REVIEW

Dietary traces of neonicotinoid pesticides as a cause of population declines in honey bees: an evaluation by Hill's epidemiological criteria

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ABSTRACT

BACKGROUND: Honey bees are important pollinators of both crops and wild plants. Pesticide regimes that threaten their sustainability therefore should be assessed. As an example, we examine the evidence that the agricultural use of neonicotinoid pesticides is a cause of the recently observed declines in honey bees. We aim to define exacting demographic conditions for a detrimental factor to precipitate a population decline and we employ Hill's epidemiological 'causality criteria' as a structured process for making an expert judgement about the proposition that trace dietary neonicotinoids in nectar and pollen cause population declines in honey bees.

RESULTS: Despite the absence of decisive experimental results, our analysis shows that while the proposition is a substantially justified conjecture in the context of current knowledge, it is also substantially contraindicated by a wide variety of circumstantial epidemiological evidence.

CONCLUSION: We conclude that dietary neonicotinoids cannot be implicated in honey bee declines, but this position is provisional because important gaps remain in current knowledge. We therefore identify avenues for further investigations to resolve this longstanding uncertainty.

Key words: agrochemicals, imidacloprid, insects, neurotoxin, pollination, pollutants

1 INTRODUCTION

Since the publication of Rachel Carson's book Silent Spring [1], there has been widespread public awareness that pesticides can have unintended detrimental effects on the non-target biota [2]. Carson's book focused on DDT and precipitated a period of scientific and regulatory scrutiny that eventually led to restrictions on the chemical's use in the USA and other countries. Carson's warning resonates today, but science-based risk appraisals normally conclude that pesticides should play a role in crop protection because the benefits of increased productivity outweigh the risks to non-target organisms [3]. The regulation of pesticides is typically based on assessing these risks. In Europe, for example, the approval of crop protection products is governed by EU Regulation 1107/2009 [4], which does not require that agrochemical pesticides are ecologically harmless, but instead specifies that member states may not authorize a crop protection product unless it has no unacceptable effect on the environment, including non-target species. A full exploration of what counts as an unacceptable effect is not in the scope of this review, but we take it as granted that a pesticide's use is unacceptable if it seriously threatens a non-target species that contributes to human wellbeing by delivering an important ecosystem service. Here, we consider the case of the neonicotinoid pesticides, which are implicated by some as a cause of the widely observed declines among honey bees populations [5, 6] and as a serious threat to valuable pollination services. We focus particularly on imidacloprid, because it is extensively used in agriculture and, among the neonicotinoids, its effect on honey bees has been the most studied.

Honey bees (*Apis mellifera* L.) are important pollinators of both crops and wild plants and they provide a highly valued ecosystem service [7]. On this basis, pesticide regimes that threaten the sustainability of honey bee populations are candidates for being deemed unacceptable. Below, we consider the acceptability of the agricultural use of neonicotinoid pesticides by examining the evidence that they contribute to the recently observed declines in honey bee populations of the USA and Europe [8, 9]. First, we briefly review the problem's context and introduce a structured procedure that can be employed to implicate a factor as a cause of a phenomenon in the absence of conclusive experimental evidence.

1.1 Context of the controversy

Systemic neonicotinoids, such as imidacloprid, are currently among the most widely used insecticides in crop protection [10]. They are neurotoxic to insects and disrupt their nervous system causing paralysis and death [11, 12]. Neonicotinoids are applied as foliar sprays and as seed dressings [13], after which the chemical pervades the plant systemically to protect it against insect pests that consume sap and tissues. Bees are non-target insects that may be harmed either by direct contact with neonicotinoid products or by ingesting them in nectar and pollen from the flowers of treated crops. Treated crops whose nectar and pollen contains neonicotinoid residues include oilseed rape (canola), *Brassica napus* L., [14, 15] and sunflower, *Helianthus annua* L. [14, 16], where the residues occur at trace levels (here defined as less than 10 μg active ingredient kg^{-1}). The existence of neonicotinoid residues in nectar and pollen suggests that trace dietary intake by the bees that forage on mass-

flowering crops is inevitable, which necessarily raises concern over the potential impact of these pesticides on bee health.

In 1994, imidacloprid was the first of the neonicotinoids to be approved for use as an agricultural pesticide, but since then its use has increased greatly (Fig. 1), as has use of other members of the family, such as clothianidin and thiacloprid. The increased use of neonicotinoids has coincided with a period of continual decline in the numbers of managed honey bee colonies in some parts of the world. In the United States, for example, the number of colonies has fallen steadily from a post-war high of almost six million to its current level of approximately 2.5 million colonies and the decline has been accompanied by high rates of annual colony loss, these being on average 30% in the years 2006–2008 [17]. More recently, Colony Collapse Disorder (CCD) has been recognized as a pathological syndrome among honey bees in the United States [18] and it accounts for up to 10% of the colonies that are lost each winter [19]. The population declines of honey bees are accompanied by widespread concern over the sustainability of pollinator services to agriculture [20, 21] and wild plants [22, 23]. The increasing imperative to protect pollination services has raised the pressure to find the factors that threaten them and pesticides are identified by some as important culprits [5]. Neonicotinoid pesticides in particular have attracted attention against this backdrop of heightened tension, as follows.

Imidacloprid was first identified publicly as a threat to bees in 1999, when its use in France was restricted by the French government after claims by bee-keepers that agricultural use of its commercial formulation, Gaucho®, was responsible for widespread losses of hives [24]. Since then, many investigations into the effects of

imidacloprid on honey bees have been published. Initially, these efforts were led by French government institutes [25] and by the laboratories of a pharmaceutical company that produces neonicotinoid products, Bayer [16]. However, there was no consensus about the potential impact of trace dietary imidacloprid on honey bees [26], because the studies were methodologically varied and produced conflicting results [27]. For example, one study indicated that mortality rates in adult bees were greatly increased at oral doses that were below the environmentally-realistic trace range [28], whereas another study failed to detect increased mortality even at much higher doses [29]. Moreover, while many laboratory tests found that trace dietary imidacloprid harmed adult honey bees, field tests found no detrimental effects on honey bee colonies due to either imidacloprid [30] or another neonicotinoid, clothianidin [31]. Outside France, government regulators rarely restricted agricultural use of neonicotinoids (but see [32, 33]), presumably giving the null results of field trials precedence over the positive findings of laboratory studies [34].

Public concern over the detrimental effects of neonicotinoids was inflamed in 2008, however, when clothianidin caused the mass mortality of honey bees in Baden-Württemberg, Germany. In contrast to the preceding controversy about trace dietary residues, this alarm was caused by improper agricultural practice, which released clouds of insecticidal dust during seed drilling of treated maize [35]. The potential for a recurrence of this catastrophe was greatly reduced by technological changes to drilling equipment and by improvement of the adhesive used to apply the neonicotinoid dressing to the seed [36]. After strengthening the legislation regulating seed drilling, the German government lifted part of its restrictions on the neonicotinoid seed dressing.

Public concern reignited in 2009, however, when a new route for exposure of honey bees was identified, namely guttation fluid in seedling maize and other crops [37]. For a few weeks after germinating, seedling maize plants exude fluid droplets along their leaf margins by the process of guttation and, in crops treated with a neonicotinoid seed dressing, the concentration of the insecticide in guttation fluid can reach levels that are lethal to a honey bee that ingests it [38]. The critical question of whether bees commonly consume the fluid under field conditions is currently unresolved [37], but concern over the potential impact of neonicotinoid residues in leaf exudates has led to restrictions on the planting of maize with neonicotinoid seed dressings in Germany[39].

In summary, the agricultural use of neonicotinoids is associated with three separate modes of exposure for bees: direct exposure by dispersal in particulate clouds during seed drilling; oral ingestion of residues in guttation fluid of seedling maize; and trace dietary residues in nectar and pollen. Here, we present a model for a risk assessment of neonicotinoids that focuses exclusively on the potential impacts of trace dietary residues in nectar and pollen.

1.2 Definition of terms used in the evaluation

Throughout, we use ‘population decline’ to refer to a decrease in the number of honey bee colonies in a particular region and ‘colony decline’ to refer to the decrease in the number of individual bees in a single hive. Before proceeding, we set out the conditions necessary for a factor to precipitate a population decline using the general

theory of population dynamics. Classically, ecologists model the dynamics of a resource-limited population by the logistic equation [40], which defines the per capita contribution to population change (units of individuals per individual per unit time) as:

$$\frac{1}{N}\cdot\frac{\delta N}{\delta t} = r\left(1-\frac{N}{K}\right) \qquad \text{Eq. 1}$$

where t denotes time, N is the population size, K is the carrying capacity of the population's environment and r is the species' intrinsic per capita capacity for population change. Normally, r takes a positive value in a sustainable population and the population declines when $r < 0$, even when $N < K$, and we are interested in the case where a detrimental factor causes r to change from positive to negative. Let r denote the intrinsic rate of increase in the absence of the factor and let r^* denote the rate in its presence. We say that a factor harms individuals when

$$r > r^* \qquad \text{Eq 2.}$$

For a factor to precipitate population decline, it requires

$$r > 0 > r^* \qquad \text{Eq 3}$$

Therefore, not all factors that cause individual harm can precipitate population decline, because the inequality of Eq. 2 does not imply the inequality of Eq. 3. For a factor to precipitate population decline, it is necessary that it causes harm (Eq. 2), but the level of harm must also be sufficient to fulfil the condition given by Eq. 3, which we term the 'sufficient harm' condition. To realize the demographic basis of

this condition, we recognize that the intrinsic growth parameter, r, reflects a difference between the intrinsic per capita birth and death rates, denoted b and d respectively, which we term collectively the demographic 'vital rates', i.e.

$$r = (b - d) \qquad \text{Eq. 4.}$$

Given Eq. 1, population decline will occur whenever

$$(b - d) < 0 \qquad \text{Eq. 5}$$

Using Eq. 3, we rewrite the 'sufficient harm' condition as:

$$(b_c - d_c) > 0 > (b_c^* - d_c^*) \qquad \text{Eq. 6}$$

where b_c and d_c denote intrinsic per birth and death rates in the absence of the detrimental agent and b_c^* and d_c^* denote them in its presence. Thus, to precipitate population decline, the advent of dietary neonicotinoids must reduce r so as to cause 'sufficient harm' (Eq. 3) despite compensatory responses by beekeepers. The impact of neonicotinoids could be obscured if beekeepers compensated for an increase in the colony death rate by increased production of new colonies. In this case, a comparison between the values of d and d^* (Eq. 6), or the rate of colony losses, provides the pertinent indicator of neonicotinoid impact rather than the values of r and r^* (Eq 2). .

We note that Eq. 6 can also be applied to an individual hive, in which case we ask whether neonicotinoids generate 'depopulation symptoms' by detrimentally affecting fecundity and survivorship (i.e. b^* and d^* in Eq. 6) sufficiently enough to cause colony decline, which creates a population decline, if prevalent [41]. In this case, the impact of neonicotinoids on the fecundity of queens (i.e. b *vs.* b^*) and on the rates of individual mortality (d *vs.* d^*) would provide the pertinent indicators.

1.3 Evaluatory procedure

Normally, the results of manipulative experiments are the hard currency of decisions about causality in natural science. In situations involving public concern over environmental change, however, decisions about causes sometimes must be made under political pressure despite scientific uncertainties, which may include the lack of experimental evidence. In such circumstances, a scientific evaluation is nevertheless possible, but it uses a different process to manage uncertainty and to validate its conclusions. An example of this alternative process is the report of the Intergovernmental Panel on Climate Change (IPCC) [42], which was unable to experimentally test the effect of fossil fuel-based emissions on global climate change because of the unavailability of a control group (i.e. Earth-like planets without anthropogenic emissions). Nevertheless, the IPCC's conclusions about the cause of global climate change are widely accepted as scientifically authoritative. Similarly, we propose to evaluate whether neonicotinoid pesticides cause population declines in honey bees without having recourse to decisive experiments. Specifically, we employ 'Hill's causality criteria' [43] as a structured process for making an expert scientific judgement that is open to critical inspection and repeatable by others. Hill's

criteria are particularly appropriate for evaluating the cause-consequence relationship between certain pesticides and honey bee declines because they were devised to address epidemiological questions, such as whether there is sufficient evidence to support the proposition that a particular detrimental agent causes a particular disease.

Sir Austin Bradford Hill, a leading 20th century epidemiologist, identified nine types of information that provide 'viewpoints' from which to judge the verity of a cause-consequence relationship. These viewpoints have since become a widely used set of criteria for arriving at a verdict of causation [44] and techniques for producing quantitative scores of 'certainty' have been developed [45]. The nine criteria include not only experimental evidence, which typically will be equivocal or lacking when the criteria are used as a resort, but also eight other kinds of evidence that fall into two categories (Table 1) as follows. First, the theoretical criteria: coherence, plausibility and analogy. Second, the associational criteria: temporality, consistency, specificity, biological gradient and strength.

Our goal has been to assign certainty scores to each of the criteria to reflect the degree to which available evidence supports the hypothesis that neonicotinoids cause honey bee declines. A brief description of the criteria and a summary our scores are given in Table 1.

To produce a quantitative score of certainty for each criterion, we adapt previously formulated descriptors [45] that describe the level of conviction with which an evaluator holds a cause-effect hypothesis to be true: slight; reasonable; substantial; clear; and

certain. We associate these descriptors with numerical values to create an eleven-point scale for each criterion that returns a positive value (maximum five) if the evidence suggests that the factor (trace dietary neonicotinoid) certainly causes population decline, a negative value (maximum minus five) if the factor certainly does not and zero if the evidence is equivocal or lacking. For example, if the evidence for i^{th} criterion gives a reasonable indication that neonicotinoids do not cause population declines in honey bees, the score for that criterion would be C_i = -2, etc. We do not attempt to present an exhaustive review of evidence. Instead, we cite the evidence that, in our opinion, best supports the score of greatest absolute magnitude for each criterion.

In our initial evaluation, we focus exclusively on the proposition that neonicotinoid pesticides are capable in their own right of causing population declines in honey bees. We take this approach initially because parsimony dictates that the more complex hypothesis that neonicotinoids act in concert with other stressors needs to be considered only once the simpler case is dismissed. We turn subsequently to the topic of interactions among stressors in our concluding discussion.

2 EVALUATION OF CRITERIA AND JUSTIFICATION OF SCORES

2.1 C_1: Experimental evidence

The question of whether the neonicotinoids cause bee population declines would be settled beyond reasonable doubt if realistically dosed honey bee colonies showed sufficient harm under field conditions at the level required by Eq.s 3 and 6. At the

time of writing, no such experimental results have been reported. One experimental investigation has investigated the effect of exposure to neonicotinoid-treated crops on colony health under field conditions [31], and it found no effect on either overwinter survival and its proxy variables (e.g. gain in colony mass) or on mortality rates of individual bees. Similarly, laboratory trails provide no evidence that dietary neonicotinoids affect vital demographic rates: environmentally-realistic trace levels do not cause elevated rates of mortality[27] and effects on fecundity are as yet unstudied. However, the laboratory trials have shown that doses of dietary neonicotinoid at trace levels are capable of harming individual honey bees. Exposure for at least six days to trace dietary imidacloprid is expected to reduce behavioural performance in adult honey bees by between 6% and 20%[27]. Two major uncertainties prevent us from linking this level of individual harm directly to population decline, however. First, even if this reduction in individual performance translates into an equivalent reduction in colony performance, none of the published field and semi-field studies had sufficient statistical power to detect it [27]. Consequently, we are unsure whether the results of laboratory trials are environmentally relevant. Second, even if a dietary neonicotinoid caused a laboratory-scale reduction in colony performance (e.g. in foraging success or fecundity) under field conditions, it is not clear that this meets the condition of sufficient harm (Eq 3).

In summary, experimental evidence to date has not demonstrated that trace dietary imidacloprid causes population decline, but neither has the testing been stringent enough under environmentally-relevant conditions to reject this causal hypothesis convincingly because of shortcomings in statistical power. Instead, the credibility of

the hypothesis is sustained to some degree by the sublethal, harmful effects that are detected in laboratory tests. Taking into account the limitations of field trials (statistical power, use of proxy response variables), we take their null results as only a slight indication that neonicotinoids are not a cause of bee population decline and score this criterion as $C_1 = -1$.

2.1 C_2: Coherence.

The coherence criterion asks whether invoking a factor as the cause of a particular phenomenon conflicts with established knowledge. We find no conflict inherent in the proposition that dietary intake of an insecticidal chemical, such as a neonicotinoid, could harm honey bees sufficiently to cause a population decline because xenobiotics have this effect on other species [46]. However, we cannot populate the parameters of Eq.s 3 and 6 with well-justified, quantitative values that specify a threshold for the generation of sufficient harm. The lack of this specific threshold means that a quantitative incoherence with existing knowledge simply cannot arise. Overall, we find no conflict between existing knowledge and the proposition that neonicotinoids cause honey bee declines, but the quantitative shortcomings in current knowledge mean that this coherence provides only a substantial indication in favour of the proposition and we score this criterion at $C_2 = +3$.

2.3 C_3: Plausibility.

The plausibility criterion asks whether a reasonable scientist would, in principle, entertain the factor as the cause of the observed phenomenon. In evaluating this criterion, we critically examine each of a series of mechanistic links in the hypothesised causal chain between dietary neonicotinoids and honey bee decline.

First, it is certain that neonicotinoid residues are present in pollen and nectar in mass-flowering crops that have been systemically treated and that these are collected by honey bees [16, 47]. It is inevitable that some of these residues are subsequently ingested by bees in nectar and pollen [15], but the precise level of dosage is uncertain, because the neonicotinoid-containing pollen and nectar may be mixed with the colony's existing stores or with pollen and nectar collected at the same time from sources other than the treated mass-flowering crop, which would dilute the dose.

Second, it is well established that adult worker honey bees are susceptible to harm by trace dietary neonicotinoids [27, 48], although there is no evidence to support the claim that honey bees are unusually sensitive among insects [49]. It is possible that larval stages are equally or more susceptible [50], but the effect on queens and drones is currently unknown.

Third, it is possible that dietary neonicotinoids may affect the demographic vital rates of honey bees. While dietary imidacloprid at trace levels does not cause increased mortality rates in adult workers [27], it could have various sublethal impacts [51], including interfering with important behaviours, such as navigation during foraging [52] or social hygiene. The resulting loss of foragers could deplete the workforce and

slow colony growth and poor hygiene may promote disease, but these effects are currently hypothetical. It is possible that a neurotoxin may disturb the bee's fundamental physiology [53, 54], perhaps by diverting resources towards detoxification and away from other necessary functions [55], and thereby increase death rates generally or reduce the fecundity of the sexual caste, but this hypothesis is also not substantiated.

Fourth, it is possible that the various potential harmful effects of a neonicotinoid (reduced foraging workforce, increased risk of disease, reduced fecundity of the sexual caste) could weaken the colony and thereby increase winter losses among hives (but see [30-31])(but see [31]), but whether these losses amount to sufficient harm (Eq. 3) is currently unknown.

The plausibility criterion is more exacting than the coherence criterion, because it must be bolstered by positive examples from existing knowledge. All of the preceding links in the causal chain are possible in principle, but the existence of several is purely conjectural, which diminishes their plausibility. We therefore conclude that the proposition that trace dietary neonicotinoids cause honey bee declines is only reasonably plausible and we score this criterion as C_3 = +2.

2.4 C_4: Analogy

The analogy criterion asks whether a judgement can be supported by an appeal to similar, well-resolved cases. It is known that other pesticides have caused population declines in non-target organisms. For example, the use of DDT was

associated with an increased frequency of addled eggs in the nests of the American bald eagle that was sufficient in magnitude to be held responsible for population decline [56]. However, this example is not a close analogy for the impact of neonicotinoids on honey bees because the effect of DDT on birds of prey was promoted through biomagnification in the food chain, which can greatly increase the effective concentration of a toxin in the diet [57]. In contrast, bioaccumulation of neonicotinoids in honey bee colonies has not been demonstrated. Investigations of the sublethal effects of pesticides on the population dynamics of insects are rare in demographic toxicology [58], but systemic neonicotinoids at sublethal doses are known to reduce the intrinsic rate of increase of aphids [59] and the fecundity of bumble bees [60, 61]. Although bumble bees are taxonomically a close match for honey bees, the experiments on them involved dosages well above the environmentally-realistic trace range, which diminishes their value as an analogy. Comparison with the available analogies provides substantial evidence that trace dietary neonicotinoids could detrimentally affect vital demographic rates in honey bees, which scores this criterion at C_4 = +3.

2.5 C_5: Temporality

The temporality criterion asks whether the putative cause precedes the consequence. We therefore ask whether the widespread use of neonicotinoid insecticides preceded the honey bee population decline. The neonicotinoids were first marketed in 1991 and imidacloprid was the first product to be launched [10]. In the USA, imidacloprid-based products were licensed for widespread use on crops pollinated by honey bees (e.g. apples, fruit, vegetables and oilseeds) in 1994 and

their usage grew rapidly thereafter (Fig 1) until they occupied a 16% share of the total pesticides market by 2005 [10].

In the USA, a substantial decline in the numbers of managed honey bee colonies preceded the introduction of neonicotinoid use; the number of colonies declined steadily from a peak of approximately 5.5 million in 1945 to approximately 3.5 million in 1990 [17]. The neonicotinoids are evidently not responsible for the population decline over this period. Furthermore, the rapid expansion in the use of imidacloprid in California 1994-2007, for example, is not associated with an increased rate of honey bee population decline in this region (Fig 1); there is no statistically significant correlation between the annual incremental rise in use of imidacloprid 1995-2007 and the incremental decrease in the number of honey bee colonies in either the same year (Kendall's correlation test, tau = -0.25, df = 12, P = 0.22 n.s.) or the succeeding year (Kendall's correlation test, tau = 0.38, df = 11, P = 0.07 n.s.). However, this finding is not equivalent to showing that neonicotinoids were not causing colony losses, because Eq 6 shows that a stable population can be maintained if an increase in death rates, d^*, is offset by a corresponding increase in birth rates, b^*. Thus, an increase in the production of new colonies by beekeepers may have compensated for the increased mortality and a detrimental effect of neonicotinoids that would be apparent in increased depopulation symptoms is invisible when inspecting overall colony numbers. To resolve this, we would need to have long-term data on rates of colony loss and/or replacement 1995-2007, but this is unavailable. Overall, the advent of trace dietary neonicotinoids clearly neither preceded nor apparently intensified the honey bee decline and on this basis we score the temporality criterion at $C_5 = -4$.

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2.6 C_6: Consistency

The consistency criterion asks whether the association between the putative cause and its consequence is repeated in space and time. We ask initially whether honey bee population declines coincide with spatial variation in the use of neonicotinoid insecticides. Worldwide, honey bee declines are not ubiquitous and according to figures produced by the United Nations' Food and Agriculture Organization the global stock of managed colonies has increased by 45% in the last 50 years despite the declines in North America and Europe [21]. Even in Europe, stocks of colonies have increased in some countries, such as Spain, where the numbers have risen by over 50% to approximately two million since the neonicotinoids were introduced in the early 1990s [21]. In principle, it is possible to use this spatial variation to test whether declines are associated with neonicotinoid usage, but we have been unable to obtain the necessary data on national application rates. However, we are not optimistic that the association would have emerged. For example, we doubt that the growth of colony numbers in Spain is due to its unusually low levels of neonicotinoid use, because it produces massive quantities of citrus fruit and tomatoes (FAO 2011: http://faostat.fao.org/site/339/default.aspx), for which imidacloprid is a standard insecticidal treatment [62].

The consistency criterion offers another opportunity for our evaluation, however. If neonicotinoids are to precipitate a population decline, we should expect that neonicotinoid residues are prevalent among the colonies of a declining population. However, a recent survey of 350 pollen samples from North America found

imidacloprid in less than 3% of samples [63]. We therefore conclude that dietary neonicotinoids are clearly inconsistently associated with honey bee decline and score this criterion at C_6 = -4.

2.7 C_7: Specificity

The specificity criterion asks whether the consequence is both unmistakably defined and uniquely associated with the putative cause. The case at hand fully meets the former requirement by having unambiguous characteristics, which are either population declines (Fig 1) or depopulation symptoms, such as changes to demographic vital rates (Eq. 6). However, an important source of uncertainty arises from the difficulty of attributing a unique cause to these phenomena. There are many potential causes of bee declines other than dietary neonicotinoids, of which we identify four main types. First, even if dietary pesticides cause bee declines, the neonicotinoids are only a few of the chemicals that could be involved. In a survey of pesticide residues in North American honey bee hives [63], 98 pesticides and degradates were identified, with an average of approximately seven different residues per colony. Similarly, co-occurrence of different residues in Spanish citrus fruit indicates that treatment with various pesticides was the norm [62]. Second, other highly detrimental agents that affect honey bee colonies have increased in prevalence coincident with the increase in the use of neonicotinoids, such as mites [64], microspridian parasites [65] and viruses [66]. Third, landscape-scale reductions in the area of suitable forage due to agricultural intensification may limit the availability of nectar and pollen to colonies and thereby detrimentally affect demographic vital rates [67]. Fourth, the dominant influence on the population dynamics of a domesticated

population, such as the honey bee, is likely to be the economically-motivated activities of beekeepers, who may either reduce, sustain, or increase their stocks [21]. The potential contemporaneous impact of these drivers makes it difficult to conclude that dietary neonicotinoids could be uniquely, or even primarily, responsible for honey bee declines. We therefore conclude that the putative cause, dietary neonicotinoids, is certainly not uniquely associated with population decline in honey bees and score the specificity criterion at C_7 = -5.

2.8 C_8: biological gradient

This criterion asks whether an increase in the power of the putative cause is reflected by an increased effect. Currently, we do not have the necessary information to test whether global variation in the severity of population declines is associated with variation in the levels of neonicotinoid use, but there is evidence at a smaller scale. In Europe, maize pollen can be a major component of the honey bee diet, comprising approximately 20% of the pollen harvested by honey bees over the entire flowering period [68]. A survey in Belgium found that the frequency of various depopulation symptoms, including colony mortality, in apiaries decreased as the neighbouring area of neonicotinoid-treated maize increased [69]. We therefore conclude that the available evidence relating to biological gradient clearly contraindicates dietary neonicotinoids as a cause of honey bee decline and score this criterion at C_8 = -4.

2.9 C_9: strength.

This criterion asks whether the magnitudes of the effects that coincide with the action of the putative cause are so uncommonly large as to defy explanation by either chance or other artefact. To clarify his original definition, Hill[43] referred to cancer rates in people engaged in a particular practice (e.g. smoking) and he proposed that the implication of a causal relation is increasingly justified as the cancer rate, or degree of harm, associated with the practice deviates further from the norm.

While neonicotinoids are capable of making a devastating impact on bees when delivered at a sufficient dosage, as was observed in the accident at Baden-Württemberg [35], their impacts on honey bees as trace dietary residues are much less evident. In laboratory trials, trace doses do not cause elevated death rates, but instead cause sublethal effects on behaviour, namely decreased performance in learning tasks in the range of 6% to 20% [27]. The largest field trial conducted to date failed to detect detrimental effects of trace dietary neonicotinoids on colonies [31]. While this trial lacked the statistical power to detect small detrimental effects on performance ($\leq$ 20%), it should have detected more substantive impacts (>33%) [27]. We therefore conclude that the failure to detect a strong detrimental impact of trace dietary neonicotinoids under field conditions is a reasonable indication against their implication in honey bee declines and we score the criterion as C_9 = -2.

3 DISCUSSION

In the absence of decisive experimental evidence (C_1 = -1), we evaluated the cause-consequence relationship between trace dietary neonicotinoids and honey bee declines using the other eight of Hill's criteria. The proposition that dietary

neonicotinoids cause honey bee declines scored positively on all three of the theoretical criteria, C_2 to C_4 (mean = 2.7, SD = 0.6, n = 3), which makes it a reasonably justified conjecture in the context of current knowledge and indicates why these pesticides have been widely viewed as credible culprits. In contrast, the proposition scored negatively on the associational criteria, C_5 to C_9, on which we judged the circumstantial epidemiological evidence as substantially contraindicative (mean = -3.8, SD = 1.1, n = 5). The residual uncertainty on the associational criteria arises largely from doubt over the strength of the putative effect. Overall, however, virtually all of the circumstantial evidence clearly contraindicates the proposition.

Weighted evenly, the scores of Hill's criteria imply that the evidence is almost equivocal (mean = -1.3), but the variation among criteria is high (SD = 3.2, n = 9), which may account for the vigour of the controversy over neonicotinoids, because different constituencies use the evidence differently [26]. There is no *a priori* reason to give equal weight to the nine criteria and, furthermore, the widespread primacy given to experimental evidence in science suggests that an unequal weighting is normal. Hill (1959) refused to provide hard-and-fast rules for weighting the criteria, but a quantitative analysis of known cause-consequence relationships [70] found that the criteria of experimental evidence, strength and consistency contributed most to the correct attribution of cause. If their findings generalize to our case, the scores on these criteria (-1, -2, and -4 respectively) begin to contraindicate a cause-consequence relationship. It is conventional to favour material evidence over conjecture, which implies that the associative criteria have precedence and these substantially contraindicate the proposition that trace dietary neonicotinoids cause population declines in honey bees, although not with clear certainty.

It may be argued that neonicotinoids act not as independent agents, but as synergists of other stressors, such as poor forage, disease and the presence of other agrochemicals. We make two responses to this. First, even if we assume that neonicotinoids are synergists, their detrimental effect still lacks epidemiological perceptibility and this will not change the negative scores on most of the associative criteria, which are determined by the lack of response of the rate of bee decline to the advent of neonicotinoid use (C_5 = -4), the low prevalence of the pesticides in honey bee hives (C_6 = -4), the multiplicity of detrimental agents (C_7 = -5), and the absence of a biological gradient (C_8 = -4). Second, it is not yet proven that neonicotinoids at environmentally-realistic trace dosages interact synergistically with other stressors. Laboratory experiments have shown that dietary imidacloprid synergizes the effect of disease (*Nosema* infection) in harming individual bees [71], but only at dosages of 70 μg active ingredient kg^{-1}, which are substantially above environmentally-realistic levels.

3.1 Directions for future work

Based on our evaluation, we conclude that trace dietary neonicotinoids are not implicated in population declines of honey bees. Our evaluation is provisional, however, because important gaps remain in current knowledge. What information will be most valuable in further reducing uncertainty? We suggest four avenues for further investigation. First, experimental investigations of the effects of dietary neonicotinoids on the vital demographic rates of colonies and individuals are needed. Second, a quantitative demographic model for honey bee population

dynamics is essential in order to evaluate the condition of sufficient harm (Eq.s 3 and 6), but although valuable progress towards a predictive model has been made [72], empirical measurements of the impacts of dietary neonicotinoids on the appropriate parameters are still required. Third, epidemiological analyses of the association between the rates of neonicotinoid application and colony loss will be incisive. Fourth, it is necessary to determine whether trace dietary neonicotinoids are synergists of co-acting stressors.

In closing, we commend the use of Hill's criteria. Since their inception over 40 years ago and subsequent widespread use, no criterion has been abandoned and none added [70], which means that they provide a stable and well-established infrastructure in which to process scientific evidence. Their use formalizes the evaluation of cause-consequence associations and the scoring method that we have proposed is subjective, but transparent and repeatable. In our experience, the nine criteria cover exhaustively the range of evidence that is used in the debate over neonicotinoids and honey bees. We very much hope that others will repeat our evaluation in the light of new evidence and that these iterations eventually will resolve this longstanding uncertainty.

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Figure legend

Fig. 1. Annual amounts of imidacloprid (tonnes) applied to honey bee-visited crops in California 1991-2008 (filled symbols, leftmost *y*-axis) and number of managed honey bee colonies (thousands) in California 1990-2008 (interpolated open symbols, rightmost *y*-axis). The dashed vertical line indicates 1995, when neonicotinoid use first began in California. The imidacloprid-treated crops are: alfalfa; almonds; apples; apricots; beans; bell peppers; blackberries; blueberries; cantaloupe; cherries; citrus; dried beans; grapefruit; lemons; limes; melons; nectarines; oranges; peaches; peas; plums; prunes; pumpkins; rape seed; succulent beans; tangelos; tangerines; and watermelons. Data on imidacloprid use was compiled from the Pesticide Action Network (PAN) Pesticide Database (see *http://www.pesticideinfo.org*), which draws on the records of the California Department of Pesticide Regulation. The data on colony numbers is from the United States Department of Agriculture's National Agricultural Statistics Service (USDA-NASS).

Criterion	Brief description	Score
1. Experimental evidence		-1
2. Coherence	Fails to contradict established knowledge	+3
3. Plausibility	Probable given established knowledge	+2
4. Analogy	Similar examples known	+3
5. Temporality	Cause precedes effect	-4
6. Consistency	Cause is widely associated with effect	-4
7. Specificity	Cause is uniquely associated with effect	-5
8. Biological gradient	Monotonic dose-response relationship	-4
9. Strength	Cause is associated with a substantive effect	-2

Table 1. The nine criteria that are evaluated in the present study, each with a brief indicative description - for a fuller exposition see Hill (1969). The rightmost column contains the evidence-based score that we gave to each criterion, with positive scores in favour of the hypothesis that dietary neonicotinoids cause population declines in honey bees (maximum score of five), negative scores against, and zero indicating that the evidence is equivocal. For a justification of the scores, see text.