Effects of spinosad on honey bees (*Apis mellifera*): Findings from over ten years of testing and commercial use

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Abstract

Background: Spinosad is widely used as an insecticide in crop protection against thysanopteran, lepidopteran and dipteran species. As such it is intrinsically toxic to insects and among them to the honey bee (*Apis mellifera*). An updated risk assessment is presented in the context of the regulatory evaluation of spinosad products and is in accordance with the latest recommendation of regulatory guidance documents.

Results: The intrinsic toxicity to the honey bee as observed in laboratory conditions through oral and contact tests on adults does not appear to impair honey bee colonies when exposed to treated attractive crops in tunnel conditions. Reasons for this could include reduced availability of residues of the product on plant surface compared to laboratory conditions, together with a fast dissipation from treated plants and the absence of active degradation products.

Conclusions: Spinosad products present a negligible impact on honey bees when used under the current label recommendations and conditions of agricultural use. This conclusion deduced from data available for the regulatory risk assessment has been confirmed by the feedback of surveys on incidents, which address the potential impact of spinosad products under realistic conditions of exposure, including other environmental and chemical factors that are common in cropped areas.

Keywords: honey bee, pesticide, risk assessment, risk management, spinosad

1. Introduction

Spinosad is an insect control agent derived by fermentation of the Actinomycete bacterium, *Saccharopolyspora spinosa*. The active ingredient is composed of two metabolites, spinosyn A and spinosyn D. Spinosyns (such as spinosad and spinetoram) have a novel mechanism of activity on nicotinic acetylcholine receptors which is identified as the primary cause of death.[1] The action of spinosyns on nicotinic receptors is unique in comparison with other insecticides and is at a different site from that of nicotine and imidacloprid.

In common with other insecticides spinosad is intrinsically toxic to honey bees. In laboratory tests worker honey bees were exposed orally (in sugar water diet) or to doses topically applied. LD_{50} values for technical material of 0.057 and 0.0036 µg a.s. bee^{-1} for oral and contact routes of administration respectively were recorded. Similar levels of toxicity were exhibited for a 480 g a.s. L^{-1} SC formulation containing spinosad with LD_{50} values of 0.049 and 0.050 µg a.s. bee^{-1} for oral and contact routes of administration respectively.[2]

Spinosad achieved its first registration 1998 in the US for control of bollworms in cotton. Since then it has been registered globally on over 150 crops in North America, Latin America, Asia, Europe and Australasia under a variety of trademarks (such as Tracer, SpinTor, Entrust, and Success; registered trademarks of Dow AgroSciences LLC.). Spinosad is used in vegetables, fruit trees, turf, viticulture and ornamental cultivation to control lepidopteran larvae, thysanopteran some dipteran, coleopteran and hymenoptera pest species.

It is important that plant protection products (PPPs) are authorised for use only in ways that do not pose an unacceptable risk of harm to honeybees. Data should be obtained to enable the safety to be evaluated. An evaluation of the risks to honey bees has thus been undertaken in every country where the product is authorized based on the data available at this time.[3,4] Rules for risk evaluation have been updated since, in Europe as well as in the United States, which require that potential effects on the development of larvae and the behaviour of adults are considered at earlier steps of the risk

assessment process.[5,6,7] Beside the regulatory risk assessment, it is useful to look at the feedback from public research and from field surveys of apiaries in the countries where the product is used.[8,9]

This paper summarises the effects of spinosad to the honeybee (*Apis mellifera*) and updates the current knowledge on this substance. Data come from a range of Dow AgroSciences reports conducted to meet regulatory requirements. This dataset has been complemented with a survey of literature data and feedback from surveys on incidents, if any, involving honey bees in the countries where the product is authorized from the past 10 years of use.

2. Experimental methods

Semi-field studies were re-analyzed with regard to mortality data with the aim to characterize and address uncertainties on this parameter as a function of the application rate. Ten trials performed in France, UK and Germany were used. These studies (tunnel + cages) were conducted according to CEB method n°129 and EPPO guideline 170.[10,11] Application rates were ranging from 10 to 540 g a.s./ha, depending on the pest, sprayed onto flowering crops. The crops (*Phacelia*, oilseed rape, wheat with sugar solution) were selected to maximize foraging and exposure. These ten trials were analyzed for mortality rates at the key use rate of 96 g a.s./ha. These studies are summarized in table 1.

Tab. 1 Description of the semi-field trials used for the analysis of mortality rates at 96 g a.s./ha.

Year	Site type	Country	Crop	Application type	Rate (g a.s./ha)	Number bees/hive	Replicates
2004	Tunnel	France	*Phacelia*	During bee activity	144 96	10,000	1 2
2002	Tunnel	France	Winter wheat with sugar	During bee activity	96 144	10,000	2 1
2003	Tunnel	France	*Phacelia*	During bee activity Evening	144 144	10,000	1
2006	Tunnel	France	Rape seed	During bee activity During bee activity During bee activity During bee activity Evening	96 50 10 20 96	10,000-15,000	1
2006	Tunnel	France	Rape seed	Evening	96	10,000-15,000	1
2010	Tunnel	Germany	*Phacelia*	Evening	96 76	3,500-4,000	3
2010	Tunnel	Germany	*Phacelia*	During bee activity Evening During bee activity Evening	76 96 76 96	3,500-4,000 3,500-4,000	3 3
2010	Tunnel	Germany	*Phacelia*	Evening	76 96	3,500-4,000	3
2002	Tunnel	France	*Phacelia*	During bee activity	96 144	10,000	2 1
2000	Tunnel	United Kingdom	*Phacelia*	Morning	144 540	3,500-4,000	1 1

To allow for a comparison across the studies a mortality index was calculated according to the CEB method:
tox = Ma/Mb x Cb/Ca.

With Ma: mortality in test substance after treatment, Mb: mortality in test substance before treatment, Ca: mortality in control after treatment, Cb: mortality in control before treatment.

The Itox index was analyzed over time, per day and up to one week after treatment as well as the influence of the time of application *i.e.* when bees are present and when bees are not present (early in the morning or late in the evening). A toxic reference treatment (dimethoate) and a water treated control were included.

New tunnel tests were analyzed as well, with the aim to further describe effects of spinosad applied at 76 and 96 g a.s./ha. These studies were conducted according to EPPO guideline 170 and OECD.[10,12] Parameters relating to these studies are summarized in table 2.

Tab. 2 Description of the semi-field trials used for the analysis of effects on bees at 96 g and 76 g a.s./ha.

Test description		**Test 1**	**Test 2**	**Test 3**
Application rate		**76 and 96 g a.s./ha**	**76 and 96 g a.s./ha**	**76 and 96 g a.s./ha**
Application time	During bee activity	✓		
	Out of bee activity	✓	✓	✓
Assessment	Mortality	✓	✓	✓
	Foraging	✓	✓	✓
	Brood development	✓	✓	✓
	Behavior	✓	✓	✓
	Colony strength at 28 days		✓	✓
	Colony strength at 60 days	✓		

3. Results

Results of the semi-field studies are presented in figures 1 to 5. Mortality records expressed as Itox index 1 day after treatment (1DAT) as a function of the application rate and the activity of honey bees at the time of treatment were calculated. Itox index values were comparable (mean ranging from 1.10 to 2.41) in honey bees for treatments performed out of foraging activity, for all application rates i.e. from 76 g a.s./ha to 540 a.s./ha. For sprays during foraging activity, Itox index ranged from 1.25 to 3.12 (mean values) for application rates ranging from 10.08 g a.s./ha to 76 g a.s./ha. Spray at 96 g a.s./ha lead to a higher mean Itox index value (4.01) but the median of 11 values was slightly below 2. Both the mean (6.26) and median Itox value for honey bees exposed to a spray with 144 g a.s./ha were ca 6. When applied out of bee activity at 96 g a.s./ha Itox values less than 2.0 were observed daily from one to seven days after exposure. In contrast, dimethoate (400 – 600 g a.s./ha) applied during bee flight resulting in an Itox value of 20, by one day after application (figure 1).

Mortality in honey bees exposed to a spray application spinosad performed out of bee flight are reproduced in figure 2, and expressed as the mean number of dead bees after a spray of 76 or 96 g a.s./ha. Mortality records were comparable to the water treatment control before and after treatment for both application rates. Peaks of mortality were recorded in bees exposed to the acute toxic standard (Perfekthion: dimethoate) on the day of 1 day after treatment, and mean mortality was significantly higher than in the control up to 3 days after treatment.

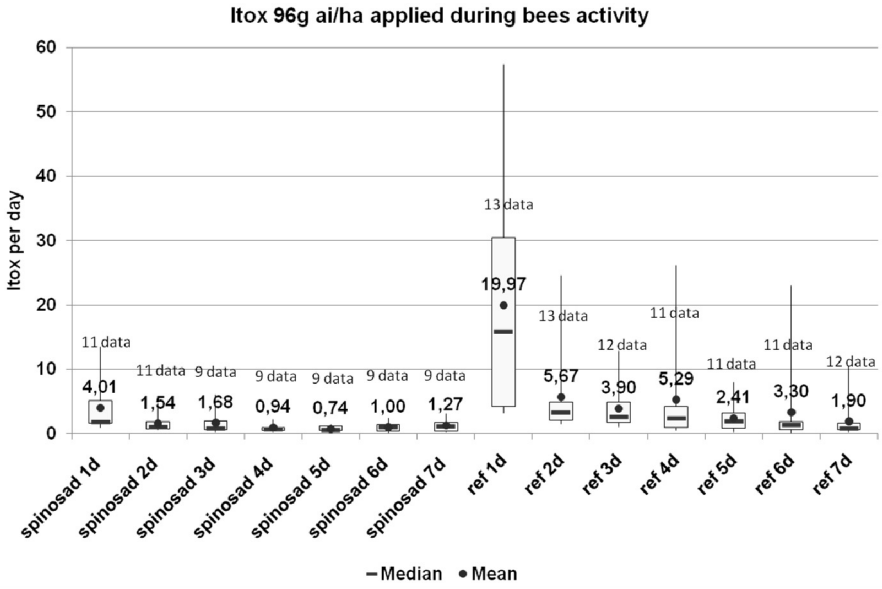

Fig. 1 Effects of spinosad on honey bees in tunnel tests when applied out of bee flight. Daily mortality expressed as a toxicity index (Itox). The ref(erence) is dimethoate. See text for explanation and calculation.

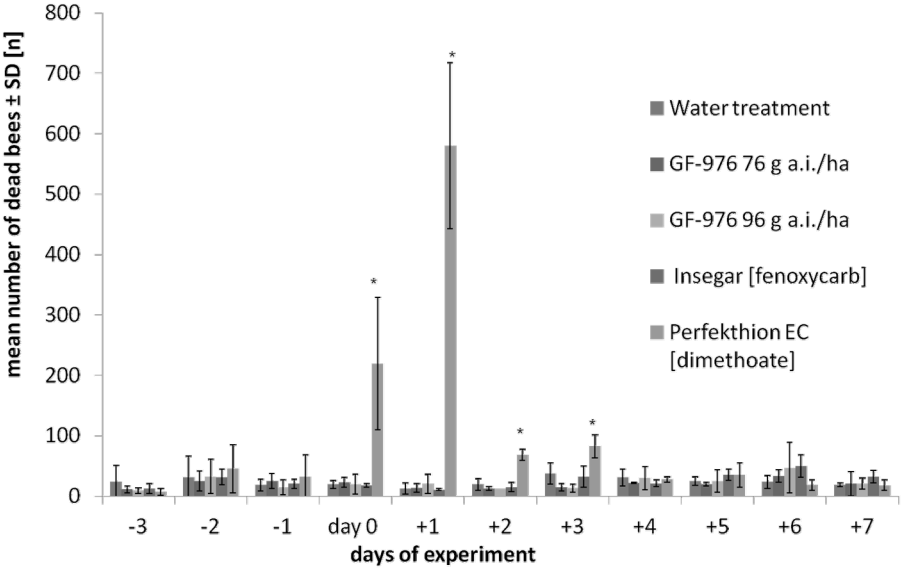

Fig. 2 Mortality to honey bees after exposure to applications of spinosad (GF-976) at 76 and 96 g a.s./ha applied out of bee flight under tunnel test conditions.

Foraging activity in honey bees exposed in the trials described in table 2 are reproduced in figure 3, and expressed as the mean number of bees /m² flowers. All records followed a similar trend with an increase in bee presence on flowers from day 3 pre-treatment to day 3 after treatment.

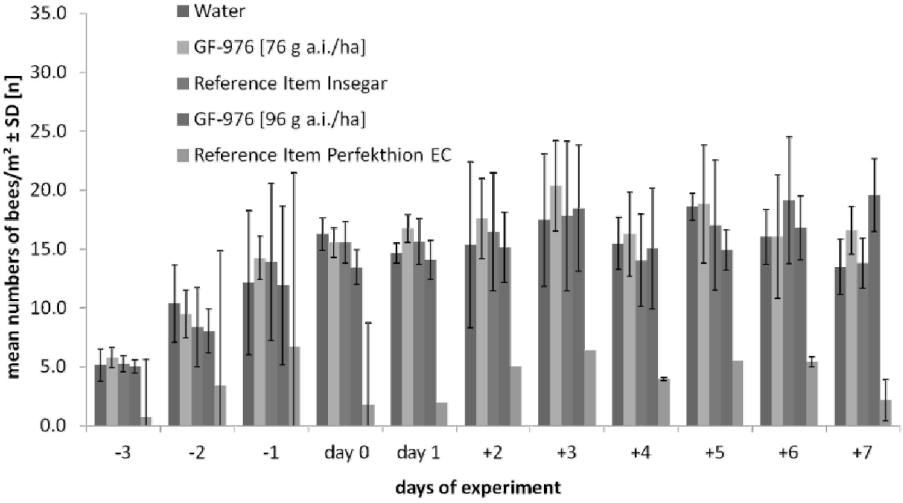

Fig.3 Foraging activity of honey bees after exposure to applications of spinosad (GF-976) at 76 and 96 g a.s./ha applied out of bee flight under tunnel test conditions.

Effects on brood were expressed as brood compensation index from day 6 to day 21 post brood fixing date. Results are reproduced on figure 4. The brood compensation index in honey bees exposed to a spray of spinosad at 76 or 96 g a.s./ha was comparable to the brood compensation index in honey bees of the water control, and as for the control it slightly increased within time. Both reference items Insegar and Perfekthion lead to a reduced brood compensation index at all assessment dates.

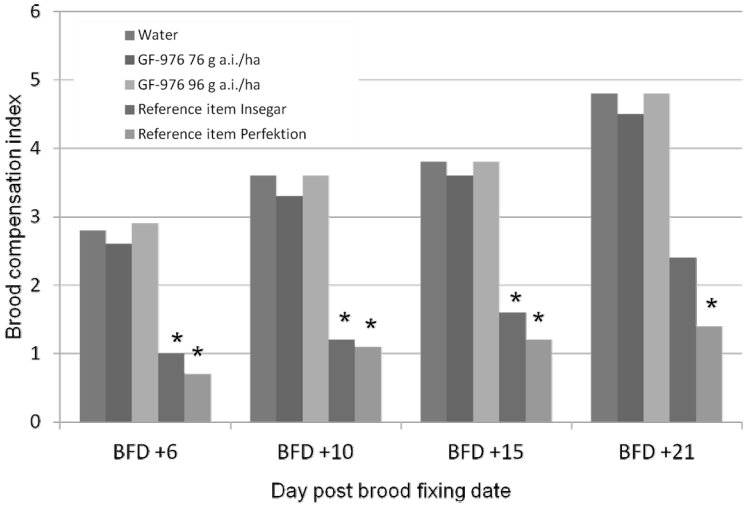

Fig. 4 Impact on brood of honey bees after exposure to applications of spinosad (GF-976) at 76 and 96 g a.s./ha applied out of bee flight under tunnel test conditions.

Finally, honey bee colony strength was measured as the mean number of bees/colony throughout the study, *i.e.* from day 6 before treatment to day 60 post treatment. Results are represented in figure 5. Colony strength was comparable in all treatments, with ca 3,000 bees/colony.

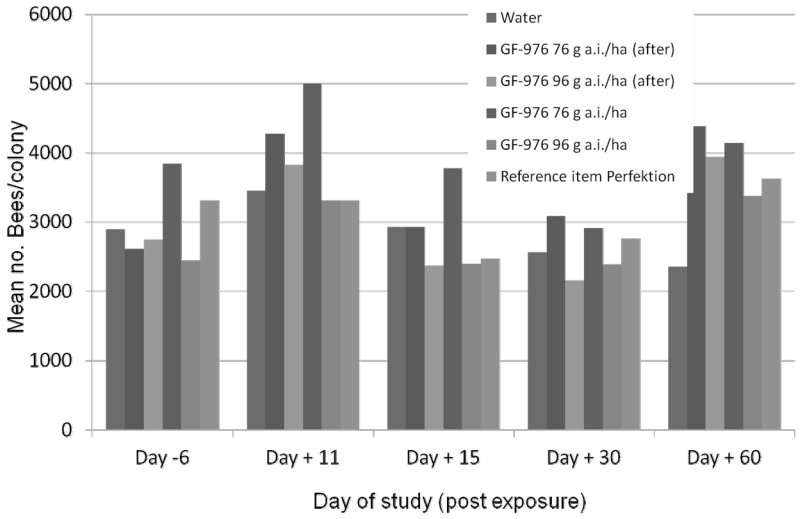

Fig. 5 Colony development and strength of honey bees after exposure to applications of spinosad (GF-976) at 76 and 96 g a.s./ha applied out of bee flight under tunnel test conditions.

4. Discussion

As previously stated, spinosad is intrinsically toxic to honey bees when tested in standard laboratory studies where worker honey bees are exposed orally (in sugar water diet) or topically to the test material. These laboratory tests are used in the screening step which is performed as an entry point in most risk assessment processes, as for example in the U.S or in Europe.[5,6] In the European risk assessment process, the screening step necessitates the calculation of Hazard Quotients (HQ), that compare toxicity to the application rate (HQ = application rate / LD_{50}). HQ values above the trigger value of 50 indicate a need for a refined risk assessment, which could involve higher tier testing (e.g. semi-field or field tests). In the case of spinosad, an application rate of 96 g a.s./ha results in HQ values ranging from 1,920 (oral HQ) to 1,950 (contact HQ) and thus indicate, in accordance with European regulation, a potential risk to honey bees "*unless it is clearly established through an appropriate risk assessment that under field conditions there are no unacceptable effects on honeybee larvae, honeybee behaviour, or colony survival and development after use of the plant protection product according to the proposed conditions of use*".[13]

The studies undertaken in tunnels on various crops, application periods and application rates aim at investigating the level of effects that are expected on honey bees under field conditions, as recommended by the European regulation. Accordingly, effects on honeybee survival, honeybee larvae, honeybee behaviour, and colony survival and development are monitored. The protocols used in the tunnel studies with spinosad allow for a more realistic appreciation of the level of acute effects that can be expected. The selection of cropped plants being attractive to honey bees and sprayed at flowering maximizes interactions of bees with the treated plants and any avoidance behavior can be observed and recorded. The inclusion of a water control and reference items allow to check that bees behaved as expected during the course of the experiment, and that effects of the product of well known toxicity are appropriately displayed during the course of the study. These tests

may or may not reproduce a direct contact of bees with spray droplets, depending on whether the spray is performed during or out of bee activity. However in any case, they reproduce the conditions of exposure of bee colonies that could be placed close to crops treated during flowering and can be considered as worse case with bees being forced to collect their food from the treated flowers.

With regards to the acute effects of fresh and dried residues of spinosad products on honeybees, Itox values indicated a dose – effect relationship when direct contact to spray droplets was allowed. In this analysis a threshold application rate for lethal effects of around 96 g a.s./ha was observed. When allowed to dry, residues of spinosad on flowers do not induce significant levels of honey bee mortality up to and including the highest application rate of 540 g a.s./ha. Assessments performed up to 7 days after treatment made during bee activity confirm the absence of mortality to foragers visiting treated flowers at 96 g a.s./ha. Therefore, this exposure rate can be considered as a threshold for immediate acute toxicity, but at which no long lasting acute toxicity is expected at this application rate or higher.

Such differences of honeybee responses in acute laboratory tests compared to tunnel test can be common and can be explained by the reduced availability of product residues on natural surfaces compared to direct contact in laboratory tests and due to the highly conservative nature of the tier I risk assessment. This phenomenon is well addressed for other non target arthropods and has lead to the development of laboratory tests exposing insects through natural substrates such as leaf discs, seedlings or soil samples as an intermediate experimental step between laboratory tests and semi-field/field tests.[14] For the two standard species used in the risk assessment procedure for non target arthropods, the parasitic wasp *Aphidius rhopalosiphi* and the predatory mite *Typhlodromus pyri*, moving from an inert surface to a natural surface reduces the residual toxicity by a factor of 9.8. Similarly, moving from inert surface exposure conditions to semi-field testing results in 45.1-fold lower residual toxicity (both factors assessed as the geometrical mean ratio of LR_{50} measured in laboratory testing on either inert or natural support and semi-field tests).[15] Spinosad is not systemic and its rapid dissipation from plant surfaces and exposure on natural surfaces (leaves and flowers) may explain the reduced residual toxicity observed.

Effects of a spinosad spray on the parameters representing sublethal effects on honey bees *i.e.* foraging behavior, brood and colony strength were also very limited in tunnel studies at the two application rates tested in detail (76 and 96 g a.s./ha). These observations are consistent with the mode of action of the substance. Spinosad acts by causing excitation of the insect nervous system, leading to involuntary muscle contractions, prostration with tremors, and finally paralysis. In addition, dissipation data indicate no persistence in environmental media and thus limited exposure duration. This is confirmed through the limited duration of residual toxicity in honey bees and other non target arthropods. Finally, the dissipation of spinosad does not lead to the formation of any active degradation products, which also limits the residual toxicity potency of the product.

The conclusions of the risk assessment appear to be confirmed by the very limited number of published data describing the potential effects of spinosad in pollinating species exposed in field conditions. With only three articles recorded in the last ten years. This could reflect the limited level of concern raised by the frequency of incidents and by the profile of the substance itself, for which the likeliness of long term or delayed effects that could have been missed in the studies having been performed in the context of the regulatory assessments is very limited.

5. Conclusions

The data presented address the potential effects of spinosad, applied as a spray to control various pests, on pollinators and more particularly to the honey bee. The analysis of higher tier studies on attractive crops having been performed for regulatory risk assessment purposes confirm the absence of significant impacts on honey bees even for treatments during flowering, provided that a delay allowing for droplets to dry is respected. Spinosad has been used all over the world for more than ten years in a wide range of crops without a recorded incident to pollinators. The feedback from field surveys is of particular importance as it reflects the potential impact of products on pollinators in realistic exposure conditions, which include interactions with other factors. Therefore the

confirmation of risk assessment outcome through survey data appears as a strong evidence of the actual limited impact of the product and its suitability for sustainable crop protection.

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