Ecosystem services, agriculture and neonicotinoids

building science into EU policy

EASAC

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EASAC covers all scientific and technical disciplines, and its experts are drawn from all the countries of the European Union. It is funded by the member academies and by contracts with interested bodies. The expert members of EASAC's working groups give their time free of charge. EASAC has no commercial or business sponsors.

EASAC's activities include substantive studies of the scientific aspects of policy issues, reviews and advice about specific policy documents, workshops aimed at identifying current scientific thinking about major policy issues or at briefing policy-makers, and short, timely statements on topical subjects.

The EASAC Council has 29 individual members – highly experienced scientists nominated one each by the national science academies of EU Member States, by the Academia Europaea and by ALLEA. The national science academies of Norway and Switzerland are also represented. The Council is supported by a professional Secretariat based at the Leopoldina, the German National Academy of Sciences, in Halle (Saale) and by a Brussels Office at the Royal Academies for Science and the Arts of Belgium. The Council agrees the initiation of projects, appoints members of working groups, reviews drafts and approves reports for publication.

To find out more about EASAC, visit the website – www.easac.eu – or contact the EASAC Secretariat at secretariat@easac.eu

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Cover image: Solitary bee (*Anthidium florentinum*), feeding on a *Lantana camara* flower.
Credit: Joaquim Alves Gaspar, Lisboa, Portugal
[https://en.wikipedia.org/wiki/File:Anthidium_February_2008-1.jpg].

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Foreword

EASAC was established to help policy-makers in European institutions deal more objectively and efficiently with the many issues that either arise from or involve science and technology. Since its formation in 2001, we have contributed analyses based on the pooled expertise of our 29 member academies in over 20 issues in the fields of energy, environment, or health and biosciences.

Science and technology issues always involve matters of interpretation and uncertainty, and when these impinge on powerful stakeholders or special interest groups, the potential for selective use of scientific evidence in support of advocacy is considerable. Seldom has this been more apparent than in the dispute over the past few years over the use of the neonicotinoid class of insecticides. Interpreting the significance of current scientific evidence, reacting to new research results and dealing with remaining uncertainty have led to unprecedented campaigns by stakeholders both in support and in opposition to continued use of these insecticides; and even involved legal action against the European Commission based on disputes over the science. Balancing the unavoidable risks associated with pesticide use against the critical role of pest control in agriculture has been the task of the Commission, and the polarisation in the dispute over science has even extended to Member States when 15 supported, 8 opposed and 4 abstained during the Appeal Committee vote on 29 April 2013 whether to restrict certain uses of these insecticides to protect bees.

The Commission's regulatory action in 2013 was based on an analysis of the available science by the European Food Safety Authority (EFSA) published in January 2013. At about that time, EASAC was also asked by the then Chief Scientific Adviser to the President of the European Commission if it could contribute to the debate. We considered reviewing the then current political issue of pollinators and neonicotinoids, but noted that pollination was just one of several ecosystem services of importance

to agriculture and our society. EASAC thus decided to conduct a study on neonicotinoids from the wider perspective of the interactions between agriculture and ecosystem services. EASAC member academies nominated 13 leading independent scientists in related fields who formed an Expert Group.

This study has not only reviewed the science available to the EFSA but also over 100 new peer-reviewed studies that have emerged since the EFSA review. Our report overviews the relations between agriculture and ecosystem services, their economic value and the trends in Europe on the key ecosystem services of pollination, natural pest control and soil ecosystems, as well as the biodiversity that contributes to such services. An analysis of the neonicotinoids and the evidence for acute, chronic and sublethal effects and field effects is then provided before considering the implications in the wider ecosystem context. I thank the members of the Expert Group for contributing their knowledge and judgement, as well as the Environment Steering Panel and EASAC Council for their guidance.

As is often the case, science is indifferent to policy and legal boundaries. In this case, too, our report points to the need to take a more systematic and holistic approach to policy analysis, including the precautionary principle, agricultural and biodiversity policies as well as considering impacts with other EU Directives. As part of its 2013 decision, the Commission committed to initiating a new scientific review within 2 years. EASAC hopes that its analysis will help policy-makers address the broader issues and not remain restricted to the narrow issue of effects on managed honey bees. We will be gratified if this report can support EU institutions and the European Parliament in developing future science-based policy in these areas.

Professor Jos W.M. van der Meer
EASAC President

Executive summary

There has been considerable controversy in recent years over the effects of neonicotinoids on honey bees, culminating in a European Commission regulation in 2013 restricting certain uses of these pesticides on flowering crops. This year (2015), the Commission will review the latest science and review the regulations accordingly.

Since restrictions were introduced, scientific research results have continued to be published at a steady pace but disputes between stakeholders continue over their interpretation; some claim that even the limited 2013 restrictions were unjustified by the science, while others see serious ecosystem-wide losses of insects and the species that depend on them. With such a critical European policy issue depending on the science, EASAC decided to conduct a detailed review of the issue. However, while the public debate has focused on honey bees and their pollination services, EASAC concluded that the wider issue should be addressed of the potential effects on organisms providing a range of ecosystem services which are critical to sustainable agriculture. EASAC Member Academies nominated 13 leading experts in related fields to form an Expert Group which worked on this study from April 2014.

This report thus overviews the relation between agriculture and ecosystem services, and what we know of their economic value. Here, even though agriculture is highly managed and simplified, it still depends on services provided by nature. The natural ecosystem provides the original genetic biodiversity used in breeding. Soils are ecologically highly diverse and provide or maintain the soil biota, which contributes to soil formation and structure, fertility, nutrient cycling, carbon capture and storage, as well as water supply. Certain crops depend on pollinators, and pests can be controlled or reduced by natural antagonists that move in to the crop from surrounding natural vegetation. Maintaining strong functional ecosystem services is a critical part of a sustainable agricultural system.

Pollination is one of the most studied of these ecosystem services. Worldwide, 75% of the crops traded on the global market depend to some degree on pollinators. Economic estimates place the monetary value of pollination in Europe at €14.6 billion. With trends to grow more crops that require or benefit from pollination, there is also an emerging pollination deficit. While bees are often the most important crop pollinators and honey bees are the most widely used managed pollinators, relying on a single species for pollination is not wise. Studies show that a diversity of pollinators can improve crop yield or fruit quality. Restoring and maintaining pollinator diversity is thus very important for agriculture as well as for natural vegetation.

Natural pest control is where predatory insects (parasitic wasps, ladybirds, some beetles, etc.) and birds consume pests sufficiently to avoid the need for chemical measures. It is estimated to be worth US$100 billion annually globally and is a fundamentally important service to agriculture. Its loss weakens agriculture's resilience and renders it less sustainable and more vulnerable to pests and diseases. Another key ecosystem service is that from **soil organisms** and their role in enhancing agricultural productivity; the value of their role just in soil formation is estimated to be US$25 billion per year globally.

Underpinning these is **biodiversity**, which is positively interlinked with the provision of ecosystem services, as well as being an objective in its own right under both global and European international agreements. Restoring and maintaining biodiversity in farmland is a particular challenge for European Union (EU) policy.

Looking at the evidence from countries of the EASAC member academies on recent trends in these aspects, we find that, while there is evidence for elevated losses of managed honey bee colonies over winter, no final conclusions can be drawn on recent underlying trends in Europe because of the confounding socio-economic factors that influence colony numbers. However, trend data on wild bee species, other pollinators, on insect species with natural pest control functions and on biodiversity indicators such as farmland birds **all show major declines** in recent decades.

Against this background, we have considered the role of neonicotinoids and their 'systemic' mode of action in the plant, which renders it toxic for insects ingesting parts of it. This makes the neonicotinoids effective for the control of a range of pests, including sap-sucking pests such as aphids. However, the insecticide's residues in the plant include pollen, nectar and guttation fluids, so non-target species harvesting these will also be exposed. Moreover, transmission across trophic levels is also possible (e.g. bees foraging on honey dew, predators exposed through ingesting prey).

The EASAC Expert Group has conducted a detailed review of the literature with particular focus on the many papers that have emerged since 2012. We find that there is a serious difference between the public perspective of the issue and the increasing scientific evidence. Public (and political) focus has been very much on honey bees, especially whether honey bee colonies are being affected by agricultural use of neonicotinoids. Yet honey bees are just one source of pollination and may not even be the most effective for some crops. Many other pollinators have a valuable role—bumble bees, solitary bees, hoverflies, butterflies and moths, etc. The declines in these have proceeded even while honey bee colony numbers have

varied according to weather, fashion and economic signals. When a wider range of ecosystem services is considered, studying honey bee colony trends does not determine what is happening to the wider ecosystem and the other key services provided. EASAC thus concludes that the policy debate should be much broader and include natural pests, biodiversity and soil issues.

The honey bee has also become the main indicator of the effects of neonicotinoids, with some field studies having failed to show detectable effects on colony survival at field level exposures. Yet the honey bee colony structure provides a resilient buffer against losses of its foragers and workers. In contrast, bumble bees are likely to be more sensitive to losses, while solitary bees have no such buffering capacity. We conclude therefore that protecting honey bees is not sufficient to protect pollination services or the other ecosystem services that we have examined.

The report also notes that as some intensive agriculture has become reliant on neonicotinoids, industry studies argue that their withdrawal would have serious economic and food security implications. On the other hand, some recent research has questioned the benefits of routine use as seed dressing against the occasional or secondary pests targeted. When combined with our strengthened and broadened understanding of risks to non-target organisms, and concerns over iatrogenic effects as a result of reduction in natural pest control services, the balance between risks and benefits for neonicotinoids requires reassessment.

Critical to assessing the effects of neonicotinoids on ecosystem services is their impact on non-target organisms: both invertebrates and vertebrates, and whether located in the field or margins, or in soils or the aquatic environment. Here, the Expert Group finds the following.

1. There is an increasing body of evidence that the widespread prophylactic use of neonicotinoids has severe negative effects on non-target organisms that provide ecosystem services including pollination and natural pest control.

2. There is clear scientific evidence for sublethal effects of very low levels of neonicotinoids over extended periods on non-target beneficial organisms. These should be addressed in EU approval procedures.

3. Current practice of prophylactic usage of neonicotinoids is inconsistent with the basic principles of integrated pest management as expressed in the EU's Sustainable Pesticides Directive.

4. Widespread use of neonicotinoids (as well as other pesticides) constrains the potential for restoring biodiversity in farmland under the EU's Agri-environment Regulation.

1 Introduction

Agriculture started when our ancestors selected plants and animals from surrounding natural ecosystems and started to exploit what we now refer to as ecosystem services. Many of the plants now grown as crops depend on other members of the ecosystem to ensure yields. For example, some flowering crops need bees and other pollinators to transfer pollen from one part of a flower to another, transferring the genetic material necessary for reproduction. As we have continuously refined and developed agriculture to produce more food for an ever-increasing population, the link between agriculture and natural ecosystems can sometimes be forgotten. Nevertheless, that link remains, as is demonstrated when an insect, plant, virus or fungus, whose populations would have probably been regulated in a natural ecosystem, takes advantage of the simplified agricultural ecosystem and becomes a pest.

Since the end of the Second World War and the 'green revolution' of the 1940–60s, synthetic chemical agents have become a critical part of the farmer's strategy for pest control. The resulting widespread use of chemicals designed to be toxic to pests (and diseases) has also led to debate over how far these should replace the natural regulation processes inherent in ecosystems, and how to avoid toxic side-effects on non-target organisms, including those that deliver ecosystem services such as pollination and natural pest control[1]. The increasing intensity of modern agriculture driven by the demand for food to support an expanding world population is not always easy to reconcile with natural systems, and the history of synthetic pesticides has been associated with unforeseen negative impacts (see, for example, EEA, 2013). The agrochemical industry has invested in continued efforts to develop new active molecules in an attempt to improve effectiveness, reduce risks and overcome resistance developed in pests, diseases and weeds as a response to previous chemical approaches.

Since 1990, a new class of synthetic chemicals, the neonicotinoids, has entered the pesticide market and its use has grown rapidly. The neonicotinoids have a 'systemic' mode of action in the plant (i.e. they are absorbed and spread through the plant's vascular system to all tissues), which becomes toxic for insects sucking the circulating fluids or ingesting parts of it. They block the neural pathways in insects and are effective in the control of a range of insect pests, including sap-sucking pests such as aphids or mealybugs. However, the systemic nature of these insecticides means that they get into other parts of the plant including pollen, nectar and guttation fluid[2]; thus non-target species harvesting these parts of the plant will also be exposed, often for a long time interval due to the chemicals' persistence. Moreover, transmission across trophic levels is also possible (e.g. bees foraging on honey dew, predators exposed through ingesting prey, or soil organisms decomposing contaminated organic matter).

In recent years, questions have emerged over the potential effects of neonicotinoids on honey bees and other pollinators such as bumble bees, and different kinds of semi-social and solitary bees, flies, beetles or butterflies. This culminated in the European Commission's action in May 2013 restricting the use of some of these compounds. However, the science on the effects of neonicotinoids remains controversial, with two manufacturers taking legal action against the Commission's actions.

In May 2013, EASAC considered reviewing the issue of pollinators and neonicotinoids but, following on from an earlier study (EASAC, 2009), noted that pollination was just one of several ecosystem services of importance to agriculture and society. These include pollination and natural pest control, but also important soil functions such as the supply of mineral nutrients; moreover biodiversity contributes to ecosystem services provision, as well as being a policy objective in its own right under both European and global international actions. EASAC Council thus decided in December 2013 to conduct a study on the science of neonicotinoids and their effects from the wider perspective of the interactions between agriculture and ecosystem services. EASAC member academies nominated 13 leading independent scientists in related fields who formed an Expert Group (Annex 1), which met three times during the preparation of this report.

This report first overviews the relations between agriculture and ecosystem services, and considers what we know of their economic value. It then assembles evidence from countries of the EASAC member academies on selected ecosystem services and recent trends. An analysis of the neonicotinoids and the evidence for acute, chronic and sublethal effects in the laboratory and the field is then provided before considering this in the wider ecosystem context. Finally, generic issues are considered related to other aspects of European Union (EU) policy, including sustainable agriculture and biodiversity. The report is intended to support EU institutions and the European Parliament in developing future science-based policy in these areas.

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[1] We use the term 'natural pest control' to refer to the role of species (insects, birds, etc.) that are present in nature and that predate or parasitise pest species as part of their natural behaviour.
[2] Guttation fluid is the xylem sap which exudes from the leaves of some vascular plants; root exudates also occur.

2 Ecosystem services and agriculture

2.1 What are 'ecosystem services' and how can they be valued?

The benefits to humankind that can be delivered by natural systems are known broadly as ecosystem services. The concept has developed over many years: through the Millennium Ecosystem Assessment (MEA, 2005) and now with a Common International Classification of Ecosystem Services (CICES) developed under the EU Framework Program (Haines-Young and Potschin, 2013). More detail is provided in Annex 2 and EASAC (2009).

A useful analysis of ecosystem services and their value can be found in 'The Economics of Ecosystems and Biodiversity (TEEB)' project, which uses the categories in Table 2.1.

As can be seen in Table 2.1, ecosystem services include 'provisioning' services such as food, fibre,

genetic materials, etc., which we obtain directly. Then there are 'regulating' services, which are derived from ecosystem processes (nutrient supply/cycling, pollination, natural pest control, etc.) or which regulate essential parts of the ecosystem (temperature and humidity regulation, air or water quality, etc.). The concept of ecosystem services also recognises that there are other values that cannot be expressed in such mechanistic or utilitarian terms yet may be very important for cultural reasons, for amenity or habitat maintenance. Ecosystem services are of enormous value to human society. Constanza et al. (1997) estimated their annual value at US$33 trillion globally which, although controversial, emphasises the point that ecosystem services represent a massive contribution to the economic well-being of all societies. Yet most of these services are not recognised in the market and are treated as free public goods[3].

Table 2.1 Classification of ecosystem services (adapted from TEEB, 2010)

Provisioning services

1	Food (e.g. meat, milk, honey)
2	Water (e.g. for drinking, irrigation, cooling)
3	Raw materials (e.g. fodder, fertiliser, bioenergy)
4	Genetic resources (e.g. medicinal purposes, gene banks)
5	Medicinal resources (e.g. biochemical products, models and test-organisms)
6	Ornamental resources (e.g. decorative plants)

Regulating (and supporting) services

7	Air quality regulation (e.g. capturing dust, chemicals)
8	Climate regulation (carbon sequestration, storage, greenhouse-gas balance)
9	Moderation of extreme events (e.g. flood prevention)
10	Regulation of water flows (e.g. natural drainage, irrigation and drought prevention)
11	Waste treatment (especially water purification, nutrient retention)
12	Erosion prevention (e.g. soil loss avoidance, vegetated buffer strips)
13	Maintenance of soil fertility (incl. soil formation)
14	Pollination (e.g. effectiveness and diversity of wild pollinators)
15	Natural regulation of pests, weeds and diseases

Habitat services

16	Maintenance of life cycles of migratory species (e.g. bio-corridors and stepping stones)
17	Maintenance of genetic diversity (especially in gene pool protection)

Cultural and amenity services

18	Aesthetic information (e.g. harmonic agricultural landscape)
19	Opportunities for recreation and tourism (e.g. agro-tourism)
20	Inspiration for culture, art and design
21	Spiritual experience
22	Information for cognitive development

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[3] A Europe-wide review of ecosystem services and their vulnerability to trends such as population growth and climate change can be found in Schröter et al. (2005).

Table 2.2   Ecosystem service and valuation projects in EU countries

Country	Activity
Belgium	Wetlands, forest, coast and grassland ecosystem services
Czech Republic	Grassland ecosystem services
Finland	National assessment of the Economics of Ecosystem Services in Finland (TEEB Finland)
France	EFESE: French national assessment of ecosystems and ecosystem services (Ministry of Ecology, Sustainable Development and Energy)
Germany	Natural Capital, ecosystems and climate change mitigation, city quality of life and ecosystems
Ireland	Irish Forum on National Capital
The Netherlands	Value to government, business and civil society of ecosystem services
Nordic countries	Socioeconomic role and significance of biodiversity and ecosystem services
Norway	Natural benefits and values of ecosystem services
Poland	Ecosystem services for sustainable development of cities
Portugal	Ecosystem services for business
Slovakia	Agricultural ecosystems, ecosystem services at national and regional level
United Kingdom	National Ecosystem Assessment, Natural Capital Committee

The Economics of Ecosystems and Biodiversity (TEEB) project (http://www.teebweb.org/) attempts to connect these free services to their economic value so that their loss is no longer invisible. It tries to help identify trade-offs between development, the environment and ecosystems, so that benefits can be maximised and damage minimised. As part of a trend towards attaching economic value to such natural 'capital', various countries are exploring ways of introducing ecosystem service values into national accounts to supplement current economic measures that ignore (*inter alia*) damage to environment and ecosystems. Activities in several EU countries are listed in Table 2.2.

2.2   How are ecosystem services important for agriculture?

The interrelationship between ecosystem services and agriculture is complex (Figure 2.1); agriculture depends on multiple and interrelated ecosystem services while, at the same time, is also responsible for altering many ecosystems and habitats, and their associated services.

Agro-ecosystems are highly managed and simplified, but they still function essentially as natural systems and depend on several services provided by nature (dark arrows in Figure 2.1). For example, the natural ecosystem provides the original genetic biodiversity used in breeding crops and livestock. Even though the above-ground species diversity is simplified in agricultural landscapes, the below-ground (decomposer) community remains ecologically highly diverse, and provides and maintains the natural ecosystem of soil biota, which contribute to soil formation and structure, fertility, nutrient cycling, carbon capture and storage, as well

as water supply (Swift and Anderson, 1994). A large proportion of crop plants in agriculture and horticulture depend on pollinators (Klein et al., 2007), and pests can be controlled or reduced by natural antagonists that move into the crop from the surrounding natural vegetation (Blitzer et al., 2012). The extent to which agriculture benefits from such central services (light arrows in Figure 2.1) on a sustainable basis or degrades them is related to the ways in which the system is managed.

Loss of natural habitats through conversion to intensive farming (to increase the yield of the primary provisioning service of food production) has major negative impacts on biodiversity, nutrient leaching, soil carbon loss, emissions of greenhouse gases, regional hydrology, loss of soil and sedimentation of waterways, as well as pesticide contamination and the danger of poisoning non-target species (Zhang et al., 2007). Rural landscapes have high cultural and heritage values acknowledged by the European Landscape Convention (Council of Europe, 2000), but intensification includes a trend towards simplification and loss of cultural values of the landscape (Agnoletti, 2014). EU agri-environment policies have been designed to ameliorate some of these negative impacts, while maintaining the primary function of agriculture and its essential provisioning services[4].

As shown in Table 2.2, work is progressing on the economic valuation of ecosystem services and, under Target 2 Action 5 of the EU Biodiversity Strategy, the state of ecosystems and their services in EU Member States should have been mapped and assessed by 2014. The economic value of such services should also be assessed, and the integration of these values into accounting and

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[4] Such Agri-Environment Schemes aim to mitigate the negative environmental effects of agricultural intensification (see Ekroos et al., 2014).

Figure 2.1   Interactions between agriculture, management of the wider landscape and ecosystem services (adapted from Power, 2010; Aisbett and Kragt, 2010).
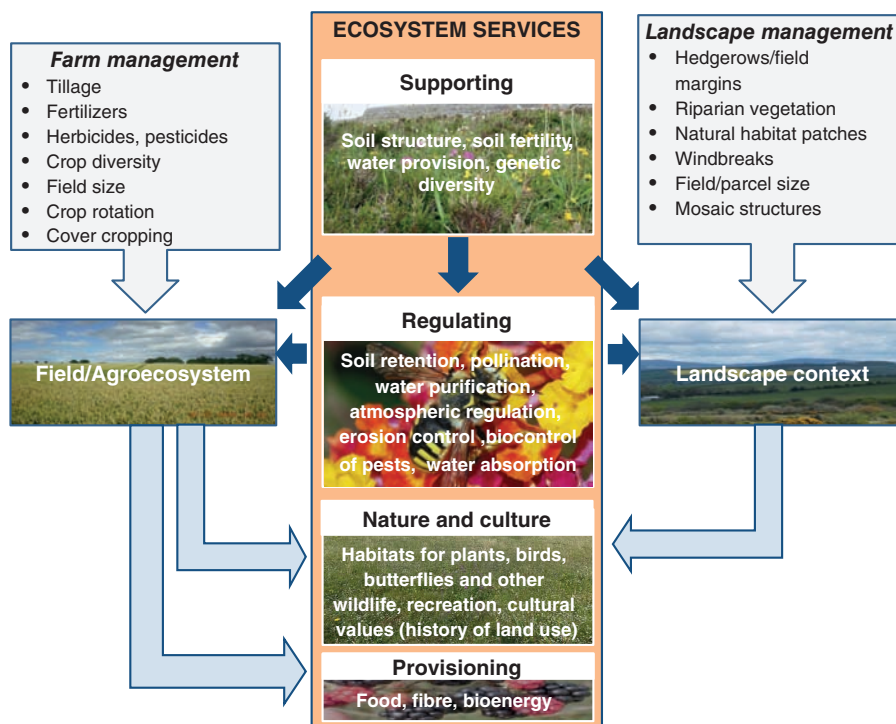
reporting systems both at EU and national levels promoted by 2020. Brouwer et al. (2013) reviewed the state of these national ecosystem assessments (NEA) and found that the UK NEA was the most comprehensive, while other Member States were at earlier stages of development in any national assessment activity. Most current lessons can thus be learnt from the UK's first comprehensive NEA, which was performed between 2007 and 2011 (UK NEA, 2011; Bateman et al., 2013) and has since been updated (UK NEA, 2014). This categorised the services and their relation to the goods received by human society, as well as making an initial estimate of their value.

2.3   Putting an economic value on ecosystem services for agriculture

Placing an economic figure on the value of specific ecosystem services is not straightforward. Economists have developed many different methods and deciding which one is appropriate to apply to a given service can be subjective. There is also debate over whether it is even appropriate or possible to attach values to some services[5.] While it is recognised that the loss of natural ecosystem services will have negative impacts on agricultural systems (whether industrial and input-

intensive commodity cropping or traditional and small-scale organic farming), it is not currently possible to recognise and capture the values of all ecosystem services. As a result, the impact of different actions on these services is not considered adequately in agricultural sector decision-making. TEEB is only just starting a project to understand better the benefits to agricultural production that are provided by biodiversity and ecosystem services.

The value of pollination services to agriculture is relatively straightforward to quantify compared with other ecosystem services; a study by Gallai et al. (2009) assessed the contribution of insect pollination to agricultural output worldwide, and concluded that the total annual economic value of pollination services amounts to approximately €153 billion. Recent studies on economic gains attributed to insect (particularly honey bee) pollination suggest that, across Europe, crop pollination by insects accounted for €14.6 billion (±€3.3 billion) annually, which equalled 12% (±0.8%) of the total economic value of annual crop production (Leonhardt et al., 2013). As shown in Figure 2.2, there is considerable variation in the value of pollination in EU countries, depending on the crops grown.

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[5]   For instance, in Constanza et al. (1997), it was impossible to value the contribution of the biota and their effects on the atmosphere where this involved basic survival of humans (aspects such as oxygen content, global temperature, etc.). Such fundamental processes also apply in agriculture: for instance, without the decomposer communities, agricultural soils would not grow crops beyond a few years, as nutrients would not be regenerated, and straw returned to the soil would accumulate. Rockstrom et al. (2009) explored nine possible planetary boundaries that provide a safe operating space for humanity yet which are either already being exceeded or in danger of being surpassed.

In the USA, Losey and Vaughan (2006) estimated that native pollinators, almost exclusively bees, were responsible for US$3.07 billion of fruits and vegetables produced. Estimates of the value of species other than the honey bee are few, but the solitary bee (*Megachile rotundata*) – the major pollinator of alfalfa – contributed US$5.26 billion to the value of alfalfa hay in 2009 (Calderone, 2012). In the UK, the value of insect pollination was estimated at £603 million per year in 2010 (Hanley et al., 2013), while the UK NEA noted that many field crops (e.g. oilseed rape, linseed), top fruits (e.g. apples, pears, plums), soft fruits (e.g. strawberries, raspberries, blackcurrants) and vegetables (e.g. tomatoes and peas) are dependent, at least in part, on insect pollination. A recent study of the value of pollination to UK apple production showed that insects were essential for the two varieties studied and contributed £36.7 million per annum (Garratt et al., 2014). In Ireland, the economic value of insect pollination to oilseed rape was estimated at an overall value of €3.9 million per annum (Stanley et al., 2013).

Globally, it has been demonstrated that wild pollinating insects (solitary bees and bumble bees, syrphids (hoverflies), etc.) are relevant for crop productivity and stability, even when honey bees are abundant. Garibaldi et al. (2011a) demonstrated that stability of flower-visitor richness, the visitation rate of wild pollinator insects and fruit set, all decreased with distance from natural areas, while honey bee visitation did not change with isolation. Moreover, wild insects often pollinate crops more effectively than honey bees, and an increase in their visitation rate enhances fruit set by twice as much as an equivalent increase in honey bee visitation (Garibaldi et al., 2013). These authors also found that visitation by wild insects and honey bees promoted fruit set independently, so that a high abundance of wild insects supplemented,

rather than substituted for, pollination by managed honey bees.

Even with crops that are not wholly dependent on insect pollination, the presence or absence of pollinators can affect the quality and/or quantity of yields (for instance some oilseed rape varieties can also be self- and wind-pollinated but yield is increased by insect pollination (Bommarco et al., 2012)). In addition to food crops, some oilseed crops for biofuels require insect pollination. Estimates of the monetary value of pollinator services may also exclude the contribution of pollinators to forage crops such as clover (which supports livestock), small-scale agriculture such as allotments and gardens, ornamental flower production, and seed production, as well as to wild flowers producing fruit and seeds which feed invertebrates, birds and mammals (Biesmeijer et al., 2006; Jacobs et al., 2009).

Another ecosystem service is the role of natural enemies in reducing the need for farmer-driven pest control. Such natural pest control is provided by a wide range of invertebrate predators and parasitoids, such as carabids, syrphids, spiders, ladybirds and parasitic wasps (Collins et al., 2002; Schmidt et al., 2003). Landis et al. (2008) and Gardiner et al. (2009) excluded natural enemies from experimental plots and measured the consequent increase in pest infestation. Based on this, Landis et al. (2008) estimated that the value of biological control against soybean aphid was at least US$239 million per year in four US States alone. Clearly the total value of biological control services across all pests and all States would be much larger, and Losey and Vaughan (2006) estimated that the annual value of pest control services provided by native insects in the whole of the USA was approximately US$13.60 billion. A wider assessment of the efficiency of natural enemies (through exclusion devices) is given by Le Roux et al. (2008), who reviewed international research (1986–2007) on cereal as well as vegetable and fruit cropping systems in tropical and temperate countries. Of these studies, 97% revealed significant and often very strong increases in pest populations (between 55% and 600%) following the exclusion of natural enemies. Overall, it has been estimated that such natural control of pests was worth US$100 billion annually and globally (Pimentel et al., 1997).

Rather than economic valuations, another approach is to evaluate the influence of natural pest control on agricultural yield. In Northern Europe, Helenius (1990) and Östman et al. (2003) consistently reported a yield reduction of around 20% as a result of even partial exclusion of generalist predators of aphids from spring cereals (using physical barriers to ground-dwelling species). The latter publication reveals that yield increases attributable to predators can be compared with yield increases from insecticide use for the evaluation of different management strategies. The conclusion from such research is that negative effects of pesticide use

Figure 2.2   Economic value for crop production of insect pollination (EVIP) in EU Member States measured in euros per square kilometre (Leonhardt et al., 2013).
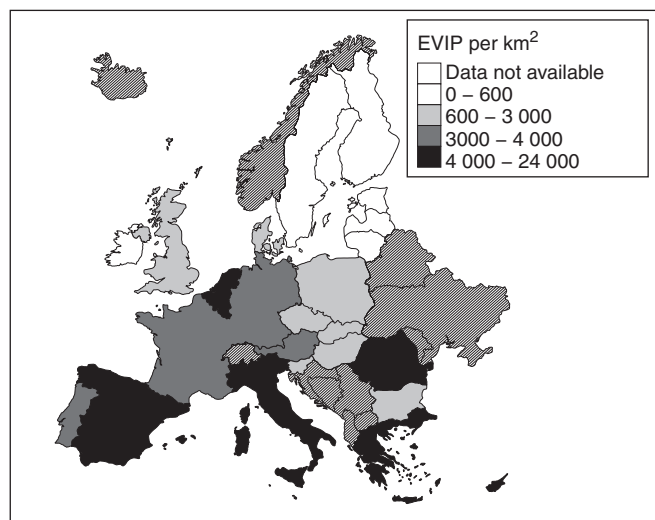
on natural pest control should be minimised to make intensive agriculture more sustainable.

There is good evidence that adequate habitat management within crop fields (such as the upkeep of grass field margins or the setting up of grassy banks or flower strips) to protect and enhance ecosystem services has positive effects on the abundance of natural enemies of many pest species (Gurr et al., 2000; Landis et al., 2000). At a wider scale, landscape heterogeneity and complexity influence beneficial predatory arthropods, and enhance their natural control activity against pests (Chaplin-Kramer et al., 2011; Veres et al., 2013).

Enhancing ecosystem services in agricultural production may require expenditures that have to be counterbalanced by economic benefits if they are to be justified. For instance, Gurr et al. (2003) demonstrated that the cost of establishing a 'beetle bank' in a 20 hectare wheat field, combined with the value of yield lost through the land taken out of production, could be US$130. On the other hand, the value of keeping aphid pest densities below the action threshold across the 20 hectare plot was estimated to be US$450, and the value of avoiding a 5% aphid-induced yield loss was US$1,000. Experiments in France (Le Roux et al., 2008) on redesigning fields to long and narrow shapes adjoining refuge zones (beetle banks and hedges) allowed savings in pesticide use worth US$19,000 per annum on a 320 hectare cereal farm.

Despite the fundamental importance of soil to agriculture (Nuti et al., 2011), estimates of economic impacts of soil degradation are limited and mostly focused on erosion and contamination, not soil biodiversity. Pimentel et al. (1997) valued the role of soil biota activity in soil formation as US$5 billion per year for the USA and US$25 billion globally.

One challenge in valuation is to estimate the *combined* value of the multiple services that ecosystems may provide to agricultural production, since valuation of individual services may underestimate the value of ecosystem services as a whole (Wam, 2010). In this context, Sandhu et al. (2008) used a combination of valuation techniques to estimate economic values for different ecosystem services on arable farming lands in New Zealand, including natural pest control, soil formation, mineralisation of plant nutrients, pollination, services provided by shelterbelts and hedges, hydrological flow, aesthetics, food provision, provision of raw materials, carbon accumulation, nitrogen fixation, and soil fertility. While there may be room for debate over the appropriateness of the economic valuations applied to so many different variables, the value of the services currently ignored by the market was calculated as approximately 15% of the value of the food produced, both via organic farming and conventional farming. This may be a conservative value, as the study assumed that conventional farming does not benefit from natural pest control.

3   Trends in ecosystem services important to agriculture

3.1   Types of ecosystem services considered

Of the ecosystem services introduced in Section 2, we now consider some that are of particular relevance to agriculture and their current trends in Europe. These are pollination, natural pest control, maintenance of soil fertility and farmland biodiversity and its supporting habitats. The last group includes flowering plants, arthropods, birds and mammals (as well as other invertebrates, fungi, protists and prokaryotes), and maintaining farmland biodiversity features prominently in the EU's agricultural policy. European agri-environment schemes (introduced following Council Regulation No (EEC) 2078/92) received nearly €20 billion in funding from 2007 to 2013, which is 22% of the total expenditure for rural development. Biodiversity and associated ecosystem processes provide ecosystem services, with many species involved in delivering more than one ecosystem service (Figure 3.1). However, overall trends in abundance and species richness for many farmland species (discussed in the following sections) are downward or uncertain.

3.2   Pollinators and trends

Many insect species contribute to pollination, including bees, syrphids, butterflies and moths, and some beetles. These insects usually visit flowers to collect food (nectar and/or pollen) and may be generalists having a wide diet and visiting many plant species, or specialists that visit only a narrow range of flower species. They may thus act as generalist or specialist pollinators, some effectively pollinating a range of species and others only a few. Since bees rely on nectar and pollen for all stages of their life cycle, provisioning their offspring with it, as well as feeding on it as adults, they are totally dependent on flowers and are frequent flower visitors. This fact, along with a range of body sizes, proboscis lengths, hairiness and capacity to forage in inclement weather, mean that bee species are the most efficient pollinators. European bee species comprise the managed western honey bee (*Apis mellifera*), 68 bumble bee species and over 1,000 semi-social and solitary bee species. However, other pollinators may have advantages over bees in some circumstances: for instance, syrphids can visit flowers at lower temperatures than bees.

Worldwide, 264 crop species are wholly or partly dependent on pollination, and production of 75% of the crops traded on the global market depends to some degree on pollinators (Klein et al., 2007). While bees are often the most important crop pollinators and the honey bee is the most widely used managed pollinator, relying on a single species for pollination is not wise. Several studies have indicated that a diversity of pollinators can improve crop yield or fruit quality (Chagnon et al., 1993; Klein et al., 2003; Greenleaf et al., 2006; Hoehn et al., 2008; Albrecht et al., 2012; Classen et al., 2014). This is because interactions between bees of different species (for example by

Figure 3.1   Ecosystem services and biodiversity components.
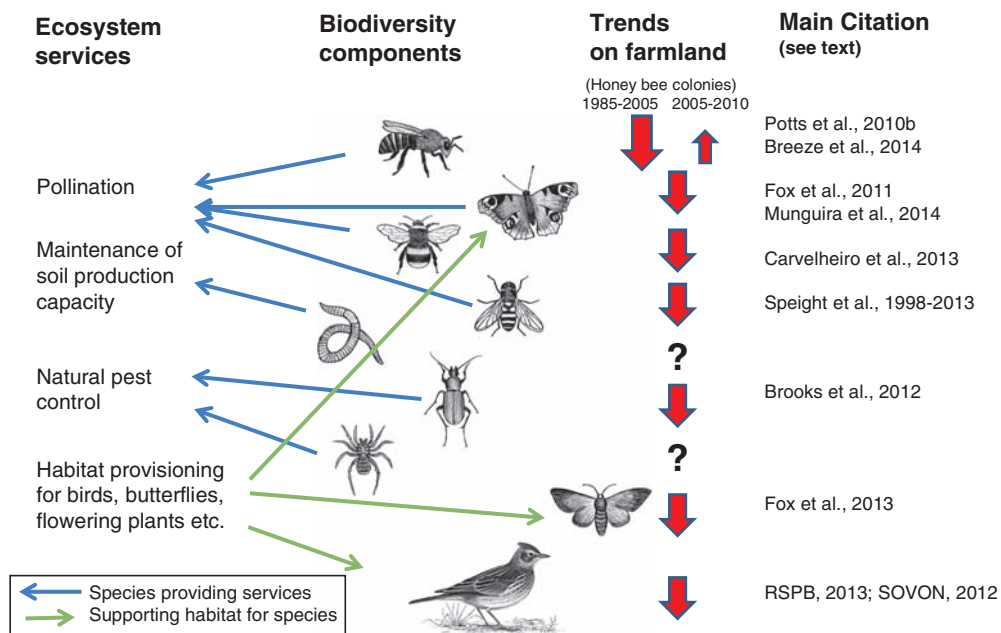
Note: examples of essential ecosystem services are in the first column, biodiversity components in the second column and trends of abundance and species richness of the involved taxa on farmland in the third column. The fourth column gives key references. The included biodiversity components are honey bees, butterflies, bumble bees, hoverflies, earthworms, carabid beetles, spiders, moths and farmland birds.

Figure 3.2 European trends (1965-85 and 1985-2005) in number of managed honey bee colonies (from Figure 1 in Potts et al., 2010b).
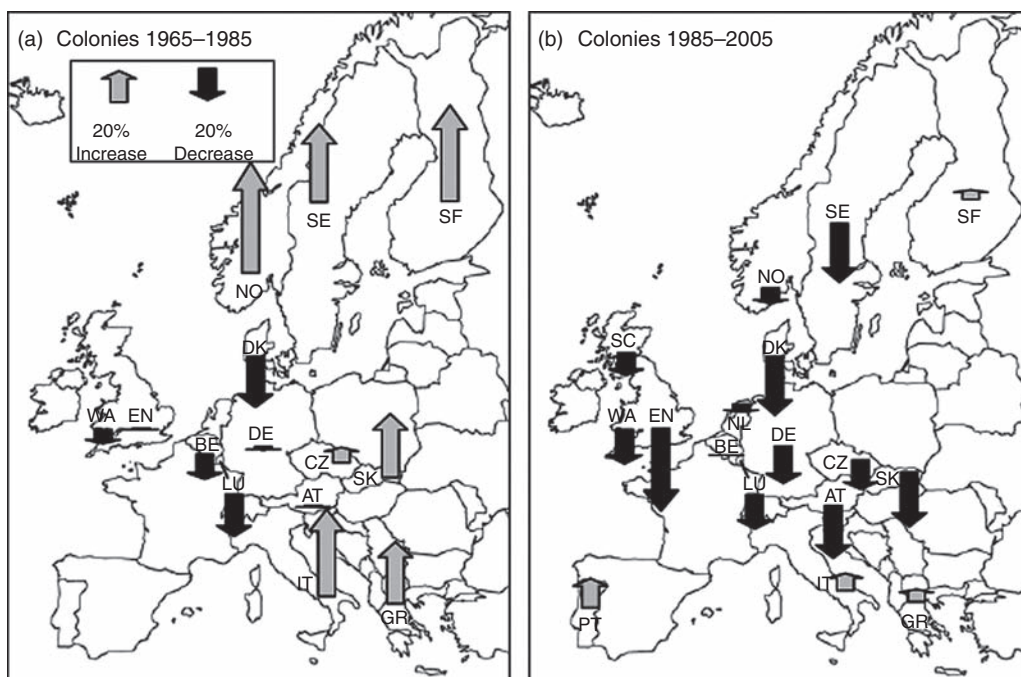
competition or because they consume floral resources in different ways) may influence foraging behaviour, change their movement patterns between flowers, and thus promote pollen dispersal and outcrossing rates in plants (Brittain et al., 2013a), or because species-specific reactions to changing environmental conditions provide a diversity of pollinators that buffers against changes in weather patterns (Brittain et al., 2013b). Additionally, different bee species visit different areas in the fruit tree canopy (Brittain et al., 2013b) or on climbing crops like pumpkins (Hoehn et al., 2008). Also, since many wild plant species, which provide food for higher trophic organisms and other ecological functions, benefit from pollination by a range of insect taxa, pollinator diversity is important not just for crop production (Ollerton et al., 2011).

3.2.1 Honey bees

Surveys of managed honey bees throughout Europe have revealed declines in both the number of colonies and the number of beekeepers in the 1985–2005 period, in contrast to overall increases in the preceding 20 years (Figure 3.2). Europe-wide data since 2005 for individual countries show that colony numbers have increased in 26 countries (up to a doubling) while they had declined (up to 47%) in 15 countries (Breeze et al., 2014), with an average increase in colony numbers of 7% between 2005 and 2010. In the UK NEA, honey bee colonies are reported to have severely declined in England, where they dropped by 54% between 1985 and 2005 (in Scotland they declined by 15%; and in Wales by 23% (Potts et al., 2010a)). In central Europe, estimated

declines since 1985 point to a 25% loss of honey bee colonies (Potts et al., 2010b). Declining numbers of honey bee colonies have also been recently reported in several countries in Europe (e.g. Belgium, Italy, Portugal, Germany, the Netherlands and the UK) (Maxim and van der Sluijs, 2013).

Drawing any conclusions about trends from such data requires a differentiation between 'losses' and 'declines'. Losses are the deaths of colonies which may occur in the temperate regions especially over winter (Neumann and Carreck, 2010); beekeepers can compensate for such winter losses by splitting strong colonies the following spring. However, declines may occur both in the number of beekeepers or in the numbers of colonies maintained by each beekeeper. The latter are particularly heavily influenced by socio-economic factors, by the price of honey, the presence or absence of subsidies, or the popularity of beekeeping as a hobby.

Quantitative assessments and comparisons between countries have been limited by lack of consistent standards for measurement and the fragmented nature of many of the surveys. An initiative to resolve this is the international network COLOSS (prevention of honey bee COlony LOSSes), which currently consists of 444 members from 70 countries (www.coloss.org). COLOSS standardises monitoring methods through its 'BEEBOOK' and collects data at a global scale on colony deaths to provide appropriate information and advice to stakeholders, including government policy-makers. The results of the COLOSS surveys in winter losses have been published (van der Zee et al., 2012a, 2012b, 2014)

and show very high variability in winter losses between countries (from zero to one-third) and mean loss rates of 12.3% (2008/9), 16.9% (2009/10), 16.1% (2012/13) and 9.1% (2013/4).

In 2012, an epidemiological surveillance programme on honey bee colony mortality informed by the German Bee Monitoring Project (Genersch et al., 2010) was implemented in 17 European countries[6] (Chauzat et al., 2014) involving a total of 3284 apiaries and 31,832 colonies, and monitored by bee inspectors, veterinarians, or bee scientists[7]. For the winter of 2012–2013, the mean rate of winter colony losses was 21.2%, ranging from 3.5% (Lithuania) to 33.6% (Belgium). A south–north geographical pattern was seen, with most southern countries experiencing losses below 10%, whereas losses in northern countries were between 20% and 30% (except for Belgium). The report noted that the rate of winter colony loss in the USA, which has been around 30% for most of the previous 12 years (van Engelsdorp et al., 2008, 2009, 2012), is generally higher than in Europe. European beekeepers consider 'normal' winter losses in Europe as being 10% or below (Haubruge et al., 2006; Charrière and Neumann, 2010; Genersch et al., 2010; Chauzat et al., 2014); this was exceeded in 2012–2013 in 6 out of the 17 member states surveyed.

Such data emphasise the high variability of losses of honey bee colonies in time and space. It appears prudent, therefore, not to base any decisions or conclusions on single-year data and to conduct surveys over several years. One example of this is the standardised COLOSS survey, the results of which, in its initial years, support the view that losses can vary substantially between years and regions, and should be monitored for long periods to be able to understand potential trends in losses of honey bee colonies.

A particularly notable phenomenon in bee management has been colony collapse disorder (CCD) in the USA, which is characterised by the following clinical symptoms (Dainat et al., 2012):

- rapid loss of adult workers;
- few or no dead bees in colonies;
- colonies dead with excess brood;
- small cluster of bees with queen present;
- presence of capped brood;
- presence of food stores, both honey and bee bread;
- no damaging levels of *Varroa destructor* or *Nosema* species.

So far, only a few cases of CCD have been reported in Europe (Dainat et al., 2012). The clinical symptoms 'few or no dead bees in colonies' as well as 'colonies dead with excess brood' strongly depend on ambient weather and are thus not suitable for diagnostics in the temperate regions. It may well be that the frequency of CCD has been underestimated in Europe (Dainat et al., 2012). In any case, CCD appears to be of minor importance for colony losses in Europe and is not listed anymore as a major factor in recent US surveys of colony losses (van Engelsdorp et al., 2013). Indeed, honey bee colonies can die in many ways and CCD is just one of them with potentially multiple causes.

3.2.2 Other pollinators

A recent study by the Status and Trends of European Pollinators (STEP)[8] project showed that of the 68 bumble bee species that occur in Europe, 46% have a declining population, 29% are stable and 13% are increasing; 24% were considered to be threatened with extinction. The numbers of bumble bee, solitary bee, butterfly, moth and syrphid species in the UK, the Netherlands and Belgium have generally declined since 1950 (Williams and Osborne, 2009; Keil et al., 2010; Fox et al., 2011; Carvalheiro et al., 2013). Three bumble bee species are thought extinct in the UK, and eight have undergone severe range contractions (UK NEA, 2011). Since 1980, wild bee diversity has declined in most landscapes, with habitat- and diet-specialist species suffering greater losses than more generalist species (Biesmeijer et al., 2006). In Ireland, more than half of wild bee species have undergone substantial declines in their numbers since 1980. The distribution of 42 species has declined by more than 50% (Fitzpatrick et al., 2006).

Moths and butterflies have declined in abundance and range too, although they make a smaller contribution to crop and wildflower pollination services. In a study of common macro-moths in Britain (Conrad et al., 2006), the abundance of two-thirds of the 337 species studied had declined over the 35-year study, and 21% (71) of the species declined by more than 30% per decade. Sixty-two moth species have gone extinct since the 1960s (Fox et al., 2013). Similar decreases have been reported from other European countries. Groenendijk and Ellis (2011) analysed 733 macro-moth species in the Netherlands, finding that 71% of Dutch species decreased in abundance and the total abundance of moths decreased by one-third (1980–2009). Mattila et al. (2006, 2008) showed significant overall decreases in the distribution of macro-moths in the families Geometridae and Noctuidae (590 species in total) in Finland.

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[6] Belgium, Denmark, Germany, Estonia, Finland, France, Greece, Hungary, Italy, Latvia, Lithuania, Poland, Portugal, Slovakia, Spain, Sweden, United Kingdom.
[7] This programme was co-financed by the European Commission (Directive 2012/362/EU) and coordinated by the European Union Reference Laboratory for bee health.
[8] http://www.step-project.net/

Long-term trends in Hungary showed opposing patterns in different habitat types. In mountainous mesophylic forests species richness and abundance of macro-moth assemblages fluctuated with the aridity of the weather, but with no overall trend. In lowland habitats, there was a significant decreasing trend both in numbers of individuals and in species which was attributable to changes both in forest habitats and in agricultural land (loss of grassland to arable lands, drainage) as well as to climatic factors (Szentkirályi, 2002; Szentkirályi et al., 2007). The latter could cause such trends to reverse in arid years, which favoured the spread of invasive pest species from the south (Szentkirályi et al., 2008).

Taken together, these studies provide overwhelming evidence of moth declines on a large geographical scale and mirror previous studies of less species-rich taxonomic groups such as butterflies. Such losses are likely to have substantial impacts at higher and lower trophic levels, because of the importance of moths as herbivores, pollinators and prey items for insectivorous birds and bats.

Fox et al. (2011) pointed out that many butterflies have continued to decline: 72% of species decreased in abundance over 10 years and 54% decreased in distribution at the UK level. Overall, three-quarters of UK butterflies showed a 10-year decrease in either their distribution or population levels. For the first time, a significant decrease in the total numbers of wider countryside butterflies was recorded with common, 'garden' butterflies dropping in abundance by 24% over 10 years. In Ireland, 18% of the native Irish butterfly fauna is under threat of extinction. A further 15% has 'near threatened' status (Regan et al., 2010). In the Netherlands, 54% of the butterfly species declined strongly between 1992 and 2013 (Munguira et al., 2014).

For syrphids, expert knowledge gathered and annually updated in the European database 'Syrph the Net' (Speight et al., 1998–2013) clearly shows an increase in the number of species that are either decreasing or threatened at national level in various countries and at the European level, as well as providing some indication of the severity of these decreases and threats.

Some of the consequences of pollinator declines are outlined in Annex 3.

3.3   Natural pest control and trends

While there are limited data, insect species that provide natural pest control also appear to have declined. Brooks et al. (2012) found that three-quarters of the carabid beetle species examined in the UK had declined in number over the past 15 years. The abundance of half of these species had fallen at a rate equivalent to 30% per decade. These are generalist predators that play a variety of important roles both in natural ecosystems and on farmland; most vitally for farmers, they eat many pests

like slugs and aphids. They provide natural pest-control services whose value is difficult to quantify and is not yet sufficiently documented, but almost certainly substantial (Section 2.3). Some ground beetles, together with some vertebrates, also consume weed seeds and help, under a strong influence of the landscape, to stop fields being overrun by unwanted plants (Meiss et al., 2010; Trichard et al., 2013). Additionally, they are themselves an important food source for everything from birds to small mammals.

There are fewer data available on natural enemies than for bees, moths, butterflies and syrphids. One survey (1982–2002) in Hungary showed no overall trends in carabids (Szentkirályi et al., 2008). Other surveys have demonstrated that natural enemies of insect pests and the intensity of the pest control they provide have declined these past decades in intensively managed agricultural landscapes (Tscharntke et al., 2005; Jonsson et al., 2012). As mentioned in Section 3.5, birds are also natural enemies of many pest insects, and both in the UK and in the Netherlands, dramatic declines in the populations of farmland breeding birds have been observed (Section 3.5) as opposed to neutral or slightly negative trends in breeding bird populations in woodland habitats (RSPB, 2013; SOVON, 2012).

3.4   Soil ecosystem services and trends

The soil decomposer food web plays an essential role in key ecosystem services such as soil formation, nutrient cycling and soil carbon storage (de Vries et al., 2013). In natural ecosystems, a substantial majority of primary production enters the soil where it is gradually decomposed and turned into nutrients and carbon dioxide which returns to the atmosphere. At the same time, the decomposer food web contributes to stabilisation of soil organic matter, which forms an important pool of carbon (soil contains about three times as much carbon as the atmosphere) and contributes to formation of soil aggregates and other structures essential for soil services such as water retention and purification, control of greenhouse gas emissions and erosion control (MEA, 2005). The extensive use of fertilisers, combined with the tillage and cultivation used in modern agriculture, replaces many of the actions provided by decomposer food webs in natural ecosystems, which in turn reduces the diversity and activity of soil biota further. This contributes to carbon loss from soil, higher erosion risks, increased sensitivity to soil-borne diseases and losses of other services provided by agriculture soils (de Vries et al., 2013).

Data on temporal trends are limited but the current situation is that, because of land use change, habitat disruption, invasive species, soil compaction, erosion, pollution and organic matter decline, soil biodiversity levels are potentially under high pressure in approximately 23% of the surface area of EU25

(excluding Sweden and Finland) and under very high pressure in 8% on this area (EEA, 2010).The biodiversity of soil food webs in four agricultural regions across Europe was examined by Tsiafouli et al. (2015) who found that '*intensive agriculture reduces soil biodiversity, making soil food webs less diverse and composed of smaller bodied organisms*'. Soil organisms with larger body sizes were especially sensitive to intensification. Although a general connection between biodiversity loss and reduction in soil ecosystem functioning has yet to be established, the losses of some groups (e.g. earthworms) is likely to reduce key functions (such as carbon and nitrogen cycling).

3.5 Biodiversity and trends

Biodiversity and its relationship to the functioning of ecosystems and their services has been the subject of considerable research since the Convention on Biological Diversity was opened for signature at the Rio Earth Summit in 1992. The current status of scientific knowledge on the influence of biodiversity on the *functioning* of ecosystems and the *services* they provide was reviewed by Cardinale et al. (2012). Scientific consensus includes the following:

- 'Biodiversity loss reduces the efficiency by which ecological communities capture biologically essential resources, produce biomass, decompose and recycle biologically essential nutrients.'

- 'Diverse communities are more productive because they contain key species that have a large influence on productivity, and differences in functional traits among organisms increase total resource capture.'

- 'There is now sufficient evidence that biodiversity *per se* either directly influences (experimental evidence) or is strongly correlated with (observational evidence) certain provisioning and regulating services.'

Biodiversity is thus an important factor when considering the supply of ecosystem services to agriculture. Biodiversity in Europe was reviewed in the 2010 State of the European Environment report (EEA, 2010). The overall picture was one of decline in species diversity, with detailed bio-geographical evaluations of the 1,182 species listed in the EU Habitats Directive showing a favourable conservation status in only 17% of cases and an unfavourable status in 52%.

Europe-wide studies on biodiversity have confirmed the impact of agriculture at different spatial scales. At the plot level, fertilisation, tillage and pesticides are negative factors, while at the broader scale, negative effects are linked to the simplification of landscapes and the loss of hedges, field margins, and other semi-natural habitats (Tscharntke et al., 2005). Alterations in hydrology and land use with the disappearance of woodland, semi-natural

grassland and ponds are further sources of biodiversity loss, together with the homogenisation of crops and the increased frequency of agricultural practices, such as mowing. Intensive agriculture in homogenous landscapes, leading to large-scale monocultures, promotes the development of populations of crop pests (as well as weeds and diseases) at the same time as reducing diversity of natural enemies of pests (Steckel et al., 2014).

However, European biodiversity straddles many climate and ecological zones, and indicators that are appropriate for measuring temporal trends over such heterogeneity are difficult to determine. Various methods to quantify farmland biodiversity are under development; for instance, Herzog et al. (2013) have proposed a 'BioBio' indicator based on a composite of 23 different measurements of biodiversity across a variety of farm types and scales in Europe. Birds (which also contribute to natural pest control) have been suggested as a simple indicator for biodiversity trends across the whole European area by Gregory et al. (2005a), who showed how supranational, multi-species indicators can provide a robust indicator for biodiversity.

The decline in farmland birds shown by Gregory et al. (2005a) up to 2003 has continued in the results of various annual bird surveys. In the Netherlands, common species like the skylark have declined by 96% since 1990, while corn bunting and grey partridge have almost completely disappeared. UK farmland bird species have declined, with several previously common species showing declines of over 75% since the 1970s (including tree and house sparrows, starling, grey partridge, wagtails, corn bunting, yellowhammer) (RSPB, 2013). There is a consistent contrast with trends for bird species in other landscapes (e.g. forests), which have exhibited no such steep declines (Figure 3.3).

3.6 Potential factors affecting agricultural ecosystem services

Factors affecting ecosystem services, especially pollination, have been extensively studied and debated (see, for example, Szentkirályi, 2002; Fox, 2013; Vanbergen et al., 2013; Fox et al., 2014). Observed changes may be the result of several pressures acting separately and in combination. While such drivers include global issues such as climate change and alien species, those most relevant to this EASAC study are described in Sections 3.6.1–3.6.4.

3.6.1 The landscape level: loss of natural and semi-natural habitats

One of the important drivers of change has been the loss of flower-rich, semi-natural landscape elements in farmland (see, for example, Tscharntke et al., 2005; Winfree et al., 2009) such as flower-rich field margins, species-rich

Figure 3.3   Trends in UK bird populations in various habitats (RSPB, 2013).
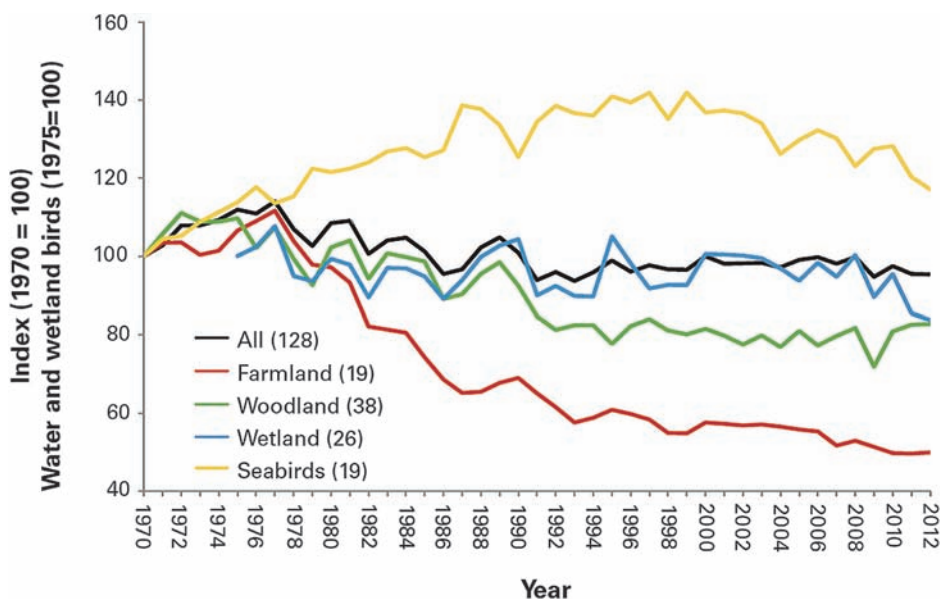
meadows and arable weeds in crops (Ricketts et al., 2008; Garibaldi et al., 2011a; Kennedy et al., 2013). The loss of grass and clover leys, and the legumes they contain, has also been important (Carvell et al., 2007). The scale of land-use change also affects pollinators in different ways, with solitary bees being particularly sensitive to local landscape destruction (Benjamin et al., 2014).

In the UK, the extinction of bee and flower-visiting wasp species has been traced back to the mid-19th century, with the highest extinction rate related to changes in agricultural policy and practice beginning in the 1920s (Ollerton et al., 2014). Since the 1930s in the UK, 97% of wildflower meadows have been lost and the number of species of flowering plants has generally declined since the 1950s, including 76% of bumble bee forage plants (Carvell et al., 2007). The remaining suitable habitat is fragmented, restricting the movement and population size of pollinators. In addition, modern practices of using herbicides to remove weeds on which pollinators feed, even in entomophilous crops (Sarthou et al., 2013), and the planting of crops as monocultures, also results in habitats poor in food sources for pollinators. Such losses of pollen host plants have been shown to be a key factor driving wild bee decline in the Netherlands (Scheper et al., 2014). The nutritional needs of individual pollinator species, and the necessary support for a diversity of pollinators, requires a variety of plant species that flower at different times of the year (Carvell et al., 2007; Araújo et al., 2010). Monocultures like oilseed rape flower *en masse* for a short time only and although they attract a large number of generalist bees and syrphids (Stanley and Stout, 2013), it is only a temporary resource, and flower resources in adjacent semi-natural habitats like hedgerows are considerably important before and after crop flowering (Kovács-Hostyánszki et al., 2013).

While this loss of natural and semi-natural habitats is a major driver of wild bee declines, urban areas and mass-flowering crops may provide important resources for bees. Semi-natural features, such as woodlands and semi-natural grasslands, have been shown to provide a spill-over of pollinators into farmland and can increase pollination services (Ricketts et al., 2008). Agri-environment schemes have sought to mitigate pollinator losses (Batáry et al., 2011; Scheper et al., 2013). Organic farming provides habitat that favours insect-pollination compared with intensive systems (Power and Stout, 2011; Batáry et al., 2013). Nevertheless, overall it has been demonstrated that with intensification and simplification of agricultural landscapes (loss of semi-natural habitats, intensive use of pesticides), pollinators, as well as natural enemies of insect pests and the intensity of the natural pest control they provide, have declined (Robinson and Sutherland, 2002; Tscharntke et al., 2005; Geiger et al., 2010; Jonsson et al., 2012). Such changes also affect farmland birds by removing food sources, nesting areas and shelter (RSPB, 2013).

3.6.2   The field level: the use of fertilisers and pesticides

The increase in the amount of fertiliser applied to arable fields has underpinned the increases in crop yield over recent decades. However, at the same time, it has led to a strong decline in species diversity and flower richness within managed fields (Kleijn et al., 2009; Kovács-Hostyánszki et al., 2011a, b) and in semi-natural habitats adjacent to fertilised fields (Berendse et al., 1992; Bakker and Berendse, 1999), which probably has led to the decline of many arthropod species as well (Kovács-Hostyánszki et al., 2011b). Insecticides applied to crops to kill insect pests can also affect insect pollinators,

natural predators as well as, potentially, soil organisms. As a result, species richness is higher in organic than non-organic production fields (Schneider et al., 2014), particularly in intensive arable regions. Fertilisers and pesticides are often added preventively, even though they do not necessarily increase crop yield (Klein et al., 2014; Motzke et al., 2014).

Few studies have attempted to differentiate between the different drivers of change. However, one study reviewed the impacts of agricultural management on species diversity of wild plants, carabids and ground-nesting farmland birds across eight European countries (Geiger et al., 2010). Negative effects of agricultural intensification were analysed and 13 potential contributing factors measured, which included the loss of landscape elements, enlarged farm and field sizes, and larger inputs of fertilisers and pesticides. Of these potential factors, the strongest correlations were those between insecticide use and reduction in natural pest control potential, and between insecticide and fungicide use and negative effects on breeding birds, carabid beetles and flowering plants.

Lüscher et al. (2014) also investigated four taxonomic groups (plants, earthworms, spiders and wild bees/bumble bees) in four European regions and assessed them against ten potential environmental drivers. The authors showed a dominant effect of geographical location in all four taxonomic groups and a strong influence of agricultural management on plants, spiders and bees, concluding that the avoidance of mineral nitrogen and pesticides is beneficial for biodiversity. Although fertilisers and pesticides are applied at the field scale, the applied chemicals are easily transported through moving air or water, so that adjacent ecosystems are also frequently affected. Aspects related to specific neonicotinoid insecticides are examined in the next section.

3.6.3 The species level: pests and diseases of honey bees

Honey bees are subject to a range of parasites and diseases that pose threats to colonies. The ectoparasitic mite (*Varroa destructor*), an invasive species from Asia (Rosenkranz et al., 2010), has contributed to the loss of most wild and feral honey bee colonies in Europe (Jaffé et al., 2010). It feeds on adult bees and juvenile stages, and can transmit several harmful viruses (Boecking and Genersch, 2008; de Miranda et al., 2010; de Miranda and Genersch, 2010) ultimately causing the loss of hives (Genersch et al., 2010; Dainat and Neumann,

2013). Unfortunately, it has also developed resistance to chemical treatments (Thompson et al., 2002). The fungal pathogen *Nosema ceranae*, another invasive species from Asia (Fries, 2010), is a microsporidium infecting the bee gut. The role of this pathogen is controversial. Some reports indicate major effects, and even losses of colonies (Higes et al., 2009), thereby suggesting an emerging serious threat to honey bee health in the past decade (Paxton et al., 2007), while other studies cannot confirm these findings (Gisder et al., 2010; Genersch et al., 2010; Dainat et al., 2012). In any case, it can also infect bumble bees (Rutrecht and Brown, 2008; Fürst et al., 2014). Two distinct bacterial infections, European foulbrood (Forsgren, 2010) and American foulbrood (Genersch et al., 2010), are serious, widespread diseases of honey bees; both infect and kill developing bee larvae. Data on diseases of wild bees are largely lacking, but it has already been shown that honey bee viruses and the microsporidian *Nosema ceranae* are able to invade multiple host species and thus infect non-*Apis* wild bees and vice versa (Genersch et al., 2006; Eyer et al., 2009; Singh et al., 2010; Fürst et al., 2014). Such diseases are not necessarily separate causes since the possibility that bees become more sensitive to infections under the influence of insecticides is a potential confounding factor (Section 4.5.3).

3.6.4 Differentiating potential drivers

The trends described in Sections 3.1–3.5 are the result of the cumulative impact of all possible drivers acting separately or in conjunction. Even where statistical associations are found correlating trends in one particular driver with a trend in a particular ecosystem service (such as that referred to above between pesticide use and some ecosystem services), **this is not proof of causality**. Nevertheless the downward trends in ecosystem services described above, declines in farmland biodiversity and the growth in the use of neonicotinoids in the past 15 years, combined with specific incidents where neonicotinoid use was associated with bee mortality events, have increased political attention. Parliamentary enquiries and regulatory action were already taken in some Member States as a precursor to the Commission's 2013 decision on restricting use of three neonicotinoids. Such action does not imply that other factors are not also important potential contributing factors to ecosystem service declines, but insecticides are one factor subject to specific regulatory control.

In Section 4 we examine the scientific evidence on the effects of neonicotinoids on ecosystem services.

4 Neonicotinoids and organisms providing ecosystem services for agriculture

4.1 Context

Since their approval 20 or more years ago, scientific information on the effects of neonicotinoids on the environment in general, and on honey bees in particular, has led to much debate in EU countries on whether the use of these insecticides should be restricted. Restrictions had already been introduced in some individual Member States (including Germany, Italy, France and Slovenia), while at the EU level the European Commission asked the European Food Safety Authority (EFSA) in 2012 to assess the safety of three neonicotinoids. The results of the EFSA's study were published in January 2013. The Commission subsequently (24 May 2013) introduced a regulation[9] restricting the use of imidacloprid, clothianidin and thiamethoxam for seed treatment, soil application (granules) and foliar treatment in crops attractive to bees. It allows some exceptions for greenhouse crops and treatment after crops have flowered, but entirely bans non-professional use. Within 2 years (due in 2015), a review of relevant scientific developments will be initiated.

The EFSA reports (EFSA, 2013a–c) provided the internal scientific advice on which the European Commission based its decision, and included scientific data published up to late 2012. The new scientific review to be initiated by the EFSA in spring 2015 will consider additional information submitted by industry, and new scientific information. In the latter context, peer-reviewed research since 2012 has continued to provide substantial new information on the fate and effects of neonicotinoids. This EASAC study provides an update on scientific knowledge up to January 2015 and includes over 100 new peer-reviewed studies that have emerged since the EFSA review. These are discussed in detail in Annex 4 (page 37). In the current section of the main report, we highlight key messages and conclusions that should be taken into account in any scientific review and risk assessment. Annex 4 and the references (listed from page 53) should be referred to for the detailed literature considered.

4.2 Methods and reviewing the evidence

In conducting any analysis, it is necessary to deal with multiple sources of data from different experimental approaches, each of which (Box 4.1) has strengths and weaknesses.

As new scientific papers are published individually, these are often assessed in isolation, and where stakeholders disagree with the results, it is not uncommon for the weaknesses to be emphasised. In evaluating the scientific evidence, the Expert Group sought to assess the totality of the evidence and how far results from one approach are supported or consistent with evidence from others.

Box 4.1   Research methods and their strengths and weaknesses

Laboratory studies assess the impacts of different exposures and pathways on whole organisms or on organism function. Such studies have the advantage of a controlled environment, and accurate measurement of concentrations and effects. They are, however, subject to criticism of being unrealistic: either through the levels of exposure used to detect effects, or in the conditions of the experiment. It is a common criticism of such results that, even where adverse effects are measured, they will be regarded as not reflecting what happens in the field, owing to mismatches between laboratory and field exposures, spatial and temporal variations in ecological systems, etc.

Field studies approach from the other perspective and start with conditions that reflect the use of the active agent under standard agricultural practice. Here the advantage is that any results may be representative of real conditions. The disadvantage is that a variable ecological context may influence findings from place to place and time to time, key variables under field conditions may be difficult to measure accurately, the comparison with controls is problematic and other confounding variables such as weather and natural variability can make it difficult to detect any effect.

Semi-field studies combine both laboratory and field approaches to reduce the variability inherent in field studies by restricting the variables in the experiment (e.g. by confining in a greenhouse) while allowing behaviour (e.g. flying, feeding from flowering plants) under more realistic conditions than in the laboratory. The advantage is that results may allow effects to be shown at a lower detection level than open field studies, but these may still be criticised for not reflecting real agricultural practice.

Descriptive studies in the field investigate possible **temporal or spatial correlations** between an indicator (for example, concentrations of a potential toxic substance) and an effect (for example, declines in species potentially susceptible to that toxin). Here the advantage is that the evidence is very relevant to the real environment. The disadvantage is that association does not prove causality (although such studies do generally attempt to differentiate between potential causative factors and offer plausible mechanisms for causality).

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[9] Regulation (EU) No. 485/2013

4.3 Sources and assessing the data

The growing body of research on the effects of neonicotinoids has already been periodically reviewed. Reviews include peer-reviewed studies (e.g. Blacquière et al., 2012), those by professional societies (e.g. Hopwood et al., 2012), parliamentary enquiries (e.g. EAC, 2014) and most recently by two comprehensive peer-reviewed analyses.

- Godfray et al. (2014) is based on a review of 259 peer-reviewed papers which have been analysed through a stakeholder engagement process to summarise the natural science evidence base relevant to neonicotinoid insecticides and insect pollinators in as policy-neutral terms as possible.

- The 'Worldwide Integrated Assessment of the Impact of Systemic Pesticides on Biodiversity and Ecosystems' (WIA) project, which has examined over 800 scientific studies spanning the past 5 years, including industry-sponsored ones[10].

Some reviews attempt to reach conclusions on the appropriateness of the current regulatory position of neonicotinoids. They can be broadly grouped into two opposing positions.

- One is to argue that there is a significant margin between the levels of exposure to non-target organisms (especially honey bees) and the levels required to cause acute toxicity; also that some field studies have not shown any significant effect in honey bee colonies feeding from crops grown from treated seeds. This line of argument goes on to assign threats to honey bees as due to other factors, as suggested in Pilling et al. (2013) and Fairbrother et al. (2014) in Annex 4.3.4.

- The other is that there is sufficient evidence of direct toxic effects on honey bees, owing to dust exposure in planting, that laboratory and semi-field studies show a range of sublethal effects at concentrations that overlap with field concentrations of nectar, pollen and guttation fluids, and that research on the wider community of pollinators and other beneficial insects also indicates sufficient risk to justify measures to restrict use of these insecticides. This is a conclusion of the WIA project (van der Sluijs et al., 2015).

There is no consensus among stakeholders (manufacturers, farmers, governments, environmental stakeholders, etc.) on which of these two views is the more appropriate. In turn, this has contributed to the different national positions within the EU.

4.4 Key information

4.4.1 Chemistry and mode of action (Annex A4.1)

The neonicotinoids are neurotoxins that mimic the naturally present neurotransmitter acetylcholine. When they bind to insect nicotinic acetylcholine receptors, this triggers an agonistic activation, not reversed by the enzyme acetylcholinesterase, which is unable to degrade these synthetic compounds; thus the impact of neonicotinoids is cumulative. Then, neonicotinoids evoke the same effect as the natural neuro-transmitter acetylcholine, but in a dysfunctional way, blocking the normal cell response and so leading to paralysis and death. Insect and mammalian receptors are structurally different; thus most neonicotinoids show much lower toxicity to mammals than insects (although there is recent evidence that degradation products of neonicotinoids might block nerve cell receptors in vertebrates as well and some reports of human effects from neonicotinoid exposure in Japan (Taira, 2014)).

4.4.2 Systemicity

Neonicotinoids are often referred to as 'systemic', which means that they are absorbed by treated plants and spread to all tissues through the vascular system (Elbert et al., 2008). This makes the plant toxic for insects ingesting parts of it or sucking its circulating fluids. This allows a more selective and effective means of targeting sap-sucking pests such as aphids or mealybugs. However, all parts of the plant including pollen, nectar and guttation fluids will contain the insecticide so that non-target species consuming these parts of the plant will also be exposed. Natural enemies, by feeding on contaminated host insects, may also come into contact with significant amounts of accumulated pesticide.

It is important to recognise that the term 'systemic' does not imply any containment of the neonicotinoid within the plant. Studies on the fate of the insecticide applied as seed dressings show that the majority of the insecticide does not enter the growing plant and thus enters the soil where its water solubility allows it to spread to other plants or to water courses (Figure 4.1). Even that part of the insecticide in the target plant will enter the soil very soon because of the rapid turnover of plant biomass (Annex A4.2). Within a growing season

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[10] This has reviewed effects of systemic insecticides (neonicotinoids and fipronil) and has published separate papers on trends, uses, mode of action and metabolites; environmental fate and exposure; impacts on invertebrates; impacts on vertebrates; impact on ecosystems and their services; alternatives: case studies; and conclusions. The Expert Group has used these review papers as a source of research results and has not relied on these papers for conclusions on the significance of impacts.

therefore, effectively all the insecticide applied will enter the environment.

### 4.4.3   Uses and application

Neonicotinoids entered the market in 1991 (Annex A4.1) and by 2010 accounted for approximately one-third of the world insecticide market. Their use in seed pre-treatment shows the fastest growth; these are routinely applied to grain and oilseed crops in developed countries; indeed untreated seeds may not be available for farmers to purchase. Imidacloprid alone had sales of US$1,091 million in 2009 (Jeschke et al., 2011), and annual world production of imidacloprid is estimated as approximately 20,000 tonnes (Simon-Delso et al., 2015). Neonicotinoids can be applied as seed coatings, granules for addition to the soil, soil drenches, foliar sprays, by injection into trees and shrubs, or by addition to irrigation water. The major effect of neonicotinoids on intensive farming has been to introduce seed pre-treatment as the standard pest management approach for large areas of major crops (including wheat, oilseed rape and maize).

4.4.4   Fate, persistence and degradation

The fate of neonicotinoids is highly dependent on methods of application. Insecticide residues are found in contaminated dust released from seed planting equipment and in weeds growing within or adjacent to treated fields, as well as in water bodies into which the water-soluble chemicals can drain (Figure 4.1 and Annex A4.2). Concentrations reported in the literature range from parts per billion (1 part per 10^9) to parts per million (in soils), parts per billion to parts per trillion (1 part per 10^{12}) (in water) and parts per billion to parts per million (in plants). The potential chronic exposure of non-target organisms is thus broad: non-pest insects and animals can be exposed through eating pollen, nectar, leaves or seeds of plants containing the insecticide transmitted from the primary crop, and via non-crop plants in areas adjoining the treated areas (hedgerows, margins and other areas of natural vegetation).

Half-life on leaves due to foliar spray is short (3–5 days) owing to photolytic degradation. However, half-life in soil ranges from days to years, depending on the insecticide, the soil conditions, exposure to sunlight and temperatures, which determine the rate of degradation through hydrolytic, photolytic and microbial degradation processes, as well as through leaching. Typically, neonicotinoid concentrations in soil decline rapidly after application, but in cool, dry and high organic-matter-containing soils, they can persist and possibly accumulate for months or years (Bonmatin et al., 2015). This has been found to be the case in some field surveys (Annex A4.2). Moreover, metabolic pathways are complex and still poorly quantified but include common metabolites that can be toxic (Simon-Delso et al., 2015) and result in prolonged toxicity. This high

Figure 4.1   Environmental fate of neonicotinoids (adapted from Goulson, 2014; Sanchez-Bayo, 2014).
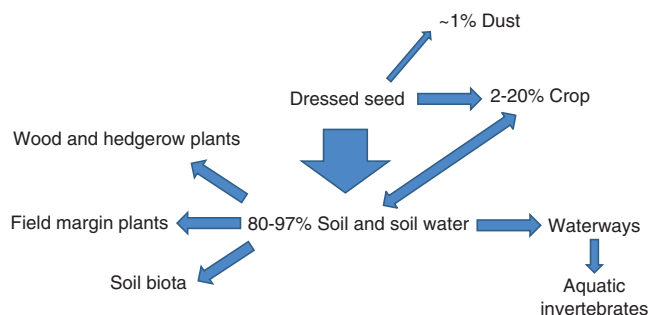
variability makes prediction of the persistence and fate of the active chemical difficult and leads to high levels of uncertainty in assessing exposure and associated risks.

4.5   Impact on non-target organisms

In the regulatory debate, most discussion has focused on honey bees. However, the honey bee species, despite its prominence in the policy debate, is not necessarily the most important pollinator (especially in the wider environment outside cropping systems) and is not a good model for the hundreds of other bee species because of its eusociality and large colony size (Annex 4.3.6). Effects on other pollinators and organisms that contribute to natural pest control and soil functioning have rarely been addressed (as seen by the large difference in available literature between these sectors in the WIA literature reviews). The effect that neonicotinoids might have on constraints to the restoration of biodiversity on farmland (one of the priorities of European agricultural policy) has been completely neglected. Annex 4 has sought to remedy these shortcomings as far as the available data allow.

4.5.1   Evidence for impacts on non-target organisms

The systemic nature of the chemicals and their persistence provide a variety of means (Annex A4.2) through which toxicologically active compounds can be taken up by non-target organisms, including the following:

- contaminated pollen and nectar (crop and non-crop plants);

- direct spray;

- residue contact (e.g. with contaminated leaves or stems);

- ingestion of treated seeds (insects, birds and mammals);

- airborne particles released in seed planting;

- contaminated nesting areas or nesting materials;

- guttation fluid and nectar producing glands outside flowers, root exudates;

- contaminated soils and water;

- consumption of contaminated prey by higher trophic consumers.

Laboratory-based research confirms the levels at which acute toxic effects are observed through standard LC_{50} (the concentration that kills half of the test organisms in the specified time period, often 24 or 48 hours) tests for honey bees (Annex 4.3.2). Laboratory-tested acute lethal effects through oral ingestion occur at concentrations of tens of parts per billion or above, and independent reviewers conclude that when applied as seed dressings, concentrations of neonicotinoids in nectar and pollen appear unlikely to reach such acutely toxic levels. However, seed dressing in spring sowing may lead to higher pollen and nectar concentrations which can overlap with acutely toxic concentrations for honey bees (Table A4.2).

The Expert Group emphasises, however, that most toxicity testing has been conducted on honey bees, which may not be useful for predicting effects on other bees. Indeed, it is likely that toxicity will be related to colony and body size, and species of smaller body sizes (occurring in most of the solitary bees) and smaller colony sizes (bumble bees) may be more susceptible to exposure (Annex A4.3.3). Differences in foraging behaviour may also affect exposure.

Direct mortality where dust from treated seed is in a bee's flight path remains a risk. Moreover, with soil drenching and/or addition to irrigation water, levels in pollen and nectar can reach much higher levels—well into the range where direct toxic effects could follow ingestion. Moreover, the Expert Group *cannot emphasise enough* that the above crude assessment is based on acute (24 hour, 48 hour) LC_{50} test results. **These are not an appropriate indicator of toxicity for prolonged exposures, which can be achieved in the case of systemic and persistent chemicals**. The impact of neonicotinoids in blocking neural pathways is not reversible because the natural enzyme that removes acetylcholine is not effective on the neonicotinoid molecule. Prolonged exposure to the chemical is thus cumulative and toxicity should be assessed over longer periods. Research on honey bees and other insect species suggests that the same amount of neonicotinoid ingested over longer periods shows greatly (orders of magnitude) increased toxicity (Charpentier et al., 2014; Rondeau et al., 2014).

This has substantial implications for risk assessment. In Annex 4 it is pointed out that the EFSA's estimate of the

exposure to toxicity ratio (ETR_{acute}) for an adult bee in 1 day ranged from less than 0.1 to more than 5 for the three neonicotinoids considered. What is already a low safety margin based on standard LC_{50} data becomes a significant risk if longer-term accumulation over days or weeks is considered as the exposure pathway.

Semi-field studies (Box A4.1) have shown a range of sublethal effects on the functions of both individual bees and colonies (cognitive abilities, communication, role behaviours, location finding and other key roles), which could result in fitness reduction. Exposure levels in these experiments have been argued to be higher than those occurring in the field, but the real exposure and cumulative effects over complete life cycles remain undetermined.

Field studies include experiments that found significant effects and experiments that have detected no difference between honey bee colonies exposed to agricultural use of neonicotinoids and controls. However, the Expert Group notes that experiments based on 'real' field conditions have to meet very difficult methodological challenges (Annex A4.3.3), so such studies have limited detection capability.

Moreover the honey bee colony structure provides a resilient buffer against losses of its foragers and workers. In contrast, bumble bees have just a few hundred workers at most and are likely to be more sensitive to losses, while solitary bees (where a single female has sole responsibility for provisioning of offspring) have no such buffering capacity. Bumble bees are thus likely to be more sensitive and solitary bees much more vulnerable to the same levels of exposure. Some studies have already demonstrated this sensitivity with bumble bees (see, for example, Whitehorn et al., 2012), and field trials to test these differences further with both bumble and solitary bees have been performed and results are expected soon.

Acute lethal or sublethal effects have also been observed on several natural pest control agents including parasitoid wasps, species such as ladybirds and lacewings, predatory mites, ground beetles, etc. (Annex A4.4). Effects vary between insecticides and between different insect taxa. The effectiveness of some species used commercially in biological pest control is also compromised by neonicotinoids. Soil organisms such as earthworms are also sensitive to neonicotinoids. Toxicity data on bird species indicate that there can be a difference in toxicity of one to two orders of magnitude, so that the risks to birds eating neonicotinoid-dressed seeds will show substantial differences between species. Calculations based on ingesting treated seeds suggest acute lethal or sublethal effects for the more sensitive species from eating just a few treated seeds. The observed negative

effects on birds will also reflect losses of insect food supplies (Annex A4.6).

Research and field measurements on persistence and mobility show that, depending on the half-life of the specific neonicotinoid and location, the active chemical may move away from the field where first applied into freshwater systems, groundwater and coastal waters (Figure 4.1). Aquatic invertebrates are important components of aquatic ecosystems (with roles as decomposers, grazers, sediment feeders and providing much of the food that fish, amphibians and birds feed upon). Surveys in the Netherlands and elsewhere have shown neonicotinoid concentrations increasing in aquatic systems and to be associated with declines in non-target invertebrate species populations (Annex A4.6). Such reductions would affect the aquatic ecosystem and its biodiversity, extending to fish and mammals (particularly birds) that depend on aquatic systems for their food supply.

4.5.2   Multifactorial interactions

Owing to the widespread use of neonicotinoids and their persistence, bees or other insects may be exposed to more than one active chemical molecule, as well as to other pesticides. Risk assessment based on interpreting results from controlled exposure to just one insecticide is thus difficult (Annex A4.3.3). Multiple exposures with other types of pesticide have also been shown to have synergistic effects (e.g. between neonicotinoids and ergosterol-inhibiting fungicides).

Several studies have demonstrated synergistic effects of neonicotinoid residues with bee parasites and viruses (Annex A4.3.5). Some effects are behavioural (e.g. blocking the ability of bees to sterilise the colony and their food). Others appear related to limiting the immune response leading either to earlier infection or to increased mortality from infection. Very recent work has shown that the limitation of the immune response after exposure to neonicotinoids can promote viral replication, allowing covert infections to become overt. Such effects reduce honey bee survival and increase developmental deformities. In view of the emphasis placed by some reviewers on assigning honey bee losses to diseases and parasites, this is a critical issue.

4.5.3   Agricultural ecosystem effects (iatrogenic pests)

Widespread reduction in natural pest control services through the use of non-selective insecticides can contribute to more serious pest outbreaks with substantial economic effects (Section 2.3). Recovery of such services, even after a decline in the concentrations of the causative agent, may be slow, leading to a period of heightened vulnerability to pest outbreaks. Such 'pest resurgence' occurs when the natural enemies are killed off by the pesticides, and the pests can build up their populations more rapidly and become more of a problem than they were in the first place. In addition, populations of pest organisms may develop resistance to the primary insecticide through rapid evolution, and secondary pest outbreaks may occur, where species that were not formerly pests become so in the absence of natural enemies.

Recent research has demonstrated that natural enemies can be affected by neonicotinoid use, even when the pests themselves are not. Douglas et al. (2014), in a study in the northeastern USA, report that slugs are unaffected by thiamethoxam but transmit the toxin to predaceous beetles, impairing or killing more than 60% of individuals in laboratory assays. In the field, thiamethoxam-based seed treatments thus depressed the activity–density of arthropod predators, thereby relaxing predation of slugs and reducing soya bean densities by 19% and yield by 5%. Ekbom and Müller (2011) also point to a possible risk of resistance in flea beetles to neonicotinoids applied through seed dressing, and that resistance of the pest could combine with negative effects on natural flea beetle predators and lead to the use of neonicotinoids increasing the flea beetle pest problem in brassicas. Research has yet to be conducted on whether such interactions may have contributed to increased flea beetle damage reported anecdotally during 2014 to brassicas not treated with neonicotinoids.

Evidence of another possible iatrogenic effect was provided by Smith et al. (2013), who examined the effects of various pesticide use strategies on combatting damage to cotton by the two-spotted spider mite (*Tetranychus schoenei*). These authors concluded after field and laboratory experiments that increased use of neonicotinoid seed treatments (replacing the previous use of aldicarb) was probably at least partly responsible for increased infestations of two-spotted spider mites in seedling cotton across the mid-south of the USA. Szczepaniec et al. (2011) also found that the use of imidacloprid for pest control on elm trees in New York removed the insect predators of spider mites, as well as increasing spider mite fecundity, so that a combination of reduced predation and enhanced reproduction promoted a non-target herbivore to pest status.

5   Implications for EU policies

The analysis in Section 4 and Annex 4 of the current state of the science on neonicotinoids has implications for several areas of EU policy. Policy development requires the parallel consideration not only of scientific issues but also of economic and social aspects involving different stakeholders: manufacturers, farmers, consumers, scientists, environmental groups and so on. In this section we briefly point to some of the policy questions raised by our analysis which stakeholders may need to consider.

The first aspect that the Commission must consider is the **future regulatory status of neonicotinoids**. Neonicotinoid insecticides raise several issues that the regulatory system did not initially sufficiently address. For instance, these include the high variability in persistence and spread to other parts of the environment, the cumulative nature of the toxic effects even at low levels, the mechanisms through which sublethal effects can affect functioning of individuals (and, for social insects, entire colonies), the differences between acute and chronic toxicity effects, the toxic effects on beneficial insects, synergistic effects with other neonicotinoids, pesticides and fungicides, immune system interactions, and wider ecosystem effects. The regulatory response on the status of neonicotinoids and how to adapt the regulatory system are major issues that are not yet resolved.

One source of contention on the regulatory process between stakeholders is how to balance the available evidence with the continued uncertainties in knowledge according to the 'precautionary principle' (EU, 2000). This principle was introduced in response to historical experiences, which showed an extended delay in acting on increasing bodies of scientific knowledge on environmental or health issues. This has been reviewed by EEA (2013), which looked at the common themes between many different issues. Whether acid rain, lead in gasoline, DDT, or the current issue of global warming, scientific knowledge of adverse health or environmental effects accumulates over time. In some cases, early concerns may be reduced by subsequent scientific findings. But in other cases the scientific body of evidence proves consistent and ultimately persuasive enough to convince governments to act. During this process, however, delays may increase adverse health and environmental impacts. Special interests may demand ever-greater burdens of proof to delay regulatory actions, and the precautionary principle was designed to help strike a balance with such pressures by legitimising regulatory action supported by scientific evidence even while uncertainties remain.

When applying the precautionary principle, the issue of economics cannot be ignored, especially when this is associated with fundamental issues such as food security. Neonicotinoids have expanded to become one of the most popular and widely used insecticides and are licensed for use in over 120 countries (Jeschke et al., 2011), in part because of the ease and flexibility of application. With the current methods for intensive food production so reliant on neonicotinoids, this perceived benefit is seen by some stakeholders as outweighing any risks to the environment and ecosystem services, and industry studies have argued that withdrawal of neonicotinoids would have serious economic and food security implications (Humboldt Forum, 2013). On the other hand, a review by Simon-Delso et al. (2015) notes that pests targeted by neonicotinoids used in seed pre-treatment are generally occasional, sporadic and secondary pests, so that benefits from the point of view of yield are '*not routinely found*'. At the same time, environmental risks may be increased due to the high proportion of the insecticide used in seed pre-treatment which enters and persists in the environment without exercising any pest control function; this also leads to concern over food security through risks of undermining vital ecosystem services that maintain food production.

One area where seed dressing has been prohibited for maize has been monitored in Italy, which reports that alternative methods for managing secondary pests exist, and agricultural practices such as crop rotation drastically reduce the need for control through neonicotinoids. Moreover, even when insecticides have to be used, alternatives to systemic and persistent insecticides are available (Apenet, 2009, 2010, 2011; Furlan and Kreutzweiser, 2015). The US Environmental Protection Agency recently released a draft for consultation of an evaluation of the benefits of neonicotinoid seed treatments to soybean production[11.] This concluded that such seed treatments with neonicotinoids provide negligible or no overall benefits to soybean production in most situations.

Such research results calling into question the benefits of prophylactic use against the occasional or secondary pests targeted have emerged while the scientific evidence has strengthened and broadened our understanding of risks to non-target organisms (Annex A4.3.5, A4.4, A4.6) and raised concerns over iatrogenic effects as a result of reduction in natural pest control services (Section 4.5.3). Pesticide use inevitably involves balancing risks and benefits, but the direction of the scientific evidence suggests that this balance for neonicotinoids (especially routine use as seed dressing) requires reassessment.

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[11]   http://www2.epa.gov/pollinator-protection/benefits-neonicotinoid-seed-treatments-soybean-production

While the major policy focus of the European Commission has been on how to regulate the use of neonicotinoids, there are wider issues that are relevant and should be carefully taken into consideration to support effective decision-making processes.

The use of neonicotinoids should be guided, as for any other insecticide, by the founding principles of Directive 2009/128/EC, which has established a framework for Community action to achieve the **sustainable use of pesticides**. Article 14 of the Directive provides specific instructions to Member States to '*take all necessary measures to promote low pesticide-input pest management, giving wherever possible priority to non-chemical methods, so that professional users of pesticides switch to practices and products with the lowest risk to human health and the environment.*'

Member States shall also '*ensure that professional users have at their disposal information and tools for pest monitoring and decision making, as well as advisory services on integrated pest management.*' And Member States shall describe in their National Action Plans how they ensure that the general principles of integrated pest management (IPM) are implemented by all professional users by 1 January 2014.

Annex III in the Directive sets out the general principles of IPM, which appear incompatible with the prophylactic use of neonicotinoids in seed dressing because of the following:

- as described earlier, only a small proportion of the insecticide enters the plant and most is released into the environment immediately;

- neonicotinoids may be applied proactively, not reactively based on just-in-time-and-place monitoring of the need for pest control; and the first priority is placed on chemicals instead of seeing them as a last resort;

- principle 5 (which specifies that '*the pesticides applied shall be as specific as possible for the target and shall have the least side effects on human health, non-target organisms and the environment*') is also inconsistent with the many toxic effects reported for neonicotinoids on non-target organisms.

Moreover, the establishment of chemically prophylactic use as standard practice has rendered redundant the necessary monitoring, threat assessment and menu of non-chemical responses to pests inherent in applying IPM. Many farmers thus lack the necessary resources, skills or experience to apply an IPM approach, which should consider all relevant and available information and provide pest control options based on actual need. Furthermore, when a need is identified, IPM applies control options such as diversifying and altering crop

rotations, planting dates, tillage and irrigation; using less sensitive crop species and cultivars in infested areas; applying biological control agents; and turning to alternative reduced risk insecticides. Shifting to an IPM model and alternative pest control options requires more monitoring, management assessment and decisions, and flexible and diverse response strategies. Providing the necessary tools, information systems and expertise will require investments in research and public extension to promote economically competitive and sustainable agriculture (Meissle et al., 2010). Furlan and Kreutzweiser (2015) also point out that adoption of IPM will require education and acceptance by regulators and practitioners, and new approaches to face the unpredictable costs of pest damage; for instance, an incentive for IPM implementation in Italy is a yield insurance scheme (mutual fund) for farmers, in which the required insurance premium is usually lower than insecticide costs (Furlan, 2014).

Secondly, on **general agricultural policy**, the EU has adopted four Basic Regulations for a reformed Common Agricultural Policy in response to external factors, which include environmental factors relating to resource efficiency, soil and water quality, and threats to habitats and biodiversity. Given the pressure on natural resources, one aim of the new policy is to improve the environmental performance of agriculture through more sustainable production methods. The EU vision on sustainable agriculture is aimed at increasing productivity without affecting the quality of soil and water; preserving ecosystems; safeguarding animal welfare; generating income for farms and improving quality of life in rural areas; supporting territorial development; and contributing to the economy. Relevant to the implementation of the reformed Common Agricultural Policy is the debate over making intensification sustainable ('sustainable intensification'). This is a complex issue which is outside the scope of this study; but the challenges and issues raised have been examined in detail elsewhere (see, for example, NAS, 2010; Buckwell et al., 2014). These have emphasised the need for a systems approach, harnessing the potential of biology-based approaches (e.g. developing pest-resistant plants: see also EASAC, 2013) and more effective application of knowledge to balance the need for maintaining and increasing agricultural productivity while at the same time managing the ecosystem services on which agriculture relies. In view of the effects on ecosystem services addressed in this study, the question is raised as to what extent widespread use of the neonicotinoids is compatible with the objectives of sustainable agriculture.

Finally, there are questions raised on interactions with **EU Biodiversity Policy**. The EU has endorsed the global target to halt, and where possible reverse, biodiversity loss and the degradation of ecosystem

services by 2020. The EU Biodiversity Strategy of 2020 includes TARGET 3 '*to increase the contribution of agriculture and forestry to biodiversity*'. This focuses on improving the integration of biodiversity conservation into key policies for agriculture and forestry. The strategy notes that intensification has had devastating consequences for biodiversity and that the reform of the Common Agricultural Policy and the new Multi-annual Financial Framework for 2014–2020 present opportunities to further enhance synergies and maximise coherence between the objectives of biodiversity conservation and those of farming and forestry. The potential effects of neonicotinoids on biodiversity described in the previous sections and in Annex A4.6 are relevant to this policy and its ability to meet its objectives.

6   Conclusions

1. Ecosystem services provide significant economic benefits to agriculture. Maintaining strong functional ecosystem services is a critical part of a sustainable agricultural system.

2. Biodiversity has significant positive impacts on the provision of ecosystem services but is also an objective in its own right under global and European international agreements.

3. Insects providing ecosystem services have shown major declines in recent decades (pollinating wild bees, natural pest control providers, etc.).

4. Protecting honey bees is not sufficient to protect pollination services and other ecosystem services. Honey bees have been the main focus in assessing the risks from neonicotinoid use, and much debate has focused on whether honey bee colonies are being affected. Yet the honey bee colony structure provides an exceptionally resilient buffer against losses of its foragers and workers. In contrast, bumble bees have just a few hundred workers at most, while solitary bees and other insects have no such buffering capacity.

5. There is an increasing body of evidence that the widespread prophylactic use of neonicotinoids has severe negative effects on non-target organisms that provide ecosystem services including pollination and natural pest control.

6. There is clear scientific evidence for sublethal effects of very low levels of neonicotinoids over extended periods on non-target beneficial organisms. This should be addressed in EU approval procedures.

7. Current practice of prophylactic usage of neonicotinoids is inconsistent with the basic principles of integrated pest management as expressed in the EU's Sustainable Pesticides Directive.

8. Widespread use of neonicotinoids (as well as other pesticides) constrains the potential for restoring biodiversity in farmland under the EU's Agri-environment Regulation.

Annex 1   Expert group members

Academia Europaea and Swiss Academies of Arts and Sciences

Chairman: Professor Peter Neumann, University of Bern (bee ecology and evolution)

Czech Republic (Czech Academy of Sciences)

Professor Jan Frouz, Czech Academy of Sciences (soil ecosystems)

Finland (Council of Finnish Academies)

Professor Juha Helenius, University of Helsinki (agroecology)

France (Académie des sciences)

Dr Jean-Pierre Sarthou, University of Toulouse (agroecology; biological conservation; landscape ecology)

Germany (German Academy of Sciences Leopoldina)

Professor Alexandra-Maria Klein, University of Freiburg (agroecology; pollination ecology; plant-animal interactions; landscape ecology; conservation ecology)

Dr Elke Genersch, Freie Universität Berlin (bee pathology and molecular microbiology/virology)

Hungary (Hungarian Academy of Sciences)

Dr Anikó Kovács-Hostyánszki, Hungarian Academy of Sciences (agroecology; wild bee ecology; pollination; conservation biology)

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(Expert group members are nominated by EASAC member academies on the grounds of their scientific expertise and are not associated with, nor do they represent, any special interests.)

Notwithstanding these very useful comments, the Expert Group emphasises that responsibility for the content rests exclusively with EASAC.

Annex 2   Ecosystem services

The concept of ecosystem services has developed over many years, but the Millennium Ecosystem Assessment (MEA) of 2005 provided the first global comprehensive analysis of ecosystem services and their use by society. This recognised that nature provides human society with a vast diversity of benefits such as food, fibre, clean water, healthy soil and carbon capture, and that our well-being is totally dependent upon the continued flow of these 'ecosystem services'. However, since they are mostly public goods with no markets and no prices, they are invisible to current economic systems, not protected by market forces, and thus continuously degraded as a result. Ecosystem services were defined in the MEA as '*the benefits people obtain from ecosystems*'. Since then, Fisher and Turner (2008) expanded this definition to '*ecosystem services are the aspects of ecosystems utilized (actively or passively) to produce human well-being*'. The concept of ecosystem services highlights the long-term role that healthy ecosystems play in the sustainable provision of human well-being, economic development, and poverty alleviation across the globe (Turner and Daily, 2008).

The MEA distinguished four groups of ecosystem services.

1. *Supporting services* are those that are necessary for the production of all other ecosystem services, such as primary production, production of oxygen, and soil formation.

2. *Provisioning services* are the products people obtain from ecosystems, such as food, water, genetic resources and fuel.

2. *Regulating services* are the benefits people obtain from the regulation of ecosystem processes, such as climate regulation, pollination, water purification, carbon storage, regulation of greenhouse gas emissions and erosion control.

3. *Cultural services* are the non-material benefits people obtain from ecosystems through spiritual enrichment, cognitive development, reflection, recreation and aesthetic experiences.

The MEA was followed by The Economics of Ecosystems and Biodiversity (TEEB) project (see main report) and since then, a Common International Classification of Ecosystem Services (CICES) has been developed under the EU Framework Program (Haines-Young and Potschin, 2013) which merges the four MEA categories into the following three categories.

1. *Provisioning services*: all nutritional, material and energetic outputs from living systems.

2. *Regulating and maintenance*: covers all the ways in which living organisms can mediate or moderate the ambient environment that affects human performance.

3. *Cultural services*: covers all the non-material, and normally non-consumptive, outputs of ecosystems that affect physical and mental states of people.

Annex 3   Consequences of pollinator declines

The consequences of declines in pollinators may be seen in reduced yields in insect-pollinated crops, and ultimately require a move to crops that do not depend on insect pollination. As the majority of wild flowering plant species benefit from insect pollination (Ollerton et al., 2011), decreases in pollinators will result in a reduced seed/fruit set and may ultimately lead to the local extinction of plant species (Ashman et al., 2004; Aguilar et al., 2006). Loss of flowering plants in turn will reduce the availability of resources for pollinators, predators and parasitoids which, in turn, will reduce insect pollination services for plants in a damaging positive feedback loop (Bascompte et al., 2006). Wild plants form key nodes in many food webs, and pollinator products, such as seeds and fruit, support a wide array of taxa including many invertebrates, mammals and birds. Loss of wild plants could, therefore, have wide-ranging impacts on multiple trophic levels and negatively impact other ecosystem services reliant on plant communities such as soil health, nutrient cycling, water quality, and pest regulation.

In addition to affecting quantity of yields, pollination is also being increasingly shown to have important influences on quality. Garratt et al. (2014) showed that insect pollination enhances fruit quality and economic value as well as just quantity in UK apples. Brittain et al. (2014) showed that with almonds in California, both fat and vitamin E composition of the nuts were highly influenced by pollination. Klatt et al. (2014) showed how ecosystem services provided by pest predators and pollinators increase quantity and quality of coffee yields. The greater pollinator abundance and diversity on organic farms also led to greater pollination success and improved both yield quantity and quality (Andersson et al., 2012).

While wind-pollinated or self-pollinated staple crops supply the vast majority of human foods by volume, insect-pollinated crops contribute vital micronutrients (e.g. vitamins, folic acid) and dietary variety (Free, 1993; Klein et al., 2007; Eilers et al., 2011). For example, plants that depend partially or wholly on insect pollinators provide 70% of vitamin A.

A global survey by Garibaldi et al. (2011b) demonstrated that the declining yield due to loss of pollination services was leading to increased land cultivation to enhance production of pollinator-dependent crops. Vamosi et al. (2006) showed that decline in pollinators in biodiversity rich areas increased competition between plants for pollination services and this could reduce species richness as a result of pollen limitation. Furthermore, Lewer et al. (2014) examined wider pollinator communities where system interactions can help resilience to some drivers of pollinator decline. However, such pollinator communities may collapse suddenly once drivers of pollinator decline reach a critical point.

There is also a contrast between pollinator trends and growth in the area cultivated with insect pollinated plants- leading to a 'pollination gap' (Breeze et al., 2014; Aizen et al., 2009). Owing to EU agricultural and biofuel policies, the demand for honey bees to provide crop pollination across Europe has increased, and honey bee stocks were insufficient in many areas. The supply/demand balance is very different between countries (Figure A3.1).

Figure A3.1   Supply of honey bees compared with demand in Europe (from Breeze et al. (2014); e is 2005 and f is 2010).
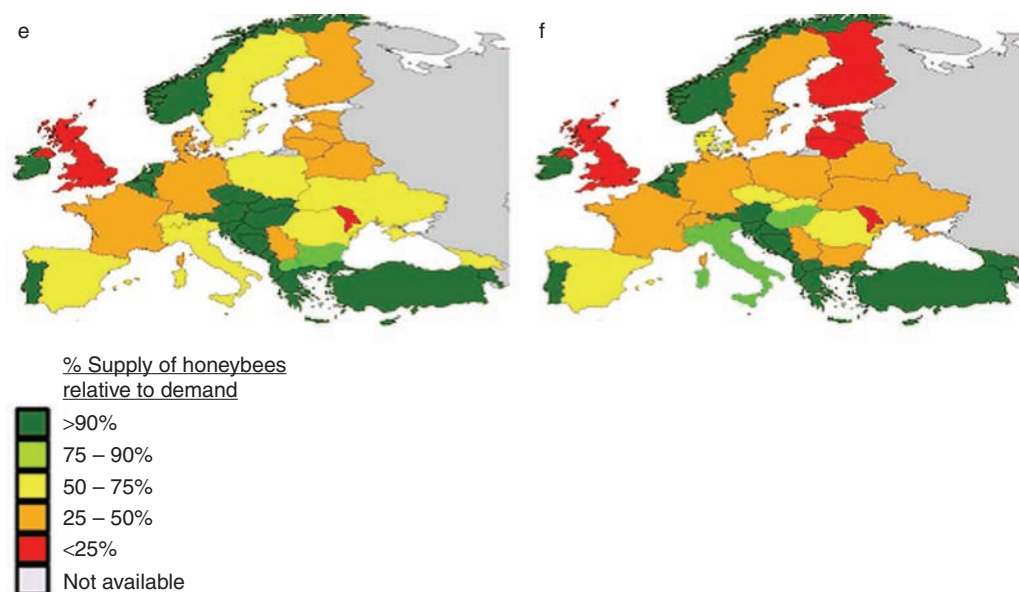
Where honey bees are in short supply, crops have to rely on other pollinators: other species of bee (wild bees and bumble bees), hoverflies and many other insects. Some crops, such as strawberries, tomatoes and peppers, are mainly pollinated by managed bumble bees. Honey bees are not as effective at pollinating some crops (e.g. field beans, apples, raspberry) as wild pollinators (Free, 1993; Willmer et al., 1994; Vicens and Bosch, 2000). Hoverflies are, for instance, the main pollinators of the semi-cropped cloudberry (*Rubus chamaemorus*) in Northern Europe (Hippa and Koponen, 1976), as well as some seed production crops such as celery, parsley and dill in Eastern Europe (Anasiewicz et al., 1989). Research also shows how much more effective at pollination are wild pollinators; Garibaldi et al. (2013) found universally positive associations of fruit set with flower visitation by wild insects in 41 crop systems worldwide. In contrast, fruit set increased significantly with flower visitation by honey bees in only 14% of the systems surveyed. Overall, wild insects pollinated crops more effectively; an increase in wild insect visitation enhanced fruit set by twice as much as an equivalent increase in honey bee visitation.

Annex 4 Overview of evidence on neonicotinoids and organisms providing ecosystem services for agriculture

A4.1   History, mode of action and use

The neonicotinoids in agricultural use and their structures are shown in Figure A4.1 as well as the manufacturers of each variant (Elbert et al., 2008).

The neonicotinoids are neurotoxins that operate by mimicking the naturally present neurotransmitter acetylcholine (Jeschke et al., 2014). Like acetylcholine, they have a positively charged nitrogen (N^+) atom which binds to the negatively charged nicotinic acetylcholine receptors in cells, triggering a response in those cells. In normally functioning cells, acetylcholine is broken down by acetylcholinesterase and the cell synaptic actions are thus reversible. However, neonicotinoids cannot be broken down in this way, and binding is irreversible and cumulative. Cell receptors thus become blocked, leading to paralysis and death. Insect and mammalian receptors are structurally different and thus insecticide research and development focused on finding chemicals that have a higher affinity for insect receptors than mammalian ones; thus most neonicotinoids show much lower toxicity to mammals than insects, and are considered less toxic to humans than previously used organophosphates and carbamates. However, some breakdown products exhibit mammalian toxicity (e.g. desnitro-imidacloprid, which is formed during metabolism and environmental degradation of imidacloprid) and reports of human effects from neonicotinoid exposure in Japan (Taira, 2014) led the author to conclude that neonicotinoids are not necessarily safer than organophosphates.

The first neonicotinoid entered the market in 1991 (Figure A4.1) and neonicotinoid use has since grown rapidly; by 2013 they had become one of the most widely used insecticide groups. The scale of the shift in agricultural practice following the introduction of the neonicotinoids can be shown by the change from 1990 when organophosphates composed 43% of the global insecticide market, pyrethroids (18%) and carbamates (16%). By 2008, neonicotinoids had gained a 24% share of the total market of €6.33 billion, mainly at the expense of organophosphates (13.6%) and carbamates (10.8%) (Jeschke et al., 2011). By 2010, neonicotinoids (and fipronil) accounted for about one-third (in monetary terms in 2010) of the world's insecticide market (Simon-Delso et al., 2015). As one example of the pace of growth, that of neonicotinoid use in the UK is shown in Figure A4.2.

The use of neonicotinoids in seed pre-treatment shows the fastest growth; these are routinely applied to grain and oilseed crops in developed countries, regardless of pest requirements; indeed untreated seeds may be unavailable. A small market of €155 million for insecticidal seed treatment in 1990 was dominated by carbamates (77.4%), but by 2008 had grown to become a €957 million market, 80% of which was for neonicotinoid insecticides. Imidacloprid, clothianidin and thiamethoxam accounted for 85% of the neonicotinoid insecticide market worth US$2,236 million in the USA in 2009. Of these, imidacloprid is the biggest selling insecticide in the world, with sales of US$1,091 million in 2009 (Jeschke et al., 2011), and annual world production of imidacloprid is estimated as approximately 20,000 tonnes (Simon-Delso et al., 2015).

A4.2   Location in plants and exposure routes

Neonicotinoids can be applied as seed coatings, soil drenches, foliar sprays, by injection into trees and shrubs,

Figure A4.1 Chemical structure of neonicotinoid insecticides and year of market introduction (Elbert et al., 2008).
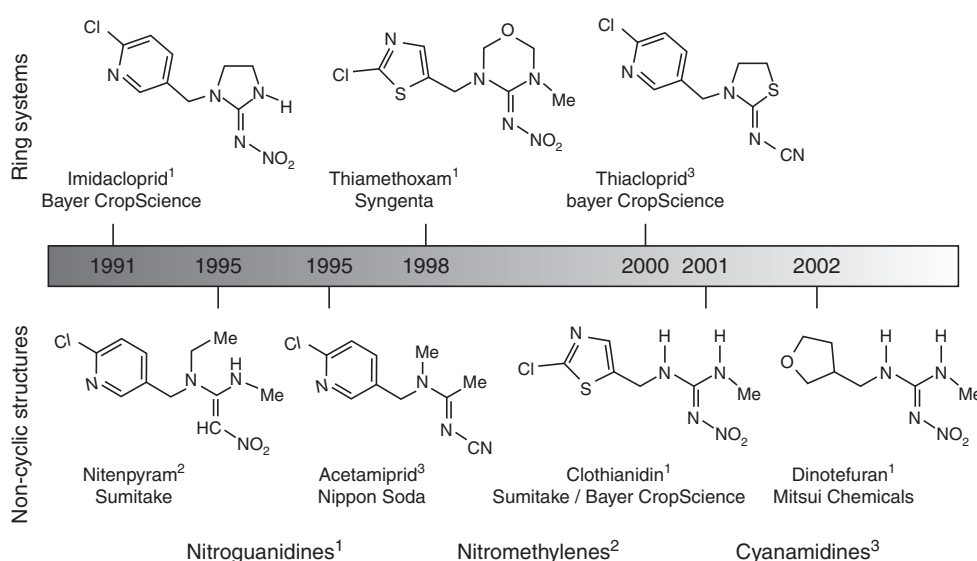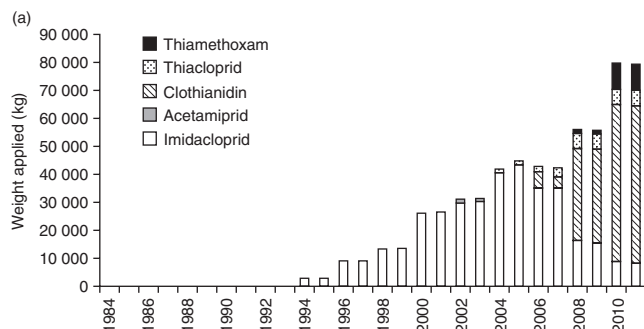
Figure A4.2   *Growth of neonicotinoid use in UK (Goulson, 2013).*

(a)

Legend:
- ■ Thiamethoxam
- ⊠ Thiacloprid
- ▨ Clothianidin
- ▨ Acetamiprid
- □ Imidacloprid

Y-axis: Weight applied (kg), from 0 to 90 000

X-axis: 1984 to 2010

Table A4.1   Half-life in soil of neonicotinoids

Neonicotinoid	Half-life in soil (aerobic soil metabolism)
Acetamiprid	1–8 days
Clothianidin	148–1,155 days
Dinotefuran	138 days
Imidacloprid	40–997 days
Thiacloprid	1–27 days
Thiamethoxam	25–100 days

Source: see references in Hopwood et al. (2012, 2013).

or by addition to irrigation water. They enter the plant as it grows, become distributed throughout it and exert their toxic effects on insects that feed on the plant: in the case of aphids via the sap they ingest, or in caterpillars through the plant tissue they consume. As a result, residues are also found in pollen, nectar and other plant exudates such as guttation fluids. Insecticide residues are also found in contaminated dust released from seed planting equipment and in weeds growing within or adjacent to treated fields, as well as water bodies into which the water-soluble chemicals can drain (Main et al., 2014).

The half-life on leaves after foliar spray is short (3–5 days) because of photolytic degradation (NPIC, 2014), but neonicotinoids may remain in plant tissues for months to over a year (e.g. Maus et al., 2003). With perennial plants and shrubs, residues can persist for several years. Some neonicotinoids can also persist for extended periods in soils. Half-lives in soil based on industry and regulatory data are shown in Table 4.1.

The half-life depends on the insecticide, the soil conditions and temperatures, and thus can be highly variable; moreover, the data on which regulatory decisions were based have been supplemented by other studies (Goulson, 2013) and reveal wider ranges than those in Table A4.1: for imidacloprid, 28–1,250 days; for thiamethoxam, 7–3,001 days; for clothianidin, 148–6,931 days (19 years). Half-lives appear to be shorter for the N-cyanoamidines (thiacloprid and acetamiprid exhibit ranges 3–74 and 31–450 days, respectively).

Goulson (2013) points out that despite the apparent targeted nature of seed dressing, only a small fraction of the insecticide is actually taken up by the plant as it grows (between 1–6% and 20%). Thus most of the insecticide enters the soil; this loss is a considerably higher fraction of the active ingredient than the foliar sprays the seed treatment approach has replaced (typically 50% of the active ingredient from a foliar spray would be absorbed by the plant tissue). In addition, even the proportion that

is taken up will have only a limited period in the plant due to the rapid turnover in plant biomass. For instance, the average lifespan of roots of *Lolium perenne*, the most common grass in fertilised pastures in Western Europe, was found to be 14 weeks, so that the total root mass would be completely replaced three to four times per year. Chemicals in the plant biomass will thus enter the soil and the plant can be viewed as a completely open system as far as organic chemicals are concerned (van der Krift and Berendse, 2002).

Persistent insecticides remaining in the soil may accumulate and be absorbed in successive crops, and increasing concentrations have been detected in the limited number of studies done. In the UK for imidacloprid used in winter wheat seed, 6–18 parts per billion (ppb)[12] remained in the soil 1 year after sowing, and after 6 years of repeated applications, it had risen to 18–60 ppb and not yet levelled off (Goulson, 2013). In France, random soil sampling for imidacloprid (Bonmatin et al., 2003; 2005) showed that while seven organic farm samples contained no imidacloprid, the insecticide was detected in 62 of 67 samples from conventional farms; 65% of these were above 1 ppb; nine samples contained 10–100 ppb; and three exceeded 100 ppb.

In soil samples taken from the central area of fields (Jones et al., 2014), concentrations of clothianidin ranged from 0.02 to 13.6 ppb. Thiamethoxam concentrations ranged from less than 0.02 to 1.5 ppb, and imidacloprid concentrations from less than 0.09 to 10.7 ppb. Concentrations of clothianidin and thiamethoxam were lower in soil samples taken from the edges of fields than from the centres. There was evidence that imidacloprid was more persistent in the soils studied than clothianidin and thiamethoxam. Neonicotinoids may also move away from treated fields because of their solubility in water and enter aquatic systems (Main et al., 2014). Imidacloprid was detected in 89% of water samples in a study in California (Starner and Goh, 2012); 19% of these exceeded 1 ppb. In the Netherlands, concentrations of up to 200 ppb in groundwater, streams and ditches have been reported (van

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[12]   Depending on the publication, units can be nanograms per gram (ng/g, ng g−1) or micrograms per kilogram (µg/kg, µg kg−1). Here they are all expressed in parts per billion (ppb; 1 part per 10^9). We also use parts per million (ppm; 1 part per 10^6) and parts per trillion (ppt; 1 part per 10^{12}).

Dijk, 2010). These high levels of persistence are important factors that need to be considered when determining the ecotoxicological properties of the neonicotinoids.

The review of environmental fate and exposure by Bonmatin et al. (2015) finds that, typically, neonicotinoid concentrations in soil decline rapidly after application, but that in cool, dry and high organic matter-containing soils, they can persist and possibly accumulate for months or years. Moreover, metabolic pathways are complex and still poorly quantified but include common metabolites that can be toxic (Simon-Delso et al., 2015) and result in prolonged toxicity. This high variability makes prediction of the persistence and fate of the active chemical difficult and leads to high levels of uncertainty in assessing exposure and associated risks.

The accumulated research and field measurements on persistence and mobility thus show that, depending on the half-life of the specific neonicotinoid, the active chemical may move away from the field where first applied, expanding contamination of agricultural soils into groundwater, streams, wetlands, non-target vegetation, etc. Bonmatin et al. (2015) note that levels reported in the literature range from parts per billion to parts per million (in soils), parts per billion to parts per trillion (in water) and parts per billion to parts per million (in plants). The potential chronic exposure of non-target organisms is thus broad: non-pest insects and animals through eating pollen, nectar, leaves or seeds of plants containing the insecticide transmitted from the primary crop, and via non-crop plants in areas adjoining the treated areas (hedgerows, margins and other areas of natural vegetation). Thus, although the main targets of the insecticides are sap feeder pests such as aphids, other beneficial insects including bees (and other pollinators) and natural pest predators or parasitoids are also vulnerable to exposure through a variety of mechanisms including the following:

- contaminated pollen and nectar;

- direct spray;

- residue contact (e.g. with contaminated leaves or stems);

- particles released in seed planting (dust from abraded coatings or talc from treated seeds);

- contaminated nesting areas or nesting materials;

- contaminated water;

- guttation fluid, extra-floral nectaries (nectar producing glands outside flowers) and root exudates;

- residues which persist in the soil and spread to adjoining areas and watercourses;

- transfer across trophic levels through predators/ parasitoids eating contaminated prey/hosts.

In the next sections we summarise the data on toxicity to honey bees and other pollinators. We then move on to other parts of the ecosystem that are potentially affected.

A4.3   Pollinators

A4.3.1   Toxic effects of direct exposure of honey bees

Insecticide-treated seeds are coated with a polymer to control release, and talc is added to avoid sticking in the planting equipment. This can be released as a dust containing insecticide and the resulting direct exposures have led to mass mortalities. In 1994, mass honey bee deaths from exposure to dust containing imidacloprid in France were revealed, while in Germany, a mass death of honey bees was caused by clothianidin. Such acute high-concentration exposures led to specific bans in some EU Member States.

Girolami et al. (2012) and Tapparo et al. (2012) have shown that honey bee foragers can acquire lethal doses of neonicotinoid residues in-flight, with concentrations up to 60 times the lethal dose. To reduce this risk, modifications have been introduced to planting equipment and seed treatment formulations improved. However, Bonmatin et al. (2015) note that modifications can be time consuming and affect seed placement, so that their use is not assured. Alternatives to talc are now being offered to reduce dust during sowing. While it is generally accepted that such improvements to machinery and stewardship practices have reduced the risk of the early mass mortality events, direct mortality reports continue: for example, in the UK, 21 incidents of poisoned bees associated with neonicotinoids were reported to the Wildlife Incident Investigation Scheme in 2012 (POST, 2013). The Incident Reporting Program of the Canadian Pest Management Regulatory Agency has reported 110 incidents involving field mortality of bees in Canada since 2007, only six of which occurred before 2012. The neonicotinoids were suspected in most incidents (Cutler et al., 2014).

A4.3.2   Acute toxic effects from plants (honey bees)

Laboratory tests of oral and/or contact LD_{50} (dose lethal to 50% of animals tested) show clothianidin, dinotefuran, imidacloprid and thiamethoxam to be highly toxic to honey bees. Acetamiprid and thiacloprid have structural differences that make them less toxic; the contact LD_{50} for thiacloprid is 816 times higher than for imidacloprid (Iwasa et al., 2004). Honey bees appear to be more susceptible through oral ingestion; a summary of results of studies on the oral ingestion route and laboratory-demonstrated effects is shown in Table A4.2. These are only general indications of concentrations causing direct toxic effects; some neonicotinoid breakdown products are less toxic than the parent while others are more so (e.g. imidacloprid); thiamethoxam breaks down to clothianidin.

Table A4.2   Effects of neonicotinoid concentrations (in parts per billion) when ingested by honey bees, and concentrations observed in pollen (P) and nectar (N) for different application methods

		Application method	
Neonicotinoid	**Honey bees**	**Seed dressing**	**Drenching**
Acetamiprid			
Lethal concentrations			
Acute exposure	>442,500		
Chronic exposure	No data		
Sublethal effects			
Acute exposure	5,000		
Chronic exposure	5,000		
Clothianidin		Canola P 3; N 3.7	
Lethal concentrations		Corn/maize P 15	
Acute exposure	190	Rape P 12, 23; N 5, 16	
Chronic exposure	NOEC 10	Sunflower P 3	
Sublethal effects			
Acute exposure	24		
Chronic exposure	No data		
Dinotefuran			Pumpkin P 44–69
Lethal concentrations			N 7.1–10.6
Acute exposure	>380		
Chronic exposure	No data		
Sublethal effects			
Acute exposure	No data		
Chronic exposure	No data		
Imidacloprid		Corn P 2.1 average; ≤18	Apple blossom 12 (at 97 days)
Lethal concentrations		Sunflower P average 3–3.9; max 36	Pumpkin N 4–13; P 30–101
Acute exposure	≥185	N average 1.9	Rhododendron 27–850
Chronic exposure	NOEC 24–48		(after 6 years; 19)
Sublethal effects			other shrubs up to 4,500
Acute exposure	LOEC 0.2–50		
Chronic exposure	NOEC 0.02–100		
Thiacloprid		Rape P range 0–199	
Lethal concentrations			
Acute exposure	>425,500		
Chronic exposure	No data		
Sublethal effects			
Acute exposure	No data		
Chronic exposure	No data		
Thiamethoxam		Corn P 1–7	Pumpkin
Lethal concentrations		Rape P1–7, 8; N 0.6–2.4, 3.8–4.2, 5	N 54.8–90.5
Acute exposure	≥250		P 7.8–12.2
Chronic exposure	No data		
Sublethal effects			
Acute exposure	No data		
Chronic exposure	25–50; (NOEC 10)		

NOEC, no observed effect concentration; LOEC, lowest observed effect concentration. Ranges of concentrations from Hopwood et al. (2012); EFSA reviews; Blacquiere et al. (2012); Godfray et al. (2014); Pilling et al. (2013); Rundlöf et al. (2014). (A more comprehensive listing of the range of concentrations observed in pollen and nectar has just been published in Bonmatin et al. (2015), but without separation by crop type.) Note that different studies use different toxicological endpoints so the figures are only indicative of the order of magnitude of concentrations that cause a range of lethal and sublethal effects.

There is a significant shortage of data on chronic toxicity and sublethal effects on honey bees, and much greater shortages of data on bumble and solitary bees. Indeed, much of the sublethal data now available have only been published in recent years (see Box A4.1). Thus, decisions on approval, and initial responses to the reports of bee mortality, relied on comparing acute lethal concentrations with concentrations likely to be encountered in the field. In general, laboratory-tested acute lethal effects through oral ingestion occur at concentrations of tens of parts per billion or above, and this has led industry to suggest a 'safe' limit of around 20 ppb for ingestion (Schmuck and Keppler, 2003). Limited data on concentrations in nectar and pollen are available, and are summarised in Table A4.2. It is clear that seed treatments (in which the insecticide spreads through and is 'diluted' by the growing plant) lead to lower pollen concentrations than drenching. In general, levels of imidacloprid and clothianidin in pollen/nectar resulting from seed treatments are below 20 ppb. Godfray et al. (2014) calculated an average from 20 studies of various seed-treated crops of 1.9 ppb (nectar) and 6.1 ppb (pollen), but persistence in soil could lead to increased residue levels as insecticides from previous seasons remain (Annex 4.2). In addition, there are substantial differences in pollen and nectar concentrations according to the time of planting; winter planting provides several months before flowering, whereas spring planting may flower within weeks. One study recorded clothianidin levels of up to 23 ppb in pollen and 16 ppb in nectar in spring-sown oilseed rape (Rundlöf et al., 2014).

Goulson (2013) concludes that, when applied as seed dressings, concentrations of the three most studied neonicotinoids (imidacloprid, thiomethoxam and clothianidin) in nectar and pollen appear unlikely to reach acutely toxic levels, so that field-realistic exposure of honey bees to neonicotinoids in nectar and pollen of seed-treated crops is unlikely to cause substantial *direct* mortality. This is also the conclusion of Godfray et al. (2014), who calculated that field-relevant concentrations and actual amounts harvested by bees are unlikely to exceed 1–10% of the acute oral LD_{50}, even under worst case assumptions of field concentrations, no dilution of contaminated pollen and nectar by other sources, and ingestion by the hive's highest pollen consumers.

The Expert Group notes these general assessments but emphasises that the use of standard lethal toxicity testing in risk assessments is not adequate, as there are sufficient indications from toxicology that sublethal (e.g. behavioural, physiological and reproductive) effects can be much more relevant to environmental situations, as discussed later. The Group also notes that only a few studies are available on the effects from neonicotinoids on pollinators other than honey bees. Bumble bees and other wild bee species comprise the vast majority of pollinating species, and the lack of information on neonicotinoid toxicity for most pollinators complicates the evaluation of the ecological impact from the use of

neonicotinoids. In general, there is a positive correlation between pesticide toxicity for *Apis mellifera* and other pollinating bee species (Arena and Sgolastra, 2014), with body size being one parameter determining impact (Devillers et al., 2003). Because of fundamental differences in life-history traits, the impacts from exposure to neonicotinoids at the colony and population levels will vary significantly, depending on the traits of the bee species (Brittain and Potts, 2011). Thus, studies on *A. mellifera* may not be useful for predicting effects from neonicotinoids on other bees.

The EFSA, in their analysis of the risk from three neocotinoids, used the exposure to toxicity ratio (ETR_{acute}) between the amount of residues that may be ingested by an adult bee in 1 day and the LC_{50} value as an indicator of risk. Depending on the crop, insecticides and type of bee, such ETRs range from less than 0.1 to more than 5. For instance, the calculations for oilseed rape using imidacloprid as a seed dressing are as in Table A4.3.

However, these figures, even when well below an ETR level of 1, are not reassuring for several reasons. Firstly, toxicological risk assessment normally regards a safety margin of one order of magnitude between exposure and toxicity levels as inadequate and requires a larger difference, particularly where there is a shortage of chronic exposure data and limited data on other species.

Secondly, longer-term accumulation over days or weeks could reach higher levels if ingested neonicotinoids are not metabolised or excreted. Moreover, with soil drenching and/or addition to irrigation water, and foliar sprays, levels in pollen and nectar can reach much higher levels (also included in Table A4.2), with pumpkins in the USA reaching over 100 ppb which is well into the range where direct toxic effects could follow ingestion. With ornamental plants, extremely high levels can persist for years inside woody plants, and have been associated with rapid mortality in bees feeding on the flowers (Hopwood et al., 2012).

The third reason is the concern over whether the standard LD_{50} test is appropriate for systemic insecticides where their persistence in the plant allows exposure to be prolonged, so that the effects of low doses over extended periods may be more relevant than the results from the 'short sharp shock' of the LD_{50} test. One early study suggested that the lethal toxicity

Table A4.3 ETR_{acute} values for authorised uses of imidacloprid on oilseed rape (EFSA, 2013a)

Application rate	ETR_{acute} forager bee	ETR_{acute} nurse bee
lowest 'maximum application rate' = 10 g a.s./ha	0.37	0.15
highest 'maximum application rate' = 52.5 g a.s./ha	1.93	0.78

Box A4.1   Research on sublethal effects on bees

Early laboratory experiments where honey bees were given a single acute contact or oral sublethal dose of a neonicotinoid had previously indicated that imidacloprid alters learning (Guez et al., 2001), impairs learning and foraging (Decourtye et al., 2003; Han et al., 2010), motor activity (Medrzycki et al., 2003), memory (Decourtye et al., 2004) and reduces ability to communicate; these could have profound effects upon social behaviour (Medrzycki et al., 2003). Clothianidin impairs foraging behaviours (Schneider et al., 2012) and acetamiprid impairs activity, memory and sucrose sensitivity (El Hassani et al., 2008). Thiamethoxam decreases sucrose sensitivity and memory (Aliouane et al., 2009). However these tests generally used doses above 20 ppb, so were regarded by the neocotinoid manufacturers, users and some government regulatory bodies as not indicating that such impacts would be triggered in the field.

Laboratory studies on bumble bees show that bees fed imidacloprid-contaminated pollen at a low dose (7 ppb) reduced foraging ability, and trembling was seen in bees fed a higher dose (30 ppb) (Morandin and Winston, 2003). In addition, reduced drone production and longer foraging times were seen in bees fed lower doses (10 ppb) of imidacloprid (Mommaerts et al., 2010). Bumble bees fed both imidacloprid-contaminated nectar and pollen (16 ppb) had lower worker survival rates and reduced brood production (Tasei et al., 2000). However, with clothianidin-contaminated pollen at doses of 6 or 36 ppb, Franklin et al. (2004) found no significant sublethal effects.

Laboratory studies also demonstrate that acute contact with imidacloprid is highly toxic to alkali bees (Stark et al., 1995; Mayer and Lunden, 1997), alfalfa leafcutter bees (Stark et al., 1995; Mayer and Lunden, 1997; Scott-Dupree et al., 2009) and blue orchard bees (Scott-Dupree et al., 2009). Acute contact with clothianidin was also toxic to blue orchard bees and alfalfa leafcutter bees (Scott-Dupree et al., 2009); *Osmia lignaria* is more sensitive to both clothianidin and imidacloprid than *Bombus impatiens*, with *Megachile rotundata* more sensitive still.

In the past few years, peer-reviewed independent studies have attempted to simulate more 'field-realistic' exposures and their effects on individuals or behaviour. These include the following:

- Sublethal concentrations of thiamethoxam can impair the function of the brain and mid-gut, and contribute to lifespan reduction (Oliveira et al., 2013).

- Imidacloprid in food affects bumble bee colony development by slowing bee growth, leading to 8–12% smaller colonies and fewer queens (one or two compared with 14 in uncontaminated control colonies) (Whitehorn et al., 2012).

- Scholer and Krischik (2014) also found a statistically significant reduction in queen survival of *B. impatiens* (at 20 ppb, there is 37% reduction for imidacloprid, 56% for clothianidin), also of worker movement, colony consumption and colony weight compared with 0 ppb treatments. At 10 ppb imidacloprid and 50 ppb clothianidin, fewer males were produced.

- Laycock et al. (2012) looked at effects of imidacloprid on reproduction in worker bumble bees and noted effects on ability to feed so that foraging efficiency is reduced, with debilitating effects on reproduction. Laycock et al. (2013) found that with exposure to field-realistic concentrations lasting about 2 weeks, brood production in worker bumble bees is more sensitive to imidacloprid than thiamethoxam.

- Mommaerts et al. (2010) found that colonies fed doses of 10 and 20 ppb were not producing offspring; only colonies fed 2 ppb exhibited no sublethal effects. Also, when workers had to walk 20 cm down a tube to gather food, there were sublethal effects on foraging activity, with a median sublethal effect concentration (EC_{50}) of 3.7 ppb.

- Cresswell et al. (2012) demonstrated that bumble bees exhibit sublethal responses to imidacloprid at 10 ppb, while honey bees were unaffected at this concentration.

- Sublethal doses of imidacloprid change the respiratory pattern of bees, and hypopharyngeal glands are smaller (Hatjina et al., 2013).

- Henry et al. (2012) showed that navigation and orientation abilities of bees eating pollen or nectar contaminated with thiamethoxam are affected; some failed to return and were more likely to die, weakening the colony.

- Imidacloprid affects honey bee foraging trips at low concentrations, causing delays in feeding trips and increased losses when bees are fed sublethal doses of the pesticide (Yang et al., 2008).

- Imidacloprid repels some pollinators from feeding on pollen (Easton and Goulson, 2013).

- Gill et al. (2012) observed impairment by imidacloprid of foraging that resulted in reduced colony productivity (reduced number of worker bees).

- One study found exposure of bee brains to imidacloprid and clothianadin inactivated bee brain tissue (Palmer et al., 2013).

- Imidacloprid affects neural development and impairs walking of newly emerged adult workers (Tomé et al., 2012).

- Sandrock et al. (2013) examined the influence of field-realistic trace residues of thiamethoxam and clothianidin in nectar substitutes on the entire lifetime fitness performance of the red mason bee, *Osmia bicornis*, and found that chronic, dietary neonicotinoid exposure has severe detrimental effects on solitary bee reproductive output.

- Sandrock et al. (2014) investigated the effects of field-realistic trace residues of thiamethoxam and clothianidin in pollen on honey bee colonies and found that chronic, dietary neonicotinoid exposure has significant negative effects on queen lifespan and on colony performance. They also found a genetic component to susceptibility to neonicotinoid pesticides.

- Lu et al. (2012) found evidence of a link between imidacloprid and CCD. A dose of 20 ppb led to CCD in 94% of colonies within 23 weeks, with no signs of virus or mites.

- Pilling et al. (2013) monitored honey bee colonies placed beside thiamethoxam-treated or control fields of maize or oilseed rape just during the crop flowering period (at other times the colonies were kept in woodland). Over 4 years, honey bees from treatment hives had higher concentrations of insecticides, but no differences in colony performance were found.

(Continues on next page)

- Thompson et al. (2013) (not peer reviewed) placed bumble bee colonies within landscapes known to contain oilseed rape treated with neonicotinoids and a second set of colonies to act as controls next to non-treated crops. They found no relationship between colony growth and neonicotinoid residues within pollen or nectar in the colonies. However, neonicotinoid residues were found in the control site, and thiamethoxam was found at the highest levels even though it was not used on the experiment's treated fields.

- Fischer et al. (2014) used a catch-and-release experimental design, in which feeder-trained bees were caught when arriving at the feeder, treated with one of the neonicotinoids tested (imidacloprid, clothianidin and thiacloprid), and released 1.5 hours later at a remote site. The flight paths of individual bees were tracked with harmonic radar. The rate of successful return was significantly lower in treated bees, the probability of a correct turn at a salient landscape structure was reduced, and fewer directed flights during homing were performed. This indicated, under field conditions, that non-lethal doses of these three neonicotinoids either block the retrieval of exploratory navigation memory or alter this form of navigation memory.

- Cutler et al. (2014) placed hives of honey bee in fields of canola treated with clothianidin for the flowering period, then maintained them in apiaries surrounded by untreated crops. Comparison with control hives showed no detectable effects on colony performance or winter survival.

(Note: further assessment of the significance of many of the recent studies can be found in the supplementary material to Godfray et al., 2014.)

of imidacloprid (including its neurotoxic metabolites) increases up to 100,000-fold when the same exposure used in the short term LD_{50} test is extended in time (Suchail et al., 2001). Such differences are beyond the ranges normally encountered (two to three orders of magnitude between acute and chronic toxicity would normally be expected for persistent compounds and less for compounds that are rapidly metabolised), and thus controversy exists over these findings. However, recent work on the effects of imidacloprid on fruit flies also revealed a substantial difference in the toxic effects of the same dose of insecticide *depending on whether it was administered in a single dose or in smaller doses over 8 days*. Charpentier et al. (2014) found that the chronic LC_{50} was much lower than the acute LC_{50}, by a factor of 29 (males) to 172 (females). This experiment also allowed the lowest observed effect concentration (LOEC) to be compared with the standard acute LC_{50}: it was found that this was 46,000 times lower for males and 115,000 times lower for females. Such nonlinear responses reflect the complex interplay of receptor binding and gene reprogramming effects, which are still being mapped out (Fagin, 2012; Charpentier et al., 2014); moreover, there remain no standardised protocols for measuring such chronic lethal effects.

Rondeau et al. (2014) reviewed toxicity data from published sources to examine time-dependent toxicity of imidacloprid for ants, termites and honey bees. They confirmed toxic effects over longer exposure periods at much lower levels than encountered in standard 1–4 day LC_{50} toxicity tests, and found a relationship between time and the level required to cause 50% mortality to be related to the square of the time of exposure. When considering exposure over the 150 days of an overwintering bee, they concluded that honey with 0.25 ppb of imidacloprid would cause long-term mortality.

Most research has focused on pollen and nectar, but a further question has recently emerged over the paths for bees to be exposed since they also collect water

from standing pools, which may be contaminated with insecticides, further increasing their exposure. Systemic insecticides may also be exuded in guttation fluid, which honey bees may collect as a source of water. Girolami et al. (2009) found concentrations of up to 100 milligrams per litre (mg/l; equivalent to parts per million) for thiamethoxam and clothianidin, and up to 200 mg/l for imidacloprid, in maize leaf guttation fluid from plants grown from coated seeds. Tapparo et al. (2011) measured up to 346 mg/l imidacloprid, 102 mg/l clothianidin, and 146 mg/l thiamethoxam. EFSA (2013c) calculated the risk by assuming an average of 46 trips a day for honey bees engaged in foraging for water. The amount carried on each trip in the crop of the bee ranges between 30 and 58 microlitres, amounting to 1.4–2.7 millilitres of water each day. The EFSA concluded that, for clothianidin, thiamethoxam and imidacloprid, there were insufficient data to evaluate fully the risks to bees posed by insecticides in guttation fluid.

A4.3.3 Delayed toxicity and sublethal effects (honey bees and other bees)

Bees (both honey bees and bumble bees) work as a group with several key roles[13], which must be performed effectively if the colony (or hive) is to survive and prosper. Chemicals that disrupt cognitive abilities, communication, role behaviours, location finding and other key roles may weaken and lead to a hive's collapse, even if individual bees are not killed. For instance, the ability of a honey bee colony to collect and store food depends on coordination and communication between workers; thus chemicals that reduce the ability of worker bees to forage and communicate may damage colony health (Desneux et al., 2007). More recent studies of honey bee response to sublethal doses of neonicotinoids have thus focused on these 'system' aspects, including measurements of foraging and feeding behaviours, learning, navigation and mobility.

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[13] Key roles include foraging, locating and returning to the hive, communicating and remembering the location of food sources, defence against predators, nursing and feeding larvae.

These studies have been reviewed by EFSA (2013a), Blacquière et al. (2012), Goulson (2013) and Godfray et al. (2014) (as well in non-peer reviewed cases (e.g. DEFRA, 2013)). Major findings and issues are summarised in Box A4.1.

Box A4.1 shows that neonicotinoids can have several sublethal effects in the laboratory and in semi-field experiments, which could potentially disrupt or incapacitate critical functions of the colony. This suggests that risk assessments should be based on neurodevelopmental and neurobehavioral endpoints; instead, the focus has been on whether such effects can be detected at organism or colony levels in the field. Up to now, it has not yet been established that concentrations in *normal field conditions* are having significant impacts at the honey bee *colony* level. However, the Expert Group notes that the difficulties of conducting experiments and obtaining appropriate data mean that this cannot be resolved satisfactorily. Studies based on 'real' field conditions have to meet very difficult methodological challenges, for instance the following:

1. Bees (both honey and bumble) forage over long distances (up to a few kilometres) so that the areas accessible to both experimental and control hives are very large and can overlap.

2. The use of neonicotinoids is so extensive it is difficult to find a location that is not already contaminated to act as a control.

3. Field conditions include so many variables, the ability of one experiment to detect subtle effects remains in doubt, and the applicability of any results to different crops, insecticides and other circumstances may also be limited.

4. The multifactorial origin of colony losses, caused by complementary and/or synergistic interactions among different stress factors, which are different over time and space, severely limits the reproducibility of field experiments aiming to assess the impact of a single stress agent, such as the exposure to a specific insecticide.

An additional problem arising from the widespread use of the neonicotinoids and their persistence is that bees may be exposed to more than a single insecticide, so that interpretation of results from controlled exposure to just one insecticide is difficult. In this context, Mullin et al. (2010) found ten pesticides in pollen at greater than one-tenth the honey bee LD_{50} level in the USA, indicating that combinations of such toxins (both

neonicotinoids and non-neonicotinoids) are likely. In particular, insecticides may be used in combination with fungicides and there may be synergistic effects that increase the toxicity of the insecticide. In the laboratory, DMI fungicides increased the toxicity of acetamiprid and thiacloprid as much as 244-fold, but not imidacloprid (Iwasa et al., 2004). An EFSA review (Thompson, 2012) found that significant synergy has been reported between EBI fungicides and both neonicotinoids and pyrethroid insecticides. Recently Sanchez-Bayo and Goka (2014) reviewed risks to bees from all pesticides found in pollen and nectar and noted that 124 pesticide residues had been found in pollen, and 77 in nectar or honey. Bees were thus seldom exposed to single active agents. Insecticides with the highest risk from observed concentrations included thiamethoxam, imidacloprid and clothianidin[14]. Synergism with ergosterol-inhibiting fungicides increased risks.

The mechanism of such synergistic effects is not fully understood. Additive toxicity of a combination of neonicotinoids is easy to understand, because the mechanism of action most probably involves the same receptor. In toxicology and pharmacology this usually leads to the default assumption of at least additivity. Synergistic effects between neonicotinoids and carbamates/organo-phosphates can also be expected from interactions of these compounds on neurotoxicological endpoints. However, the mechanism of the synergy observed with fungicides is not yet clear, especially if exposure levels are significant below that of overt toxicity (e.g. LD_{50}) values[15]. It is thus important to acquire a better understanding of such synergy through mixture toxicity studies to improve our understanding and allow a realistic risk assessment. Depending on whether compounds in the mixture act as a (partial) agonist or (partial) antagonist, deviations can be found from the presumed additivity, in which actually antagonism is usually more common.

A4.3.4   Evaluating toxicological risk to honey bees

Conclusions on the degree of risk involve judgements on many aspects: on the nature of the effect being considered (individual or colony); on the species of pollinator (data are very poor or non-existent on other than *Apis mellifera* and *Bombus terrestris*); on the nature of the insecticide use (dressing, spray or drench); on the extent to which actual application follows or does not follow the manufacturers' instructions; and other factors. This gives ample scope for different conclusions about risk. The basis of judging the acceptability of the risk includes two approaches used by EFSA.

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[14] They found that imidacloprid poses the highest risk to bumble bees (31.8–49% probability to reach the median lethal cumulative dose after 2 days feeding on field-realistic dose in pollen) and thiamethoxam the highest risk to honey bees (3.7–29.6% probability to reach median lethal cumulative dose).

[15] Emerging research indicates that the fungicide reduces detoxification mechanisms in the bee, making the neonicotinoid more toxic.

The first is the 'exposure to toxicity ratio' (ETR), which has already been mentioned. While the figures for exposure and toxicity will tend to be upper limits and thus probably worst-case scenarios, a ratio of 1 nevertheless suggests that there is no safety margin between the levels of the insecticide that may be encountered by the pollinating insect and the levels that have been shown to be toxic. In their evaluation of clothianidin, imidacloprid and thiamethoxam, calculations of ETR close to or even above 1 were calculated for some scenarios of treatment method and crop, while other combinations showed levels of below 0.1.

The other approach is the reverse, which is to compare the residue levels in nectar (or pollen) the bee may consume with the toxicological endpoint of 'no observed effect concentration' (NOEC). When this is applied to imidaclropid (Tables A4.4 and A4.5), the maximum expected residue levels in rape are still a factor of 2.9 below the NOEC; and for other crops the factor is higher.

Similar uncertainties abound in the potential route through guttation fluid. For clothianidin, concentrations in guttation fluid from maize have been measured as high as 717 mg/l and, as noted by the EFSA, a honey bee would have to consume only 0.005 microlitres of such a fluid to reach the acute oral LD_{50}. When the amounts of water actually carried by bees is considered (the EFSA estimated 1.4–2.7 millilitres of water per day), it is clear that contamination of guttation fluid could be toxic *if* that fluid is a significant source of the bee's water supply. The latter point, however, will depend on location of alternative supplies, which in turn depend on the circumstances of each individual bee, weather, time of day, etc.; so sufficient uncertainties remain to support opposite conclusions of risk or no risk. Some experiments suggest bees rarely collect guttation fluid, in which case, as the EFSA observes, the risk may be considered low.

Nevertheless the existence of such high concentrations raises significant uncertainties.

The EFSA's assessment of the risk to honey bees was the basis of the European Commission's 2013 decision on use restrictions. As one example of a conflicting view, Fairbrother et al. (2014) conclude that '*It is not reasonable, therefore, to conclude that crop-applied pesticides in general, or neonicotinoids in particular, are a major risk factor for honey bee colonies, given the current approved uses and beekeeping practices*'. In their critique, they cite general reviews such as by Staveley et al. (2014) and UNEP (Kluser and Peduzzi, 2007), which conclude that the major factor in reduced survival of bee colonies is *Varroa* mites (neonicotinoid pesticides were rated as unlikely to be the sole cause but could not be excluded as a contributing factor). Evidence that genes responsible for the immune response are downregulated during mite feeding are also cited as suggesting increased bee susceptibility to viral infection as the result of a compromised immune system. However, the key role proposed for *Varroa* in the induction of immunosuppression (Yang and Cox-Foster, 2005) has been questioned (Gregory et al., 2005b; Navajas et al., 2008; Zhang et al., 2010; Ryabov et al., 2014), while the deformed wing virus appears to exert that action only when attaining high levels of infection (Nazzi et al., 2012). In any case, the close association and interaction between these two stress agents severely limit the immune barriers of the honey bee.

Contrasting the results of LD_{50} tests with likely field concentrations, Fairbrother et al. (2014) point to the many variables that influence toxicity outcomes (e.g. sensitivity to insecticides can vary with the age of the test bees, subspecies, season, nutrition, physiological condition and handling during testing). They also highlight inconsistencies between various studies (e.g. on the

Table A4.4   Comparison of the residue levels in nectar to the toxicological endpoint for foragers (EFSA, 2013a)

	Oilseed rape	Sunflower	Cotton
Residue level for the lowest 'maximum application rate'	1.59 µg/kg	1.9 µg/kg	3.45 µg/kg
Residue level for the highest 'maximum application rate'	8.35 µg/kg		4.6 µg/kg
Chronic endpoint (NOEC)		**24 µg/kg**	
Ratio (NOEC/residue) for the lowest 'maximum application rate'	15.1	12.6	7.0
Ratio (NOEC/residue) for the highest 'maximum application rate'	2.9		5.2

Table A4.5   Calculated residue levels in the mixed diet of nurse bees and the comparison of these levels to the toxicological endpoint for foragers (EFSA, 2013a)

	Oilseed rape	Sunflower	Maize	Cotton
Residue level (RC) for the lowest 'maximum application rate'	1.58 µg/kg	2.27 µg/kg	0.56 µg/kg	3.45 µg/kg
Residue level (RC) for the highest 'maximum application rate'	8.32 µg/kg		2.8 µg/kg	4.6 µg/kg
Chronic endpoint (NOEC)		**24 µg/kg**		
Ratio (NOEC/RC) for the lowest 'maximum application rate'	15.1	10.6	42.6	7.0
Ratio (NOEC/RC) for the highest 'maximum application rate'	2.9		8.6	5.2

interactions between neonicotinoid residues and *Nosema* infection); and they emphasise that the concentrations of exposure in some sublethal field trials (e.g. on homing behaviour) are at concentrations significantly higher than expected from field exposure. At the same time, they emphasise the important influences of other factors, including pathogens and nutrition management. They cite a major study of honey bee losses in Germany (Genersch et al., 2010) as finding that the main factors that had a statistically significant association with colony losses were (1) high infestation levels of *Varroa destructor*, (2) infection with deformed wing virus and acute bee paralysis virus in autumn, (3) queen age and (4) weakness of the colonies in autumn. No association could be observed for *Nosema* species or pesticides[16].

Concerning the meta analysis by Cresswell (2011), which suggested that dietary intake of imidacloprid at field realistic levels could have sublethal effects and reduce honey bee performance by 6–20%, Fairbrother et al. (2014) countered with other field studies (Maus et al., 2003; Stadler et al., 2003; and the same Cresswell study), which suggested that neonicotinoids in nectar and pollen do not cause acute toxic effects on foraging honey bees or significant health effects to colonies when applied in conformity with label directions[17]. Another study by Nguyen et al. (2009) of apiaries in a radius of 3,000 metres of maize fields treated with imidacloprid had also concluded that high mortality rates were more probably the result of mite infestations and that imidacloprid seed-treated maize had no measurable impact on honey bees.

Vanbergen et al. (2013) focused on the key pressures on pollinators and their *interactions* with various risk or stress factors. They highlighted five critical interactions:

1. *Land-use intensification and associated pesticide use*; with honey bees chronically exposed to a cocktail of different chemicals that can subtly interact, sometimes synergistically, with detrimental effects on bee survival, learning and navigation behaviours (Johnson et al., 2009; Cresswell, 2011; Henry et al., 2012).

2. *Climate change*, where plant and pollinator ranges are shifting, also interacts with habitat fragmentation.

3. Interaction between *nutrition and pathogens*, where management practices or reduction in diversity of bee diet (caused by monoculture agriculture) may weaken resistance to pathogens.

4. *Nutrition* may also interact with *pesticides* because variations in diet, and changes in beekeeping practices

or land use management that affect bee nutrition have the potential to reduce or enhance the ability of the honey bees to detoxify pesticides.

5. Combined impacts of *pathogens and pesticides* have physiological implications for bee health both at individual and at colony levels.

A4.3.5 Combination effects with diseases and parasites

There has been much recent research on combination effects relevant to Vanbergen et al. (2013)'s fifth interaction. Honey bee colonies may be affected by a range of diseases and parasites (Potts et al., 2010a), and several studies have looked at possible synergy between insecticide exposure and susceptibility. Such interactions could have a role to play in CCD, through the hypothesis that insecticide exposure could interact with viruses or parasites to weaken colony health and increase susceptibility to the disorder.

In one study, combined exposure to imidacloprid and *Nosema ceranae* was found to weaken honey bees significantly through high individual mortality and stress, blocking the ability of bees to sterilise the colony and their food (Alaux et al., 2010). Bees reared from brood comb with high levels of pesticide residues were also found to become infected with *N. ceranae* at a younger age (Wu et al., 2011). Pettis et al. (2012) found that sublethal imidacloprid exposure in brood food fed to honey bee larvae led to increases in *N. ceranae* spores in adult bees. Similarly, Vidau et al. (2011) found a synergistic interaction between infection with *N. ceranae* and exposure to sublethal levels of thiacloprid that increased honey bee mortality. Retschnig et al. (2014) demonstrated that a synergistic effect on mortality by thiacloprid and *N. ceranae* is dependent on the pesticide dose. Fauser-Misslin et al. (2014) investigated how laboratory exposure over 9 weeks to neonicotinoid insecticides (thiamethoxam and clothianidin) with a gut parasite, *Crithidia bombi*, affected various crucial colony traits of the bumble bee *Bombus terrestris*. They showed that chronic dietary exposure, from an early stage of colony development, to doses of thiamethoxam and clothianidin that could be encountered in the field truncated worker production, reduced worker longevity and decreased overall colony reproductive success. Further, they demonstrated a significant interaction between neonicotinoid exposure and parasite infection on mother queen survival.

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[16]   However, the significant association between colony losses and parasites/pathogens does not rule out a role for neonicotinoids (see Section A4.3.6).
[17]   This, however, from a toxicological viewpoint is like 'comparing apples and pears'; sublethal effects (e.g. behaviour, reproduction) and acute effects (lethality) are likely to have different dose–response relationships, and risk assessment principles require that the most sensitive be used.

Di Prisco et al. (2013) found that exposure to neonicotinoids, by limiting the immune response, can promote viral replication in honey bees bearing covert infections. Such studies emphasise the central role of the immune system in the modulation of pathogen infections, in particular of deformed wing virus, which is one of the most widespread viruses and often present asymptomatically in the large majority of colonies. This covert virus can rapidly replicate under stress conditions, to generate overt infections. Whatever the stress promoting deformed wing virus replication, the resulting high viral loads determine honey bee reduced survival and the appearance of developmental deformities, which are reliable predictive markers of overwintering colony losses.

The impact of multiple stress factors on honey bee health has been analysed at the physiological and molecular levels, allowing a functional model to be defined (Nazzi et al., 2012; Di Prisco et al., 2013; Nazzi and Pennacchio, 2014), which accounts for the multifactorial origin of colony losses. The major factors found associated with colony losses exert their action on honey bee colonies that are almost always infected by deformed wing virus and infested by *Varroa destructor*. Deformed wing virus covert infections, resulting from the balance between the control action exerted by the honey bee immune system and the immune-suppressive effect of the virus, are constantly exposed to environmental modulation. Given the high degree of cross-talk among different pathways activated by concurrent environmental challenges, this delicate equilibrium can be disrupted by any stress factor that may interfere with antiviral defence. These may include, for example, *Varroa destructor* mites, poor and/or unbalanced nutrition, extreme temperatures and pesticides such as neonicotinoids; these can synergistically interact as they can all have an impact on honey bee immunocompetence. Therefore, neonicotinoids can contribute to the complex network of interactions underpinning bee defence against pathogens, aggravating the impact of the latter.

This effect on bee health could be due to the disruption by acetylcholine agonists of a neural reflex circuit controlling the immune response, to prevent detrimental overzealous reactions, through mechanisms that appear to be quite conserved in animals, including vertebrates (see Tracey, 2009; Olofsson et al., 2012). Therefore, the existence of subtle cross-modulatory pathways between the nervous and immune systems sets the stage for future studies aiming to shed light on possible similar effects of neonicotinoids on immunity in higher animals, a risk not considered so far.

Collectively, the experimental data provided by Di Prisco et al. (2013) further corroborate the stress model proposed by Nazzi et al. (2012), and allow the conclusion that although neonicotinoids cannot be considered as the only 'cause' of colony losses, they can certainly contribute by aggravating the impact of viral pathogens stably associated with honey bee populations all over the world (Nazzi and Pennacchio, 2014). Moreover, based on this model, their impact will be different on bee populations exposed to different stress combinations and characterised by different starting levels of pathogen infection. This may partly account for the variability of neonicotinoid effects observed under field conditions.

Lu et al. (2014) showed that sublethal exposure to the neonicotinoids imidacloprid or clothianidin affected the winterisation of healthy honey bee colonies that subsequently leads to CCD. Both control and neonicotinoid-treated groups progressed almost identically through the summer and fall, and no acute morbidity or mortality was observed in either group until the end of winter. However, bees from 6 of the 12 neonicotinoid-treated colonies had abandoned their hives, and were eventually dead with symptoms resembling CCD. By contrast, the control colonies were re-populated quickly with new emerging bees.

Looking at broader ecosystem impacts, Mason et al. (2013) examined trends in outbreaks of infectious diseases in honey bees, fish, amphibians, bats and birds in the past two decades and pointed to a coincidence between outbreaks and increasing use of systemic insecticides, notably the neonicotinoids and fipronil. This is not currently supported by other evidence, which indicates that mammals are much less sensitive to neonicotinoids. However, understanding of the effects on immune systems is still developing and the effects of these insecticides on a wider range of species' immune responses has yet to be established.

Finally, there is some evidence that there may be internal reactions by the plants themselves to the presence of neonicotinoids. Szczepaniec et al. (2013) discovered that the application of neonicotinoids suppressed expression of plant defence genes when applied to cotton and tomato plants. Such a response could render the plant susceptible to other pests and could be a factor contributing to the recent infestation increases of two-spotted spider mite occurring in various crops across the mid-south of the USA.

A4.3.6 Resilience of honey and other bees to environmental stressors

Honey bees are highly eusocial insects with a well-developed reproductive division of labour between the workers and the queen, which usually monopolises reproduction. Colonies can consist of 3,000–30,000 bees depending on season, are perennial (in theory immortal) and constitute so-called superorganisms, with individual

bees cooperating in the colony like cells in multicellular organisms (Moritz and Southwick, 1992). Owing to this reproductive division of labour, losses of workers resemble losses of somatic cells and can be tolerated to an impressive extent. Since bees are highly flexible, young workers can start foraging earlier and old workers can return to hive duties, if this is required (Winston, 1987). Therefore, losses of hundreds or even thousands of individual bees can be compensated for without even being noticed by beekeepers. Moreover, an increase in mortality or inhibition of feeding by worker bees may actually increase honey stores due to the decline in consumption.

Honey bee colonies compensate for losses by producing many workers daily; thus colonies are resilient against significant losses of individuals, especially during spring and summer, and may also survive the winter. Thus, honey bees appear to be a rather unsuitable model system for evaluating effects of environmental stressors such as insecticides because effects at the colony level may be overseen due to this buffering capacity. However, the usually long-living queens may be more vulnerable than the colony as a whole, and may indeed be more often replaced if colonies are under chronic exposure, e.g. by neonicotinoid pesticides (Sandrock et al., 2014).

Bumble bee colonies in the temperate regions are initiated by overwintered queens in the spring. Such queens forage by themselves until a sufficient number of worker offspring can take over this risky task (about 10 workers). Then, colonies build up over the season (up to a few hundred workers depending on species and region) and eventually start to produce sexuals, males and queens. While the workers usually take part in male production, queens can only be produced by the queens as the sole mated females in the colonies. Given that queens die early, the production of new queens is obviously constrained in bumble bee colonies (Fauser-Misslin et al., 2014). Owing to the smaller number of workers, bumble bee colonies can obviously tolerate lower losses of bees than honey bees. Moreover, only the mated queens overwinter by themselves to start new colonies next spring, which constitutes another crucial bottleneck. It appears as if bumble bee colonies are less able to buffer stressors than highly eusocial honey bees.

Semi-social and solitary wild bees represent a large range of different life histories; they may winter as eggs, pupae or adults. Each female may or may not reproduce, depending on environmental or intrinsic stressors. Indeed, the fitness of solitary bees can be severely compromised by neonicotinoid pesticides (Sandrock et al., 2014); however, without actual data collection it is not possible to give reliable predictions of how exposure to neonicotinoid pesticides will affect populations of solitary bees.

Recently, it has been suggested that there may also be differences in the rate at which ingested neonicotinoids are cleared between honey and bumble bees. Cresswell et al. (2014) found that honey bees maintained much lower body levels of imidacloprid than bumble bees, and this caused bumble bees to be more sensitive to the experimental doses.

In conclusion, owing to their life history, honey bees appear to be an inappropriate model system to evaluate the role of environmental stressors for populations of pollinating bees.

A4.4 Effects on natural pest predators

The current political debates (and the EFSA assessments) have addressed only one aspect of the potential impacts of neonicotinoids on ecosystems: that on pollinators and primarily honey bees. Recent research and literature reviews not considered by the EFSA have raised broader concerns over effects on the wider ecosystem, which includes beneficial insects that act as natural enemies of the pest species being targeted by insecticides. While natural predators/parasitoids have been less in the regulatory spotlight than pollinators, they provide a very important ecosystem service, and evidence in Section 3 also suggests declines in many species, although on the basis of limited quantitative studies. As outlined in Section 2.3, the economic value of natural pest control is high. Losses of beneficial insects can lead to increased pest outbreaks and more pesticide use. Although predator/parasitoid insects prey/lay eggs upon other insects during most of their life cycle, many are omnivorous and feed on pollen, nectar or plant tissues when prey/hosts are scarce or during certain life stages. Moreover, parasitoids are carnivorous during larval development, while adults may feed upon different sources, including pollen and nectar. The same is true for some predators that are nectar/pollen feeders as adults, such as syrphids and chamaemyiids. Carnivorous insects are thus potentially exposed to the same sources of neonicotinoids as pollinators and, when feeding on insect hosts, they are exposed to insecticide residues circulating in the food chain and accumulating in the upper levels.

A review of the peer-reviewed literature by the US Xerces Society for Invertebrate Conservation provides one compilation of current knowledge of the effects of neonicotinoids on such species (Hopwood et al., 2013). This has been supplemented recently by WIA review papers (Pisa et al., 2015). Studies have looked into the impacts of contact due to spray applications, or residues of insecticides in vegetation or in soils, and found acute lethal or sublethal effects on several beneficial insects including parasitoid wasps, predatory plant bugs, stink bugs, ladybirds, predatory mites, ground beetles, pirate bugs and green lacewing adults. Effects vary between insecticides and between different insect species.

A limited number of experiments have compared predator species richness and abundance in fields planted with and without imidacloprid-treated corn seed. In one study, populations of spiders, ladybirds (Coccinellidae), and ground beetles (Carabidae) in treated fields were not significantly different from untreated fields[18], but populations of rove beetles (Staphylinidae) and some predatory true bugs (Hemiptera) were significantly smaller in treated fields (Albajes et al., 2003). In cotton fields treated with either acetamiprid or imidacloprid foliar sprays, numbers of predatory big-eyed bugs (*Geocoris*) were similar to control fields. However, populations were significantly lower in fields treated with foliar applications of thiamethoxam (Kilpatrick et al., 2005). A study by Mullin et al. (2010) exposed 18 different carabid species to corn seedlings treated to field-relevant doses of either imidacloprid, thiamethoxam or clothianidin. Nearly 100% mortality was observed for all species over 4 days.

Biological control of pest insects using natural antagonists is an important component of integrated pest management, and some research suggests that the effectiveness of some such agents can be compromised by neonicotinoids. For instance, the parasitoid wasp *Tiphia vernalis* was introduced to North America to control the Japanese beetle (*Popilla japonica*). Exposure to imidacloprid applied to the soil for Japanese beetle control did not increase adult wasp mortality or reduce longevity but it did reduce their ability to parasitise beetle grubs, compromising the biological control capability of the beetle (Rogers and Potter, 2003). Poletti et al. (2007) found that imidacloprid significantly increased the time it took for predatory mites (another biological control agent) to find, identify and attack their spider mite prey. Consequently, the predatory mites consumed significantly fewer pest mites. Kramarz and Stark (2003) demonstrated that imidacloprid alone had no impact on *Aphidius ervi*, a parasitoid antagonist of the aphid *Acyrtosiphon pisum*, but in combination with cadmium (from phosphate fertilisers) it had the highest negative impact on this beneficial insect.

With their environmental persistence and multiple pathways of exposure, the balance of evidence thus indicates that neonicotinoids may be harmful to beneficial insects, as well as to other beneficial invertebrates (Chagnon et al., 2015).

A4.5   Effects on soil organisms

Soils provide many ecosystem services related to water and nutrient movement in the landscape: water purification, water provisioning, flood protection and other services. Provisioning of these services is fully (for example in the case of mineralisation of organic residues for release of

plant nutrients back to mineral forms available to plants) or at least partly dependent on soil biota (de Vries et al., 2013). Although detailed historical data across large scales are not available, there is good evidence showing that increasing agricultural intensity has adverse effects on soil biota, which may reduce the ability of agricultural landscapes to provide key ecosystem services (Bjorklund et al., 1999). However, the effects of agriculture on soil biota are complex and relate to landscape changes, cultivation, plant community changes as well as agrochemicals; thus distinguishing the effect of one particular component, such as one group of chemicals, is difficult.

Earthworms and other invertebrates that dwell in soil or leaf litter can also be exposed to neonicotinoids applied as soil drenches, granules or seed dressings. Extensive use of neonicotinoids thus raises concerns about the broad impact of these chemicals on soil health, soil food webs and soil invertebrate communities. Earthworms are often used as a model test organism and are among the better-studied soil invertebrates for non-target effects of neonicotinoids. Imidacloprid, clothianidin, thiacloprid and acetamiprid are more toxic to earthworms than other modern synthetic insecticides, including novel types of carbamates, organophosphate esters and pyrethroids (Wang et al., 2012). Of the four neonicotinoids tested, acetamiprid and imidacloprid were the most toxic to earthworms.

In an extensive review, Chagnon et al. (2015) provide many examples of neonicotinoids having a deleterious effect on soil organisms in field realistic concentrations. Peck (2009a, b) assessed the impacts of the neonicotinoid imidacloprid applied to turfgrass for scarab beetle control and found direct and indirect long-term effects on some arthropods. Kreutzweiser et al. (2008, 2009) found reduction of worm-driven litter decomposition caused by avoidance of litter treated by neonicotinoids. Also, burrowing behaviour of earthworms was negatively affected by neonicotinoids (Capowiez and Bérard, 2006). Pisa et al. (2015) conclude that such studies show that operational applications of neonicotinoids can result in soil concentrations that are likely to pose a high risk of sublethal effects and potential risk of lethal effects to earthworms.

Beside effects on soil fauna, there are also some reports indicating effects on microbial activity in soil. Microbial decomposition of leaves from maple (*Acer saccharum*) trees was significantly inhibited at concentrations expected from systemic treatments to control wood-boring insects (Kreutzweiser et al., 2008). Other studies (Singh and Singh, 2005) found an increase in some microbial enzymatic activity. These effects may potentially correspond with provisioning of key ecosystem services,

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[18]   However, the significance of such findings is in doubt because of the pitfall trap methods used, which measure not just population density but also activity. Other studies have shown that under conditions of starvation caused by killing off the usual prey, activity initially increases and leads to more trap capture, which can compensate for population declines that are taking place (Sunderland et al., 1995).

but there is lack of studies providing empirical evidence about reduction in service provisioning by effects of neonicotinoids on soil biota.

A4.6   Biodiversity effects

In the main report (Section 3.5), we introduced birds as an indicator of biodiversity. These have in the past revealed overall ecosystem effects such as those from organochlorine insecticides in the 1950s and 1960s (Newton, 1995). Insecticides may theoretically have an impact on birds and other vertebrates by direct toxic effects or by broader ecosystem effects such as a reduction in food supply. As noted in Section 3.5, populations of farmland birds in Europe have shown substantial declines (in some cases local extinction) in the past 10–20 years. Researchers have thus looked at both direct toxicological effects through ingestion of neonicotinoid insecticide-containing food supplies and indirect effects through declines in the food supply.

Mineau and Palmer (2013) (not peer-reviewed although much of the source material was) reviewed both open literature results and data from industry submitted under the US regulatory process. The overview is summarised in Box A4.2, which notes that although neonicotinoids show lower toxicity to vertebrates than insects, seed-eating animals and birds could still ingest toxicologically significant amounts of insecticide if they eat treated seeds, either from spills or directly after planting.

Since Mineau and Palmer's analysis was published, a peer-reviewed analysis has been published as part of the WIA project (Gibbons et al., 2015), which reviewed 150 studies of their direct (toxic) and indirect (e.g. food chain)

effects on vertebrate wildlife: mammals, birds, fish, amphibians and reptiles. Their analysis confirms that one of the serious failings of current risk assessments is the underestimation of interspecies variation in insecticide susceptibility that is now apparent. For instance the LD_{50} for birds ranges from 284 milligrams per kilogram for mallard (what the US Environmental Protection Agency (US EPA) classifies as 'moderately toxic') to 13.9 milligrams per kilogram for grey partridge (the Agency's 'highly toxic' classification) for imidacloprid. This review also observed that sublethal effects (for instance house sparrows becoming uncoordinated and unable to fly) are detected at levels an order of magnitude below lethal doses. Reviews of available laboratory data suggest that some effects can be detected at even lower doses (1/1,000).

Treated seeds contain some of the highest concentrations of neonicotinoids, with a typical individual canola (oilseed rape), beet or corn seed calculated to contain 0.17, 0.9 or 1 milligram of active ingredient, respectively (Goulson, 2013). The US EPA modelled the estimated daily intake of clothianidin, assuming that mammals and birds only eat a diet of treated seeds. This risk modelling approach showed that clothianidin, at least when used to treat oilseed rape and cotton seeds, could reduce the survival of small birds and mammals (DeCant and Barrett, 2010). The potential risk can be further illustrated by calculating the relative risk for two granivorous species, a grey partridge (mass about 390 grams) and a house sparrow (mass about 34 grams), feeding on a field recently sown with imidacloprid-treated beet seed, each containing 0.9 milligrams of imidacloprid. Gibbons et al. (2015) calculate that ingestion of just six and one and a half seeds, respectively, would have a 50% chance of killing

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Box A4.2   Potential effects of pesticides on birds

Mineau and Palmer (2013) consider two potential mechanisms through which birds may be affected: one is direct toxic (acute or chronic) toxicity to a bird, which eats food contaminated with neonicotinoids; the second is through overall ecosystem effects leading to a reduction in food supply, particularly for birds reliant on insect populations.

Representative species of birds are included in the preclearance toxicity testing protocols (in the USA the two species are mallard duck and the bobwhite), where acute toxicity (LD_{50}) for imidacloprid was 152 and 283 milligrams per kilogram respectively. However, for grey partridge, the LD_{50} is only 15 milligrams per kilogram. Since non-lethal effects (ataxia) have been observed at 10% of lethal doses, the report concludes a real risk to birds.

Calculations based on the likely neonicotinoid concentrations in seeds and their methods of application suggest risk of lethal effects from ingestion of just a few seeds treated with imidacloprid, clothianidin or thiamethoxam for corn, and risks of sublethal effects from ingestion of fewer than two seeds treated with imidacloprid (corn, rapeseed, wheat), clothianidin (corn, wheat) or thiamethoxam (corn). Scenario calculations based on seeds having approximately 0.2–1 milligram of insecticide per seed suggest that a grey partridge only needs to eat 5–30 seeds to receive an LD_{50} dose, out of a typical daily consumption of several hundred seeds. Typical sowing rates are approximately 7.5–12 seeds per square metre for maize and 80 seeds per square metre for oilseed rape, so even a 99% injection rate for seed into the soil leaves substantial numbers available within the range of foraging birds.

In one recent experiment, Lopez-Antia et al. (2013) fed imidacloprid-dressed wheat seed to red-legged partridge (Alectoris rufa) for 10 days and obtained 58% mortality, with the survivors exhibiting a range of sublethal effects. Birds given the choice did not show any preference between untreated seeds and seeds treated with imidacloprid.

The mobility and persistence of the neonicotinoids already referred to may allow spread to other (non-crop) plants, leading to exposure by non-target insects. Any resulting decline in insect populations would reduce the food supply for insect-eating birds. Invertebrate-dependent bird species in the Netherlands have been declining on a massive scale in recent times, in all kinds of habitats (grasslands, marshes, heathlands, at the coast, woodlands, settlements, farmlands). These evaluations point to broader ecosystem effects through declines in general populations of insects, which has recently been analysed by Hallmann et al. (2014); see main text.

an individual foraging partridge and sparrow. Less than a quarter of a seed could have a sublethal effect on a house sparrow.

The broader ecosystem implications of insect loss have been the focus of a recent study in the Netherlands, which demonstrated an association of declines in insectivorous birds with high neonicotinoid concentrations (Hallmann et al., 2014). Trends in local bird populations were significantly more negative in areas with higher surface-water concentrations of imidacloprid. At imidacloprid concentrations of more than 20 nanograms per litre, bird populations tended to decline by 3.5% on average annually. Additional analyses revealed that this spatial pattern of decline appeared only after the introduction of imidacloprid to the Netherlands in the mid-1990s. These findings are also supported by research (Boatman et al., 2004) that has shown reductions in invertebrate food abundance caused by insecticide to lead to reductions in reproductive success of at least four farmland passerines in the UK: corn bunting (*Miliaria calandra*), yellowhammer (*Emberiza citronella*), whinchat (*Saxicola rubetra*) and reed bunting (*Emberiza schoeniclus*).

Aquatic ecosystems may be the ultimate repository for neonicotinoids persisting in the soil because of their water solubility. Surveys of neonicotinoids in aquatic systems in nine countries show the majority of the surface waters surveyed contain residues at up to 18 ppb (Main et al., 2014; van Dijk et al., 2013). Aquatic organisms are exposed to contaminants throughout their life, so the appropriate toxicological endpoint is that resulting from continuous low-level exposure. Beketov and Liess (2008) found delayed and cumulative lethal effects on a range of aquatic organisms, and experiments in model ecosystems with imidacloprid found that aquatic insects would not survive at levels above 1 ppb. Nyman et al. (2013) show that imidacloprid inhibits movement and feeding of a freshwater amphipod, but this is insufficient to explain the mortality observed. Surveys in the Netherlands show neonicotinoid concentrations increasing in aquatic systems and associated with declines in non-target invertebrate species populations across aquatic ecosystems (van Dijk et al., 2013; Roessink et al., 2013).

In field studies, Beketov et al. (2013) found a general reduction of stream biodiversity across sites in Germany, France and Australia, with up to 42% fewer species in streams with high levels of pesticide contamination. Biodiversity loss was observed even where pesticide

levels met European water quality criteria. For neonicotinoids, Pisa et al. (2015) performed a species sensitivity distribution of acute toxicity data and predicted a hazardous concentration for 5% of aquatic species for imidacloprid concentrations in water of 1.04–2.54 ppb (Sanchez-Bayo and Kouchi, 2012). van Dijk et al. (2013) have found a correlation between the abundance of aquatic macroinvertebrate species and nearby imidacloprid concentrations in Dutch surface waters. Macroinvertebrate abundance consistently declines along the gradient of increasing median nearby imidacloprid concentrations; imidacloprid concentrations as low as 0.01 ppb led to significant reductions of macroinvertebrates in surface waters. Most studies have used imidacloprid, but one that used thiacloprid found that strong effects on sensitive, long-lived insects were detectable at levels of 0.1 ppb (Beketov and Liess, 2008; Liess and Beketov, 2011). These researchers also found that the aquatic organism used in regulatory testing (*Daphnia magna*) is several orders of magnitude less sensitive, with an acute LC_{50} of 7,000 ppb.

Aquatic invertebrates are important components of aquatic ecosystems (with roles as decomposers, grazers, sediment feeders and providing much of the food that fish, amphibians and birds feed upon). Their reduction will thus have wide effects on aquatic ecosystem and their biodiversity, extending to fish and mammals (particularly birds) that depend on aquatic systems for their food supply.

A4.7 Concerns and uncertainties

This Annex has attempted to integrate the many more detailed overviews on various aspects of ecosystem services that have been referenced. In the process, not all the details present in the original references can be provided, but from a system-wide and toxicological risk assessment viewpoint, there are many points of potential concern. Those that are particularly notable include the persistence and mobility of the neonicotinoids, the significant difference in toxicity between species (even within the same genera), the very large difference that is indicated between acute and chronic toxicity, the possibility of effects within non-agricultural ecosystems and on higher trophic levels, mixture toxicity and synergism with other pesticides, and immune system effects. The main report considers the implications of these and related aspects for EU policy.

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