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Registration of systemic insecticides and European and Mediterranean Plant Protection Organisation (EPPO) guidelines

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Abstract

French bee scientists and experts involved in the registration of agrochemicals have a special concern in the assessment of the toxicity and hazards of systemic substances to which bees are exposed. EPPO guidelines and the decision making scheme have been established only to estimate the effects of spray treatments on honey bees and they do not refer to any specific method for assessing indirect effects due to systemic insecticides and to low doses modifying adult bee behaviour or causing delayed actions. Therefore three new laboratory methods have been set up by several scientists for estimating indirect effects of low concentrations of compounds on bees:

1/ a sub-chronic test on adults,
2/ a proboscis extension reflex test followed by a cage test on artificial flowers,
3/ a brood feeding test.

These methods should be regarded as sentinel tests, which implies that further testing in realistic conditions is also required before registration.

Key words: Systemic insecticide, *Apis mellifera*, lethal and sublethal effect, test method, larva, adult.

Introduction

Systemic insecticides may be applied to ground, seeds, or aerial parts of plants before bloom. Bees may be affected by these treatments in a different way compared to contact insecticides currently applied to crops by spray. Therefore specific procedures and test methods should be recommended for risk assessment.

Characteristics of systemic insecticides

An insecticide is regarded as systemic when it penetrates into the treated plants through the vascular system or leaf cuticle and kills piercing and sucking pest insects which feed on sap or cell content. The first of these compounds with such properties has been dimethoate, followed by phorate and aldicarb, then later by imidacloprid. Some of their formulations are sprayed and will intoxicate insects via dermal and oral route. (formulated dimethoate and acephate), whereas others are always incorporated to the ground (aldicarb and carbofuran). Imidacloprid can be used either as seed dressing treatment or foliar spray according to the crop and the target pests. If the usage is a seed or ground application, the only risk to be considered derives from the ingestion by bees of contaminated nectar and pollen. This contamination depends on the solubility of the compound in water. The solubility of acephate, which is a typical systemic insecticide, is very high (790 g l^{-1}) whereas those of imidacloprid and fipronil used as a seed dressing, are only 0.6 g l^{-1} and 0.0019 g l^{-1} respectively. The third of these compounds which is not effective for aphid control is not considered as systemic.

Overview of the impact of systemic insecticides on honey bees

Oral toxicity

A number of articles report on the oral toxicity of various systemic active substances, assessed by laboratory feeding tests. Considering the LD_{50} figures ranging from 3.7 ng/bee (Suchail *et al*., 2001) to 1370 ng/bee (Atkins and Kellum, 1986) it is concluded that theses compounds are highly toxic to honey bees by ingestion.

Residues in nectar and pollen

Chemical analysis of samples of nectar and pollen from treated plants showed they contained residues of the systemic compounds applied to ground or to plants (onion, lemon-tree, apple-tree, lucerne, rape). For most of the systemic substances the residues ranged from 0.02 mg kg $^{-1}$ aldicarb (Knapp and Ansonmoye, 1988) to 8 mg kg^{-1} acephate (Fiedler and Drescher, 1984). For dimethoate the range was 0.1 - 7 mg kg^{-1} according to Waller *et al*. (1979, 1984). In the case of imidacloprid special analysis techniques with low determination levels, showed that residues in sunflower florets reached 0.002 mg /kg^{-1} in nectar and 0.004 mg kg^{-1} in pollen (Schmuck *et al*., 2001).

Effects of sublethal concentrations on honey bees

On adult:

Laboratory feeding tests showed that low concentrations of systemic compounds e.g. 0.25 mg kg^{-1} dimethoate, acephate or methamidophos applied to adults and causing no acute mortality, killed 50% of the bees exposed for two weeks to contaminated sugar solutions

(Fiedler, 1987a). Besides, sublethal effects with some substances have been reported on:

- The consumption of food contaminated with acephate or aldicarb sulfoxide at 0.25 mg kg-¹ (Fiedler, 1987b; Nigg *et al*., 1991)
- The egg laying of queens when colonies were fed syrup contaminated with dimethoate at 0.2-0.4 mg kg-¹ (Lensing, 1987).

On eggs and larvae:

- Feeding colonies with syrup contaminated at 0.1 mg kg-¹ acephate or dimethoate affected larval survival (Davis and Shuel, 1985; Ferguson, 1987; Davis *et al*., 1988).
- Absorption of sugar solution at 1 mg kg-¹ dimethoate inhibited egg hatching (Waller and Barker, 1979).

These data show that honey bees may be at risk via the oral route when registered doses of dimethoate, acephate and methamidophos were applied to various crops. When adult bees ingested sugar solution at low concentrations similar to those determined in nectar of treated crops, they did not show acute mortality but either died after a 2 week exposure or affected larval development through nursing and possible concentration process of the insecticide. Some of the sublethal effects mentioned above, such as the alteration of egg laying and hatching, had a direct impact on the colony development.

Standardized tests on survival and behaviour alteration

In France the use of insecticides belonging to the former chemical families (organophosphorus, carbamates, organochlorines, pyrethroids), has been reduced by 50% in weight over the last ten years, whereas that of neonicotinidoids with systemic properties (imidacloprid) has grown rapidly. Imidacloprid is characterized by a new mode of action on the nervous system, the molecular target being the nicotinic acetylcholine post-synaptic receptor. Due to their generalization, the seed treatments with imidacloprid have been suspected of endangering honey bees through the ingestion of nectar and pollen from sunflower and maïze, contaminated at low concentrations. Intoxications have been assumed to cause lethal or sublethal effects on colonies. Considering the various symptoms reported by a number of French beekeepers, scientists investigated the effects of low concentrations on adult longevity, behaviour and larval survival according to the EPPO decision scheme where it is recommended to study indirect effects of insecticides such as systemic effects, delayed action, alteration of behaviour through "special tests" preferably conducted in cage or field. Unfortunately no reference of "special test" is given in the scheme.

It has been evidenced that alteration of orientation capabilities could hardly be tested in enclosure and in addition field testing experiments have been criticized and the proceeding results rejected on the argument that the low dissipation rate of imidacloprid impeded access to potential control areas deprived of soil residues. Therefore laboratory methods seemed more appropriate to collect relevant data under standardized conditions. Three tests have been selected:

1- A sub-chronic feeding test on adults.

2- A larval feeding test.

3- A test on the Proboscis Extension Reflex (P.E.R.).

These tests should provide an LD_{50} (1 and 2), a LOEC (1-2-3) and a NOEC (1-2-3).

The sub-chronic feeding test on adults has been described by Pham-Delègue *et al*. (2000) and Suchail *et al*. (2001).

The larval feeding test has been inspired from the method published by Peng *et al*. (1992). It is notable that thanks to a special handling and artificial diet , control mortality can be less than 10% during the predefecation period.

The P.E.R. (Decourtye and Pham-Delègue, 2002) is based on an associative learning. It is performed with workers restrained in a harness during trials where they are conditioned to an odour thanks to a sugar reward. When the reward is no longer offered the rate of positive responses to the odour measures the alteration of the learning process and olfactive memory.

These laboratory tests should be validated by conducting trials in more realistic conditions. In the case of the P.E.R. test a complementary cage method has been established to test the modifications of foraging behaviour (Pham-Delègue *et al*., 1993; Decourtye *et al*., 2001). This test uses a free flying colony having access to six artificial flowers. Each flower comprises a feeder containing a sugar solution and two odour dispensers on its sides. This device enables associative learning as in the P.E.R. test. A conditioning period with control or treated syrup is followed by a test period where bees have to discriminate scented and unscented feeders without food.

Conclusion

The tests mentioned above have been set up because they were adapted to sub-chronic exposure of bees to sublethal concentrations of systemic compounds resulting from plant treatments. The larval feeding test should serve in the screening procedure before registration, whereas the sub-chronic feeding test on adults and the P.E.R. test should be used to investigate the toxicity of systemic insecticides if residues proved to be translocated to nectar and pollen of treated plants.

Thanks to their standardization and sensitiveness they allow comparisons between systemic compounds. As they should be regarded as sentinel tests, further testing in realistic conditions are also required before the test compound is registered.

Additional methods should be set up to assess the synergy of systemic insecticides with other compounds or with bee diseases.

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