The dose makes the poison: have “field realistic” rates of exposure of bees to neonicotinoid insecticides been overestimated in laboratory studies?

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REVIEW ARTICLE

The dose makes the poison: have "field realistic" rates of exposure of bees to neonicotinoid insecticides been overestimated in laboratory studies?

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Summary

Recent laboratory based studies have demonstrated adverse sub-lethal effects of neonicotinoid insecticides on honey bees and bumble bees, and these studies have been influential in leading to a European Union moratorium on the use of three neonicotinoids, clothianidin, imidacloprid, and thiamethoxam on "bee attractive" crops. Yet so far, these same effects have not been observed in field studies. Here we review the three key dosage factors (concentration, duration and choice) relevant to field conditions, and conclude that these have probably been over estimated in many laboratory based studies.

La dosis hace el veneno: ¿se han sobreestimado las tasas "realistas de campo" de exposición de las abejas a los insecticidas neonicotinoides en estudios de laboratorio?

Resumen

Recientes estudios de laboratorio han demostrado efectos subletales adversos de los insecticidas neonicotinoides en la abeja de miel y abejorros, y estos estudios han sido de gran influencia en la consecución de una moratoria de la Unión Europea sobre el uso de los tres neonicotinoides, clotianidina, imidacloprid, tiametoxam y en " cultivos amigables con las abejas". Sin embargo, hasta ahora estos mismos efectos no se han observado en estudios de campo. En este artículo revisamos tres factores de dosis (concentración, duración y elección) y concluimos que estos han sido probablemente sobreestimado sen muchos estudios basados en laboratorio.

Keywords: honey bees, bumble bees, clothianidin, imidacloprid, thiamethoxam, concentration, duration, choice, laboratory studies, field studies

Introduction

In December 2013, the European Commission imposed a two year moratorium on the use of three neonicotinoid insecticides, imidacloprid, clothianidin and thiamethoxam as a seed dressing on certain "bee attractive crops" (European Commission, 2013). This followed intensive lobbying from pressure groups claiming that bee populations were being harmed (Ratnieks and Carreck, 2010). The debate has become highly charged and polarised. Great weight (European Food Safety Authority, 2013a,b,c) was attached by the European Commission to a few laboratory-based studies recently published in high impact journals which showed sub-lethal effects on honey bees or bumble bees at the colony or individual level.

Laboratory-based toxicology studies of bees (Medrzycki *et al.*, 2013) provide important information for policy makers, but for the results to be of maximum relevance to field conditions, appropriate doses must be used. Here we discuss three key factors affecting field exposure of neonicotinoids to bees that appear to have been systematically overestimated in these laboratory studies: concentration, duration, and choice. A recent review of the effects of

neonicotinoids on bee disorders (van der Sluijs *et al.,* 2013) states that: "at field realistic doses, neonicotinoids cause a wide range of adverse sublethal effects in honey bee and bumble bee colonies, affecting colony performance through impairment of foraging success, brood and larval development, memory and learning, damage to the central nervous system, susceptibility to diseases, hive hygiene etc.". But what actually are "field realistic doses"?

In this article we review the primary source studies for the information on field realistic doses used in these laboratory based studies.

Concentration

The laboratory based studies that were key to the EU Moratorium aimed to use concentrations representative of those in the pollen or nectar of crops whose seeds were treated with neonicotinoids. This is not as simple as it sounds. Data on field concentrations vary greatly, and may have been collected under circumstances not relevant to EU agriculture.

The UK study by Whitehorn *et al.* (2012) found that dietary exposure of bumble bee (*Bombus terrestris*) colonies to imidacloprid in the laboratory for two weeks reduced subsequent queen production when the colonies were placed in the field. "Field realistic" concentrations of 6 and 12 ppb imidacloprid in pollen and 0.7 and 1.4 ppb in sugar syrup were used that "represented the levels found in oilseed rape". To justify this, they cited the paper of Bonmatin *et al.* (2005) concerning maize (a species that does not produce nectar) although Bonmatin *et al.* (2005) noted that their levels in maize were "comparable with oilseed rape", and themselves cited Scott Dupree *et al.*'s (2001) study of spring-sown oilseed rape in North America. In that study, four honey bee hives were placed at each of two fields seed-treated with imidacloprid or clothianidin. On two occasions, pollen and nectar were collected from the hives and pooled. Imidacloprid was found at 4.4-7.6 ppb in pollen and 0.60-0.81 ppb in nectar, and clothianidin at 1.6-3 ppb in pollen and 0.9-3.7 ppb in nectar. Although these levels were similar to the doses used by Whitehorn *et al.* (2012), these figures may not be relevant to the UK, because seeds in the Canadian study were treated at three times the UK recommended rate. In addition, samples were collected only 50-68 days after spring sowing. In the UK, spring flowering of winter oilseed rape occurs approximately 200 days after autumn sowing. Winter rape plants are also larger than spring-sown rape (NC personal observation), which should lead to greater dilution, and are generally sown at a lower seed rate (Home-Grown Cereals Authority, 2012); between 2008-13, yields of UK spring rape were half those of winter sown oilseed rape (Home-Grown Cereals Authority, 2013). A recent second study by the same research group (Feltham *et al.,* 2014) also used 6 ppb imidacloprid in pollen and 0.7 ppb in sugar syrup.

In the UK study by Gill *et al.* (2012), bumble bee colonies were exposed to 10 ppb imidacloprid in sugar syrup plus a synthetic pyrethroid insecticide. Effects on foraging and longevity were quantified. The authors stated that these rates "could approximate field-level exposure", and cited Cresswell (2011) and Blacquière *et al.* (2012) in justification. Both these papers are, however, reviews. Cresswell (2011) cited the study by Bonmatin *et al.* (2005), which, as already noted, in turn cited Scott Dupree *et al.*'s (2001) study of spring sown rape, and the paper by Rortais *et al.* (2005). Rortais *et al.* (2005) contains **estimates** of exposure to bees foraging on imidacloprid-treated sunflowers and maize, but does not mention oilseed rape at all, and uses only the data from Bonmatin *et al.* (2001; 2002). The Blacquière *et al.* (2012) paper in turn cites results on oilseed rape of Genersch *et al.* (2010), and Cutler and Scott Dupree (2007). The Genersch *et al.* (2010) study considered 215 samples of pollen collected in Germany between 2005 and 2007. Clothianidin was not detected in any sample, and imidacloprid at a level of 3 ppb in only one sample. The study by Cutler and Scott-Dupree (2007) found maximum levels of clothianidin of 2.59 ppb in pollen and 2.24 ppb in nectar.

In total, therefore, the basis for the "field realistic rates" used in these two UK studies (Whitehorn *et al.,* 2012; Gill *et al.*, 2012) were the results of four samples collected during an oilseed rape trial in Canada that was not representative of UK pesticide use , and one sample collected in Germany.

The above are, however, not the only studies quantifying neonicotinoids in pollen and nectar. Chauzat *et al.* (2011) analysed 187 honey bee-collected pollen samples from France between 2002 and 2005. They detected imidacloprid in 40.5 % of them, at up to 5.7 ppb but with a mean of only 0.9 ppb. Samples of spring sown rape flowers, nectar, pollen and foraging honey bees from Sweden, France and the UK had residues of less than 10 ppb, the then level of quantification (Schmuck, 1999). In later studies, levels of <1.5 and 5 ppb were recorded in nectar and pollen (Maus *et al.*, 2003), whilst other studies found no detectable residues (Schöning and Schmuck, 2003). Other papers published since 2012 confirm these results. In a four-year study at three sites in France, median thiamethoxam levels in oilseed rape plant tissue were <1 ppb, were between <1 and 1 ppb in honey bee collected pollen, and were between 0.7 and 1.7 ppb in honey bee collected nectar (Pilling *et al.*, 2013). In a recently published Canadian study (Cutler *et al.*, 2014) of honey bee colonies adjacent to oilseed rape fields many samples of nectar, honey and beeswax contained no detectable residues, but some contained low levels (0.5-2.0 ppb) of clothianidin.

Overall, these field studies show that neonicotinoid residues are extremely variable. Many found no detectable residues, and others found levels lower than 10 ppb. The rates used in some of these recent laboratory based studies, therefore, appear not to be typical of concentrations in nectar and pollen. Rather than "field realistic", they

seem more representative of a "worst case" scenario. This problem is compounded by the fact that a single dose rate is often used, rather than the normal toxicological procedure of determining effects over a range of doses to generate a response curve (e.g. di Prisco *et al.*, 2013; Laycock *et al.*, 2014). This means that the dose chosen is critical.

Duration

When an individual bee is treated experimentally, it may receive at one time a dose equivalent to much longer field exposure. In the French study by Henry *et al.* (2012) individual honey bees were fed 1.34 ng of thiamethoxam at 67 ppb in 20 µl of syrup at one time. To justify this, Henry *et al.* (2012) cited Rortais *et al.* (2005), which contains similar **estimates** of exposure to imidacloprid, 1.1-4.3 ng per bee, via nectar foraging. Rortais *et al.* (2005) does not include any information about thiamethoxam at all, and again is based only on the figures for imidacloprid of Bonmatin *et al.* (2001; 2002). Furthermore, the Rortais *et al.* estimate noted that it would take 7 days of foraging for a bee to receive this dose. Henry *et al.* (2012) demonstrated that this large single dose of thiamethoxam affected individual homing ability, and from this they went on to infer colony mortality using a honey bee colony population model. As honey bees can detoxify poisons (Hodgson, 2004; Cresswell *et al.,* 2014), effects of chemical exposure over a short versus long period may be very different. One researcher compared this to the different effects on a human of drinking a bottle of whisky over 1 hour, 24 hours, or longer.

A further complication is that a foraging bee unloads its honey stomach on returning to the hive, so may suffer reduced effects from any chemical contamination of its nectar load. Depending on the time of year, and the demands of the colony, this nectar may be immediately used by the workers, or may be stored as honey, which involves the removal of water and hence the concentration of any dissolved contaminant, but equally it may be mixed with other uncontaminated nectar, leading to dilution before it is consumed. Pollen may similarly be consumed in its unmixed state or mixed with uncontaminated pollen from other plants. It is thus by no means straightforward to estimate or predict actual consumption of contaminants in nectar or pollen.

Choice

When a bee colony is treated in the laboratory, it is frequently assumed that the entire colony would forage on a treated crop. Gill *et al.* (2012) stated that "workers were exposed to the amount of active ingredient that would be present if they foraged for nectar exclusively on a crop field with 5 ppb imidacloprid in the nectar". Similarly, Bryden *et al.* (2013) fed 10 ppb imidacloprid *ad libitum* to bumble bee colonies, and found that they died out in the following six weeks. Bryden *et al* (2013) stated that this concentration was "near the upper end of the field realistic range reported for nectar and pollen in agricultural species". But in the field, bees generally have a choice of food sources.

In the UK, the major mode of exposure of neonicotinoids to bees has been via oilseed rape, as it is by far the most important bee-visited crop plant whose seeds have been treated with neonicotinoids. In 2012, over 3 % of the UK land area was oilseed rape. A field of blooming oilseed rape, with bright yellow flowers, is a dramatic sight. It is perhaps understandable, therefore, that the inference is made that all the bees must be foraging on this apparent bonanza. But this is not the case.

In Hertfordshire, UK, honey bee colonies were studied in an agricultural landscape containing many flowering oilseed rape fields. But pollen loads collected from traps on the hives were only 0.5-50.6 % (mean 12 %) oilseed rape (Osborne *et al.*, 2001). In a study in Sussex, UK, honey bee foraging was determined by decoding waggle dances and mapping oilseed rape fields. The study area had many rape fields within foraging range, with a total area close to the national average. Across two study years, only 0.00 % and 0.02 % and 2.2 % and 26.1 % of dances indicated oilseed rape fields, for hives in the rural area and the neighbouring urban area, respectively (Garbuzov *et al.*, 2015). Pollen was also collected. Dandelion, a common wildflower, was actually the main pollen collected by the rural hives; oilseed rape was fourth at just 13 %, similar to the Hertfordshire study.

Field studies

The laboratory based studies discussed above show that neonicotinoid insecticides can have adverse sub-lethal effects on bees, but so far these effects have not been observed in the field. Indeed many beekeepers deliberately place bee hives beside oilseed rape fields for honey production. It has been suggested in the media that no field studies have been carried out, but this is not so. A three-year French study quantified pesticides in honey, pollen and beeswax, and honey bee colony mortality and bee and brood populations (Chauzat *et al.*, 2009, 2010, 2011). A four-year German study measured pest and disease incidence together with environmental factors including pesticides (Genersch *et al.,* 2010). A large scale field experiment carried out in Canada in 2012-13 in which honey bees were exposed to clothianidin in the field (Cutler *et al.*, 2014) failed to find adverse effects on colony weight gain, honey production, pest incidence, bee mortality, number of adults, amount of brood and winter survival. None of these studies showed statistically significant evidence that neonicotinoids harmed honey bee colonies in the field.

A study of free-foraging bumble bee colonies in the UK (Thompson *et al.,* 2013) failed to detect the reduced queen production found in laboratory based studies (Whitehorn *et al.*, 2012) even though the colonies all foraged on treated oilseed rape. Indeed the mean numbers of queens produced by the colonies exposed to imidacloprid or clothianidin in the Thompson *et al.* (2013) study were greater than those of even the control (unexposed) colonies in the Whitehorn *et al.* (2012) study, in which queen production seems unduly low compared to previous published studies (e.g. Műller and Schmid-Hempel, 1992).

Why is there this discrepancy between laboratory based and field studies? If a field experiment finds no significant effects, it could indicate poor design or execution. It is inherently difficult to carry out controlled experiments in the field. In this instance, a truly replicated study to produce sufficient statistical resolution to detect a small effect would require multiple fields with treated and untreated crops, at a sufficient distance apart to avoid bees flying between, ideally also on identical soils and other environmental conditions. Even if such conditions were available, the costs would be prohibitive, so no such studies have been carried out to date. The studies that have been carried out may, therefore, easily be criticised for their imperfect design. For example the study by Thompson *et al.* (2013) was heavily criticised because it was not published in a peer-reviewed journal, because it was not replicated, and because the bumble bee colonies placed beside an untreated "control" field clearly had access to other treated fields. Nonetheless, if the effects in the field were of the magnitude implied by the laboratory studies, surely these effects should have been seen despite the imperfections in the design? In particular, colonies were placed next to treated fields of oilseed rape and foraged on it.

An alternative explanation for the discrepancies between the laboratory and field studies is that that the doses used in the laboratory studies overestimate field exposure. As shown above, the basis for the "field realistic" concentrations were four samples collected during an oilseed rape trial in North America of doubtful applicability to UK conditions, and one sample collected in Germany. Other studies, including those showing lower or zero amounts, appear not to have been used in determining field realistic concentrations.

Policy issues

Perhaps the most careful overview on the issue of neonicotinoids and bees is that of Cresswell *et al.* (2012a), which received virtually no publicity in the media. This used Hill's Epidemiological Criteria, normally used to evaluate clinical trial data, to weigh the hypothesis that "dietary neonicotinoids can be implicated in honey bee declines". The hypothesis was justified, as neonicotinoids can certainly kill bees, but was contraindicated by circumstantial epidemiological evidence. For example, decline in the number of honey bee colonies in the US

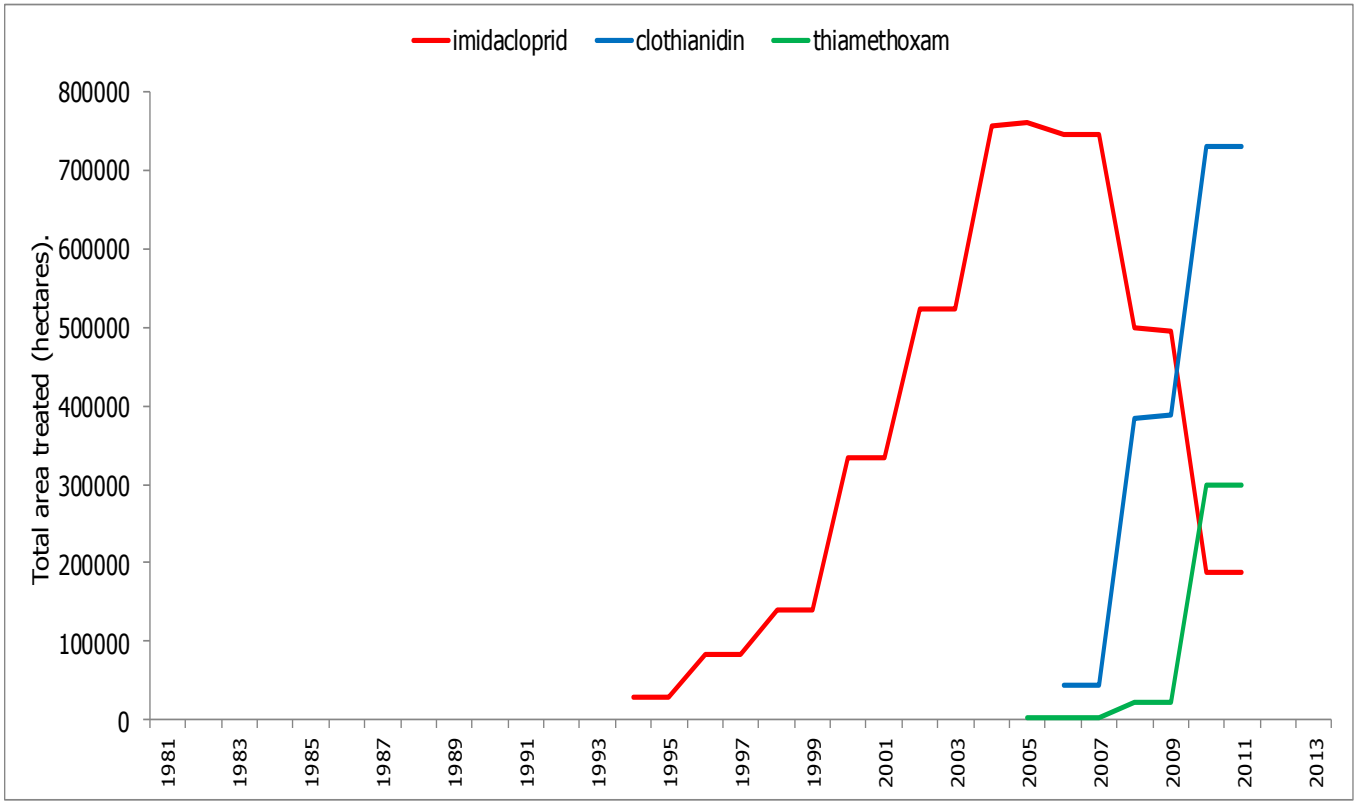

Fig. 1. Area of all crops in Great Britain seed treated with neonicotinoid insecticides. Data from Fera Pesticide Usage Survey: http://www.fera.defra.gov.uk/landUseSustainability/surveys/

preceded the introduction of neonicotinoids. Cresswell *et al* (2012a) concluded that "trace dietary neonicotinoids are not implicated in population declines of honey bees. The evaluation is provisional, however, because important gaps remain in our current knowledge". Similarly, using a formal causal analysis approach, Stavely *et al.* (2014) concluded that "neonicotinoid pesticides were judged to be 'unlikely' as the sole cause of reduced overwinter survival of honey bee colonies, although they could possibly be a contributing factor".

The neonicotinoid compounds used to treat seeds have changed greatly in recent years (Fig. 1). Many of the laboratory studies used imidacloprid, a compound that was no longer widely used to treat seeds in the countries in which the studies were conducted (Walters, 2013). Newer neonicotinoids, thiamethoxam and clothianidin, may have different effects on bees than imidacloprid (Laycock *et al.*, 2014). In addition, bumble bees, honey bees and solitary bees may not be affected in the same way or at the same dose (Cresswell *et al.*, 2012b; Walters, 2013).

The EU moratorium aims to help bees. If neonicotinoid seed dressings on bee-visited crops are harming bees or the environment, then prohibiting their use is clearly of value. The moratorium has been justified using the Precautionary Principle (Tosun, 2013), but if done unnecessarily it could make matters worse. Faced with the need to control pests, farmers will use other control measures, including insecticide sprays, which could harm bees (Carreck and Ratnieks, 2013). Another principle guiding EU policy is the Substitution Principle (Lofstedt, 2013), in which one set of compounds is replaced by newer, safer alternatives. Over the past decades, insecticides harmful to bees, humans, and the environment, such as organochlorine, organophosphorus and carbamate compounds have been replaced by pyrethroids and neonicotinoids. The incidence of pesticide poisoning of bees in England and Wales that can be attributed to the approved use of agricultural chemicals has seen a large reduction, as recorded by incidents reported to the UK Government's Wildlife Incident Investigation Scheme (Fig. 2). The moratorium will result in reverse substitution, that is, the use of older compounds and application methods whose effects have not been subject to modern rigorous registration procedures, to replace a group of chemicals which have been more closely studied than any other. If older classes of insecticides were to be similarly tested, sub-lethal effects on bees would also probably be detected. Indeed, sub-lethal effects of

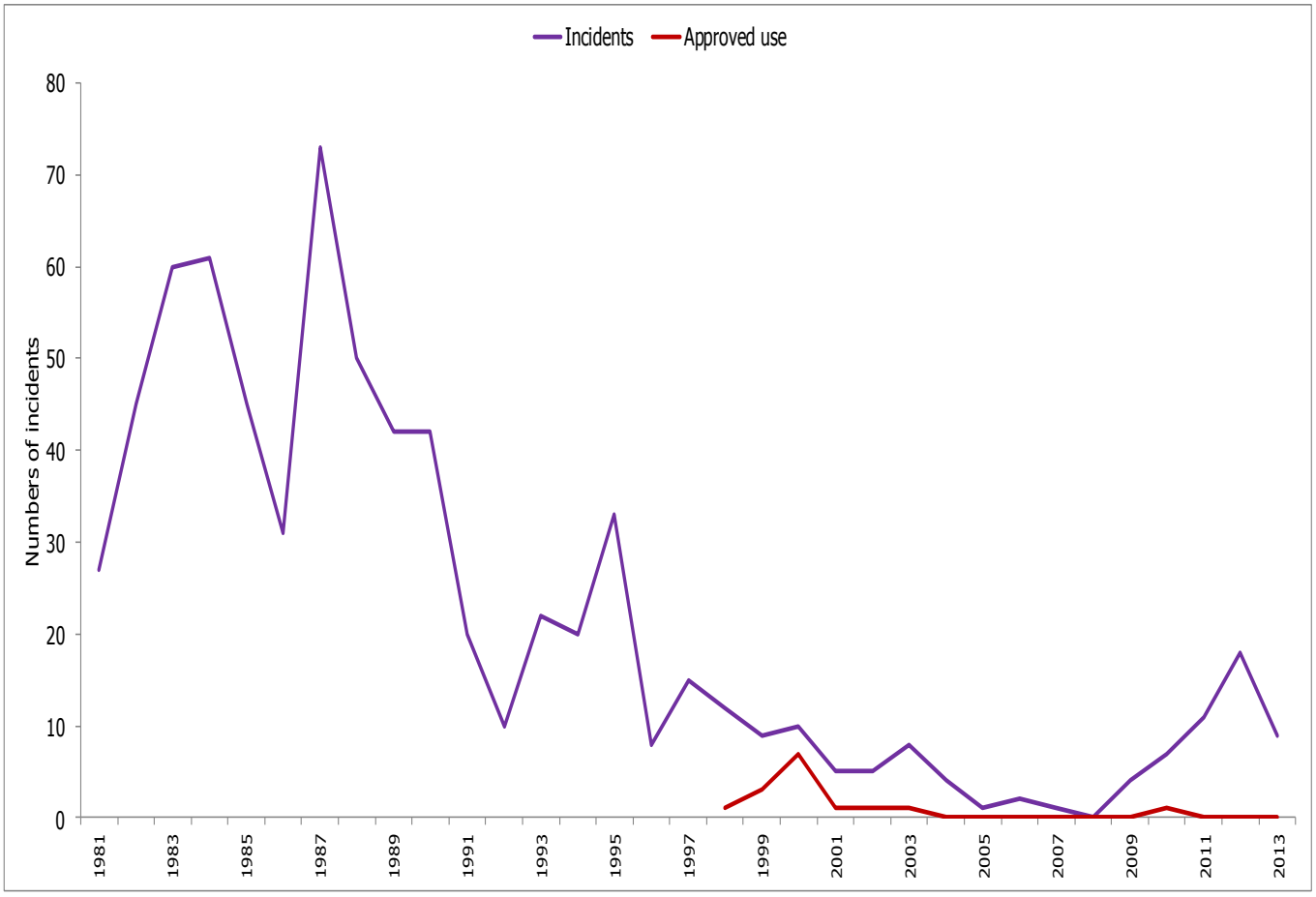

Fig. 2. Incidents involving honey bees investigated by the UK Wildlife Incident Investigation Scheme, and those confirmed to have been due to the approved use of a compound. There has not been a confirmed incident involving honey bees and the approved use of an agricultural pesticide since 2003. The single incident in 2010 involved a beekeeper who had treated his hives with wood preservative. The majority of poisoning incidents in recent years have involved misuse of compounds such as bendiocarb for destroying wild honey bee colonies. Data from WIIS: http://www.pesticides.gov.uk/guidance/industries/pesticides/topics/reducing-environmental-impact/wildlife

pyrethroid insecticides on bumble bee colonies have recently been reported (Baron *et al.,* 2014).

The moratorium could also hinder what we consider to be the crucial gaps in current knowledge: good data under field conditions on the actual amounts of neonicotinoids in nectar and pollen and their effects on bees. Although there is provision in the moratorium Regulation (European Commission, 2013) for a dispensation for research purposes, making it theoretically possible to perform replicated field experiments using large areas of treated seed, confident that control areas would not use treated seed, it is difficult to see who would fund such expensive studies.

It is self-evident that insecticides can kill insects, and it is unsurprising that sub-lethal doses can weaken colonies or disorient individual bees. But, as noted by Paracelsus, the dose makes the poison.

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