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Impacts of Pesticides on Honey Bees

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Additional information is available at the end of the chapter

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Abstract

This chapter focuses on the detrimental effects that pesticides have on managed honey bee colonies and their productivity. We examine first the routes of exposure of bees to agrochemicals used for crop protection and their application to crops, fate and contamination of water and plants around the fields. Most of the time, the exposure of bees to pesticides is through ingestion of residues found in the pollen and nectar of plants and in water. Honey bees are also exposed to pesticides used for the treatment of *Varroa* and other parasites. The basic concepts about the toxicity of the different kinds of pesticides are explained next. Various degrees of toxicity are found among agrochemicals, and emphasis is given to the classic tenet of toxicology, “the dose makes the poison,” and its modern version “the dose and the time of exposure makes the poison.” These two factors, dose and time, help us understand the severity of the impacts that pesticides may have on bees and their risk, which are analysed in the third section. Sublethal effects are also considered. The final section is devoted to some practical advice for avoiding adverse impacts of pesticides in beekeeping.

Keywords: residues, toxicity, exposure, sublethal effects, risk management

1. Introduction

For centuries, beekeepers have been aware of the environmental conditions that help prosper their honey bee colonies: a diversity of flowers from trees, shrubs, the so-called weeds and even crop plants. A healthy, diverse floral environment has always been the recipe for a healthy, bumper honey production. Perhaps the only problems they faced were the occasional infection by microorganisms, diseases and parasites that could kill the bees and their colonies [1] or the unpredictable vagaries of weather that could affect flower production on particular bad years.

In the past few decades, however, beekeepers have had to cope with a new threat to their business: agrochemical pesticides, which are scattered over large areas of crops, fruit groves, forests and other environments for the control of insect pests, weeds, vermin and plant diseases. There was no doubt, from the beginning, that chemical insecticides could represent a serious threat to bees for the simple reason that bees are insects and, therefore, susceptible to any poison designed to kill insect pests. Consequently, strict toxicity testing was and still is required before such chemicals can be registered for use in crop protection [2, 3], at least in developed countries. Despite these regulations, the number of managed honey bee (*Apis mellifera* L.) colonies in the United States declined from 6 million in 1947, when DDT was introduced in agriculture, to less than 3 million in 2010 [4]. Similar trends have been observed in Europe, where the number of apiaries declined 14% in Scandinavia and 25% in central Europe between 1985 and 2005, although they increased 13% in the Mediterranean countries in order to counteract the lower production in the north [5].

But, what about other pesticides, such as herbicides and fungicides? Could they also affect honey bee productivity? If the target of such chemicals is not the insects, many argued, they are probably safe to the bees. Research conducted in the past few years in countries with a long history of pesticide usage suggests differently. It is now acknowledged that the extensive and prolonged use of herbicides leads to a reduced diversity of flowering plants [6, 7] that inevitably affect the bees' colonies [8] and their productivity. Moreover, the combination of some fungicides with insecticides has been revealed more deadly to the bees than either chemical alone [9]. Lately, the indiscriminate use of acaricides in apiaries for the control of parasites, such as *Varroa destructor*, has added one more threat for the beekeepers, as these chemicals are also toxic—although to a lesser degree—to the honey bees. Not surprisingly, the colony collapse disorder (CCD) has been linked by some authors not only to parasites and diseases but also to pesticide usage [10].

In these circumstances, a new management approach is needed for successful beekeeping. Production of honey and wax is no longer dependent on the availability of flowers in the surrounding environment, but rather appears to be intimately linked to the quality of food that the bees collect. It is now clear that pesticide-contaminated flowers affect the health of the honey bee colonies to the extent that their productivity declines [11]. In order to better manage this situation, we must first understand how bees are exposed to pesticides and what are the consequences of such exposure for the health of the individual bees, the colony and their overall productivity.

2. Exposure of bees to agrochemicals

Most insecticides are applied as sprays over the crop canopy, but sprays of herbicides and fungicides are usually applied directly on the soil before the planting of crops. In all these cases, droplets and dust from the applications can fall directly on the bees that fly across the treated fields or nearby because wind can carry the tiny droplets and dust particles hundreds of metres away from the crop [12]. A single droplet of insecticide may be sufficient to kill a bee

because the spray solutions contain concentrated doses of these chemicals—this is the most common cause behind the bee incidents reported in the literature [13, 14]. Granular pesticides that are incorporated into soil (e.g., herbicides) have no direct exposure to bees.

The so-called systemic insecticides are usually applied as seed coatings. The treated seeds are introduced into the soil using pneumatic drilling planters, and the friction of the seeds in the machinery produces dust particles that are heavily loaded with the insecticides. These poisonous particles can also cause a great deal of mortality among bees, if they happen to be in the surroundings [15]. Systemic insecticides applied this way are taken up by the crop plants as they grow and their residues are present in all parts of the treated plant, including the flowers, pollen and nectar [16]. Not only the crop plants but also the weeds and bushes that grow in the vicinity are affected [17, 18] because they also take up small amounts of residues that spread through the soil through lateral water flow [19] or are contaminated through dust/spray drift. In addition, some plants can produce guttation drops in the early hours of the morning (e.g. maize, strawberries), and systemic insecticides appear in such drops in elevated concentrations [20] that are capable of killing the bees.

Most of the time, the exposure of bees to pesticides is through ingestion of residues found in the pollen and nectar of contaminated plants, whether from the crop plants or from the weeds around the fields [21]. It is important to realise that bees forage everywhere they can and search for the most suitable flowers that produce pollen and nectar in abundance. Thus, some crops are more attractive than others; for example, the yellow flowers of canola (rape seed oil), sunflowers and many weeds that grow in and around the crops are more attractive to bees than the flowers of potato plants. Pesticide residues in pollen and nectar are taken by the forager bees to their colonies and remain in the bee bread and honey for quite some time [22, 23]. These residues are then fed to the larvae and the queen, which are affected in similar ways as the forager bees.

In addition to food, bees also drink water to keep their body temperature under control [24]. Pesticide residues in soil eventually move into the water and appear in the streams, creeks and ponds of agricultural areas and beyond, which are thus contaminated with a mixture of agrochemicals [25]. Some water contamination is also due to drift from spray applications, particularly from insecticides [26, 27]. Honey bees, bumblebees and wild bees like to drink from puddles, irrigation ditches, ponds and streams, and if these waters are contaminated with pesticide residues, the forager bees ingest them as well [28].

Apart from the pesticides used in agricultural production, honey bees are also exposed to the acaricides used for the control of *Varroa* and other parasites. In this case, bees come in contact with the high residue levels present on the waxy cells of the comb [29], affecting mainly the developing larvae [30] and presumably the adult honey bees and the queen.

Given the enormous variety of agrochemicals used in crop production, it is not surprising that, to date, residues of 173 different compounds have been found in apiaries [21]. It should be realised that through the various routes of exposure to pesticides in the environment (**Figure 1**), bees are not threatened by one or two chemicals alone but by cocktails of many agricultural compounds.



Figure 1. Routes of exposure of bees to agricultural pesticides.

3. Toxicology of pesticides

Pesticides are toxic chemicals with specific mode of action, meaning they are designed to specifically control a target group of organisms by interfering with particular metabolic pathways. Thus, insecticides and acaricides kill insects and mites by disrupting their neuronal activity, their moulting process or other specific metabolism of these arthropods; herbicides and algicides kill plants and algae by disrupting their photosynthetic capacities or the synthesis of essential organic compounds and fungicides kill fungi by inhibiting the formation of their cell membranes or another metabolism specific of these organisms. There are other kinds as well, like rodenticides that kill small mammals, bird repellents, etc. The term biocide is reserved for broad-spectrum poisons that kill any organism, mainly microbes, but also large animals.

The toxicity of each kind of pesticide, however, is not exclusive to the target group of organisms: other species that share similar metabolism are affected as well, although usually to a lesser degree. The potency of a pesticide to any species is defined by the dose of toxic chemical that is lethal to 50% of individuals of that species (LD50), and such dose varies from species to species. Doses lower than the LD50 are considered 'sublethal', but they can also cause mortality on a certain proportion of the species population, i.e., 20 or 30% of individuals may die. In general, sublethal doses cause toxic effects that do not kill the organisms but still affect their

normal functioning and health. For example, exposure of bees to sublethal doses of neurotoxic insecticides may cause stress [31], paralysis or abnormal behaviours without killing the bees [32].

By their very nature, insecticides are the most toxic compounds to bees, whereas herbicides are largely innocuous (**Table 1**). Beekeepers should be wary of any insecticide application in the vicinity of their hives because spray drift could certainly inflict a heavy toll on the bees. Pesticide applicators are aware of this danger and, in many countries, are required to inform beekeepers before they apply insecticides to a crop [33]. Also, while acaricides are less toxic to bees than to the target parasites, excessive amounts of their residues in the combs may have unpleasant consequences for the health of the bees [34].

Pesticide type	Chemical name	Contact* LD50(µg/bee)	Oral* LD50 (µg/bee)	Half-life† (days)
Acaricides	Acrinathrin	0.17	0.12	22
	Amitraz	50	-	1
	Coumaphos	20	4.6	-
	Fenpyroximate	11	-	49
	Tau-fluvalinate	8.7	45	4
	Tetradifon	1250	-	112
Fungicides	Azoxystrobin	200	25	78
	Boscalid	200	166	118
	Captan	215	91	4
	Carbendazim	50	-	22
	Chlorothalonil	135	63	44
	Myclobutanil	40	34	35
	Propiconazole	50	77	214
	Quintozone	71	-	210
	Tebuconazole	200	83	47
Herbicides	Metolachlor	-	110	90
	Norflurazon	1485	-	225
	Simazine	879	-	90
Insecticides	Beta-cyfluthrin	0.031	0.050	13
	Bifenthrin	0.015	0.20	87
	Carbofuran	0.16	-	14
	Chlorpyrifos	0.072	0.24	50
	Clothianidin	0.039	0.004	121
	Cypermethrin	0.034	0.064	69

Pesticide type	Chemical name	Contact* LD50(µg/bee)	Oral* LD50 (µg/bee)	Half-life† (days)
	DDT	8.8	5.1	6200
	Diazinon	0.38	0.21	18
	Endosulfan	6.4	21	86
	Fenthion	0.22	-	22
	Fipronil	0.007	0.001	142
	Imidacloprid	0.061	0.013	174
	Malathion	0.47	9.2	1
	Mevinphos	0.094	-	1
	Pyrethrum	0.18	0.057	-

*Source: ECOTOX and AGRITOX databases, compiled in Sánchez-Bayo and Goka [21].

†Source: Footprint database (IUPAC). <http://sitem.herts.ac.uk/aeru/iupac/>

Table 1. Toxicity of common pesticides to bees (LD50 at 48 hours) by contact or oral exposure and their persistence in soil (half-life)

All animals, including bees, are endowed with detoxification mechanisms that transform and eliminate most toxic chemicals. Currently, the majority of organic pesticides are degradable either in the organisms themselves or in the environment. The exception is the organochlorine pesticides (e.g. insecticides like DDT and lindane), which are very persistent and recalcitrant. Because they were applied in large quantities in the past decades, their residues are still present —although at low levels—in the soils of many countries, even if nowadays are banned from use in agriculture. Due to their low solubility in water, organochlorine residues are not taken up by the plants growing in contaminated soils, and so they do not appear in the pollen or nectar of the flowers.

The persistence of pesticides is evaluated by their half-life ($t_{1/2}$), which is defined as the time required for half the amount of a chemical to disappear from a medium, that is, water, soil, air or biological tissues. Half-lives longer than 90 days indicate that the pesticide may accumulate, since more than 5% of the amount applied will remain in the environment after 1 year [35]. Residues of persistent pesticides found in pollen or nectar (Table 1) will, therefore, remain in the beebread throughout the entire season of honey production.

Systemic insecticides, such as neonicotinoids (e.g. imidacloprid) and fipronil, are more toxic and persistent than the majority of organophosphorus (e.g. malathion), carbamates (e.g. carbofuran) and pyrethroids (e.g. cypermethrin) (Table 1). Given their high solubility in water, their residues also appear in water bodies of agricultural areas and the rivers they drain into [36, 37]. As they are applied consistently as seed dressings, their residues may remain in the soil for years and are taken up by the crop and weeds, ending up in the nectar and pollen of all plants in the treated landscape [16]. This poses a risk to bees, not only because of their high toxicity and availability but also due to their particular mode of action. For example, neonicotinoids show delayed toxicity at low doses, so apart from various sublethal effects

they cause [38], they end up killing the bees if they are exposed to the residues for a long period [39]. Both neonicotinoids and fipronil also produce immune suppression on honey bees [40, 41] and, consequently, they predispose bees to *Nosema* infections [42] and outbreaks of viral diseases that are commonly transmitted by *Varroa* mites [43, 44]. As a result, colonies feeding on honey and pollen contaminated with these neurotoxic insecticides may succumb to the combined effects of chemicals and diseases [45].

The toxicity of certain insecticides can be enhanced in the presence of ergosterol-inhibiting fungicides (e.g. propiconazole, myclobutanil), which act as synergists. Indeed, this type of compounds inhibits the detoxification system in bees [46, 47], so the insecticide and acaricide residues are not metabolised or eliminated as fast as they should. Furthermore, the toxicity of insecticides and acaricides used for *Varroa* control is often additive or synergistic [9]. Since the food that forager bees collect is usually contaminated with a mixture of both insecticides and fungicides, and because most managed apiaries are treated with acaricides, the combined toxicity and synergism of all these chemicals pose a real threat to the health and survival of honey bee colonies and all other species of wild bees.

Sublethal exposure to pesticides, including fungicides and some herbicides, often produce stress in animals, because the organisms try to metabolise and get rid of the toxic chemicals quickly using large amounts of energy. Apart from stress, bees experience other negative effects when exposed to sublethal doses of pesticides. For example, under conditions of chronic exposure, honey bee larvae fed on pollen contaminated with chlorpyrifos produced very few queens [48]. Wild bees (*Osmia bicornis*) exposed to sublethal levels of thiamethoxam and clothianidin had their reproductive success reduced by 50% [49], while honey bee queens experienced unusually high rates (60%) of supersedure [50]; bumble bees (*Bombus terrestris*) colonies exposed to sublethal levels of thiamethoxam failed to perform and produced 85% less queens than normal [51]. Sublethal doses of neonicotinoid insecticides also cause disorientation and memory loss in forager bees [38], contributing to less efficiency in the collection of pollen by bumble bees [52]. Sublethal doses of the acaricide coumaphos also produce abnormal mobility in the exposed honey bees [53]. Undoubtedly, all these effects disturb the performance of the individual bees and that of the colony [54].

Finally, the indirect effects caused by herbicides cannot be ignored. Herbicides are not toxic to bees, but they disturb the environment in which bees and other pollinators live. Plant biodiversity, and its associated arthropod communities, have certainly decreased in areas that have been treated with herbicides for many years [55, 56]. The lack of certain plant species, mainly weeds, implies an impoverishment of the natural environment that sustains pollinators, including honey bees. Consequently, bees find more difficult to collect the variety of pollen that is required for a healthy bee diet [57]. Poor bee nutrition due to scarcity of flowers is the indirect result of continuous herbicide applications in crops and forestry areas over many decades.

4. Risk of pesticides to bees

Having explained above the routes of exposure to pesticides and their various impacts on bees, an evaluation of the actual risks that current pest control products and acaricides used for treating hives pose to honey bees is needed. The main risk derives from the acute toxicity of the chemicals to the bees, which produce their mortality in the short or middle term. Other risks include sublethal effects that may harm the performance of hives and the long-term viability of the colonies, as mentioned above.

Risks are typically estimated as probabilities of harm and are based on the acute toxicity and the frequency with which a chemical may affect the bees. Three scenarios can be considered: (1) risks from spraying of pesticides over agricultural fields; (2) risks posed by ingestion of agrochemical residues found in pollen, honey and water, which are collected and ingested by the forager bees and transported to the hive, where they are processed into honey and bee bread and fed to the other bees, the larvae and the queen; and (3) risks from exposure to combs treated with acaricide products.

4.1. Risk from exposure to sprays

For the first scenario, the only data required are the concentrations of the active ingredients in the spray solutions applied and their acute toxicity, i.e., LD50 values for each chemical, since the probability of a bee being sprayed on can be considered 100% if the bee flies directly through the spray cloud in the field, or if a hive is placed downwind and within the normal range of spray drift by aerial or ground-rig applications, i.e., less than 1 km. This kind of risk is estimated using the typical hazard quotient HQ

$$HQ = \frac{\text{Exposure}(\mu\text{g})}{LD50(\mu\text{g} / \text{bee})} \quad (1)$$

where the exposure term can be determined by the concentration of active ingredient in the spray droplets and the volume received by the bees, according to the following expression

$$HQ = \frac{\text{Concentration}(\mu\text{g} / \text{ml}) \times \text{vol.droplets}(\text{ml})}{LD50(\mu\text{g} / \text{bee})} \quad (2)$$

In this case, the HQ can be indicative of high risk when its value is 1 or more, since 50% or more bees exposed would die; moderate risk is when HQ values are between 0.1 and 1 and low risk when it is less than 0.1, as fewer than 10% (similar to a natural mortality rate) of bees would be threaten.

Estimates of risks are typically done by considering the spray drift [58, 59] and the exposure to the flying bees [60]. For example, to compare the risk posed by different products under the same conditions, the spray drift volume can be fixed, e.g. 500 droplets for a bee crossing the spray cloud in a few minutes, at 5×10^{-4} μl for a standard droplet would result in 0.25 μl /

bee. **Table 2** shows a comparison of the risk that commonly applied pesticides would have in such situations.

Pesticide type	Chemical name	Droplet concentration (µg/ml)	LD50 (µg/bee)	HQ	Risk evaluation
Acaricide	Amitraz	200	50.0	0.001	Low
	Dicofol	240	19.0	0.003	Low
	Propargite	600	62.1	0.002	Low
Fungicide	Azoxystrobin	75	200.0	<0.001	Negligible
	Fludioxonil	12.5	50.3	<0.001	Negligible
	Mancozeb	750	226.2	<0.001	Negligible
	Tolclofos-methyl	500	100.0	0.001	Low
Insecticide	Abamectin	18	0.03	0.15	Moderate
	Acetamiprid	225	7.9	0.007	Low
	Beta-cyfluthrin	25	0.031	0.20	Moderate
	Bifenthrin	100	0.015	1.70	High
	Carbaryl	500	0.84	0.15	Moderate
	Chlorantraniliprole	350	4.0	0.022	Low
	Chlorpyrifos	300	0.072	1.04	High
	Difenthiuron	500	1.5	0.083	Low
	Dimethoate	400	0.12	0.85	Moderate
	Endosulfan	350	6.35	0.014	Low
	Esfenvalerate	50	0.026	0.48	Moderate
	Fipronil	200	0.007	6.8	High
	Imidacloprid (spray)	200	0.061	0.81	Moderate
	Imidacloprid (dust)	24*	0.061	0.1	Moderate
	Indoxacarb	150	0.58	0.064	Low
	Lambda-cyhalothrin	250	0.048	1.3	High
	Methidathion	400	0.27	0.37	Moderate
	Methomyl	225	0.50	0.11	Moderate
	Pririmicarb	500	35.7	0.004	Low
	Spirotetramat	240	242	<0.001	Negligible
	Thiamethoxam (spray)	250	0.025	2.5	High
	Thiamethoxam (dust)	36.8*	0.025	0.37	Moderate

*Data for dust particles from conventional pneumatic planters [61, 62].

Table 2. Risk of common agricultural pesticides to honey bees that fly across a spray cloud (ppm) and receive a total dose of 0.25 µl/bee

The example in Table 2 reveals that the insecticides fipronil, thiamethoxam, bifenthrin, lambda-cyhalothrin and chlorpyrifos are the most dangerous to bees when sprayed to agricultural crops. The microencapsulated formulation of lambda-cyhalothrin is particularly hazardous because bees can carry the microcapsules containing the concentrated chemical to the hive. In general, dust particles of neonicotinoid-treated seeds and spray droplets of pyrethroids, organophosphorus and carbamate insecticides pose moderate or high risks, whereas other insecticides and acaricides present low risks in comparison. The fungicides shown here, and possibly most others applied as foliar sprays, pose low or negligible risks to bees by direct contact with spray droplets. This evaluation is in agreement with the reported incidents of pesticides on bees in the United Kingdom [63] and Canada [64]. Obviously, the most toxic insecticides are the most dangerous to bees.

4.2. Risks by oral exposure

For the second scenario, ingestion of contaminated food, data on the concentration and frequency of residues in each media are essential, as well as information on the dietary intake of pollen, honey and water by each caste of bees, that is, foragers, nurses, larvae and queen. Oral exposure to contaminated food is considered the typical exposure of bees in the hive. The risk expression in this case would take the form

$$Risk = \frac{Frequency(\%) \times residue\ dose(\mu g)}{LD50(\mu g / bee)} \quad (3)$$

where the residue dose of a given pesticide can be estimated for different bees as the product of the concentration of residues in pollen, honey or water by the total intake of a particular caste of bee [21]. In turn, total intakes are estimated from daily intakes and the life span of bees, which vary from 5–6.5 days for larvae, 8–10 days for brood attendants and nurses, to 30 or more days for foragers [65]. The food intake by queens is hard to estimate, as they are fed royal jelly (a particular combination of pollen and honey), can live several years and vary their intake — which is unknown — throughout the reproductive and winter seasons. For the toxicity, oral LD50s are used in this case. The risk estimated by expression (3) can be interpreted as the probability that a given pesticide residue has of causing 50% mortality among the bees that ingest the contaminated pollen or nectar.

In recent years, a number of studies have reported the residue levels of agricultural pesticides found in pollen [66, 67] and nectar of flowers [68, 69], in water bodies of agricultural areas [28], as well as in beehive matrices, such as beebread, honey and wax [70, 71]. Based on these reports, we estimated the average and maximum residues for each pesticide as well as their frequency of appearance in those matrices. This information allowed us to calculate the risks that bees encounter when feeding on such contaminated food or drinking sources. A summary of results for the compounds that pose the highest risks by oral exposure of combined food and drink is shown in **Table 3**.

Chemical name	Residues (µg/kg)			Larvae	Nurses		Foragers		
	Pollen	Honey	Water*	Risk (%)	T50 (days)	Risk (%)	T50 (days)	Risk (%)	T50 (days)
Thiamethoxam	28.9	6.4	4.1	2.77	23	4.80	27	276	7
Gamma-HCH (lindane)	7.6	176.5	-	0.62	9	0.01	979	200	3
Clothianidin	9.4	1.9	2.6	1.02	54	1.91	58	39.5	13
Imidacloprid (total)	19.7	6.0	0.9	1.19	68	1.57	103	25.4	25
Cypermethrin	13.9	18.1	-	0.13	119	0.04	711	4.00	44
Coumaphos (total)	128.3	105.5	-	0.11	1444	0.06	5524	2.62	545
Dinotefuran	45.3	13.7	-	0.10	49	0.13	74	1.50	20
Quinalphos	-	9.6	-	<0.01	253	-	-	1.29	91
Methiocarb	1.4	15.0	-	<0.01	1080	<0.01	>5000	1.08	391
Chlorpyrifos	32.6	3.9	-	0.04	1605	0.13	1118	0.86	764
Carbaryl	58.9	23.4	-	0.41	202	0.42	392	0.54	80
Beta-cyfluthrin	2.2	9.0	-	0.10	190	0.01	3497	0.43	69
Dimethoate	2.3	4.8	-	0.01	1198	<0.01	>5000	0.40	440
DDT (total)	31.2	44.2	-	<0.01	3871	<0.01	>5000	0.29	1432
Pirimiphos ethyl	-	19.0	-	<0.01	401	-	-	0.21	144
Diazinon	8.5	17.0	-	0.04	426	0.01	3869	0.19	156
Malathion	17.1	98.0	-	<0.01	3218	<0.01	>5000	0.15	1167
Pirimicarb	-	38.0	-	<0.01	3500	-	-	0.10	1261
Phosmet	339.3	-	-	0.07	991	0.79	168	-	-
Fipronil (total)	1.6	-	-	0.02	596	0.27	101	-	-
Acrinathrin (total)	146.8	-	-	0.01	719	0.17	122	-	-

*From the survey by Samson-Robert et al. [28].

Table 3. Average pesticide residue levels in food and water (ppb) and their risk by oral exposure to worker honey bees and larvae. The time to reach the oral lethal dose (T50, days) is also shown for a comparison

Despite the high risk of some chemicals, namely neonicotinoids, most insecticide residues in pollen and honey present a moderate risk to bees (1 to 5%), especially those of pyrethroid and organophosphorus compounds. Overall, 21 of the 113 pesticide residues in food for which toxicity data are available pose some kind of risk to honey bees, but only 8% of the chemicals are of concern. Residues in water are more variable from place to place: the data shown in

Table 3 are from one survey in Canada where only neonicotinoids, fungicides and herbicides were found—the risks posed by the latter two groups were negligible nonetheless, so they are not shown in the table.

4.3. Risks by contact exposure

Apart from oral exposure, bee larvae may also be in contact with residues deposited on the walls of the comb cells, in particular, the acaricides used for controlling *Varroa*. Although the highest loads of pesticide residues in a hive are found in the wax [23], the availability of such chemicals is thought to be minimal except for the fumigated acaricides. The risk of the latter products to bee larvae should be estimated not as oral intake, as some authors do [30], but rather as contact exposure. The expression (3) can be used, with the maximum residue dose in this case estimated as 5 mg of active compound per cell for a single larva and the contact LD50 instead of the oral one. The results of the risk analysis for a number of acaricides to honey bee larvae are shown in **Table 4**.

Pesticide	Residues in wax (µg/kg)	Risk by contact (%)	T50 (days)
Acrinathrin (total)	139.0	0.03	247
Amitraz (total)	585.5	<0.01	>5000
Bromopropylate	16.4	<0.01	>5000
Carbofuran (total)	19.4	<0.01	1649
Chlorfenvinphos	1156	0.14	709
Coumaphos (total)	1352	0.02	3003
Dicofol	6.8	<0.01	>5000
Pyridaben	5.4	<0.01	1957
Spirodiclofen	28.5	<0.01	>5000
Tau-fluvalinate	3144	0.15	551
Tau-fluvalinate+amitraz	3730	21.25	11
Tau-fluvalinate+coumaphos	4496	122.6	11
Tetradifon	7.9	<0.01	>5000

Table 4. Average acaricide residue levels in comb wax (ppb) and their risk to larvae of honey bees. The time to reach the lethal dose (T50, days) is also shown for a comparison

As it can be seen, the risks of acaricides to bee larvae are below 1% for all individual chemicals, but increases dramatically for synergistic mixtures, such as tau-fluvalinate with amitraz or coumaphos. Except for the latter mixtures, the overall risk to bee larvae of the individual products is very low or negligible compared to that of the same compounds by oral ingestion of contaminated food and water (Table 3).

4.4. Novel approaches to risk assessment

Another way of estimating risks, particularly for oral exposures, is by calculating the time that would take for a bee to reach the LD50 of a given pesticide, based on the daily intake of contaminated food and water. This estimate is made using the expression

$$T50(days) = \frac{LD50(\mu g / bee)}{Daily\ intake(\mu g)} \quad (4)$$

where T50 is the time to reach the median lethal dose (LD50), also termed median time to death. As it can be expected, there is a good correlation between the T50 values estimated using equation (4) and the risk values calculated using equation (3)—see Tables 3 and 4.

Neonicotinoid insecticides, however, can cause delayed mortality due to their agonistic mode of action [39]. This particularity means that their acute oral LD50s, which are usually estimated for exposures of 48 hours, are insufficient to estimate accurate risks of these insecticides, because the actual dose that causes the death of the bees decreases as the time of exposure increases [72]. Consequently, the mathematical function that relates the median time to death (T50) with the median lethal dose (LD50) is used to estimate the risk, as follows

$$\ln T50(days) = a + b \ln LD50(\mu g / bee) \quad (5)$$

where *a* (intercept) and *b* (slope) are empirical parameters specific to each chemical and species tested (in this case honey bees). The approach estimates the cumulative mortality with exposure time with more precision than the standard equation (4), as explained in a previous study [21].

4.5. Risk from synergistic mixtures of pesticides

The above tables help determine the pesticides that pose the greatest danger to bees, whether by exposure to spray droplets or dust, oral ingestion of contaminated food and water or contact with chemicals used for mite control in the hives. It is clear that the majority (92%) of pesticides registered for agricultural production do not pose significant or measurable risks to honey bees, but this is only when considering the exposure to individual compounds.

Recent developments, however, indicate that combination of certain chemicals, in particular insecticides and acaricides with fungicides or mixtures of acaricides, is more toxic to bees than the individual compounds on their own. The additive and synergistic effects of those mixtures have already been mentioned above, and estimation of the risks they pose needs to be calculated using the same approaches but modifying the toxicity of the insecticide or acaricide by a synergistic factor [21]. These factors are calculated experimentally for several combinations of fungicides with insecticides and/or acaricides [73], and some examples are shown in **Table 5**.

Insecticide or acaricide	Fungicide	Synergistic factor	Risk to larvae (%)		Risk to nurses (%)	Risk to foragers (%)
			Wax	Food	Food	Food
Acetamiprid	Propiconazole	104.7	<0.01	<0.01	<0.01	<0.01
Acetamiprid	Fenbuconazole	4.5	<0.01	<0.01	<0.01	<0.01
Coumaphos	Fenpyroximate	20.0	<0.01	0.77	<0.01	<0.01
Cyhalothrin	Propiconazole	16.2	2.16	<0.01	<0.01	<0.01
Cyhalothrin	Myclobutanil	10.9	<0.01	<0.01	<0.01	<0.01
Cyhalothrin	Penconazole	4.4	<0.01	<0.01	<0.01	<0.01
Tau-fluvalinate	Myclobutanil	50.0	<0.01	0.01	<0.01	<0.01
Thiacloprid	Propiconazole	559.4	0.89	0.08	0.30	<0.01

Table 5. The synergistic effect of some fungicides with insecticides or acaricides and their risks to honey bees

Although the increases in risk are obvious, only the interaction of the pyrethroid insecticide cyhalothrin with propiconazole points to a moderate concern for bee larvae; even the risk of thiacloprid appears to be low under these circumstances. However, the risk of certain acaricide mixtures, such as tau-fluvalinate with amitraz or coumaphos, used in *Varroa* treatments, can be very high for the larvae (see Table 4).

5. Management in order to avoid pesticide impacts

The various risks estimated above give us some clues about the type of exposure most dangerous to the different castes of bees in the hives. Spray drift is the main cause of incidents involving mortality of forager worker bees [63, 74], whereas ingestion of contaminated pollen, nectar and water is at the root of the CCD malady that affects many apiaries of the world [45], affecting mainly the nurse workers and the queen in particular [49, 51]. In addition, the acaricides used in *Varroa* treatment pose a significant risk mainly to the bee larvae, and consequently to the long-term sustainability of the colonies. Awareness of these threats can help beekeepers and farmers draw specific management plans to avoid pesticide impacts.

Beekeepers should be aware of the landscape environment on which their managed bees forage, bearing in mind that a large proportion of the land in developed and developing countries is used for agricultural production where pesticides of all kinds are used on a regular basis. Since usage of these plant protection products cannot be stopped, as they are necessary for agricultural production, a rational approach must look at minimising the risks of such agrochemicals to bees.

Chemical companies are obliged by law to state on the labels whether their products are dangerous to bees or not. If so, they must specify the risks they pose and the specific actions to take, such as “DO NOT spray any plants in flower while they [the bees] are foraging.”

However, label warnings are ineffective unless there is proper communication among the applicators, farmers and beekeepers. It is the responsibility of the former to ensure that beekeepers are informed of any spraying operations, so that hives are moved to a safe location during the spraying season. Moving hives usually takes more than 24 hours, so farmers must notify their neighbouring beekeepers with sufficient time in advance. Only thus damage by drift to the hives can be avoided.

Bees are generally active between sunrise and an hour or two before sunset, and most honey bees forage within a 2–4 km radius of their hive, although may travel as far as 7 km or more in search of pollen and nectar when their local sources are scarce [75]. Therefore, pesticide risk to bees can be reduced by spraying the crops in the evening, when bees are not foraging.

Despite all precautions, if an area in which the crop or weeds were in flower has been sprayed inadvertently, the farmer should notify the affected beekeepers in order for them to take appropriate action. This should ensure the managed bees are kept out of that sprayed area for a while. As well as the cropping areas, damage may occur when pesticides drift over the neighbouring vegetation that is foraged by bees, including hedges, road-side weeds and trees, such as fruit trees, eucalypts, etc. For example, coolibah trees (*Eucalyptus microtheca*) grow on plains along many river courses in the cotton growing areas of Australia and are a primary source of nectar and pollen for wild and honey bees. The Australian cotton industry has produced a best management practices manual in which, among other recommendations, indicates to the cotton growers how to deal with this issue and be aware of the possible damage to beekeepers. “With good communication and good will,” says the manual, “it is possible for apiarists and cotton growers to work together to minimize risks to bees, as both the honey industry and cotton industry are important to regional development.” [33].

In summary, awareness of the problems that pesticides have for bees should prompt appropriate actions by all parties involved in order to minimise the chemical impacts on bees and the productivity of the apiarist industry. Such actions must aim, first of all at managing the use of agrochemicals in ways that do not harm other producers of the land. In addition, farmers should minimize the contamination of the surrounding landscapes, including water bodies, with pesticides, because not only honey bees but a large array of pollinator species (e.g. butterflies, bumblebees, hoverflies, etc.) may also be affected.

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