Intoxications

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Intoxications

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General considerations

The close relationship between bees and plants does not always favour the perenniality of bee species. In agroecosystems the bee is often a sensitive indicator, sometimes suffering from the presence of residues of agrochemicals. If the nature and level of residues are unfavourable for the honeybee, toxicity can be observed, either within the bee as an individual, or within the network of relations between the thousands of individuals forming the colony.

For this social insect acute toxicity, indicated by death, is obviously important, but there may also be effects of sub-acute or chronic toxicity. In these latter cases, the individual survives but it does not fulfil its normal social function and thereby the social organisation of the colony progressively decays, especially if exposure persists for a long time.

The particular susceptibility of the bee colony must be understood when surveying the quality of the environment. The honeybee is a beneficial insect fulfilling a vital role in both agricultural and natural landscapes. In accordance with the proposal of Fossato (cited by Accorti *et al.*, 1991), the bee fulfils three criteria of a good ecological indicator: (i) the criterion of presence-absence, which means the absence of the species in a particular biotope is correlated with severe disturbance; (ii) the criterion of collector-accumulator, which means that the 30,000 bees of one colony can hoard contaminated food foraged from an area of a dozen square kilometres; and (iii) the criterion of reliability, which shows consistency in the type and the intensity of reaction to a certain environmental contaminant.

The aim of this chapter is not to provide details of the range and type of possible pollutants or the amount the bee can hoard in the hive, but to propose some general schemes of poisoning in order to facilitate appropriate sampling and to highlight the difficulties in accurate diagnosis. This diagnosis is generally based on three criteria: (i) absence of major disease; (ii) colony history and field symptoms; and (iii) the identification and quantification of residues found in the dead bees, in the hive or in the crop. The confirmation or denial of poisoning based on only one of these criteria is always questionable.

Epidemiology

The frequency of intoxications is obviously correlated with that of the use of agrochemicals. At the present time the forager bee is considered the main pollinator of crops, thereby its protection becomes a prime necessity in a coherent agrosystem.

Since the forager bee may be exposed to agrochemicals whilst visiting flowers and collecting pollen or nectar, the best recommendation is to avoid the application of chemicals during flowering or, at least, to use compounds which are less toxic for adult bees. When these recommendations are followed, the loss of whole apiaries on the day of crop treatment or the following day, does not occur.

Such accidents can still occur when areas where pest insects such as mosquitoes, flies and locusts reproduce, are sprayed by plane with insecticides toxic for adult bees. The use of agrochemicals less toxic for adult bees (for instance the insect growth regulators) sometimes results in contaminated food being carried back to the hive, and may thus affect another caste of adult bees or immature bees. Such cases of sub-acute intoxication are most likely responsible for a general weakening of colonies without symptoms of disease.

Etiology

Toxins

Plant toxins

Poisoning by plants is generally rare because bees usually avoid the sources of toxic nectar or pollen. However, in the absence of familiar nectariferous or polliniferous plants, bees are forced to forage on the poisonous ones. For example, when the season is particularly dry bees may forage exclusively on the pollen of *Ranunculus auricornus,* a flowering plant present in moist meadows, that impairs the ability of bees to fly. Among the main toxic plants, the more frequently cited are *Aesculus* spp., *Digitalis* spp., *Ranunculus* spp. for their poisonous pollen and *Tilia* spp., *Aesculus* spp., *Rhododendron* spp. for their poisonous nectar. The toxic substances from plants include compounds such as alkaloids and saponins or sugars such as galactose.

Mineral toxins

Environmental pollution arising from industrial gases or smoke has become rare because of the general improvement in the efficiency and widespread use of anti-fog devices. Industrial exhaust gases contained a variety of mineral acids and metals such as lead, arsenic and fluoride. These compounds were deposited as dust on flowers, and contaminated the pollen or nectar collected and stored in the hive, which was consumed by the adult bees or fed to the brood immediately after storage or some time later. In these cases the poisoning was often chronic, inducing daily small losses of adult bees or larvae. Some arsenical salts used as insecticides or acaricides also produced similar effects.

Agrochemicals

The majority of the agents toxic to bees are found in the compounds used as insecticides, and less frequently, in acaricides, fungicides, or herbicides. The testing of insecticidal compounds against beneficial insects is often a prerequisite of registration, which explains our better knowledge of the side effects of insecticides on the honeybee. This chapter deals mainly with insecticides and occasionally with other agrochemicals. Some chemical families contain insecticide molecules, but other molecules of the same family may have different properties.

The main chemical families of insecticides

Organochlorines: Although the DichloroDiphenylTrichloroethan (DDT) molecule was synthesised in 1874, its insecticidal properties were not fully recognized until 1939. The molecule is of remarkable chemical stability, which gives it an extraordinary permanency in the environment and in the fatty tissues of animals. These properties were considered an advantage in the initial use of DDT but they favour the accumulation of the compound in the fat bodies of carnivorous or predatory animals and even the human consumer. If the animal undergoes a period of starvation the fatty tissue is metabolised, releasing DDT into the general circulation where it causes various disorders. After the synthesis of some DDT by-products, gamma-hexachlorocyclohexane (lindane) gained considerable success in pest control. Containing at least two chlorine atoms, the cyclodiene compounds display a strong insecticidal activity associated with their remarkable stability. Dieldrin, which belongs to the same family, is still used in the control of the migratory locust in the Sahel area. However the drawbacks are similar to that of DDT and generally the use of these molecules is no longer permitted.

Organophosphorus compounds: These compounds were first studied and used as an asphyxiating gas during the second world war because of their strong anticholinesterase activity. After Schrader discovered the insecticidal properties of methylparathion, less or non-volatile compounds were synthetized and used until 1944. At the present time, more than a hundred molecules are used in pest control. The methyl- or ethyl-esters of orthophosphoric, thionophosphoric, thionothiophosphoric and pyrophosphoric acids are the most active.

These molecules are less stable than the organochlorines. However the stability of some molecules in the environment can exceed three months. Their degradation in vertebrates occurs more readily than in the case of organochlorines.

Carbamates: The dimethylesters of carbamic acid were the first to be discovered in 1947, but only the methyl-esters are of practical use. Although the biological target of the carbamates is the same as that of the organophosphorus compounds, their insecticidal activity is more selective and depends to a certain extent on the insect species, which is of more interest and relevance for pest control. Generally their insecticidal properties are targeted against the adult insect. However, the exception is the fenoxycarb molecule, which is an antagonist of juvenile hormone and its action inhibits the metamorphosis of larvae. Some fungicides and herbicides belong to this family.

Pyrethroids: The insecticidal properties of powders or extracts of *Chrysanthemum* spp. or *Pyrethrum* spp. have been known since antiquity. The pyrethrin and cinerin molecules having these properties are destroyed within a few days by sunlight. The first photostable compound of this family was permethrin synthetized in 1973. The stability and the reactivity of the natural molecules were further improved by adding various atoms or groups (Cl, Br, aromatic rings, etc.) to the chrysantemic acid. From a chemical or pharmacological point of view, two sub-groups of pyrethroids are distinguished: with or without the cyano group.

Urea derivatives: Diflubenzuron was the first molecule of this family to be developed. It acts by inhibiting the synthesis of chitin at the end of the larval moult. Other molecules of the family are also larvicidal.

Benzhydrazide: At the present time, few molecules of this group are registered for use. The activity of the molecule mimics ecdysone, the moulting hormone, lethally accelerating the moulting process, thus it is considered as larvicidal.

Formulation and penetration into the insect

The aim of the formulation is twofold: (i) to make the molecule easily dispersible on the crop and available for the insect; and (ii) to aid the extensive and rapid penetration into the insect. To make the commercial product, the active ingredient is first diluted in solvents such as aromatic hydrocarbons, glycols or acetones. In addition, surface active agents are included to increase the anti-static and lubricant properties and dispersal. In some formulations, molecules specifically inhibiting the degradation of the active ingredient by the insect increase the toxicity of the formulation, for instance, pyperonyle butoxid for the pyrethroids. In general, the adjuvants in formulations often have insecticidal properties.

The hazard for bees comes from agrochemicals sprayed onto crops principally. The volumes sprayed by terrestrial devices, range between 1000 l ha^{-1} to 50 l ha^{-1} depending on the crop. There is an increasing trend to reduce the volume of the solution applied in the field (Reduced Volume Application) which means more concentrated solutions are used, especially for aerial spraying. With this type of application, the volume sprayed per hectare can be lower than 5 l (Ultra Low Application). The fineness of the sprayed solution is positively correlated with the concentration, which increases the persistence and the toxicity of the insecticide. The insecticidal effect is enhanced when the formulations are not diluted in water but in vegetable or mineral oils. Since these aerosols need some minutes to deposit on the ground, drifting on air currents may lead to an increased deposition on non-target areas.

The formulation of the insecticide in polyamide micro-capsules is also particularly hazardous for the honeybee. These micro-capsules are porous envelopes with diameters ranging between 10 to 50 microns, filled with approximately one microgram of active ingredient, which is very slowly released through the pores. The dimensions and the weight of the micro-capsules are similar to those of a pollen grain, so that foragers may collect them and add them to their pollen loads.

Three main means of penetration are recorded for insects: (i) per os; (ii) by contact; and (iii) through the respiratory spiracles.

(i) The bee hoards nectar and pollen from the flowers on which it forages. Poisoning can occur by ingestion during the return flight, only if the toxin is at high concentration in the nectar and if it rapidly crosses the epithelium of the bee crop. Often the contaminated food is stored in the hive and is subsequently directly consumed by adult bees or fed to larvae in the secretions provided by the nurse bees. Thus the nurse bees, the queen and the larvae are generally poisoned per os.

(ii) Contact can occur in two ways:

- A large area of the body surface can be contaminated when foragers are present in the crop during the spraying of the agrochemical; or

- Small areas of the body surface frequently contact the sprayed plant surface during foraging activity.

In the first case the toxin can cross the cuticle over the whole body surface of the insect. In the second case, the toxin enters through the areas which are the most frequently in contact with the sprayed surfaces; the tarsi and the antennae. These surfaces bear many sense organs which enhance and aid the penetration of toxins acting on the nervous system.

(iii) The toxin can penetrate the bee through the spiracles and the tracheae when the droplet size in sprays is less than ten microns in diameter. These fine droplets, called aerosols, are produced when the application method of the agrochemical solution is by ultra low volume spray.

Factors affecting the severity of poisoning

Environmental factors

Temperature

In the case of organophorous or carbamate compounds, the higher the temperature, the more toxic is the compound. The converse is true for pyrethroids and cyclodienes.

Humidity, light

The toxicity of various organophosphorous compounds is positively correlated with humidity. Light influences the activity of many insects, including bees, and thereby affects their exposure to poisoning.

Chemical degradation

Some metabolites of the active ingredient can be more toxic than the original compound. A well known example is the -oxon form of some organophosphorus compounds.

Factors dependent on application

Formulation

The distribution of the toxin in the environment, its stability, its penetration into the insect and its metabolism depend primarily on the type of formulation.

Mixture and synergy

In order to reduce the number of field applications, farmers often mix different types of agrochemicals. If the formulations are chemically compatible, a synergistic effect occurs in some cases, greatly increasing the toxicity of each individual component of the formulation. This synergy was defined by Macht (1929) as "the phenomenon exhibited by the combination of two or more drugs in which the pharmacodynamic effect produced by the mixture is not a simple summation of the effects produced by the two or more individual components. Such combinations produce a pharmacological effect of an unexplained nature in that the effect of one component may be greatly heightened or potentiated by the other".

Concentration

Generally, the more concentrated the formulation, the more toxic is its effect. However, in the case of per os penetration, some insecticides can be repellent at high concentration.

Exposure time

This depends on the mode of application, on the environmental stability of the formulation and on the accessibility of the treated surfaces to bees. Puddles, present in the field before spraying or created by run-off when it rains just after spraying, can be contaminated with agrochemicals and provide a source of toxins when bees need water.

Droplet size and nature

As discussed earlier, toxicity is inversely correlated with the diameter of the droplets and oil formulations are generally more toxic. The means of penetration through the respiratory ducts can become important if the droplet diameter is less than ten microns. Moreover, aerosols can be dispersed by the wind and reach non-target crops.

Factors dependent on the insect

Age

There is great variability in the effect of agrochemicals on bees which depends not only on the type of active ingredient but also on the life stage or age. It is often difficult to distinguish between adult bee mortality due to natural ageing or exhaustion due to intense foraging activity and mild poisoning. Newly emerged bees are the most susceptible to DDT and carbaryl. By contrast, older bees are more susceptible to malathion and methylparathion. The youngest stages of larvae are generally the most susceptible.

Nutrition

Starvation significantly increases susceptibility to toxins, probably because the fat bodies, which are an important site of detoxification, are considerably reduced in size under such circumstances.

Sex and caste

The primary role of drones is to mate with virgin queens. Excluding their reproductive role, their contribution to the life of the colony is considered negligible. By contrast, the queen fulfils an essential role as the only reproductive female. Adequate nutrition of the queen is essential to enable her to lay up to 2000 eggs a day, but the particular susceptibility of queens to toxins is not known.

Resistance

Bees may have the ability to develop resistance to certain insecticides.

Group effect

This effect was first described in *Aedes aegypti*. The greater the number of insects in a defined volume, the greater the susceptibility of individuals to the toxin. Although similar effects have not been demonstrated for bees, one can imagine that 5000 adult bees per litre is a very high concentration of insects.

In conclusion, the additive action of each individual factor which is harmful for the insect, can increase the toxicity more than 100 times.

Targets

Nervous system

Until recently, the main toxic activity of insecticides focused on nervous targets. Various structures and functions of the nervous system are affected, leading to a disruption of transmission of nervous signals from one neuron to another, to a neurosecretory neuron or to a muscle.

Axonic conduction

When the nerve signal moves along the nerve axon, the change in the membrane potential triggers firstly the opening, and secondly the closing, of the sodium ion channels. A sodium current crosses the membrane into the axon and then outwards within a few milliseconds. The action of pyrethroids causes a dysfunction of the voltage sensitive channel and the inward sodium current is not fully stopped, producing a residual current, called a tail current. The DDT analogues and the pyrethroids (mainly the type II) produce a prolonged tail current. The amplitude of the tail current is positively correlated with the degree of toxicity and the gravity of the intoxication symptoms. Due to the depolarisation of the nerve terminal and action on the voltage sensitive calcium channel, the release of the neurotransmitter, contained in the synaptosomes, is improperly regulated. The enhanced neurotransmitter release causes a block in the conduction of the nerve impulse. These phenomena are observed not only in the central nervous system but also in the sensory structures and in the muscles. The release of neurohormones can also occur at the terminals of the neurosecretory neurons, increasing the deleterious effects of the intoxication.

Synaptic transmission

Acetylcholinesterase

This enzyme hydrolyses the acetylcholine neurotransmitter, inhibiting the transmission of the nervous signal. Organophosphorous and carbamate compounds block the enzyme. However, five different forms of the enzyme are recognized in the central nervous system of the bee. Three forms are bound to the synaptic membrane and two are free in the synaptic space. The forms display various physical and biochemical properties, including a variable resistance to the action of organophosphorus compounds and carbamates. The relative proportion of the different forms varies with the developmental stage, especially with foraging and learning activities, which suggests a precise dose of one of these compounds may have variable effects.

GABA inhibitory synapses

The antagonistic effect of the inhibitory neurotransmitter gamma amino butyric acid causes a hyperexcitation of the whole nervous system. The GABA receptor-chloride ionophore complex is blocked by molecules belonging to the organochlorine family such as the cyclodienes and lindane. The type II pyrethroids are also blockers but only at high concentrations. The complex is also blocked by a new phenylpyrazole molecule, named fipronil. The avermectins also affect synaptic transmission by increasing the conductance of chloride ions.

Nicotinic acetylcholine receptor

Pyrethroids probably interact with a site of the nicotinic acetylcholine receptor without affecting ion transport.

Intracellular homeostasy

DDT and pyrethroids have an inhibitory action on various ATP-ases: the calcium ATP-ases involved in the regulation of intracellular calcium, the sodium and potassium ATP-ases involved in the neurone ion pumps and the magnesium-ATP-ases of the mitochondria.

Endocrine system

Some insecticides have larvicidal effects because of their action on the process of moulting. The urea derivatives inhibit chitinase, which prevents the synthesis of the cuticle of the new larval stage. Fenoxycarb mimics the action of the hormone JH III, which maintains the juvenile features of the larvae. It inhibits the triggering of moulting of early instar larvae and disrupts metamorphosis. By contrast, tebufenozide, an ecdysone antagonist which binds to the receptor protein, accelerates larval and imaginal moults.

Symptoms

Individual level

If the dose of pyrethroids is near the LD_{50} they rapidly produce symptoms of intoxication in adult bees: loss of coordinated movements, periods of convulsive activity and ultimately paralysis, rapidly leading to death. Moreover, the type II pyrethroids, containing a cyano moiety, cause a profuse salivation and a sinuous writhing.

In the case of organophosphorus compounds, the bees become irritable and easily excited. The symptoms are remarkable considering the low doses (ten times or more lower than the LD_{50}) of the agrochemicals.

In the case of pyrethroids, Cox and Wilson (1984) give some detailed features of intoxication with sub-lethal doses of permethrin. Abnormal behaviour is observed such as trembling, rolling, curling of the abdomen, self-cleaning of the abdomen and leg rubbing. Normal behaviour patterns are modified; activities in the cells, walking over the comb surface, trophallaxis and antennal communications are less frequent. In contrast, the time devoted to normal self-cleaning activity is increased.

Colony level

The first individuals of the colony that come into contact with treated plants are foraging bees. If the agrochemical has been applied recently or if the bee comes into direct contact with the spray, it can lead to overacute toxicity, indicated by the death of bees in the treated field. At slightly lower doses, the foragers are sometimes able to return from the field and they generally die near the hive.

With pyrethroid poisoning, the foragers are disorientated and unable to fly home. Consequently, only a few bees die near the hive. The colony is subject to a progressive loss of foragers and if this persists for one or two weeks, an imbalance between the number of adult bees and the amount of brood occurs. It may appear to beekeepers that there is "too much brood" in the colony. Brood at the outer edges of the comb is inadequately heated, which creates optimal conditions for the development of brood diseases such as ascosphaerosis.

In the case of organophosphorous compounds and carbamates, acute toxicity causes the death of foragers in the field, anywhere between the field and the hive, and at the entrance of the hive. At lower doses these compounds can enter the hive and come into contact with another age class of bee that performs different duties. If the hive bees are severely affected, then all hive activities cease and the colony rapidly collapses. If they are only partially affected, the brood temperature cannot be regularly maintained over the whole brood surface. Brood chilling is often followed by an outbreak of mycosis.

When the foragers are only slightly affected, either because the agrochemical is relatively harmless for the adult bees or when the doses are very low, contaminated nectar or pollen can be stored in the hive. After a variable period, adult bees have the opportunity to feed on contaminated honey. One can imagine then that sometimes the contaminated bees are unable to regulate the temperature of the winter cluster. This could be one explanation for the mortality of colonies previously exposed to agrochemicals. Contaminated stored pollen, converted to brood food by the nurse bees, can reach either young larvae or the queen, leading to the death of the brood when insect growth regulators are involved. Concerning the queen, she can either accept or reject the contaminated food and may solicit food from healthy bees. In the former case, the queen often ceases egg-laying and is removed by the bees. Requeening in these conditions is always hazardous.

Sampling and dispatching

In order to find the cause of the intoxication, sampling consists of gathering freshly dead bees, the treated or visited plant and the agrochemical. The majority of bees die near the hive entrance when the poisoning is due to organophosphorus compounds or carbamates. On the other hand, bees poisoned by pyrethroids can die in the treated field or anywhere between there and the hive. Since pesticide degradation is often rapid, sampling should ideally occur as soon as possible after the crop has been treated. After packing in aluminium foil, the samples must be frozen immediately and maintained at this temperature during dispatch to the laboratory.

Diagnosis

Keeping in mind that the best diagnosis is founded on three bases, as outlined in the Section on General considerations, this section is mainly devoted to residue detection in bees, hive products and plants.

The detection of agrochemical residues is generally conducted by means of gas chromatography coupled to mass spectrometry. The preparation of the bee extract is generally similar whatever the agrochemical, whereas the conditions for gas chromatography are highly variable, and dependent on the nature of the molecule.

As an example, we give the method for detecting deltamethrin. Five grams of bees (45 bees) were homogenized with an Ultra-Turrax mixer in 50 ml of an acetone:hexane (2:1, v/v) solution. The homogenate was filtered through a Whatman GF/A filter covered with a thin layer of Hyflo-Supercel. The fraction retained on the filter was extracted in an acetone:hexane (1:2, v/v) solution and filtered as above. The resulting filtrates were pooled and evaporated to dryness. Fatty material was removed by extraction with 30 ml of hexane-saturated acetonitrile and 90 ml of acetonitrile-saturated hexane. The acetonitrile phase was subjected to liquid partitioning by the addition of 200 ml of water and 20 ml of NaCl saturated water, followed by three extractions with 50 ml of hexane. The three organic phases were pooled and dried with anhydrous sodium sulphate for 2 h with constant agitation and evaporated to dryness. The sample was dissolved in 10 ml of hexane and loaded on a Sep-pack Florisil Plus cartridge (Waters, Milford, MA). The cartridge was washed with 10 ml of hexane, and the sample eluted with 25 ml of a hexane:ethyl ether (90:10, v/v) solution, evaporated to dryness, and resuspended in 5 ml of hexane. Using this method, the recovery of deltamethrin was 95% of the initial dose present in the bees, as determined in preliminary experiments. Samples were analysed with a Girdel 30 gas chromatograph equipped with an electron-capture detector and a JWDB 17 column (J & W Scientific, Folsom, CA). The temperatures of the injector, the column, and the detector were 280°C, 260°C, and 290°C, respectively. The sample volume injected for analysis was 3 µl. The detection limit was 2 pg of deltamethrin representing 0.7 µl of deltamethrin per kg of tissue (i.e. 0.074 ng.bee-1).

In conclusion, the detection of pesticide residues in bees provides only one element to solve the problem posed by poisoning, because several factors affect the reliability of analysis of material collected from the field: (i) the specificity of the conditions for gas chromatography makes uncertain the identification of an agrochemical when no information on the field treatment is available; (ii) a level of residue below the detection limit does not always mean the absence of toxic compounds because the pesticide can be metabolised or degraded in the period between the collection of bees from the field and the arrival of the samples at the laboratory; and (iii) several active compounds can be detected in the same sample.

Therefore, the presence of an agrochemical in dead bees only means that the insect has been in contact with it, but it does not give any information on the dose received by the bee and eventually brought back to the hive.

Treatment

Today, no antidote to bee poisoning is available. Sometimes management methods can arrest the development of the poisoning. If the intoxication exclusively affects the foraging bees, the colonies have to be moved quickly to another site. If the adult bees are only slightly affected, but the toxin is present in the hive reserves, the adult bees and the brood have to be transferred to a clean hive and moved from the area. However, when the social structure is severely disturbed, for instance by the complete disappearance of the foraging bees, any stress such as moving the colony, can be fatal. Requeening after poisoning always favours recovery.

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