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REVIEW

An overview of the environmental risks posed by neonicotinoid insecticides

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Summary

1. Neonicotinoids are now the most widely used insecticides in the world. They act systemically, travelling through plant tissues and protecting all parts of the crop, and are widely applied as seed dressings. As neurotoxins with high toxicity to most arthropods, they provide effective pest control and have numerous uses in arable farming and horticulture.
2. However, the prophylactic use of broad-spectrum pesticides goes against the long-established principles of integrated pest management (IPM), leading to environmental concerns.
3. It has recently emerged that neonicotinoids can persist and accumulate in soils. They are water soluble and prone to leaching into waterways. Being systemic, they are found in nectar and pollen of treated crops. Reported levels in soils, waterways, field margin plants and floral resources overlap substantially with concentrations that are sufficient to control pests in crops, and commonly exceed the LC_{50} (the concentration which kills 50% of individuals) for beneficial organisms. Concentrations in nectar and pollen in crops are sufficient to impact substantially on colony reproduction in bumblebees.
4. Although vertebrates are less susceptible than arthropods, consumption of small numbers of dressed seeds offers a route to direct mortality in birds and mammals.
5. *Synthesis and applications*. Major knowledge gaps remain, but current use of neonicotinoids is likely to be impacting on a broad range of non-target taxa including pollinators and soil and aquatic invertebrates and hence threatens a range of ecosystem services.

Key-words: bee, clothianidin, environmental fate, half-life, imidacloprid, non-target wildlife, soil water, systemic insecticide

An introduction to neonicotinoids

Neonicotinoids were developed in the 1980s, and the first commercially available compound, imidacloprid, has been in use since the early 1990s (Kollmeyer *et al.* 1999). They are nicotinic acetylcholine receptor agonists; they bind strongly to nicotinic acetylcholine receptors (nAChRs) in the central nervous system of insects, causing nervous stimulation at low concentrations, but receptor blockage, paralysis and death at higher concentrations. Neonicotinoids bind more strongly to insect nAChRs than to those of vertebrates, so they are selectively more toxic to insects (Tomizawa & Casida 2005). They can be classified into one of three chemical groups, the *N*-nitroguanidines (imidacloprid, thiamethoxam, clothianidin and dinotefuran), nitromethylenes (nitenpyram) and *N*-cyanoamidines (acetamiprid and thiacloprid; Jeschke *et al.* 2011). They are generally toxic to insects in minute quantities; for example, the LD_{50} (dose that kills 50% of individuals) for ingestion of imidacloprid and clothianidin in honeybees is 5 and 4 ng per insect, respectively, which for comparison is approximately 1/10 000th of the LD_{50} for dichlorodiphenyltrichloroethane (DDT; Suchail, Guez & Belzunces 2000). Neonicotinoids are water soluble and are readily absorbed by plants via either their roots or leaves and then are transported throughout the tissues of the plant. This provides many advantages in pest control, for they protect all parts of the plant; for example, they are effective against boring insects and root-feeding insects, both of which cannot easily be controlled using foliar sprays of non-systemic compounds. Concentrations in plant tissues and sap between 5 and 10 ppb (parts per billion) are generally regarded as sufficient to provide protection against pest insects (Castle *et al.* 2005; Byrne & Toscano 2006). For example, in citrus trees treated with imidacloprid via irrigation water, 5 ppb in xylem fluids was sufficient to

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control the sap-sucking insect *Homalodisca coagulata* (Castle *et al.* 2005).

In developed countries, neonicotinoids are predominantly used as seed dressings for a broad variety of crops such as oilseed rape, sunflower, cereals, beets and potatoes (primarily imidacloprid, clothianidin and thiamethoxam). For example, in the UK, use as a seed dressing accounted for 91% of all neonicotinoid use in farming in 2011 (Defra 2012a; note that this does not include garden or amenity use). Globally, 60% of neonicotinoids are used in this way (Jeschke *et al.* 2011). One attraction of seed dressings is that they require no action from the farmer, prophylactically protecting all parts of the crop for several months following sowing, and they are also regarded as providing better targeting of the crop than spray applications (Jeschke *et al.* 2011). However, the widespread adoption of neonicotinoids is partly down to their flexibility of use, for they can be applied in many other ways (Jeschke *et al.* 2011); they are commonly used as foliar sprays on horticultural crops such as soft fruits and on some arable crops such as soya, and they are sold for garden use as a spray on flowers and vegetables. They are used in bait formulations for domestic use against cockroaches and ants and also as granular formulations for the treatment of pasture and amenity grasslands against soil-dwelling insect pests. They can be applied as a soil drench or in irrigation water to defend perennial crops such as vines, and they can be injected into timber to combat termites or into trees to protect them against herbivores, where a single application can provide protection for several years (e.g. Oliver *et al.* 2010). Finally, they are commonly used in topical applications on pets such as dogs and cats to control external parasites.

Their advantages of low toxicity to vertebrates, high toxicity to insects, flexible use and systemic activity led to neonicotinoids swiftly becoming among the most widely used pesticides globally; they are now used more than any other class of insecticides and comprise approximately one quarter of all insecticides used. They are licensed for use in more than 120 countries and have a global market value of ~$2·6 billion, with imidacloprid alone comprising 41% of this market and being the second most widely used agrochemical in the world (Jeschke *et al.* 2011; Pollack 2011). Detailed data on use by country are generally not available, but figures for the UK illustrate the rapid adoption of neonicotinoids in the last 20 years, with UK use rising from three tonnes in 1994 to nearly 80 tonnes in 2011 (Fig. 1a).

The widespread adoption of neonicotinoids as seed dressings has led to a move away from integrated pest management (IPM), a philosophy of pest management predicated on minimizing use of chemical pesticides via monitoring of pest populations, making maximum use of biological and cultural controls, applying chemical

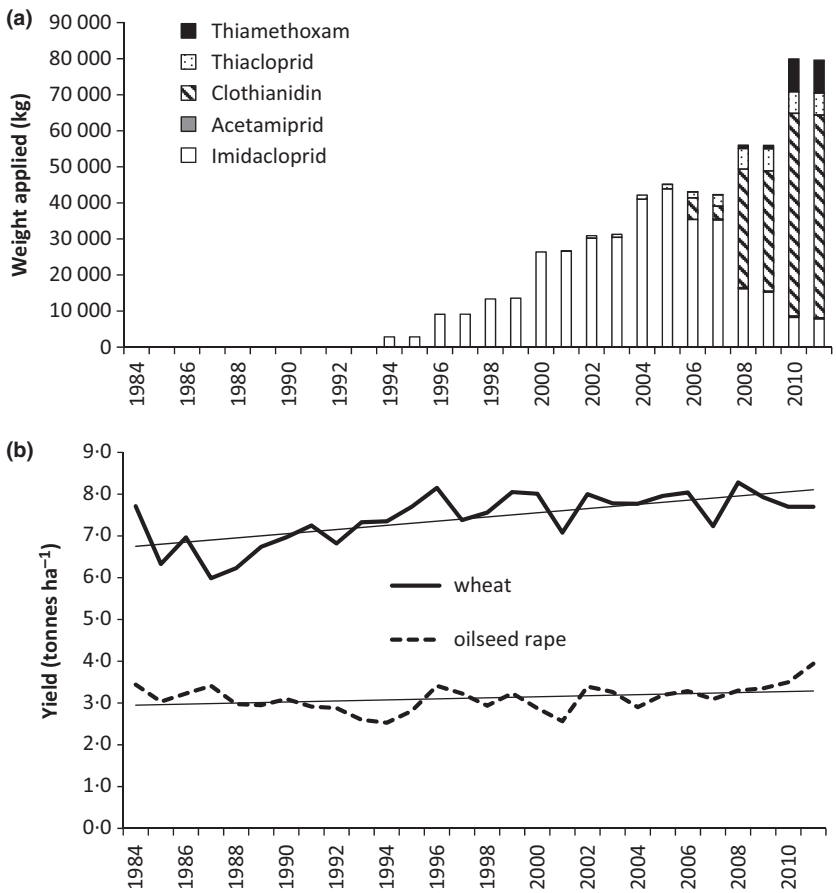

Fig. 1. (a) Annual usage (kg) of neonicotinoids in agriculture and horticulture in the UK, one of few countries from which detailed records are available (Defra 2012a). Note that these figures do not include garden or amenity use, or use for treatment of pets. In 2011, the area of land treated was approximately 1·3 million ha. (b) UK yields of two crops that are now widely treated with neonicotinoids as a seed dressing (Defra 2012b). There has been no significant rise in oilseed rape yield since its introduction, while winter wheat yields have risen slightly (linear regressions, $F_{1,26} = 4{\cdot}01$, ns and $F_{1,26} = 21{\cdot}1$, $P < 0{\cdot}001$, respectively).

pesticides only when needed and avoiding broad-spectrum, persistent compounds (Metcalf & Luckmann 1994). Of necessity, seed dressing has to be applied prophylactically to crops before any information is available on likely pest problems in the coming year.

Economic benefits of neonicotinoids

There is abundant evidence that neonicotinoids can provide effective control of a broad range of insect pests (reviewed in Jeschke *et al.* 2011). It is less clear to what extent the widespread adoption of neonicotinoids has contributed to yield increases in farming or whether neonicotinoids offer economic benefits compared to alternatives. Yields per hectare of almost all arable crops have increased markedly over the last 60 years as a result of many changes, including improved crop varieties, widespread use of artificial fertilizers, new agronomic techniques and the development of successive generations of pesticides. However, the pace of yield increases has slowed, and yield increases in the last 20 years in developed countries have been modest, with some crops such as oilseed rape showing no increase coincident with the introduction of neonicotinoids; for example, in the UK, yields of oilseed rape were the same pre-1994 (when no neonicotinoids were available) as they are today, when close to 100% of crops are treated (Parry & Hawkesford 2010; Defra 2012a,b; Fig. 1b). Where yield increases have occurred in recent years, it is hard to disentangle the contribution of neonicotinoids from the effects of other changes in agronomic practices.

Given their widespread use, it is surprising that few studies have attempted to compare the effectiveness of neonicotinoids with alternative means of pest control. Bueno *et al.* (2011) compared managing soya pests in Brazil using either an IPM approach or prophylactic use of insecticides (the latter primarily based on imidacloprid). Crop yields were indistinguishable in the two treatments, but pesticide use and costs were much lower in the IPM treatment, demonstrating that this remains the best alternative in this system. In North America, Seagraves & Lundgren (2012) compared yield of either imidacloprid or thiamethoxam seed dressings on soya with untreated controls and found no difference in yield in either of the 2 years of their study, but populations of beneficial natural enemies were depressed in treated plots. In this system, the evidence would suggest that the cost of seed treatment (~\$30 ha^{-1}) is not being recouped by the farmer. This is in accordance with a several similar studies of soya which found either no yield benefits (McCornack & Ragsdale 2006; Cox, Shields & Cherney 2008; Ohnesorg, Johnson & O'Neal 2009) or yield benefits below those which could be achieved more economically using foliar insecticides applied only when pests exceeded a threshold (McCornack & Ragsdale 2006; Johnson *et al.* 2009). Similarly, studies of the efficacy of imidacloprid dressing of winter wheat in North America suggest that yield benefits are small (compared to unprotected, control crops) and often exceeded by the cost of the pesticide (Royer *et al.* 2005). In contrast, in Western Australia, McKirdy, Jones & Nutter (2002) demonstrated that application of an imidacloprid seed dressing to spring wheat is cost-effective compared to using no pest control, but that using foliar applications of alpha-cypermethrin (which is much cheaper) provided a significantly higher economic return.

There is clearly a need for further studies of other crops and geographical regions to establish in which instances use of neonicotinoids is cost-effective and whether alternatives such as pyrethroid sprays or IPM systems offer a more cost-effective approach. Such studies would need to incorporate the additional labour and application costs associated with crop monitoring and responsive spray applications.

Persistence of neonicotinoids in soils

Studies of the uptake of neonicotinoid seed dressings into the target crop suggest that between 1·6 and 20% of the active ingredient is absorbed by the crop (Sur & Stork 2003). Thus, although seed dressings are often stated to provide accurate targeting of the crop (e.g. Jeschke *et al.* 2011), they result in a considerably smaller proportion of the active ingredient ending up in or on the crop than do traditional spray applications to foliage, which commonly exceed 50% efficiency (Graham-Bryce 1977).

Of the 80–98% of the active ingredient in seed dressings, which is not absorbed by the crop, a small proportion (<2%) is lost as dust during sowing (Tapparo *et al.* 2012). This aerial dust can be sufficient to cause direct mortality in honeybees flying nearby (Marzaro *et al.* 2011; Tapparo *et al.* 2012) and is deposited on field margin vegetation at concentrations ranging from 1 to 9 ppb (Krupke *et al.* 2012). Release of active ingredient in dust is exacerbated when talcum powder or graphite is added to the seeds to lubricate their flow, as is common practice in North America (Krupke *et al.* 2012). Deflectors can be fitted to drilling equipment which direct this dust at the soil surface and reduce the amount of powder drifting in the air by 50–95%, although of course the active ingredient is then on the soil surface (Biocca *et al.* 2011).

By far the bulk of the active ingredient, typically more than 90%, enters the soil. Neonicotinoids are water soluble and have a half-life in soil, which varies greatly among compounds, soil type and across studies. No systematic attempt has been made to understand what factors affect their persistence or why published values are so variable. The primary sources of data are commonly not available for inspection since they are studies commissioned by industry to comply with regulatory requirements. For the most commonly used seed treatments, reported half-lives in soil typically range from 200 to in excess of 1000 days (range 28–1250 days for imidacloprid; 7–3001 days for thiamethoxam; 148–6931 days for clothianidin; Table 1). Half-lives appear to be shorter for the *N*-cyanoamidines

Table 1. Estimated dissipation times (DT_{50}) for neonicotinoids in soil

Compound	DT_{50} (days)	Laboratory or field study	Soil type	Location	Reference
Acetamiprid	450	Laboratory	Silty clay loam	NA	Reported in Anon (2004)
Acetamiprid	388	Laboratory	Clay loam	NA	Reported in Anon (2004)
Acetamiprid	Mean 31	Field	Various	Europe	Reported in Anon (2004)
Dinotefuran	82	Laboratory	NA	NA	PPDB (2013)
Dinotefuran	75	Field	NA	NA	PPDB (2013)
Imidacloprid	990–1230	Laboratory	Sandy loam	Australia	Baskaran, Kookana & Naidu (1999)
Imidacloprid	455–518	Laboratory	Sandy loam	Spain	Fernández-Bayo, Nogales & Romero (2009)
Imidacloprid	233–366	Laboratory	Silty clay loam	Spain	Fernández-Bayo, Nogales & Romero (2009)
Imidacloprid	34–45	Laboratory	Alluvial	India	Sarkar *et al.* (2001)
Imidacloprid	28–44	Laboratory	Lateritic	India	Sarkar *et al.* (2001)
Imidacloprid	36–46	Laboratory	Coastal alkaline	India	Sarkar *et al.* (2001)
Imidacloprid	1250	Field	Loam	UK	Calculated from data in Anon (2006)
Clothianidin	6931	Laboratory	Fuquay loamy sand	USA	Rexrode *et al.* (2003)
Clothianidin	1386	Field	Clay loam	North Dakota	Reported in De Cant & Barrett (2010)
Clothianidin	1155	Laboratory	Elder loam	USA	Rexrode *et al.* (2003)
Clothianidin	990	Laboratory	Howe sandy loam	USA	Rexrode *et al.* (2003)
Clothianidin	693	Laboratory	Susan silt loam	USA	Rexrode *et al.* (2003)
Clothianidin	578	Laboratory	Crosby silt loam	USA	Rexrode *et al.* (2003)
Clothianidin	533	Laboratory	Sparta sand	USA	Rexrode *et al.* (2003)
Clothianidin	533	Laboratory	Quincy loamy sand	USA	Rexrode *et al.* (2003)
Clothianidin	495	Laboratory	Loamy sand	Germany	Rexrode *et al.* (2003)
Clothianidin	365	Field	Silt loam	Ontario	Reported in De Cant & Barrett (2010)
Clothianidin	315	Field	Silt loam	Ohio	Reported in De Cant & Barrett (2010)
Clothianidin	277	Field	Sandy soil	Wisconsin	Reported in De Cant & Barrett (2010)
Clothianidin	239	Laboratory	Laacher Hof AII silt loam	Germany	Rexrode *et al.* (2003)
Clothianidin	148	Laboratory	Hofchen silt	Germany	Rexrode *et al.* (2003)
Clothianidin	Negligible dissipation in 25 months	Field	Silty clay loam	Saskatchewan	Reported in De Cant & Barrett (2010)
Nitenpyram	8	Laboratory	NA	NA	PPDB (2013)
Thiacloprid	>1000	Laboratory	NA	NA	Reported in Anon (2009b)
Thiacloprid	74	Laboratory	Sandy loam	Australia	Reported in Anon (2001b)
Thiacloprid	3·4–27	Field	NA	Australia	Reported in Anon (2001b)
Thiamethoxam	294–353	Laboratory	Sandy loam	USA	Reported in Anon (2001c)
Thiamethoxam	34–233	Laboratory	Silty loam	NA	Reported in Anon (2001c)
Thiamethoxam	7–109	Field	NA	NA	Reported in Anon (2001c)
Thiamethoxam	46–3001	Laboratory	NA	NA	Gupta, Gajbhiye & Gupta (2008)

(thiacloprid and acetamiprid, ranges 3–74 and 31–450 days, respectively).

Given these estimates, we would expect repeated applications of neonicotinoids in successive years to result in accumulating concentrations in soils, but data here are sparse. The only studies available, from spray applications of imidacloprid to orchard soil in Germany and when used as a seed treatment on winter wheat in the UK, do show significant accumulation (Fig. 2, Anon 2006). For example, in the UK study, concentrations ranging from 6 to 18 ppb remained in the soil 1 year after sowing. After 6 years of repeated applications, soil concentrations 1 year after the final application ranged from 18 to 60 ppb, depending on the application rate. Concentrations may have continued to rise, but the experiment was terminated (Fig. 2).

Given their long life and potential for accumulation in soil, we would expect most arable soils to contain detectable, variable quantities of neonicotinoids, depending on cropping history, rainfall and soil properties. Bonmatin *et al.* (2005) randomly sampled 74 farmland soils in France and screened them for imidacloprid. Seven soils from organic farms contained no imidacloprid. Of the remaining 67 samples, 62 contained detectable imidacloprid (>0·1 ppb) and 65% of samples contained >1 ppb. Some of these positive samples had not been treated with imidacloprid in the previous 2 years, and only ten of the positive samples were from fields treated in the current year. Nine samples contained between 10 and 100 ppb, and three exceeded 100 ppb. They did not screen for other neonicotinoids, but given their widespread use and similar persistence, we would expect broadly similar levels of clothianidin and thiamethoxam. Since Bonmatin *et al.*'s study, neonicotinoid use has increased greatly – in the UK, it has approximately doubled – so current levels in arable soils are likely to be higher. It seems likely that most soil-dwelling organisms in conventional arable farmland are chronically exposed to fluctuating concentrations

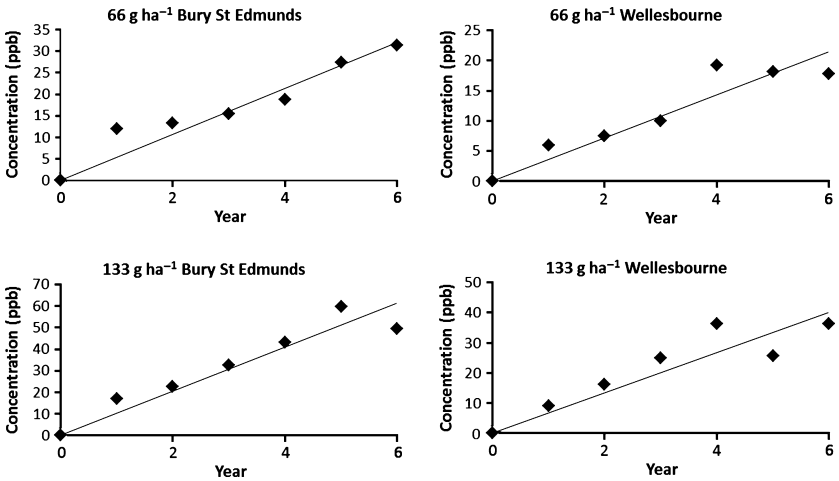

Fig. 2. Levels of imidacloprid detected in soil into which treated winter wheat seeds were sown each autumn (1991–1996). Both study sites are in the east of England. Treatment rates were 66 and 133 g a.i. ha^{-1} except in the first year, when it was 56 and 112 g, respectively. Data from Placke, FJ, reported in Anon (2006).

and mixtures of neonicotinoids in the range from 1 to >100 ppb.

Contamination of other environments

Loss of neonicotinoids from agricultural soils is presumably via degradation or leaching in soil water, but the relative importance of these routes cannot be clearly established from existing data. The pattern of loss is commonly biphasic, with an initial rapid phase followed by a much slower second phase, probably reflecting sorption of a proportion of the active ingredient onto soil particles which then slows dissipation (Gupta, Gajbhiye & Gupta 2008). This biphasic pattern will lead to an underestimation of persistence if dissipation studies are performed over short periods. Leaching is lower and sorption is higher in soils with high organic matter content (Cox, Koskinen & Yen 1998; Selim, Jeong & Elbana 2010). Before they become bound to soil, neonicotinoids readily leach so that significant levels might be predicted in groundwater and run-off immediately after application, particularly if there is heavy rainfall at this time, where the soil organic content is low, and on steep slopes (Scorza *et al.* 2004; Anhalt, Moorman & Koskinen 2008; Selim, Jeong & Elbana 2010; Thuyet *et al.* 2012). For example, Gupta, Gajbhiye & Gupta (2008) leached 79% of applied thiamethoxam from soil by simulating 65 cm of rainfall in the laboratory. Dissolved organic carbon appears to compete with neonicotinoids for soil sorption sites, increasing leaching (Flores-Céspedes *et al.* 2002). Accordingly, neonicotinoids have been detected in groundwater, streams, storm-water ponds and tidal creeks (Anon 2007; Lamers *et al.* 2011; DeLorenzo *et al.* 2012). For example, Starner & Goh (2012) detected imidacloprid in 89% of water samples taken from rivers, creeks and drains in California, with 19% of samples exceeding the US Environmental Protection Agency guideline concentration of 1·05 ppb. In the Netherlands, concentrations of up to 200 ppb in groundwater, streams and ditches have been reported (van Dijk 2010). However, neonicotinoids are absent from many groundwater and run-off samples collected in areas where they are deployed (e.g. Anon 2007). This may be because they are only present for a short period after application and so are likely to be missed by most sampling regimes and also because imidacloprid, clothianidin and thiamethoxam (but not thiacloprid and acetamiprid) rapidly degrade through photolysis in clear water (Anon 2007; Pena, Rodriguez-Liebana & Mingorance 2011). Many water-monitoring programmes do not screen for the metabolites of neonicotinoids such as imidacloprid olefin, but these can be as toxic as the parent compound (Anon 2007). Notably, no neonicotinoids feature in the EU Water Framework Directive's list of priority substances for aquatic pollution monitoring (Anon 2001a), and so they are not specifically targeted, and screening methods may not be well suited to their detection.

One aspect of the environmental fate of neonicotinoids for which few data are available is with regard to their uptake from soil and soil water by non-target plants. Given their persistence and accumulation in soils, we might predict hedgerow plants and trees, field margin vegetation and naturally regenerating fallows to take up neonicotinoids. Data on persistence of neonicotinoids once taken up by plants are sparse. However, vines treated in spring via irrigation maintain levels of imidacloprid sufficient to control pests through the growing season (Byrne & Toscano 2006), and levels of imidacloprid and thiamethoxam in citrus trees remain sufficient to suppress pests for 5 months following a single application (Castle *et al.* 2005). Similarly, a single application of imidacloprid to maple trees protected them against insect pests for 4 years (Oliver *et al.* 2010). Hence, there is the potential for non-target vegetation growing near arable crops to be contaminated for much or all of the year via uptake from roots, supplemented annually by neonicotinoid dust deposition during sowing. This could deliver chronic exposure to herbivorous insects. However, other than the isolated study of Krupke *et al.* (2012) (which describes concentrations up to 9 ppb in dandelions in field margins), such vegetation does not appear to have been screened for

neonicotinoids, so it is not possible to evaluate exposure of non-target organisms via this route.

Patterns of toxicity across taxa

Given the scale of use of neonicotinoids, their persistence in soils, leaching into waterways and their systemic nature within plants, there is no doubt that most organisms inhabiting arable environments will be exposed to them. The key question is whether typical levels of exposure are likely to lead to significant individual- or population-level impacts.

Many studies have examined the toxicity of neonicotinoids to both target and non-target organisms, including mammals, birds, fish, insects, crustacean, molluscs and annelids (Table S1 in Supporting Information). Insects are consistently among the most sensitive taxa, whether exposed via contact or ingestion. Typical LD_{50} values vary from 0·82 to 88 ng per insect, with much of the variation between species due to the size of the insect (Table S1, Supporting Information). For example, the most sensitive species, the brown planthopper, *Nilaparvata lugens*, weighs approximately 1 mg, while the least sensitive, the Colorado potato beetle, *Leptinotarsa decemlineata,* weighs approximately 130 mg, so that the LD_{50} values expressed as ng mg body per weight are similar (0·82 and 0·67, respectively). LC_{50} values (the concentration which kills 50% of individuals) for aquatic insects vary from 0·65 to 44 ppb (Table S1, Supporting Information). Here, the variation between studies is partly explained by differences in the duration of exposure. For example, the LC_{50} for the mayfly *Epeorus longimanus* falls from 2·1 ppb at 24 h to 0·65 ppb at 96 h (Alexander *et al*. 2007). Most studies assess only mortality and are carried out over short periods, but it is clear that important sublethal effects (such as reduced feeding, movement and reproduction) can be elicited by much lower doses. For example, feeding of *E. longimanus* nymphs was reduced for 4 days following exposure to water containing 0·1 ppb of imidacloprid for 24 h (Alexander *et al*. 2007).

The widespread prophylactic use of neonicotinoids has led to some insect pests developing resistance (e.g. Horowitz, Kontsedalov & Ishaaya 2004; Szendrei *et al*. 2012). For example, Szendrei *et al*. (2012) describe Colorado potato beetle populations with a 26-fold increase in resistance to thiamethoxam and a 100-fold increase in resistance to imidacloprid. The first strains with increased resistance to imidacloprid were detected in 1998, just 3 years after the chemical was first used against this pest. Given the increasing ubiquity of neonicotinoids and their persistence, insect populations in arable ecosystems are likely to be chronically exposed to them, a situation which will inevitably lead to increasing resistance in pest species (which tend to have large populations and short generation times).

Studies of toxicity to crustaceans are few, but they appear to be highly variable in their susceptibility to neonicotinoids, with LC_{50} values ranging from 7·1 ppb (over 28 days) in the amphipod *Hyalella azteca* to 361 000 ppb (over 48 h) in the brine shrimp *Artemia* sp. (Table S1, Supporting Information). Most crustaceans are considerably less susceptible than insects. Studies of annelids are also scarce, but suggest lower susceptibility than insects (Table S1, Supporting Information).

Toxicity to vertebrates is also low compared to insects, but varies greatly among neonicotinoids; for example, the LD_{50} value in rats varies from 140 mg $kgbw^{-1}$ (mg of active ingredient per kilogram body weight) for acetamiprid up to 5000 mg $kgbw^{-1}$ for clothianidin (Table S1, Supporting Information). Birds appear to be generally more susceptible than rats, with LD_{50} values ranging from 14 mg $kgbw^{-1}$ for imidacloprid in grey partridge up to 1333 mg $kgbw^{-1}$ for clothianidin in mallard ducks. Fish are markedly less susceptible than aquatic insects, with LC_{50} values ranging from 16 to 177 ppm (parts per million; Table S1, Supporting Information).

Risks to granivorous vertebrates

Although neonicotinoids do show relatively low toxicity to vertebrates, we might expect seed-eating vertebrates to be exposed to lethal doses if they consume treated seeds spilled during sowing. Typically, maize seeds are treated with ~1 mg of active ingredient per seed, beet seeds with 0·9 mg and the much smaller oilseed rape seeds with 0·17 mg (Rexrode *et al*. 2003; Anon 2012; Krupke *et al*. 2012). A grey partridge, typically weighing approximately 390 g, therefore needs to eat ~5 maize seeds, six beet seeds or 32 oilseed rape seeds to receive an LD_{50}. A grey partridge typically consumes ~25 g of seeds per day (Liukkonen-Anttila, Putaala & Hissa 1999), equivalent to ~600 maize seeds, so clearly there is the potential for birds to swiftly consume a lethal dose. By a similar calculation, three maize seeds treated with imidacloprid would deliver more than the LD_{50} to a mouse. The US Environmental Protection Agency estimated that ~1% of drilled seeds remain accessible to granivorous vertebrates (i.e. they are not buried during drilling), and this does not include spillages which may occur, for example, when transporting grain or loading hoppers. With typical sowing rates of ~50 000 seeds ha^{-1} for maize and 800 000 seeds ha^{-1} for oilseed rape, we might expect sufficient seed to be available on the soil surface to deliver an LD_{50} to 100 partridge or 167 mice for every hectare sown.

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. This mortality rate, although considerable, is less than we might expect from the calculations above. Lopez-Antia *et al*. report anecdotally that partridge did not avoid dressed seed when offered both dressed and undressed, but speculate that treated birds ate less than control birds and so received a lower dose than expected. This requires further investigation, in

this and other species, to determine how much treated seeds vertebrates actually consume in the field. De Snoo, Scheidegger & de Jong (1999) describe incidents of poisoning of wild partridge, pigeon and duck by seed dressed with imidacloprid, reported by members of the public in France in 1994–1995 (a time when neonicotinoid use was very low), but other evidence for effects in the field is lacking, and it is unclear whether public reporting is an efficient means of detecting such incidents.

There are other knowledge gaps. Susceptibility of most granivorous vertebrates that occur in farmland, which includes various rodents and a large number of bird species, has not been evaluated. Sublethal effects on invertebrates are poorly understood, although in birds they are known to include hyporeactivity, ataxia, wing drop, diarrhoea, opisthotonos (rigidity and severe arching of the back), immobility, intoxication, eggshell thinning, reduced egg hatching rate and low weight in chicks; and in mammals, they include reduced reproduction, premature deliveries and deformities in foetuses (Rexrode *et al.* 2003; Anon 2007; Lopez-Antia *et al.* 2013). Bal *et al.* (2012a) report reduced sperm production in rats exposed to imidacloprid at 2 mg kgbw^{-1} day^{-1}, a dose representing ~1/250th of the LD_{50} per day, equivalent to a rat eating one treated maize seed (see also Bal *et al.* 2012b for a related study on clothianidin). Thus, one might expect doses considerably lower than the LD_{50} (which is derived from short-term laboratory tests) to have significant impacts on the long-term survival or reproductive success of vertebrates living in natural environments where they are exposed to other stressors. For example, many treated crops are sown in October; birds or mammals that consume seeds at this time will shortly have to survive the winter, and any factors that reduce their fitness at this time are likely to result in substantially reduced overwintering survival.

Impacts on pollinators

Much of the controversy over the use of neonicotinoids has focussed on their effects on bees. Neonicotinoids are routinely used to dress seeds of oilseed rape, sunflower and maize, and these crops are major forage sources for both managed honeybees and wild pollinators in arable landscapes. Being systemic, small concentrations of neonicotinoids are found in both pollen and nectar of seed-treated crops. Neonicotinoids are also routinely applied as foliar sprays to fruit crops such as raspberries (mainly thiacloprid), which are visited by both managed and wild pollinators (Lye *et al.* 2011; Defra 2012a). Widespread but unquantified use of neonicotinoids as foliar sprays in gardens, where they are recommended for use on both vegetables and flowers, provides a further route of exposure for pollinators.

Limited information is available on the actual concentrations of neonicotinoids typically found in pollen and nectar of treated crops (reviewed in EFSA 2012 and USEPA 2012; see also Stoner & Eitzer 2012). Concentrations in nectar are generally lower than those in pollen. When applied as seed dressings, concentrations in nectar range from <1 to 8·6 ppb (mean maximum level ±SE from 20 studies = 1·9 ± 0·5 ppb, EFSA 2012), with concentrations in pollen ranging from <1 to 51 ppb (mean maximum level ±SE from 20 studies = 6·1 ± 2·0 ppb). Generally higher concentrations are found when neonicotinoids are applied directly to the soil (e.g. in irrigation water), ranging from 1 to 23 ppb in nectar and 9 to 66 ppb in pollen (USEPA 2012). The highest concentrations recorded in nectar and pollen appear to result from foliar applications; Dively & Kamel (2012) report concentrations in pollen of 36 to 147 ppb for dinotefuran and 61 to 127 ppb for thiamethoxam when sprayed on pumpkin, plus significant concentrations of toxic metabolites. Concentrations in nectar were approximately 10-fold lower, ranging from 5 to 11 ppb for dinotefuran and 6 to 9 ppb for thiamethoxam.

Given the oral LC_{50} value for imidacloprid in honeybees of 5 ng bee^{-1} (Suchail, Guez & Belzunces 2000), and taking the mean values for seed-treated crops calculated here, a bee would need to consume nearly 1 g of pollen or 2·6 ml of nectar to obtain an LC_{50} dose. This seems unlikely in the short term for a honeybee, which weighs ~0·1 g, but could easily be accumulated over a number of days or weeks, so the actual effect of field exposure on mortality is likely to depend on the rate at which neonicotinoids are metabolized or excreted. A recent meta-analysis based on 13 studies of the impacts of imidacloprid on honeybees found that field-realistic doses (for seed-treated crops) under laboratory and semi-field conditions had no significant lethal effects (Cresswell 2011). Overall, the balance of evidence at present suggests that field-realistic exposure of bees to neonicotinoids in nectar and pollen of seed-treated crops is unlikely to cause substantial direct mortality (although exposure to dust released during drilling can cause direct mortality, Marzaro *et al.* 2011; Tapparo *et al.* 2012). However, only honeybees and bumblebees have been investigated; no information is available of susceptibility of other pollinating taxa such as hoverflies or butterflies. Also, if pollinators forage on crops treated with neonicotinoids via irrigation water or as a foliar application, direct mortality is likely; this has not yet been investigated, with attention largely focussed on exposure of bees to seed-treated crops.

Although there is little convincing evidence for direct mortality in bees, there is strong evidence for important sublethal effects. Exposure to sublethal doses of neonicotinoids is known to reduce learning, foraging ability and homing ability in both honeybees and bumblebees (Yang *et al.* 2008; Han *et al.* 2010; Mommaerts *et al.* 2010; Henry *et al.* 2012). Such effects will not be revealed in standard safety-testing protocols that typically involve laboratory or cage trials with *ad lib* food, but would be much more marked under natural conditions when colonies rely on their workers to locate patches of flowers

across the landscape. However, very few studies have been carried out in which bees that have been exposed to pesticides have to navigate across realistic distances.

In one such study, Henry *et al.* (2012) showed that honeybees, after being fed with sublethal doses of the neonicotinoid thiamethoxam, had a lower chance of finding their home colony than control bees. Importantly, the effect was much stronger when foragers had to return from an unfamiliar location at 1 km from their hive, compared to familiar locations or when closer to the hive. However, the dose given was higher than that bees might commonly be expected to receive in a single feed. Recently Gill, Ramos-Rodriguez & Raine (2012) found that bumblebee *Bombus terrestris* workers from colonies exposed to field-realistic concentrations of imidacloprid in nectar suffered from impaired foraging ability when gathering food in a natural setting, particularly when collecting pollen. As a result, treated colonies grew more slowly.

In the only well-replicated field study that has looked at the impacts of neonicotinoids on bee colony reproduction, Whitehorn *et al.* (2012) first simulated exposure of bumblebee colonies to a crop of treated flowering oilseed rape in the laboratory using realistic concentrations (6 ppb in pollen and 0·7 ppb in nectar). Colonies were then allowed to develop naturally in the field, gathering food for themselves. They recorded reduced nest growth and an 85% drop in queen production resulting from exposure to imidacloprid compared to control colonies. This study and Gill, Ramos-Rodriguez & Raine (2012) provide complementary evidence that reduced foraging efficiency following exposure to realistic levels of imidacloprid can result in a strong colony-level effect, which is likely to impact upon bumblebee populations in the long term. However, both studies placed treated food in the nests (and in the case of Whitehorn *et al.,* no other food was provided during the exposure phase), so we cannot be certain that the concentrations to which bees were exposed are representative of what happens under field conditions. For example, if bees detect and avoid neonicotinoid-treated crops, they may be exposed to less that we would otherwise expect. Easton & Goulson (2013) demonstrate that pollinating flies and beetles avoid pan traps containing imidacloprid at as low as 0·01 ppb, but whether bees avoid contaminated crops is unknown. If they do, this could have consequences for crop pollination.

Studies to date have focussed almost exclusively on exposure of adult bees. However, Yang *et al.* (2012) recently showed that learning of adult bees was impaired if they had been treated with 0·04 ng larva^{-1} of imidacloprid in the larval stage (<1/100th of the LC_{50} for adult bees). It seems highly likely that bee larvae are routinely exposed to such very low concentrations, but we have no data on whether this has long-term repercussions for colony fitness. This also raises the interesting question as to whether the exposure of other insects to low levels of neonicotinoids during development has effects on adult behaviour, an area which has not been investigated.

In summary, there is clear evidence that exposure of bees to field-realistic levels of neonicotinoids has significant sublethal impacts and that in the case of bumblebees, this has been demonstrated to have major impacts on colony success. To understand how widespread these effects are, further studies are needed to determine the range of concentrations of neonicotinoids to which wild bumblebee colonies and managed honeybee colonies are actually exposed in different environments (especially in urban areas for which we have no data). We also have a poor understanding of how the effects of neonicotinoids interact with other stressors, such as other pesticides, diseases and food stress, all of which undoubtedly influence bee health (Goulson, Lye & Darvill 2008; Moritz *et al.* 2010). At present, we have no data on impacts on pollinators other than bees. The major knowledge gaps concerning possible impacts of neonicotinoids on pollinators are usefully summarized in recent reviews of this issue conducted by the European Food Standards Agency (EFSA 2013a,b,c).

CONCLUSIONS

The adoption of prophylactic use of neonicotinoids as seed dressing has led to the abandonment of the long-established principles of IPM, an approach which uses monitoring of pest populations to indicate when treatment is necessary, avoids broad-spectrum pesticides wherever possible and avoids use of pesticides that persist in the environment (Metcalf & Luckmann 1994). This minimizes pesticide use, reduces the likelihood of the development of resistance in pests and minimizes impacts on non-target organisms.

At the Convention on Biological Diversity in 2002, world leaders committed to achieving a significant reduction in the rate of loss of biodiversity. By almost all indices, we have failed to reach this target (Butchart *et al.* 2010). In many developing countries, the reasons for this are clear: ongoing loss and degradation of species-rich habitat. Continuing declines of biodiversity in the European Union are more surprising, particularly given the real-term increase in spend on conservation, notably through a range of agri-environment schemes intended to boost biodiversity on farmland. For example, in England alone in 2009, 58 000 farmers were paid a total of £400 million per year to farm in a more environmentally sensitive manner (Anon 2009a). Despite this, UK indices for bees, butterflies, moths, carabid beetles and birds (the groups for which good data are available) all show significant overall declines in recent years, particularly in farmland (Biesmeijer *et al.* 2006; Fox *et al.* 2006; Wilson, Evans & Grice 2010; Brereton *et al.* 2011; Brooks *et al.* 2012). Although data are sparse for many taxa, similar ongoing declines are evident across Europe (e.g. De Heer, Kapos & Ten Brink 2005; Gregory *et al.* 2005; Van Dyck

Table 2. Knowledge gaps and suggestions for further research

Gap	Required Research
Acute toxicity to most taxa has not been investigated: for example, almost all pollinators apart from honeybees; many soil arthropods; non-target herbivores such as butterfly larvae; most farmland vertebrates	Further LD/LC_{50} studies conducted over long time-scales, for example 28 days
Sublethal impacts on learning, behaviour and fecundity unstudied for almost all taxa	Studies including behavioural and fecundity assays under realistic scenarios
Impacts of chronic exposure during development on neuronal development and adult behaviour are largely unknown	Assessment of adult fitness of insects following exposure as a larvae
Possible synergies between neonicotinoids and other stressors such as disease are largely unknown	Trials exposing insects to multiple stressors
Consumption of treated seeds by vertebrates has not been quantified	Trials to establish whether treated seeds are consumed, and if so what mortality this causes
Very few data are available on actual levels of neonicotinoids in arable soils and on whether accumulation with repeated application is common	More sampling of soils, long-term accumulation studies
No data from most countries on levels of neonicotinoids found in waterways	Sampling of waterways, particularly in the period following sowing of treated seed
No data are available on the extent to which field margin vegetation and hedgerow plants draw up neonicotinoids from arable soils	Screening of non-target vegetation, manipulative studies
No data are available on the extent of use of neonicotinoids in gardens	Collection of data via gardening outlets or random sampling of gardens
Few data are available on the agronomic or economic benefits of neonicotinoids	More field trials to compare the efficacy of alternative control strategies

LD_{50}, dose that kills 50% of individuals; LC_{50}, concentration that kills 50% of individuals.

et al. 2009). The reasons for these declines remain unclear and are the subject of ongoing debate.

The evidence presented here suggests that the annually increasing use of neonicotinoids may be playing a role in driving these declines. The concentrations accumulating in soil (1 to >100 ppb), waterways (often in excess of 1 ppb, sometimes up to 200 ppb), field margin plants (1–9 ppb) and nectar and pollen of flowering crops (1–50 ppb) exceed levels in crop tissues needed to control pest insects (5–10 ppb) and overlap with LC_{50} values for a range of non-target insects. They would appear to be sufficient to cause both direct mortality in the more sensitive non-target species and chronic sublethal effects in many more. The groups most at risk are likely to include soil-dwelling insects, benthic aquatic insects, granivorous vertebrates and pollinators. Herbivorous insects feeding on field margin and hedgerow plants may also be exposed.

Of course all pesticides are harmful to non-target organisms to some degree. Reconciling conserving biodiversity with food production requires a balance to be found. If it is not, then biodiversity loss will threaten vital ecosystem services upon which food production depends. Use of neonicotinoids appears to pose a particular threat to pollination services and also to soil health which depends on soil invertebrates that play major roles in nutrient cycling and maintaining soil structure. However, there are major knowledge gaps at present, so it is not possible to fully evaluate these threats (Table 2). Overall, there is an urgent need to re-evaluate whether current patterns of usage of neonicotinoids provide the optimum balance between meeting the demands of food production and farming profitability in the short term, vs. the need to sustainably manage global biodiversity to ensure the long-term health of ecosystems (including farmland) upon which all life depends.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Additional data and references on toxicity of neonicotinoids to various taxa.