Evaluatie

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Translation of the Study Report

The effects of sublethal doses of imidacloprid on the foraging behaviour and orientation ability of honeybees.

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Summary:

This paper examines the possible effects of sublethal doses of the insecticide imidacloprid on the behaviour and orientation performance of foraging honeybees. Sucrose solutions containing imidacloprid was fed to bees, and changes in behaviour were found for imidacloprid concentrations of 20 ppb to 100 ppb after comparison with the control groups. No effect was observed at 10 ppb. In the sublethal concentration range indicated above, imidacloprid causes a reduction in the foraging activity of the treated bees and induces trembling dances by which the foraging bees discourage other worker bees from foraging, which in turn reduces the foraging activity of the bees in the nest. In addition, the effectiveness of the waggling dances used to attract bees to such food sources is reduced as the direction and the distance information as communicated by the waggling dance is less precise. Although these effects on the behaviour of the bees were observed to start at imidacloprid concentrations of 20 ppb, no damage to the test populations was observed for the range of concentrations tested up to 100 ppb.

Although this experiment did not examine whether the observed effects will affect the population development, such effects appear not very likely unless bee hives without any food stores are exposed to such food sources at concentrations where the foraging activity decreases. Should concentrations above 20 ppb occur in nectar, it has to be verified whether or not a decrease in honey yield is observed under practical conditions.

Imidacloprid is a chloronicotinyl insecticide which was developed by Bayer. Imidacloprid acts on various types of nicotinic acetylcholine receptors. It is used amongst others as a seed dressing agent to control pest species. Following reports from French beekeepers of 'disoriented' honey bees that had been foraging in treated sunflower fields, and preliminary trials carried out by Bayer which showed possible effects on foraging behaviour of bees fed with an 100 ppb imidacloprid sucrose solution, further specifically designed experiments were performed in summer 1998 to find out whether feeding of imidacloprid in the sublethal concentration range from 10 ppb to 100 ppb could affect the foraging behaviour and orientation ability of honey bees.

Comprehensive research has been undertaken on various aspects of the foraging behaviour of honeybees. In contrast to many other insect species that feed on flowering plants, foraging behaviour of honey bees is to a large extent regulated by social interactions with the dance communication system as the main element in regulating the collection of nectar and pollen (surveys in von [redacted] 1965, [redacted] 1995, [redacted] 997). This means that potential effects observed on the foraging intensity for nectar or pollen observed at the population level may not solely be based on direct effects on the foraging behaviour of individual bees but may also be triggered by the social communication system. In other words, if a reduction in foraged food is recorded, this may possibly be due to the fact that the frequency and/or duration of round and waggling dances used to attract conspecifics in the hive to food sources are reduced.

Indications of such complex effect arised from observations in preliminary trials carried out by Bayer that trembling dances appeared to be more frequent at high concentrations of imidacloprid in sugar solution. The honey bee trembling dance, whose function was not understood for a long time (von Frisch 1965), regulates the balance between the amount of nectar brought in by foraging bees and the amount accepted and processed by worker bees inside the hive ([redacted] 1992, [redacted] 1993, [redacted] 1993, [redacted] 1994, [redacted] et al. 1996). If so much nectar is brought in that the foraging bees have to wait for a long time in the hive before they can deliver the food, some of these foraging bees start to perform trembling dances. These dances reduce the number of recruited foraging bees (foraging activity) due to a decreased frequency of waggling dances and increase the recruitment of hive bees which take the nectar from the foraging bees. [redacted] (1994) found that

even when there was not an oversupply of food, trembling dances could be triggered in experiments by a wide range of conditions which caused longer waiting times for nectar delivery. Reports from [redacted] (1949) and [redacted] 1953) state that feeding various toxic substances also triggered trembling dances. It therefore seemed that a detailed investigation of the effects of imidacloprid on dance behaviour and the frequency of trembling dances would be a sensible approach to explaining the fall in foraging activity observed at the population level if high concentrations of the compound are fed to the bees.

At the same time, a detailed investigation of bee dance behaviour will also allow us to characterise any impairment of orientation more accurately. When honeybees find a good source of food they learn its smell, colour and visual appearance, and also its position relative to the hive [redacted] 1965, [redacted] 1995). They do not only return on a direct route from the food source to the hive and find the food source directly when they next leave the hive, but they also communicate the direction and distance between the hive and the food source to their conspecifics in the hive via dancing. Any impairment of solar compass orientation, estimation of distance and route integration can therefore be quantified by assessing the direction and distance information coded in the bee dance [redacted] 1994).

The purpose of our study was therefore to quantify the possible effects of imidacloprid on the behaviour and orientation ability of individual bees, and in particular the behaviour of individually marked bees returning to the hive from a food source. The concentrations of the active ingredient examined were limited to a range from 10 ppb to 100 ppb.

Materials and methods

The experiments were performed on two honeybee populations of the strain *Apis mellifera carnica*. Each population contained about 5,000 bees. The test hives were placed in twin-comb observation hives as described by [redacted] (1965). One of the populations had access to a flight room at the beginning of the experiment. Later on in the experiments both colonies were given the opportunity to forage out-doors. All bees returning to the hive were directed to one side of the comb so that all individually marked foraging bees could be observed.

The tests in the flight room were performed between April and June and the out-door tests from June to the end of August. In the flight room, groups of individually marked foraging bees were fed one metre from the hive with a solution that contained either 2M sucrose solution or 2 M sucrose solution mixed with 100 ppb (w/v) of imidacloprid (calculated on the basis of the 70% concentration of Gaucho WS 70 used in this series of tests) or with 0.5 M table salt (as an additional control). Records were made of the frequency of trembling dances according to the method described in [redacted] 1994, the search time until a foraging bee met a hive bee which accepted the harvested food, and the number of trophallactic contacts.

These experiments were continued out-door with the same population and a food source 10 metres from the hive. In this set of experiments imidacloprid was used at concentrations ranging from 10 ppb to 100 ppb derived from Confidor (containing 98.3% imidacloprid). The observations also covered the frequency of waggling dances (for the traditional distinction between round dances and waggling dances at close distances see [redacted] et al. 1988).

The second colony was used to investigate the precision in the communication of direction and distance as given in the waggling dances. The food source was located 500 metres away from the hive. The tests were performed using imidacloprid concentrations ranging from 10 ppb to 100 ppb derived from imidacloprid (98.3% a.i. content). The dances of the returning foraging bees were recorded in the dark (room lit only with a red darkroom light that is invisible to bees) on an infrared-sensitive video camera. Subsequent evaluation of the dances allowed us to determine the direction information communicated with each waggling dance to the nearest 1°, and the speed of waggling movements (which codes the distance of the food

source) to the nearest 20ms. A calibration method derived from previous tests was used to calculate the distance indicated by the speed of the waggling movements.

For detecting any persistent effects, control runs were conducted before and after each test run and temporal trends were analyzed. However, the relative low longevity of fouragier bees restrict the possibility to monitor chronic effects. In the field, the average longevity of fouragier bees is about 8 to 10 days. Fouragier bees which were marked on the food source will, therefore, live on average only further 4 - 5 days. In the experiments examining the frequency of trembling dances individual bees were tested for up to 10 days. In the experiments examining the precision of communication, the imidacloprid-containing sucrose solutions were fed typically over three subsequent days. The days before and after the feeding period were used to perform the control runs.

The preparation of the test solution was done according to the following procedure:
100 mg a.i. (i.e. either 142.8 mg Gaucho (WS 70 uncoloured, NTN 33893 70 WS) or 101.7 mg imidacloprid tech. (98.3%) was pre-solved in 1 L A. dest. and stirred for 4 hrs (results in 100 ppm). 10 ml of this solution was then diluted with 490 ml (2 ppm). A lot of either 2.5 ml, 5 ml, 12.5 ml or 25 ml of this dilution was then filled up to 500 ml into a 2 molar sucrose solution (resulting in 10, 20, 50 and 100 ppb (w/v) imidacloprid in 2 M sucrose solution). The ready-to-use 2 M sucrose solutions were stored in a cooler at 4°C and used for a maximum of 1 week.

Circular statistical methods were used in the statistical evaluation of direction informations coded in the bee dances ([redacted] 1981).

Results and discussion

Trembling dances

Foraging bees returning from foraging flights to food sources containing imidacloprid were observed to perform trembling dances more frequently, both in the field and in the flight chamber; these dances are rare except in very special circumstances (Fig. 1). The effect can be observed for concentrations of 20 ppb and above, and is statistically significant (chi-squared test, $p<0.01$).

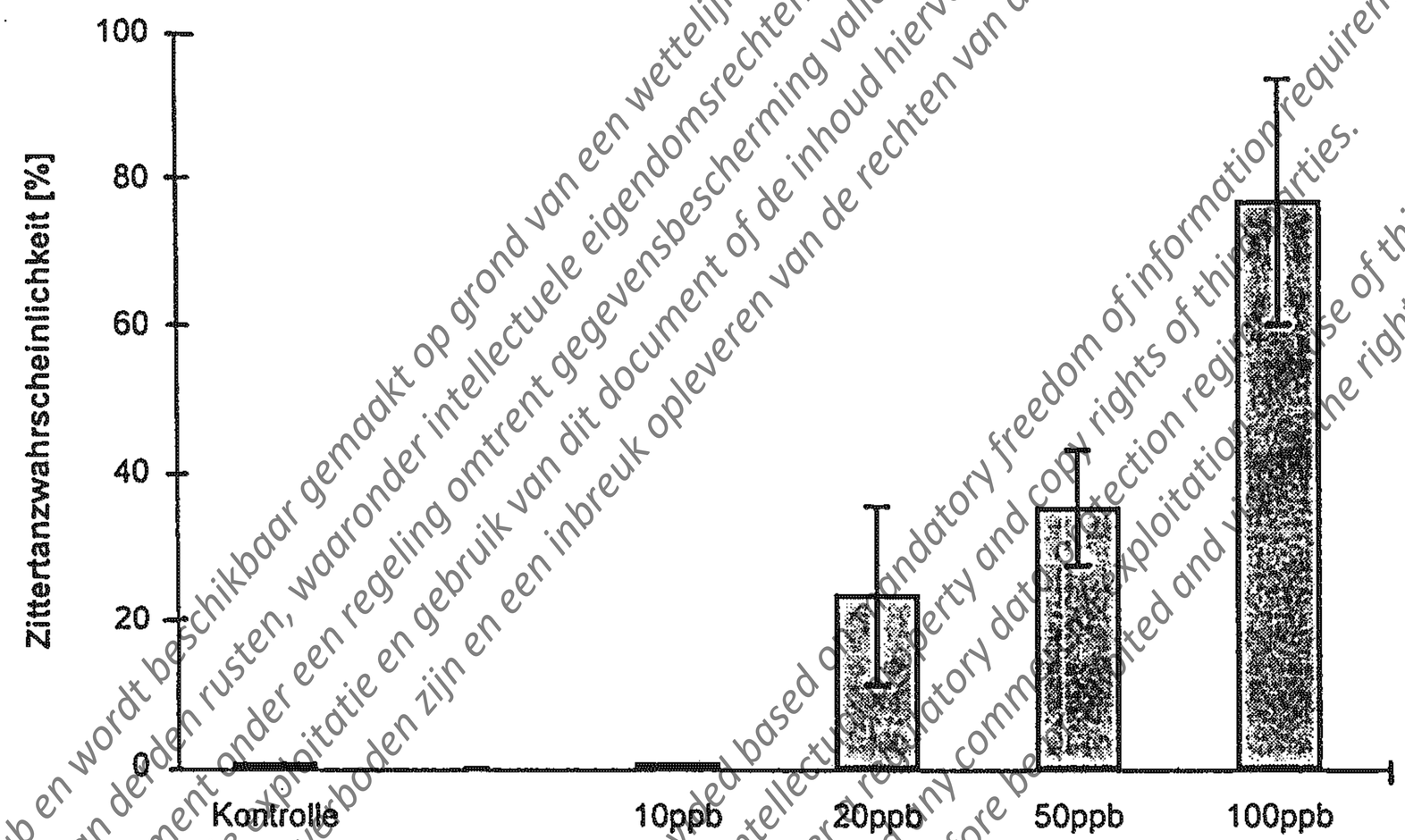

Fig. 1: Frequency of trembling dances performed by individually marked foraging bees on their return to the hive. The test food consisted of a 2 M sucrose solution containing imidacloprid at the concentrations shown in the diagram (except for the control group where the food contained no imidacloprid). The figures relate to mean values and standard deviations for sets of 5 tests on a total of 375 bees. The frequency of trembling dances increased significantly at concentrations of 20 ppb and higher.

This effect is similar to that described by [redacted] in 1994 following the addition of a high concentration of table salt to the sugar solution, which was examined in the present test run as an additional control. Trembling dances were likely to be performed by about 50% of bees that had collected 2 M sucrose solution containing 0.5 M NaCl. Contact with imidacloprid also affected the search time of foraging bees for hive bees which take over the nectar and the number of trophallactic contacts made; for doses of 50 ppb and above (t-test, $p<0.01$) the search time was significantly extended and the number of trophallactic contacts was significantly greater. The same effects can be triggered by the addition of 0.5 M NaCl. The results indicate that imidacloprid affects the social transfer of food, the

trophallaxis, in a similar manner as high concentrations of table salt. The existing data do not allow us to determine whether this is due to the rejection of the food by the bees in the hive, as in the case of table salt, or whether there is a pharmacological effect on the foraging bees which leads to changes in behaviour during social food transfer. Feeding tests did not find indications of a sensory perception of imidacloprid at the low concentrations used, and so we cannot exclude the possibility of a direct effect on the foraging bees at concentrations of 50 ppb and higher.

Foraging activity

The frequency of visits made by individually marked bees to a food source located 500 metres away from the hive fell significantly during a two-hour observation period when imidacloprid was fed at 100 ppb in a 2 M sucrose solution (t-test, $p<0.01$). Lower concentrations of 20 ppb and 10 ppb of imidacloprid were not found to have any effect on the frequency of visits (Fig. 2)

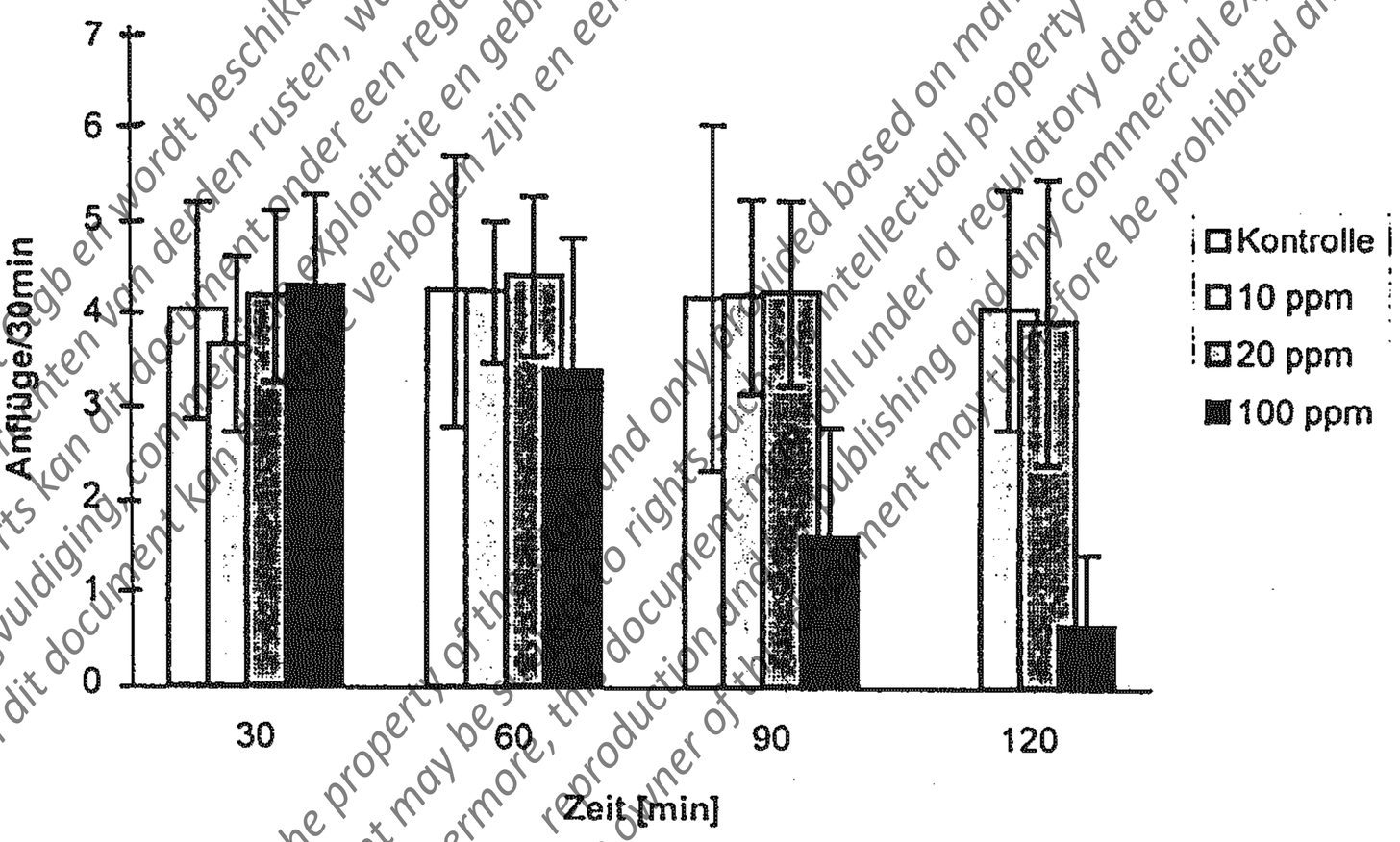

Fig. 2: The number of visits to a food source located 500 metres away from the hive decreased significantly during a two-hour observation period when the provided 2 M sucrose solution contained imidacloprid at 100 ppb (t-test, $p<0.01$). Lower concentrations of 20 ppb and 10 ppb of imidacloprid were not found to have any impact on this endpoint. The graph shows the number of flights per 30 minute intervals. Mean values and standard deviations are given for groups of 40 - 60 bees.

A reduction in foraging activity is often observed in bees performing trembling dances, and this reduction is at least in part directly influenced by the duration of the trembling dances, a parameter, which we did not measure. Trembling dances vary considerably in duration, but

are on average much longer than waggling dances. However, also bees, which do not perform trembling dances, reduces the frequency of flights to the food source.

Recruitment of conspecifics via waggling dances

The frequency of waggling dances (performed to attract conspecifics to a food source) fell sharply following feeding with imidacloprid (Fig. 3). The effect can be observed at concentrations of 20 ppb and above, and is statistically significant (chi-squared test, $p<0.01$).

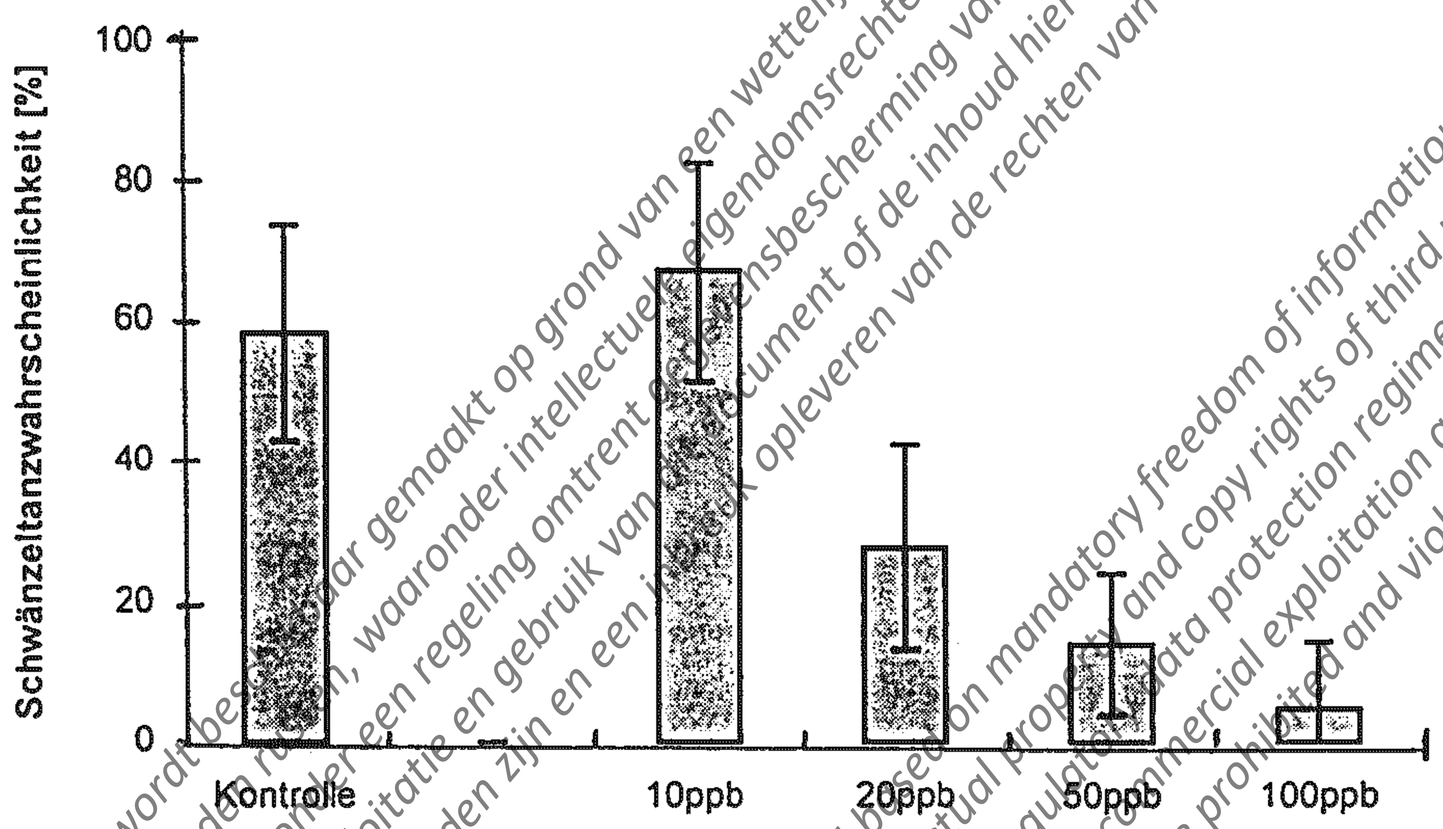

Fig. 3: Frequency of waggling dances performed by individually marked foraging bees on their return to the hive. The food consisted of a 2 M sucrose solution containing imidacloprid at the concentrations shown in the diagram (except for the control group where the food contained no imidacloprid). The figures relate to mean values and standard deviations for sets of 5 tests on a total of 375 bees. The frequency of waggling dances decreased significantly at concentrations of 20 ppb and higher.

This is, as shown by [redacted] in 1993 and [redacted] in 1994, a consequence of the increased frequency of trembling dances. The function of the trembling dance is to reduce the rate of recruitment via waggling dances. Under natural conditions this occurs if honey bees discover a particularly rich food source and bring so much food to the hive at one time that there are not enough bees in the hive to take and process this amount of nectar immediately. As a direct result of this the foraging bees' search time for nectar transfer to hive bees becomes longer, and they understand this as a signal that there is no benefit in performing waggling dances to encourage other bees to forage. Some of the foraging bees perform trembling dances instead, and this has the effect of reducing the frequency and length of waggling dances performed by other foraging bees. Therefore the reduction in the

frequency of wagging dances caused by imidacloprid should not be interpreted as a specific direct effect on the behaviour of the forager bees, but rather as a consequence of the effect on social food transfer which has been discussed above; this effect causes trembling dances. It must however be mentioned that this feedback mechanism is not limited to reducing attraction to a specific food source (if this were so, then bees would be less likely to visit treated areas and so any collection of plant protection products would be reduced), but is non-specific and affects the overall foraging activity of the entire hive. Under unfavourable conditions, for example if populations did not have sufficient honey stores and if the reduction in foraging activity is strong and long enough, this could even lead to bee losses caused by food deprivation.

Precision of informations on direction in the waggling dance

The precision of direction informations to the food source in the waggling dance decreases after the bees were fed with imidacloprid, i.e. there was an increase in the variance of the direction informations from one wagging movement to the next, expressed as the circular standard deviation of direction information (Fig. 4). The effect is significant for imidacloprid concentrations of 20 ppb and above (p<0.01).

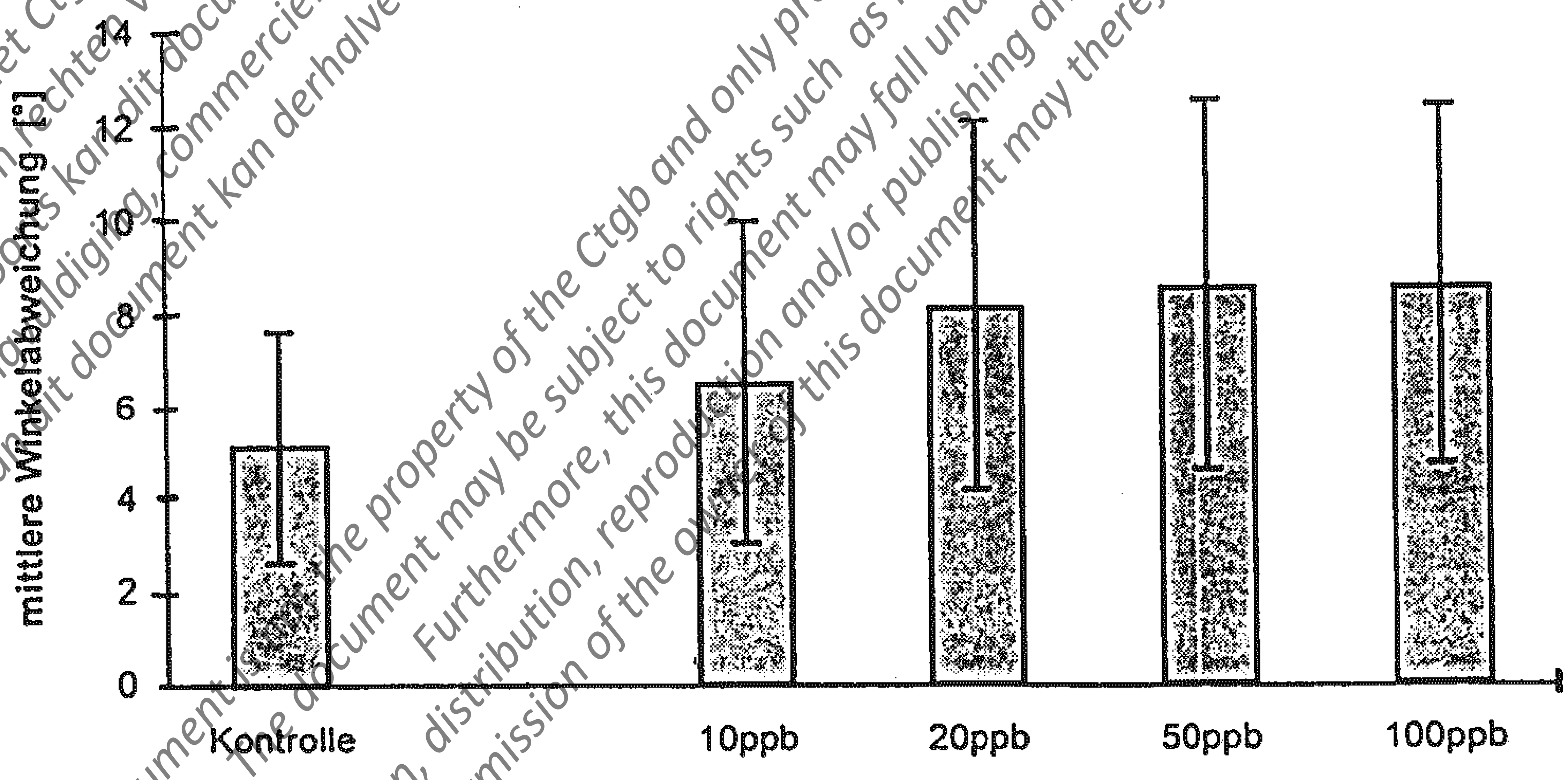

Fig. 4: Mean deviation from the azimuth angle as communicated by the waggling dance. The food consisted of a 2 M sucrose solution containing imidacloprid at the concentrations shown in the diagram (except for the control group where the food contained no imidacloprid). The figures relate to mean values and standard deviations for the mean angle deviations of groups of 16 dancers (approximately 2,000 azimuth angle informations). The mean deviation from the correct azimuth angle increased significantly for doses of 20 ppb and higher.

However, in the light of what we know about the function of bee dances in recruiting conspecifics to a food source, and in particular in the light of recent findings on the role of olfactory orientation in the search for food ([redacted] 1998, [redacted] 1998), the lower precision in the direction information should not have a significant effect on the rate of recruitment. The effect could be explained for instance as an indication of an impairment in the bees' orientation ability, since the accuracy of direction informations in the dance depends on the accuracy of perception and integration of the direction in which the bees have flown, or by other impairments, for example of movement coordination or slower movements during the dance.

Precision of distance informations in the waggling dance

The distance of food sources is communicated by the duration of the waggling dances and the simultaneously produced dance vibrations. The bees that had been fed with imidacloprid gave distance informations that distinctly differed from those given by the control group (Fig. 5). The treated bees underestimated the distance significantly for imidacloprid concentrations of 50 ppb and above (t-test, $p<0.01$).

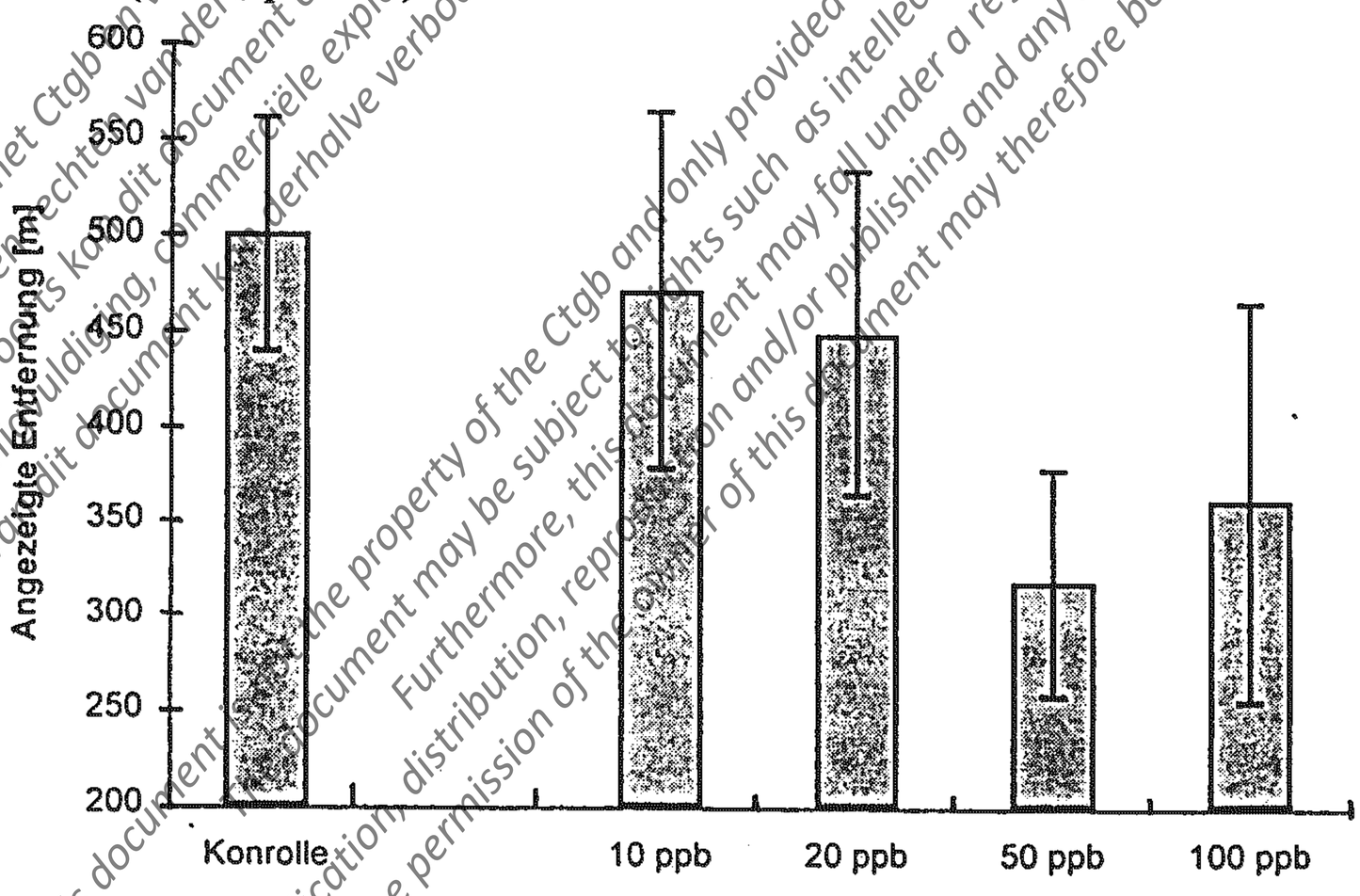

Fig. 5: The distance of a food source located 500 metres from the hive as communicated by the waggling dance. The food consisted of a 2 M sucrose solution containing imidacloprid at the concentrations shown in the diagram (except for the control group where the food contained no imidacloprid). The figures relate to mean values and standard deviations for the distances indicated in sets of 20 - 30 dances. The distance indicated in the dance decreased significantly at concentrations of 50 ppb and higher.

Long-term observations of behaviour and of symptoms of intoxication

In all of the tests performed effects of imidacloprid fed at concentrations of up to 100 ppb did not persist beyond the day of the test. Control values recorded on the days following the tests with imidacloprid were in no case different from control values recorded before testing. In tests involving the administration of imidacloprid over a period of several days, we did not observe any trends over time that might have been caused by substance or effect accumulation. Also no damage was observed to the populations used in the tests, which took more than 4 months in total. Colony strength, reproductive performance of the bee queen and brood development were normal until the end of the tests, and during and later checks, they were also not found affected until end of September. However, it should be noted that in contrast to situations where large quantities of sugar solution are fed or where bees forage on natural nectar sources potentially containing imidacloprid, only a limited number of bees were trained to forage on the food source in our tests. The number of foraging bees was rarely greater than 20, and there were rarely more than 20 flights to the food source containing imidacloprid per foraging bee and per day. As honeybees collect about 50 mg of nectar on each foraging flight, this means that the average volume of imidacloprid brought into the hive each day was below 1 µg (considering the average of all test concentrations). If all the other bees foraging in the field on natural food sources harvest only 100 g of nectar per day in total, this would result in imidacloprid concentration in the nectar stores of less than 10 ppb.

Conclusions

Imidacloprid fed via sucrose solutions has recordable effects on the behaviour of foraging honeybees at concentrations of 20 ppb and above. Foraging on nectar that contains imidacloprid at concentrations of 10 ppb or less has no effect on the behaviour parameters we have examined. As the observed effects of imidacloprid also relate to the bees' communication system, they also affect the behaviour of those bees in the hive that do not come into direct contact with the substance. In particular there is a significant reduction in the rate of recruitment of conspecifics to food sources containing imidacloprid. According to our knowledge about the honey bee communication, this will concern not only the nectar sources containing imidacloprid but also all other available sources of nectar since the trembling dances inhibit recruitment of foraging bees unspecifically. The inhibition of recruitments lasts for hours, often (at higher concentrations) for the rest of the day. Depending on the availability of alternative food sources, this may lead in practice to a reduction in honey yield if the bees forage on nectar sources containing high concentrations of imidacloprid. The effects on behaviour described above are not likely to cause damage to bee populations where the concentration of imidacloprid is between 20 ppb and 100 ppb, provided that they are not completely deprived from stored food. When completely deprived from food bee losses cannot be excluded due to a lack of food following the reduction in the amount of nectar collected as a result of the effects described above at the corresponding higher test concentrations.

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06.01.2003 19:41

An: [redacted]
Kopie:
Thema: Re: Rückfragen aus Frankreich

Which was the age of the tested bees?

The data presented in the 1999 report are exclusively from forager bees, i.e. individuals which were marked outside the nest at a feeding site. Honeybees start foraging at an age of 2-3 weeks and forage then typically for less than two weeks.
The same is true for most of the data in the 2000 report: All field experiments and most of the proboscis extension reflex tests were performed on foragers, just the longterm effect of imidacloprid on learning and memory
was investiged using bees hatched in an incubator which were tested at an age of 10-12 days.

Which are the statistical results?

The test statistics used in our studies are t-tests for the comparisons of means, chi-square tests for frequency data and circular statistical methods following Batschelet (1981) for the data on dance orientation.

Was the feeder containing imidacloprid protected from sunlight ?

All test solutions were kept in the dark at 4°C. When bees were trained to visit feeders providing solutions containing imidacloprid, these feeders were never exposed to direct sunlight, the test solutions were exposed to ambient temperature for a maximum of two hours.

Where are the results on the part concerning the sublethal effects measured in an indoor cage (hive placed at 1m of the feeder and measures on the search time in the hive, number of trophallactic contacts and trembling dance frequency)?

The indoor series of tests was restricted to just one concentration (100ppb imidacloprid). The data were therefore not included in the 1999 report.
However, the effects were significant and similar to the results of the outdoor series.

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