Australian Government
Australian Pesticides and
Veterinary Medicines Authority

OVERVIEW REPORT

NEONICOTINOIDS AND THE HEALTH OF HONEY BEES IN AUSTRALIA

FEBRUARY 2014

If new information particularly relevant to neonicotinoid use in Australia becomes available, the APVMA may update this overview report. Any revision of the document will be indicated on the cover page.

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Comments and enquiries regarding copyright:

The Manager, Public Affairs
Australian Pesticides and Veterinary Medicines Authority
PO Box 6182
KINGSTON ACT 2604 Australia

Telephone: +61 2 6210 4701

Email: communications@apvma.gov.au

This publication is available from the APVMA website: www.apvma.gov.au.

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EXECUTIVE SUMMARY

This overview report was prepared as part of a project undertaken by the APVMA to establish whether:

a) the use of the neonicotinoid insecticides in Australia is presenting any more of a risk to the health of honeybees than other pesticides which have been in use for many years; and

b) the current APVMA data requirements for testing of insecticides are adequate to address scientific concerns about subtle effects of neonicotinoids (and other pesticides) on honey bees which have been suggested as impacting their ability to pollinate plants and collect honey.

The APVMA is aware of concerns that insecticides, especially those of the neonicotinoid ('neo-nicotin-oid') class, may be contributing to a decline in honeybee populations in Europe and the USA. Our current reading of the scientific literature is that there is lack of consensus on the causes of these regional declines, with a wide range of possible causes being actively investigated including pesticides, parasites, viruses, climate change, bee nutrition, lack of genetic diversity, and bee keeping practices. Furthermore, these declines are not universal, with evidence that global populations of honey bees are increasing (eg. Aizen & Harder, 2009a, b).

Information and advice available to the APVMA suggests that, in Australia, honey bee populations are not in decline and insecticides are not a highly significant issue, even though they are clearly toxic to bees if used incorrectly. Incidents of beekeepers losing bee colonies as a result of insecticide use do occur, but this most often arises because there has been a break-down in communication between the farmer and the affected beekeeper.

Different crops vary in how much they rely on pollination by bees - some crops such as apples, pears, cherries and almonds depend almost totally on bees for fruit and nut production. In Australia, the Rural Industries Research and Development Corporation (RIRDC) estimates that around 35 different crops are dependent on honeybee pollination for most of their production. However, because of the large number of wild (or feral) European honeybee colonies in Australia, RIRDC notes that the important role of managed pollination by honey bees is not widely recognised or valued and thus only a relatively small proportion of agricultural producers pay for pollination services from commercial beekeepers.

In Australia, beekeeping is undergoing a significant shift away from honey production and into pollination services. For example, increasing almond production will require a significant increase in the need for hives. However, as Australian beekeepers move away from traditional chemical-free sources of nectar and pollen (native scrub and forest) into providing agricultural and horticultural pollination services, there is a commensurate increase in the risk of exposure to agricultural chemicals.

While the neonicotinoids are acutely toxic to honey bees (ranging from slightly to highly toxic, dependent upon the neonicotinoid), this is not a property which differentiates them from many other insecticides which have been in use for many years. Other classes of insecticides will cause problems for bees and other insect pollinators, depending on how much is applied and when and how they are applied. Furthermore, some fungicides and herbicides used in agriculture and horticulture can be toxic to bees. It is not necessarily just the active constituent in pesticide products that can cause toxicity - other product excipients (ie. non-active

constituents in products), especially surfactants (wetting agents), can also be hazardous to bees (eg. Goodwin, 2012).

In general, monitoring data collected from a number of countries suggest that, despite a number of laboratory and semi-field studies describing sub-lethal effects of neonicotinoids on foraging behaviour, learning and memory, few adverse impacts have been observed at doses to which pollinators might be exposed in the field – with the exception of those well-documented cases in several European countries and in Canada of bee mortality caused by acute exposure of bees to neonicotinoid dusts generated during planting of insecticide-coated maize seed. Thus, other than dust generation during planting, there has been only a limited number of cases of poisoning of bees with neonicotinoids in countries where monitoring (either passive or active) has been carried out. Neonicotinoids appear to be less frequently involved in bee poisoning incidents than many other insecticide classes eg. pyrethroids, carbamates and organophosphorus insecticides. However, there are concerns that repeated exposures to sub-lethal concentrations of neonicotinoid residues in plants may make bees more susceptible to other stressors in the environment, including bee pests and diseases. Whereas tests to study the lethal effects of pesticides on bees are well defined and have been adopted into regulatory guidelines, studies related to sub-lethal effects are less well-defined and developed, and have generally not been incorporated into regulatory risk assessment schemes to date.

The introduction of the neonicotinoid insecticides has brought a number of benefits, including that they are considerably less toxic to humans (and other mammals) than the organophosphorus and carbamate insecticides they have significantly replaced. Furthermore, because of the physicochemical properties of a subset of the neonicotinoids, they can be used to coat crop seeds; this insecticide coating protects the seeds and the young plants while they are growing. This means that there is much less need for farmers to apply chemicals to the growing crops using in-field sprays (applied by ground boom or aerial spraying) which have the potential to lead to a greater spread of the pesticide in the environment. Nevertheless, care will have to be taken not to use seed treatments prophylactically, in the absence of a reasonable assessment of the likelihood of insect-pest attack during the development of the crop.

Conversely, there are some potential negatives. For a compound to be effective as a seed-treatment insecticide it needs to be able to be translocated from the seed into the growing plant and to have a reasonable level of stability in the soil and in the plant tissues. The greater environmental stability of certain neonicotinoids (ie. clothianidin, imidacloprid and thiamethoxam) and their ability to translocate within plants means that they do have the potential to present a greater environmental hazard than other less persistent and/or less mobile insecticides. This report summarises the main uses of neonicotinoids in different crops in Australia and the potential for exposure of managed honeybee colonies to these chemicals; this information should allow better targeting of local research on neonicotinoids.

On the basis of information available to it, the APVMA is currently of the view that the introduction of the neonicotinoids has led to an overall reduction in the risks to the agricultural environment from the application of insecticides. This view is also balanced with the advice that Australian honeybee populations are not in decline, despite the increased use of this group of insecticides in agriculture and horticulture since the mid-1990s.

Nevertheless, the APVMA recognises the importance of bees and other pollinators to Australian agriculture and ecosystems, and the risks that pesticides may pose. Thus it will continue to follow the research into

pesticide effects on bees, especially that on the neonicotinoids and related insecticides which act via the nicotinic acetylcholine receptor (nAChR); should research be generated that provides good evidence that a change in the risk management of any chemical is required, the regulatory system will respond accordingly.

The APVMA is working cooperatively with other government agencies, the chemical industry, the agricultural and horticultural industry and the bee-keeping industry to encourage more research and surveillance, to improve bee protection statements on product labels, to promote the use of our adverse experience reporting program (AERP), to encourage product stewardship, to ensure that suitable products are available for treatment of bee diseases, and to help prevent and manage incursions of exotic bee pests and parasites. The APVMA believes that the risks posed by currently-registered neonicotinoid use patterns can be appropriately managed by adopting a range of regulatory, industry stewardship, and educational measures.

Accepting that it is prudent policy to strengthen a range of risk management measures, this report makes a number of recommendations and research suggestions for consideration; some are addressed to the agricultural and horticultural industries, some to the chemical industry, and some to government agencies which support relevant research and extension services, and others to apiarists. This report also considers several recommendations made to the APVMA by a consultant contracted to review the adequacy of (1) the current battery of regulatory tests designed to investigate the effects of new pesticides on bees; and (2) bee protection statements on existing product labels,

As an overall conclusion, current scientific opinion is that pollinator declines in some parts of the world are likely to be caused by multiple interacting pressures including habitat loss and disappearance of floral resources, honeybee nutrition, climate change, bee pests and pathogens, agricultural/horticultural pesticides, miticides and other chemicals intentionally used in hives, and bee husbandry practices. Effective risk mitigation measures will be aided by ongoing collaborative research and monitoring which is directed at identifying and understanding the key risk factors. This will include research to translate test results on sub-lethal effects of pesticides on bees in a controlled laboratory setting to colonies/populations in the natural environment eg. what is the significance of a behavioural effect seen in the laboratory to the health of bees in the field?

If more information comes to light which suggests that there may be a concern with a particular use or uses of a pesticide (or group of pesticides), there are a number of regulatory options available to the APVMA, including a formal chemical review of the neonicotinoid insecticides, using its powers under the Agricultural and Veterinary Chemicals Code Act 1994. Alternatively, it may conduct a more limited label review of insecticide products; this will depend on the outcomes of APVMA's consideration of its current labelling requirements, recommended standard statements and information with respect to bee warnings and product use instructions on existing product labels.

1 INTRODUCTION

For several years now there has been a high level of research activity and a plethora of media articles and web blogs about the apparent severe decline of honeybee populations in the USA and parts of Europe. Reference is often made to 'Colony Collapse Disorder' (CCD), a syndrome first reported in the USA in late 2006.

Much of the focus of this recent research and commentary has been on a group of insecticides commonly known as 'neonicotinoids', with a lack of consensus on whether or not they present any more of a risk to insect pollinators than other insecticides. The focus on the neonicotinoid insecticides as the prime suspect arose from European reports of bee deaths coincident with planting corn seeds coated with these insecticides. In particular there were several reports from Italy in the early 2000's about problems arising during maize planting and in 2008 there was a mass poisoning of bee colonies in a maize-growing area of the Upper Rhine Valley in Germany. This incident, which gained widespread attention, resulted from neonicotinoid dusts emitted from vacuum seeders during planting operations depositing on nearby flowering crops and weeds.

Maini et al. (2010) noted that:-

> "… many hypotheses are available on the problem of declining bee populations …. The problem is made worse by some in the media who sensationalize and report unsupported data and opinion. Such sensationalizing and the use of unsupported data are, unfortunately, not restricted to the media and can be made by scientists who report data that are not sufficiently verified, come from suspected sources, and/or fail to cite relevant research … in some published manuscripts neither the author(s), reviewer(s), [or] editor(s) were acquainted with competing literature".

These comments convey quite well the level of discussion and debate that is occurring at the moment.

The Pesticide Action Network UK aptly summarised it in the comment that "environmental risk decision making is undoubtedly difficult in the context of scientific uncertainty, disputed values, high socioeconomic stakes and political pressure, as is the case for bees and neonicotinoids" (www.bees.pan-uk.org/assets/downloads/Bee_factsheet4.pdf).

Thus, in mid-2012 the APVMA commenced an investigation which sought to establish:-

- whether the use of neonicotinoid insecticides (clothianidin, imidacloprid, thiamethoxam) in Australia is presenting any more of a risk to the health of honey bees than other pesticides which have been in use for many years.

- whether the current APVMA data requirements for testing of insecticides are adequate to address scientific concerns about subtle effects of neonicotinoids (and other pesticides) on honey bees, which have been suggested as impacting their ability to pollinate plants and collect honey.

These investigations have been summarised in this overview report. Key questions considered included:-

- Is there a bee problem overseas?
- Is there a problem here?
- Does Australia's regulatory framework need to be revised in order to better protect insect pollinators?

The report also briefly summarises regulatory actions taken in Europe and North America to date, in response to concerns about bee declines and the suggestion that neonicotinoid insecticides are the key culprits.

The collapse of bee colonies is a very significant economic and environmental concern because of the role both commercial and feral bees play in pollinating crops and many plant species in the environment. Therefore it is important to try and gain an understanding of the cause, or causes, so that appropriate steps can be taken to help ensure their continued contribution to plant reproduction and agricultural production systems.

It should be noted that the APVMA's regulatory 'reach' is limited by the powers given to it under the Agvet Code. Following an appropriate evaluation the APVMA can approve new active constituents and register agricultural and veterinary chemical products (and approve their associated labels); review existing active constituents and agvet chemical products; issue permits for uses of agvet chemicals not included on the label; licence the manufacture of chemical products; and regulate the supply of chemical products. In summary, the APVMA is the Australian Government statutory authority responsible for the assessment and registration of pesticides and veterinary medicines, and for their regulation up to and including the point of retail sale. Thus, while a number of factors can impact bee health, the APVMA's risk management reach cannot address them all. For example, the APVMA does not manage the use of pesticides and veterinary medicines once they are sold (this control-of-use is the responsibility of the states and territories), it is not a research-funding organisation, and it does not have jurisdiction over industry codes-of-practice.

2 BEE COLONY DECLINES OVERSEAS

Examination of historical records shows that reports of significant regional declines in honeybee populations are not uncommon (vanEngelsdorp & Meixner, 2010; OPERA Research Centre, 2013). One such event was reported by beekeepers on the Isle of Wight in 1906. Within a few years all losses of bees in Britain were being ascribed to the "Isle of Wight Disease" (Neumann & Carreck, 2010).

More than a decade ago in Europe, French beekeepers suggested that hive weaknesses of a new type (viz. massive mortality within the few days of the first visits of foragers to sunflower crops or during the following winter) coincided with the 1994 introduction of the neonicotinoid insecticide imidacloprid as a coating for sunflower seeds (Aubert et al, 2004).

Concerns about the neonicotinoid insecticides increased in the 2000s when a number of incidents of bees being acutely poisoned by neonicotinoid dusts generated during planting of insecticide-coated maize seeds using vacuum seeders were reported in Austria, Germany and several other European countries.

Then in 2007 the term 'Colony Collapse Disorder' or CCD was first applied to a drastic increase in losses of honeybee colonies in parts of the USA in late 2006. Even though the term was coined to describe a specific set of symptoms (see below), subsequent bee colony losses in Europe and in several Asian countries were reported in the media as being part of a worldwide CCD 'epidemic'.

Now there are regular media reports, both nationally and internationally, about the problem of declining honeybee populations, with many of these concluding that insecticides of the neonicotinoid class are the prime causal agents.

In summary, Aizen & Harder (2009b) note that claims of global bee disappearance are based on regional examples which are not necessarily representative of global trends. These examples usually come from parts of Western Europe and the USA where limited natural or semi-natural habitat remains. Any declines in stocks of domesticated honey bees in Western Europe and the USA over the 20th century have been more than offset by strong increases in Eastern Europe, Asia, Latin America and Africa. Indeed, it is reported that the number of managed honeybee hives worldwide is estimated to have increased by about 45% in the past five decades. Large bee losses resulting from CCD in the USA and the global spread of Varroa mites present significant problems but they are unlikely to be drivers of any long-term trend. Instead, the decline seen over many decades in the USA and Western Europe, in particular, are consistent with the economic dynamics of the honey industry, which is shifting to developing countries in search of cheaper production.

2.1 Colony Collapse Disorder (CCD)

CCD describes the abrupt disappearance of worker bees from beehives or colonies of the European honey bee. It should be noted that CCD as described in USA has not been observed in Europe (Hendrikx et al, 2009; Genersch et al, 2010a; OPERA Research Centre, 2013). In the USA, CCD is characterised by no adult bees and no corpses, with significant amount of capped brood and stores of honey and pollen (bee

bread) left in the hive, but a lack of healthy adult worker bees inside the hive (Ellis et al., 2010; vanEngelsdorp et al, 2009)[1]. A collapsing colony shows too small a workforce for colony maintenance and that workforce is made up of young bees; this bee cluster seems reluctant to feed on either stored honey or pollen. A reported peculiar symptom is the lack of robbing behaviour by surviving colonies of colonies that have died out (Kevan et al, 2007).

Furthermore, there is little evidence for the occurrence of CCD in Canada (Kevan et al, 2007). There are differences between commercial beekeeping practices in the USA and Canada that may help explain this. For example, migratory beekeeping for pollination services is not such an important part of commercial beekeeping in Canada as it is in the USA, and hive moves are fewer and over shorter distances. And, by and large, Canadian beekeepers probably use fewer chemical and antibiotic control agents against pests and diseases than do their US counterparts and those chemicals that are used are applied more conservatively.

The observations commonly described as CCD are likely to be due to a combination of many factors rather than any single cause. Research by the US Department of Agriculture (USDA) suggests that a combination of environmental stressors may set off a cascade of events and contribute to colonies in which weakened worker bees are more susceptible to pests and pathogens (USDA, 2010; 2011; 2012).

2.2 Bee declines - possible contributing factors

A large number of factors negatively impact honeybee health. A summary list of honeybee stressors is given in the report of an EFSA bee health colloquium held in May 2013 (EFSA, 2013d). Some of these have been suggested as contributing to CCD, including:-

- *Varroa* mite: *Varroa destructor* is an external parasitic mite that attacks honey bees. (Note: Australia has been fortunate to date to avoid any incursion of *Varroa* which presents a major threat to the health of honey bees. Strategies are in place in case any outbreaks are detected.)

- *Nosema* fungus: Nosema (or nosemosis) is probably the most widespread of the adult honeybee diseases. *Nosema apis* and *Nosema ceranea* are microsporidia, small, unicellular parasites recently reclassified as fungi.

- Other fungal diseases of bees: These include 'Chalkbrood' (*Ascosphaera apis*) that infests the gut of bee larvae and 'Stonebrood', caused by *Aspergillus fumigatus, Aspergillus flavus* and *Aspergillus niger*.

- Viral diseases: Eighteen viral diseases of honey bees have been described including: Israel Acute Paralysis Virus (IAPV); Deformed Wing Virus (DWV); and Invertebrate Iridescent Virus type 6 (IIV-6). Little is known about some of these viruses which are often associated with *Varroa* or nosema.

[1] In 2012 the US Department of Agriculture's Agricultural Research Service (ARS) noted that "The defining characteristic of CCD is the disappearance of most, if not all, of the adult honey bees in a colony, leaving behind honey and brood but no dead bee bodies. This definition has recently been revised to include low levels of Varroa mite and other pathogens, such as Nosema, as probable contributing factors (USDA-ARS, 2012).

- Small hive beetle (*Aethina tumida*)
- Acarine mites: *Acarapis woodi* is a small parasitic mite that infests the airways of honey bees.
- Bacterial diseases: American Foulbrood (AFB) and European Fouldbrood are caused by *Paenibacillus larvae* ssp. larvae and by *Melissococcus plutonius*, respectively.
- Stresses related to environmental and climate changes
- Malnutrition: There is some reasonably strong evidence that CCD may not occur in healthy, well-nourished colonies. In the USA, honeybee colonies used for pollination services on large monocultures such as almonds, blueberries and alfalfa may be located in environments where little or no food choice is available to them. It is known that a diverse diet of a mixture of pollens from different plant sources is beneficial to bees, and the same would be true for nectar (Schmidt et al, 1987, 1995). Nutritional imbalance could explain, at least in part, some of the observed symptoms of CCD in the USA. Moreover, the situation for almonds is complicated by the potential toxicity of pollen and nectar from almond flowers, especially in large quantity and for prolonged durations (Kevan & Ebert, 2005; Kevan et al, 2007).
- Lack of genetic diversity: In the USA at least, it has been suggested that a limited gene pool from which nearly all queen bees have descended may be leading to a lack of 'hybrid vigour'.
- Pesticides: Any disappearance of an insect species would implicate pesticides as a potential cause, and CCD is no exception.
- Migratory beekeeping: Relocation of honey bees on a regular basis is likely to be stressful, possibly rendering them less resistant to pathogens. Furthermore, moving hives around the country (as routinely occurs in the USA) aids in the spread of bee pathogens. Migratory beekeeping involves the packing of large numbers of colonies onto the backs of trucks for transport over long distances. Mingling of bees between the hives increases transmission of pathogens. Transportation itself causes colony death - 10% to 30% losses are not uncommon as a result of moving colonies for pollination; migratory beekeepers then split hives into less numerically-sustainable colonies to compensate for the losses. These splits change the natural age structure of the colonies which is itself a further colony stressor. Rapid long-distance movement of bee colonies (e.g. across the USA) may cause disturbances equivalent to "jet-lag" since bees have diurnal rhythms of activity and do sleep. During long-distance moves, hives may be kept in 'staging apiaries' where hundreds of hives are placed cheek-by-jowl - often there is not enough food within the flight ranges of the foragers, hive robbing is common (leading to disease transmission), and hives become weakened despite the efforts of the beekeepers to provide food (pollen or pollen substitute and syrup). Other stressors include confinement in the hives (with stale air, higher carbon dioxide levels, and fluctuating temperatures and moisture levels) and mechanical vibrations. Even moving colonies short distances is well known by apiarists to cause the bees to become upset, so moves taking several days over thousands of kilometers would be considerably more stressful (Kevan et al, 2007; see references cited therein).

- Bee-keeping practices: Bee husbandry practices including the application of chemical miticides and antibiotics may compromise bee health. The difficult task for the apiarist is to differentially kill the pest while not killing the host.

- Electromagnetic radiation / mobile phone signals: This appears to have been largely discounted for a number of reasons, including the observations that CCD occurred in areas without mobile phone coverage and that many apiaries immediately adjacent to mobile phone towers and under high-voltage electrical transmission lines thrive without problem.

- Genetically modified (GM) crops with pest control characteristics

More recently (January 2014) researchers from the USA and China reported that Tobacco ringspot virus (TRSV), a viral pathogen that typically infects plants, has been found in honeybees and can replicate in this host, resulting in detections throughout the body. On the basis of their results, they concluded that the observed negative correlation between the level of TRSV infections and size of host bee populations suggests that this RNA virus, in combination with other viruses, is likely to be a contributing factor to poor survival of honeybee colonies and winter colony collapse (Lian et al, 2014).

An informative document on diseases of honey bees by Plant Health Australia and a number of other national organisations provides information and pictures of the diseases referred to above (PHA, 2012; see www.animalhealthaustralia.com.au/programs/biosecurity/biosecurity-planning/honey-bees/).

Other conditions of bees, not usually suggested as being possible contributing factors in CCD, include:-

Dysentery: This refers to a condition resulting from a combination of long periods of inability to make cleansing flights (generally due to cold weather) and food stores which contain a high proportion of indigestible matter.

Chilled brood: This is not a disease but can be caused by a sudden drop in temperature or when a beekeeper opens a hive and inadvertently prevents nurse bees from clustering over the brood to regulate temperature.

Wax moths (*Aphomia sociella*) and Greater wax moths (*Galleria mellonella*) cause destruction of the honeycomb and may kill bee larvae.

Environmental toxins: Chemicals other than those used in agriculture/horticulture or deliberately applied as miticides/acaricides cannot be discounted as having an impact on bee health; foraging bees may be impacted by urban, household or industrial chemicals.

Naturally-occurring toxins: There is an extensive literature on plants that have pollen which is toxic to honey bees[2] (eg. Vieira de Melo, 2013). There is also some evidence that under conditions of stress,

[2] Almond (*Amygdalus communis*) is commonly cited as a plant which is toxic to honey bees because its nectar and pollen contains the cyanogenic glycoside amygdalin. However, research (University of Haifa, 2010) suggests that even though amygdalin is toxic to mammals, it is not poisonous for honey bees, but rather is an insect attractant.

some plants can produce toxic components in pollen and/or nectar (e.g. some Australian Eucalyptus species may do so on occasion). Under certain weather conditions nectar can ferment to produce alcohol (ethanol) which can cause alcohol poisoning in bees [see Report 2 below under the section on 'Adverse Experience Reports (AERs)'].

2.3 Pesticides

With respect to pesticides, a number of scientists have been concerned that insecticides and possibly some fungicides may have sub-lethal effects on bees, not killing them outright but instead impairing their development, behaviour and immunity to parasites and diseases. While the N-nitroguanidine neonicotinoids (clothianidin, imidacloprid and thiamethoxam) are the focus of current attention with respect to sub-lethal effects (and have been for the past decade or so), there is an extensive literature on such effects of a number of other pesticides on honey bees eg. Maini et al. (2010) refer to studies on the insecticides acetamiprid, cyhalothrin, deltamethrin, fipronil and parathion, and the fungicides captan, chlorothalonil, myclobutanil and propiconazole, among others. Furthermore, it is quite likely that interactions (additive, synergistic, or antagonistic) may occur between different pesticides; in the UK, for example, advice has been issued not to spray pyrethroid insecticides together with EBI[3] fungicides (Barnett et al, 2007). Laboratory studies indicate a synergistic effect occurring between EBI fungicide and a neonicotinoid insecticide in honey bees (Schmuck et al, 2003a). In laboratory studies Isawa et al. (2004) found that a number of fungicides (applied one hour before dosing with insecticides) significantly increased the 24-h acute topical toxicity of several neonicotinoids.

Mullin et al. (2010) found 121 different pesticides and metabolites within 887 wax, pollen, honeybee and associated hive samples taken in the USA, which indicates that attributing findings in bees to any one pesticide will prove to be a difficult task.[4]

Honey bees may be affected by neonicotinoids when they are used as a seed treatment because they are known to translocate through the plant up into the flowers and leave residues in the nectar and pollen, albeit at very low levels. The doses taken up by bees are not acutely toxic but it is feasible that there may be sub-lethal effects or chronic problems caused by cumulative exposure. Concerns about neonicotinoids have also arisen from direct poisoning of bees flying into dust from pneumatic seeding ('drilling') machines, and from dust drifting onto flowering plants near the field being planted. (These and other routes of exposure of bees to pesticides are discussed in more detail below.)

It also needs to be borne in mind that the chemicals deliberately used within hives to treat Varroa mites are insecticides; it is quite possible that the very nature of the Varroa treatments themselves are causing significant problems. For example, Williamson & Wright (2013) report that coumaphos, a commonly-used Varroa control chemical, affected important bee behaviours involved in foraging. In many countries (including the USA) treatments for Varroa have been taking place over many years. If beekeepers don't regularly

[3] Ergosterol biosynthesis inhibitor

[4] By contrast, a sample of beeswax foundation purchased from a supplier in Brisbane and tested for 171 different chemicals in the same USDA laboratory only found one (1) chemical, chlorpyrifos, at 165 ppb (J Draper, 2011; *pers. comm.*)

change their brood combs – reports suggest that some can stay in a hive for many years – then there will be a build-up chemicals which have an affinity for beeswax, leading to increasing hive health problems over time.

3 WHAT ARE NEONICOTINOIDS?

Neonicotinoids are a class of insecticides which act on acetylcholine receptors (AChR) in the nervous system of insects. These receptors have been historically divided into two broad types, those activated by nicotine and those by muscarine. Neonicotinoids activate nicotinic acetylcholine receptors. These receptors are located in both the central (CNS) and peripheral nervous systems (PNS) of mammals but are limited to the CNS in insects. In normal synaptic transmission between nerve cells the naturally-occurring transmitter acetylcholine is broken down by an enzyme called acetylcholinesterase (AChE) and this ends nerve signalling. However, AChE cannot break down neonicotinoids which bind to AChR, leading to overstimulation of the insect nervous system, paralysis and death.

It is important to note that most neonicotinoids bind much more strongly to insect AChRs than to mammalian AChRs and thus are selectively more toxic to insects than to mammals (eg. Jeschke & Nauen, 2010).

Table 1 lists the neonicotinoid insecticides plus two related compounds which, while not classified as neonicotinoids (see footnote to Table 1), also act via nicotinic acetylcholine receptors. Neonicotinoid insecticides can be chemically classified as N-nitroguanidines (imidacloprid, thiamethoxam, clothianidin and dinotefuran), nitromethylenes (nithiazine, nitenpyram), and N-cyanoamidines (acetamiprid and thiacloprid) (Jeschke et al, 2011).

Clothianidin, imidacloprid and thiamethoxam are used as seed-treatment insecticides while the soil stability of dinotefuran, the other N-nitroguanidine neonicotinoid, is too limited for use as a seed treatment. The limited soil stability of nitenpyram and acetamiprid also mean that these compounds are not used as insecticide seed coatings.

In terms of bee toxicity, the N-cyanoamides acetamiprid and thiacloprid have a significantly more favorable profile than the N-nitroguanidine neonicotinoids and are usually applied to crops as foliar sprays.

Table 1 List of neonicotinoids and related compounds

GENERIC NAME	ORIGINATOR COMPANY	NOTES
Neonicotinoids		
Acetamiprid	Aventis Crop Sciences	
Clothianidin	Takeda Chemical Industries* & Bayer	
Dinetofuran	Mitsui Chemicals	
Imidacloprid	Bayer CropScience	
Nitenpyram	Novartis Animal Health	Veterinary uses only
Nithiazine	Shell Development Co.	Prototype neonicotinoid – early 1970s
Thiacloprid	Bayer CropScience	
Thiamethoxam	Syngenta	Active metabolite is clothianidin
Other related insecticides acting at nicotinic AChRs		
Sulfoxaflor[5]	Dow AgroSciences	
Flupyradifurone	Bayer CropScience	

**Takeda's agrochemical interests were transferred to Sumitomo Chemical Co. Ltd in 2007*

[5] Like the neonicotinoids, sulfoxaflor acts as a nicotinic acetylcholine (nAChR) receptor agonist. Because of its chemical structure, the novel way it interacts with the nAChR, and its lack of insecticidal cross-resistance with the neonicotinoids, sulfoxaflor is not normally referred to as a neonicotinoid. Because of its lack of cross-resistance to neonicotinoids the international Insecticide Resistance Action Committee (IRAC) has separately categorised it within mode-of-action (MoA) Group 4 [nicotinic acetylcholine receptor agonists]; it has been placed in Group 4C while the neonicotinoids are in Group 4A (see the Mode-of-Action classification table (v 7.2; February 2012) at www.irac-online.org/content/uploads/MoA-classification.pdf).

4 REGISTERED USES OF NEONICOTINOIDS IN AUSTRALIA

Attachment 1 lists the neonicotinoids approved in Australia and their registered label uses (application method and crop type). In agriculture, the main routes of application include seed treatment, foliar spraying, soil spraying, and soil incorporation of granules.

The N-nitroguanidine neonicotinoids imidacloprid and thiamethoxam are used as seed dressings for a number of different crops. Imidacloprid[6] and thiamethoxam products are registered for seed treatment of canola, cereals, cotton, maize, sweet corn, sorghum and sunflower; imidacloprid products are also registered for seed treatment of lentils and lupins, faba beans, field peas, pulses, forage and seed pasture (eg. red clover, subterranean clover, strawberry clover, white clover, ryegrass, phalaris, lucerne, medics), and forage brassicas (Kale, turnip, rape and swedes). At the time this report was prepared there were no registered seed-treatment uses for clothianidin in Australia.

In addition to seed-treatment uses, there are a number of neonicotinoid products approved for use as foliar or trunk sprays eg. products containing acetamiprid, clothianidin, imidacloprid and thiamethoxam are approved for foliar spraying of cotton while products containing clothianidin, imidacloprid and thiacloprid are approved as sprays for stone and pome fruit.

[6] APVMA's PubCRIS lists at least 24 different imidacloprid products sold in Australia as seed treatments for canola.

5 AGRICULTURAL AND ECONOMIC IMPORTANCE OF NEONICOTINOIDS

Neonicotinoids are registered globally in more than 120 countries, and they are among the most effective insecticides available for control of sucking insect pests such as aphids, white flies, leaf- and plant-hoppers, thrips, some micro-lepidoptera, and a number of coleopteran (beetle) pests. They have broad-spectrum activity, with contact, stomach and systemic activity. Their physicochemical properties mean that they are very versatile in terms of application methodology, being used for foliar, seed treatment, soil drench and stem applications in a wide range of crops.

The neonicotinoids are the most significant chemical class of insecticides introduced to the global market since the synthetic pyrethroids.

Imidacloprid was the first neonicotinoid insecticide to come into commercial use, in 1991. It is currently the most widely used insecticide worldwide. It is applied against pests in/on soil, seed, timber and animals, and is in products for foliar application to turf and food crops including cereals, cotton, legumes, potatoes, pome fruits and vegetables. It is a systemic insecticide with particular efficacy against sucking insects and has quite a long residual activity. Because of its water solubility it can be added to the water used to irrigate plants.

In 1990 (before the launch of imidacloprid), the insecticide market was dominated by organophosphorus insecticides ('organophosphates' or OPs) (ca. 43%), pyrethroids (ca. 18%) and carbamates (ca. 16%). By 2008, neonicotinoids had gained around 24% share of the total insecticide market, mainly at the expense of the OPs and carbamates (which fell to around 13.6% and 10.8% of the insecticide market, respectively, at this time). In the past several years the use of neonicotinoid insecticides has grown further; in 2009 imidacloprid was estimated to account for ca. 41.5% of the global neonicotinoid market, making it the largest selling insecticide in the world. Thiamethoxam was second in terms of sales, followed closely by clothianidin. Together, the N-nitroguanidine class of neonicotinoids account for around 85% of the neonicotinoid insecticide market. Nevertheless, sales of other neonicotinoids have grown as well (Jenschke et al, 2011).

The application rates for neonicotinoid insecticides are commonly much lower than older classes of insecticides (eg. Jeschke et al, 2010).

When compared with OPs and carbamates, neonicotinoids pose lower risks to humans and other mammals.

As a result of their mechanism of action, there is no cross-resistance to other insecticide classes, including the so-called organochlorines (now superseded), OPs, carbamates and pyrethroids. Their introduction has increased the insecticide armentarium available to farmers and horticulturalists, thus helping to prevent the build-up of resistance of insect pests to OPs and pyrethroids. Nevertheless, pests can evolve resistance to neonicotinoids; the first reported example was the silverleaf whitefly, Bemisia tabaci (Thany, 2010).

One problem with systemic insecticides is that the selection pressure placed on pests from the ongoing presence of the insecticide, coupled with their site-specific, mode of insecticidal action, may result in the development of resistant insect genotypes. Conversely, the benefits of using systemic insecticides like the neonicotinoid seed-treatment insecticides include (1) plants are continuously protected throughout most of the growing season without the need for repeat spray applications of insecticides; (2) these insecticides are

not susceptible to UV light degradation or 'wash off' during watering; (3) there is no surface residue on the crop and hence a reduced risk to agricultural workers performing in-crop activities.

A comprehensive review of the value of neonicotinoid seed treatments has been published by the Humboldt Forum for Food and Agriculture e.V. (Noleppa & Hahn, 2013). It investigated the socioeconomic and environmental contribution made by this technology to the European Union across major crops and key countries, and the significant economic and social impact should the technology no longer be available because of bans or suspensions.

An analysis of collated sales figures held by the APVMA suggest that the value of sales of neonicotinoid insecticide products is approximately 1/5th of total insecticide sales (including domestic-use insecticides). Over the past five financial years (from FY 2007/08) there has been a slight decline in this proportion because the value of overall insecticide sales has increased at a faster rate than the value of sales for insecticide products containing neonicotinoids. It should also be noted that more than half the sales value of neonicotinoid-containing products is made up of animal-treatment products (including domestic pet care products).

6 EXPOSURE OF POLLINATORS TO NEONICOTINOIDS

Arising from the registered uses of neonicotinoids, pollinators may be exposed to them by:-

- contact with neonicotinoid dusts arising during planting of seeds coated with the insecticide
- intake of systemic residues in nectar, pollen and guttation fluid of the plant, arising from neonicotinoid treatment of the seed used to grow the plant or from application of a neonicotinoid insecticide (as a spray or granule) to the soil in which the plant was grown
- contact with foliar sprays applied to the flowering plant (e.g. canola).

Since neonicotinoids are used in the same way around the world, these possible exposure routes could occur in Australia as in other countries.

6.1 Seed-treatment dusts

More than a decade ago there were reports from various European countries including Austria, Germany, Italy and Slovenia of honeybee poisonings which occurred at the same time as the spring sowing of maize seeds coated with neonicotinoids (see eg. Forster, 2011). In the most reported incident, approximately 12,000 bee colonies in Germany were poisoned in 2008 (Pistorius et al, 2008; Forster, 2009); these extensive poisonings in the Upper-Rhine Valley were attributable to high quantities of clothianidin-contaminated dusts from coated maize seeds being emitted into the air by vacuum-pneumatic seeders and depositing onto nearby flowering plants (eg. oilseed rape, fruits, weeds). It was subsequently established that the quality of the seed dressing was poor (inadequate stickers and binders), allowing the release of the abraded seed coating as fine dust. It appears that these corn seeds had been treated in controlled industrial facilities since there was negligible on-farm treatment of corn seeds in Central and Western Europe at the time. However there have been substantial improvements in seed treatment practices and quality standards since then (see below).

An Italian study found clothianidin and imidacloprid in the exhaust of pneumatic seeding equipment (Girolami et al, 2012). In the USA almost all Bt corn seed[7] is treated with neonicotinoids and a 2010/11 study conducted in the USA found high levels of clothianidin and thiamethoxam in pneumatic planter exhaust and in the soil and on dandelions in unplanted fields nearby (Krupke et al, 2012). There is evidence from Canada that dusts from improperly formulated or applied seed treatments can acutely kill bees; in 2012 several provinces in Canada reported bee poisonings during the sowing of neonicotinoid-treated corn seed.

[7] Bt corn is a genetically-modified corn containing a gene which codes for a protein from a naturally-occurring soil bacterium, *Bacillus thuringiensis*. This protein (called *Bt* delta endotoxin) is expressed in the corn plant and is highly effective at controlling caterpillars feeding on the corn but is generally not harmful to other insects such as beetles, flies, bees and wasps. Growers use *Bt* corn as a safer alternative to spraying insecticides.

Since 2008, regulations and product stewardship in Europe have largely eliminated this dust problem by controlling both the process of seed treatment and of planting (see eg. Nikolakis et al, 2009). For example, in Germany:-

- Seed treatment can only be performed in professional seed treatment facilities which are registered, have a quality control regime in place (covering staff training, improved coating technology, compliance with maximum permissible levels of dust, appropriate packing, storage and transport of treated seeds) and are subject to independent inspection.
- Packages of treated seed must bear a label advising that:-
    - the seed can only be sown using a pneumatic seeding machine of a type tested and registered by the German Federal Research Institute for Cultivated Plants
    - the seed can only be sown if the wind speed at planting does not exceed 5 m/sec
    - the treated seeds and any dust they produce must be completely incorporated in the soil
    - at least 48 h before seeding, the farmer/farm manager must notify beekeepers with hives located within 60 m of the sowing area (Forster, 2011).

However, concerns have been expressed that equipping maize planters with deflectors may still not have completely solved the dust problem arising during planting (Krupke et al, 2012; Sgolastra et al, 2012).

In Canada, product stewardship measures have been put in place (2012/13) to deal with the issue of neonicotinoid dusts arising from planting coated seeds.

The APVMA has not received any reports of bee poisonings arising from the generation of neonicotinoid dusts during planting of coated seeds in Australia. Saul Cunningham, a bee researcher and Group Leader in Ecology, Ecosystem Sciences / Sustainable Agriculture Flagship, the Commonwealth Scientific and Industrial Research Organisation (CSIRO) also advised that he was not aware of any such instances (*Pers. comm*, 4 Dec. 2013). [Further information about planting of treated seeds for specific crops can be found in Section 7 of this report.]

6.2 Systemic residues in plants

Pollinators may be exposed to neonicotinoids as systemic residues in plant tissues. These residues can arise from uptake via the roots of neonicotinoids applied as seed treatments, soil sprays or soil-incorporated granules or tablets. The absorbed insecticide is then translocated by the plant xylem from the roots to the foliage. Systemic residues may also arise following foliar application of neonicotinoid sprays. The following sections discuss systemic residues following these different insecticide application methods.

6.2.1 Seed treatments

There has been an extensive debate in the literature on whether the systemic levels of neonicotinoids occurring in nectar and pollen following seed-treatment uses of this class of insecticides are adequate to

acutely impair honey bees or other insect pollinators. The evidence is that they do not present an acute poisoning problem, but whether the levels are high enough (either alone or in combination with other pesticides to which bees might be exposed) to subtly affect bee behaviour (and ultimately affect colony health) is a subject of intense ongoing research (as the extent of the reference list associated with this overview report attests).

Frazier et al (2011) summarised a number of studies and noted that acute LD50s for imidacloprid and clothianidin in bees were around 28 and 24 ng/bee respectively, with sub-lethal effects reported at much lower levels. The lowest observed sublethal effects for imidacloprid in laboratory studies occur at doses around at 1 ng/bee, equivalent to 10 ppb for an average-size bee (100 mg). Achieving a 10 ppb dose would require consuming pollen with residues of 250 ppb imidacloprid at a consumption rate of 4 mg pollen/day (4% of bee's body weight). This level of residue is never found when label rates of 'Gaucho' (trade name for a key imidacloprid seed-treatment product) are used as a seed treatment (generally 1-5 ppb in pollen). Nectar residues of imidacloprid are usually less than in pollen, although more nectar than pollen is consumed over the bee's life. However, even if forager bees ingest 10% of their body weight in nectar per day, it would require 100 ppb of imidacloprid in the nectar to achieve a 10 ppb dose per day. Imidacloprid is known to be rapidly metabolised by bees and is excreted with a half-life of about 5 hours.

Thus, more than double the above doses of imidacloprid in the food would be required to maintain a level that keeps up with its rapid clearance from the bee. Thus, it appears unlikely that doses of neonicotinoids from routine systemic seed treatments would attain the necessary >100 ppb levels in pollen or nectar to acutely impair honey bees. However, it is possible that concentrations of neonicotinoids in guttation water from glandular exudations on young plants[8] grown from treated seed[9] could acutely kill bees.

Following the mass bee poisoning incident in the Upper-Rhine Valley in Germany in 2008 (in which clothianidin dust arising from planting of neonicotinoid-treated maize seed deposited on nearby flowering plants and poisoned ca. 12,000 bee colonies - see 'Seed-treatment dusts' section above), beekeepers in the region were concerned that when the maize flowered, systemically-translocated residues might cause new poisoning incidents. However, residue analyses following several poisoning reports concluded that they were not linked with maize but resulted from pesticide spray applications to other crops (Pistorius et al, 2009). Monitoring of damaged bee colonies in the region did not show any adverse effects on bee health during and after flowering of the maize, and there were no effects on overwintering strength, overwintering success and colony strength in the spring of 2009 (Liebig et al, 2008).

[8] In corn, neonicotinoid (thiamethoxam, clothianidin, imidacloprid) concentrations in guttation drops have been shown to progressively decrease during the first 10-15 days after the emergence of the plant from the soil (Tapparo et al, 2011).

[9] There appears to be a paucity of published information on guttation levels after other routes of application. Neonicotinoids move in the xylem sap flow of the plant in an upward direction and have most complete plant penetration by root uptake (seed or soil application). Theoretically, if a young plant is sprayed, any part of the plant (including guttation fluid) at or above the application point could contain the applied insecticide.

6.2.2 Soil-incorporated granules, soil sprays etc.

Systemic plant residues may arise from the use of neonicotinoids in granules incorporated in the soil at planting and from soil sprays and drenches.

Studies indicate that clothianidin degraded moderately under field conditions (Stupp & Fahl, 2003). Field trials in Europe and the USA on the degradation of imidacloprid demonstrate that it does not accumulate in the soil following repeated yearly applications (Krohn &Hellpointer, 2002). Thiamethoxam showed a moderate to fast degradation rate under field conditions (Maienfisch et al, 1999a). For thiacloprid, half-lives in soil measured under field conditions of northern Europe ranged from 9 - 27 days, and in southern Europe, from 10 - 16 days (Krohn, 2001).

Nevertheless, Krupke et al (2012) found neonicotinoids in the soil of sampled fields, including unplanted fields, near maize and soybean production fields in north western Indiana. Dandelions growing near these fields were also found to contain neonicotinoids. While this could have arisen from insecticide deposition on the flowers, uptake by the root system of neonicotinoids in the soil was suggested as a possibility.

Laboratory studies and field observations suggest that there could be conditions under which, depending on the neonicotinoid, a greater (or lesser) degree of soil persistence might occur. A 2005 field study (cited in Goulson, 2013) which randomly sampled farmland soil in France for imidacloprid found that nearly all soil samples from conventional farms contained detectable levels, even those that had not applied the chemical in the previous year; of the 67 samples from these farms, 9 contained between 10 and 100 ppb imidacloprid, and 3 exceeded 100 ppb.

Note that applications to the APVMA for the registration of plant protection products (including the neonicotinoids) require the submission of data from trials to determine the nature and amount of pesticide residues in crops (so-called follow crops) grown in soil previously used to grow a crop which was treated with the pesticide. Commonly, three different rotational intervals are used to be representative of immediate replanting after failure of the treated crop, a typical crop rotation following harvest of the treated crop, and a typical rotation in the following year.

6.2.3 Foliar sprays

As well as being systemic insecticides, neonicotinoids have trans-laminar or local systemic activity. Trans-laminar movement is the ability of the compound to penetrate the leaf cuticle and move into the leaf tissue, thus controlling insects feeding on the unexposed side of the leaf not directly exposed to the foliar spray, as well as sucking insects that feed on plant juices inside the leaf. Whether there is only local systemic activity in the leaf receiving the spray or whether there is xylem movement resulting in a broader distribution of residues in the whole plant will depend on the stage of the plant's growth when the spray is applied. In general, there is likely to be both trans-laminar activity and xylem movement when actively-growing plants are sprayed. If the plant has reached maturity and no more meristems are actively growing, then the movement of the insecticide will be trans-laminar and limited ie. the residues will be largely confined to the leaves.

6.3 Contact with foliar sprays

Bees and other pollinators can be acutely poisoned by contact with insecticide sprays, either during spraying or to the un-dried spray on plant surfaces. Most insecticides are likely to be problematic in this regard, not just neonicotinoid sprays. The Rural Industries Research and Development Corporation (RIRDC), which has supported extensive research on crop pollination by honey bees[10], notes (at www.rirdc.infoservices.com.au/items/10-116) that:-

> One of the biggest drawbacks of placing bees near any agricultural crop is the possibility of colonies or field bees being affected by pesticides. Pesticides should be kept to a minimum while hives remain on the property. Most poisoning occurs when pesticides are applied to flowering crops, pastures and weeds.
>
> It is strongly recommended that growers take the following steps to prevent or reduce bee losses:
>
> - follow the warnings on pesticide container labels
> - select the least harmful insecticide for bees and spray late in the afternoon or at night
> - do not spray in conditions where spray might drift onto adjacent fields supporting foraging bees
> - dispose of waste chemical or used containers correctly
> - always warn nearby beekeepers of your intention to spray in time for steps to be taken to protect the bees; give at least two days' notice
> - always advise nearby farmers.

[10] See RIRDC's pollination website at www.rirdc.gov.au/research-programs/rural-people-issues/pollination for a large amount of extension information for farmers and bee keepers.

7 EXPOSURE OF POLLINATORS TO NEONICOTINOIDS IN AUSTRALIA

In considering which agricultural/horticultural situations in Australia might lead to insect pollinators being exposed to neonicotinoids, the following list summarises the possible scenarios:-

- plant/crop is grown from treated seed (or in treated soil) and is attractive to pollinators
- plant/crop has foliar spray applied but not at a time near flowering
- plant/crop has foliar spray applied at or near flowering
- plant/crop is treated but is not attractive to pollinators and/or (in the case of hive bees) is not used by apiarists - doesn't produce the required honey, or pollen/nectar is of poor nutritional quality
- plant/crop is not treated with neonicotinoids

The following section provides a brief overview of the main crops on which neonicotinoids may be used during the growing season, either as seed treatments, soil applied treatments, or foliar sprays. Where information is available to determine which of the above scenarios applies to a particular crop, the likelihood of insect pollinators being exposed to neonicotinoids from their use in that crop is assessed.

In addition to information about agricultural and horticultural uses of neonicotinoids (ie. crops applied to; method of application; see above under 'Registered uses of neonicotinoids in Australia'), it is important to have information about the importance of insect pollinators to the production of various crops ie. how dependent is a particular crop on pollination by insect pollinators and thus how likely is it that hives will be placed in or near the crop? This is summarised in the following summary table reproduced from the website Australian Government the Department of Agriculture (The Department of Agriculture, 2011) – see www.daff.gov.au/animal-plant-health/pests-diseases-weeds/bee_pests_and_diseases/honeybees-faqs).

Table 2 Dependence of different crops on pollination by insects (from Klein et al, 2007)

LEVEL OF BIOLOGICAL DEPENDENCE ON INSECT-MEDIATED POLLINATION				
ESSENTIAL	GREAT	MODEST	LITTLE	NONE
Kiwifruit	Apple	Cotton	Capsicum	Sugar cane
Passionfruit	Mango	Coffee	Tomato	Corn/Maize
Macadamia	Blackberries & related berries	Faba bean	Kidney bean	Wheat
Watermelon	Cherries	Soya bean	Peanut	Rice
Rockmelon	Plums	Sunflower	Papaya	Barley
Pumpkin, squash & zucchini	Avocado	Chestnut		Sorghum
	Almonds			Chickpea
	Canola			Grapes
	Cucumber			

Essential = pollinators essential for most varieties (production reduction by 90% or more comparing experiments with and without animal pollination)
Great = animal pollinators are strongly needed (40-90% reduction)
Modest = animal pollinators are clearly beneficial (10-40% reduction)
Little = Some evidence suggests that animal pollinators are beneficial (0-10% reduction)
None = no production increase with animal mediated pollination

Neonicotinoids are used in the following crops, as seed treatments and/or foliar sprays.

7.1 Canola

In Australia canola seems to be a crop of particular concern with respect to neonicotinoids and bee feeding because (1) it is now Australia's third-largest broad-acre crop after wheat and barley (>970,000 ha sown in 2008); (2) it is a crop which significantly relies on insect pollinators for good yields; (3) canola blossom is frequently one of the earliest floral species available in spring to commercial honey bees in the southern areas of Australia, flowering from September to October; (4) it produces abundant quantities of nectar and pollen with high protein content and thus is an important floral resource for beekeepers; and (5) almost all canola seed in Australia is treated with a neonicotinoid prior to planting.

There has been a difference of opinion over the need for insect pollination of canola. Some reports claim that canola is largely self-pollinated and does not need honey bees, whereas the weight of evidence indicates greater seed yields (more pods per plant, more seeds per pod, and higher rates of germination of resultant seed) when honey bees are present (Keogh et al, 2010b and references cited in this RIRDC 'Pollination

Aware' note; see also the Department of Agriculture's Q&A website at www.daff.gov.au/animal-plant-health/pests-diseases-weeds/bee_pests_and_diseases/honeybees-faqs). Honey bees in particular, whether feral or hive bees, represent a beneficial and important pollen vector for optimal canola yield. An informative NSW Agriculture 'Agnote' brochure titled Honey bees on canola (Somerville, 2002; available at www.dpi.nsw.gov.au/__data/assets/pdf_file/0013/117112/bee-on-canoloa.pdf) provides a concise overview of some key facts relating to bees and canola.

Insect pollinators working canola could potentially be exposed to neonicotinoids from (1) dust from seed coating arising during planting; (2) residues in nectar, pollen and guttation fluid arising from systemic absorption of the insecticide into the plant from the coated seed; and (3) direct exposure to foliar insecticide sprays applied around the time of canola flowering.

The risk of neonicotinoid dusts arising during canola planting appears to be very low because in Australia the low pressure air seeders which are used to plant canola vent directly into the furrow and there is no generation of dust clouds.

There has been considerable debate about whether the low levels of residues in canola nectar and pollen arising from seed treatment are having any impact on pollinators; this issue is considered elsewhere in this report (see above under 'Systemic residues in plants'). Advice provided to the APVMA during the preparation of this report suggests that, in the main, honey bees are doing well on canola in Australia. [See also further discussion on this issue in section 15.2 of this report ('Research Suggestions') under 'Canola'.]

There is no doubt that foliar application of neonicotinoid sprays (and indeed any other insecticide sprays) around the time of canola flowering will present a risk to pollinators. This is a risk that can be addressed, at least in part, by appropriate label warnings and by increasing grower awareness of the value of insect pollinators to improved canola yields – they will then be more inclined to consider whether there is a need to spray and, if so, the timing of spraying in relation to pollinator activity in the crop. In Australia there are currently no approved foliar spray uses in canola for any of the neonicotinoid insecticides listed in Table 1. A product (Dow AgroSciences 'Transform') containing sulfoxaflor which is related to the neonicotinoids (it targets the same receptors; see footnote to Table 1) is approved as a foliar spray in canola - its label contains the bolded caution that 'this product is highly toxic to bees: read the PROTECTION OF LIVESTOCK[11] section in this booklet before use'. The use instructions also advise that 'if honeybees are present in the target area during flowering, see the PROTECTION OF LIVESTOCK directions'. The text under the PROTECTION OF LIVESTOCK heading is as follows:-

> Highly toxic to bees. Will kill foraging bees directly exposed through contact during spraying and while spray droplets are still wet. May harm bees in hives which are over-sprayed or reached by spray drift.
>
> **Do Not** apply this product while bees are foraging in the crop to be treated.
>
> Treatments made to crops in flower or upwind of adjacent plants in flower that are likely to be visited by bees at the time of application, should not occur during the daytime if temperatures within an hour

[11] Since honey bees are not native to Australia, they are classified as livestock.

after the completion of spraying are expected to exceed 12°C. It is recommended that orchard floors containing flowering plants be mown just prior to spraying. Beekeepers who are known to have hives in, or nearby, the area to be sprayed should be notified no less than 48 hours prior to the time of the planned application so that bees can be removed or otherwise protected prior to spraying.

7.2 Cereals

Imidacloprid and thiamethoxam seed treatment products are approved for treating cereal seeds. However, wheat, barley, oats, rice, corn etc. (the world's staple food crops ie. they provide more food energy than any other type of crop) are pollinated by wind and thus are not reliant on honey bees for seed set. Since they do not need to expend energy to produce colourful petals, nectar, or an attractive odour, they are not bee-attractive crops and are of no value to apiarists.

Therefore the risks to insect pollinators from neonicotinoid treatment of cereal crops are likely to be very low.

7.3 Clover & other pasture

Products containing imidacloprid are approved in Australia for coating forage and pasture seeds, including red clover, subterranean clover, strawberry clover, white clover, ryegrass, phalaris, lucerne and medics.

A wide range of clovers and sub-clovers are grown in Australia, in temperate, sub-tropical and tropical regions. Pollination of these mostly self-incompatible species is necessary for seed set. Pollination has consistently been identified as a major limiting factor to higher, more reliable clover seed yields and improved seed quality. Research in Victoria suggested that honey bees made up 88% of all insect visitors to white clover (Goodman & Williams, 1994). Red clover flowers are almost completely self-sterile and require cross-pollination to produce seed; it appears that honey bees are satisfactory pollinators of red clover provided that they are sufficiently numerous and that competing bloom is not too abundant. Little is known about the pollination of purple clover although the bright flower colour and observations of extensive bee activity suggest that it is also pollinated by bees (Keogh et al, 2010c and references cited in this RIRDC 'Pollination Aware' note).

Different clover species grown represent significant nectar resources for beekeepers, particularly in the New England area of NSW, Victoria and Tasmania. Somerville (2001) noted that pollen of white clover is of very reasonable quality and white clover produces choicest quality honey. Beekeepers are quite keen to work lucerne (T Weatherhead, AHBIC, 2013; *pers. comm.*)

Planting of pasture seed is by broadcasting (including aerial broadcasting) or shallow drilling.

The APVMA has no information about whether neonicotinoid seed treatment of clover and other forage and seed pasture (eg. ryegrass, phalaris, lucerne, medics) is causing any problems, arising either from dust generation during broadcasting or shallow drilling of coated seed, or from systemic residues occurring in the pollen and nectar. The potential for exposure of bees cannot be discounted.

7.4 Corn/Maize

In the USA corn is the number one broad-acre crop in terms of area under cultivation and economic value; the USA is by far the largest producer of corn in the world, producing over 30% of the world's corn.

In Australia sweet corn/maize[12] is a relatively minor crop, both in area and production compared to other summer crops such as sorghum and cotton. However, it has the widest geographical spread of all the field crops, being grown from tropical North Queensland to as far south as Tasmania. The total area of production varies from season to season, but over the 5 seasons to 2004 it averaged 72,000 ha (Maize Association of Australia website at www.maizeaustralia.com.au/austoverview.html).

The majority of the grain production is used domestically by a wide range of industries, ranging from stockfeed to human food uses including breakfast cereals, snack foods and for starch extraction. A significant area of maize is also grown for whole plant silage, especially for dairy farmers and cattle feedlots.

Over 90% of USA seed corn is treated with neonicotinoids (either clothianidin or thiamethoxam) and a similar statistic would apply to corn planted in Australia. As for canola, insect pollinators could potentially be exposed to neonicotinoids (1) from dust from seed coating arising during planting; (2) from residues in nectar, pollen and guttation fluid arising from systemic absorption into the plant from the coated seed; and (3) direct exposure to foliar insecticide sprays applied to the maturing crop. There is a significantly greater potential for dust generation during corn seeding (as cf. canola) because of the increasing use of vacuum seeders; fans which create a vacuum to 'suck' seeds onto a rotating seed plate vent directly to the air and hence there is a risk of generation of dusts which could poison flying pollinators directly or deposit on nearby flowering plants.

However, it appears that corn growing in Australia will present only a low risk to pollinators. As indicated in Table 1 (above) corn is not reliant on insect pollination for seed set or yield improvement. Furthermore, published research (eg. Höcherl et al, 2012) indicate that feeding bees on a pure maize pollen diet is linked to a reduction in brood rearing and lifespan; as a general rule any pollen from plants that are wind pollinated is low in protein and corn/maize has been confirmed as having low quality pollen. There is no nectar in corn and beekeepers report that bees on corn go backwards in hive strength if that is the only source of pollen (T Weatherhead, 2013; *pers comm*). Therefore Australian bee keepers advise that corn is not a crop that they are interested in putting their hives on.

The possible deposition of neonicotinoid dusts (arising from corn seeding) on flowering plants adjacent to the field may needs to be investigated; since corn can be planted from August through to April/May depending on the region, it is likely that various other plants may be flowering sometime during this extended planting season.

[12] Maize is harvested at maturity when its kernels are dry and can be stored for stockfeed. Sweet corn is maize which is high in sugar and is harvested before maturity for human consumption; it must be eaten shortly after picking.

The risks to pollinators from direct foliar application of insecticides to maturing corn is likely to be low because it is not favoured as a nectar and pollen source by Australian beekeepers. Furthermore, there are currently no products containing neonicotinoids approved for foliar spraying of corn crops in Australia.

7.5 Cotton

Cotton is grown in southern and central Queensland and northern NSW. Although a relatively small producer on the world scale, Australia is the world's third-largest cotton exporter and thus cotton is a significant agricultural crop, with over 300,000 ha in production in 2008 (Keogh et al, 2010d and references therein).

In Australia imidacloprid and thiamethoxam seed treatment products are approved for cotton while acetamiprid, clothianidin, imidacloprid, thiacloprid and thiamethoxam products are approved as foliar sprays.

Since the introduction of GM cotton, there has been a very significant reduction in the total amount of pesticides applied to cotton – 96% of cotton planting in Australia is now 'Bollgard II' and the total amount of insecticide active constituent (ac) applied has decreased from ca. 6.24 kg ac/ha in 2000-01 to 0.54 kg ac/ha in 2010-11 (information provided by Cotton Australia, 7 Feb 2013). In Bollgard II cotton, foliar applications of the neonicotinoids acetamiprid, clothianidin, imidacloprid and thiamethoxam made up about 7.5% of the total foliar insecticide applications in 2010-11, with about 0.04 kg ac/ha of neonicotinoids applied by spray during this particular season.

Cotton Seed Distributors (CSD) advised (through Cotton Australia) that 92% of cotton seed planted in the 2012 season was treated with a neonicotinoid seed coating; CSD is currently the sole supplier of cotton planting seed in Australia and there does not appear to be any on-farm treatment of planting seed. The coating process for cotton appears to be tightly controlled, with industrial application of insecticide, fungicide and a colour polymer coating (for cotton variety identification purposes) that acts as an adhesive to improve uniformity of the coating and to reduce insecticide/fungicide dust.

Cotton is commonly regarded as being a partially cross-pollinated crop, and largely self-fertile and self-pollinating (McGregor 1976), although introducing insect pollinators into the crop during flowering has resulted in increased quantity and quality of cotton lint and seed (Keogh et al, 2010d and references cited therein; Rhodes, 2000). However, while cotton is a high-value crop for the grower, it is not considered to be a primary resource by apiarists and is not particularly attractive to bees. The nutritional value of cotton for bees is said to be 'acceptable' for honey bees although there appears to be limited research on its pollen and nectar characteristics.

The presence of bee-attractive extra-floral nectaries on cotton plants outside of flowering potentially provides an additional attraction to insect pollinators, particularly in the absence of other food sources[13]. Extra-floral nectaries are nectar-producing epidermal glands that are located on vegetative plant parts or on reproductive parts without being involved in pollination. Early in the season, when floral nectar is not yet available in

[13] Little is known about the function and ecological role of extra-floral nectaries; they may help sustain beneficial insect parasitoids sufficiently to help reduce plant damage by sucking and chewing insects.