients, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs,	(Thompson and Wilkins, 2003) (Defra PN0945)
Flumethrin	Flusilazole max field application rate 1 mg/ml	mixture	19.1 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Active ingredients, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs,	(Thompson and Wilkins, 2003) (Defra PN0945)
Flumethrin	Prochloraz max field application rate 2.5 mg/ml	mixture	10 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Active ingredients, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003) (Defra PN0945)
Flumethrin	Propiconazole max field application rate 0.63 mg/ml	mixture	4.1 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Active ingredients, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs	(Thompson and Wilkins, 2003) (Defra PN0945)
Flumethrin	Tebuconazole max field application rate 1.25 mg/ml	mixture	3.1 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start	Active ingredients, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs,	Thompson & Wilkins 2003 (Defra PN0945)

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
					of study		
Flumethrin	thiophanate-methyl max field application rate 2.3 mg/ml	mixture	1.9 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Active ingredients, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003) (Defra PN0945)
Flumethrin as Bayvarol strip	Carbendazim 12.5-100 mg/ml	Mixture or sequential (6hrs)	Slight increase in mortality	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 4 reps per dose, 10 bees per rep.Dose of pyrethroid causing 30% mortality Mortality at 4, 24, 48, 72 and 96hrs,	(Thompson and Wilkins, 2003) (Defra PN0945)
Flumethrin as Bayvarol strip	Difenoconazole 2.6 mg/ml	Mixture or sequential (6hrs)	Slight increase in mortality	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 4 reps per dose, 10 bees per rep.Dose of pyrethroid causing 30% mortality Mortality at 4, 24, 48, 72 and 96hrs,	(Thompson and Wilkins, 2003) (Defra PN0945)
Flumethrin as Bayvarol strip	Flusilazole 0.33-2.6 mg/ml	Mixture or sequential (6hrs)	Slight increase in mortality	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 4 reps per dose, 10 bees per rep.Dose of pyrethroid causing 30% mortality Mortality at 4, 24, 48, 72 and 96hrs,	(Thompson and Wilkins, 2003) (Defra PN0945)
Flumethrin as Bayvarol strip	Propiconazole 2.8 mg/ml	Mixture or sequential (6hrs)	Slight increase in mortality	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 4 reps per dose, 10 bees per rep.Dose of pyrethroid causing 30% mortality Mortality at 4, 24, 48, 72 and 96hrs,	(Thompson and Wilkins, 2003) (Defra PN0945)
Flumethrin as Bayvarol strip	Tebuconazole 4.9 mg/ml	Mixture or sequential	Slight increase in mortality	Apis mellifera	No antibiotic no varroacide	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 4 reps per	(Thompson and Wilkins,

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
		(6hrs)			within 4 weeks of start of study	dose, 10 bees per rep.Dose of pyrethroid causing 30% mortality Mortality at 4, 24, 48, 72 and 96hrs,	2003) (Defra PN0945)
Flumethrin as Bayvarol strip	thiophanate-methyl 50-100 mg/ml	Mixture or sequential (6hrs)	Slight increase in mortality	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 4 reps per dose, 10 bees per rep.Dose of pyrethroid causing 30% mortality Mortality at 4, 24, 48, 72 and 96hrs,	(Thompson and Wilkins, 2003) (Defra PN0945)
Fluvalinate	Carbendazim max field application rate 4.4 mg/ml	mixture	3 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Active ingredients,, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs,	(Thompson and Wilkins, 2003) (Defra PN0945)
Fluvalinate	Difenconazole max field application rate 0.63 mg/ml	mixture	2.5 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Active ingredients,, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs,	(Thompson and Wilkins, 2003) (Defra PN0945)
Fluvalinate	Flusilazole max field application rate 1 mg/ml	mixture	19 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Active ingredients, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs,	(Thompson and Wilkins, 2003) (Defra PN0945)
Fluvalinate	Prochloraz max field application rate 2.5 mg/ml	mixture	53 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Active ingredients, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs,	(Thompson and Wilkins, 2003) (Defra PN0945)

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
Fluvalinate	Propiconazole max field application rate 0.63 mg/ml	mixture	2.5 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Active ingredients,, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs, Students t test vs additive toxicity	(Thompson and Wilkins, 2003) (Defra PN0945)
Fluvalinate	Tebuconazole max field application rate 1.25 mg/ml	mixture	6.1 fold increase in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Active ingredients, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs,	(Thompson and Wilkins, 2003) (Defra PN0945)
Fluvalinate	thiophanate-methyl max field application rate 16.2 mg/ml	mixture	1.9 fold decrease in toxicity	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Active ingredients, 25 ±1 C, 65±5%RH, OECD contact method, 3 reps per dose, 10 bees per rep 5 dose rates. Mortality at 4, 24, 48hrs,	(Thompson and Wilkins, 2003) (Defra PN0945)
Fluvalinate as Apistan strip	Carbendazim 100 mg/ml	Mixture or sequential (6hrs)	No change in mortality	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 4 reps per dose, 10 bees per rep.Dose of pyrethroid causing 30% mortality Mortality at 4, 24, 48, 72 and 96hrs,	(Thompson and Wilkins, 2003) (Defra PN0945)
Fluvalinate as Apistan strip	Difenoconazole 2.6 mg/ml	Mixture or sequential (6hrs)	No change in mortality	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 4 reps per dose, 10 bees per rep.Dose of pyrethroid causing 30% mortality Mortality at 4, 24, 48, 72 and 96hrs,	(Thompson and Wilkins, 2003) (Defra PN0945)
Fluvalinate as Apistan strip	Flusilazole 0.03-2.6 mg/ml	Mixture or sequential (6hrs)	increased mortality	Apis mellifera	No antibiotic no varroacide within 4	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 4 reps per dose, 10 bees per rep.Dose of	(Thompson and Wilkins, 2003) (Defra

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
					weeks of start of study	pyrethroid causing 30% mortality Mortality at 4, 24, 48, 72 and 96hrs,	PN0945)
Fluvalinate as Apistan strip	Propiconazole 2.8 mg/ml	Mixture or sequential (6hrs)	No change in mortality	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 4 reps per dose, 10 bees per rep.Dose of pyrethroid causing 30% mortality Mortality at 4, 24, 48, 72 and 96hrs,	(Thompson and Wilkins, 2003) (Defra PN0945)
Fluvalinate as Apistan strip	Tebuconazole 4.9 mg/ml	Mixture or sequential (6hrs)	No change in mortality	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 4 reps per dose, 10 bees per rep.Dose of pyrethroid causing 30% mortality Mortality at 4, 24, 48, 72 and 96hrs,	(Thompson and Wilkins, 2003) (Defra PN0945)
Fluvalinate as Apistan strip	thiophanate-methyl 12.5-100 mg/ml	Mixture or sequential (6hrs)	Inc mortality	Apis mellifera	No antibiotic no varroacide within 4 weeks of start of study	Formulations, 25 ±1 C, 65±5%RH, OECD contact method, 4 reps per dose, 10 bees per rep.Dose of pyrethroid causing 30% mortality Mortality at 4, 24, 48, 72 and 96hrs,	(Thompson and Wilkins, 2003) (Defra PN0945)
+ other pesticides/varroacides							
Fluvalinate LD50 6.75 ug/bee contact	Coumaphos 0.1, 0.3, 1, 3, 10 ug/bee contact	Synergist 1hr prior	LD50 6.14, 3.29, 2.68, 1.53, 0.21	Apis mellifera	Terramycin and fumadil used in colonies	Active ingredients, no temp/humidity/lighting information, mortality (incl knocked down) at 24hrs statistics probit analysis	(Johnson et al., 2009)
Coumaphos LD50 20 ug/bee contact	Fluvalinate 0.1, 0.3, 1, 3 ug/bee contact	Synergist 1hr prior	LD50 20.9, 12.8, 6.1, 6.1 ug/bee	Apis mellifera	Terramycin , Apilife Var (Thymol plus Eucalyptus Oil, Menthol and	Active ingredients, no temp/humidity/lighting information, mortality (incl knocked down) at 24hrs statistics probit analysis	(Johnson et al., 2009)

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Pesticide	Pesticide	Time between treatments	Effect	Strain	Pre-exposure treatments	Design	Reference
					Camphor) and fumadil used in colonies		
Fluvalinate (queen tabs)	Bifenthrin contact	48h preexposure	1.9 fold inc in toxicity	Apis mellifera	Not stated	20 C in dark, Six replicates of 10 bees, six dose levels 24hr mortality, Probit analysis	(Ellis et al., 1997)
Fluvalinate (queen tabs)	Carbaryl contact	48h preexposure	No change in toxicity	Apis mellifera	Not stated	20 C in dark, Six replicates of 10 bees, six dose levels 24hr mortality, Probit analysis	(Ellis et al., 1997)
Fluvalinate (queen tabs)	Methyl parathion contact	48h preexposure	No change in toxicity	Apis mellifera	Not stated	20 C in dark, Six replicates of 10 bees, six dose levels 24hr mortality, Probit analysis	(Ellis et al., 1997)

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Table 2.17: Effect of concurrent exposure to fungicide formulations at their maximum application rate on the toxicity of varroacide treatments (Apistan – tau-fluvalinate, Bayvarol – flumethrin) and the tau-fluvalinate pesticide Mavrik,; (- no increase, + lowest increase in mortality, +++ highest increase in mortality) (Defra PN0945).

Fungicide formulation	**Flumethrin fold increase**	**Bayvarol (flumethrin)**	**Tau-fluvalinate**	**Apistan (tau-fluvalinate)**	**Mavrik (tau-fluvalinate) (agrochemical)**
Carbendazim	6.4	+	3	-	-
Difenoconazole	6.0	+	2.5	-	+++
Flusilazole	19.1	+	19	+++	+++
Iprodione +thiophanate methyl	1.9	-	1.9	++	++
Propiconazole	4.1	+	2.5	-	+++
Tebuconazole	3.1	+	6.1	-	+++

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Table 2.18: Effects of mixtures of pesticides and antibiotics

Pesticide	Medicines	Acute effect	Strain	Pre-treatment	Method	Reference
Coumaphos 2ug contact	Oxytetracycline 1.4mM oral 2 days pre-exposure	Inc mortality from 7% to 51%	Apis mellifera	Untreated colonies(fresh frames and new combs)	Newly emerged bees, fed at day1-3 33± 2C and (70–80%) RH, repeated measures of analysis. 5–10 replicate cohorts of 25 bees were tested for each acaricide - pretreatment combination	(Hawthorne and Dively, 2011)
Fluvalinate 3ug contact	Oxytetracycline 1.4mM oral 2 days pre-exposure	Inc mortality from 5.6% to 39%	Apis mellifera	Untreated colonies(fresh frames and new combs)	Newly emerged bees, fed at day1-3 33± 2C and (70–80%) RH, t-test. 5–10 replicate cohorts of 25 bees were tested for each acaricide - pretreatment combination	(Hawthorne and Dively, 2011)

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3. Interactions between diseases and susceptibility of bees to pesticides.

The database searches yielded 112 references of which 71 related to honeybees, 7 for bumble bees and 34 on other insects. As in Chapter 2 although the searches were not primarily directed at other insects (and therefore should not be regarded as comprehensive for other species) these references were used as a comparison where they provided additional data.

Honeybees are known to suffer from a wide array of bacterial, fungal and viral pathogens as well as ecto- and endo-parasites (Cantwell, 1974; Genersch et al., 2010; Morse and Nowogrodski, 1990; Mouches et al., 1982; Evison et al., 2012; Macfarlane et al., 1995). Multiple infections are common and the impact of some pathogens can be far higher in the presence of other associated pests and diseases (Fries, 2010; Conte and Ellis, 2008). Honeybees have a well-developed immune system for coping with bacterial and fungal infections (Bekesi, 2005; Evans, 2004; Evans et al., 2006; Glinski and Jarosz, 2001) although their immune response to viral pathogens is less well understood (Che, 2007) and there has been significant interest recently on the potential for pesticides to affect the susceptibility of bees to diseases (Desneux et al., 2007; Wang et al., 2009; Oliver, 2010; vanEngelsdorp et al., 2009b; vanEngelsdorp et al., 2010; AFSSA, 2008; Faucon et al., 1992; Maini et al., 2010; Moritz et al., 2010). This has been highlighted by high pesticide residues reported in honeybee colonies in the USA (Anon, 2008; Mullin et al., 2010) and the importance of microbial communities within the hive which may be affected by pesticide residues (Andersen et al., 2011; Mattila et al., 2012) as well as impacting on the bees directly.

This literature review is directed at the major pests and diseases of honeybees to assess the potential role of pesticides in increasing susceptibility to pathogens. Both laboratory studies on direct interactions between pesticide exposure and susceptibility to disease in honeybees (which are very limited in number, e.g. Alaux et al. (2010b) and at directed surveys of the incidence of disease in honeybee colonies related to pesticide usage, e.g. Genersch et al. (2010), are included in the review.

3.1. Bee immune defence systems

The dense crowding within social and eusocial bee colonies together with the relatively homeostatic nest environment with stored resources of pollen and nectar/honey results in conditions conducive to increased susceptibility to disease (Evison et al., 2012). This has resulted in the evolution of both individual and social immunity in the honeybee and bumble bee (Wilson et al., 2003).

Individual immune defences in the honeybee parallel the innate immune systems of vertebrates (James and Xu, 2011) (Figure 3.1). The primary defences are the cuticle, the spiracles and trachea and the alimentary canal including the intestinal epithelium and peritrophic membrane (Aronstein and Murray, 2010; Glinski, 2000; Glinski and Kauko, 2000). If these are breached cellular immune defences include the cellular response represented by phagocytosis by haemocytes and melanisation. The humoral response is represented by secretion of antimicrobial peptides (inducible antibiotic peptides such as apidaecins) (Casteels et al., 1989; Li et al., 2006) from within the fat body due to the activation of one or several intracellular signalling pathways (Toll, Imd, JNK and JAK/STAT) which degrade pathogens as well as the action of reactive oxygen and nitrogen species (Figure 3.1).

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When compared to the sequenced *Drosophila* and *Anopheles* genomes, honey bees possess only roughly one-third as many genes in 17 gene families implicated in insect immunity (Evans et al., 2006). However this reduction in individual immunity may be compensated for by honeybee social immunity (Evans et al., 2006), e.g. grooming, nest hygiene (examples include the removal of dead adults and varroa infested larvae (hygienic behaviour)) and the use of the anti-microbial agents propolis (collected tree resin) and glucose oxidase in salivary secretions.

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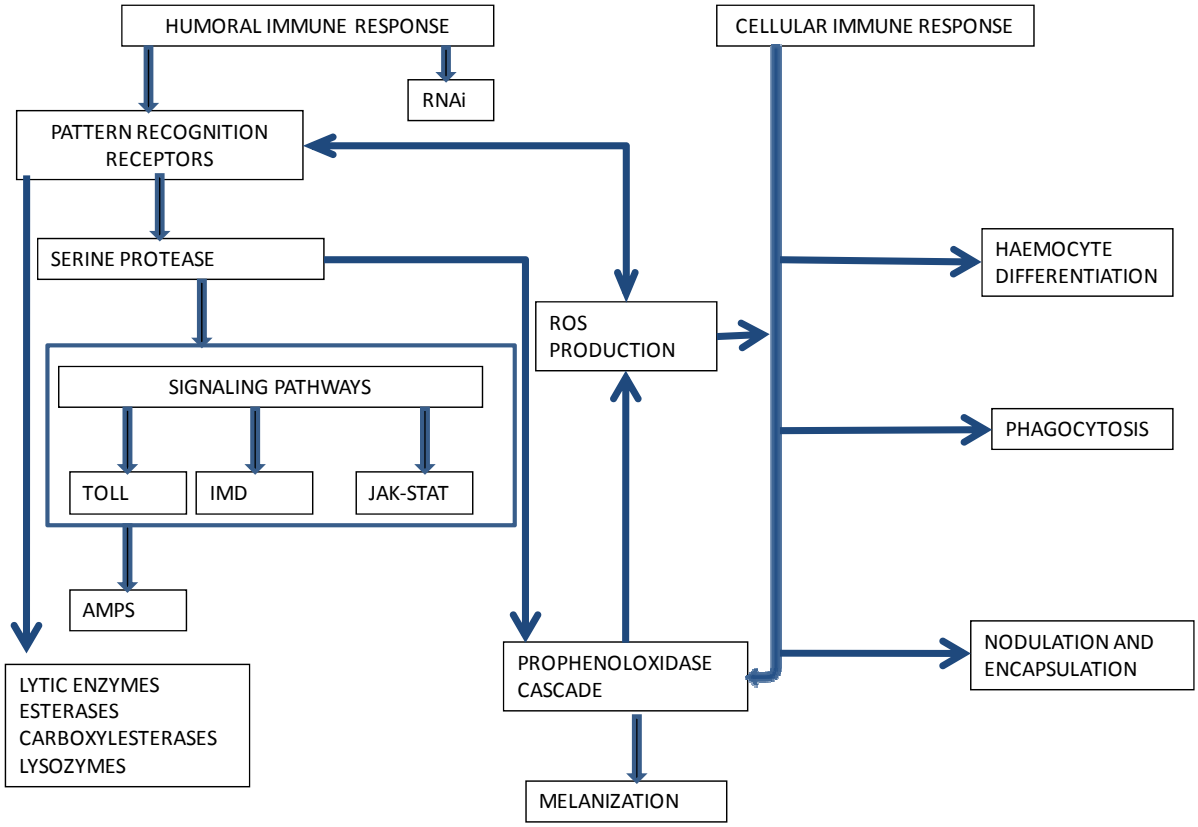

Figure 3.1 Overview of the immune system of bees (after (James and Xu, 2011) (AMP – antimicrobial peptide, ROS –reactive oxygen species)

The capacity for immune function varies with age in honeybees but not in bumble bees (James and Xu, 2011). While there are no differences in encapsulation response between developmental stages (Wilson-Rich et al., 2008) honeybee larvae and pupae have the highest total haemocyte counts and lowest levels of phenoloxidase (PO) activity but high levels of catalytically inactive pro-PO (pro-phenoloxidase) (Laughton et al., 2011) that is proteolytically activated upon infection (Chan et al., 2009). Phenoloxidase activity increases post- emergence reaching a plateau within the first week and does not decline with age in workers despite the reported reduction in haemocyte numbers (Schmid et al., 2008). PO activity increased with adult age but decreases following the challenge, possibly due to utilisation and failure to replenish (Laughton et al., 2011). In queens phenoloxidase activity continuously increases with age, reaching levels twice as high as those found in workers, and slightly declines with age in drones (Schmid et al., 2008). It has been suggested that honeybee foragers show a pronounced reduction in haemocyte number when compared with nurse bees and an almost total loss of the capability to form nodules in response to bacterial challenge. Antimicrobial peptides have been shown to increase in adult honeybees following an immune challenge and show signs of scenescence.

This reported loss of immune competence is age and not task-related and has been regarded as advantageous with respect to an already high mortality rate due to foraging and to redistribution of energy costs at the colony level (Laughton et al., 2011). However immune strength has also been reported to be most vigorous in older, foraging bees and weakest in young bees suggesting induced shifts in behavioural roles may increase a colony's susceptibility to disease if nurses begin foraging activity prematurely (Wilson-Rich et al., 2008).

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In the eusocial bumble bees, which differ from honeybees in their hygienic behaviour and the antibacterial conditions in nests, do not show a reduction in haemocyte number with age but do show a decreasing ability to encapsulate and melanise invading parasites and haemocyte number decrease following parasite challenge (Doums et al., 2002).

3.1.1. Factors affecting immunocompetence

Factors other than pesticides can impact on the immune system in honeybees and increase susceptibility to disease, e.g. other diseases (Brodsgaard et al., 2000; Navajas et al., 2008; Romano and Moncecchi, 1988; Glinski and Kauko, 2000) the immunsuppressive effects of Varroa destructor, antibiotics, sulphonamides (Glinski and Kauko, 2000) and metals (Buczek et al., 2008) and immunostimulators have been proposed (Luft et al., 2007). Confinement of colonies can result in immune suppression and oxidative stress in colonies (Morimoto et al., 2011) and poor habitat quality may result in lowered immune response (Naug, 2009). One key factor is that although colonies show qualitatively similar immune responses the colony is a significant factor in the level of the response, i.e. there are large variations between colonies on the level of the response (Laughton et al., 2011; Wilson-Rich et al., 2008). There have also been suggestions that stress, e.g. isolation, weakens individual immunocompetence. This may explain why some immune competence effects are evident in under laboratory conditions but not in colonies (Pettis et al 2012). The encapsulation response, or haemocyte concentration as a correlate, has been shown by some authors to be highly variable (Wilson-Rich et al., 2008) and varies considerably with host age, temperature, stress condition (e.g. simultaneous parasitic infections), nutritional status, and genotype (Schmidt-Hempel and Schmidt-Hempel, 1998; Szymas and Jedruszuk, 2003). Thus, in studies on the effects of pesticides on immunocompetence it is important that as many of these factors are controlled as possible.

Pollen is the primary source of protein in honeybees is well established to affect longevity, development of the hypopharyngeal glands and ovaries and the susceptibility to pathogens. Pollen quantity does not affect individual immunity (measured by haemtocyte concentration, fat body content and phenoloxidase activity) or social immunity (glucose oxidase activity). However, diet diversity (polyfloral pollen) increases glucose oxidase activity which has a key role in social immunity (Alaux et al., 2010a) and protein quality has been reported to affect melanisation, e.g. of the gut wall. This parallels observations of immune function in other insects where antibacterial activity was higher for individuals fed high-quality diets (Lee et al., 2008). Post-ingestive utilization of nitrogen was reduced for larvae on the low-quality protein diet implying that protein quality had a significant influence on the nitrogen pool potentially available for investment in both melanin production and immune function.

Pollen is also important for haemocyte function in honeybees (Szymas and Jedruszuk 2003) and has been shown to be a key parameter in bumble bees (Tasei and Aupinel, 2008). A lack of dietary protein in 7-8 day old bees caused a significant increase in the percentage of granular haemocytes, a significant decrease of other types of haemocytes and a lower metabolic activity. The lack of protein also resulted in a large decrease in the proportion of metabolically active cells, which suggests a reduced ability to phagocytose. Pollen quality also affects sensitivity of bees to pesticides (Wahl and Ulm, 1983) with the amount and quality of pollen in the first few days after emergence affecting the pesticide sensitivity of young and older bees whereas the quality of pollen fed to larvae does not appear to affect the sensitivity of the emerging adults.

3.2. Fungal diseases

There are a number of fungal pathogens of honeybees including the microsporida *Nosema apis* and *Nosema ceranae* (*Nosema* is a microsporidian fungal pathogen which has a number of hosts (Cadeddu, 2000), primarily affect adult bees, has been associated with bee losses in the USA and Spain and

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causes nutritional stress and increases food intake - for a review see Fries, 2010) and *Ascophaera apis* (chalkbrood) which affects larvae (for a review see Aronstein and Murray, 2010; Glinski and Buczek, 2003). *N. bombi* is a pathogen of bumble bees and when infection occurs at an early stage of colony development virtually all individuals are infected, with spores being found in a number of tissues, and the functional fitness of males and young queens is reduced to zero. Furthermore, the survival of workers from infected colonies is reduced (Otti and Schmid-Hempel, 2007).

Nosema sp. have two primary stages in the lifecycle, a vegetative stage and a sporogenic phase in which spores are produced (Higes et al., 2010). The infective stage is the spore which is ingested by the host and infects host cells in the gut lumen and Malpighian tubules and then spreads within the host; fresh spores are capable of surviving outside of the host and are spread via faeces or a decaying host. *Nosema* is transferred via food in adult honeybees and therefore only infection of adults is likely to occur. When spores of *Nosema* are ingested by bees they germinate within 30 minutes inside the stomach. The organism then penetrates cells of the stomach lining. It continues to grow and multiply rapidly, using the cell contents as its food supply. Large numbers of spores are produced in the host cell in 6 to 10 days. Bees can be exposed to *Nosema* by cleaning contaminated comb and through trophallaxis or via infected bees that are crushed by beekeepers (Higes et al., 2010; Malone et al., 2001). *N. ceranae* spores have also been found in corbicular pollen, honey and royal jelly (Higes et al., 2008; Cox-Foster et al., 2007). It usually takes 20-90 spores to infect a bee but in inclement weather when bees are prevented from taking defaecating flights spore numbers within a bee can build up to over 200 million (Delaplane, 1998).

N. apis infection in honeybees causes spring dwindling in colonies, due to decreased lifespan of adult workers, is associated with digestive disorders and has been correlated with queen supersedure (Mattila and Otis, 2006). *N. ceranae* is an increasingly prevalent parasite of the honeybee which has moved host from the Asian honeybee, *A. cerana*, that shortens the lifespan of adults and has been associated with heavy European honeybee colony losses in some countries, e.g. Spain (Fries, 2010). Initially *N. ceranae* was considered to be present throughout the year but recent evidence suggests there may be some seasonal variations (Traver et al., 2012). Although the spores of *N ceranae* appear less durable than those of *N apis,* it is thought to be more virulent and is increasingly prevalent within Europe (Fries, 2010). *N ceranae* is therefore an emerging threat to honeybee colonies which may pose additional threats to other pollinators since many microsporidia have multiple hosts (Alaux et al., 2010b; Evison et al., 2012).

The use of spore counts to diagnose the health of bees infected by *N ceranae* has been reported to be unreliable (Meana et al., 2010). The *N. ceranae* spore count has been used as a marker of the severity of nosemosis and as an indicator of the health status of CCD colonies in the USA (Cox-Foster et al., 2007), but has been rejected as a marker of health status in naturally infected colonies (Higes et al., 2008). Nosematosis involves not only the parasite but also its host and the use of spore counts alone as a measure of the response of bees to infection by *Nosema* is probably inadequate (Rinderer and Dell Elliott, 1977). Spore counts cannot measure possible differences in tolerance by the host to a given level of infection. The mean spore count for *N. cerenae* varies between the in-hive and forager bees throughout the study, and even on the same day (Meana et al., 2010). For example, a maximum spore count in a sample of foragers of 10.3 million spores / bee whilst other in-hive bees contained no spores and there were no signs of illness and indeed, the colony was apparently as healthy (asymptomatic) (Meana et al., 2010).

Spore counts in bees infected by either *N. apis* or *N. cerenae* may therefore not be related to the number of parasites in the bee. The higher proportion of immature stages of *N. ceranae* (70%) than of *N. apis* (50%) in a comparative study of infected honey bees (Martin-Hernandez et al., 2009) indicated that there are clear differences in the total number of parasites inside the gut. As such, the real number of affected cells in bees cannot be accurately evaluated through the total spore count. Therefore it is

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reported that *N. cerenae* spore count, unlike *N. apis*, is not a useful measure of the state of a colony's health and in-hive bees are unsuitable as indicators of the degree of infection of the colony (Higes et al., 2008). Foragers collected at the sealed entrance of the hive are a more reliable source for sampling when studying nosemosis due to *N. ceranae*, as shown for *N. apis*. The mean proportion of infected bees may be a more reliable method to establish colony health (Antúnez et al., 2009; Higes et al., 2010; Higes et al., 2008; Meana et al., 2010) and consideration should be given to using methods which assess all growth stages.

Rinderer and Dell Elliott, (1977) showed that bees fed *N apis* spores and pollen lived longer than those fed *Nosema* spores alone but in-hive use of pollen to rear brood limits the applicability of pollen feeding to prolong longevity (Mattila and Otis, 2006). Gontarski and Mebs (1964) (cited by Rinderer and Dell Elliott, 1977) suggested that protein feeding on emergence results in an expansion of the honey bee midgut which leads to more infection sites and thereby causes greater spore production. They suggested that a 3-fold increase in midgut volume from newly-emerged bees to pollen-eating bees caring for brood could account for the 2-fold increase in *N. apis* spores.

There are significant differences between effects of the two major *Nosema* infections of honeybees. *N. ceranae* infection but not *N apis* infection appears to significantly suppresses the honey bee immune response (Antúnez et al., 2009). Such immune suppression would also increase susceptibility to other bee pathogens. After infection with *N. apis* the honey bee immune system quickly activates defence mechanisms, which includes the increase in the expression of genes encoding antimicrobial peptides and other immunity-related enzymes. Whereas *N. ceranae* infection seems to suppress the immune response by reducing the transcription of some of these genes (Antúnez et al., 2009).

Only a small number of a few ventricular epithelial cells are infected 3 days after *Nosema* infection, while the majority of cell infection and degeneration occurs after 7 days (Higes et al., 2007). Four days after *N. apis* infection, the expression of the antibacterial peptides with broad antibacterial activity, abaecin, denfesin and hymenoptaecin increases. The expression of phenoloxidase also increases 7 days after *N. apis* infection, favouring nodulation, encapsulation and phagocytosis (Glinski and Jarosz, 2001). In contrast 4 days after *N. ceranae* infection antibacterial peptides were not expressed even though the pathogen had already invaded the ventricular epithelium (Higes et al., 2007) and by 7 days antibacterial peptide expression was significantly depressed suggesting partial suppression of humoral and cellular defence mechanisms favouring further infection by other pathogens. This effect of *N cerenae* on susceptibility to other pathogens is supported by the detection of both *N cerenae* and viruses in Greece in 2009 when "sudden deaths, tremulous movements and population declines" of adult honey bees were reported by the beekeepers in the region of Peloponnesus (Mt. Mainalo), Greece. Symptomatic adult honey bees tested positive for Varroa destructor, Nosema ceranae, Chronic bee paralysis virus (CBPV), Acute paralysis virus (ABPV), Deformed wing virus (DWV), Sacbrood virus (SBV) and Black queen cell virus (BQCV), but negative for Acarapis woodi. Chemical analysis revealed that amitraz, thiamethoxam, clothianidin and acetamiprid were all absent from symptomatic adult bees, sugar and sugar patty samples (some bee samples, contained imidacloprid residues) (Bacandritsos et al., 2010).

3.2.1. Interactions of fungal disease and pesticides in bees

There are few studies investigating the interactions of pesticides and fungal diseases in bees and all relate to *N. apis* or *N cerenae.*

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3.2.1.1. Effects of diseases on the toxicity of pesticides

N. apis infection has been shown to affect the susceptibility of bees to pesticides (DDT and Dipterex) decreasing the LD50 for DDT from 45.7 to 33.4 µg/bee (Ladas, 1972).

An increase in mortality was observed when bees (five days after emergence) were infected with 125,000 spores of *N ceranae* and after a further 10 days were exposed to sublethal (LD_{50}/100) doses of thiacloprid or fipronil for 10 days (Vidau et al., 2011). The bees clearly showed energetic stress after *Nosema* infection by their increased consumption of sucrose with infected bees consuming approximately twice the amount of sucrose compared with uninfected bees. Although there was no apparent difference in overall daily intake when exposed to the insecticides, the total insecticide intake was 106% in the infected bees (26.9 pg fipronil /bee/day) compared with uninfected bees (25.3 pg fipronil /bee/day) and 136% in infected bees (153 ng thiacloprid/bee/day) compared with uninfected bees (112 ng thiacloprid/bee/day). There were large difference in intake on day 1 of the pesticide exposure period (although statistics were not reported) with bees infected with *N ceranae* consuming 39 pg fipronil or 174 ng thiacloprid on the first day of exposure compared with 25 pg fipronil or 97 ng thiacloprid in uninfected bees, i.e. 156% and 179% respectively. Mean intake in *Nosema*/ thiacloprid treated bees was consistently but not significantly higher than thiacloprid only treated bees.

Mortality in the *Nosema* only infested bees was 47%, in fipronil only treated bees 15% and in thiacloprid only treated bees 12% (control was 10%). Mortality of the *Nosema*/fipronil dosed bees was 82% and the *Nosema*/thiacloprid bees 71%. Behavioural effects were observed following both treatments in the infected but not in the uninfected bees. Therefore, additive toxicity would account for 62% of the 82% mortality in the *Nosema*/fipronil dosed bees and 59% of the 71% mortality in the *Nosema*/thiacloprid treated bees, suggesting additional mortality occurred when the bees were exposed to the combination.

Unfortunately although the consumption data are reported with standard deviations the mortality data are not and the only mortality statistics reported were the difference between infected bees with fipronil and thiacloprid. Therefore the variability in mortality between test units could not be assessed. However, although not identified in the paper, the additional mortality in the thiacloprid treated bees may be accounted for by the higher overall treated sucrose intake at 136% of the thiacloprid only treated bees.

Fipronil reduced the total *N. cerenae* spore count from a mean of 112 x 10^6 to 74.8 x 10^6 by the end of the study whereas thiacloprid significantly increased it to 157 x 10^6 although the authors suggest this would not impact on mortality. As identified above, spore count may not be a reliable indicator of impact in *N ceranae* infection.

N ceranae infection was shown to significantly increase glutathione-S-transferase activity in the mid-gut and fat body 1.6 fold and 1.7 fold respectively but had no effect on 7-ethoxycoumarin-O-deethylase activity, a measure of P450 activity. Unfortunately the authors authors prepared microsomal fractions for mid-gut P450 activity assessment which has inherent problems relating to the presence of P420 forms in microsomes and inhibitors in gut wall (Johnson et al. 2009). Therefore it is difficult to interpret the midgut enzyme activity data. The authors did not assess effects on the immune system parameters and therefore it is unclear whether the pesticides affected tolerance to the disease or vice versa. These factors make it difficult to determine the mode of interaction between the pesticide and *N. ceranae*.

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3.2.1.2. Effects of pesticides on disease susceptibility

Exposure of adult bees

Gilliam et al. (1977) demonstrated that a combination of stress (caging) and 2,4,5-T (1000ppm) exposure resulted in higher levels of yeasts and 2,4,5-T residues in honeybee midguts than in free-flying bees fed 2,4,5-T. The high level of dosing and toxicity of the 2,4,5-T was demonstrated by the loss of larvae within the free-flying colonies within 6 weeks. Gilliam (1978) (cited by Bendahou et al., 1997) reported an increase in chalkbrood after nearby insecticide treatment but there was no mechanism proposed.

There have been conflicting reports over the interactions between imidacloprid and Nosema. (Alaux et al., 2010b; Wehling et al., 2009). In a short report by Wehliing et al (2009) no effects under laboratory conditions on the sensitivity of bees to imidacloprid are reported. However Alaux et al (2010) reported the effects of combined exposure to imidacloprid and Nosema cerenae on individual mortality, and energetic demands (sucrose consumption), individual immunity (total haemocyte count provides an indirect measure of basal cellular immunocompetence and involved in phagocytosis and phenoloxidase plays a key role in the encapsulation of foreign objects by melanisation, the fat body is a major site of antimicrobial peptide synthesis) and social immunity (glucose oxidase activity which is mainly expressed in hypopharangeal glands in which is catalyses the conversion of glucose to gluconic acid and hydrogen peroxidase which has antiseptic properties and is secreted into larval food and honey).

Bees were Apis mellifera mellifera x Apis mellifera ligustica and experiments were repeated with bees from 3 colonies. The studies were established so that bees were exposed to untreated sucrose only, to N. cerenae spores only, to imidacloprid only or to a combination of Nosema spores and imidacloprid. For the mortality assays 3 replicates of 30 bees were used and for the immune parameters groups of 120 bees were dosed. As required 1-day-old bees were dosed individually with 200,000 Nosema spores (a mixture of N apis and N ceranae) in 2µl freshly prepared 50% w/v sucrose. Imidacloprid was fed for 10 days in 50% w/v sucrose containing 0, 0.7, 7 (the field realistic rate) or 70 µg/Kg for 10 hours each day and consumption was recorded. The remainder of the time untreated sucrose was provided ad libitum and the consumption of this was not reported. Bees were held at 28^{o}C and 70%RH in the dark. Bees were also provided with untreated pollen to ensure normal development.

Control mortality was approximately 5% and increased mortality was observed in all the treatment groups. Additive mortality was observed in all the Nosema + imidacloprid treatments. Bees treated with Nosema consumed significantly more sucrose than control or those treated with imidacloprid alone and appears to be the basis of the increased mortality reported in the highest imidacloprid treatment rate. Reading from the figures presented in the paper the mean intake of sucrose per day was similar for the Nosema treated bees and the imidacloprid treated bees but in the 0.7 µg/kg dose the presence of Nosema increased mean intake to 129% of the imidacloprid alone; in the 7 µg/kg increased the mean intake to 141% of the imidacloprid alone and in the 70 µg/kg dose group Nosema increased mean intake to 161% of the imidacloprid alone. Thus the increased mortality in the Nosema + imidacloprid dose groups is related to the increased intake of imidacloprid rather than increased susceptibility due to the presence of Nosema. The authors recognise this effect in the paper but refer to it as interaction whereas the true effect is through increased intake of sucrose and was additive. In fact in the early stages of the study the intake of the sucrose treated with imidacloprid at the realistic environmental rate of 7 µg/kg was lower than that of the Nosema only treated bees which may suggest a reduced intake of treated feed in the absence of an alternative.

The number of Nosema spores increased in all groups, including the control but it was significantly lower in the imidacloprid treated bees (again the caveat about the reliability of spore counts for N

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ceranae applies). Other immune parameters assessed phenoloxidase activity and total haemocyte count showed no effects of Nosema, imidacloprid or the combined treatment at any dose rate. The only effect observed was on the activity of glucose oxidase activity (antiseptic properties through production of hydrogen peroxide) and there was no mechanism proposed for this effect.

Exposure of brood

The effects of pesticide exposure on susceptibility to N. cerenae of adults emerged from comb containing high levels of pesticides (Table 3.1) has been assessed (Wu et al., 2011) (Wu et al., 2012)

Table 3.1 Residue levels in combs used to rear brood by Wu et al (2012) compared with reported residue levels in beeswax in the EU (note all pesticides were not found in combination in the EU)

	Comb Y µg/kg	**Comb G µg/kg**	**EU range (section 1) µg/kg**
2,4,dimethylphenyl formamide (a metabolite of amitraz)	142	147	
chlorpyrifos	8.5		14.9
coumaphos	7230	281	<LOD-4700
coumaphos oxon	231	10.2	
chlorothalonil		65.7	
endosulfan	3.7		51-370
esfenvalerate	12.3		
fluvalinate	6800	11280	35-5100
phosalone	31.7		
THPI (a metabolite of captan)	98.7		
permethrin		103	
pyrethrin		229	

A control comb contained low levels of pesticides (21 ppb coumaphos).

A queen was allowed to lay in each of the brood combs and bees were emerged in the laboratory, tagged and placed within experimental colonies. One treatment colony received 10 ml/week of sucrose containing 5 million spores/ml and the other colony received sucrose solution in the absence of spores. The infection levels in the colonies was shown to be 555,000 spores/bee in the treated colony and 32,000 spores/bee in the control colony before the start of the study.

Twenty tagged bees were randomly sampled from each colony 2, 3 and 4 weeks post release. Spore levels were quantified in each bee and showed a higher proportion of bees reared from pesticide treated comb were infected with *Nosema* spores than those emerged from low residue comb. However, the spore inoculation of the colony had no effect on the proportion of infected bees but higher spores levels were identified in those that did contain spores. Bees from high residue combs contained 5,059,000 from the spore dosed colony and 142,000 from the undosed colony, whereas bees from low residue comb contained 1,925,000 spores in the spore dosed colony and 67,000 in the undosed colony. The reduction in longevity resulting from the combination of high pesticide residues and *N ceranae*

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was shown by only one bee surviving from one comb 4 weeks after treatment. The proportion of control bees infected by 2 weeks after introduction into the colonies was 2% compared with 20% in the bees reared from high residue comb.

However, as highlighted above there are indications in several studies that the use of *N.cerenae* spores counts alone are not a good correlate of infection (Meana et al., 2010) (Higes et al., 2008) and thus in the above study the use of the proportion of infected bees is probably a better assessment than spore numbers. The doubts over the use of spore numbers is also raised by the results of a study reported in which colonies of bees were exposed colonies of bees to 0, 5 or 20ppb imidacloprid in patties for 5 or 8 weeks and then bees taken to the laboratory and artificially exposed to high levels of *Nosema apis* and *N. cerenae* for 2 days and then spore counts assessed after 10 days (Pettis et al., 2012). The residues of imidacloprid in the hive bees was 1.58 ± 0.68 ppb in 5ppb dosed colonies and 3.67 ± 1.48 ppb in 20 ppb dosed colonies (control colonies contained 0.6 ± 0.31 ppb showing cross-contamination) and no residues were detected in newly emerged bees which received a challenge of approximately 333,000 spores. No more than 20% of the treated bees died over the 12 day laboratory observation period. Spore numbers in each of 10 bees from each treatment were assessed on day 12. In the July study the bees emerged from the two.5 ppb colonies sampled and four 20ppb colonies samples contained higher spore counts after *Nosema* dosing than the bees emerged from the three control colonies. In the August study with different colonies but dosed at the same level similarly had higher spore counts in the dosed bees and a further study showed there was no effect of spore dosing level (0.1 or 1 million spores) on the number of spores present. However there was a large difference in the numbers of spores present in the dosed bees in the two studies in July and August. Control bees dosed with 333,000 spores in July contained 0.17 x 10^6 spores whereas those dosed in August with 0.1 or 1 million spores contained 1.1 x 10^6 spores which was higher than those reported for the imidacloprid dosed bees in July. It was shown that *N cerenae* was the predominant strain within the spores used to dose the bees and it has been reported that spore count may not be a reliable measure of infection in *N cerenae* affected bees (Meana et al., 2010; Higes et al 2008). In the colonies used for the study eight of the 30 treated colonies tested positive for *Nosema* after 10 weeks and there was no relationship between infection and imidacloprid treatment. Of the total of thirty treated colonies (10 per treatment) three control colonies contained 4.3 million spores per bee, three 5ppb contained 2.9 million spores/bee and two of the 20 ppb treated colonies contained 0.5 million spores/bee respectively. This confirms the need for greater understanding of *N cerenae* at the colony level and the most robust measure of infection within both individual bees and the colony in order to more fully understand the interactions between pesticides and *N cerenae*.

As *N .ceranae* impacts on the immune system it is possible it also results in adverse effects on the activity of midgut enzymes. Unfortunately the bee midgut contains P450 inhibitors and microsomes prepared from midgut contain the inactive P420 form. Therefore studies on effects of *N ceranae* should be performed on intact midgut preparations to avoid such confounding factors. In addition, given the effects of sucrose on the ability of bees to detoxify pesticides with the importance of components such as quercetin in honey inducing P450s within the mid-gut (Johnson et al., 2012), it is important to ensure that the diet replicates as closely as possible that within the hive when determining effects of pesticides and disease on bees. The use of alterantive methods to assess disease status would also improve understanding of impacts compared with *N. ceranae* spore counts which have been questioned over their reliability in interpreting disease status.

It is also vital to understand the disease status of individuals used in pesticide assessments since both the honeybee (*Apis mellifera*) and the bumble-bee (*Bombus terrestris*) perform poorly in proboscis extension reflex (PER) memory tests when their immune systems were challenged by lipopolysaccharide (LPS) (Mallon et al., 2003; Alghamdi et al., 2008). *Nosema apis* appears to act on the corpora allata to increase the juvenile hormone titres in infected bees and this induces earlier

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foraging in bees (Huang and Lin, 2004). Thus bees collected at the entrance may not be mature foragers but young bees infected with *Nosema apis*.

3.2.2. Interactions of fungal disease and pesticides in other insects

Interactions between fungal diseases and pesticides have been widely used in biological control systems to reduce pesticide inputs. For example, malathion has been reported to have a synergistic effect on the action of *Nosema whitei* in the stored product pest *Tribolium castaneum* (Al-Hafidh and Selman, 1983). Formulations of profenofos, imidacloprid, acetamiprid+abamectin and azadirachtin at 50% recommended application rates with a formulation of *Beauvaria bassiana* achieved good protection of whitefly with more than additive effects (Ibrahim et al., 2009). Synergistic interactions were observed in Colorado potato beetle larvae between imidacloprid, and *B. bassiana* when they were applied together or when *B bassiana* was applied 24 hours after imidacloprid but not when imidacloprid was applied 24 hours after *B. bassiana*. In this case no effects on the rate at which conidia were removed from the cuticle were observed but feeding was inhibited when larvae were fed imidacloprid. Decreased resistance to disease is well established to increase susceptibility to pesticides (Furlong and Groden, 2001). A similar non-synergistic effect of co-exposure to imidacloprid and *B. bassiana* is reported in whitefly that was suggested to be due to *B bassiana* reducing feeding and thus intake of imidacloprid (James and Elzen, 2001).

Combinations of low concentrations of buprofezin with *Metarhizium anisopliae* increased mortality of the brown planthopper (Geng and Zhang, 2005). Effects on the cuticle of insects can enhance the effects of fungal biocontrol agents and examples include the effects of diflubenzuron and *Metarhizium anisopliae* in *Manduca sexta* and in grasshoppers (Neves et al., 2002). Similarly cyromazine affects insect cuticle formation and acts synergistically with nematodes in insect larvae (Yildrim and Hoy, 2003). Spinosad has been reported to act synergistically with *M anisopliae* in the house fly (*Musca domestica*) but there was no detailed data available (Sharififard et al., 2011).

Imidacloprid has been identified to have a range of effects in termites (Boucias et al., 1996) including reduced social interactions such as grooming, trophalaxis and tunnel construction. Pesticides also cause changes to the cuticle of the gut wall may enhance germination and penetration causing increased infection rates in some cases, e.g. chitin synthesis IGRs (Boucias et al., 1996). Other effects of pesticides that may increase invasion by mycopathogens include the gut transit time, imidacloprid inhibits food passage in termites (Boucias et al., 1996) and effects on gut microbiota. Imidacloprid has been shown to interact with entomopathogenic fungi in termites, soil inhabiting *Curculionidae* larvae and cockroaches. Termites rapidly succumbed to soil borne *Conidiobulus coronatus* and *M anisopliae* in imidacloprid treated soils and this was concluded to be due to reduced social grooming (Boucias et al., 1996). *B.bassiana* and *M anisopliae* conidia were removed by termites within 6 hours after application of the fungi alone but imidacloprid treated termites failed to remove the conidia. Therefore the recommended dosages of imidacloprid to treat termites could be reduced by 99% and the conidial concentrations by 75% to achieve the same level of control as either component alone (Neves et al., 2002). Similarly synergism between imidacloprid and *M. anisopliae* and *B. bassiana* can be observed in a arrange of other soil inhabiting insects and effects have also be ascribed to reduced locomotion and mobility within the soil resulting in reduced shearing of the conidia from the insect cuticle (Jaramillo et al., 2005; Neves et al., 2002). Asian long- horned beetles treated with imidacloprid produced significantly fewer *Metarhizium* conidia compared to beetles not treated with imidacloprid (Russell et al., 2010). Similar synergism was observed in wireworms (Ericsson et al., 2007). Cockroaches (B*latella germanica*) were killed significantly faster when fed on imidacloprid or propetamphos after a topical application of *M anisopliae* whereas chlorpyrifos and cyfluthrin were additive (Neves et al., 2002). The synergistic interaction between *B bassiana* and imidacloprid has

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been modelled in the homopteran *Nilaparvata lugens* (Feng and Pu, 2005),between *B bassiana* and a nereistoxin analague insecticide in the diamondback moth ((Tian and Feng, 2006) and between *B bassiana* and imidacloprid in the aphid *Macrosiphoniella sanboni* and *Myzus persicae* (Ye et al., 2005). It was concluded that conventional variance analysis of mortality data from factorial experiments is probably unsuitable for revealing fungal/chemical interactions as it is unable to estimate continuous time–concentration interactions that play an important part in the lethal activity of both agents.

A single study was identified in the hemipteran *Rhodnius prolixus* following exposure to azadirachtin, administered via a blood meal, which affected the immune reactivity as shown by a significant reduction in numbers of hemocytes and reduction in ability to produce antibacterial activity in the hemolymph when injected with bacteria, as well as decreased ability to destroy the infection caused. (De Azambuja and Garcia, 1992).

3.3. Bacterial diseases

There are two main bacterial diseases of honeybees *American foulbrood* caused by the spore forming bacteria *Paenibacillus larvae* and European foulbrood caused by *Melissococcus plutonius*. *Paenibacillus larvae* (American foulbrood) infects honey bee larvae when they consume spores in their food. The spores germinate in the gut; bacteria then move into the tissues, where they multiply in number. Infected larvae normally die after their cell is sealed, and millions of infective spores are formed in their remains. These remains dry to form 'scales' which adhere closely to the cell wall and cannot easily be removed by bees. Consequently brood combs from infected colonies are inevitably severely contaminated with bacterial spores. If infected combs are subsequently used and distributed or moved from colony to colony during routine beekeeping management then infection has the potential to spread quickly. The spores are very resistant to extremes of heat and cold, and to disinfectants and can survive for many years in honey, in old combs kept in store, or in derelict hives. Once a colony is infected the disease will usually progress until most of the brood is affected. The colony then becomes unable to replace the ageing adult bee population, causing it to become weakened, and finally to die out. The disease may develop for months before the colony succumbs, and death may occur at any time of the year.

European foul brood is caused by the bacterium called *Melissococcus plutonius*. The bacteria multiply in the mid-gut of an infected larva, competing with the larva for its food. They remain in the gut and do not invade the larval tissue; larvae that die from the disease do so because they have been starved of food. This normally occurs shortly before their cells are due to be sealed. Subsequently other species of bacteria may multiply in the remains of dead larvae as 'secondary invaders', such as *Paenibacillus alvei, Enterococcus faecalis, Brevibacillus laterosporus*, and *Lactobacillus eurydice.* The development of the disease within a colony is complex, and still not fully understood. It appears that infection can develop over a period of months or years, often debilitating but not killing the colony. During this time, signs of the disease may become more or less severe, or disappear altogether. Frequently there is a seasonal pattern, with signs becoming most obvious in late spring.

3.3.1. Interactions of bacterial disease and pesticides in bees

There are very few studies assessing the combined effects of pesticides and bacterial diseases on honeybees and most are anecdotal. For example, Morse et al 1965 cited by (Bendahou et al., 1997) reported an increase in the larval disease American foulbrood after treatment with carbaryl. Although larval honeybees demonstrate low inherent levels of immune parameters with catalytically inactive prophenoloxidase (proPO), lysozyme and the antimicrobial peptides, e.g. abaecin and hymenoptaecin

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present in the hemolymph these are activated and royal jelly (food) and energy storage proteins were downregulated when the immune system is challenged. Phenoloxidase (PO) enzyme activity is undetectable in one or two-day-old larvae but increases rapidly during development and has been shown to parallel the age-related ability of larvae to resist infection when challenged, e.g. by the American foulbrood organism *Paenibacillus larvae.* (Chan et al., 2009; Evans, 2004). This has been identified in colonies dosed with fenoxycarb which was observed to predispose the treated hives to European foulbrood and sacbrood (a virus) (Marletto et al., 1992).

3.3.2. Interactions of bacteria and pesticides in other insects

There are a number of studies which assess the impacts of bacterial based control agents with pesticides, all relating to *Bacillus thuringiensis*. Combinations of *Bacillus thuringiensis* H14 with chlorpyrifos showed synergistic actions on mosquitos whereas *Bt* and permethrin were antagonistic (Foo and Yap, 1987). The antagonistic effects were ascribed to the knockdown effects which may prevent the mosquito larvae from taking up a lethal dose. Similar synergistic effects of neem and *Bacillus thuringiensis* Israelensis have been observed in mosquitos (Murugan et al., 2002). Fenvalerate interacts with *Bacillus thuringiensis* in the Bihar hairy caterpillar *(Spilarctia obliqua)* which was particularly apparent when fenvalerate was applied 5 days after *Bt* but the scale of the synergy depended on the developmental stage of the insect and the concentrations of the components (Kandru and Dhingra, 2002; Sudhakar and Swaran, 2002). This may be due to the effect of the fenvalerate on the ability of *Bt* to sporulate. Effects on *Bt* toxicity have also been reported in a range of insects including the Colorado potato beetle (Dobrincic, 1996; Unal et al., 1997), Mediterranean fruit fly (El-Sebae et al., 1990), tobacco budworm (Atwood et al., 1996) with low doses of a range of organophosphorus, carbamate and pyrethroid insecticides and an IGR but no detailed data were available.

3.4. Viruses and bees

Honeybees are the target of a large number of viruses with a total of 18 identified to date including Deformed wing virus (DWV), Black queen cell virus (BQCV), Sacbrood virus (SBV), Kashmir bee virus (KBV), Acute bee paralysis virus (ABPV), Israeli acute paralysis virus (IAPV) and Chronic bee paralysis virus (CBPV) (Che, 2007). Often vectoring by Varroa, which is a significant stressor in honeybee colonies by feeding on the haemolymph causes a variety of physical and physiological effects on the colony, results in infections from viruses which are otherwise present as covert infections and can result in severe disease and mortality within the colony. In addition viruses can be transmitted within the colony by trophallaxis, contact, faeces and salivary gland secretions (Locke et al., 2011).

3.4.1. Interactions of viruses and pesticides in bees

Viruses have been shown to affect pesticide toxicity. (Bendahou et al., 1997) showed that chronic paralysis virus decreased the LD_{50} of cypermethrin from 0.16 ± 0.03 ug/bee to 0.06 ±0.01 ug/bee, i.e. an increase in toxicity of 2.7 fold with a decrease in sucrose consumption in the latter stages of the study. (Bendahou et al., 1997) ascribed this effect to several possible factors:

Pyrethroids altering lipid metabolism and the intestinal lipid bilayers so that CPV can cross more readily through the intestinal membrane into the haemolymph

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Pyrethroids decrease lysozyme concentration and phagocytosis capabilities

Locke et al., (2011) reported the effects of acaricide treatment using Apistan (tau-fluvalinate) on the infection dynamics of deformed wing virus (DWV), sacbrood virus (SBV), and black queen cell virus (BQCV) in adult bees, mite infested pupae, their associated *Varroa* mites, and uninfested pupae. Titres of DWV in adult bees, mite infested pupae and uninfested pupae increased initially with the onset of the acaricide application and then slightly decreased progressively coinciding with the removal of the *Varroa* mite infestation but remained higher in treated colonies than in untreated colonies. The subsequent decrease in DWV titers after the initial increase was, at least partly, due to the removal of *Varroa*-mediated transmission, as implied by the strong correlation between DWV titers and *Varroa* infestation rates in both adults and pupae. The titres of SBV and BQCV did not show any direct relationship with mite infestation and showed a variety of possible effects of the acaricide treatment.

The initial increase of DWV titres in adult bees and pupae of the acaricide-treated colonies coincided with the most potent chemical effect of the treatment and could therefore was considered to potentially have been a consequence of debilitating direct effects of tau-fluvalinate on honey bee physiology and/or immune system responses, causing increased host susceptibility to DWV infection. The authors considered the action of fluvalinate at the cellular and biochemical levels as a possible mode of action. Fluvalinate interferes with the voltage-gated sodium ion transport channels and these channels regulate the osmotic potential of the cell. The same channels are therefore frequently a target for virus infections that use osmotic pressure to burst the cell to release newly-formed virus particles which may result in the increased titres observed. However, the authors also highlighted the limited level of information available on the effects of the tau-fluvalinate treatment on host immunity and pathogen virulence in both bees and mites.

3.4.2. Interactions of virus and pesticides in other insects

Botanical insecticides such as neem affect the mortality caused by nuclear polyhedrosis virus on bollworms (*Helicoverpa armigera*) (Murugan et al., 1998; Kumar et al., 2008) and the mode of action was postulated as damage to the gut tissues by the insecticides which facilitated the proliferation of the virus. The insecticides appeared to disrupt the gut muscles, possibly altering peristaltic movement, and alter the gut epithelium together with decreased gut secretions leading to disruption of gut physiology. The interacting effect of injections of nuclear-polyhedrosis virus, DDT, rotenone and groundnut oil on fourth- and fifth-instar larvae of Galleria mellonella (L.) was determined in laboratory tests. DDT acted slightly more rapidly than the virus and essentially the same population phenotype was susceptible to both, indicating that application of the compounds alone was as effective as application in combination. However, virus appeared to enhance the effect of rotenone, and groundnut oil appeared to enhance the effect of the virus (Hsieh et al., 1974). The effect of combined application of *Heliothis* nuclear polyhedrosis virus and azadirachtin or imidacloprid on larvae of *H. virescens* showed combinations do not always show synergism. None of the combinations significantly increased mortality compared to the better of the respective single agents. Interactions between the agents were additive, except for an antagonistic interaction between imidacloprid and azadirachtin with 2nd-instar larvae (Koppenhofer and Kaya, 2000)

In tests with neonate larvae of *Heliothis virescens*, interactions were characterized for combinations of a recombinant Autographa californica nuclear polyhedrosis virus (AcAaIT) that expresses an insect-

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selective neurotoxin (AaIT) and wild-type AcNPV when combined with low concentrations of allethrin, cypermethrin, DDT, endosulfan, methomyl and profenofos. All combinations of the recombinant virus AcAaIT and insecticides showed a positive interaction (decrease in the median lethal time (LT50) compared with the LT50 for either component alone). A pyrethroid (cypermethrin, which modifies currents of sodium channels) and a carbamate (methomyl, an inhibitor of acetylcholinesterase) were synergistic in combination with AcAaIT. Other insecticides also showed a positive interaction when tested in combination with the recombinant virus, but joint activity was slightly antagonistic (i.e., less than predicted activity when combined) with wild-type AcNPV (McCutchen et al., 1997).

3.5. Other interactions

Pyrethroids may affect the ability of insects to evade nematodes due to their knockdown activity. Tefluthrin increased the susceptibility of the western corn rootworm larvae to two nemamode species (Nishimatsu and Jackson, 1998)

3.6. Monitoring studies

There are a number of monitoring studies in Europe which have assessed both the residues of pesticides within colonies and the presence of pests/pathogens. So far none have clearly identified an interaction between pesticides and disease.

Bacandritsos et al. (2010) assessed the levels of imidacloprid in dead bees at the entrance of affected hives at 5 apiaries in Greece during June/July 2009. They investigated levels of a range of pesticides together with pathogens (*Nosema ceranae* and viruses) and demonstrated amitraz, thiamethoxam, clothianidin and acetamiprid were all absent but imidacloprid was present in some samples from apiaries associated with olive, citrus and fruit cultivations and near pine and fir forests. Residues in the bees ranged from 14-39 ng/g bees (1.4-3.9 ng/bee) however in all cases *Nosema ceranae* was also present in the samples of dead bees from all 5 apiaries (but not in samples of live bees taken from within the same colonies) and several viruses were also present

A four-year study involving more than 1200 bee colonies from about 120 apiaries in Germany has been reported in which bee samples were collected twice a year to analyze various pathogenic factors including the ectoparasitic mite *Varroa destructor*, fungi (*Nosema* spec., *Ascosphaera apis*), the bacterium *Paenibacillus larvae*, and several viruses. Data on environmental factors, beekeeping management practice, and pesticides were also collected. All data were statistically analyzed in respect to the overwintering mortality of the colonies. Several factors were significantly related to the observed winter losses of the monitored honey bee colonies: (i) high varroa infestation level, (ii) infection with deformed wing virus (DWV) and acute bee paralysis virus (ABPV) in autumn, (iii) queen age, and (iv) weakness of the colonies in autumn. However no effects could be observed for *Nosema* spec. or pesticides (Genersch et al., 2010).

A three-year field survey was carried out in France, from 2002 to 2005, to study honey bee colony health in relation to pesticide residues found in the colonies. When all apicultural matrices were pooled together, the number of pesticide residue detected per sampling period (four sampling periods per year) and per apiary ranged from 0 to 9, with the most frequent being two (29.6%) and no pesticide residues were detected during 12.7% of the sampling periods. Residues of imidacloprid and 6- chloronicotinic acid were the most frequently detected in pollen loads, honey, and honey bee

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matrices. Several pairs of active ingredients were present concurrently within honey bees and in pollen loads but not in beeswax and honey samples.No statistical relationship was found between colony mortality and pesticide residues. When pesticide residues from all matrices were pooled together, a mixed model analysis did not show a significant relationship between the presence of pesticide residues and the abundance of brood and adults, and no statistical relationship was found between colony mortality and pesticide residues. Thus, although certain pesticide residues were detected in apicultural matrices and occasionally with another pesticide there was no data reported on the incidence of particular diseases within the colonies. A previous study into colony mortality in France (Chauzat et al., 2010a) involved diagnosis of the main honeybee diseases assessment of colony management and pesticide residues in colony matrices. Poor *Varroa destructor* treatments together with *Nosema* disease and brood diseases were frequent in apiaries with high colony mortalities. The absence of any preventive treatment against *V. destructor* was the main risk factor.

Some beekeepers in Spain have suggested that sunflower seeds treated with the insecticide fipronil could be an important factor in causing the high losses and strong depopulation of honey bee colonies (Bernal et al., 2011). Neither fipronil residues nor its metabolites were detected in any of the samples analysed, indicating that short-term or chronic exposure of bees to fipronil and/or its metabolites could be ruled out in the apiaries surveyed. However *Varroa destructor* and *Nosema ceranae* were found to be very prevalent and it was concluded that the combination of the two pathogens could augment the risk of colony death in infected colonies, without fipronil residues exerting a significant effect in the given field conditions.

3.7. Conclusions

The only reports of interactions of pesticides and disease were for honeybees.

There are a small number of studies in honeybees which suggests that infection by *Nosema* or viruses may increase the susceptibility to pesticides. The reported levels of increase in toxicity are less than 3 fold to date (but the number of reported studies are small and the reported levels of apparent infection are high).

There are data that may demonstrate increased spore counts of *N.ceranae* in bees previously chronically exposed to pesticides but there are also reports that spore count may not be a reliable indicator of the impact of *N ceranae* infection in bees. There is a need for improved methods of assessment for some pathogens, e.g. N cerenae which more clearly link to the impact of the disease on the individual.

There are a wide range of factors which affect the immunocompetence of bees including the presence of other diseases, such as *N ceranae*, or pests, e.g. Varroa, or in-hive treatments such as antibiotics. In addition the confinement of colonies or individuals may result in stress leading to immunosuppression.

It is important that these factors are taken into account in determining the effects of pesticides on both individual and social immunity.

The effect of the diet on the immunocompetence and the xenobiotic metabolising enzymes within the gut are important and pathogens may also impact on some measures of sublethal effects of pesticides. It is therefore important that the disease status of bees used in pesticide studies is fully understood.

Currently there is no clear evidence from field based studies that exposure of colonies to pesticides results in increased susceptibility to disease or that there is a link between colony loss due to disease and pesticide residues in monitoring studies.

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CONCLUSIONS & RECOMMENDATIONS

There are a variety of other routes of exposure where there is currently insufficient data to fully quanitfy their contribution to total exposure and further research is required:

If dusts are produced during sowing of treated seeds this may be a significant source of exposure and may result in residues in pollen and nectar of nearby flowering weeds or crops further work is required to develop robust methods to fully quantify this.

Inhalation may be a significant route of exposure for compounds with high vapour pressure and present in stored pollen or collected in water and further data are required.

Beeswax may be a significant route of exposure for highly lipophilic chemicals and more information is required to evaluate transfer to brood.

Water may be sourced from puddles or guttation droplets which may contain high residues for periods of days-weeks and further data is required on the relative importance of these routes.

There was insufficient data available to assess the exposure of bumble bees or solitary bee species. More data are required to fully evaluate the importance of differing routes of exposure for bumble bees and other non-Apis bees.

Other bees may be exposed to mixtures of pesticides through multiple applications, overspray of residues already present, e.g. systemic pesticides, collection of pollen and nectar from a variety of sources and stored within the nest. As previously there is a need to quantify this for non-Apis bees.

There is evidence in the literature of multiple residues of pesticides detected in honeybees, honey and pollen and wax within the hive but this is limited by the direction of the analysis to chemicals of interest to the researchers and rarely are levels of individual components reported. More data are required on realistic levels and combinations of pesticides at the individual colony level within the EU to more fully evaluate the effects of multiple pesticide exposure.

There are a large number of studies that have investigated the interactions between pesticides in honeybees. By far the majority have related to the interactions involving EBI fungicides and can be related to their inhibition of P450. The scale of the synergy is shown to be dose and season-dependent in acute exposures but there are few data relating to the effect of time between exposures or on chronic exposure effects at realistic exposure levels.

The vast majority of the studies have concentrated on the contact toxicity of the combinations. However the exposure section shows that a significant proportion of the exposure may be through ingestion of contaminated nectar. It appears that pesticides which induce P450s in other insects do not induce these enzymes in honeybees but natural chemicals, such as quercetin present in honey and propolis do induce P450s and reduce the toxicity of some pesticides. Given the role of the midgut enzymes in the metabolism of xenobiotics the shortage of data following oral exposure of mixtures is a major gap in our understanding of the potential interactions between chemicals, particularly those present in pollen and nectar, and the effects of diet quality in maintaining xenobiotic metabolising capacity within the gut.

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Greater synergy is observed in the laboratory between EBI fungicides at field rates application rates and pyrethroids used as varroacides (flumethrin and fluvalinate) and between coumaphos and fluvalinate varroacides. Given the persistence of residues of varroacides detected in monitoring studies further evaluation of the combined effects of these with agricultural pesticides is warranted.

As effects are dose-dependent synergism between pesticides may be an area where modelling is applicable both from toxicokinetic/toxicodynamic and QSAR approaches but also needs to take into account formulation differences in affecting rate of uptake.

More recently data has shown that antibiotics used in hives may increase the susceptibility of bees to organophosphorus, pyrethroid and neonicotinoid insecticides through interaction with the membrane bound transporter proteins and further work is required to more fully understand the implications of these findings. It is therefore important that all treatments used on colonies used in studies are reported.

The exposure data demonstrate that bees are often exposed directly through applications of multiple active ingredients or indirectly through consumption of stored pollen and nectar to several pesticides over a period of time. Data are required to determine the effects of such long term low level exposure to multiple pesticides on the health and functioning of honeybee colonies foraging in agricultural environments.

There are data that may demonstrate increased spore counts of *N.ceranae* in bees previously chronically exposed to pesticides but there are also reports that spore count decreased following exposure to some pesticides. However, spore count may not be a reliable indicator of the impact of *N ceranae* infection in bees. There is a need for improved methods of assessment for some pathogens, e.g. *N cerenae* which more clearly link to the impact of the disease on the individual and the colony.

There are a wide range of factors which affect the immunocompetence of bees including the quality of the pollen diet, the presence of other diseases, such as *N ceranae*, or pests, e.g. Varroa, and in-hive treatments, such as antibiotics. In addition, the confinement of colonies or individuals may result in stress leading to immunosuppression. It is important that these factors are taken into account in studies determining the effects of pesticides on both individual and social immunity.

The effect of the diet on both the immunocompetence and the xenobiotic metabolising enzymes within the gut are important and impact on both the effects on the toxicity of other pesticides and the impacts on disease susceptibility. Pathogens may also impact on some measures of sublethal effects of pesticides. It is therefore important that the realistic routes of exposure are used in mixture studies, i.e. oral for contaminated pollen and nectar, and that the disease status of bees used in pesticide studies is fully understood.

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REFERENCES

Aajoud A, Raveton M, Aouadi H, Tissut M, and Ravanel P, 2006. Uptake and xylem transport of fipronil in sunflower. Journal of Agricultural and Food Chemistry 54, 5055-5060.

Abdel-Hafez HF, and Mohamed E, 2009. Joint Action of Reduced-risk Insecticides Spinetoran with Chlorofluazuron or Fenpropathrin Against Cotton Leafworm, Spodoptera littoralis (Boisd. (Lepidoptera, Noctuidae)). Egyptian Journal of Biological Pest Control 19, 25-30.

Adams SJ, Fussell RJ, Dickinson M, Wilkins S, and Sharman M, 2009. Study of the depletion of lincomycin residues in honey extracted from treated honeybee (Apis mellifera L.) colonies and the effect of the shook swarm procedure. Analytica Chimica Acta 637, 315-320.

Adams SJ, Heinrich K, Hetmanski M, Fussell RJ, and Wilkins S, Thompson HM, Sharman M, 2007. Study of the depletion of tylosin residues in honey extracted from treated honeybee (Apis mellifera) colonies and the effect of the shook swarm procedure. Apidologie 38, 315-322.

Adams SJ, Heinrich K, Fussell RJ, Wilkins S, Thompson HM, Ashwin HM, and Sharman M, 2008. Study of the distribution and depletion of chloramphenicol residues in bee products extracted from treated honeybee (Apis mellifera L.) colonies. Apidologie 39, 537-546.

Adler LS, 2000. The ecological significance of toxic nectar. Oikos 91, 409-420.

AFSSA, 2008. Weakening, collapse and mortality of bee colonies, AFSSA. pp. 155.

AFSSA, 2007. OPINION of the French Food Safety Agency (Afssa. on the conclusions of the THE DIRECTOR GENERAL Cruiser assessment regarding the long-term risk to bee colonies AFSSA Request no. 2007-SA-0393 subject related to no. 2007-3845 – Cruiser

Al-Fattah MA, and El-Shemy AAM, 1990. Eight methods for ventilating confined honeybee colonies during the application of insecticides. Journal of Apicultural Research 29, 214-220.

Al-Hafidh EMT, Selman BJ, 1983. The interaction of Nosema whitei and malathion on Tribolium castaneum, 10th International Congress of Plant Protection 1983.Volume 2.Proceedings of a conference held at Brighton, England, 20-25 November, 1983.Plant protection for human welfare. pp. 791.

Alaux C, Ducloz F, Crauser D, and Le Conte Y, 2010a. Diet effects on honeybee immunocompetence. Biology Letters.

Alaux C, Brunet JL, Dussaubat C, Mondet F, Tchamitchan S, Cousin M, Brillard J, Baldy A, Belzunces LP, and Le Conte Y, 2010b. Interactions between Nosema microspores and a neonicotinoid weaken honeybees (Apis mellifera. Environmental Microbiology 12, 774-782.

Alferis KA, and Jabaji S, 2011. Metabolomics - a robust bioanalytical approach for the discovery of modes of action of pesticides, A review. Pesticide Biochemistry and Physiology 100, 105-117.

Alghamdi A, Dalton L, Phillis A, Rosato E, and Mallon EB, 2008. Immune response impairs learning in free-flying bumble-bees. Biology Letters 4, 479-481.

Alptekin S, Bass C, and Paine M, 2011. Microarray analysis of P450 upregulation in honey bee following neonicotinoid treatment. Current Opinion in Biotechnology 225, G17, S152.

Andersen KE, Sheehan TH, Eckholm BJ, Mott BM, and DeGrandi-Hoffman G, 2011. An emerging paradigm of colony health, microbial balance of the honey bee and hive (Apis mellifera.. Insectes Sociaux, 431-444.

Anderson TD, and Lydy MJ, 2002. Increased toxicity to invertebrates associated with a mixture of atrazine and organophosphate insecticides. Environmental Toxicology and Chemistry 21, 1507-1514.

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Andreescu ME, Crivineanu V, Goran GV, and Codreanu MD, 2008. Studies on cypermethrin poisoning in bees. Lucrari Stiintifice - Universitatea de Stiinte Agricole a Banatului Timisoara, Medicina Veterinara 41, 494-503.

Anon, 2008. Pesticide build-up could lead to poor honey bee health. American Bee Journal 148, 861-861.

Anonymous, 1988. Study of the short-term toxicity of mixtures of insecticide and fungicide Sportak PF + Mavrik (prochloraze + carbendazime + fluvalinate., Tilt C + Decis (propiconazole + carbendazime + deltamethrin, Sportak PF + Decis|. Revue Francaise d'Apiculture 477, 383-386.

Anonymous, 1989. Pesticides and honeybees. Study of the short term toxicity under tunnels, mixtures of pyrethrinoids and of fungicides inhibiting ergosterol biosynthesis (deltamethrin, fenpropimorph, prochloraz, propiconazole, bifenthrin, Experiments 1987-1988 Plant Protection Service ACTA. pp. 24-28.

Antúnez K, Martín-Hernández R, Prieto L, Meana A, Zunino P, and Higes M, 2009. Immune suppression in the honey bee (Apis mellifera following infection by Nosema ceranae (Microsporidia). Environmental Microbiology 11.

Aronstein KA, and Murray KD, 2010. Chalkbrood disease in honey bees. Journal of Invertebrate Pathology 103, S20-29.

Atwood DW, Young SY, III, and Kring TJ, 1996. Interactions of Cotesia marginiventris parasitization and field applied Bacillus thuringiensis, Thiodicarb, and their combination on tobacco budworm mortality and parasitoid emergence, 1996 Proceedings Beltwide Cotton Conferences, Nashville, TN, USA, January 9-12, 1996, pp. 905-908.

Babendrier D, Kalberger N, Romeis J, Fluri P, and Bigler F, 2004. Pollen consumption in honeyb bee larvae, a step forward in the risk assessment of transgenic plants. Apidologie 35, 293-300.

Bacandritsos N, Granato A, Budge G, Papanastasiou I, Roinioti E, Caldon M, Falcaro C, Gallina A, and Mutinelli F, 2010. Sudden deaths and colony population decline in Greek honey bee colonies. Journal of Invertebrate Pathology 105, 335-340.

Badiou A, and Belzunces LP, 2008. Is acetylcholinesterase a pertinent biomarker to detect exposure of pyrethroids? A case study with deltamethrin. Chemico-Biological Interactions 175, 406-409.

Balayiannis G, and Balayiannis P, 2008. Bee honey as an environmental bioindicator of pesticides' occurrence in six agricultural areas of Greece. Archives of Environmental Contamination and Toxicology 55, 462-470.

Barker RJ, Lehner Y, and Kunzmann MR, 1980. Pesticides and honey bees, nectar and pollen contamination in alfalfa treated with dimethoate. Archives of Environmental Contamination and Toxicology 9, 125-133.

Bekesi L, 2005. Infection and immunity of the honeybee (Apis mellifera.. Literature review. Magyar Allatorvosok Lapja 127, 594-602.

Belanger A, and Rivard I, 1980. Residues of dimethoate in the nectar of apple flowers. Resume des Recherches, Station de Recherches, Saint-Jean, Quebec 9, 32-33.

Belliardo F, and Nano GM, 1975. The presence of dithiocarbamates in honeybees killed by treatment with zineb and sulphur. Apicoltore Moderno 66, 189-192.

Belliardo F, Nano GM, and Vidano C, 1975. Detection of alpha -naphthyl-methylcarbamate (carbaryl. in vine pollen stored by honeybees. Apicoltore Moderno 66, 193-196.

Belzunces LP, and Colin ME, 1993. Synergies between insecticides and fungicides applied to honey bees at sublethal doses. Experimental approach in the laboratory. Phytoma 446, 20-24.

The present document has been produced and adopted by the bodies identified above as author. This task has been carried out exclusively by the author in the context of a contract between the European Food Safety Authority and the author awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Bendahou N, Bounias M, and Fleche C, 1997. Acute toxicity of cypermethrin and fenitrothion on honeybees (Apis mellifera mellifera. according to age, formulations and (chronic paralysis virus./insecticide interaction. Journal of Environmental Biology 18, 55-65.

Bernal J, Martin-Hernandez R, Diego JC, Nozal MJ, Gozalez-Porto AV, Bernal JL, and Higes M, 2011. An exposure study to assess the potential impact of fipronil in treated sunflower seeds on honey bee colony losses in Spain. Pest Management Science 67, 1320-1331.

Bernal J, Garrido-Bailon E, del Nozal M, Gonzalez-Porto AV, Martin-Hernandez R, Diego J, Jimenez J, and Higes M, 2010. Overview of Pesticide Residues in Stored Pollen and Their Potential Effect on Bee Colony (Apis mellifera. Losses in Spain. Journal of Economic Entomology 103, 1964-1971.

Bernard JL, 1990. Studies on honeybees with the lambda-cyhalothrin + pyrimicarb (Karate K mixture.. Phytoma - Defense des Vegetaux 44, 21-28.

Boelter AM, and Wilson WT, 1984. Effect of methyl parathion vapors from contaminated pollen on honey bees Apis-mellifera Hymenoptera Apidae within a hive. Environmental Entomology 13, 1233-1236.

Bogdanov S, 2006. Contaminants of bee products. Apidologie 37, 1-18.

Bogdanov S, Kilchenmann V, and Imdorf A, 1998. Acaricide residues in some bee products. Journal of Apicultural Research 37, 57-67.

Bonmatin JM, Moineau I, Charvet R, Fleche C, Colin ME, and Bengsch ER, 2003. a LC/APCI-MS/MS method of analysis of imidacloprid in soils, plants and in pollens. Analytical Chemistry 75, 2027-2033.

Bonmatin JM, Marchand PA, Charvet R, Moineau I, Bengsch ER, and Colin ME, 2005. Quantification of imidacloprid uptake in maize crops. Journal of Agricultural and Food Chemistry 53, 5336-5341.

Bonmatin JM, Marchand PA, Cotte JF, Aajoud A, Casabianca H, Goutailler G, and Courtiade M, 2007. Bees and systemic insecticides (imidacloprid, fipronil. in pollen, subnano-quantification by HPLC/MS/MS and GC/MS. Environmental fate and ecological effects of pesticides, 827-834.

Bonnet J, Corbel V, Darriet F, Chandre F, and Hougard J-M, 2004. Topical applications of pyrethroid and organophosphate mixtures revealed positive interactions against pyrethroid-resistant Anopheles gambiae. Journal of the American Mosquito Control Association 20, 438-443.

Bonnet J, Pennetier C, Duchon S, Lapied B, and Corbel V, 2009. Multi-function oxidases are responsible for the synergistic interactions occurring between repellents and insecticides in mosquitoes. Parasites and Vectors 2.

Bonzini S, Tremolada P, Bernardinelli I, Colombo M, and Vighi M, 2011. Predicting pesticide fate in the hive (part 1., experimentally determined tau-fluvalinate residues in bees, honey and wax. Apidologie 42, 378-390.

Boucias DG, Stokes C, Storey G, and Pendland JC, 1996. The effects of imidacloprid on the termite Reticulitermes flavipes and its interaction with the mycopathogen Beauveria bassiana. Pflanzenschutz-Nachrichten Bayer (English ed.. 49, 103-144.

Brasse D, 2001. Overview about the poisoning incidents in honeybee populations and their clarification in Germany from 1996-1998, in, L. P. Belzunces, et al, Eds.., Hazards of Bees to Pesticides, INRA, Avignon, France. pp. 141-147.

Brattsten LB, Berger DA, and Dungan LB, 1994. In vitro inhibition of midgut microsomal P450s from Spodoptera eridania caterpillars by demethylation inhibitor fungicides and plant growth regulators. Pesticide Biochemistry and Physiology 49.

The present document has been produced and adopted by the bodies identified above as author. This task has been carried out exclusively by the author in the context of a contract between the European Food Safety Authority and the author awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Brobyn PJ, 2001. Possible synergistic effects on honeybees of pyrethroids and fungicides, the UK regulatory consideration, in, L. Belzunces, et al, Eds.., Hazards of Pesticides to Bees, INRA, Avignon, France.

Brodsgaard CJ, Ritter W, Hansen H, and Brodsgaard HF, 2000. Interactions among Varroa jacobsoni mites, acute paralysis virus, and Paenibacillus larvae larvae and their influence on mortality of larval honeybees in vitro. Apidologie 31, 543-554.

Buchler R, and Volkmann B, 2003. Residues of Carbendazim and other fungicides in honey due to blossom application in canola. Gesunde Pflanzen 55, 217-221.

Buczek K, Chelminski M, and Kauko L, 2008. Immunotoxic and immunosuppressive action of environment on the honey bee (Apis mellifera L.., Poland. pp. 21-26.

Burgett M, and Fisher GC, 1980. Recovery of Penncap-M from foraging honey bees and pollen storage cells. Environmental Entomology 9, 430-431.

Buss DS, and Callaghan A, 2008. Interaction of pesticides with p-glycoprotein and other ABC proteins, a survey of the possible importance to insecticide, herbicide and fungicide resistance. Pesticide Biochemistry and Physiology 90, 141-153.

Cabras P, Martini MG, Floris I, and Spanedda L, 1994. Residues of Cymiazole in Honey and Honey-Bees. Journal of Apicultural Research 33, 83-86.

Cadeddu, 2000. The heuristic function of “error” in the scientific methodology of Louis Pasteur , the case of silkworm diseases.. History and Philosophy of the Life Sciences 22, 3-28.

Cang T, Wu C, Wang X, Wu S, Yu R, Chen L, Zhang Z, and Zhao X, 2008. The toxicity of fipronil and trichlorfon on Italian honey bees (Apis mellifera.. Zhejiang Nongye Kexue, 473-475.

Cantwell GE, 1974. Honey bee diseases, parasites, and pests, in, G.E.Cantwell, Ed.., Insect diseases, Vol.II,, New York. pp. 501-547.

Casteels P, Ampe C, Jacobs F, Vaeck M, and Tempst P, 1989. Apidaecins, antibacterial peptides from honeybees. EMBO Journal 8, 2387-2391.

Cedergreen N, Kamper A, and Streibig JC, 2006. Is prochloraz a potent synergist across aquatic species? A study on bacteria, daphnia, algae and higher plants. Aquatic Toxicology 78, 243-252.

Celli G, and Porrini C, 1988. Flower morphology and pesticide contamination of nectar (some theoretical considerations.. Preliminary report, Atti XV Congresso Nazionale Italiano di Entomologia, L'Aquila, 13-17 Giugno 1988. pp. 1039-1045.

Celli G, Porrini C, and Tiraferri S, 1985. Connections between beekeeping and the environment. The bee as a biological indicator of pesticides (with particular reference to the Province of Forli., Preliminary note.. Bollettino dell'Istituto di Entomologia 'Guido Grandi' della Universita degli Studi di Bologna 39, 231-241.

Celli G, Porrini C, Baldi M, and Ghigli E, 1991. Pesticides in Ferrara Province - 2 Years Monitoring with Honey-Bees (1987-1988.. Ethology Ecology & Evolution, 111-115.

Chalvet-Monfray K, Belzunces LP, Colin ME, Fleche C, and Sabatier P, 1996. Synergy between deltamethrin and prochloraz in bees, modeling approach. Environmental Toxicology and Chemistry 15, 525-534.

Chalvet-Monfray K, Belzunces LP, Colin ME, Fleche C, and Sabatier P, 1995. Modelling synergistic effects of two toxic agents in the honeybee. Journal of Biological Systems, Vol 3, Nos 1-4, 1995, Special Issue, 2Nd Ecmbm-Lyon, Pts 1-4, 253-263.

The present document has been produced and adopted by the bodies identified above as author. This task has been carried out exclusively by the author in the context of a contract between the European Food Safety Authority and the author awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Chan QWT, Melathopoulos AP, Pernal SF, and Foster LJ, 2009 The innate immune and systemic response in honey bees to a bacterial pathogen, Paenibacillus larvae. BMC Genomics 10, 387 doi, 10.1186/1471-2164-10-387.

Chaney WE, 1988. The effect of synthetic pyrethroid insecticides on honey bees in Indiana, laboratory studies and a survey of beekeepers and pesticide applicators. pp. xi-pp.

Chauzat MP, Faucon JP, Martel AC, Lachaize J, Cougoule N, and Aubert M, 2006. A survey of pesticide residues in pollen loads collected by honey bees in France. Journal of Economic Entomology 99, 253-262.

Chauzat MP, Martel AC, Zeggane S, Drajnudel P, Schurr F, Clement MC, Ribiere-Chabert M, Aubert M, and Faucon JP, 2010a. A case control study and a survey on mortalities of honey bee colonies (Apis mellifera. in France during the winter of 2005-6. Journal of Apicultural Research 49, 40-51.

Chauzat MP, Martel AC, Cougoule N, Porta P, Lachaize J, Zeggane S, Aubert M, Carpentier P, and Faucon JP, 2011. An Assessment of Honeybee Colony Matrices, Apis Mellifera (Hymenoptera Apidae. to Monitor Pesticide Presence in Continental France. Environmental Toxicology and Chemistry 30, 103-111.

Chauzat MP, Carpentier P, Martel AC, Bougeard S, Cougoule N, Porta P, Lachaize J, Madec F, Aubert M, and Faucon JP, 2009. Influence of Pesticide Residues on Honey Bee (Hymenoptera, Apidae. Colony Health in France. Environmental Entomology 38, 514-523.

Chauzat MP, Martel AC, Blanchard P, Clement MC, Schurr F, Lair C, Ribiere M, Wallner K, Rosenkranz P, and Faucon JP, 2010b. A case report of a honey bee colony poisoning incident in France. Journal of Apicultural Research 49, 113-115.

Che S, 2007. Honey bee viruses. Honey bee viruses. 70, 33-80.

Choudhary A, and Sharma DC, 2008. Dynamics of pesticide residues in nectar and pollen of mustard (Brassica juncea (L.. Czern.. grown in Himachal Pradesh (India.. Environmental Monitoring and Assessment 14, 143-150.

Chukwudebe AC, Cox DL, Palmer SJ, Morneweck LA, Payne LD, Dunbar DM, and Wislocki PG, 1997. Toxicity of emamectin benzoate foliar dislodgeable residues to two beneficial insects. Journal of Agricultural and Food Chemistry 45, 3689-3693.

Claudianos C, Ranson H, Johnson RM, Schluer MA, Berenbaum MR, and Feyereisen R, 2006. A deficit of detoxification enzymes, pesticide sensitivity and environmental response in the honeybee. Insect Molecular Biology 15, 615-636.

Colin ME, and Belzunces LP, 1992a. Impact of the synergism of pesticides on bees, biological effect of a combination of an insecticide with a fungicide, in, E. Bruneau (Ed.., Bees for pollination.Proceedings of an EC workshop, Brussels, Belgium, 2-3 March 1992. pp. 167-170.

Colin ME, and Belzunces LP, 1992b. Evidence of synergy between prochloraz and deltamethrin in Apis mellifera L., a convenient biological approach. Pesticide Science 36, 115-119.

Colliot EA, 1999. Combinations of a fungicide containing an azole group with an insecticide containing a pyrazole, pyrrole or phenylimidazole group, USA.US Patent 5,877,194

Conte Yl, and Ellis M, 2008. Mortality and depopulation in domesticated bee colonies, the American case. Biofutur, 49-53.

Corbel V, Stankiewicz M, Bonnet J, Grolleau F, Hougard JM, and Lapied B, 2006. Synergism between insecticides permethrin and propoxur occurs through activation of presynaptic muscarinic negative feedback of acetylcholine release in the insect central nervous system. Neurotoxicology 27, 508-519.

The present document has been produced and adopted by the bodies identified above as author. This task has been carried out exclusively by the author in the context of a contract between the European Food Safety Authority and the author awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Cox-Foster DL, Conlan S, Holmes E, Palacios G, Evans JD, Moran NA, Quan P, Briese T, Hornig M, Geiser DM, Martinson V, Van Engelsdorp D, Kalkstein A, Drysdale A, Hui J, ZhaiI J, Cui L, Hutchison SK, Simons JF, Egholm M, Pettis JS, and Lipkin WI, 2007. A metagenomic survey of microbes in honey bee Colony Collapse Disorder.. Science USA 318, 283=287.

Cox RL, Wilson WT, and Moffett JO, 1986. Residues on foliage honey bees and bee products following ec applications of ethyl parathion to commercial sunflowers. American Bee Journal 126, 828.

Cutler GC, and Scott-Dupree CD, 2007. Exposure to clothianidin seed-treated canola has no long-term impact on honey bees. Journal of economic entomology 100, 765-72.

Danka RG, Williams JL, Harmon CW, Rinderer TE, and Morris HF, 1991. Doses and residues of acephate baits used to eradicate undesirable honey bees, a hazard assessment. Bulletin of Environmental Contamination and Toxicology 47, 422-427.

Davis AR, and Shuel RW, 1988. Distribution of 14C-labelled carbofuran and dimethoate in royal jelly, queen larvae and nurse honeybees. Apidologie 19, 37-50.

Davis AR, Shuel RW, and Peterson RL, 1988. Distribution of carbofuran and dimethoate in flowers and their secretion in nectar as related to nectary vascular supply. Canadian Journal of Botany 66, 1248-1255.

De Azambuja P, and Garcia ES, 1992. Effects of azadirachtin on Rhodnius prolixus, immunity and Trypanosoma interaction. Memorias do Instituto Oswaldo Cruz 87, 69-72.

de Batista GC, Dias E, and Amaral E, 1976. Synergistic action of piperonyl butoxide mixed with carbaryl in various proportions for Apis mellifera ligustica X Apis mellifera adansonii. Anais da Sociedade Entomológica do Brasil 5, 69-73.

deGrandi-Hoffman G, and Hagler J, 2000. The flow of incoming nectar through a honey bee (Apis mellifera L. colony as revealed by a protein marker. Insectes Sociaux 47, 302-306.

Defra PN0945 2004. Assessing the impact of mixtures of pyrethroids and fungicides on honeybees.

Defra PS2354 2011. Background information for considering risk of exposure to multiple pesticides

Defra PS2368 2012. Potential impacts of synergism between systemic seed treatments and sprayed fungicides in crops

Delaplane KS, 1998. Nosema disease and its control. American Bee Journal 138, 343-344.

Deneer JW, 2000. Toxicity of mixtures of pesticides in aquatic systems. Pest Management Science 56, 516-520.

Desneux N, Decourtyre A, and Delpuech J-M, 2007. The sublethal effects of pesticides on beneficial arthropods. Annual Reviews of Entomology 52, 81-106.

Dieckmann EA, 2010. Systemicity enhancers, in, U. S. P. Application (Ed...

Dinter A, Brugger KE, Frost N-M, and Woodward MD, 2009. Chlorantraniliprole (Rynaxypyr., A novel DuPont insecticide with low toxicity and low risk for honeybees (Apis mellifera. and bumble bees (Bombus terrestris. providing excellent tools for uses in integrated pest management. Julius Kuhn Archive 423, 84-96.

Dobrincic R, 1996. Investigations of interactions between different groups of insecticides in the control of the Colorado potato beetle (Leptinotarsa decemlineata Say.., Istrazivanje interakcije razlicitih skupina insekticida u suzbijanju krumpirove zlatice (Leptinotarsa decemlineata Say... pp. 23-43.

The present document has been produced and adopted by the bodies identified above as author. This task has been carried out exclusively by the author in the context of a contract between the European Food Safety Authority and the author awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Donnarumma L, Pulcini P, Pochi D, Rosati S, Lusco L, and Conte E, 2011. Preliminary study on persistence in soil and residues in maize of imidacloprid. Journal of Environmental Science and Health.Part B, Pesticides, Food Contaminants, and Agricultural Wastes 46, 469-472.

Doums C, Moret Y, Benelli E, and Schmid-Hempel P, 2002. Senescence of immune defence in Bombus workers. Ecological Entomology 27, 138-144.

El-Sebae AH, Komeil AM, and Thabet AAM, 1990. Interaction of conventional insecticides with Bacillus-thuringiensis against the mediterranean fruit fly ceratitis-capitata weid, british crop protection council. brighton crop protection conference, pests and diseases, 1990, vols. 1, 2 and 3; International Conference, Brighton, England, UK, November 19-22, 1990. British Crop Protection, Farnham, England, UK. illus. maps. paper. pp. 241-244.

Ellis M.D, Siegfried BD, and Spawn B, 1997. The effect of Apistan on honey bee (Apis mellifera L.. responses to methyl parathion, carbaryl and bifenthrin exposure. Apidologie 28, 123-127.

Erickson EH, Jr, Erickson BJ, and Wyman JA, 1994. Effects of honey bees of insecticides applied to snap beans in Wisconsin, Chemical and biotic factors. Journal of Economic Entomology 87, 597-600.

Ericsson JD, Kabaluk JT, Goettel MS, and Myers JH, 2007. Spinosad interacts synergistically with the insect pathogen Metarhizium anisopliae against the exotic wireworms Agriotes lineatus and Agriotes obscurus (Coleoptera, Elateridae.. Journal of Economic Entomology 100, 31-38.

EU Commission, 2009. State of the Art Report on Mixture Toxicity.

Evans JD, 2004. Transcriptional immune responses by honey bee larvae during invasion by the bacterial pathogen, Paenibacillus larvae.. Journal of Invertebrate Pathology 85, 105-111.

Evans JD, Aronstein K, Chen Y-P, Hetru C, Imler J-L, Jiang H, Kanost M, Thompson GJ, Zou Z, and Hultmark D, 2006. Immune pathways and defence mechanisms in honey bees Apis mellifera. Insect Molecular Biology 15, 645-656.

Evison SEF, Robertts KE, Laurenson L, Pietravalle S, Hui J, Biesmeijer JC, Smith JE, Budge G, and Hughes WOH, 2012. Pervasiveness of Parasites in Pollinators. PLoS One 7, e30641.

Faucon JP, Vitu C, Russo P, and Vignoni M, 1992 Diagnosis of acute paralysis application to epidemic honeybee diseases in France during 1990. Apidologie 23, 139-146.

Faucon JP, Mathieu L, Ribiere M, Martel AC, Drajnudel P, Zeggane S, Aurieres C, and Aubert M, 2002. Honey bee winter mortality in France in 1999 and 2000. Bee World 83, 14-23.

Feng MG, and Pu XY, 2005. Time-concentration modelling of the synergistic interaction of Beauvaria bassiana and imidacloprid against Nilaparvata lugens. Pest Management Science 61, 363-370.

Fiedler L, 1987. Acephate residues after pre-blossom treatments, effects on small colonies of honey bees. Bulletin of Environmental Contamination and Toxicology 38, 594-601.

Floch M, 2003. Bees under treatments. PHM Revue Horticole 452, 27-29.

Foo AES, and Yap HH, 1987. Interactive effects of Bacillus-thuringiensis h-14 with chlorpyrifos and permethrin. Tropical Biomedicine 4, 47-50.

Free JB, and Ferguson AW, 1980. Foraging of bees on oil-seed rape (Brassica napus L.. in relation to the stage of flowering of the crop and pest control. Journal of Agricultural Science, UK 94, 151-154.

Fries I, 2010. Nosema ceranae in European honeybees (Apis mellifera.. Journal of Invertebrate Pathology 103, S73-79.

The present document has been produced and adopted by the bodies identified above as author. This task has been carried out exclusively by the author in the context of a contract between the European Food Safety Authority and the author awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Fries I, and Wibran K, 1987. Effects on honey-bee colonies following application of the pyrethroids cypermethrin and PP 321 in flowering oilseed rape. American Bee Journal 127, 266-269.

Furlong MJ, and Groden E, 2001. Evaluation of synergistic interactions between the Colorado potato beetle (Coleoptera, Chrysomelidae. pathogen Beauveria bassiana and the insecticides, imidacloprid, and cyromazine. pp. 344-356.

Gaal F, and Herczeg T, 1972. Investigation of the efficiency of warm-fog control with gas-oil containing dichlorvos on Culex pipiens, Musca domestica and Apis mellifera. Parasitologia Hungarica 5, 297-303.

Garcia M.A, Fernandez MI, and Melgar MJ, 1995. Contamination of honey with organophosphorus pesticides. Bullentin of Environmental Contamination and Toxicology 54, 825-832.

Gary NE, and Mussen EC, 1984. impact of Mediterranean fruit fly malathion bait spray on honey bees. Environmental Entomology 13, 711-717.

Genersch E, von der Ohe W, Kaatz H, Schroeder A, Otten C, Buechler R, Berg S, Ritter W, Muehlen W, Gisder S, Meixner M, Liebig G, and Rosenkranz P, 2010. The German bee monitoring project, a long term study to understand periodically high winter losses of honey bee colonies. Apidologie 41, 332-352.

Geng B, and Zhang R, 2005. Interactive effect of low concentration buprofezin and Metarhizium anisopliae var. acridum on the mortalities of Nilaparvata lugens (Homoptera, Delphacidae. nymphs and adults in laboratory bioassays. Acta Phytophylacica Sinica 32, 53-56.

Georghiou GP, and Atkins Jr EL, 1964. Temperature coefficients of toxicity of certain n-methyl carbamates against honeybees, and the effect of the synergist piperonyl butoxide. Journal of Apicultural Research 3, 31-35.

Ghini S, Fernandez M, Pico Y, Marin R, Fini F, Manes J, and Girotti S, 2004. Occurrence and distribution of pesticides in the province of Bologna, Italy, using honeybees as bioindicators. Archives of Environmental Contamination and Toxicology 47, 479-488.

Gilliam M, Morton HL, Prest DB, Martin RD, and Wickerham LJ, 1977. The mycoflora of adult worker honeybees, Apis mellifera, effects of 2,4,5-T and caging of bee colonies. Journal of Invertebrate Pathology 30, 50-54.

Girolami V, Mazzon L, Squartini A, Mori N, Marzaro M, Bernardo AD, Greatti M, Giorio C, and Tapparo A, 2009. Translocation of neonicotinoid insecticides from coated seeds to seedling guttation drops, a novel way of intoxication for bees. Journal of Economic Entomology 102, 1808-1815.

Glinski Z, 2000. Immuno-suppressive and immuno-toxic action of contaminated honey bee products on consumers. pp. 634-638.

Glinski Z, and Kauko L, 2000. Problems of immunosuppression and immunotoxicolgy in respect to the honeybee protection against microbial and parasitic nvaders. Apiacta 2, 65-76.

Glinski Z, and Jarosz J, 2001. Infection and immunity in the honey bee Apis mellifera. Apiacta 1.

Glinski Z, and Buczek K, 2003. Immune response impairs learning in free-flying bumble-bees. Apiacta 38, 183-189.

Greatti M, Sabatini AG, Barbattini R, Rossi S, and Stravisi A, 2003. Risk of environmental contamination by the active ingredient imidacloprid used for corn seed dressing. Preliminary results. Bulletin of Insectology 56.

The present document has been produced and adopted by the bodies identified above as author. This task has been carried out exclusively by the author in the context of a contract between the European Food Safety Authority and the author awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Greatti M, Barbattini R, Stravisi A, Sabatini AG, and Rossi S, 2006. Presence of the a.i. imidacloprid on vegetation near corn fields sown with GauchoReg. dressed seeds. Bulletin of Insectology 59, 99-103.

Greig-Smith PW, Thompson HM, Hardy AR, Bew MH, Findlay E, and Stevenson JH, 1994. Incidents of poisoning of honeybees (Apis mellifera. by agricultural pesticides in Great Britain 1981-1991. Crop Protection 13, 567-582.

Hagler JR, Waller GD, and Lewis BE, 1989. mortality of honeybees Hymenoptera Apidae exposed to permethrin and combinations of permethrin with piperonyl butoxide. Journal of Apicultural Research 28, 208-211.

Haouar M, Cormis LD, and Rey J, 1990. Fluvalinate applied to flowering apple trees, contamination of honey-gathering bees and hive products. Agronomie 10, 133-138.

Harbo JR, 1986. Effect of population size on brood production, worker survival and honey gain in colonies of honeybees. Journal of Apicultural Research 25, 22-29.

Hawthorne DJ, and Dively GP, 2011. Killing Them with Kindness? In-Hive Medications May Inhibit Xenobiotic Efflux Transporters and Endanger Honey Bees. PLoS One 6, e26796.

Hepburn HR, Bernard RTF, Davindson BC, Muller WJ, Lloyd P, Kurstjens SP, and Vincent SL, 1991. Synthesis and secretion of beeswax in honeybees. Apidologie 22, 21-36.

Heuer EA, 1999. Fungicidal active compound combinations, USA.Patent 5,972,971

Higes M, Martin-Hernandez R, and Meana A, 2010. Nosema ceranae in Europe, an emergent type C nosemosis Apidologie 41, 375-392.

Higes M, García-Palencia P, Martín-Hernández R, and Meana A, 2007. Experimental infection of Apis mellifera honeybees with the Microsporidia Nosema ceranae. Journal of Invertebrate Pathology 94, 211-217.

Higes M, Martin-Hernandez R, Botias C, Garrido-Bailon E, Gomnzalez-Porto AV, Barrios L, Jesus del Nozal MJ, Bernal JL, Jimenez JJ, Garcia-Palencia MP, and Meana A, 2008. How natural infection by Nosema ceranae causes honey bee colony collapse. Environmental Microbiology 10, 2659-2669.

Hsieh ML, Collins WJ, and Stairs GR, 1974. Interaction of nuclear-polyhedrosis virus, DDT, rotenone and peanut oil in Galleria mellonella larvae. Environmental Entomology 3, 567-569.

Huang ZY, and Lin R, 2004. JH titers, biosynthesis and metabolism in honey bee workers infected by a microsporidia parasite, Nosema apis. Journal of Insect Science 4, 7.

Hurny J, and Zieba S, 1980. Toxicity of carbamate insecticides to honeybees under field conditions. Organika, 121-131.

Ibrahim AA, Shalaby HH, and El-Saadany HM, 2009. Interaction between the entomopathogenic fungus, Beauveria bassiana and some insecticides against the whitefly, Bemisia tabaci (Genn.. (Homoptera, Aleyrodidae.. Egyptian Journal of Biological Pest Control 19, 41-48.

Ibrahim SA, and Eshbah HM, 1989. Toxicity and bioresiduality of selected insect growth regulators (IGRs./insecticdes mixtures to honey nees (Hymeoptera, Apidae.. Minia Journal of Agricultural Research and Development 11, 1891-1906.

Imdorf A, Kilchenmann V, Maquelin C, and Bogdanov S, 1994. Optimization of the use of 'Apilife VAR' to combat Varroa jacobsoni Oud in honey bee colonies. Apidologie 25, 49-60.

Iwasa T, Motoyama N, Ambrose JT, and Roe RM, 2004a. Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, Apis mellifera. Crop Protection 23, 371-378.

The present document has been produced and adopted by the bodies identified above as author. This task has been carried out exclusively by the author in the context of a contract between the European Food Safety Authority and the author awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Iwasa T, Motoyama N, Ambrose JT, and Roe RM, 2004b. Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, Apis mellifera. pp. 371-378.

James RR, and Elzen GW, 2001. Antagonism Between Beauveria bassiana and Imidacloprid When Combined for Bemisia argentifolii (Homoptera, Aleyrodidae. Control. Journal of Economic Entomology 94, 357-361.

James RR, and Xu J, 2011. Mechanisms by which pesticides affect insect immunity. Journal of Invertebrate Pathology.

Jamet EA, 2011. Pesticidal mixtures. US Patent US2011/0046123

Jaramillo J, Borgemeister C, Ebssa L, Gaigl A, Tobon R, and Zimmermann G, 2005. Effect of combined applications of Metarhizium anisopliae (Metsch. Sorokin (Deuteromycotina, Hyphomycetes. strain CIAT 224 and different dosages of imidacloprid on the subterranean burrower bug Cyrtomenus bergi Froeschner (Hemiptera, Cynidae.. Biological Control 34, 12-20.

Jimenez JJ, Bernal JL, Del Nozal MJ, and Martin MT, 2005. Residues of organic contaminants in beeswax. European Journal of Lipid Science and Technology 107, 896-902.

Johansen CA, and Kious CW, 1978. Bee poisoning characteristics of microencapsulated methyl parathion. Gleanings in Bee Culture 106, 382-385.

Johansen CA, Mayer DF, Eves JD, and Kious CW, 1983. Pesticides and bees. Environmental Entomology 12, 1513-1518.

Johnson RM, 2008. Toxicogenomics of Apis mellifera, Graduate College of the University of Illinois at Urbana-Champaign, 2008.

Johnson RM, 2009. Managed pollinator CAP - Coordinated Agricultural Project , When varroacides interact. American Bee Journal 149, 1157-1159.

Johnson RM, Pollock HS, and Berenbaum MR, 2009. Synergistic interactions between in-hive miticides in Apis mellifera. Journal of Economic Entomology 102, 474-479.

Johnson RM, Wen Z, Shuler MA, and Berenbaum MR, 2006. Mediation of Pyrethroid Insecticide Toxicity to Honey Bees (Hymenoptera, Apidae. by Cytochrome P450 Monooxygenases. Journal of Economic Entomology 99, 1046-1050.

Johnson RM, Ellis MD, Mullin CA, and Frazier M, 2010. Pesticides and honey bee toxicity - USA. Apidologie 41, 312-331.

Johnson RM, Mao W, Pollock HS, Niu G, Schuler MA, and Berenbaum MR, 2012. Ecologically Appropriate Xenobiotics Induce Cytochrome P450s in Apis mellifera. PLoS One 7, e31051.

Jullien J, 2009. Bees and phytosanitary products. PHM Revue Horticole 509, 22-27.

Kandru S, and Dhingra S, 2002. Interactive effect of sublethal concentrations of fenvalerate and various microbial insecticides to larval instars of Spilarctia obliqua (Walker.. Annals of Plant Protection Science 10, 31-37.

Kezic N, Lucic D, and Sulimanovic D, 1992 Induction of mixed function oxidase activity in honey bee as a bioassay for detection of environmental xenobiotics. Apidologie 23, 217-223.

Koch H, and Spieles M, 1993. Effect of concentration of plant protection products in spray fluids on the retention on honey bees (Apis mellifera.. Gesunde Pflanzen 45, 61-66.

Koch H, and Weisser P, 1997. Exposure of honey bees during pesticide application under field conditions. Apidologie 28, 439-447.

Konig B, 1985. Plant sources of propolis. Bee World 66, 136-139.

The present document has been produced and adopted by the bodies identified above as author. This task has been carried out exclusively by the author in the context of a contract between the European Food Safety Authority and the author awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Koppenhofer AM, and Kaya HK, 2000. Interactions of a nucleopolyhedrovirus with azadirachtin and imidacloprid. Journal of Invertebrate Pathology 75, 84-86.

Kostromytska OS, Buss EA, and Scharf ME, 2011. Toxicity and neurophysiological effects of selected insecticides on the mole cricket, Scapteriscus vicinus (Orthoptera, Gryllotapidae.. Pesticide Biochemistry and Physiology 100, 27-34.

Krohn EA, 2008. Synergistic mixtures exhibiting insecticidal and fungicidal action. US Patent US2008/0261811

Kruype CH, Hunt GJ, Eitzer BD, Andino G, and Given K, 2012. Multiple routes of pesticide exposure for honey bees living near agricultural fields. PLoS One 7, e29268.

Kubik M, Pidek A, Goszczynski W, Nowacki J, and Michalczuk L, 1991. Can 'Sumilex' applied to raspberry plantations be the source of contamination of bee honey? Fruit Science Reports 18, 119-124.

Kubik M, Pidek A, Nowacki J, Warakomska Z, Michalczuk L, and Goszczynski W, 1992. Pesticide residues in pollen and bee-honey collected from strawberry plantation protected with fungicides Sumilex and Euparen. Fruit Science Reports 19, 63-72.

Kubik M, Nowacki J, Pidek A, Warakomska Z, Michalczuk L, and Goszczynski W, 1999. Pesticide residues in bee products collected from cherry trees protected during blooming period with contact and systemic fungicides. Apidologie 30, 521-532.

Kubik M, Nowacki J, Pidek A, Warakomska Z, Michalczuk L, Goszczynski W, and Dwuznik B, 2000. Residues of captan (contact. and difenoconazole (systemic. fungicides in bee products from an apple orchard. Apidologie 31, 531-541.

Kuhn R, 1985. Toxicity of insecticides and fungicides to larval and adult honeybees in separate and combined application. Apidologie 16, 201-203.

Kumar NS, Murugan K, and Zhang W, 2008. Additive interaction of Helicoverpa armigera Nucleopolyhedrovirus and Azadirachtin. BioControl (Dordrecht. 53, 869-880.

Ladas A, 1972. Effects of certain internal and external factors on the resistance of honeybees to insecticides. Apidologie 3, 55-78.

Ladurner E, Bosch J, Kemp WP, and Maini S, 2008. Foraging and nesting behaviour of Osmia lignaria (Hymenoptera, megachilidae. in the presence of fungicides, Cage studies. Journal of Environmental Entomology 101, 647-653.

Lagier RF, Johansen CA, Kleinschmidt MG, Butler LI, McDonough LM, and Jackson DS, 1974. Adjuvants decrease insecticide hazard to honey bees. Washington Agri Exp Stn Bulletin 801, 107.

Laughton AM, Boots M, and Siva-Jothy MT, 2011. The ontogeny of immunity in the honey bee, Apis mellifera L. following an immune challenge Journal of Insect Physiology 57, 1023-1032.

Laurent FM, and Rathahao E, 2003. Distribution of [14C] Imidacloprid in sunflowers (Helianthus annuus L.. following seed treatment. Journal of Agricultural & Food Chemistry 51, 8005-8010.

Lee KP, Simpson SJ, and Wilson K, 2008. Dietary protein-quality influences melanization and immune function in an insect. Functional Ecology 22.

Lefebvre B, and Bassand D, 2001. Bee selectivity of tau-fluvalinate in tank mix with difenoconazole. Short, medium and long term effects under semi-field conditions, Proceedings of the Seventh International Symposium of the ICP-BR Bee Protection Group. pp. 71-77.

Li W-F, Ma GX, and Zhou XX, 2006. Apidaecin-type peptides, Biodiversity, structure-function relationships and mode of action. Peptides (New York. 27, 2350-2359.

The present document has been produced and adopted by the bodies identified above as author. This task has been carried out exclusively by the author in the context of a contract between the European Food Safety Authority and the author awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Li X, Schuler MA, and Berenbaum MR, 2007. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics.. Annual Review of Entomology 52, 231-253.

Lienau FW, 1990. Effect of varroacide and pesticide treatment on honeybees. Apidologie 21, 375-377.

Liu MY, Lanford J, and Casida JE, 1995. Relevance of [3H]imidacloprid binding site in house fly head nicotinic acetylcholine receptor to insecticidal activity of 2-nitromethylene- and 2-nitroimino-imidazolidines. Pesticide Biochemistry and Physiology 46.

Liu X., Liang P., Gao X., Shi X, 2006. Induction of the cytochrome P450 activity by plant allelochemicals in the cotton bollworm, Helicoverpa armigera (Hubner.. Pesticide Biochemistry and Physiology 84, 127-134.

Locke B, Forsgren E, Fries I, and de Miranda JR, 2011. Acaracide treatment affects viral dynamics in Varroa destructor-infested honey bee colonies via both host physiology and mite control. Applied Environmental Microbiology.

Lodesani M, Costa C, Serra G, Colombo R, and Sabatini AG, 2008. Acaricide residues in beeswax after conversion to organic beekeeping methods. Apidologie 39, 324-333.

Lodesani M, Pellacani A, Bergomi S, Carpana E, Rabitti T, and Lasagni P, 1992. Residue determination for some products used against Varroa infestation in bees. Apidologie 23, 257-272.

Lodesani M, Carpana E, Bassini A, Dottori M, Mascher A, and Lavazza A, 1994. Oxytetracycline residues in hives treated by two different administration methods. Apicoltura, 51-66.

Lord KA, May MA, and Stevenson JH, 1968. The secretion of the systemic insecticides dimethoate and phorate into nectar. Annals of Applied Biology 61, 19-27.

Luft D, Zon MT, and Mikolajczak M, 2007. The honey bee natural and synthetic immunestimulators - characteristics. Annales Universitatis Mariae Curie-Sklodowska.Sectio DD, Medicina Veterinaria 62, 23-31.

Maccagnani B, Ferrari R, Zucchi L, and Bariselli M, 2008. Measures against grasshoppers while safeguarding bees. Informatore Agrario 64, 53-56.

Macfarlane RP, Lipa JJ, and Liu HJ, 1995. Bumble bee pathogens and internal enemies. Bee World 76, 130-148.

Maini S, Medrzycki P, and Porrini C, 2010. The puzzle of honey bee losses, a brief review. Bulletin of Insectology 63, 153-160.

Mallipudi MM, 1978. Toxicity and mode of action of N-sulfenated derivatives of insecticidal methylcarbamate esters in the honey bee, University of California Riverside.

Mallon EB, Brockmann A, and Schmid-Hempel P, 2003. Immune response inhibits associative learning in insects. Proceedings of the Royal Society of London B 270, 2471-2473.

Malone LA, Gatehouse HS, and Tregidga EL, 2001. Effects of time, temperature, and honey on Nosema apis (Microsporidia , Nosematidae., a parasite of the honeybee, Apis mellifera (Hymenoptera , Apidae.. Journal of Invertebrate Pathology 77, 258-268.

Mao W, Schuler MA, and Berenbaum MR, 2011. CYP9Q-mediated detoxification of acaricides in the honey bee (Apis mellifera.. Proceedings of the National Academy of Sciences of the United States of America 108, 12657-12662.

Marletto F, Arzone H, and Dolci M, 1992. Action of fenoxycarb on honey bee brood. Apicoltore Moderno 83, 209-218.

The present document has been produced and adopted by the bodies identified above as author. This task has been carried out exclusively by the author in the context of a contract between the European Food Safety Authority and the author awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Martin-Hernandez R, Meana A, Garcia-Palencia MP, Marin P, Botias C, Garrido-Bailon E, Barrios L, and Higes M, 2009. Temperature effect on biotic potential of honey bee microsporidia.. Applied and Environmental Microbiology 75, 2554-2557.

Marzaro M, Vivan L, Targa A, Mazzon L, Mori N, Greatti M, Toffolo EP, Di Bernardo A, Giorio C, Marton D, Tapparo A, and Girolami V, 2011. Lethal aerial powdering of honey bees with neonicotinoids from fragments of maize seed coat. Bulletin of Insectology 64, 119-126.

Mattila HR, and Otis GW, 2006. Effects of pollen availability and Nosema infection during the spring on division of labor and survival of worker honey bees (Hymenoptera , Apidae.. Environmental Entomology 35, 708-717.

Mattila HR, Rios D, Walker-Sperling VE, Roeselers G, and Newton ILG, 2012. Characterization of the Active Microbiotas Associated with Honey Bees Reveals Healthier and Broader Communities when Colonies are Genetically Diverse. PLoS One 7, e32962.

McCutchen BF, Hoover K, Preisler HK, Betana MD, Herrmann R, Robertson JL, and Hammock BD, 1997. Interactions of recombinant and wild-type baculoviruses with classical insecticides and pyrethroid-resistant tobacco budworm (Lepidoptera, Noctuidae.. Journal of Economic Entomology 90, 1170-1180.

Meana A, Martín-Hernández R, and Higes M, 2010. The reliability of spore counts to diagnose Nosema ceranae infections in honey bees. Journal of Apicultural Research 49, 212-214.

Meled M, Thrasyvoulou A, and Belzunces LP, 1998. Seasonal variations in susceptibility of Apis mellifera to the synergistic action of prochloraz and deltamethrin. Environmental Toxicology and Chemistry 17, 2517-2520.

Menkissoglu-Spiroudi U, Tsigouri AD, Diamantidis GC, and Thrasyvoulou AT, 2001. Residues in honey and beeswax caused by beekeeping treatments. Fresenius Environmental Bulletin 10, 445-450.

Moffett JO, Harvey J, and Cox R, 1986. Effect of a sticker on the toxicity of Penncap-M and Furadan to honey bees when these insecticides were sprayed on flowering alfalfa. Journal of Entomological Science 21, 294-300.

Moosbeckhofer R, Pechhacker H, Unterweger H, Bandion F, and Heinrich-Lenz A, 2003. Investigations on the oxalic acid content of honey from oxalic acid treated and untreated bee colonies. European Food Research & Technology 217, 49-52.

Morimoto T, Kojima Y, Toki T, Komeda Y, Yoshiyama M, Kimura K, Nirasawa K, and Kadowaki T, 2011. The habitat disruption induces immune-suppression and oxidative stress in honey bees. Ecology and Evolution.

Moritz RF, de Miranda J, Fries I, Le Conte Y, Neumann P, and Paxton RJ, 2010. Research strategies to improve honeybee health in Europe. Apidologie 41, 227-242.

Morse RA, and Nowogrodski R, 1990. Honey bee pests, predators and diseases Comstock Publishing Associates, New York.

Morton HL, Moffett JO, and Martin RD, 1974. Influences of water treated artificially with herbicides on honey bee colonies. Environmental Entomology 3, 808-812.

Mouches C, Bove JM, Albisetti J, Clark TB, and Tully JG, 1982. A Spiroplasma of Serogroup-Iv Causes A May-Disease-Like Disorder of Honeybees in Southwestern France. Microbial Ecology 8, 387-399.

Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, vanEngelsdorp D, and Pettis JS, 2010. High Levels of Miticides and Agrochemicals in North American Apiaries, Implications for Honey Bee Health. PLoS One 5.

The present document has been produced and adopted by the bodies identified above as author. This task has been carried out exclusively by the author in the context of a contract between the European Food Safety Authority and the author awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Murugan K, Thangamathi P, and Jeyabalan D, 2002. Interactive effect of botanicals and Bacillus thuringiensis subsp. israelensis on Culex quinquefasciatus Say. Journal of Scientific & Industrial Research 61, unpaginated.

Murugan K, Sivaramakrishnan S, Kumar NS, Jeyabalan D, and Nathan SS, 1998. Synergistic interaction of botanicals and biocides nuclear polyhedrosis virus on pest control. Journal of Scientific & Industrial Research 57, 732-739.

Nakajima C, Okayama A, Sakogawa T, Nakamura A, and Hayama T, 1997. Disposition of ampicillin in honeybees and hives. Journal of Veterinary Medical Science 59, 765-767.

Nakajima C, Sakogawa T, Okayama A, Nakamura A, and Hayama T, 1998. Disposition of mirosamicin in honeybee hives. Journal of Veterinary Pharmacology and Therapeutics 21, 269-273.

Nauen R, Ebbinghaus-Kintscher U, and Schmuck R, 2001. Toxicity and nicotinic acetylcholine receptor interaction of imidacloprid and its metabolites in Apis mellifera (Hymenoptera, Apidae.. pp. 577-586.

Naug D, 2009. Nutritional stress due to habitat loss may explain recent honeybee colony collapses. Biological Conservation 142, 2369-2372.

Navajas M, Migeon A, Alaux C, Martin-Magniette ML, Robinson GE, Evans JD, Cros-Arteil S, Crauser D, and Le Conte Y, 2008. Differential gene expression of the honey bee Apis mellifera associated with Varroa destructor infection. BMC Genomics 9, 301.

Nelson D, Sporns P, Kristiansen P, Mills P, and Li M, 1993. Effectiveness and residue levels of 3 methods of menthol application to honey bee colonies for the control of tracheal mites. Apidologie 24, 549-556.

Neves PMJO, Alves SB, Almeida JEM, and Moino Junior A, 2002. Interactions between entomopathogenic fungi and chemical pesticides. Documentos - Embrapa Soja 184, 41-45.

Nguyen B, Saegerman C, Pirard C, Mignon J, Widart J, Tuirionet B, Verheggen F, Berkvens D, De Pauw E, and Haubruge E, 2009. Does Imidacloprid Seed-Treated Maize Have an Impact on Honey Bee Mortality? Journal of Economic Entomology 102, 616-623.

Nicolson SW, 2009. Water homeostasis in bees, with emphasis on sociality. The Journal of Experimental Biology 212.

Nielsen SA, Brodsgaard CJ, and Hansen H, 2000. Effects on detoxification enzymes in different life stages of honey bees (Apis mellifera L., Hymenoptera, Apidae. treated with a synthetic pyrethroid (flumethrin.. ATLA, Alternatives to Laboratory Animals 28, 437-443.

Nishimatsu T, and Jackson JJ, 1998. Interaction of insecticides, entomopathogenic nematodes, and larvae of the western corn rootworm (Coleoptera, Chrysomelidae.. Journal of Economic Entomology 91, 410-418.

Niu G, Johnson RM, and Berenbaum MR, 2011. Toxicity of mycotoxins to honeybees and its amelioration by propolis. Apidologie 42, 79-87.

Nixon HL, and Ribbands CR, 1952. Food transmission within the honeybee community. Proceedings of the Royal Society 140, 43-50.

Nozal M, Bernal J, Martin M, Alvaro A, Martin R, and Higes M, 2008. Trace analysis of fumagillin in honey by liquid chromatography-diode array-electrospray ionization mass spectrometry. Journal of Chromatography A 1190, 224-231.

Oliver R, 2010. Sick Bees. American Bee Journal 150, 767-772.

The present document has been produced and adopted by the bodies identified above as author. This task has been carried out exclusively by the author in the context of a contract between the European Food Safety Authority and the author awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Orantes-Bermejo JF, Gomez Pajuelo A, Megias Megias M, and Torres Fernandez-Pinar C, 2010. Pesticide residues in beeswax and beebread samples collected from honey bee colonies (Apis mellifera L.. in Spain. Possible implications for bee losses. Journal of Apicultural Research 49, 243-250.

Ortiz de Motellano PR, and De Voss JJ, 2005. Substrate oxidation by cytochrome P450 enzymes, in, O. d. M. P. R, Ed.., Cytochrome P450, structure, mechanism, and biochemistry,, Kluwer Academic/Plenum Publishers, New York. pp. 183-245.

Otti O, and Schmid-Hempel P, 2007. Nosema bombi, A pollinator parasite with detrimental fitness effects. Journal of Invertebrate Pathology 96, 118-124.

Pankiw T, and Jay SC, 1992. Aerially Applied Ultra-Low-Volume Malathion Effects on Caged Honey Bees (Hymenoptera, Apidae., Caged Mosquitoes (Diptera, Culicidae., and Malathion Residues Journal of Economic Entomology 85, 687-691.

Papaefthimiou C, and Theophilidis G, 2001. The cardiotoxic action of the pyrethroid insecticide deltamethrin, the azole fungicide prochloraz and their synergy on the semi-isolated heart of the bee Apis mellifera macedonica. Pesticide Biochemistry and Physiology 69, 77-91.

Pereboom JJM, 2000. The composition of larval food and the significance of exocrine secretions in the bumblebee Bombus terrestris. Insectes Sociaux 47, 11-20.

Pettis JS, Wilson WT, Shimanuki H, and Teel PD, 1991. Fluvalinate treatment of queen and worker honey bees (Apis mellifera L.. and effects on subsequent mortality, queen acceptance and supersedure. Apidologie 22, 1-7.

Pettis JS, VanEngelsdorp D, Johnson J, and Dively G, 2012. Pesticide exposure in honey bees results in increased levels of the gut pathogen Nosema. Naturwissenschaften.

Pilling ED, 1992. Evidence for pesticide synergism is the honeybee (Apis mellifera.. Aspects of Applied Biology 31, 43-47.

Pilling ED, and Jepson PC, 1993. Synergism between EBI fungicides and a pyrethroid insecticide in the honeybee (Apis mellifera.. Pesticide Science 39, 293-297.

Pilling ED, Bromley-Challoner KAC, Walker CH, and Jepson PC, 1995. Mechanism of synergism between the pyrethroid insecticide □-cyhalothrin and the imidazole fungicide prochloraz in the honeybee (Apis mellifera L... Pesticide Biochemistry and Physiology 51, 1-11.

Plapp FW, 1979. Synergism of pyrethroid insecticides by formamidines against Heliothis pests of cotton. Journal of Economic Entomology 72, 667-670.

Porrini C, Celli G, and Radeghieri P, 1998. Monitoring of pesticides through the use of honeybees as bioindicators of the Emilia-Romagna coastline (1995-1996.. Annali di Chimica 88, 243-252.

Prakash A, and Srivastava BP, 1997. Potentiation of carbaryl with some fungicides against green leafhopper Nephotettix Cincticeps (Uhler.. National Academy Science Letters-India 20, 124-126.

Ramoutar D, Cowles RS, Requintina E, and Alm SR, 2010. Synergism between demethylation inhibitor fungicides or gibberellin inhibitor plant growth regulators and bifenthrin in a pyrethroid-resistant population of Listronotus maculicollis (Coleoptera, Curculionidae.. Journal of Economic Entomology 103, 1810-1814.

Reetz JE, Zuehlke S, Spiteller M, and Wallner K, 2011. Neonicotinoid insecticides translocated in guttated droplets of seed-treated maize and wheat, a threat to honeybees? Apidologie 42, 596-606.

Reinhard A, Janke M, Ohe WD, Kempf M, Theuring C, Hartmann T, Schreier P, and Beuerle T, 2009. Feeding deterrence and detrimental effects of pyrrolizidine alkaloids fed to honey bees (Apis mellifera.. Journal of Chemical Ecology 35, 1086-1095.

The present document has been produced and adopted by the bodies identified above as author. This task has been carried out exclusively by the author in the context of a contract between the European Food Safety Authority and the author awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Rinderer TE, and Dell Elliott E, 1977. Worker Honey Bee Response to Infection with Nosema apis, Influence of Diet. Journal of Economic Entomology 70, 431-433.

Robertson LN, and Rhodes JW, 1992. Honey bee (Apis mellifera L.. deaths near sprayed cotton and observations on bee foraging behaviour in flowering cotton (Hymenoptera, Apidae.. Journal of the Australian Entomological Society 31, 243-246.

Romano G, and Moncecchi AG, 1988. Interaction and additive effects of varroasis and European foul brood, Interaccion y potenciacion entre varroasis y loque europea. pp. 675-685.

Rortais A, Arnold G, Halm MP, and Touffet-Briens F, 2005. Modes of honeybees exposure to systemic insecticides, estimated amounts of contaminated pollen and nectar consumed by different categories of bees. Apidologie 36, 71-83.

Rouas G, 1987. Innocuity for bees of fluvalinate alone or mixed with a fungicide. Phytoma 387, 9.

Russell CW, Ugine TA, and Hajek AE, 2010. Interactions between imidacloprid and Metarhizium brunneum on adult Asian longhorned beetles (Anoplophora glabripennis (Motschulsky.. (Coleoptera, Cerambycidae.. Journal of Invertebrate Pathology 105, 305-311.

Santas LA, 1985. Parthenolecanium corni (Bouche. an orchard scale pest producing honeydew foraged by bees in Greece. Entomologia Hellenica 3, 53-58.

Satta A, Floris I, Caboni P, Cabras P, Eguaras M, and Velis G, 2008. New experimental data on use of rotenone as an acaricide for control of Varroa destructor in honey bee colonies. Journal of Economic Entomology 101, 1075-1080.

Schmid-Hempel P, Kacelnik A, and Houston A, 1985. Honeybees maximise efficiency by not filling their crop. Behavioural Ecology and Sociobiology 17, 61-66.

Schmid MR, Brockmann A, Pirk CWW, Stanley DW, and Tautz J, 2008. Adult honeybees (Apis mellifera L.. abandon hemocytic, but not phenoloxidase-based immunity. journal of Insect Physiology 54, 439-444.

Schmidt-Hempel R, and Schmidt-Hempel P, 1998. Colony performance and immunocompetence of a social insect, Bombus terrestris, in poor and variable environments. Functional Ecology 12, 22-30.

Schmuck R, Nauen R, and Ebbinghaus-Kintscher U, 2003a. Effects of imidacloprid and common plant metabolites of imidacloprid in the honeybee, toxicological and biochemical considerations, in, C. Porrini (Ed... pp. 27-34.

Schmuck R, Stadler T, and Schmidt HW, 2003b. Field relevance of a synergistic effect observed in the laboratory between an EBI fungicide and a chloronicotinyl insecticide in the honeybee (Apis mellifera L, Hymenoptera.. Pest Management Science 59, 279-286.

Schmuck R, Schoning R, Stork A, and Schramel O, 2001. Risk posed to honeybees (Apis mellifera L, Hymenoptera. by an imidacloprid seed dressing of sunflowers. Pest Management Science 57, 225-238.

Schoning R, and Schmuck R, 2003. Analytical determination of imidacloprid and relevant metabolite residues by LC MS/MS. Bulletin of Insectology 56, 41-50.

Sharififard M, Mossadegh MS, Vazirianzadeh B, and Zarei-Mahmoudabadi A, 2011. Interactions between entomopathogenic fungus, Metarhizium anisopliae and sublethal doses of spinosad for control of house fly, Musca domestica. Iranian Journal of Arthropod-Borne Diseases 5, 28-36.

Shawki MAA, Titera D, Kazda J, Kohoutkova J, and Taborsky V, 2006. Toxicity to honeybees of water guttation and dew collected from winter rape treated with Nurelle DReg. Plant Protection Science 42, 9-14.

The present document has been produced and adopted by the bodies identified above as author. This task has been carried out exclusively by the author in the context of a contract between the European Food Safety Authority and the author awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Skerl MIS, Kmecl V, and Gregorc A, 2010. Exposure to Pesticides at Sublethal Level and Their Distribution Within a Honey Bee (Apis mellifera) Colony. Bulletin of Environmental Contamination and Toxicology 85, 125-128.

Skerl MIS, Bolta SV, Cesnik HB, and Gregorc A, 2009. Residues of Pesticides in Honeybee (Apis mellifera carnica). Bee Bread and in Pollen Loads from Treated Apple Orchards. Bulletin of Environmental Contamination and Toxicology 83, 374-377.

Smirle MJ, 1988. Insecticide resistance mechanisms in the honey bee, Apis mellifera L. pp. xii-xpp.

Smirle MJ, and Winston ML, 1987. Intercolony Variation in Pesticide Detoxification by the Honey-Bee (Hymenoptera, Apidae.. Journal of Economic Entomology 80, 5-8.

Sonnet PE, 1978. Controlled-release pesticides. Bee World 59, 112-114.

Sonnet PE, Lye TL, and Sackett RR, 1978. Effects of selected herbicides on the toxicity of several insecticides to honey bees. Environmental Entomology 7, 254-256.

Spencer WF, Shoup TD, Cliath MM, and Farmer WJ, 1979. Vapor pressures and relative volatility of ethyl and methyl parathion. Journal of Agricultural & Food Chemistry 27.

Stanger W, and Winterlin W, 1975. Residues of the insecticides carbaryl and monocrotophos in honeybee colonies. Journal of Apicultural Research 14, 131-135.

Suchail S, Debrauwer L, and Belzunces LP, 2004. Metabolism of imidacloprid in Apis mellifera. pp. 291-296.

Sudhakar K, and Swaran D, 2002. Interactive effect of sublethal concentrations of fenvalerate and various microbial insecticides to larval instars of Spilarctia obliqua (Walker.. Annals of Plant Protection Sciences 10, 31-37.

Suh YT, and Shim JH, 1988. Enzyme activities of a honeybee Apis-mellifera l. associated with the degradation of some insecticides. Agricultural Chemistry & Biotechnology 31, 241-248.

Szymas B, and Jedruszuk A, 2003. The influence of different diets on haemocytes of adult worker honey bees, Apis mellifera. Apidologie 34, 97-102.

Tapparo A, Marton D, Giorio C, Zanella A, Solda L, Marzaro M, Vivan L, and Girolami V, 2012. Assessment of the environmental exposure of honeybees to particulate matter containing neonicotinoid insecticides coming from corn coated seeds. Environmental Science and Technology.

Tasei JN, and Aupinel P, 2008. Nutritive value of 15 single pollens and pollen mixes tested on larvae produced by bumblebee workers (Bombus terrestris, Hymenoptera, Apidae.. Apidologie 39, 397-409.

Thompson HM, 2001. Assessing the exposure and toxicity of pesticides to bumblebees (Bombus sp... Apidologie 32, 305-321.

Thompson HM, 2010. Risk assessment for honey bees and pesticides - recent developments and 'new issues'. Pest Management Science 66, 1157-1162.

Thompson HM., and Folkard-Ward H, 2001. Toxicity of realistic combinations of pyrethroids and fungicides to honeybees. Hazards of Pesticides to Bees, 83-89.

Thompson HM, and Wilkins S, 2003. Assessment of the synergy and repellency of pyrethroid/fungicide mixtures. Bulletin of Insectology 56, 131-134.

Thompson HM, Waite RJ, Wilkins S, Brown MA, Bigwood T, Shaw M, Ridgway C, and Sharman M, 2005. Effects of European foulbrood treatment regime on oxytetracycline levels in honey extracted from treated honeybee (Apis mellifera. colonies and toxicity to brood. Food Additives and Contaminants 22, 573-578.

The present document has been produced and adopted by the bodies identified above as author. This task has been carried out exclusively by the author in the context of a contract between the European Food Safety Authority and the author awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Tian L, and Feng M, 2006. Evaluation of the time-concentration-mortality responses of Plutella xylostella larvae to the interaction of Beauveria bassiana with a nereistoxin analogue insecticide. pp. 69-76.

Traver BE, Williams MR, and Fell RD, 2012. Comparison of within hive sampling and seasonal activity of Nosema ceranae in honey bee colonies. Journal of Invertebrate Pathology 109, 187-193.

Tremolada P, Bernardinelli I, Colombo M, Spreafico M, and Vighi M, 2004. Coumaphos distribution in the hive ecosystem, Case study for modeling applications. Ecotoxicology 13, 589-601.

Tremolada P, Mazzoleni M, Saliu F, Colombo M, and Vighi M, 2010. Field Trial for Evaluating the Effects on Honeybees of Corn Sown Using Cruiser(A (R.. and Celest xl(A (R.. Treated Seeds. Bulletin of Environmental Contamination & Toxicology 85, 229-234.

Tremolada P, Bernardinelli I, Rossaro B, Colombo M, and Vighi M, 2011. Predicting pesticide fate in the hive (part 2., development of a dynamic hive model. Apidologie 42, 439-456.

Twinn DC, Lacy JC, and Floyd MA, 1984. The safety to honeybees of an iprodione-phosalone tank mix applied to flowering winter oilseed rape. Aspects of Applied Biology, 311-321.

Ucar T, Hall FR, Tew JE, and Hacker JK, 2003. Wind tunnel studies on spray deposition on leaves of tree species used for windbreaks and exposure of honey bees. Pest Management Science 59, 358-364.

Unal G, Benlioglu K, and Klc B, 1997. Studies on the interaction of widely used insecticides with Bacillus thuringiensis var. tenebrionis against Colorado potato beetle (Leptinotarsa decemlineata Say.. Bitki Koruma Bulteni 37, 67-78.

van der Steen JJM, and Dinter A, 2007. A monitoring study to assess the acute mortality effects of indoxacarb on honey bees (Apis mellifera L.. in flowering apple orchards. Pest Management Science 63, 1095-1099.

Vandame R, and Belzunces LP, 1998a Erratum to "Joint actions of deltamethrin and azole fungicides on honey bee thermoregulation" [Neurosci. Lett. 251 (1998. 57â€“60]. Neuroscience Letters 255, 61. DOI, 10.1016/s0304-3940(98.00728-9.

Vandame R, and Belzunces LP, 1998b. Joint actions of deltamethrin and azole fungicides on honey bee thermoregulation. Neuroscience Letters 251, 57-60. DOI, 10.1016/s0304-3940(98.00494-7.

vanEngelsdorp D, Evans JD, Donovall L, Mullin C, Frazier M, Frazier J, Tarpy DR, Hayes J, Jr, and Pettis JS, 2009a. "Entombed Pollen", A new condition in honey bee colonies associated with increased risk of colony mortality. Journal of Invertebrate Pathology 101, 147-149.

vanEngelsdorp D, Evans JD, Saegerman C, Mullin C, Haubruge E, Nguyen BK, Frazier M, Frazier J, Cox-Foster D, Chen Y, Underwood R, Tarpy DR, and Pettis JS, 2009b. Colony Collapse Disorder, A Descriptive Study. PLoS One 4, Article-No, e6481.

vanEngelsdorp D, Speybroeck N, Evans JD, Nguyen BK, Mullin C, Frazier M, Frazier J, Cox-Foster D, Chen Y, Tarpy DR, Haubruge E, Pettis JS, and Saegerman C, 2010. Weighing Risk Factors Associated With Bee Colony Collapse Disorder by Classification and Regression Tree Analysis. Journal of Economic Entomology 103, 1517-1523.

Verbruggen EMJ, and Van den Brink PJ, 2010. Review of recent literature concerning mixture toxicity of pesticides to aquatic organisms

Vidano C, 1975. Preliminary investigations of high honeybee mortality associated with spraying of fungicides on vineyards by helicopter. Apicoltore Moderno 66, 81-86.

Vidau C, Diogon M, Aufauvre J, Fontbonne R, Vigues B, Brunet JL, Texier C, Biron DG, Blot N, El Alaoui H, Belzunces LP, and Delbac F, 2011. Exposure to Sublethal Doses of Fipronil and

The present document has been produced and adopted by the bodies identified above as author. This task has been carried out exclusively by the author in the context of a contract between the European Food Safety Authority and the author awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Thiacloprid Highly Increases Mortality of Honeybees Previously Infected by Nosema ceranae. PLoS One 6.

Viuda-Martos M, Ruis-Navajas Y, Fernandez-L´Opez J, and Perez-´Alvarez JA, 2008. Functional Properties of Honey, Propolis, and Royal Jelly. Journal of Food Science 73, R117-R124.

Volckaert M, and van Laere O, 1984. Toxicity of an MCPP/2,4-D mixture for the larval stage of the honeybee (Apis mellifera L... Mededelingen van Faculteit Landbouwwetenschappen Rijksuniversiteit, Gent 36, 751-757.

Wahl O, and Ulm K, 1983. Influence of Pollen Feeding and Physiological Condition on Pesticide Sensitivity of the Honey Bee Apis-Mellifera-Carnica. Oecologia 59, 106-128.

Walker CH, 1998. The use of biomarkers to measure the interactive effects of chemicals. Ecotoxicology and Environmental Safety 40, 65-70.

Waller GD, Barker RJ, and Martin JH, 1979. Effects of dimethoate on honey bee foraging. Chemosphere, 461-463.

Waller GD, Erickson BJ, Harvey J, and Martin JH, 1984. Effects of dimethoate on honey bees (Hymenoptera, Apidae. when applied to flowering lemons. Journal of Economic Entomology 77, 70-74.

Wallner K, 1997. Honeybee intoxication caused by chemical plant protection in vineyards. Mitteilungen der Deutschen Gesellschaft fur Allgemeine und Agewandte Entomologie, Band 11, Heft 1-6, Dezember 1997, Entomologists Conference 11, 205-209.

Wallner K, 2009. Sprayed and seed dressed pesticides in pollen, nectar and honey of oilseed rape. Julius Kuhn Archive 423, 152-153.

Walorczyk S, and Gnusowski B, 2009. Development and validation of a multi-residue method for the determination of pesticides in honeybees using acetonitrile-based extraction and gas chromatography-tandem quadrupole mass spectrometry. Journal of Chromatography A 1216, 6522-6531.

Wang C-H, Lo CF, Nai YS, Chih-Yuan W, Chen YR, Huang WF, Chien TY, and Wu CY, 2009. Honey Bee Colony Collapse Disorder. Formosan Entomologist 29, 119-138.

Wang C-J, Qiu L-H, Zheng M-Q, Tao C-J, Jiahg H, Zhang W-J, and JLi X-F, 2006. Safety evaluation of abamectin and its mixtures to honey bees (Apis mellifera L... Journal of Agro-Environment Science 25, 229-231.

Warne MSJ, 2003. A Review of the ecotoxicity of mixtures, approaches to, and recommendations for, their management., in, A. Langley, et al, Eds.., Proceedings of the 5th national workshop on the assessment of site contamination, NEPC, Adelaide. pp. 253-276.

Wehling M, Ohe Wvd, Brasse D, and Forster R, 2009 Colony losses - interactions of plant protection products and other factors, in, P. A. T. H. M. Oomen (Ed.., Hazards of pesticides to bees. 10th International Symposium of the ICP-Bee Protection Group. Bucharest, Romania, 8-10 October, 2008., JKI, Germany. pp. 153-154.

White JB, Park Y-L, West TP, and Tobin PC, 2009. Assessment of potential fumigants to control Chaetodactylus hrombeini (Acari, Chaetodactylidae. associated with Osmia cornifrons (Hymenoptera, Megachilidae.. Journal of Economic Entomology 102, 2090-2095.

Wiest L, Bulete A, Giroud B, Fratta C, Amic S, Lambert O, Pouliquen H, and Arnaudguilhem C, 2011. Multi-residue analysis of 80 environmental contaminants in honeys, honeybees and pollens by one extraction procedure followed by liquid and gas chromatography coupled with mass spectrometric detection. Journal of Chromatography A 1218, 5743-5756.

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Wilkinson CF, Christoph GR, Julien E, Kelley JM, Kronenberg J, McCarthy J, and Reiss R, 2000. Assessing the risks of exposures to multiple chemicals with a common mechanism of toxicity, How to cumulate? Regulatory Toxicology and Pharmacology 31, 30-43.

Wilson-Rich N, Dres ST, and Starks PT, 2008. The ontogeny of immunity, Development of innate immune strength in the honey bee (Apis mellifera.. Journal of Insect Physiology 54, 1392-1399.

Wilson K, Knell R, Boots M, and Koch-Osborne J, 2003. Group living and investment in immune defence, an interspecific analysis. Journal of Animal Ecology 72, 133-143.

Winterlin W, Walker G, and Luce A, 1973. Carbaryl residues in bees, honey, and bee bread following exposure to carbaryl via the food supply. Archives of Environmental Contamination and Toxicology 1, 362-374.

Woyciechowski M, 2007. Risk of water collecting in honeybee (Apis mellifera. workers (Hymenoptera, Apidae.. Scociobiology 50, 1059-1068.

Wu JY, Anelli CM, and Sheppard WS, 2011. Sub-lethal effects of pesticide residues in brood comb on worker honey bee (Apis mellifera. development and longevity. PLoS One 6, e14720.

Wu JY, Smart MD, Anelli CM, and Sheppard WS, 2012. Honey bees (Apis mellifera. reared in brood combs containing high levels of pesticide residues exhibit increased susceptibility to Nosema (Microsporidia. infection. Journal of Invertebrate Pathology.

Yamamoto I, Tomizawa M, Saito T, Miyamo to T, Walcott EC, and Sumikawa K, 1988. Structural factors contributing to insecticidal and selective actions of neonicotinoids. Archives of Insect Biochemistry and Physiology 37, 24-32.

Ye S, Dun Y, and Feng M, 2005. Time and concentration dependent interactions of Beauveria bassiana with sublethal rates of imidacloprid against the aphid pests Macrosiphoniella sanborni and Myzus persicae. pp. 459-468.

Yildrim E, and Hoy CW, 2003. Interaction between cyromazine and the entomopathogenic nematode Heterorhabditis bacteriophora Poinar "GPS11" for control of onion maggot, Delia antiqua (Meigen.. Crop Protection 22, 923-927.

Yu SJ, Robinson FA, and Nation JL, 1984. Detoxication capacity in the honey bee, Apis mellifera L. Pesticide Biochemistry and Physiology 22, 360-368.

The present document has been produced and adopted by the bodies identified above as author. This task has been carried out exclusively by the author in the context of a contract between the European Food Safety Authority and the author awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Appendix 1 Database search terms

Search | Journals | Books | My Workspace

Search History (52 searches) (Click to close) — Remove Duplicates | View Saved

# ▲	Searches	Results	Search Type	Actions
1	agrochemical.mp. or pesticides.sh. or chemical control.sh. or herbicides.sh. or agricultural chemicals.sh. or fungicides.sh. or pesticide residues.sh. or insecticides.sh.	331881	Advanced	Display More »
2	plant protection product*.mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw]	2010	Advanced	Display More »
3	plant protection compound*.mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw]	40	Advanced	Display More »
4	plant protection chemical*.mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw]	483	Advanced	Display More »
5	(Pesticid* or insecticid* or Acaricid* or Nematicid* or Molluscicid*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw]	370996	Advanced	Display More »
6	(Herbicid* or Fungicid* or antifungal* or anti-fungal*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw]	343095	Advanced	Display More »
7	1 or 2 or 3 or 4 or 5 or 6	665665	Advanced	Display More »
8	veterinary medicine*.mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gl, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw]	207220	Advanced	Display More »
9	veterinary pharmaceutical*.mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gl, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw]	361	Advanced	Display More »
10	(varroacid* or miticid*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gl, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw]	1111	Advanced	Display More »
11	(antibacterial* or antibiotic*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gl, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw]	406419	Advanced	Display More »
12	7 or 8 or 9 or 10 or 11	1237215	Advanced	Display More »
13	(honeybee* or honey bee*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gl, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw]	45120	Advanced	Display More »
14	Apis mellifera.mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gl, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw]	27595	Advanced	Display More »
15	13 or 14	49947	Advanced	Display More »
16	12 and 15	5387	Advanced	Display More »
17	(toxic* or sublethal* or sub-lethal*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gl, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw]	1557109	Advanced	Display More »
18	(ecotox* or nontarget* or non-target*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gl, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw]	61995	Advanced	Display More »
19	((additiv* or cummulativ* or synergis* or mixture* or sequent*) adj5 effect*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gl, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw]	83243	Advanced	Display More »
20	(multiple adj exposur*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gl, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw]	850	Advanced	Display More »
21	(sublethal* or sub-lethal*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gl, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw]	27164	Advanced	Display More »
22	19 or 20 or 21	110640	Advanced	Display More »
23	17 or 18	1590139	Advanced	Display More »
24	16 and 23	2496	Advanced	Display More »

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25	16 and 22	228	Advanced	Display More »
26	remove duplicates from 25	168	Advanced	Display More »
27	route*.mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gl, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw]	271675	Advanced	Display More »
28	(oral* or pollen* or nectar* or water*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gl, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw]	2166686	Advanced	Display More »
29	(contact* or spray* or overspray* or systemic*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gl, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw]	634153	Advanced	Display More »
30	(dust* or guttation*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gl, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw]	48822	Advanced	Display More »
31	(inhation* or vapor* or vapour*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gl, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw]	49688	Advanced	Display More »
32	(adult* or larv* or brood*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gl, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw]	1824833	Advanced	Display More »
33	27 or 28 or 29 or 30 or 31 or 32	4559284	Advanced	Display More »
34	24 and 33	1439	Advanced	Display More »
35	remove duplicates from 34	1251	Advanced	Display More »
36	35 not 26	1156	Advanced	Display More »
37	from 36 keep 1001-1156	156	Advanced	Display More »
38	(insect* or arthropod*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gl, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw]	1913216	Advanced	Display More »
39	15 or 38	1919098	Advanced	Display

39	15 or 38	1919098	Advanced	Display More »
40	23 and 39	135415	Advanced	Display More »
41	(foulbrood* or bacillus* or leissococcus* or pathogen* or disease* or fungus* ort fungal* or bacteria* or biocontrol*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gl, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw]	5168802	Advanced	Display Delete More »
42	(nosema* or microsporidia* or varroa* or mite* or acarine* or virus* or viral* or parasit*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gl, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw]	2099151	Advanced	Display More »
43	41 or 42	6113163	Advanced	Display More »
44	39 and 43	550199	Advanced	Display More »
45	12 and 44	101534	Advanced	Display More »
46	interact*.mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gl, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw]	1143071	Advanced	Display More »
47	12 and 39	260970	Advanced	Display More »
48	43 and 47	101534	Advanced	Display More »
49	46 and 48	4075	Advanced	Display More »
50	"interact*".m_titl.	259224	Advanced	Display More »

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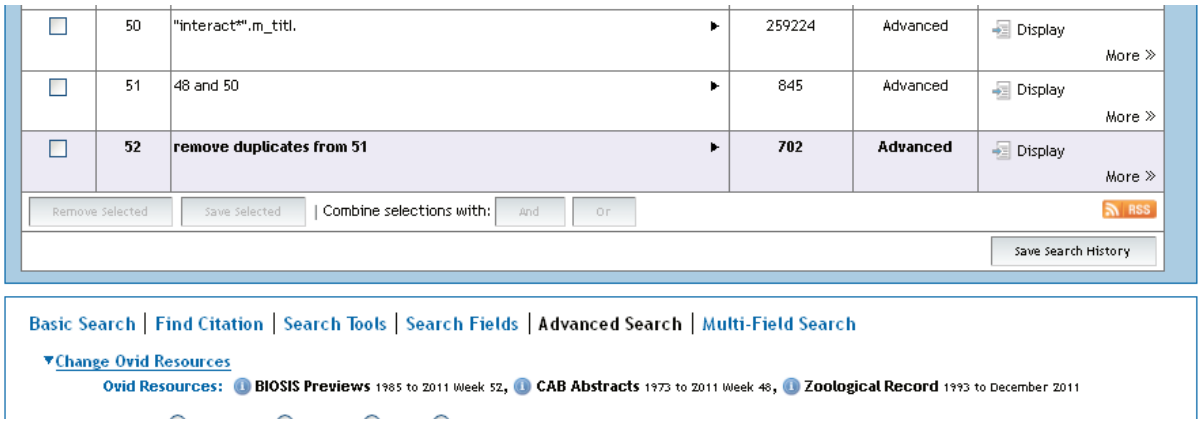

Databases:

BIOSIS Previews <1985 to 2011 Week 52>, CAB Abstracts <1973 to 2011 Week 48>, Zoological Record <1993 to December 2011>

All searches included the terms in the title, abstract and/or keywords

revision 2, 13th Feb 2012

1. agrochemical.mp. or pesticides.sh. or chemical control.sh. or herbicides.sh. or agricultural chemicals.sh. or fungicides.sh. or pesticide residues.sh. or insecticides.sh.

2. plant protection product*.mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw, nm, rs, ui]

3. plant protection compound*.mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw, nm, rs, ui]

4. plant protection chemical*.mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw, nm, rs, ui]

5. (Pesticid* or insecticid* or Acaricid* or Nematicid* or Molluscicid*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw, nm, rs, ui]

6. (Herbicid* or Fungicid* or antifungal* or anti-fungal*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw, nm, rs, ui]

7. 1 or 2 or 3 or 4 or 5 or 6

8. veterinary medicine*.mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw, nm, rs, ui]

9. veterinary pharmaceutical*.mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw, nm, rs, ui]

10. (varroacid* or miticid*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw, nm, rs, ui]

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11. (antibacterial* or antibiotic*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw, nm, rs, ui]

12. 7 or 8 or 9 or 10 or 11

13. (honeybee* or honey bee*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw, nm, rs, ui]

14. Apis mellifera.mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw, nm, rs, ui]

15. 13 or 14

16. 12 and 15

17. (toxic* or sublethal* or sub-lethal*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw, nm, rs, ui]

18. (ecotox* or nontarget* or non-target*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw, nm, rs, ui]

19. ((additiv* or cumulativ* or synergis* or mixture* or sequent*) adj5 effect*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw, nm, rs, ui]

20. (multiple adj exposur*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw, nm, rs, ui]

21. (sublethal* or sub-lethal*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw, nm, rs, ui]

22. 19 or 20 or 21

23. 17 or 18

24. 16 and 23

25. 16 and 22

26. remove duplicates from 25

27. route*.mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw, nm, rs, ui]

28. (oral* or pollen* or nectar* or water*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw, nm, rs, ui]

29. (contact* or spray* or overspray* or systemic*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw, nm, rs, ui]

30. (dust* or guttation*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw, nm, rs, ui]

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31. (inhation* or vapor* or vapour*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw, nm, rs, ui]

32. (adult* or larv* or brood*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw, nm, rs, ui]

33. 27 or 28 or 29 or 30 or 31 or 32

34. 24 and 33

35. remove duplicates from 34

36. 35 not 26

37. (insect* or arthropod*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw, nm, rs, ui]

38. 15 or 37

39. 23 and 38

40. (foulbrood* or bacillus* or leissococcus* or pathogen* or disease* or fungus* or fungal* or bacteria* or biocontrol*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw, nm, rs, ui]

41. (nosema* or microsporidia* or varroa* or mite* or acarine* or virus* or viral* or parasit*).mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw, nm, rs, ui]

42. 40 or 41

43. 38 and 42

44. 12 and 43

45. interact*.mp. [mp=ab, bc, bo, bt, cb, cc, ds, ge, gn, mc, mi, mq, or, ps, sq, st, ti, tm, tn, ot, hw, nm, rs, ui]

46. 12 and 38

47. 42 and 46

48. 45 and 47

49. "interact*".m_titl.

50. 47 and 49

51. remove duplicates from 50

52. 36 use mesz

53. 51 use mesz

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15th February 2012

*** It is now 2012/02/15 16:22:06 ***

(Dialog time 2012/02/15 11:22:06)

Subaccount is set to W8JZ_HONEYBEES.

Notice = $10.00

? b155,50,5,185,10,203,40,156,76,41,34,434

SYSTEM:OS - DIALOG OneSearch

File 155:MEDLINE(R) 1950-2012/Feb 13

(c) format only 2012 Dialog

*File 155: MEDLINE has been reloaded. Please see HELP NEWS154

for details.

File 50:CAB Abstracts 1972-2012/Feb W1

(c) 2012 CAB International

File 5:Biosis Previews(R) 1926-2012/Feb W1

(c) 2012 The Thomson Corporation

File 185:Zoological Record Online(R) 1864-2012/Feb

(c) 2012 The Thomson Corp.

File 10:AGRICOLA 70-2012/Feb

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File 203:AGRIS 1974-2012/Dec

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File 40:Enviroline(R) 1975-2008/May

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*File 40: This file is closed and will no longer update. For

similar data, please search File 76-Environmental Sciences.

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File 156:ToxFile 1965-2012/Feb W2

(c) format only 2012 Dialog

*File 156: The last daily update of Medline records for 2011 was

UD20111114. Updates resumed with the 2012 MeSH with UD20120105.

File 76:Environmental Sciences 1966-2012/Jan

(c) 2012 CSA.

File 41:Pollution Abstracts 1966-2012/Jan

(c) 2012 CSA.

File 34:SciSearch(R) Cited Ref Sci 1990-2012/Feb W2

(c) 2012 The Thomson Corp

File 434:SciSearch(R) Cited Ref Sci 1974-1989/Dec

(c) 2006 The Thomson Corp

Set Items Description

S1 1690058 AGROCHEMICAL? OR PESTICID? OR CHEMICAL()CONTROL? OR HERBIC-ID? OR AGRICULTURAL()CHEMICAL? OR FUNGICID? OR INSECTICID?

S2 4562 PLANT()PROTECTION()PRODUCT? OR PLANT()PROTECTION()COMPOUND? OR PLANT()PROTECTION()CHEMICAL?

S3 63739 ACARICID? OR NEMATICID? OR MOLLUSCICID?

S4 232227 ANTIFUNGAL? OR ANTI-FUNGAL?

S5 1882500 S1 OR S2 OR S3 OR S4

S6 360277 VETERINARY()MEDICINE? OR VETERINARY()PHARMACEUTICAL?

S7 2530 VARROACID? OR MITICID?

S8 1199988 ANTIBACTERIAL? OR ANTIBIOTIC?

S9 3314429 S5 OR S6 OR S7 OR S8

S10 116545 APIS()MELLIFERA OR HONEYBEE? OR HONEY()BEE?

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S11 11752 S9 AND S10

S12 4460623 TOXIC? OR SUBLETHAL? OR SUB-LETHAL?

S13 137777 ECOTOX? OR NONTARGET? OR NON-TARGET?

S14 2732942 ADDITIV? OR CUMULATIV? OR SYNERGIS? OR MIXTURE? OR SEQUENT?

S15 284151 S14(3N)EFFECT?

S16 23078 MULTIPLE()EXPOSURE? OR REPEATED()EXPOSURE?

S17 78386 SUBLETHAL? OR SUB-LETHAL?

S18 344571 (S12 OR S13) AND (S14 OR S16 OR S17)

S19 123044 (S12 OR S13) AND (S15 OR S16 OR S17)

S20 736 S11 AND S18

S21 486 S11 AND S19

S22 500316 ROUTE?

S23 8063047 ORAL? OR POLLEN? OR NECTAR? OR WATER?

S24 2399654 CONTACT? OR SPRAY? OR OVERSPRAY? OR SYSTEMIC?

S25 277125 DUST? OR GUTTATION?

S26 442968 INHALATION? OR VAPOR? OR VAPOUR?

S27 7976748 ADULT? OR LARV? OR BROOD?

S28 18030575 S22 OR S23 OR S24 OR S25 OR S26 OR S27

S29 5568 S11 AND S28

S30 397 RD S20 (unique items) – ITEMS PRINTED FROM DATABASES NOT PREVIOUSLY SEARCHED

S31 15336 EXPOSURE?(2N)ROUTE?

S32 57 S29 AND S31

S33 22 RD S32 (unique items) – ALL ITEMS PRINTED

S34 4351537 INSECT? OR ARTHROPOD?

S35 941 S9 AND S31 AND S34

S36 35 S35 AND REVIEW?/TI,DE **– ALL ITEMS PRINTED**

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S37 20481533 FOULBROOD? OR BACILLUS? OR LEISSOCOCCUS? OR PATHOGEN? OR D-ISEASE? OR FUNGUS? OR FUNGAL? OR BACTERIA? OR BIOCONTROL?

S38 5977919 NOSEMA? OR MICROSPORIDIA? OR VARROA? OR MITE? OR ACARINE? - OR VIRUS? OR VIRAL? OR PARASIT?

S39 6010 S11 AND (S37 OR S38)

S40 72 S39 AND INTERACT?/TI,DE

S41 48 RD S40 (unique items) – ALL ITEMS PRINTED

SearchSave "SD915239102" stored

Results

The first searches yielded 168 results for mixtures, 702 for interactive effects and 1156 for exposure. The raw output is available as text files (4 files) if supporting information is required.

The wording was corrected to Cumulative and the mixtures searches rerun including Medline and the results incorporated into the databases and duplicates removed,

Additional Medline searches for exposure routes yielded 13 references and for interactions with disease yielded 64 additional references.

The dialog searches yielded a further 65 relevant references of which only 5 were not duplicates.

Duplicates have been removed and clearly apparent irrelevant references also moved to a separate database to produce the EndNote databases. The EndNote databases are being updated with further references as these are identified during the project, e.g. cited in papers/reports or as a result of further searches on Web of Science/OVID and cited references in downloaded papers.

The databases held are:

Exposure: 386 references of which148 contain directly relevant data and 238 contain no specific relevant residue data (see databases)

Mixtures: 103 references of which 84 are specific to honeybees, and 19 relate to other insects

Interactions with disease: 112 references of which 71 are specific to honeybees, 7 to bumble bees and other insects 34

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