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A restatement of the natural science evidence base concerning neonicotinoid insecticides and insect pollinators

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There is evidence that in Europe and North America many species of pollinators are in decline, both in abundance and distribution. Although there is a long list of potential causes of this decline, there is concern that neonicotinoid insecticides, in particular through their use as seed treatments are, at least in part, responsible. This paper describes a project that set out to summarize the natural science evidence base relevant to neonicotinoid insecticides and insect pollinators in as policy-neutral terms as possible. A series of evidence statements are listed and categorized according to the nature of the underlying information. The evidence summary forms the appendix to this paper and an annotated bibliography is provided in the electronic supplementary material.

1. Introduction

Neonicotinoid insecticides are a highly effective tool to reduce crop yield losses owing to insect pests. Since their introduction in the 1990s, their use has expanded so that today they comprise about 30% by value of the global insecticide market [1]. They are commonly applied to crops as seed treatments, with the insecticide taken up systemically by the growing plant, so that it can be present in all plant parts, including nectar and pollen that bees and other pollinating insects collect and consume. Pollinators can potentially be exposed to neonicotinoids in other ways, for example through plant exudates, dust from planting machines and contamination of soil and water.

There is evidence that in Europe and North America many species of pollinators are in decline; both in abundance and distribution. There is a long list of potential causes for these declines, including parasites, disease, adverse weather and loss of habitat [2,3]. However, there has been particular concern about the impact on pollinators of the relatively recently introduced neonicotinoids and the European Union (EU) imposed a partial restriction on their use in December 2013. This decision has been criticized on the grounds that the benefits of neonicotinoid use outweigh any detriment they might cause.

The tension between the agricultural and environmental consequences of neonicotinoid use, and the recent EU restriction, has made this topic one of the most controversial involving science and policy. Here, we describe a project that aimed to provide a 'restatement' of the relevant natural science evidence base expressed in a succinct way that is comprehensible to non-expert readers. We have tried to be policy-neutral though are aware that complete neutrality is

impossible. The evidence restatement forms appendix A to this paper and is accompanied in the electronic supplementary material by a detailed annotated bibliography that provides an entry into the technical literature. The restatement is divided into six sections: after a description of the methodology and the importance of pollinators and insecticides, successive sections consider evidence for exposure paths, laboratory evidence for lethal and sublethal effects, the occurrence of residues in pollinators and their products in the environment, experiments conducted in the field, and consequences for pollinators at colony and population levels.

Experiments to establish the effect of defined doses of insecticides upon individual pollinators are required by regulatory authorities and can be carried out under laboratory conditions. These laboratory studies have the strength of allowing carefully controlled experiments to be performed on individual insects subjected to well-defined exposure. However, because they are conducted under artificial conditions, it is hard to assess a number of processes that may be relevant in the field. For example, neonicotinoids may affect the sensitivity of insects to other stressors; pollinators may actively avoid food contaminated by insecticide and responses at the colony or population level may mitigate or exacerbate the loss or impairment of individual insects. Nevertheless, such experiments provide important information about the range of concentrations where death or sublethal effects are to be expected.

Purely observational surveys in the field are used to establish the levels of exposure that occur under normal use. A number of large surveys in different countries have measured neonicotinoid residues in wild-foraging honeybees and unmanaged pollinators, as well as in nectar, pollen, honey and wax within bee colonies. These data are heavily weighted towards honeybees, and long time series are seldom available.

Experiments in the field are used to establish the impact of different doses of insecticide on pollinator behaviour, mortality and colony performance. They may be conducted as part of the registration process or for general research. One class of experiment involves bees artificially exposed to neonicotinoids and then observed to forage in the field. These are designed to discover whether neonicotinoids affect the performance of individual pollinators (and where appropriate their colonies) under field conditions. The critical issue here is whether the experimental exposure to insecticides is representative of what pollinators are actually likely to experience. The second class of experiment involves placing bee colonies in the environment in situations where they are exposed to crops treated with neonicotinoids, with suitable controls. These are large, difficult experiments where the unit of replication is typically the field site and where there are potentially many confounding factors to be taken into consideration. So far only one such study has been concluded successfully. The statistical power of this type of experiment is likely to be constrained by the expense and logistics of high levels of replication.

To understand the consequences of changing neonicotinoid use, it is important to consider pollinator colony- and population-level processes, the likely effect on pollination ecosystem services, as well as how farmers might change their agronomic practices in response to restrictions on neonicotinoid use. While all these areas are currently being researched, there is at present a relatively limited evidence base to guide policy-makers.

2. Material and methods

The literature on pollinators and neonicotinoids was reviewed and a first draft evidence summary produced by a subset of the authors. At a workshop, all authors met to discuss the different evidence components and to assign to each a description of the nature of the evidence using a restricted set of terms. We considered several options to describe the nature of the evidence we summarize including the GRADE [4] system widely used in the medical sciences, or the restricted vocabulary used by the International Panel on Climate Change [5]. However, none precisely matched our needs and instead we used a scoring system based on one previously developed for another 'restatement' project concerning bovine tuberculosis [6]. The categories we used are:

- [D_{ata}] a strong evidence base involving experimental studies or field *data* collection, with appropriate detailed statistical or other quantitative analysis;
- [E_{xp_op}] a consensus of *expert opinion* extrapolating results from related ecological systems and well-established ecological principles;
- [S_{upp_ev}] some *supporting evidence* but further work would improve the evidence base substantially; and
- [P_{rojns}] *projections* based on the available evidence for which substantial uncertainty often exists that could affect outcomes.

These categories are explicitly not in rank order.

A revised evidence summary was produced and further debated electronically to produce a consensus draft. This was sent out to 34 stakeholders or stakeholder groups including scientists involved in pollinator research, representatives of the farming and agrochemical industries, non-governmental organizations concerned with the environment and conservation, and UK government departments and statutory bodies responsible for pollinator policy. The document was revised in the light of much helpful feedback. Though many groups were consulted, the project was conducted completely independently of any stakeholder and was funded by the Oxford Martin School (part of the University of Oxford).

3. Results

The summary of the natural science evidence base concerning neonicotinoid insecticides and insect pollinators is given in appendix A, with an annotated bibliography provided as the electronic supplementary material.

4. Discussion

The purpose of this project is not to conclude whether neonicotinoids are 'safe' or 'dangerous' but to try to help set out the existing evidence base. When neonicotinoids are used as seed dressing on crops visited by pollinators there is no doubt that these systemic insecticides are typically present in pollen and nectar and so bees and other pollinators can be exposed to them [7,8]. The concentrations in pollen and nectar are nearly always some way below those that would cause immediate death. The great problem is to understand whether the sublethal doses received by pollinators in the field lead to significant impairment in individual performance, and whether the cumulative effect on colonies and populations affects

pollination in farmed and non-farmed landscapes and the viability of pollinator populations [3].

For this topic, the published literature is a small fraction of the evidence that has been collected. The process of registering a new insecticide requires the production of detailed environmental risk assessments (see <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:093:0001:0084:EN:PDF> and <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:093:0085:0152:EN:PDF>). These include substantial evidence on toxicity to non-target organisms (including honeybees) and a range of further studies that will, in some cases, escalate to full-scale field trials of toxicity. The data generated in such studies are not typically in the public domain, or only in a form summarized by the regulatory agencies, and hence we have not been able to include reference to them. There are understandable commercial reasons for the withholding of this information, though the chief reason is not that it contains proprietary intellectual property but that the information would be commercially advantageous to a competitor in registering the compound when it is out of licence. We wonder if registration rules might be amended to allow this type of data to be published, a clear public good, without disadvantaging companies that had invested in its collection.

If neonicotinoids are not available, then farmers will have to choose alternative pest-management strategies, alternative crops or accept greater losses. The impact upon pollinators of withdrawing neonicotinoids will be greatly influenced by such choices. Farmers' likely strategies when faced with restrictions on the use of neonicotinoids are being researched, but there is currently only limited evidence to guide policy-makers in what changes to expect. This is just one aspect of human behaviour, economics and other social science that may be relevant to questions about threats to pollinators. However, it was not the purpose of this review to summarize the social science literature in this area (the annotated bibliography provides an entry into this literature).

There is clear evidence of the great value of neonicotinoids in agriculture [1] as well as the importance of the ecosystem services provided to agriculture by managed and wild pollinators [9]. Pollinators also have intrinsic importance as components of natural biodiversity that cannot, or can only inexactly, be accorded economic value. In some cases, intelligent regulation of insecticide use can provide 'win-wins' that improve both agricultural and biodiversity outcomes but in other cases there will be trade-offs, both within and between different agricultural and environmental objectives. Different stakeholders will quite naturally differ in the weightings they attach to the variety of objectives affected by insecticide use, and there is no unique answer to the question of how best to regulate neonicotinoids, an issue that inevitably has both economic and political dimensions. But economic and political arguments need to be consistent with the natural science evidence base, even though the latter will always be less complete than desirable. We hope that our attempt to set out this evidence base in as policy-neutral a manner as possible will stimulate discussion within the science community about whether our assessments are fair and where investment most needs to be made to strengthen them. We hope it will also make the evidence base less contested and so help stakeholders from all perspectives develop coherent policy and policy recommendations.

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Appendix A. A restatement of the natural science evidence base concerning neonicotinoid insecticides and insect pollinators

For an annotated bibliography of the evidence supporting each statement, see the electronic supplementary material.

(a) Introduction and aims

- (1) Wild and managed insect pollinators play a critical role in the production of a variety of different foods (and in the case of honeybees also produce various 'hive products' of which the most important is honey) and are an important functional and cultural component of biodiversity. Insecticides are applied to crops to control insect pests and make a very important contribution to achieving high yields. Insecticides kill insects and thus clearly have both positive and negative effects on different aspects of food security and the environment. Concern has been expressed by a number of bodies that neonicotinoid insecticides may be harming pollinators and a partial restriction on their use in the EU came into force across all 28 member states in December 2013 (to be reviewed after 2 years). Other bodies have criticized this decision, arguing that the benefits of neonicotinoid use outweigh their costs.
- (2) The aim here is to provide a succinct summary of the evidence base relevant to policy-making in this area as of April 2014. It also provides a consensus judgement by the authors on the nature of the different evidence components; a consensus arrived at using the studies listed in the annotated bibliography. We use the following descriptions, which explicitly are not a ranking, indicated by abbreviated codes. Statements are considered to be supported by:
 - [Data] a strong evidence base involving experimental studies or field *data* collection, with appropriate detailed statistical or other quantitative analysis;
 - [Exp_op] a consensus of *expert opinion* extrapolating results from related ecological systems and well-established ecological principles;
 - [Supp_ev] some *supporting evidence* but further work would improve the evidence base substantially; and
 - [Projns] *projections* based on the available evidence for which substantial uncertainty often exists that could affect outcomes.
- (3) The review focuses on the natural science evidence relevant to pollinator policy in the EU but includes relevant data from other regions; its scope does not include

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evidence from social sciences and economics. The statements are based on the evidence in the peer-reviewed scientific literature, though the annotated bibliography also notes the existence of information in non-reviewed reports and industry studies.

(b) Pollinators and neonicotinoid insecticides

- (4) Insect pollinators are required to achieve optimum yield and quality for a number of important food crops. The most economically significant crops in the UK include oilseed rape (canola), soft fruits (strawberry, raspberry, etc.), top fruits (apple, pear, plum, etc.) and vegetables (courgettes, runner beans, tomato, etc.), whereas in continental Europe sunflower, peaches, melon and other crops are also important. Insect pollinators are important for both field crops and those grown under glass, though in their absence some crops can, to differing extents, be wind- or self-pollinated without the involvement of insects. Many plant species in pastureland and non-agricultural habitats require insect pollinators for successful reproduction [D_{ata}].
- (5) A lack of pollinators can reduce crop yields and quality [D_{ata}], and there is some evidence that pollinator diversity can reduce the variance in pollination and hence improve crop yield stability [S_{upp_ev}]. Where insect-pollinated crops are grown in glasshouses or 'polytunnels' the introduction of pollinators can be particularly important for both quality and quantity of yield [D_{ata}]. There is emerging evidence for the potential of economically significant pollination deficits in some UK field crops in some years [S_{upp_ev}], but data do not currently exist to determine whether observed changes in pollinator abundance and diversity (see para. 7) have affected the economic value of crop yields [E_{xp_op}].
- (6) Pollination may be carried out by wild or managed insects. The most important pollinators for crops include honeybees, which are native to Europe (their status in the British Isles is unclear [E_{xp_op}]) but are now almost entirely managed, bumblebees, solitary bees and true flies (including hoverflies).¹ Other pollinators such as butterflies and moths are not as important for crop pollination, particularly in northern temperate regions, but do pollinate wild plant species. Wild pollinators can be viewed as an element of natural capital² that provides (with managed species) pollination, an ecosystem service of economic importance to society. Pollinators are also an important component of a nation's biodiversity [D_{ata}].
- (7) Data from volunteer recording schemes that record species presence (but not abundance or absence) have revealed changes in the diversity and distribution of pollinators. In Great Britain, The Netherlands and Belgium (where the best data exist) the average numbers of species of bumblebees, butterfly and moths, and solitary bees in different areas have declined since the 1950s [D_{ata}]. There is some evidence of a recent slowdown in the rate of decline in species richness (for bumblebees in all three European countries) and also some increases (solitary bees in Great Britain and The Netherlands but not in Belgium where the decline continues) [D_{ata}]. The data for hoverflies are more complex with species richness reported to have increased, decreased or remained unchanged depending on location and the geographical scale of the analysis.
- (8) Honeybees throughout Europe (and elsewhere) have been severely affected by the introduction of the *Varroa destructor* mite which both parasitizes bees and acts as a vector for a number of debilitating and paralytic honeybee viruses [D_{ata}]. In addition, honeybee colony losses have increased in frequency across Europe and the USA because of overwintering mortality [D_{ata}] which is thought to arise from multiple factors, including adverse weather, poor nutrition as well as parasites and disease [S_{upp_ev}]. Some of these losses in the USA have been ascribed to a particular syndrome, colony collapse disorder, though its precise nature is debated [E_{xp_op}]. Not all parts of the world have experienced recent increases in overwintering colony mortality [D_{ata}].
- (9) Neonicotinoids are a relatively new class of insecticide, introduced in the early 1990s. They target the nicotinic acetylcholine receptor (nAChR) with high affinity for insect receptors and low affinity for mammalian receptors and have relatively low (but not zero) mammalian and bird toxicity. They can be used as sprays, applied to soils as drenches or in granular form, introduced into irrigation water or injected into trees. However, they are most frequently (approx. 90% by volume in the UK) applied as seed treatments with the insecticide being taken up systemically by the growing plant. The convenience and cost-effectiveness of seed treatments, the development of resistance to other classes of insecticide by many insect pests, and restrictions on the use of other compounds, have resulted in neonicotinoids capturing 28.5% of the global insecticides market (2011; worth US\$3.6B) and their wide use in Europe [D_{ata}].
- (10) Five neonicotinoids are approved for use in the EU: three from the *N*-nitroguanidine group—clothianidin, imidacloprid and thiamethoxam (metabolized to clothianidin in the plant, insect and environment); and two from the *N*-cyanoamidine group: thiacloprid and acetamiprid. Concern over their possible effects on pollinators has focused on the first three because they are the most used compounds, they have greater honeybee toxicity and they are used as seed treatments so can be present in the pollen and nectar of treated crops [D_{ata}].
- (11) In Europe (and elsewhere), environmental risk assessments of pesticides including all neonicotinoids are required before a product can come to market. A tiered approach has been adopted to ensure cost-effectiveness and proportionality. The tiers start with laboratory tests to determine hazard to a standard set of seven non-target organisms (including honeybees) and, if potential hazards are identified, may progress through more complex semi-field experiments and modelling to simulate exposure under different more realistic conditions, culminating with full-scale toxicity assessments to identify potential risks in the field. Field trials were conducted during the original environmental risk assessment process

for neonicotinoids. Extensive data are often generated during the registration process but typically is not placed in the public domain, except in summary form [D_{ata}].

(c) Exposure of pollinators to neonicotinoid insecticides

- (12) Neonicotinoids have been widely used in Europe as a seed treatment for oilseed rape, sunflowers, maize, potato, soya bean (and other crops such as cereals and beets not visited by pollinators).
- (a) A single treated oilseed rape seed is typically treated with approximately 35 µg neonicotinoids and a maize seed with 1.2 mg (see Endnote 3) [D_{ata}].
- (b) Pollinators may be exposed to neonicotinoids applied as sprays. The use of *N*-nitroguanidine neonicotinoids at flowering time is restricted in most countries though acetamiprid and thiacloprid (from the less toxic *N*-cyanoamidine group) are sprayed on raspberries, fruit trees and oilseed rape at flowering time [D_{ata}].
- (13) The plant absorbs some of the insecticide from the seed treatment and as it grows the insecticide spreads to all plant parts including the nectar and pollen that bees and other pollinators collect and consume [D_{ata}].
- (a) Estimates of the concentration of neonicotinoids in the pollen and nectar of seed-treated crops vary considerably with average maximum levels (from 20 published studies) of 1.9 (nectar) and 6.1 (pollen) ng g⁻¹. Concentrations vary across crops and can be appreciably higher if neonicotinoids are applied as foliar sprays, soil drenches or through drip irrigation [D_{ata}].
- (14) Some plants secrete droplets of liquid (xylem sap) called guttation fluid at leaf tips or margins. High concentrations of neonicotinoids have been measured in the guttation fluid of seed-treated plants (up to 10⁴–10⁵ times that in nectar), especially when plants are young [D_{ata}]. There has been concern that were pollinators to use guttation fluid as a source of water they would ingest highly toxic levels of insecticides. The available evidence does not suggest that pollinators collect guttation fluid containing neonicotinoids to any great extent, in part because it chiefly is present at times of the year when crops are unattractive to pollinators and other sources of water are present [E_{xp_op}].
- (15) Dust emitted from seed drilling machines can contain high concentrations of neonicotinoids; as well as being deposited on the soil, the dust can drift to contaminate neighbouring flowering crops and natural vegetation as well as surface waters. Sporadic incidents of mass honeybee mortality in several EU countries, the USA and Canada have been caused by dust from seed drilling machines [D_{ata}].
- (a) Issues concerning dust chiefly involve the formulation of the insecticide, in particular, how it is made to 'stick' to the seed. EU and national regulations on formulation and seed drilling have been introduced to reduce the risks of these problems [D_{ata}].
- (16) Neonicotinoids introduced into the environment as seed treatments can affect soil insects and other invertebrates, effects considered in insecticide evaluation and registration. They persist in the environment with typical half-lives estimated to be of the order 15–300 days (with some longer estimates from laboratory studies and in the field under drought and freezing conditions). There is evidence that neonicotinoids can accumulate in soils when treated crops are grown repeatedly in the same field. Neonicotinoids can sometimes, but not always, be detected in weeds or in subsequent crops grown in the same soil, though when present the concentrations are considerably lower than in the target crop. Neonicotinoids have been detected in surface or groundwater around fields where they have been used as seed treatments [S_{upp_ev}].
- (17) Bees bring pollen and nectar (which in social bees is often extensively modified post-ingestion) to their hives or nests to feed their developing larvae [D_{ata}] which thus may have different patterns of exposure and susceptibility compared with adults (see also para. 24) [S_{upp_ev}].
- (18) The risk of exposure to neonicotinoids for different pollinator species will be influenced by many aspects of their biology and ecology including body size, flower preference, whether they are a social species, and whether the time of year at which they are active (or in the case of social species experiencing rapid colony growth) coincides with the flowering of neonicotinoid-treated crops. There may also be differences in the physiological susceptibility of different pollinator species to neonicotinoids [E_{xp_op}].
- (19) The exposure of pollinators to neonicotinoids will be affected by the distribution of flowering crops in the landscape, the fraction that are treated with neonicotinoids, the length of time the treated crops are in flower, and the availability of alternative, suitable floral resources (including weeds and managed resources in floral strips, wildflower headlands, untreated crops, etc.) and whether they are contaminated with insecticide. Over multiple years the frequency of treated crops in agricultural rotations will affect long-term population exposure [E_{xp_op}].
- (20) The distance between treated fields and nest sites or honeybee hives will affect insect exposure to neonicotinoids [E_{xp_op}].
- (a) Pollinators can forage over a large area: the maximum foraging distance for bumblebees is 2–3 km from the colony (though with considerable variation) and for honeybees 10–15 km (median distances are 1–6 km); some solitary bees may only forage a few hundred metres or less. Observed foraging distances are strongly influenced by the distribution of flowering plants [D_{ata}].
- (21) *Summary*. There are several proven pathways through which pollinators may be exposed to neonicotinoid insecticides applied as seed treatments (or in other ways). Quantitative information about the extent and significance of these different routes in the published literature is poor [E_{xp_op}].

(d) Laboratory studies of lethal and sublethal effects of neonicotinoids

- (22) Estimates of LD₅₀s (see Endnote 4) for different neonicotinoid-pollinator combinations are available,

although a majority of the studies have considered only the honeybee [D_{ata}].

- (a) The acute oral LD₅₀s for the major neonicotinoids have been estimated (by EFSA⁵) to be 3.7 ng per honeybee for imidacloprid, 3.8 ng per honeybee for clothianidin and 5.0 ng per honeybee for thiamethoxam (these estimates are used in the calculations below). A meta-analysis of 14 studies of imidacloprid estimated an LD₅₀ of 4.5 ng per honeybee (95% confidence limits 3.9–5.2 ng) [D_{ata}].
 - (b) Equivalent acute contact LD₅₀s have been estimated (by EFSA) to be 81 ng per honeybee for imidacloprid, 44 ng per honeybee for clothianidin and 24 ng per honeybee for thiamethoxam [D_{ata}].
 - (c) There is considerable variation among LD₅₀s measured across different bee species, and this is influenced by type of neonicotinoid and mode of application [D_{ata}]. This complicates simple comparison with honeybee data [E_{xp_op}].
 - (d) A honeybee, returning to the hive after foraging, typically carries 25–40 mg nectar or 10–30 mg pollen. If nectar or pollen is contaminated with insecticide at the concentrations described in Para. 13a, then these loads will contain approximately 0.06 ng (nectar) or 0.12 ng (pollen) of insecticide. Depending on the type of neonicotinoid this is 1–3% of the LD₅₀ acute oral dose (though note that none of the pollen and hardly any of the nectar is metabolized by the forager). A colony of 10 000 workers was observed to store 750 g of pollen in four days. If all the pollen was similarly contaminated this equates to 8–11% of the acute oral LD₅₀ [P_{rojns}].
 - (e) Maximum pollen consumption is found among nursing honeybees that can consume 7.2 mg d⁻¹. If the pollen contains 6.1 ng g⁻¹ neonicotinoid the daily intake is 0.044 ng or, depending on the compound, 0.8–1.1% of the acute oral toxicity LD₅₀. Maximum nectar consumption is found among nectar-foraging honeybees and can be 32–128 mg d⁻¹. If nectar contains 1.9 ng g⁻¹ neonicotinoid the daily intake is 0.061–0.243 ng, or 1.2–6.7% of the LD₅₀ acute oral [P_{rojns}].
 - (f) Honeybee colonies collect pollen and nectar from multiple sources, which dilutes the effects of foraging on neonicotinoid-treated crops [D_{ata}]. For this reason and because they are based on the average maximum neonicotinoid concentrations in Para. 13a, the calculations in subparagraphs d and e above should be viewed as a worst-case scenario [E_{xp_op}].
- (23) Prolonged exposure of pollinators in the laboratory to doses of neonicotinoids that do not cause immediate death can reduce longevity (chronic toxicity). Because chronic effects can be estimated in many different ways, comparisons are harder than for acute toxicity [D_{ata}].
 - (a) For honeybees and bumblebees, chronic lethal effects have typically been reported when bees are fed diets containing 10–20 ng g⁻¹ neonicotinoid over 10–20 days, although some studies with higher doses have not observed such effects [D_{ata}].
 - (b) These neonicotinoid concentrations are higher than the worst-case assumptions of maximum insecticide consumption in para. 22e [P_{rojns}].
 - (24) Effects of neonicotinoids on adult pollinators have been detected in the laboratory at doses substantially below those that cause death. At the lowest doses responses involve metabolic changes (for example, in acetylcholinesterase activity) and subtle neurological and behavioural responses. As doses increase (including concentrations in food similar to that observed in the nectar and pollen of treated crops) olfactory learning, memory and feeding behaviour can be affected, though there is considerable variability in the results reported in different studies. When doses approach lethal concentrations substantial neurological and locomotory impairment can occur [D_{ata}].
 - (a) The majority of studies have involved honeybees; where comparisons of honeybees with bumblebees and solitary bees have been made differences are frequently observed, although these depend on species, assay and type of neonicotinoid and general patterns are difficult to discern [S_{upp_ev}].
 - (b) There has been debate in the literature as to the extent that neonicotinoids accumulate in pollinators; recent studies have suggested that bees have a substantial capacity to extrude neonicotinoids from cells and tissue (honeybees were estimated to clear 2 ng d⁻¹ imidacloprid from their body—approximately 50% of oral LD₅₀—and larger bumblebees 7 ng d⁻¹) [D_{ata}].
 - (25) Sublethal effects on larval development and colony productivity have been identified in the laboratory.
 - (a) Delayed larval and pupal developments have been observed in honeybees though at neonicotinoid concentrations higher than those expected to occur in the field [D_{ata}].
 - (b) Increases in development time, and reductions in worker egg laying, worker production, worker longevity and male and new queen (gyne) production have been observed in bumblebee colonies when food is provided containing concentrations of neonicotinoids towards the high end of those observed in nectar and pollen in treated crops in the field. Similar results have been found for larval development and reproductive output in solitary bees [S_{upp_ev}].
 - (26) Stressed pollinators tend to be more susceptible to neonicotinoids (and vice versa), although data are largely restricted to honeybees [S_{upp_ev}].
 - (a) Honeybees stressed by disease are more susceptible (lethal and sublethal effects occur at lower doses) to neonicotinoids, whereas in bumblebees synergistic effects of neonicotinoids and parasites on queen longevity, but not other colony parameters, have been observed. Neonicotinoids can modulate insect innate immunity negatively affecting anti-viral and other defences [D_{ata}].
 - (b) Laboratory molecular biological studies show a potential for the presence of other pesticides (targeted at fungi and *Varroa*) to exacerbate the effects of neonicotinoids though there is limited evidence for such effects from studies with live insects [S_{upp_ev}].
 - (c) It is likely that pollinators exposed to poorer diets are more susceptible to neonicotinoids (and other stressors) [E_{xp_op}].
 - (27) In interpreting these laboratory results, the following issues need to be considered:
 - (a) There is extensive information on the acute lethality of major neonicotinoids in honeybees, but data on other effects, on other pollinators and with the full range of neonicotinoids, are more limited [E_{xp_op}].

- (b) Stress affects insect responses to neonicotinoids and laboratory conditions may be more or less stressful than in the field, an effect that is probably pollinator-species specific and rarely directly assessed in experiments [E_{xp_op}].
 - (c) Laboratory experiments normally involve feeding pollinators with sugar solution or mixed pollen which may affect insects differently to naturally collected food [E_{xp_op}].
 - (d) Chronic and sublethal effects will depend on the pattern of dietary consumption and the rate at which ingested neonicotinoids are cleared from the body [E_{xp_op}]. In addition, neonicotinoids can act as anti-feedants and hence may affect pollinators through reduced food intake, though typically at concentrations higher than expected in the field. How insecticide treated food is presented to pollinators in laboratory experiments, and whether the insects have access to alternative foods, will thus influence the observed responses [S_{upp_ev}].
 - (e) It is challenging to study the impacts of neonicotinoids on entire colonies in the laboratory (particularly for honeybees). As a result, the majority of laboratory studies examine effects on individual bees or queenless groups (often referred to as micro-colonies in bumblebee studies). These results need careful interpretation when assessing how they might translate to whole colony impacts for social bees in the field [E_{xp_op}].
- (28) *Summary.* The strengths of laboratory studies are that they allow carefully controlled experiments to be performed on individual insects subjected to well-defined exposure. The weaknesses are that they are conducted under very artificial conditions (which may affect tolerance to external stress), any avoidance response by the insect is limited and hence the exposure dose and form is determined solely by the experimenter, and responses at the colony or population level are both difficult to study and to extrapolate to the field. Nevertheless, they provide important information about the range of concentrations where death or sublethal effects may be expected to occur [E_{xp_op}].

(e) Neonicotinoid residues observed in pollinators in the field

- (29) Nectar and pollen collected from bees constrained to feed on treated crops have similar insecticide concentrations to those found in samples taken from the plant [D_{ata}].
- (30) There have been few surveys of pesticide and metabolite levels in honeybees in the field. Two studies in Belgium (sample size, $n = 48$ and 99) and one in the USA ($n = 140$) found no honeybees with residues, while a survey in France conducted in 2002–2005 ($n = 187$) detected imidacloprid in 11% of honeybees (at concentrations of 0.03–1.0 ng per bee) [D_{ata}]. We are aware of no data on other pollinators [E_{xp_op}].
- (31) Insecticide residues are more likely to be found in nectar and pollen collected by honeybees and in honey than in the insects themselves. Thus, the French study that found imidacloprid residues in 11% of the bees sampled

also found residues in 22% of honey samples and 40% of pollen samples (mean and range: 0.9, 0.2–5.7 ng g⁻¹). Some large surveys (e.g. a Spanish study with $n = 1021$) found no contaminated pollen; a German study that surveyed hives ($n = 215$) after oilseed rape flowering found low incidence of those neonicotinoids used in seed treatments (though higher incidence of thiacloprid); an American study found imidacloprid in 3% of pollen ($n = 350$) and 1% of wax samples ($n = 208$) [D_{ata}].

(32) *Summary.* Neonicotinoids can be detected in wild pollinators as well as honeybee and bumblebee colonies but data are relatively few and restricted to a limited number of species. Studies to date have found low levels of residues in surveys of honeybees and honeybee products. Observed residues in bees and the products they collect will depend critically on details of spatial and temporal sampling relative to crop treatment and flowering [E_{xp_op}].

(f) Experiments conducted in the field

- (33) This section discusses recent studies that have explored the consequences of providing bee colonies placed in the field with food containing insecticide, as well as experiments where the performance of colonies placed adjacent to fields treated or not treated with neonicotinoids are compared. Some earlier studies with limited statistical power are listed in the annotated bibliography [E_{xp_op}].
- (34) Schneider *et al.* 2012 [10]. Individual honeybees were given single sublethal doses of imidacloprid or clothianidin and their foraging behaviour was monitored. Reductions in foraging activity and longer time foraging flights were not observed at field-relevant doses although negative effects were seen at doses greater or equal to 0.5 ng per bee (clothianidin) or 1.5 ng per bee (imidacloprid) [D_{ata}].
 - (a) These doses are higher than those likely to be encountered by honeybees foraging on nectar from treated plants (see calculations in para. 22e) [E_{xp_op}].
- (35) Henry *et al.* 2012 [11]. Honeybees fed a single high dose of thiamethoxam (1.34 ng, equivalent to 27% of the LD₅₀) and then released away from the hive were significantly less likely to return successfully than controls. The return rate depended on the local landscape structure and the extent of the honeybees' experience of the landscape. The failure to return per trip was estimated to be up to twice the expected background daily mortality [D_{ata}].
 - (a) The rate of forager loss per trip (15%) was analysed as if it were excess daily mortality but as foraging honeybees make 10–30 trips per day real loss rates would be very much higher, reflecting the high dose of insecticide used in the experiment (see para. 22e for calculation of likely field doses) [E_{xp_op}].
 - (b) Assuming honeybees were exposed every day to this dose rate (much higher than expected from observed residues in pollen and nectar), mathematical modelling of colony development predicted severe decline within a season though this conclusion depends critically on poorly understood aspects of honeybee colony dynamics [P_{rojns}].
- (36) Whitehorn *et al.* 2012 [12]. Bumblebee (*Bombus terrestris*) colonies fed exclusively on imidacloprid-treated sugar water (at two concentrations: 0.7 or 1.4 ng g⁻¹) and

pollen (either 6 or 12 ng g⁻¹) for two weeks in the laboratory before being placed in the field (for six weeks) showed reductions in growth rate and queen production. A subsequent study [13] using the same concentrations of imidacloprid found the bumblebees' capacity to forage for pollen (but not nectar) was impaired [D_{ata}].

- (a) The concentrations of insecticide are at the high end of those observed in the nectar and pollen of treated plants (Para. 13a) and are likely to be greater than most bees will receive in the field because alternative food sources were not available [E_{xp_op}].
- (37) Gill *et al.* 2012 [14]. Bumblebee (*B. terrestris*) colonies given access to sugar water containing imidacloprid (10 ng g⁻¹) and allowed to forage for pollen and nectar in the field grew more slowly than controls; individual foragers from imidacloprid-treated colonies were less successful at collecting pollen, and treated colonies sent out more workers to forage and lost more foragers, compared to controls. Combined exposure to imidacloprid and a second pesticide of a different class (a pyrethroid) tended to reduce further colony performance and increase the chances of colony failure [D_{ata}].
- (a) The concentration of insecticide in the sugar water is within the range observed in nectar in the field but considerably higher than the average (1.9 ng g⁻¹; Para. 13a). The actual amount of imidacloprid consumed by individual bumblebees was not measured but will be diluted through foraging from other sources (no pollen was provided). Although it is difficult to make precise comparisons, the pyrethroid concentrations used were towards the upper end of recommended application rates for field or fruit crops [E_{xp_op}].
- (38) Thompson *et al.* 2013 [15]. Bumblebee (*B. terrestris*) colonies were placed adjacent to single oilseed rape fields grown from seeds that were treated with clothianidin, imidacloprid or had no insecticidal seed treatment. No relationship between the oilseed rape treatment and insecticide residues was observed, presumably because the bees were foraging over spatial scales larger than a field. Insecticide residues varied among colonies and the authors reported no evidence of a correlation with colony performance [D_{ata}].
- (a) The experimental design, in particular the lack of replication at field level and absence of a clear effect of treatment, allows only limited inference about the effects of neonicotinoids in the field [E_{xp_op}].
- (39) Pilling *et al.* 2013 [16]. Over a 4 year period, honeybee colonies (six per 2 ha field) were placed beside thiamethoxam-treated or control fields of maize (three replicates) or oilseed rape (two replicates) for between 5 and 8 days (first 3 years) or 19 and 23 days (fourth year) to coincide with the crop flowering period (at other times the colonies were kept in woodland presumed to have no local exposure to insecticides). Honeybees from treatment hives had higher concentrations of insecticide residues, but no differences in multiple measures of colony performance or overwintering survival were observed [D_{ata}].
- (a) Levels of replication precluded formal statistical analysis though the lack of any differences between treatment and control was reasonably consistent across field sites [E_{xp_op}].

- (40) *Summary.* The experiments described in Paras. 33–37 involve bees artificially exposed to neonicotinoids and observed to forage in the field. They show the potential for neonicotinoids to affect the performance of individual pollinators and pollinator colonies in the field. The main issue for their interpretation is the extent to which the doses received by the bees are representative of what they will receive under normal use of neonicotinoids in the field. It appears that most studies have used concentrations at the high end of those expected in the field. The experiments described in Paras. 38 and 39 are true field experiments in the sense that the treatments involve the normal use of neonicotinoids, though only the Pilling *et al.* [16] study was successfully concluded and found no effects of neonicotinoids, but with limited statistical power to detect differences [E_{xp_op}].

(g) Consequences of neonicotinoid use

- (41) At the colony or population level, there may be processes that can compensate for the deaths of individual insects which would mitigate the potential effects of mortality caused by neonicotinoid insecticides. Thus, the deaths of individual pollinators may not lead to a simple proportionate decrease in the overall numbers of that pollinator species. In the case of rare species, extra mortality caused by insecticides could lead to a threshold population density being crossed below which the species declines to extinction, hence magnifying their effects. However, there is a weak evidence base to help understand the presence and magnitude of these effects in the field. Models of honeybee and bumblebee colony dynamics, as well as population-level models of all pollinators, are important tools to explore these effects [E_{xp_op}].
- (42) There is evidence that some crops do not always receive sufficient pollination [D_{ata}], and further limited evidence that this has increased in recent decades [S_{upp_ev}]; but the information available does not allow us to determine whether or not this has been influenced by the increased use of neonicotinoids [E_{xp_op}]. Whether pollination deficits in wild plants have increased is not known [E_{xp_op}].
- (43) Declines in the populations of many insect species in general and pollinators in particular have been observed (para. 7) although the decline in bees predate by some decades the introduction of neonicotinoid insecticides, and there is some evidence of a recent abatement in the rate of decline for some groups [D_{ata}]. Habitat alteration (especially in farmland) is widely considered to be the most important factor responsible. The evidence available does not allow us to say whether neonicotinoid use has had an effect on these trends since their introduction [E_{xp_op}].
- (44) There have been marked increases in overwintering mortality of managed honeybee populations in recent decades (para. 8) [D_{ata}]. It has been suggested that insecticides (particularly neonicotinoids) may be wholly or partly responsible. The weak evidence base cannot at present resolve this question although honeybee declines began before the wide use of neonicotinoids and there is poor geographical correlation between neonicotinoid use and honeybee decline [E_{xp_op}]. Two studies using different structured methodologies have explored this question.

- (a) Cresswell *et al.* 2012 [17]. Used ‘Hill’s epidemiological “causality criteria”’ and concluded that the evidence base did not currently support a role for dietary neonicotinoids in honeybee decline but that this conclusion should be seen as provisional [E_{xp_op}].
- (b) Staveley *et al.* 2014 [18]. Used ‘causal analysis’ methodology and concluded that neonicotinoids were ‘unlikely’ to be the sole cause of honeybee decline but could be a contributing factor [E_{xp_op}].
- (45) Neonicotinoids are efficient plant protection compounds and if their use is restricted farmers may switch to other pest-management strategies (for example, different insecticides applied in different ways or non-chemical control measures) that may have effects on pollinator populations that could overall be more or less damaging than neonicotinoids. Alternatively, they may choose not to grow the crops concerned, which will reduce exposure of pollinators to neonicotinoids but also reduce the total flowers available to pollinators [E_{xp_op}].
- (46) *Summary.* To understand the consequences of changing neonicotinoid use, it is important to consider pollinator colony-level and population processes, the likely effect on pollination ecosystem services, as well as how

farmers might change their agronomic practices in response to restrictions on neonicotinoid use. While all these areas are currently being researched there is at present a limited evidence base to guide policy-makers [E_{xp_op}].

Endnotes

¹The honeybee is *Apis mellifera* (Apidae); bumblebees are *Bombus* species (Apidae), while solitary bees belong to a number of different, related families (Apiformes). Bees belong to the order Hymenoptera, while true flies are in the order Diptera (hoverflies are in the family Syrphidae) and butterflies and moths in the order Lepidoptera.

²Natural capital describes the components of the natural environment that produce value (directly and indirectly) for people; the actual benefits are called ecosystem services (which can be thought of as the flows that arise from natural capital stocks).

³A milligram (mg) is one thousandth (10^{-3}) of a gram (g); a microgram (μg) is one millionth (10^{-6}) of a gram and a nanogram (ng) is one billionth (10^{-9}) of a gram. We express concentrations as nanograms insecticide in 1 g of substance and hence in units of ng g^{-1} (the equivalent metrics ‘one part per billion’ or $1 \mu\text{g kg}^{-1}$ are frequently used in the literature). Concentrations are also sometimes expressed per volume ($\mu\text{g l}^{-1}$); for neonicotinoids 1 ng g^{-1} is approximately $1.3 \mu\text{g l}^{-1}$ in a 50% weight for weight sugar solution.

⁴The LD₅₀ (lethal dose 50%) is the amount of a substance that kills 50% of exposed organisms.

⁵European Food Safety Authority.

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Annotated bibliography to accompany:

A restatement of the natural science evidence base concerning neonicotinoid insecticides and insect pollinators

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Paragraph numbering corresponds to those in the main document; full references at end. Website URLs were accessed 7 March 2014. Any corrections and clarification will be posted at <http://www.futureoffood.ox.ac.uk/news/neonics>.

(a) Introduction and aims

- (1) References below where topics discussed in more detail. EU partial restriction is Regulation (EU) No. 485/2013 European Commission (2013). For concerns about the restriction of neonicotinoid use see Campbell (2013), Walters (2013).
- (2) Categories developed by authors, influenced by scheme in Godfray *et al.* (2013).
- (3) For an introduction to the social science and economic literature on pollinators see Kevan and Phillips (2001), Losey and Vaughan (2006), Kremen *et al.* (2007), Zhang *et al.* (2007), Gallai *et al.* (2009), Keitt (2009), Kuldna *et al.* (2009), Osgathorpe *et al.* (2011), UK National Ecosystem Assessment (2011), Noleppa and Hahn (2013), Vanbergen and Insect Pollinators Initiative (2013). Goulson (2013) reviews studies comparing yields on crops protected by neonicotinoids or through other means.

(b) Pollinators and neonicotinoid insecticides

- (4) UK data from UK National Ecosystem Assessment (2011) (updated in Vanbergen *et al.*, 2014) which also estimates economic importance of pollinator services. For summary of European crops dependent on pollinators see Table 2 of the SOM in Leonhardt *et al.* (2013) and for US see Calderone (2012). Ollerton *et al.* (2011) estimate that 87% of all plant species are animal pollinated; Klein *et al.* (2007) calculate that 87 of the 115 most important food plant species for man require animal pollination, and though these make up only 35-40% of human food by volume; Eilers *et al.* (2011) show that some essential human nutrients come largely from pollinated crop plants; Lautenbach *et al.* (2012) map global distribution of crops needing pollination and hence likely benefits of pollinators; Aizen *et al.* (2008) note proportion of pollinating crops increasing.
- (5) Benefits of insect pollination for crops shown by Klein *et al.* (2007), Hoehn *et al.* (2008), Garibaldi *et al.* (2011b), Bommarco *et al.* (2012b), Brittain *et al.* (2013a), Brittain *et al.* (2013b), Garratt *et al.* (2014a, 2014b); and in enclosures by Dag (2008). Aizen and Harder (2009) show that globally crop demand for pollination is growing faster than the supply of honeybees.
- (6) For overview see Free (1993). Importance of wild pollinators to crops discussed by Greenleaf and Kremen (2006a), Greenleaf and Kremen (2006b), Winfree *et al.* (2007), Jauker *et al.* (2012), Klein *et al.* (2012), Brittain *et al.* (2013b), Garibaldi *et al.* (2013). Pollination as an ecosystem service is explored by Losey and Vaughan (2006), Boyd and Banzhaf (2007), Kremen *et al.* (2007), Zhang *et al.* (2007), Aizen *et al.* (2008), Brosi *et al.* (2008), Aizen *et al.* (2009), Lonsdorf *et al.*

- (7) Biesmeijer *et al.* (2006), Committee on the Status of Pollinators in North America (2007), Goulson *et al.* (2008), Potts *et al.* (2010a), Potts *et al.* (2010b), Gonzalez-Varo *et al.* (2013), Lebuhn *et al.* (2013), Vanbergen and Insect Pollinators Initiative (2013) discuss the evidence for pollinator declines, and the multiple factors that may be responsible. See also Grixti *et al.* (2009), Cameron *et al.* (2011) (US bumblebees) and Bommarco *et al.* (2012a) (changes in bumblebee community composition in Sweden). Evidence of historic declines and recent slowdowns and reversals is in Carvalheiro *et al.* (2013). For butterfly and moth data see The State of Britain's Larger Moths 2013 (<http://butterfly-conservation.org/files/state-of-britains-larger-moths-2013-report.pdf>), Conrad *et al.* (2004) and Asher *et al.* (2001).
- (8) Rosenkranz *et al.* (2010) review the consequences of the introduction of *Varroa* to Europe; see also Carreck *et al.* (2010). Elevated overwintering mortality in Belgium: Nguyen *et al.* (2010); in the US: VanEngelsdorp *et al.* (2011), VanEngelsdorp *et al.* (2012). Martin *et al.* (2012) showed massive increase of a single strain of a bee virus after *Varroa* introduction to Hawaii, indicating that *Varroa* is both vectoring viruses but also driving selection for higher viral virulence. US beekeepers have compensated for overwintering losses by initiating more colonies; VanEngelsdorp *et al.* (2011). For colony collapse disorder in the US and for discussion about its contribution to colony losses as well as its presence or absence in Europe see vanEngelsdorp *et al.* (2009), Ratnieks and Carreck (2010), vanEngelsdorp *et al.* (2010), Williams *et al.* (2010), Frazier *et al.* (2011), Smith *et al.* (2014). For global patterns in honeybee numbers see Aizen and Harder (2009).
- (9) Kollmeyer (1999), Maienfisch *et al.* (2001b) & Jeschke *et al.* (2013) describe neonicotinoid discovery; mode of action discussed by Maienfisch *et al.* (2001a), Matsuda *et al.* (2001), Elbert *et al.* (2008) and uptake by plant by Sur and Stork (2003); invertebrate/vertebrate toxicity by Tomizawa *et al.* (2000), Tomizawa and Casida (2003), Tomizawa and Casida (2005), Goulson (2013) Table S1. Rise and current use of neonicotinoids described by Jeschke and Nauen (2008), Jeschke *et al.* (2011), Jeschke *et al.* (2013). For details of pesticide usage on UK crops in 2012 see <http://pusstats.fera.defra.gov.uk/>. Nauen and Denholm (2005) discuss the evolution of resistance.
- (10) See the EU pesticides database at http://ec.europa.eu/sanco_pesticides/public/?event=activesubstance.selection, thiamethoxam metabolism: Nauen *et al.* (2003).
- (11) For Europe see European Food Safety Authority (2013e) and the US Environmental Protection Agency. Kohler and Triebkorn (2013) review general issues of wildlife

ecotoxicology. The data required for pesticide active substances and associated products under Regulation 1107/2009 are outlined in <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:093:0001:0084:EN:PDF> and <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:093:0085:0152:EN:PDF>.

(c) Exposure of pollinators to neonicotinoid insecticides

- (12) Klein *et al.* (2007), Leonhardt *et al.* (2013), Vanbergen and Insect Pollinators Initiative (2013).
- (a) Velasco *et al.* (1999) estimate an average rapeseed weighs 5.9 mg and Environment Protection Agency (2003) give figures of 600g active ingredient per 100kg seeds giving 35µg per seed. Typical figures quoted for much larger maize seed (~200 mg) are 1.2mg (Environment Protection Agency (2003)).
- (b) Larson *et al.* (2013) show that if clothianidin is sprayed on turf with clover in bloom (against label recommendation) to control lawn pests, the growth of colonies of bumblebees that forage on the clover is impaired, see also Gels *et al.* (2002). Zimmer and Nauen (2011) on use of thiacloprid on oilseed rape. We are aware of on-going studies of the consequences to bees of spraying *N*-cyanoamidine neonicotinoids on flowering raspberries and oilseed rape and when published we shall note at <http://www.futureoffood.ox.ac.uk/news/neonics>.
- (13) For action of neonicotinoids see Maienfisch *et al.* (2001a), Matsuda *et al.* (2001), Elbert *et al.* (2008). Sur and Stork (2003) estimate 1-20% neonicotinoids absorbed by plant.
- (a) Blacquièrre *et al.* (2012) review studies on sunflower and maize; see also Schmuck *et al.* (2001), Bonmatin *et al.* (2005), Rortais *et al.* (2005), Halm *et al.* (2006), Cutler and Scott-Dupree (2007), Cresswell (2011), Krupke *et al.* (2012), Pohorecka *et al.* (2012). Concentrations may differ on other crops and be influenced by application method, for example see work by Stoner and Eitzer (2012) on squash and Dively and Kamel (2012) on pumpkin who found relatively higher concentrations compared to seed treatments.
- (14) For studies of guttation fluid see Girolami *et al.* (2009), Reetz *et al.* (2011), Schenke *et al.* (2011), Tapparo *et al.* (2011), Hoffmann and Castle (2012), Pistorius *et al.* (2012) and EFSA (2012).
- (15) Bortolotti *et al.* (2003), Alix *et al.* (2009), Girolami *et al.* (2009), Pistorius *et al.* (2009), Thompson (2010), Tremolada *et al.* (2010), Marzaro *et al.* (2011), Girolami *et al.* (2012), Krupke *et al.* (2012), Pochi *et al.* (2012), Tapparo *et al.* (2012), Girolami *et al.* (2013).
- (a) Nuyttens *et al.* (2013) review relevant issues of formulation and sowing equipment configuration; see EFSA (2013) for regulations; air deflectors have become mandatory for certain products in the Netherlands, France, Belgium and Germany.
- (16) Goulson (2013), Table 1, lists 35 estimates from 12 published studies of soil half-lives (dissipation times) for different neonicotinoids (of which 10 were conducted in the field, the rest in the laboratory). More information has been collected by industry for regulatory purposes but is not in the public domain though summarised in regulatory documents (EFSA). For accumulation in soil see Anon (2006) discussed by Goulson (2013) in Figure 2. For leaching and contamination of groundwater see Gupta *et al.* (2008), Selim *et al.* (2010), Starner and Goh (2012) and Goulson (2013). Krupke *et al.* (2012) found weed flowers (dandelion, *Taraxacum*) visited by pollinators near treated feeds contained neonicotinoids (though whether through dust or soil is not known). Schmuck *et al.* (2001); Charvet *et al.* (2004) found untreated sunflower and maize grown on soils containing imidacloprid from earlier treatments do not have detectable residues in pollen and nectar.
- (17) Winston (1987), Seeley (1995), Brodschneider and Crailsheim (2010); studies of larval mortality discussed in para. (24).
- (18) Thompson and Hunt (1999), Hoyle *et al.* (2007), Cresswell *et al.* (2012b) for seasonal susceptibility of honeybees.
- (19) Authors' summary.
- (20) Jha and Kremen (2013) discuss bumblebee foraging distances and behaviour, see also Osborne *et al.* (1999), Darvill *et al.* (2004), Knight *et al.* (2005), Osborne *et al.* (2008), Hagen *et al.* (2011), Carvell *et al.* (2012). For honey bees see Visscher and Seeley (1982); Beekman and Ratnieks (2000) (which includes maximum 15km estimate); Steffan-Dewenter and Kuhn (2003). For solitary bees Gathmann and Tschardt (2002), Greenleaf *et al.* (2007), Zurbuchen *et al.* (2010a), Zurbuchen *et al.* (2010b).
- (21) Authors' summary.

(d) Laboratory studies of lethal and sublethal effects of neonicotinoids

- (22) Recent review in Blacquièrre *et al.* (2012), see also Decourtye and Devillers (2010) and meta-analysis by Arena and Sgolastra (2014).
- (a) The oral sensitivities reported here come from European Food Safety Authority (2013c), European Food Safety Authority (2013b), European Food Safety Authority (2013a) though we note that not all the studies upon which these assessments are based are in the public domain. The meta-analysis is Cresswell (2011) which included 13 peer-reviewed and one non-peer-reviewed studies.
- (b) The contact sensitivities reported here also come from European Food Safety Authority (2013c), European Food Safety Authority (2013b), European Food Safety Authority (2013a). A study by Iwasa *et al.* (2004) compared honeybee acute contact LD₅₀s for imidacloprid (18 ng bee⁻¹), clothianidin (22 ng bee⁻¹), thiamexotham (30 ng bee⁻¹) and, for neonicotinoids from the cyano-substituted group, acetamiprid (7 µg bee⁻¹), thiacloprid (15 µg bee⁻¹). These figures differ somewhat from those in EFSA though are in the same general range.
- (c) A meta-analysis by Arena and Sgolastra (2014) compares the differential sensitivity of bee species to different classes of insecticides and includes data on neonicotinoid exposure to nine bee species including the honeybee; the latter comes near the middle of the range of sensitivities with studies on bumblebees consistently reporting higher sensitivities (a similar pattern was found with other insecticide classes). In a direct comparison of acute contact toxicity across non-*Apis* bee species, Scott-Dupree *et al.* (2009) showed variation of up to 19 times for imidacloprid (up to 4 times for clothianidin); see also Stark *et al.* (1995), Biddinger *et al.* (2013).
- (d) For general honeybee economics see Winston (1987), Seeley (1995), Brodschneider and Crailsheim (2010); Rortais

- et al.* (2005) observed the (relatively small) colony of 10,000. We have calculated these figures and those in the next two subparagraphs to provide a general guide to the relative concentrations involved. Since most of the nectar and pollen in the foraging honeybee stomach is not metabolized (Fournier *et al.* 2014) the insect is only exposed to a small fraction of any insecticide it contains, almost all of which will be carried to the hive.
- (e) Winston (1987), Seeley (1995), Brodschneider and Crailsheim (2010) and authors' calculations.
- (f) Winston (1987), Seeley (1995), Brodschneider and Crailsheim (2010), Decourtye *et al.* (2010) and authors' conclusions.
- (23) Recent review in Blacquière *et al.* (2012).
- (a) See Decourtye *et al.* (2001), Suchail *et al.* (2001), Decourtye *et al.* (2003), and European Food Safety Authority (2013c), European Food Safety Authority (2013b), European Food Safety Authority (2013a) for studies of chronic lethality in the laboratory.
- (b) Authors' conclusions.
- (24) Recent review in Blacquière *et al.* (2012) and see Decourtye and Devillers (2010) for general issues of sublethal effects on non-target insects Desneux *et al.* (2007); see also European Food Safety Authority (2012), European Food Safety Authority (2013c), European Food Safety Authority (2013b), European Food Safety Authority (2013a). See Belzunces *et al.* (2012) and Boily *et al.* (2013) for imidacloprid stimulation of acetylcholinesterase activity; for neuronal inactivation Palmer *et al.* (2013), on learning performance and behaviour, Decourtye *et al.* (2004a), Decourtye *et al.* (2005a), Decourtye *et al.* (2004b), El Hassani *et al.* (2008), Yang *et al.* (2008), Aliouane *et al.* (2009), Matsumoto (2013a), Matsumoto (2013b). The proboscis extension response is discussed by Bitterman *et al.* (1983) and Giurfa and Sandoz (2012).
- (a) For sublethal effects on bumble bees see Tasei *et al.* (2000), Tasei *et al.* (2001), Morandin and Winston (2003), Franklin *et al.* (2004), Mommaerts *et al.* (2010), Cresswell *et al.* (2012b), Laycock *et al.* (2012), Bryden *et al.* (2013), Elston *et al.* (2013), Fauser-Misslin *et al.* (2013) (the last two of which found no effects at estimated field concentrations), Laycock *et al.* (2014); and on solitary bees Abbott *et al.* (2008), Thompson and Hunt (1999), Scott-Dupree *et al.* (2009), Brittain and Potts (2011), Biddinger *et al.* (2013), Sandrock *et al.* (2014) compare risk factors for different bee species.
- (b) For debate see Suchail *et al.* (2004a), Suchail *et al.* (2004b), Tennekes (2010), Maus and Nauen (2011), Tennekes and Sánchez-Bayo (2012); figures from Cresswell *et al.* (2013). Hawthorne and Dively (2011) describe how multi-drug resistance transporters actively remove neonicotinoids from the cytoplasm so reducing their toxicity.
- (25) Recent review in Blacquière *et al.* (2012).
- (a) Studies on honeybees: Decourtye *et al.* (2005b) (imidacloprid added at 5 ng g^{-1} ; at the high end of what observed in field prior to any processing of the food by the adult), Yang *et al.* (2012) (observed effects when the doses given to larvae were near the LD_{50} reported from larger adults); for effects on cellular physiology see Gregorc and Ellis (2011). Wu *et al.* (2011) studied larvae reared in old contaminated combs; the combs contained many different chemical residues, the most abundant of which were acaricides (used by the beekeepers against *Varroa*); three types of neonicotinoid were present at low levels, but in only one out of the 13 replicates. Retarded larval development and reduced longevity of emerging bees was observed. This study has been cited as evidence of the effects of neonicotinoids on larval honeybees, in the authors' view incorrectly.
- (b) Bumblebees: Elston *et al.* (2013) found thiamethoxam reduced worker consumption of sugar water solution and their production of wax cells at both 1 and 10 ng g^{-1} , and at the higher dosage workers laid fewer eggs and no (male) larvae were produced in micro-colonies; see also Laycock *et al.* (2012), Laycock and Cresswell (2013). Fauser-Misslin *et al.* (2013) studied chronic dietary exposure of whole bumblebee colonies. Solitary bees: Abbott *et al.* (2008), Sandrock *et al.* (2014).
- (26) European Food Safety Authority (2012), Thompson (2012).
- (a) Abrol (2007), Alaux *et al.* (2010), Vidau *et al.* (2011) showed honeybees infected by the microsporidian *Nosema ceranae* have lower neonicotinoid LD_{50} , and Pettis *et al.* (2012) that prior exposure to neonicotinoids in the hive led to a higher *N. ceranae* spore load in artificially infected bees in the lab (though no effect in the hive). Pettis *et al.* (2013) found that honeybees fed pollen contaminated by neonicotinoids were significantly less likely to become infected by *Nosema* (fungicides used in hives had the opposite effect). Fauser-Misslin *et al.* (2013) found chronic exposure of bumblebees to thiamethoxam and clothianidin increased the negative effects of infection by the trypanosome *Crithidia bombi*. Di Prisco *et al.* (2013) showed neonicotinoids reduced antiviral defences via effects on $\text{NF-}\kappa\text{B}$ signalling pathways in honeybees. See also European Food Safety Authority (2012, p. 108). Doublet *et al.* (2014) find that in the laboratory sublethal doses of the *N*-cyanoamidine group neonicotinoid thiacloprid can increase the detrimental effects on honeybees of microsporidian and viral pathogens.
- (b) Review in Blacquière *et al.* (2012); see also Iwasa *et al.* (2004), European Food Safety Authority (2012, p. 113). Hawthorne and Dively (2011) show that honeybees from colonies treated with the in-hive antibiotic oxytetracycline (not allowed in the EU) may be sensitised to acaricides and insecticides (including neonicotinoids); oxytetracycline inhibits multi-drug resistance transporters that clear cells of toxins. Williamson *et al.* (2013) found little additive acute effects of imidacloprid and the organophosphate acaricide coumaphos (a modest improvement in memory) while chronic multiple exposure caused significant impairment Williamson and Wright (2013).
- (c) For increased sensitivity to pesticides (lower LD_{50}) when honeybees are stressed, for example when raised on protein deficient diets, see Von der Ohe and Janke (2009), Wehling *et al.* (2009).
- (27) This paragraph chiefly authors' judgment.
- (a) Walters (2013) comments on lack of data on full range of neonicotinoids.
- (b) See Visser and Blacquière (2010) for discussion and preliminary experiments.
- (c) Authors' comment.
- (d) For issue of insecticide clearance see Para. (24)(b); for anti-feedant behaviour see Suchail *et al.* (2004a), Department for Environment (2007), Cresswell *et al.* (2013), and in bumblebees Laycock *et al.* (2012), Elston *et al.* (2013).
- (e) Authors' comment.
- (28) Authors' summary.

(e) Neonicotinoid residues observed in pollinators in the field

- (29) Pilling *et al.* (2013).
- (30) Comprehensive review in Blacquière *et al.* (2012); the Belgium studies are by Pirard *et al.* (2007) and Nguyen *et al.* (2009); the US study by Mullin *et al.* (2010); and the French study by Chauzat *et al.* (2006), Chauzat *et al.* (2009), Chauzat *et al.* (2011); see Wiest *et al.* (2011) for recent technical advances.
- (31) Comprehensive review in Blacquière *et al.* (2012); French studies see para. (30); Spanish study is by Bernal *et al.* (2010), the German by Genersch *et al.* (2010). Škerl *et al.* (2009) found pollen collected from hives near recently sprayed apple trees had high levels of thiacloprid (a neonicotinoid less harmful to bees) after apple tree spraying, a compound found relatively frequently in the surveys of Škerl *et al.* (2009), Genersch *et al.* (2010). As part of an experiment Thompson *et al.* (2013) placed bumblebee colonies near treated fields and recorded residues in nests (though often not of the substance applied to the adjacent field). Mullin *et al.* (2010) found neonicotinoid residues in less than 2% of wax samples ($n = 208$) and less than 5% of pollen samples in North America ($n = 350$) though relatively high levels of agricultural chemicals were observed. See also Bonmatin *et al.* (2003), Brittain and Potts (2011), Krupke *et al.* (2012).
- (32) Authors' summary.

(f) Experiments conducted in the field

- (33) For earlier studies see Schmuck *et al.* (2001), Faucon *et al.* (2005), Cutler and Scott-Dupree (2007); see also Cresswell (2011) for a meta-analysis of 13 laboratory and semi-field studies of imidacloprid that concluded field-realistic doses did not cause honey bee death but could reduce colony performance by 6-20%.
- (34) Schneider *et al.* (2012).
- (a) Authors' comment.
- (35) Henry *et al.* (2012a) gave bees an acute dose of thiamethoxam and released them 1km away from the colony carrying Radio Frequency Identification (RFID) tags that were read at the hive entrance (the use of RFID tag technology to monitor bee foraging activity is described by Streit *et al.* (2003) and Molet *et al.* (2008)).
- (a) As foragers typically make multiple trips from the hive to collect food each day, reported homing failure rates of 10.2-31.6% per trip would rapidly reduce the forager workforce. Henry *et al.* (2012a) measured the dose given to bees but Food and Environment Research Agency (2013) questioned the realism of exposing the bees to the equivalent of a daily dose in one meal.
- (b) Assumptions in original model in Henry *et al.* (2012a) were debated by Cresswell and Thompson (2012) and Henry *et al.* (2012b).
- (36) Whitehorn *et al.* (2012) allowed bumblebees to feed on pollen and sugar water containing 6 and 0.7 ng g⁻¹ imidacloprid respectively (low dose) and double this (high dose) and measured colony mass and queen production in the field. The insecticide affected both measures of performance with little difference between low and high doses. Feltham *et al.* (2014) used RFID tag technology to assess foraging success of dosed and non-dosed bees. Whitehorn *et al.* (2012) and Gill *et al.* (2012) provided food containing neonicotinoids at rates informed by those observed in pollen and nectar though Food

and Environment Research Agency (2013) has argued that in practice these are higher than would occur in the field.

- (a) Authors' comment. There was considerable variability in queen production among colonies, with many colonies producing no queens; the replication was sufficient to detect differences despite this variability that might reflect that the colonies were stressed. Imidacloprid can cause anti-feedant behaviour affecting energy intake but typically at higher concentrations than used here.
- (37) Gill *et al.* (2012) placed bumblebees in nests with an antechamber where they had access to sugar water and monitored colony size and foraging (by RFID tagging workers and measuring pollen loads) over a 28-day period. The experiment had four treatments: sugar water containing imidacloprid, antechamber with surface sprayed with pyrethroid (λ -cyhalothrin); the two combined, and a control.
- (a) Authors' comment. The bumblebees walked over filter paper sprayed with pyrethroid at a concentration that if extrapolated to the whole environment would be equivalent to an application rate of about 40g ha⁻¹. This is considerably higher than the 7.5 g ha⁻¹ EU maximum for oilseed rape though higher concentrations (up to 30 g ha⁻¹) are allowed on some field crops and up to 125g ha⁻¹ in grapes, cane fruit and hops (http://ec.europa.eu/food/fs/sc/scp/out01_ppp_en.html). Exposure by walking over filter paper (through tarsal contact) may be less than an animal encounters in the field, for example through spray drift.
- (38) Thompson *et al.* (2013) is a non-peer reviewed report published on the web in March 2013 with the version currently (March 2014) available containing a supplementary explanatory note added in June 2013. The study involved placing ten bumblebee nests adjacent to three different fields (hence there was no replication of treatments) though these could not be carried out at the same time (introducing the possibility of systematic error).
- (a) Authors' comment. The design of the study was criticised by several commentators and in a detailed review by European Food Safety Authority (2013d). The addendum added on 14th June 2013 states "This study was not designed as a definitive statistically robust study" but to look quickly for "major effects". Reanalysis of some of the data reported to the Advisory Committee on Pesticides has questioned the lack of a colony-level relationship between neonicotinoid residues and colony performance (<http://www.pesticides.gov.uk/guidance/industries/pesticides/advisory-groups/acp/ACP-News/ACP-359-29-January-2013-Detailed-Record-of-Discussion>) but this too has not been peer-reviewed.
- (39) Pilling *et al.* (2013). Neonicotinoid treatments were typical for France where the experiments were carried out. The short exposure time in the first three years coincided with oilseed rape flowering. It is possible that bees from a single hive exploit asynchronously flowering fields and so experience longer exposure.
- (40) Authors' summary.
- ### (g) Consequences of neonicotinoid use
- (41) The observed abundance of a species is influenced by factors that have the same effect on death rates (or birth rates) irrespective of population size (density-independent factors, a

- typical example would be the effects of bad weather) and those whose magnitudes depend on population size (density-dependent factors; a typical example would be starvation due to competition for food). The reduction in pollinator population size caused by insecticides (a density-independent factor) may be less if they result in reduced density-dependent mortality through factors acting subsequently. Such effects are likely to be common in pollinator population dynamics though very difficult to predict in the absence of detailed study. "Allee effects" are density-dependent factors that increase in severity at low densities (a typical example would be reduced fecundity due to failure to mate at low population densities). If insecticide application reduced population density to a level where an Allee effect occurred then population extinction could occur. We do not know of examples of Allee effects from pollinator populations. Kohler and Triebkorn (2013) review population consequences of pesticide use. For population dynamic models of honeybee colonies see Khoury *et al.* (2011), Becher *et al.* (2013), Khoury *et al.* (2013) and bumblebee colonies see Bryden *et al.* (2013).
- (42) Garratt *et al.* (2014a), Garratt *et al.* (2014b) report deficits in UK apple pollination and Breeze *et al.* (2014) suggest a lack of availability of honeybees may affect pollination of oilseed rape in Europe. Bommarco *et al.* (2012b) provide evidence of a decrease in clover seed yield through time linked to pollinator loss. Garibaldi *et al.* (2011b) show decreases in crop yield and stability with distance from native vegetation. Aizen *et al.* (2008) for global comparison of yield growth of insect- and wind-pollinated plants (no difference suggesting no pollination deficit). Dicks *et al.* (2013) survey main evidence needs for pollinator conservation. Ashman *et al.* (2004) review pollination deficits in wild flowers and Holzschuh *et al.* (2011) shows mass-flowering crops can attract pollinators away from wild species. Our assessment of available evidence.
- (43) See references in Para. (7); importance of habitat loss: Carvell *et al.* (2006), Ricketts *et al.* (2008), Brown and Paxton (2009), Goulson *et al.* (2010), Carvalho *et al.* (2011), Kennedy *et al.* (2013), though see Gonzalez-Varo *et al.* (2013) and Vanbergen (2013) for importance of considering multiple effects. Authors' assessment of available evidence.
- (44) See references in Para. (8). Authors' assessment of available evidence.
- (a) Cresswell *et al.* (2012a); see also Maxim and van der Sluijs (2010).
- (b) Staveley *et al.* (2014).
- (45) Authors' assessment of available evidence.
- (46) Authors' summary.

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