INTERNATIONAL COMMISSION
FOR PLANT-BEE RELATIONSHIPS

Proceedings of the 6th International Symposium on Hazards of Pesticides to Bees

September 17 - 19, 1996
Federal Biological Research Centre
for Agriculture and Forestry
Braunschweig, Germany

BBA

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INTERNATIONAL COMMISSION FOR PLANT-BEE RELATIONSHIPS

- Founded in 1950 by Anna MAURIZIO 1900-1993-

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I congratulate everyone concerned with the organisation of the Sixth Symposium of the ICP-BR Bee Protection Group and with the production of this report. In particular we are grateful to the President and staff of the Biologische Bundesanstalt für Land- und Forstwirtschaft for providing all facilities for the meeting, and to the following companies for generous financial support:

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Once again the Symposium provided a forum where representatives of National Regulatory Authorities, Government and University Research Departments and Industry can come together to discuss the assessment of the hazards to bees of crop protection operations. I am particularly pleased that the principles established by the Bee Protection Group since it began work in 1980 are increasingly being adopted as models for the study of other beneficial organisms.

Ingrid H. Williams

Professor Ingrid H Williams PhD
Chairman ICP-BR

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1. Opening Session

Dr. STEVENSON, Chairman of the ICPBR Bee Protection Group, opened the meeting expressing thanks to the Biologische Bundesanstalt für Land- und F orstwirtschaft (BBA), Braunschweig for hosting the Sixth International Symposium on the Hazards of Pesticides to Bees. In particular, he thanked Dr. Brasse and his colleagues for the excellent organisation that had gone into the meeting.

He reported that Prof. Ingrid Williams (ICPBR President) sent her best wishes and informed the meeting that membership forms for the ICPBR could be obtained from him. He introduced Dr. Oomen to the meeting as the vice-Chairman of the Bee Protection Group and also Dr. Lewis who has taken over as Secretary to the group from Dr. Harrison, as she is unable to continue due to a change of job and he thanked her for her past contributions.

Prof. Dr. PES TEMER, deputy director and head of the Institute for Ecological Chemistry, then welcomed everyone to the meeting and delivered the kind regards of Prof. Dr. Klingauf, president of the BBA who was unable to attend due to prior engagements. He then presented a brief review of the structure and working of the BBA and its constituent institutes. The majority of the BBA personnel work in Braunschweig while about 30% are in Berlin and Kleinmachnow (near Berlin) and another 10% are at smaller institutes elsewhere in Germany. Most of the scientists are engaged in various aspects of research concerning the biology and control of plant pests and diseases, including the protection of honey bees against the use of plant protection products.

With regard to the European harmonisation process for the testing of pesticide side effects on non-target organisms, he paid tribute to the work of the ICPBR which had resulted in the harmonised scheme for honey bees being at the forefront in this area. However, he recognised that there was still work to be done and he posed a series of questions which might be considered, as an example:

> are the current regulations sufficient for the protection of wild bee species or is additional work necessary to investigate the risk to them from the use of pesticides and ways in which they may be protected?

- do we have sufficient information about the possible synergistic effects of bee diseases and the methods used for their control and pesticides as well as between different pesticides occurring in the environment?
- what further can be done to reduce the number of bee incidents involving pesticides every year?

Prof. Dr. Pestemer concluded by wishing everyone a successful and enjoyable meeting.

A transcript of the welcoming address by Prof. Dr. Pestemer can be found in **Appendix 3**.

2. Summary of the meeting

The papers presented at the meeting were divided into a series of sessions covering a range of distinct areas (the full meeting agenda is presented in **Appendix 2**):

2.1 Test methodology
2.2 Development of established methods (including laboratory, semi-field and field)
2.3 Residue testing
2.4 Larval toxicity and growth regulators
2.5 Other problems and techniques
2.6 Poisoning incidents and monitoring schemes
2.7 Varroa and varroacides
2.8 Work with other bee species

2.1 Test methodology

Dr. OOMEN (Dutch Plant Protection Service) leader of the EPPO/CoE honey bee sub-group described the history and current status of the honey bee risk assessment scheme to the meeting (**Appendix 4**).

The EPPO/CoE honey bee scheme was published in 1993 being the most developed of a series of 11 guidelines for environmental risk assessment and the first to be finished. It subsequently formed the basis of the EU requirements in this area under Council Directive 91 / 414/EEC. At

the fifth International Symposium of the ICPBR Bee Protection Group (W ageningen, October 1993) discussions were held concerning the different testing methods now in use and a number of recommendations were agreed for updating EPPO/CoE guideline 170 which provides the recommended methodology for the honey bee risk assessment scheme. Dr. Oomen presented these changes to the meeting. They have now been submitted to EPPO as a proposal to update guideline 170 although this has not yet resulted in an official review.

Following the fifth ICPBR Symposium in Wageningen the EPPO/CoE honey bee sub-group was asked to decide about the number of tests required for the regulatory scheme. It had been agreed that a minimum of two tunnel or cage tests and one field trial per country/climatic zone were needed, which was in agreement with the ICPBR proposals from the 1993 meeting. After much discussion it had also been decided that one good contact and oral test were required. It was considered that the normal variation in test results was sufficiently accounted for by the various safety margins used in the scheme. However, where the hazard ratio is very close to the decision threshold of 50, an 'analysis of uncertainty' would be triggered requiring expert judgement by the evaluating authority to decide whether or not additional tests are required. Again, a series of modifications to the EPPO scheme, incorporating these decisions, have been submitted.

Finally, Dr. Oomen reported on a validation exercise that had been conducted in 1995 by the EPPO Environmental Risk Assessment Panel of the environmental risk assessment schemes including that for the honey bees. The European regulatory authorities had conducted an evaluation of standard data sets following the schemes and their results reviewed by EPPO. The results from the honey bee scheme were found to be both consistent and accurate. A number of minor problems were found, primarily with inexperienced assessors e.g. the relative importance of different routes of exposure and the need to calculate the hazard ratio using amounts expressed as active ingredient or formulated product throughout. Overall it was considered that the honey bee scheme was seen to give reliable results with the need for some minor revisions to make the scheme more robust.

Dr. Brasse said that the BBA was now using both the BBA and EPPO schemes in order to familiarise itself with the latter and he asked what the situation was in other European countries. UK and the Netherlands reported that the EPPO scheme was now fully implemented, Hungary to a large extent while in Sweden it was well known and in use but not

consistently. National schemes are still in use in France and Poland, although the latter was reported to be similar to the EPPO one.

Dr. TIBERG of the Swedish National Chemicals Inspectorate then gave a presentation on the draft OECD honey bee guideline, which she was helping to prepare (**Appendix 5**). She introduced the OECD Test Guidelines Programme (TGP) which covers physical/chemical properties, bioaccumulation and degradation as well as effects on biological organisms. Under the Mutual Acceptance of Data, tests conducted in one of the 25 OECD member countries according to theses guidelines has to be accepted in all the others. The honey bee acute toxicity test guidelines were drafted in 1994 on the basis of existing guidelines, primarily EPPO 170, and following circulation to member countries, revised drafts were produced in 1996 which were due to be considered at a TGP National Co-ordinators meeting on 18 September 1996.

Dr. Tiberg described a number of differences in the OECD compared to the EPPO guideline. These were mainly concerned with giving greater detail e.g. specifying an acceptable relative humidity of 50 ± 10%, the use of young hatching bees and specifying the use of 50% (w/v) sucrose solution as food. Both schemes agreed on the need for the use of a toxic standard, although the OECD guideline includes an acceptable range of LD_{50} values in order to allow the evaluator to assess the validity of the test. In the OECD test, the use of solvents e.g. acetone, for technical material and substances of low solubility is specified, while for water-soluble formulations wetting agents are preferred which clarifies an ambiguity in the EPPO guideline. She concluded that tests conducted according to the OECD and EPPO guidelines would be equally acceptable if some of the details were checked. Dr. Tiberg finished with a number of issues which might still need to be considered including the incorporation of a limit test, the acceptable duration of anaesthetisation and the validity criteria for the toxic standard.

In subsequent discussions, concern was expressed that the OECD guideline was too prescriptive which could cause problems regarding the GLP acceptability of a study i.e. recommendations would not be seen as subject to interpretation. For example: the specification of a cage size of 0.5 dm^3 was associated with the BBA spray test but was not necessary with topical application; the acceptable relative humidity range was specified as 50 ± 10% while at Wageningen the ICPBR had decided a specific range was not necessary; what was the definition of a young bee. In particular, while it was generally agreed that a toxic

standard was necessary, Prof. Künast was concerned that the toxic standard acceptability range given for guidance would be seen as absolute validation criteria. Dr. Lewis, one of the authors of the paper referred to in connection with this, agreed and pointed out that the results presented had been from one laboratory and should be used as the basis of a wider validation exercise if this was to be appropriate for international purposes.

Prof. Dr. KÜNAST presented a summary of regulatory studies conducted with plant protection products using the BBA guidelines (**Appendix 6**). A data base was assembled from three companies; BASF, Bayer and AgrEvo, comprising laboratory, tent (cage) and field tests, as appropriate, for a large number of products. In most cases there were three laboratory tests per product, one to three tent tests and two field tests, conducted at different test facilities. The results were assessed according to the following classification: homogeneously 'not hazardous' from all laboratories; homogeneously 'hazardous' from all laboratories and thirdly a mixture of results. It was found that for herbicides, fungicides, bioregulators, Insect Growth Regulators (IGRs) and acaricides, the laboratory results were generally either uniformly 'not hazardous' or a mixture while 'not hazardous' predominated in the tent and field except for IGRs where the situation was unchanged at all stages. Insecticides were mostly uniformly 'hazardous' in the laboratory, uniformly 'hazardous' or a mixture in the tent and a mixture in the field. Comparing between the tiers of testing, there were only five cases where a more hazardous result was obtained at the higher tier and four of these were IGRs. Comparing between the test facilities on the basis of the average results and results more/less favourable than this, for compounds that were classified as 'not hazardous' there was considerable variation which was not related to geographical distribution but was probably attributed to possible differences in testing procedures between the laboratories.

Prof. Dr. Künast concluded that while the BBA guidelines presented a valid sequential testing scheme for environmental risk assessment, harmonisation of regulatory requirements in different countries was desirable and so the LD_{50} test should be adopted in Germany. Dr. Schmidt pointed out that the variability in results obtained from different laboratories using the same method emphasised the need for the validation of international guidelines.

Mr. HINTZEN presented a review of factors which might influence the LD_{50} values obtained from honey bee acute toxicity test (**Appendix 7**). The state of health of the bees is a factor already taken into account in the EPPO 1 70 guideline. Food has been found to be important

with penetration of a pesticide through the honey bee gut increasing with decreasing sucrose concentration. While feeding has been found to influence body weight there is no clear relationship and so it was not considered necessary to relate the LD_{50} to unit body weight. The age of the bees used had been found to affect sensitivity to pesticides with young bees generally being more sensitive than older ones. Genetic origin had been found to sometimes influence the outcome e.g. Africanised bees being generally more tolerant while *Apis mellifera ligustica* is more variable than *A. mellifera mellifera*. Seasonal aspects e.g. summer vs. winter had also been reported to affect susceptibility although Mr. Hintzen had found little difference. A number of factors in the conduct of the test itself were considered to be of potential importance e.g. inclusion of a acclimatisation phase (24 hours) before using the bees in the tests and having a period of food deprivation (60 - 100 minutes) before oral dosing. The site of topical application had not been found to significantly affect the outcome.

Mr. Hintzen asked if the information available concerning factors affecting the LD_{50} is sufficient to allow us to assess the risk of pesticides to bees. Dr. Pilling said that it would be important to consider the effect of this variation on the hazard ratio and in particular the threshold of 50. Mr. Hintzen suggested that a deviation ratio could be calculated, comparing the LD_{50} values produced from different test and he suggested a value of 3 might be acceptable. However, it was pointed out that there was currently no basis for deciding on what was acceptable and that consideration of the confidence interval for a test might be a better way of assessing the significance of test variation.

Mrs. BARRETT was unable to attend the meeting due to a recent car accident and the meeting sent her their best wishes. Dr. Pilling reported that the presentation that she had been due to give was concerned with the use of solvents in honey bee toxicity tests. While solvents e.g. acetone, may be necessary for tests with technical material, they were not appropriate with formulated products. Dr. Brasse agreed and said that such solvents could affect the activity of the formulation. Some people reported that they had used acetone with formulations in order to get the droplet to spread (i.e. where they were greatly diluted) but Dr. Lewis said that in such cases it was better to use a wetter (which would also need to be in the control).

2.2 Development of established methods (including laboratory, semi-field and field)

Dr. KOCH presented the results of five years of experiments investigating the exposure of bees to plant protection products in the field (**Appendix 8**). Hives were placed in orchards or fields of *Phacelia* and a fluorescent dye applied to the crop at a rate of 20 g/ha using the appropriate application technique. After application the hives were closed up and bees returning to the hives were collected at the entrance and the amounts of dye on individual bees measured. In the orchard trials the average deposit varied between 1 0 and 25 ng/bee compared to 20 and 40 ng/bee in *Phacelia*. Between 0 and almost 100% of bees sampled were contaminated. The proportion of contaminated bees decreased so that 20 minutes after the end of application, levels were about 1 0%. In the *Phacelia* trials the overall variability between colonies was similar but decline over time was slower. Dr Koch discussed these findings in the context of laboratory and field test methods.

Dr. VESELY described a bee flight room at the Bee Research Institute in Dol, Czech Republic (**Appendix 9**). Small colonies of bees are introduced into a mesh-covered tunnel (8 x 3 x 3m) where temperature, humidity and daylength can be regulated. After an acclimatisation period (7 - 1 0 days or until the queen starts laying, in winter) the bees can be used for up to two months. Two to three frames of bees are shaken onto an area in front of the hive and sprayed with the test substance (known concentration of active ingredient) so that they have to fly up in order to return to the hive. Mortality and any 'knockdown' effect are observed before and for up to 7 - 10 days after treatment, together with brood rearing and behaviour. Products resulting in mortality after treatment which is within two standard deviations and with no effects on behaviour or the brood are classified as relatively non-toxic. Where there is increased mortality after treatment (more than two standard deviations on at least two days) but the sum of dead bees is not greater then the number treated and there are no other effects then the product is classified as medium toxic. Toxic compounds give rise to more dead bees than those treated and brood effects. In answer to questions, Dr. Vesely said that flowering plants had not been tried in the flight room but that the results were consistent with those obtained in the field.

Dr. KOVACS reported on a sequential series of experiments conducted in Hungary to investigate the effects of Fury 1 0 EC (zeta-cypermethrin) on honey bees (**Appendix 10**):

(1) In standard laboratory tests (similar to EPPO 170) the contact and oral LD_{50} values obtained were 0.013 and 0. 13 3 µg/bee for the contact and oral routes, respectively, indicating that Fury 10 EC is highly toxic to bees. At the lower doses in the contact test there was 'knockdown' with affected bees recovering after six hours.

(2) In a residual toxicity test, Fury 10 EC (0.1 l/ha) was applied to alfalfa in the evening and bees were exposed to the foliage after various weathering intervals: Twenty four hour mortality increased for up to eight hours weathering but then decreased after 24 hours so that the LT_{50} was between eight and 24 hours.

(3) In a cage test, Fury 10 EC (0.1 l/ha) was applied to flowering *Phacelia* in the early morning (before bee flight). While there was no increase in mortality there was a strong reduction in foraging for up to three hours before numbers started to increase, reaching control levels after about seven hours. This foraging effect was also seen in the morning of days 1 and 2.

(4) In five field trials, Fury 10 EC (0.1 l/ha) was applied aerially to fields of flowering oilseed rape or sunflower (at least 17 ha). In two cases, application was in the evening and no adverse effects were observed. In two, application was in the morning (at least 30 minutes before bee flight) and again there were no adverse effects although in one of the trials there was marked repellency with foraging levels down to about one-third of the day before In a fifth trial, application was during the day (late morning) and there was up to 10% mortality (in the field and in dead bee traps) from bees directly sprayed, as demonstrated by caged bees, so that the application was classified as moderately toxic.

Dr. Kovacs concluded that Fury 10 EC showed no hazard to honey bees if applied outside their activity period, including early morning spraying, with repellency of up to one day reducing exposure so that the potential residual toxicity is not manifested and the bees are able to recover in the hive.

Dr. Oomen pointed out that this series of experiments was a good validation of the EPPO scheme. It was suggested by Dr. Schmidt that the one significant effect observed, repellency, would not reduce pollination as the flowers would remain open until the effect was gone and they were pollinated.

Dr. MAYER looked at the effects of pyriproxyfen, a pyriden insect growth regulator (IGR), on three bee pollinators, the alfalfa leafcutter bee, the alkali bee and the honey bee, using a similar sequential testing scheme (**Appendix 11**).

(1) In an acute toxicity test conducted by Dr. Mayer, adult bees were dosed by topical application at field application rates and there was no increase in mortality compared to the controls in all three species. This is consistent with the mode of action of pyriproxyfen, which acts as a juvenile hormone mimic preventing metamorphosis in pupae but without affecting other stages.
(2) In a colony feeding experiment, pyriproxyfen was fed to colonies at field application concentration using in-hive feeders. Twelve days later 300 capped cells were examined and there was about 35% mortality (mostly pupal) compared to 0% in the controls.
(3) Similarly, no increase in mortality was found when adult bees were exposed for 24 hours residues of pyriproxyfen which had been applied to field plots of alfalfa and then allowed to weather for intervals up to 24 hours.
(4) In field trials conducted with honey bees on flowering pears and white clover, pyriproxyfen was applied at 124 g ai/ha and the number of foraging honey bees counted later that day or early the next together with dead bee counts from hive traps and an assessment of the different brood stages. No repellency was observed and trap mortality showed no increase in adult mortality or immatures/deformed adults. Up to about 10% of cells marked as eggs or young larvae prior to treatment were found not to be capped (empty or containing honey) 11/12 days after treatment and of a sample of capped cells that were opened only a few dead adults/pupae/larvae were found.
Dr. Mayer concluded that while pyriproxyfen showed some effect on brood mortality under severe exposure conditions (direct feeding), when applied to flowering crops at 124 g ai/ha it does not harm bees and can be classified as 'non-hazardous'.

Dr. SCHMIDT reported on work that had been carried out to investigate the effects of the insecticide imidacloprid on honey bees (**Appendix 12**). He reported the following results:
(1) the compound is toxic to honey bees in laboratory tests, especially by the oral route, so that the hazard quotient indicates that it has a potentially high risk to bees.
(2) a series of cage and field trials had been conducted to see to what extent the prediction based on laboratory toxicity was manifested in practice. Following application of imidacloprid (formulated as Confidor) at a rate of 100 g ai/ha to flowering *Phacelia* at midday either in a cage or in the open field, there was an increase in mortality for up to two days after treatment amounting to about 5% (tent) and 2% (field) of all bees. Dr. Schmidt concluded that imidacloprid is hazardous to bees and should not be applied to flowering crops although he noted that the colonies did not suffer, as indicated by colony weight, due to bees remaining

active on the alternative forage available (in the field tests). He pointed out that a strong repellent effect had also been seen, for at least four days after application, and he suggested that this was due to exposed bees returning to the hive and quickly communicating the danger to other bees via the 'tremble dance'.

(3) when application was carried out in the evening, mortality was reduced to about half that of the daytime applications but the reduction in foraging was of a similar magnitude and duration as for the midday application.

(4) no effects were seen on brood development in field trials with *Phacelia* at rates up to 125 g ai/ha.

(5) application 10-14 days before flowering in apple (green tip stage) resulted in no effects on foraging or mortality when the bees were subsequently active during flowering.

(6) imidacloprid is used as a seed treatment and shows strong systemic activity: when used in winter crops e.g. oilseed rape, the time between sowing and planting is more than 200 days so that there will be no honey bee exposure. However, for spring crops this interval is about 60 days and so a trial was conducted in which bees were exposed to flowering sunflower which had been treated as seeds at a rate equivalent to 50 g ai/ha. Mortality was slightly higher than the control although still within the 'normal' range and no effects were seen on flower visitation or hive development.

Asked why mortality was seen over two days if imidacloprid is repellent, Dr. Schmidt said that it was due to the initial exposure but that the effect was slow acting.

2.3 Residue testing

Dr. OOMEN reported on the residue testing sub-group that had been set up at the ICPBR Symposium in Wageningen in 1993 (**Appendix 13**). The group comprises Brasse (BBA, Germany), Forster (BBA, Germany), Lewis (Covance, UK), Oomen (Plantenziektenkundige Dienst, The Netherlands), Schmidt (Bayer, Germany) and van der Steen (Ambrosiushoeve, The Netherlands). The sub-group had concluded that residue testing is important because:

- residues are a potentially important route of pesticide exposure to bees.
- some risk management practices require information on residual toxicity.
- residual toxicity information is required by the EU although accepted testing methods and the subsequent risk assessment are not yet available and technical advice has been requested. The sub-group had produced a number of comments with regards to the incorporation of residual toxicity testing into the risk assessment scheme:

- it would be helpful for risk management and a better understanding of the risks of residues.
- it should have an advantage to the applicant e.g. faster decision making in the overall scheme.
- it should be optional.
- the development of an appropriate test method and the subsequent risk assessment is worthwhile.

A proposal was made for the subsequent steps that need to be taken:

- develop a suitable method.
- collect test results for several pesticides.
- collect field data on residual toxicity to calibrate the laboratory tests (set decision thresholds).

Dr. Oomen said that it was the conclusion of the residual toxicity sub-group that it should continue its work, following the steps outlined above and report back at the next ICPBR meeting.

Following on from this report, Dr. PILLING presented a method for testing residue toxicity to honey bees (**Appendix 14**). He identified the following main requirements for a suitable method: providing realistic exposure; representing pick-up from a leaf surface; practically feasible; repeatable and statistically valid. The current methods available include the US chopped leaf test (unrealistically severe), the Gerig flower test (variable results) and the BBA box test (low variability but unrealistic residue exposure). Dr. Pilling then described a new test using leaf discs: dwarf french bean leaves are sprayed and discs (8.5 cm diameter) cut out and placed in plastic boxes with a sucrose feeder placed at the centre of the disc. Twenty bees are added to each box and exposed to the leaf residues for 72 hours after which mortality and sub lethal effects are assessed. For aged residues the leaves are put in water for appropriate periods before the discs are cut out. Results were presented for three pesticides, cypermethrin, endosulfan and triazophos, comparing the box, flower and leaf-disc method. The box method was found to be more sensitive than the flower one. The leaf-disc method required at least 48 hours to get the full expression of toxicity but was found to be as sensitive as the box method, could detect ageing e.g. showed a reduction in mortality after 24 hours ageing of triazophos and although it was more variable than the box method the results were still acceptable. Dr. Pilling proposed the use of leaf discs as a suitable method and suggested that for pesticides with a hazard ration >2500, a high risk classification could be reduced to a medium risk if the LT_{50} were < 8 hours.

There followed much discussion on the question of residue testing and the proposed methodology. While it was generally agreed that the test should remain optional, Dr. Brasse pointed out that the decision pathway for its use should be clearly defined presenting a benefit for companies. Dr. Candolfi said that it should be available to companies for risk mitigation e.g. allowing evening applications. However, while the option of going straight to cage or field testing would be available, Dr. Schmidt pointed out that the residue toxicity test should be able to replace this if it were to have real benefit. The importance of the plant species used in the leaf-disc test was raised and Dr. Brasse asked whether a standardised species should be used or a species relevant to each use while Dr. Candolfi wondered if the use of a leaf substrate were 'worst-case' as required for a laboratory test. Dr. Oomen pointed out that while the US chopped leaf test could be considered as 'worst-case' it was unrealistically severe and that what was needed was an appropriate degree of realism and standardisation as provided by the leaf-disc method.

2.4 Larval toxicity and growth regulators

Mr. VAN DEN EIJNDE presented a report on the honey bee larvae/bee brood feeding test sub-group that had also been set up at the ICPBR Symposium in Wageningen in 1993 (**Appendix 15**). The group comprises de Ruijter (Ambrosiushoeve, The Netherlands), van den Eijnde (Ambrosiushoeve, The Netherlands), Czoppelt (Max Planck Institut, Germany), Calis (Amsterdam University, The Netherlands), Hughes (National Bee Unit, UK), Urban (Novartis, Switzerland), de Wael , Tornier (GAB, Germany), Dustmann (Celle, Germany), Wittmann (Germany) and Colin (INRA, France). No international guidelines have been agreed to test insect growth regulators (I GRs) on honey bee brood although a number of methods are available and Mr. van den Eijnde reviewed these:

- an *in vitro* laboratory test in which the larvae are reared on an artificial diet in an incubator. While this method can provide detailed information it is complicated and the artificial diet requires royal jelly which is not available as a standard product and so its composition affects reproducibility.
- another laboratory feeding test involves feeding royal jelly and the test substance to larvae in the comb allowing an LC_{50} to be produced. However, a lot of skill and experience is needed to perform the test and again reproducibility is poor. Also, extrapolation of the results to the field is difficult.

- an alternative approach is to feed the test substance (in sucrose solution) into small colonies so that the adult bees are used to transfer it to the brood. However, currently the method suffers from high control variability and lack of reproducibility and an improved method is not available.
- the same approach is adopted in the method of Oomen *et al*, with the test substance being fed into normal colonies of free-flying bees. This has proved fairly quick and easy to perform with low control mortality and reproducible results.
It was proposed that this last method should be used as the standard screening test for larval honey bees although a number of improvements were proposed and discussed.

In the discussion that followed, Ms. Hughes asked why the final assessment was carried out after the marked brood had emerged. Mr. van den Eijnde replied that there may be affects in early emerged bees so it is necessary to leave the brood and pick up these effects in the dead bee tray. However, Dr. Lewis pointed out that this information would not be lost if the marked brood were examined pre-emergence, which would provide more specific information on its fate, as the dead bee tray information was provided by all the brood. Dr. Schmidt asked how the number affected could be assessed and it was pointed out that while the method provides precise quantitative counts ultimately it could not be used to assess the significance of any effects observed in the field but was a screening test to determine the need for further testing under field conditions. Mr. van der Steen said that fenoxycarb and five or six other compounds had so far been tested by this method.

Dr. MÜHLEN reported on semi-field and field tests that he had conducted with the IGR Alsystin (active ingredient triflumuron) (**Appendix 16**). The brood assessments carried out as part of the standard BBA semi-field and field tests are not considered adequate as lost brood may be compensated for by increased breeding activity of the queen and so not detected. Accordingly a ring test had been initiated by the German Bee Protection Group to look at brood effects under field conditions. In the cage tests, nuclei were placed in a tunnel (12 x 4 x 1.8 m) containing flowering *Phacelia* and the colonies were removed one week after treatment to allow better feeding. In the field tests, colonies were placed next to small fields (3000 m^2) of flowering *Phacelia*. In both cases mortality was assessed for up to 22 days after treatment using dead bee trays and from in front of the hive/paths in the crop (field) or from around the edge of the crop (tunnels). Foraging was assessed before application, to demonstrate exposure, as well as after. Brood assessments using marked cells and overall assessments of

levels of brood stages and amounts of food were carried out on days -1, 7, 15 and 22. Following application at 800 g/ha (higher than the recommended field rate) increased mortality was seen in both test and field from about days 12 to 17 (higher in the field), coinciding with the emergence of young and old larvae. In both cases marked losses were seen in the brood development of control colonies and the additional reduction in the treated colonies was from less than 10 to 14 % but the high variability did not allow statistical comparison. It was concluded that the current methods are not sufficiently sensitive to detect effects in the field (as identified in the mortality assessments) but that following marked cells is the right way to assess the hazard of IGRs.

Dr. Urban suggested that as IGRs have different modes of action it might be necessary to have flexible timing of brood assessments to accommodate this. Dr. Mühl en said that one of the main problems was the practicality of opening the hive which leads to high control mortality which would be exacerbated if, for example, assessments were needed at less than weekly intervals in order to look at egg mortality. However, it was pointed out that the Oomen test was a Tier 1 screening test which can remove products showing no IGR activity at an early stage while only triggering further testing (Tier II) under more realistic conditions of exposure, as considered here, where necessary. It was suggested by Dr. Kovacs that a simple quantitative assessment could be carried out using grid counts of brood comb although Dr. Tornier thought that this could also greatly disturb the colony. Dr. Oomen pointed out that the cage and field tests were general tests which could identify brood effects of non-I GR compounds but were not specifically designed for IGR testing although they could be modified for this purpose. Dr. Lewis suggested that it is important to define what a significant effect would be in advance for a given method taking into account the background mortality and its variability.

2.5 Other problems and techniques

Dr. VON DER OHE considered the sensitivity of *Bacillus* larvae to Plantomycin (**Appendix 17**). In recent years there has been a marked increase in the incidence of fireblight in Germany and in order to control this the use of the antibiotic Plantomycin has been allowed. However, while there is no evidence of effects on adult bees there are concerns that unintended exposure of bees e.g. via nectar, could lead to residues in honey and cross-resistance in other pathogens.

The residues in honey could also result in low level infection of diseases such as American foulbrood without clear clinical symptoms but with an increase in the spores of the pathogens responsible. Dr. van der Ohe described laboratory tests in which *Paenibacillus larvae* cultures on agar were exposed to various concentrations of streptomycin sulphate (ai) and Plantomycin. This pathogen was found to be inhibited at concentrations less than the maximum application concentration. It was thus concluded that if contaminated nectar or pollen is taken in, low level infections could develop without clinical symptoms being detected but with an increase in the spores present which could then be spread e.g. by robbing, especially where colonies are placed in close proximity e.g. orchards.

A second paper on the treatment of fireblight by streptomycin was given by Dr. ROSENKRANZ (**Appendix 18**) and this looked at the implications for honey quality. The potential problem has been caused by repeated treatments of apple and pear orchards during blossom when the bees are active and cannot be easily removed as they are needed for pollination. While the bees are unlikely to be affected they, or the nectar they collect, may become contaminated with the antibiotic leading to residues in honey. Twenty nine honey samples from beekeepers in commercial orchards in the Bodensee region were analysed at Hohenheim and none were found to contain streptomycin (50 µg/kg detection limit) although it was found in freshly collected nectar. Analysis at another laboratory did detect residues in two out of 35 samples collected from within Germany (10 µg/kg detection limit). In order to investigate the conditions affecting the levels of residues going into a hive a number of field experiments were conducted in which apple trees within a cage and in a 10 ha orchard were sprayed with streptomycin and vinclozolin (as a marker) together. Bees were collected from the hive entrance during and for up to 30 hours after application. Residues were detected at all time points with the proportion of positive bees remaining constant although the level was variable and not related to honey sac weight. The results were discussed in the context of public concern with regard to streptomycin residues in honey.

It was suggested that the streptomycin molecule would be neutralised by fermentation products within the honey but Dr. Rosenkranz said that residues had been detected up to 3 - 4 months at room temperature after collection and although heating the honey was an option there remained the problem of public perception. It was felt that the problem arose from the availability of streptomycin to treat fireblight which would inevitably lead to some residues in honey, even with dilution from other nectar sources. In addition to this, there is an increasing

problem of fireblight together with developing resistance to streptomycin. Dr. Brasse said that in Germany more than three or four applications could be used during flowering while in the US large areas were affected and daily applications might be carried out for three weeks (Dr. Mayer). Dr. Oomen said that in the Netherlands the use of streptomycin was very controlled but he felt that even if residue levels were reduced improved methods would also result in decreases detection limits. It was felt that a certain amount of streptomycin residues in honey would be inevitable and therefore that either the affected honey was not sold to the public or there would have to be acceptance of the situation.

2.6 Poisoning incidents and monitoring schemes

Dr. BRAS SE presented a report on another of the working groups that had been set up at the ICPBR Symposium in Wageningen in 1993, this one being concerned with the development of a monitoring scheme for poisoning incidents of honey-bees by pesticides (**Appendix 19**). The group comprises Brasse (BBA, Germany), Fletcher (CSL, UK), Oomen (Plantenziektenkundige Dienst, The Netherlands) and Stark (Uppsala, Sweden). Similar schemes already operated in the UK and Germany and a number of common components for a suitable scheme had been identified:

1. Assessment of the poisoning incident
2. Sampling - it was emphasised that this did not involve a systematic collection of data on individual products but providing 'monitoring' on incidents as reported.
3. Storage, packing an transport of samples
4. Biological investigations e.g. bee diseases, pollen present on bees etc.
5. Chemical investigations e.g. pesticide residue analysis
6. Reporting and collation of results

It was recognised that currently such a scheme would not include IGRs due to the delayed nature of their action. It was proposed to develop a standardised form for the recording of bee incidents.

Dr. Oomen asked about the possible wider development of the scheme with a view to presenting it to the EU for implementation on a European-wide scale. Dr. Brasse said that additional members from other countries e.g. France and Italy, were needed before it would be ready to submit to the EU.

A new classification scheme for the risk assessment of pesticides to honey bees that has recently been implemented in the UK was described by Mr. CLOOK of the Pesticides Safety Directorate (**Appendix 20**). The previous scheme involved products being classified on the basis of their toxicity but the resultant label phrases were seen as potentially contradictory and confusing e.g. in the case of pyrethroids which are very toxic but field data indicates no adverse effects. The new scheme is risk based, taking into account the use of the product, and therefore more relevant. The initial step involves an assessment of whether exposure of bees is possible or not followed by, if necessary, acute toxicity tests which are assessed using the hazard ratio. If the ratio is > 50, a 'High Risk' label results which can be removed by the provision of further data to demonstrate acceptability of specific uses e.g. cage or field tests. Soil drenches, seed treatments, pellets and granules will not normally be labelled unless the active substance shows strong systemic activity and is present when bees are active, while IGRs will initially be labelled as 'High risk' unless brood test data is presented. The scheme has been through a consultation exercise with farmers, beekeepers etc and was implemented on 1 July 1996 and would be assessed through the Wildlife Incident Monitoring Scheme and feedback from beekeepers, companies, etc.

In reply to questions, Mr. Clook said that cage tests would be accepted but the requirements would not be prescriptive, rather it would be left to companies to present a supportable case. Dr. Brasse suggested that a positive label would be useful in order to provide farmers with the necessary information on how to use a product with regard to bee safety. Mr. Clook said that this information would be provided by a product's GAP and farmer training e.g. the use of evening/early morning applications.

Miss. BARNETT gave a presentation on the UK Wildlife Incident Monitoring Scheme. looking at the changing patterns of bee pesticide poisoning incidents in England and Wales in recent years (**Appendix 21**). The scheme involves a number of stages:
(1) field investigations looking at the number and condition of the colonies involved, potential forage crops available, pesticide use, etc.
(2) residue analysis using a multi-residue method which detects most compounds
(organophosphates, carbamates, pyrethroids etc)
(3) interpretation, involving consideration of the residue levels found, LD_{50} data, nature and extent of any pesticide use, the pattern of beekeeping, etc.

(4) reporting and collation, informing beekeepers and other interested parties of the evaluation of individual incidents as well annual reviews of all incidents.
Over the period 1993 to 1995, 134 suspected bee poisoning incidents were investigated and of these, 60 were found to involve pesticides (main compounds identified - 30% bendiocarb, 20% dimethoate, 12% pirimiphos-methyl and 12% chlorpyrifos). While the number of incidents reported and poisoning identified had declined from 1990 to 1992, for the last few years they had remained fairly constant. Factors accounting for differences in the compounds associated with bee poisonings were discussed and included weather, time of spraying, state of the crop, proximity of bees, reporting rate of incidents, adherence to label instructions. It was concluded that post-registration surveillance was necessary in order to validate risk assessment schemes, detect unanticipated effects and provide evidence for enforcement action.

Dr. WALLNER considered the use of insecticides as an unexpected source of honey bee intoxications (**Appendix 22**). There has been a long history of bee damage in German vineyards despite not being considered a high exposure crop for bees. When this was realised, pesticides that were dangerous to bees were not allowed for use during flowering which reduced the number of incidents, but not completely. The main vine pest is the grape berry moth which is first treated in June (pre-flowering) using mist blowers with high drift potential. A residual toxicity test was developed which showed that the persistence of pesticides was similar on leaves and flower clusters and that in some cases the duration was much longer than on other crops especially where deposits were high near to the application source. In addition, pollen traps showed that vines were an important pollen source for bees in the area. The structure of the vine flower cluster is such that buds and opened flowers are very close to each other so that bees collecting pollen come into contact with any residues present on the buds even though the pesticide is applied pre-flowering. Thus a route of honey bee exposure to pesticides used on vines was established. The potential for this route to lead to honey bee poisoning was confirmed using a feeding station which simulated bee foraging on vines. It was concluded that persistent pesticides should not be used on vines pre-flowering in order to avoid this new route of bee poisoning that had been identified.

Dr. Urban asked why the situation was particularly bad in the Baden-Baden area compared to other vine-growing areas in Germany. Dr. Wallner said that the treatment practice resulting in this risk was more prevalent in this area and also that there was less alternative forage available together with a high density of beekeepers attracted to the neighbouring Black Forest. When

asked which pesticides were involved, he said that they were all insecticides and that while individual compounds had been found to be particularly persistent e.g. azinphos-methyl, combinations were present resulting in the possibility of interactions between them. Dr. Canez asked about the influence of the uneven distribution of the pesticides resulting from the application technique used and Dr. Wallner replied that residue persistence was indeed very long close to the source of application where the deposits were higher (effects having been seen for up to 50 days).

Dr. STARK was unable to attend the meeting but he has kindly submitted his offered paper concerning bee poisoning incidents in Sweden and the possibility of γ-HCH residues in rainwater (**Appendix 23**).

2. 7 Varroa and varroacides

Dr. BOECKING presented a review of Varroosis and its control (**Appendix 24**). He identified four main types of control: (1) chemical e.g. Apistan, Perizin, etc; (2) 'gentle' chemicals e.g. formic and lactic acids; (3) physical e.g. thermo-box; (4) biotechnical e.g. trap comb. Six chemical products are currently approved for use in Germany, although non-approved products are also used. Dr Boecking highlighted the problem of residues in wax and honey that can result from the use of these products. Lipophilic compounds, in particular, are liable to accumulate in wax and in some cases high levels have been found to occur e.g. bromophylate in German wax and fluvalinate in foreign wax. Depending on the strength of binding these residues may then migrate into honey e.g. bromophylate, coumaphos and to some extent fluvalinate. It was pointed out that even those which do not tend to migrate may accumulate in the wax and small wax particles subsequently contaminate the honey. Residues may also be spread to other hives through the use of re-cycled wax. One of the consequences of the increased exposure of Varroa mites to these compounds, particularly at sub-lethal levels, is the development of resistance and Dr. Boecking gave the example of the reduced effectiveness of fluvalinate in some parts of Italy.

In response to questions, Dr. Boecking said that the problem of varroacide residues was exacerbated by the use of re-cycled wax from outside of Germany resulting in their accumulation, particularly in the case of certain compounds e.g. fluvalinate and flumethrin.

Dr. Oomen asked about the movement of the lipophilic compounds from wax into honey or into man. Dr. Boecking replied that with honey it was variable but in some cases had been shown to be marked e.g. coumaphos. While he did not know in the case of man, he pointed out that beeswax is used in pharmaceuticals and cosmetics so providing additional opportunities for exposure.

A new chemical treatment for Varroa mite was described by Dr. SZALAI (**Appendix 25**). The Varroa mite is becoming an increasing problem in Hungary resulting in the need for effective control measures, with the specific aim of minimising the use of chemicals. Experiments were conducted in 1994 and 1995 comparing a new treatment, Gabon, with Apistan and a 'home-made' material. Application took place in August for one month. Gabon was found to give similar results to Apistan while a significantly weaker effect was found with the 'home-made' material. It was concluded that Gabon gave a significant reduction in Varroa mite numbers without any side-effects.

Asked about the active ingredient of Gabon, Dr. Szalai replied that it is an acrinathrin compound. With regards to the composition of the 'home-made' material, he said that Hungarian beekeepers produced mixtures of all readily available products in order to obtain a cheap treatment but he agreed with Dr. Muhlen that it would have been mainly fluvalinate (the agricultural product 'Mavrik-Flo'). Dr. Vesely added that the illegal use of fluvalinate is an increasing problem where the official products are too expensive, resulting in increased resistance. Dr. Brasse said that in Germany compounds are also used for Varroa-control, which are not officially registered and that many beekeepers use a mixture of different compounds. He supposed that the real reason for many poisoning incidents is a synergistic effect of these mixtures together with the effects of plant protection products to honey bee colonies which are already weakened by Varroa.

Mr. GEFFCKEN reviewed the mode of action and efficacy of the varroacide 'Apitol' (**Appendix 26**). This product contains the active ingredient cymiazol hydrochloride which is water soluble (under acid conditions) and lipophilic. It produces very effective knockdown in mites and may also cause hyperactivity and paralysis. There is no evidence that it has acetylchloinesterase activity and so it may be particularly useful for use against OP-resistant mites. It is systemic in action and can be applied either by a drip-on method or fed to bees (in sucrose solution) although some evidence of distastefulness was found. Mr. Geffcken

provided a comparison of the efficacy of Apitol with other varroacides e.g. 'Folbex' and 'Perizin' using different treatment regimes. He concluded that 'Apitol' provided an alternative treatment to the organophosphate compounds for the control of V arroa, having a different mode of action, particularly where there were resistance problems.

Dr. Stevenson asked if ' Apitol' was registered yet and Mr. Geffcken said that it was not yet on the market. Dr Wallner added that it had residue problems, as with other varroacides, and therefore may only be appropriate for use where the other treatments were not effective.

2.8 Work with other bee species

Mr. VAN DER STEEN gave a report of the work of the fourth working group that had been set up at the ICPBR Symposium in Wageningen in 1993, "Toxicity Test for Bumble bees" (**Appendix 27**). He said that the aim of the group was to develop methods for testing the toxicity of pesticides to bumble bees starting with the honey bee guideline EPPO 170. It was clear that bumble bees were not just big honey bees as pesticide toxicity had been found to differ between the two, although there was no clear relationship with weight. In the oral test, the animals have to be fed individually as there is no trophollactic feeding with bumble bees, but if the bees are held individually they become lethargic and will not feed. Also, there is the problem of contamination from the large quantities of faeces that bumble bees produce. At the second of two meetings held by the group, draft methods had been presented for acute oral and contact tests with bumble bees (see below). These had been discussed, together with workers involved in this area from other institutes and bumble bee suppliers, and it had been agreed to continue to exchange information and to meet again when the formal protocols had been prepared. A number of people had been involved in these discussions: L. de Wael, M. de Greef, H. Schaefer, S. Aldershof, C. Gretenkord, K. Bolckmans, N. Steeghs, A. de Ruijter, A. van den Eijnde and A. van der Steen.

Mr. VAN DER STEEN then went on to describe the methods to determine the acute oral and contact toxicity of pesticides to bumble bees that had been developed jointly at the Dutch bee research institute at "Ambrosiushoeve", the University of Bonn and IPSAB, Münster (**Appendix 28**).

(1) Oral toxicity: Worker bees are collected from young colonies (before males appear), avoiding young bees, and their mean weight determined. They are caged individually and after a starvation period, the bees are fed a range of concentrations of the test material in 50% sucrose solution (a few drops of sucrose solution are placed near the feeder in order to stimulate feeding). The bees are given two hours to consume the test solution and then each treatment group housed together with assessments being made after 24, 48 and 72 hours. There should be two replicate tests with at least 36 bees at each of five concentrations, together with a toxic standard (dimethoate or parathion) and a control. The test is considered valid if at least 30 bees at each concentration consume the test dose, control mortality is less than 10% and the toxic standard LD_{50} is within prescribed limits.
(2) Contact toxicity: the test is conducted in the same way as for the oral test except that there is no starvation period for dosing the bees are anaesthetised with CO_2 (period kept as short as possible). For administration, 1 µl of the test solution is delivered to the ventral thorax between the second and third pair of legs.

In the discussion that followed, Mr. van der Steen agreed that the LD_{50} value should be presented as µg/g bee. Dr. Lewis asked if the test had been validated yet and Mr. van der Steen replied that so far only three laboratories had used the test but there was good consistency between them. Dr. Urban agreed that it was important to validate the test in order to set the test criteria and in particular thought that the number of bees required was high compared to the honey bee test. Other details of the test method were raised including test dose consumption (bees generally consume all the dose and any not doing so should be recorded) and the need to standardise the bees used in the test possibly by buying them in from a number of recognised sources. The regulators were asked if they would use this test and Mr. Clook (PSD) said that while they do not currently request this information they would if bumble bees were shown to be more sensitive. Dr. Brasse (BBA) agreed that a test for bumble bees might be required as the data requirements refer to 'bees' as a whole and honey bees were currently the only representative species on the assumption that others were not more sensitive. However, it was stressed that it was first necessary to show that honey bee data was not sufficient to protect this group and that a comparison of the sensitivity of the two species to pesticides should be made. It was agreed that a ring test was necessary, with as many laboratories as possible involved, in order to validate the test and to make the comparison between honey bees and bumble bees.

Mr. GRETENKORD described laboratory and cage test methods for the evaluation of the effects of IGRs on the bumble bee, *Bombus terrestris* (**Appendix 29**).

(1) In the laboratory tests, larvae of various ages (1, 4 and 6 days), together with a fixed number of attendant nurse bees, were fed the test substance in pollen and sucrose solution at the highest recommended concentration and a series of dilutions if necessary. In addition to LC_{50} values, food consumption was used to estimate the test dose per larva so that LD_{50} values could be produced. With Insegar, there was no mortality or malformations at the highest concentration of 100 ppm but with Dimilin there was increasing mortality with decreasing age so that the LC_{50} values ranged from about 1 ppm (1 day old larvae) to 100 ppm (6 day old larvae).

(2) Bumble bee colonies were connected to cages containing flowering *Phacelia* and when sufficient flight activity had been reached the colonies were removed (leaving the foraging workers in the cage) and reduced in size to leave the queen and a small number of all brood stages. The colonies were then returned to the cages and the test substance was applied to the *Phacelia* on the following day. They were left in the cages for about two weeks before being transferred to the laboratory for a further two weeks to look for any effects on the emerging adults. Insegar had no effect at up to twice the recommended rate. Dimilin on the other hand killed nearly all the brood within two days of application, except for some of the older larvae, and this effect continued on brood produced during the first week after the colonies had been brought into the laboratory. Residue analysis of stored pollen tied in with the laboratory toxicity levels.

It was concluded that the methods described allowed the effects of IGRs on bumble bees to be assessed in both the laboratory and semi-field. While Insegar was not hazardous to bumble bees this did not apply to Dimilin. He finished by asking if field experiments confirmed the assessment for Dimilin should it be classified as hazardous to bumble bees (with associated restrictions) despite the fact that it was considered non-hazardous to honey bees

Dr. Stevenson asked what would regulators do in this situation and Mr. Clook replied that they would need to look at the field data and then consider both the risks and benefits. Dr. Brasse agreed and pointed out that in Germany the classification of Dimilin (a.i. Diflubenzuron) as not hazardous for honey bees was best proved by its use in practice without causing any damage to honey bee populations. On the other hand Insegar (a.i. Fenoxycarb) produced a lot of serious poisoning incidents in honey bee populations. This makes evident that honey bees cannot serve

as the only representative of hymenopterous pollinators in testing side effects of plant protection products.

The effects of IGRs under field conditions was the subject of the presentation of Mrs. SCHÄFER (**Appendix 30**). Alsystin was applied to field plots (2400 m^2) of flowering *Phacelia* at a rate of 800 g/ha. Bumble bee colonies had been placed adjacent to the field and were assessed for brood mortality, numbers of workers and their activity at the hive and in the field and identity of collected pollen. The treatment had no effect on the bumble bee flight activity at the colonies or in the field. Pollen was collected from a number of sources but all colonies collected from the *Phacelia* at some time during over the course of the experiment. Dead larvae were difficult to detect due to workers carrying them away or removal by scavengers but there was a reduction in the number of larvae and also egg cups present in the colonies (without an associated increase in cocoons). It was concluded that Alsystin affected bumble bee colony development and that as this could be more significant than for honey bee colonies (given the relative importance of individual larvae) it was necessary to consider the effects of IGRs on both. However, it was recognised that it was difficult to work with bumble bees in the field as it was hard to follow them and to detect effects in the colonies. One development that was needed was the development of a trap for dead larvae.

Dr. Brasse pointed out that it was not possible to say that Alsystin was harmful to bumble bees with this study as application had been at four times the recommended rate. Mrs. Schäfer agreed and pointed out that the main aim had been to develop appropriate methodology.

3. Recommendations of the meeting

3.1 Regulatory guidelines

A number of issues had been raised with regard to the EPPO/CoE honey bee risk assessment scheme and it was considered appropriate that the guidance notes should be clarified to improve its operation e.g. better guidance on the routes of exposure. Similar consideration was needed for a number of technical issues relating to the recommended test methodology (EPPO 170) e.g. the use of appropriate carriers with technical material, formulated products and biological agents. It was agreed that these points should be considered by the EPPO/ CoE

honey bee sub-group and submitted, as appropriate, to the proposed revision of the EPPO/ CoE risk assessment schemes.

Action: Dr. Oomen and the EPPO/CoE honey bee sub-group

Dr. Tiberg said that she was leaving the ICPBR symposium to attend a meeting of the OECD Test Guidelines Programme in Paris and that she would include notes on this in the paper of her presentation (**see Appendix 5**).

One issue in particular that was seen as being important in the context of the regulatory testing schemes was the use of toxic standards in the laboratory tests. While it was agreed that these were necessary, concern was expressed over the inclusion of specific validatory requirements for the LD_{50} values produced for them. The OECD draft guideline incorporates a specific range of values for dimethoate which have to be met in order to validate each test. These ranges are based on an exercise conducted at one laboratory and it was considered that these do not necessarily represent the variability shown by the various strains of bees used in different laboratories and countries. Accordingly, it was agreed that it is necessary to conduct a similar exercise to that referred to, but using data drawn from as wide a range of sources as possible. It was decided that the ICPBR 'Bee Protection Group' would co-ordinate this exercise and anyone who wishes to contribute data should contact Gavin Lewis.

Action: Dr. Lewis. Any members able to contribute data should send this to Dr. Lewis.

3.2 Residue testing

- it was agreed that there is a role for residual toxicity testing within the risk assessment scheme but that the management option for utilising the information produced should be clarified e.g. the possibility of an evening application where the duration is very short or where it is longer, determining the re-entry period for honey bee colonies into treated crops.
- it was proposed that the name of the sub-group should be changed to persistence testing.
- it was also agreed that the sub-group should continue to develop a suitable validated method, according to the steps proposed by Dr. Oomen, which should be presented to the ICPBR 'Bee Protection Group' for approval at its next meeting.

Action: Dr. Oomen and members of the persistence testing sub-group

3.3 Bee brood testing

- the honey bee brood testing group had recommended that the EPPO method of Oomen *et al* should be used as the standard screening test for honey bee larvae, although a number of improvements had been considered appropriate, and this approach was endorsed by the meeting.
- in addition, it was felt that there was a need for guidance on how to carry out testing of IGRs under semi-field and field conditions. The experiences of Dr. Mühlen, presented to the meeting, were considered to be particularly useful in this respect.

Action: Mr. de Ruijter and members of the honey bee brood testing group

3.4 Monitoring schemes

- the main recommendation of the monitoring scheme sub-group was the need to extend such schemes to as many countries as possible and the meeting agreed that new participants should be invited to join.
- it was recognised that monitoring schemes were not necessarily effective for IGR compounds as these often have a delayed action which would make identification of cause and effect difficult. It was suggested that someone from the bee brood testing sub-group should be asked to join this sub-group to help with consideration of how this area should be dealt with.
- given the development of harmonised regulatory requirements in Europe, it was considered that the implementation of a European-wide monitoring scheme should be recommended and it was proposed that the European Commission could be approached to discuss this possibility.

Action: Dr. Brasse to approach European Commission and to identify bee brood testing sub-group representative.
All monitoring scheme sub-group members to identify potential new members.

3.5 Bumble bee testing

- it was considered important that now the bumble bee sub-group has developed appropriate methodology for assessing pesticide effects on bumble bees, there should be testing of the hypothesis that there is a good correlation between honey bee and bumble bee data i.e. information on the former is sufficient to protect the latter. It was therefore recommended that there should be a comparison between the toxicity values generated for a range of compounds with bumble bees and the corresponding values for honey bees.
- one problem raised in connection with this is the cost of the work involved in ring-testing the method and conducting the toxicity comparison. As this work is important for the implementation of EU Directive 91 / 414 it was felt that an approach should be made to the European Commission to see if it could help with funding.

Action: Mr. van der Steen and members of the bumble bee sub-group.

- a question was also raised about the effects of pesticides on other bee species and comparison with the toxicity values for honey bees. Dr. Mayer was asked if he could provide comparative data on honey bees and other bee species that he has worked with e.g. the alkali bee (*Nomia melanderi*), and the alfalfa leafcutter bee (*Megachile rotundata*).

Action: Dr. Mayer

3.6 Closing

All the participants agreed that it had been a very constructive and enjoyable meeting enabling many old friendships to be renewed. In particular, it continued the successful development of the honey bee risk assessment scheme, providing an example for all other areas of ecotoxicology. It was unanimously agreed that the ICPBR Bee Protection Group should continue with its work. Dr. Stevenson announced that Dr. Brasse would be joining Dr. Oomen as a Vice-Chairman of the group. Dr.Oomen led a vote of thanks to Dr. Stevenson for chairing the sessions and the meeting closed.

Anyone wishing to join the International Commission for Plant Bee Relationships (free membership) should contact Dr J-N Tasei (INRA Laboratoire de Zoologie, 86600 Lusignan,

France). Participation in any of the sub-groups referred to in this report is welcomed and anyone wishing to do so should contact the appropriate sub-group co-ordinator.

3.7 Next meeting

A kind invitation to host the next meeting of the ICPBR 'Bee Protection Group' in two to three years in France was extended by Gilbert Maurin (Association de Co-ordination Technique Agricole). Details on the timing and venue of the meeting will be announced at the beginning of 1998, giving notice of at least one year.

Action: All

Additional copies of this report of the meeting are available for purchase from the International Bee Research Association (IBRA, 18 North Road, Cardiff, CF1 3DY, UK). Any further information on the ICPBR Bee Protection Group can be obtained from Dr. Stevenson, Dr. Oomen, Dr. Brasse or Dr. Lewis.

Appendix 1

List of registered persons

List of registered persons for the 6th ICP-BR Symposium on Hazards of Pesticides to Bees BBA Braunschweig, 17 - 19 September 1996	
NAMES	ADDRESSES
Adrian, Philippe (Dr)	FMC Europe NV Avenue Louise 480 B 9 B-1050 Bruxelles Belgium
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Appendix 2

The Agenda for the Sixth Symposium

Agenda for the sixth symposium of the ICP-BR Bee Protection Group, 17 - 19 September 1996 at Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA) Braunschweig, Germany

Tuesday, 17 th September,

Introduction

9.00 Welcome by
Dr. J. H. Stevenson, Chairman and Prof. Dr. F. Klingauf, President of the BBA
Technical informations by Dr. D. Brasse, BBA.

Session:

Test methodology

9.30 EPPO/Council of Europe risk assessment scheme-report on developments since the last meeting
(P. A. Oomen, Wageningen - NL)

10.00 Development on an OECD-honey-bee testing guideline - differences to the EPPO-guideline
(Ebba Tiberg, Solna - S)

10.20 Discussion

10.50 Coffee

11.20 Experiences in Germany with honey-bee registration testing programme according to BBA guideline (23-1) from the view of the chemical industry (Ch. Kühnast, Limburgerhof - D)

11.40 Factors determining the LD_{50} for honey-bees (Apis mellifera L:)
(R. Hintzen and W. Mühlen, Münster- D)

12.00 Assessing the acute toxicity of formulated plant protection products
(Katie Barrett, Huntingdon - GB)

12.20 Discussion

13.00 Lunch

Developments of established methods (including laboratory, semi -field and field tests)

14. 00 Contamination of bees during the application of pesticides
(H. Koch and P. Weißer, Mainz - D)

14.20 Test in the bee flight room
(V. Vesely, D. Titera and L. Bohacek, Libcice - CZ)

14.40 Effects of the Fury 10EC (Zeta-Cypermethrin)-insecticide to honey-bees in laboratory and field tests
(G . Kovacs, F acankert-
H)

15. 00 Coffee

15.30 Effects of pyriproxifen on bees
(D. F. Mayer, Prosser WA - USA)

15. 50 The reaction of bees under the influence of the insecticide Imidacloprid
(H. W. Schmidt, Leverkusen - D)

16.20 Discussion

Residue Testing

16.50 Report from the discussion group „Residue Testing“

(P. A. Oomen , Wageningen - NL)

17. 10 Method for testing residue toxicity to honey-bees
(E. D. Pilling, Jealott` s Hill - GB)

17.30 Discussion

Wednesday, 18th September, start 8.30 a. m.

Larval Toxicity and Growth Regulators

8.30 „Toxicity tests for honey-bee larvae and bee brood feeding test" (J.H.P.M. van den Eijnde,Hilbarenbeek - NL)

9.00 Implications of the IGR Alsystin on the development of honey-bee colonies under field and semi-field conditions
(W. Mühlen, Münster - D)

9.20 Discussion

9.40 Coffee

Other Problems and Techniques

10.10 Sensitivity of Bacillus larvae to Plantomycin
(W. von der Ohe, Celle - D)

10.30 Treatment of fireblight by use of Streptomycin: A problem for the honey quality
(P. Rosenkranz, K. Wallner and Th. Held, Hohenheim - D)

10.50 Discussion

Poisoning Incidents and Monitoring Schemes

11.10 Report from the discussion group on „Monitoring Schemes"
(D. Brasse, Braunschweig - D)

11.30 Lunch

12.30 Bus departure
for excursion

Return at about 22.00 h

Thursday, 19th September, start at 8.30 a.m.

continuing with: **Poisoning Incidents and Monitoring Schemes**

8.30 Label phrases regarding the risk to honey-bees: a UK approach (M.A. Clook, York - GB)

8.50 Changing patterns of pesticides poisoning incidents of bees in England and Wales in recent years
(E. A. Banett, Slough - GB)

9.10 Insecticides in vineyards an unexpected honeybee intoxications
(K. Wallner, Hohenheim - D)

9.50 Residues of Lindane in rain water and intoxication of bees (J. Stark, Uppsala - S)

9.50 Discussion

10.20 Coffee

Varroa and Varroacides

10.50 Control of Varroasis - a necessity for beekeepers, Why?, how?, difficulties!
(O. Boecking, Bonn and K. Wallner, Hohenheim - D)

11.10 New chemical control against Varroa-mite
(E. M. Szalai, M. E. Molnar, Z.S. Pacs and L. Lennert, Gödollo - H)

11.30 Spreading of Varroa jacobsoni Oud. influenced by low effect of the preparation Sanvar
(Violeta Ceksteryte, Kedainiai- Lt)

11. 50 Mode of action and efficiacy of Apitol against Varroa
(H. Geffken, Celle - D)

12. 10 Discussion

Work with other bee species

12.30 Report from the discussion group on „Toxicity tests for Bumble bees"
(J.J.M. van der Steen, Hilvarenbeek - NL)

13.00 Lunch

14. 00 Methods to determine the acute oral LD_{50} and acute contact LD_{50} of pesticides for Bombus terrestris L.
(J.J.M. van der Steen, Hilvarenbeek - NL, C.Gretenkord, Bonn - D and Heike Schäfer, Münster - D)

14.20 Laboratory and cage test methods for the evaluation of the effects of insect growth regulators (Insegar, Dimilin) on the brood of Bombus terrestris L. (C. Gretekord and W. Drescher, Bonn -D)

14. 40 First experiences to test side-effect of Alsystin on bumble-bees (Bombus terrestris L.) in the field (Heike Schäfer and W. Mühlen, Münster - D)

15. 00 Discussion

15.20 Coffee

15. 40 -Conclusions and future plans
- Next meeting
- Any other points

End about 16.00 h

Appendix 3

A statement from Professor Dr. W.Pestemer, welcoming the participants of the VI. ICP BR Symposium on Hazards of Pesticides to Bees

Welcome of the Participants of the VI. ICP-BR Symposiums on Hazards of Pesticides to Bees
by Prof. Dr. Wilfried Pestemer (inplace of Prof. Dr. Klingauf, President of the BBA)

Mister Chairman,
Ladies and Gentlemen,
it is a great pleasure for me to welcome you for the 6th symposium of the ICP-BR Bee-Protection Group at Braunschweig as representative of our president Prof. Klingauf. I beg your pardon, that he is not in the fortunate position to give the welcoming speech by himself, due to other engagments in this time of great changes in our ministry in Bonn do need his presence in person. But he sends his kindest regards to all participants of this meeting.

Unfortunately, it is not possible to hold the meeting on the site of the Federal Biological Research Centre for Agriculture and Forestry itself as planned in the beginning. In the meantime the great conference hall of the BBA was started to be rebuilt for the purpose of holding international conferences, especially regarding the registration of pesticides in the European Community. So we are planning, e.g. to install cabins for simultaneous translation, which might have been useful for your symposium too.

But nevertheless, I really hope that the solution to hold the meeting in the hotel, in which most of you found accommodation, will be convenient for you.

But now let me give a short overview of the goals and objectives of the place, which is the host of this year's symposium.

This is a map of the reunified Germany with the institutes of the BBA, which are distributed over Germany. The main centre is Braunschweig and the 2nd one Berlin, where the former Imperial Biological Reseach Centre for Agriculture was founded some 1 00 years ago in this building, where I am working as head of the Institute for Ecological Chemistry and the chemical examination of bees are carried out by Dr. Koßmann, who is participating this symposium too.

So you see, that in two years we will celebrate the 1 00th anniversary day of the BBA, which is one of the oldest research centres in Germany. Originally the BBA was part of the federal investigation center for public health. But from the beginning on it was the task of the BBA to investigate plant pests and diseases and methods of their control.

This is a list of the current institutes of the BBA. Most of the employees, among some 300 scientists, are working in Braunschweig; about 30 % in Berlin and Kleinmachnow near Berlin in the former GDR and another 10 % at the other institutes near Heidelberg, Darmstadt and a little town on the river Moselle. The biggest institute of the BBA with more than 70 scientists is the "Department of Plant Protection Products and Application Techniques" with 3 Divisions for Chemistry, Biology and for Application Technique. There, all pesticides are tested and in case they are found efficient and do not having danger to humans and the environment they get a license for marketing in Germany. The next transparency shows some aspects covered in the authorization procedure for PPP in Germany. But most of the scientists are working on biology and of pests and diseases of plants and methods of control, that means for instance threshods of pests as shown in this transparency.

Already at the beginning of this century well developed methods existed for a chemical control of plant pests and diseases and it was obvious that already at that time scientists looked for possible side effects of pesticides to the environment and especially to honey-bees. The fact that honey-bees are the best pollinators in agriculture was well known since many, many years. But in the years with bad economical situation people realize this much more intensive than in times, in which you can buy all kind of food in a plenty. This was the situation in Germany at the end of the twenties and logically the aims of honey-bee research in that time were changed.

While in the beginning of the century most of the honey-bee investigations in the BBA were carried out with the aim to detect honey-bee diseases and their control, this day the aim is to protect bees against the use of potential toxic substances in agriculture mainly.

The first result of these efforts worth of mentioning was that in 1934 a first decree for the protection of honey-bees against the use of pesticides was issued by the government. This makes evident that honey bee protection has a long tradition in German plant protection and therewith in the BBA.

But I believe, that in many other European countries a similar development in bee protection took place. The fact that here today a group of nearly 50 scientists from 13 states of Europe and America came together in order to discuss about a better honey-bee protection does express this in an evident manner.

With regard to the progress of the unification of Europe and because of the special problems in agriculture linked with the unification, it seems to be necessary to harmonize many things, which is

the usual standard in some countries for a long time, but not yet introduced to other countries. This concerns for example to the use of plant protection products and their undesirable effects to human health and the different parts of the environment, which are continuously standing in political discussion, in any case in Germany. In the harmonizing process the testing procedure for side effects of pesticides to non target organisms the test with honey-bees always played the role of the out-rider. The harmonization of the testing procedure for honey-bees has made in my information the best progress in the system of harmonizing tests for side effects of pesticides. This is, as I believe, the result of the intensive work, which had been made on the foregoing symposia of the ICP-BR. As I am informed the ICP-BR has already started in 1980 on their first symposium at Wageningen to define the criteria of testing side effects of pesticides to honey-bees in the different European countries with regard to a later harminization.

I believe, that you have fulfilled herewith a great deal of work in harmonizing plant protection in Europe.

But nevertheless it seems that the work of harmonization and investigation regarding the undesirable effects of pesticides to bees is not yet been finished. As I can see from your todays programme wide spheres of activity are open, for example:

- How and in what extent are wild bee species put at risk by the use of plant protection products and how can we protect them. Do we need new regulations or are the existing ones sufficient?
- Are we good enough informed about synergism effects between bee diseases and their control and the use of plant protection products, or the synergism between different chemical agents in the environment and its effect to bees.
- What can we do to drive back the every years number of damages to honey-bee populations by the use of pesticides.

Especially in Germany we made sorrowful experiences with great losses of honey-bee populations caused by the use of pesticides in the years between 1970 and 1985. In this time we had areas in Germany, which had been nearly emptied from honey-bee populations and I do wish that such situation should not come back again, neither in Germany nor in any other country. For this reason I feel that it is very important that you can keep pace in the development of methods for testing and evaluation of pesticides with the development of new pesticides.

A look to your todays programme makes me sure that you are on a promising way or the solution of these problems. In that sense I wish you a very successful meeting and some nice days in Braunschweig, especially with a pleasant excursion tomorrow.

Thank you very much.

Appendix 4

P. Oomen
The EPPO/Council of Europe: Subgroup Honeybees Report on developments since the last meeting of the ICPBR

EPPO / Council of Europe: Subgroup Honeybees
Members: Belzunces FR, Forster D, Greig-Smith UK, Kovács CZ, Lewis GIFAP, Oomen NL, Stevenson UK, Svendsen DK

Report on developments since the last meeting of ICPBR
Pieter A. Oomen, Coordinator, 16 August 1996

Since the last meeting of ICPBR in Wageningen in October 1993 the following developments occurred in the field of work of the EPPO/Council of Europe Subgroup on Honey Bees. The main decelopments are elaborated in separate annexes to this paper.

1. Proposal to EPPO from the coordinator for updating the EPPO Guideline 170 to accommodate the recommendations of the Wageningen Symposium 1993 (annex 1).

2. Discussion in the Subgroup on the number of tests to be required for decision making in the Honey Bee Scheme (annex 2).

3. Validation exercise of the Panel on Environmental Risk Assessment, done by European Plant Protection Authorities of the Honey Bee Risk Assessment Scheme (annex 3).

4. Start of an initiative by OECD for developing laboratory test guidelines for an oral and a topical LD50 test method (see contribution by Ebba Tiberg). OECD is expecting from this ICPBR-symposium a recommendation for a range of values for acceptability of the LD_{50} values of the positive controls.

5. EU discussion and decision on data requirements and risk assessment on honey bees for the European registration of pesticides, to implement directive EEG/91/414. The EPPO/CoE Group was not directly involved, but its members were through their national authorities.

6. The Panel on Environmental Risk Assessment decided and confirmed in its meeting in Paris of March 1996 that
    - a revision of the separate chapters of the scheme now is possible and desirable. The results of this can be published separately;
    - a workshop will be held in 1997, probably in Wageningen, to advertise the existence of the schemes, to instruct representatives of registration authorities in its use, and to obtain the opinion of practitioners on its usefulness and ease of use. The honey bee scheme will certainly be presented as one of the best examples of a scheme that is known to work well.

Report of EPPO / Council of Europe Subgroup Honeybees
Request to EPPO for updating guideline 170.
Pieter A. Oomen, Coordinator, 16 August 1996

EPPO-guideline 170 (Bulletin OEPP/EPPO Bulletin 22, 203-215, 1992)
Guideline on test methods for evaluating the side effects of plant protection products on honeybees.

Background:
In October 1993 the ICPBR (International Commission for Plant Bee Relationships), which is concerned with international harmonization of test methods for effects of pesticides on honey bees and bumble bees, met in Wageningen for its fifth International Symposium. The ICPBR reviewed the different testing methods in use now and made a number of recommendations. These have resulted in the following proposal to update EPPO guideline 170, dated January 5, 1995. This proposal has not yet resulted in an offical update of the scheme.

Proposed changes:
p.203, line 17 (line counting includes the title):
' .. and Harpenden, GB 1985)'
Add: Rez, CZ (1990) and Wageningen, NL (1993)

p. 203, line 31:
' .. is currently developing such schemes, including .. '
Change for:
' .. has developed such schemes (EPPO/CoE 1993, 1994), including .. '

p.204, § 1.2
Delete: ' .. and a high relative humidity (about 60-70% RH).'
Add: 'Relative humidity during the test should be recorded.'

p.204, § 1 .3, 2nd line
Insert after ' .. queen-right
colonies. The last varroacide treatment should be identified and the timing recorded. The treatment should have ended at least 4 weeks before the start of the test.'

p. 205, § 4
The first line should read:
'Repeat tests where control mortality is above 15%.

p. 206, § 3
The first line should read:
'Record effects *at least twice* before, and at several intervals, . .'

p. 207, § 1.5
First sentence should read:
'Treatments: product(s) to be tested, reference product known to present a low hazard to bees or an untreated control; reference product known to present a high hazard to bees (e.g. parathion, dimethoate). If a toxic reference treatment is not possible or undesirable, honey bee exposure should be demonstrated otherwise, e.g. by evidence based on observations such as:
- Counting foraging bees before and after application;
- Collecting pollen
- Marking bees in the field.'
p. 208, § 3.2.2
First sentence should read:
'Pre-treatment assessment: at least twice, of which the last one day or just before treatment.'

p. 208, § 4
Insert after first sentence:
' .. treatment is low, or when exposure cannot be convincingly demonstrated.'

p. 215, References: Add:
ICPBR (1990) Proceedings of the Fourth International Symposium on the Harmonization of Methods for Testing the Toxicity of Pesticides to Bees. May 15-18, 1990, Rez, Czechoslovakia.
ICPBR (1993) Discussion and Recommendations of the Fifth Meeting, p.10-14 in:
Harrison, E.G. (1993) Proceedings of the Fifth International Symposium on the Hazards of Pesticides to Bees. October 26-28, 1993, Wageningen, The Netherlands.
Gough, H.J., McIndoe, E.C., Lewis, G.B. (1994) The use of dimethoate as a reference compound in laboratory acute toxicity tests on honey bees (Apis mellifera l.) 1991-1992. Journal of Apicultural Research 33 (2) 119-125.

Report of EPPO / Council of Europe Subgroup Honeybees

Number of tests required for decision making

Pieter A. Oomen, Coordinator, 16 August 1996

Background:
At the fifth International Symposium of the ICPBR in Wageningen in October 1993 the EPPO/Council of Europe Subgroup on Honey Bees was asked to decide about the number of tests to be required for decision making. The group discussed this matter extensively and came to the following conclusion. Herewith its report to the ICPBR Symposium in Braunschweig:

Number of tests required

Without much discussion the group came to an agreement about the number of cage, tunnel and field tests. More discussion was needed to decide on the number of laboratory tests. For decision making at the different levels of the scheme, all members agreed that it is sufficient to have:

- **one *good* (oral *and* contact) laboratory test**;
- **a minimum of two tunnel and cage tests** (according to the ICPBR proposal in 1993)
- **one field trial per country/climatic region** (according to the ICPBR proposal in 1993)

Explanation on the number of laboratory tests

Normal variation in measurements is sufficiently accounted for by the different safety margins used in the decision making scheme, while hazard ratios very near to the decision threshold (i.e. slightly below 50) usually will activate the *'analysis of uncertainty'* of the scheme. This may result, depending of the expert judgement of the evaluating authority, in a need for more information, e.g. more test results. This decision of the subgroup has led to the following proposal to update the decision making scheme for the environmental risk assessment of plant protection products (Chapter 1 0 Honey Bees, Bulletin OEPP/EPPO Bulletin 23 (1), 151-165, 1993). This proposal has not yet resulted in an offical update of the scheme.

Proposed changes in scheme:

1. (p. 153, step 16, 1st line)
Add: 'see Note 10'

2. (p. 156, note 2, last line)
Add: 'In principle a single good laboratory test of both the contact and the oral toxicity is sufficient for decision making. See also Note 10.'

3. (p. 156, note 3, 3rd line)
Replace: 'described by Gerig & Oomen (1993)' by 'being developed by ICPBR'.

4. (p. 156, note 6, last line)
Add: 'In principle two good cage trials are sufficient for decision making.'

5. (p.157, note 7, last line)
Add: 'In principle one good field test per country or cimatic region is sufficient for decision making.'

6. (p. 157, below note 9)
Add*: 'Note 10. Analysis of uncertainty*
The measurements used for decision making in this scheme may vary as a consequence of their biological and practice-derived character. The suggested thresholds for decision making contain a safety margin that accounts for the normal variation in measurements. In situations where slight variations in a measurement would change the risk category (e.g. hazard ratio slightly below 50), additional information such as more test results may be used to confirm the final risk classification.'

Annex 3

Report on the validation of the EPPO/CoE Scheme on Environmental Risk Assessment, Bulletin OEPP/EPPO Bulletin 23 (1), 151-165, 1993: Chapter 10 Honeybees by Pieter A. Oomen, Coordinator, 16 August 1996

Validation exercise

The EPPO Panel on Environmental Risk Assessment organised in 1995 an international 'validation exercise' in order to try and validate the different environmental risk assessment schemes among which the Honey Bee Scheme. The exercise consisted of five complete pesticide data sets that were made available to the European Plant Protection Authorities, together with the schemes. The authorities were asked to evaluate the datasets according to the schemes and send their findings. The results of the validation exercise were studied and evaluated by the coordinators of the EPPO/CoE Panel and of the Subgroup on Honey Bees.

General comment

This sub-scheme on honey bees was the most consistent and accurate in the way that assessors interpreted the results of laboratory toxicity tests, probably because the test methods are already standard and widely used. Interpretation of chances for exposure, and results of field trials appeared less consistent. The final classifications, actual risk assessments, were consistent. The following aspects need comments.

Specific comments

One type of error in the calculation of the hazard ratio (or Toxicity Exposure Ratio, TER) was made by several assessors. This is the ratio between dose rates (in g/ha) and LD_{50} (in µg/bee). It should be calculated by using either quantities of *active ingredient* in dose rate *and* LD_{50}, or quantities of *formulated product* in both. Remarkably, errors made here did not lead to errors in final classifications. These errors can be avoided by a clearer explanation in the scheme.

In the cases that the main route of exposure was not through direct sprays but, for example, by systemic intoxication such as with granular nematicides, the assessors remained puzzling long with calculating the hazard ratio. It is, however, only in more realistic studies such as cage tests that risks of this kind can be reliably assessed. It led to attention spent to less important aspects, but not to incorrect use of the scheme, or wrong conclusions. Better guidance in the scheme on the relevance of different routes of exposure (but also more experience of the assessors) may prevent these spilled efforts.

Estimation of the possibility for exposure appeared to be variable. For instance, use of pre-emergence herbicides is usually considered not to expose honeybees. Risk assessment of such pesticides can be done fast and easily by dismissing realistic chances for exposure before entering a toxicity evaluation. Better guidance to help assessors in estimating chances for exposure dependent of the type of pesticide and type of crop seems desirable.

One of the aspects addressed by the scheme is the persistence of pesticides on foliage (question 4). It appeared that hardly any information is available to answer this question. Pesticides with a low chance of exposure and a high toxicity (such as the systemic nematicide, product A) may be wrongly classified as high risk if persistence on foliage would be estimated as high. The assessor in question, however, indicated correctly that field test results were considered to prevail over this conclusion.

Conclusion

Most of these points can be solved by a minor review of the guidance notes in the scheme. In spite of these potential sources of error, it was remarkable that nearly all classifications were correct. This is likely to be due both to the expertise of the assessors and the robustness of the scheme.

Appendix 5

E. Tiberg
Development of an OECD Honey Bee Test Guideline - Difference to the EPPO Guideline

Development of an OECD Honey Bee Test Guideline
Difference to the EPPO Guideline

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Introduction

OECDs Test Guidelines Programme (TGP) develops guidelines for the testing of chemicals. It is an activity under the Chemicals Programme within the OECD and includes the development of test guidelines for physical/chemical properties, bioaccumulation and the degradation of chemicals as well as for the testing of effects of chemicals on biological systems and human health. The decision on Mutual Acceptance of Data (MAD) is important for the OECD member countries. It means that a test which has been carried out with a chemical substance in one member country has to be accepted in all member countries, thereby saving money and effort on testing. The member countries therefore prefer to develop OECD guidelines instead of using national or other international standardised methods. However, the guidelines have to be developed and adopted in consensus between the member countries, which often is a relatively long procedure (1).

The request for a guideline for the testing of acute toxicity to honey bees came from the secretariat of the TGP but was also highly prioritised by the Pesticide Task Force within OECD. Guidelines for acute oral and contact toxicity were drafted in 1994 on the basis of existing test methods. At that time the European and Mediterranean Plant Protection Organization (EPPO) method was the one most recently published, i.e. in 1992 (2), and was used as a base for the work together with the suggestions for its updating (3). However, other test methods, like the BBA-guideline from 1991 (4) and the US-EPA guideline for contact toxicity from 1995 (5), were taken into account, as well as the older UK method (6) and recommendations from SETAC (7). After the circulation of the first draft versions in 1995, the guideline proposals were revised according to the member countries comments in spring 1996. These revised drafts are now the subject for my comparison with the original EPPO guideline mentioned above. The purpose of the comparison is to investigate if the two test methods are significantly divergent and if there are still questions in the test procedure, which could be answered by further research.

Comparison between the EPPO test from 1992 and the present OECD proposal from 1996

In Table 1, details of the EPPO guideline from 1992 with the suggestions for changes from the I CB PR-meeting in 1993 (3) are listed to the left. The corresponding test stage in the existing OECD draft guidelines (both the oral and the contact test) are listed to the right. Only details which seemed to be crucial, either from a practical or scientific viewpoint, as identified when drafting the guidelines and from the comments from member countries, have been listed. However, only the words and sentences in italics are *different* in the two guidelines.

The items which have been most discussed during the development of the OECD drafts are the anti-varroa (or other chemotherapeutical) treatment of bees and the use of a toxic standard. It was concluded that, as probably most of the European honey bee strains are infected with varroa, and subsequently treated, it is most relevant to use treated bees. As stated in Table 1, bees that are used should have been free of chemotherapeutical treatment of any kind for four weeks before testing. This is valid for both tests. However, it has to be stressed that bees

treated with chemicals probably have a different physiology and it is therefore not adequate to compare older test results achieved with untreated bees with test results from the treated bees. This is especially true a chemical used for the treatment of bee diseases can have a synergistic effect with the pesticide tested.

The use of a toxic standard, preferably dimethoate or parathion, is given in both tests but is also included in other test methods. However, if a toxic standard has to be used, an LD_{50}-value for this substance has to be given in the test guideline in order for the evaluator to be able to verify the test results. The LD_{50}-value presently included in the OECD draft is derived from Gough *et al.* 1994 (8). However, ICBPR has been asked by the TGP to make a larger survey of LC_{50}-values for dimethoate to be included in the guideline. The survey should compile data from different countries and laboratories using different bee strains.

Table 1. Comparison between the EPPO test from 1992 and the OECD draft guideline 1996. The numbers refer to the respective paragraph in the test guideline.

EPPO 1992 (2) incl. suggestions for changes 1993 (3)	**OECD Draft 1996**
1.2 Trial conditions -well ventilated and easy to clean test cages, disposable plastic, sterilised wooden cages, should not cause control mortality -25 °C ± 2 °C, RH should be recorded 1.3	8. Test cages/18. Test conditions - stainless steel, wire mesh, plastic or disposable wooden cages (one side transparent) - the *size of the cage* is given (0.5 dm^3) - 25 °C ± 2 °C, *RH: 50% ± 10%*
Preparation of bees -collection from frames without brood, flight board entrance -avoid collection in early spring, late autumn -in contact test CO_2 can be used for anaesthetisation -bees treated with antibiotics, anti-varroa subst. etc. should not be used for four weeks from the end of the last treatment	7. Collection of bees - frames without brood, *young hatching bees* - avoid *winter*, early spring, late autumn - collection time: evening before or morning for test - *nitrogen* is also allowed, many questions about the time for anaesthetisation - bees treated with chemical substances (against varroa e.g.) should not be used for four weeks from the end of the last treatment
1.4 Design of the trial - include appropriate reference product to check consistency of results (e.g. parathion, dimethoate) - concentrations in order to provide a regression line and LD_{50}	16. Toxic standard/ 13. Test and control groups - a toxic standard should be included in the test series (dimethoate is preferred) - some more advice for how many concentrations and which, inclusion of NOEL
2.1 Oral tox. test - 2.1.1 Test products - formulated product or a.i. in 20-50% sucrose solution - dissolve formulations without solvents if possible (acetone permitted)	9.Handling and feeding conditions - food: *50% (w/v) sucrose solution*, use a bee feeder 11-12. Preparation of doses - for technical products and substances of low water solubility, organic solvents, emulsifiers or dispersants of low toxicity to bees may be used (however, then with appropriate controls)

2.1.2 Mode of application - dose 10-20 µl of test solutions per bee through glass tubes - group feeding	10. Preparation of bees/17. Administration of doses - bees are randomly allocated to test cages, which are randomly placed in the experimental room - each test group is provided with 100-200µl of test substance in 50% sucrose solution with a bee feeder (with a recommendations when the lower or higher amount is needed)
2.2 Contact test: Test products - dissolve compound in acetone	11. Preparation of doses - acetone is preferred as organic solvent - use a wetting agent for the contact test (Agral, Citowett, Lubrol, Triton, Tween)
3. Mode of assessment - count the number of dead and affected bees at 24h-intervals for up to 48 h, or longer if mortality is still increasing	19. Duration/20-22 Observations - duration of test: 48 h. If mortality increases more than 10 %, test duration should be extended to max. 96 h - mortality is recorded after 4 h, 24h, 48h (72h, 96h) - diet consumed should be estimated
4. Results - control mortality: 15%	24. Data/25. Test report - control mortality: 10% *(BBA 15%, EPA 20%, NL 15%)* - report: a long list of data

The comments received from the member countries when the first OECD draft was circulated made the guidelines more detailed. This is not a desired development as the guidelines should include more general descriptions than national test methods. It should be possible for the laboratories to follow a national test method, but still be working according to the OECD test guideline. Examples of the details are the description of test cages, the range for relative humidity and the use of 50% sucrose as food. The use of honey solution as nourishment has also been a demand from the member countries.

In the EPPO test, it is clearly stated that when the doses are prepared, solvents should be avoided even if acetone is permitted if necessary. According to the OECD test, the use of solvents for technical products is mentioned, while for water-soluble formulations, wetting agents are preferred, especially for the contact test for better wetting capacity.

Conclusions

It can be concluded that the OECD draft guidelines are much more detailed than the EPPO guideline. It is possible to conduct the test according to the OECD draft and still be working under the umbrella of the EPPO test if one is aware about some details in the OECD drafts, e.g. the relative humidity and the concentration of sucrose. Data on which influence e.g. different food sources, light, bee strain or age have on the toxicity could probably be found in existing publications. Validation studies would be of interest.

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Note

The day after this presentation, the 18 September 1996, it was decided at the TGP National Co-ordinators meeting that some changes to the guidelines should be made. The more detailed descriptions of test cages, the range of relative humidity etc., should be deleted, leaving it more open to the laboratories for how to work. The toxic standard should prevail, and a relevant range for a LD_{50}-value for dimethoate for oral and contact toxicity should be found with the help of an inventory and an evaluation of LD_{50}-values from different countries, carried out by the ICBPR-Bee hazard group (as soon as possible). A limit test should be included. As these are major changes of the draft guidelines, they will be circulated on a quick commenting round to the National Co-ordinators of the TGP for approval through written procedure. The guidlines can thereafter be directly forwarded to the OECD Joint Meeting for adoption.

Appendix 6

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Summarizing data analysis of registration study results with crop protection compounds on honey bees (*Apis mellifera*) from Germany

Summarizing Data Analysis of Registration Study Results with Crop Protection Compounds on Honey Bees (Apis mellifera) from Germany

Künast, C. (BASF AG); Bock, K.D. (AgrEvo); Schmuck, R. (Bayer AG)

I INTRODUCTION

Various European countries differ regarding the test methods which are used to carry out studies on regulatory testing procedures of crop protection compounds with honey bees. In Germany there is an extensive fund of data available according to (11). The main principles of this testing guideline are:

° The laboratory test is divided into four different procedures, namely vapour phase, prolonged contact, wetting or dusting, oral uptake test.

° With the exception of the oral uptake test these trials are limit tests in which only one dose (twice the maximum recommended concentration) is applied.

° The oral uptake test specifies threshold concentrations, between which the experimentally determined LD_{50} lies.

The laboratory studies, also tent and field studies, were generally carried out several times per compound at different test institutes. If a trigger in the laboratory test - 15% after 24 h, or 30% after 72 h in the mortality rating - was exceeded, it was recommended to continue the investigation in the tent and, as the next step, in the field. Thus each stage of the sequential testing scheme (laboratory, tent and field) generated a series of data that flowed into the overall assessment of the crop protection compound.

II AVAILABLE DATA

The data records kept by BASF AG, Bayer AG and AgrEvo over a period of many years were evaluated. Although the records of the three companies are structured differently, what is substantially common to them all is that they contain the following information:

- Codes of the test substances

- Class of each test substance (herbicide, fungicide, insecticide, bioregulator, acaricide or insect growth regulator [IGR]).

- Stage of the sequential testing scheme (laboratory/tent/field trial)

- Result of each study (not hazardous to bees - yes/no)

  (This study-specific assessment is independent of the overall classification (B 1 - B 4 according to (2)) of the substance. The code "not hazardous to bees - no" means the exceeding of a trigger value and the recommendation that the study must be carried out at the next stage of the sequential testing scheme [with the exception of the field trial as final stage of the sequential testing scheme]).

- Testing institute

Table 1

Number of Available Studies of the Companies Involved

	LABORATORY	TENT	FIELD
BASF AG	274	89	7
Bayer AG	273	143	99
AgrEvo	124	*	*

* Only laboratory results were available from AgrEvo.

Altogether the data sets derive from 95 herbicides, 79 fungicides, 44 insecticides, 5 bioregulators, 5 acaricides and 3 insect growth regulators. The oldest dated studies are from 1982 and the most recent ones from 1995.

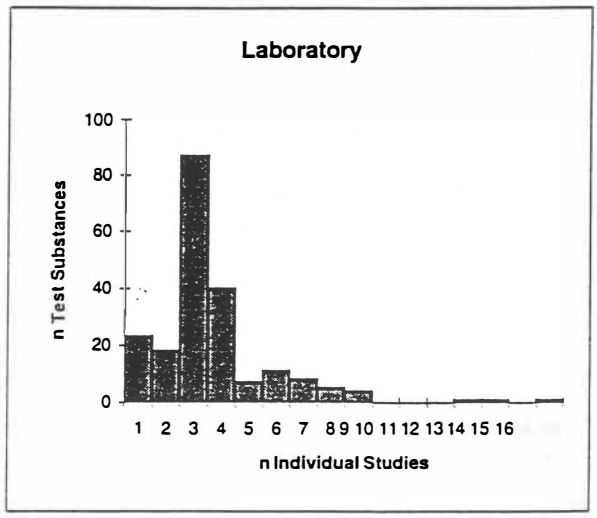
III SUMMARY AND ANALYSIS OF THE DATA

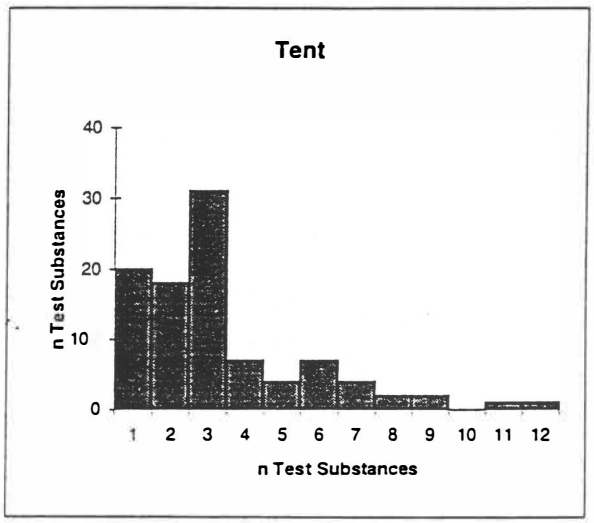
1. Number of Studies per Test Substance

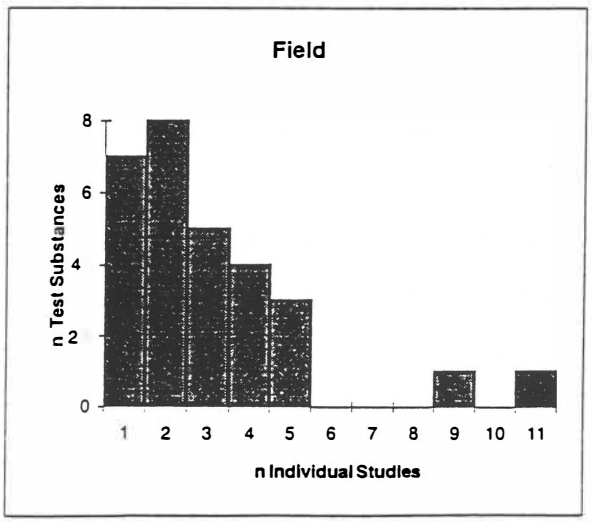
Regularly, several studies were reported per test substance on each level of the sequential testing scheme. Isolated single studies were carried out exceptionally, for example for development formulations, sometimes also for new formulations of known active ingredients. Greater numbers of studies are often documented for substances where individual results were not uniform (Fig. 1).

Fig. 1

Number of Individual Studies per Test Substance







As expected the highest absolute number of studies is existing from the laboratory. Mostly 3 test replicates per compound, conducted at different institutes, have been recorded in laboratory and tent.

2. Structure of Results in Laboratory, Tent and Field Studies

At each level of the sequential testing scheme, as Fig. 1 shows, a series of data was generated per test substance. Each individual result was recorded in the files alternatively either positively or negatively - "not hazardous to bees - yes" or "not hazardous to bees - no" (as already mentioned, this study-specific coding is independent of the overall assessment of the substance that is conducted by the national registration authorities). The results of these individual studies of a series conducted at different test institutes were not always identical. For the following evaluations the series of results for the same substance and the same level of the sequential testing scheme are divided up into three groups:

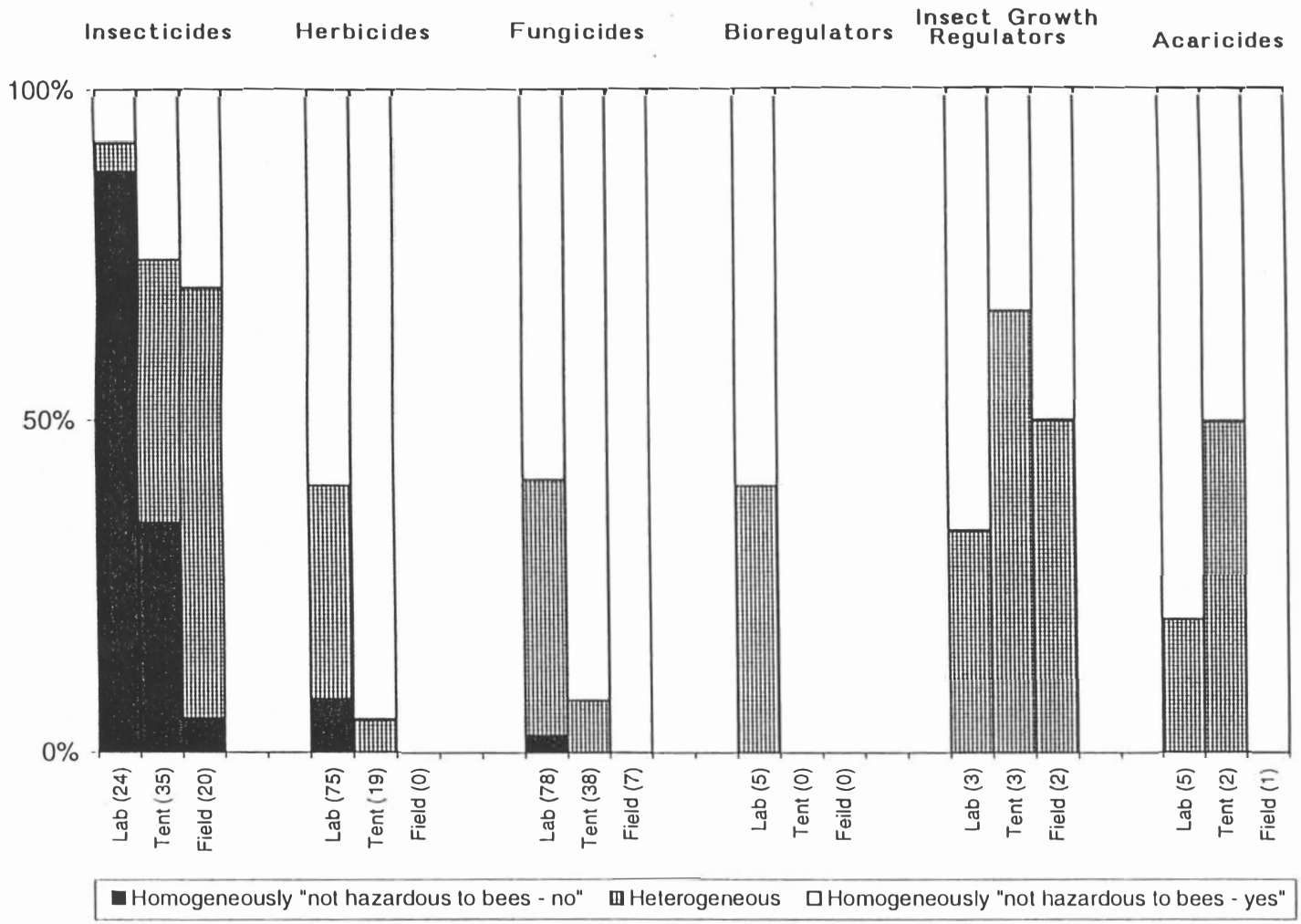
I) Results from all test institutes homogeneously "not hazardous to bees - yes"

II) Non-uniform results from the test institutes

III) Results from all test institutes homogeneously "not hazardous to bees - no"

If the data are broken down according to these criteria and assigned to classes of substances and to the three levels of the sequential testing scheme, the following pattern results (Fig. 2).

Fig. 2
Structure of Results Specific to the Classes of Test Substances in the Laboratory, Tent and Field



The figures in brackets indicate the absolute numbers of the test substances.

In view of the large number of test substances, the data for insecticides, herbicides and fungicides can be considered to be more representative than those for bioregulators, IGRs and acaricides. It is obvious in the case of the first three classes mentioned that the laboratory study may be regarded as worst-case situation inasmuch as the data relativised in favor of the substances in the course of the test cascade from laboratory to tent to field.

Generally unfavorable tent and field results for one test substance ("0 % not hazardous to bees - yes")occurred only in the case of insecticides.

3 IGR substances have been recorded and evaluated. This absolute figure is low, but, in contrast to all other classes of substance, no further differentiation during tent and field study level comparing to the laboratory study level could be established. All three levels of the sequential testing scheme show a similiar distribution of unhomogeneous results. This can be regarded as an indication that the experimental procedures of the guideline requirements are not optimiced for IGR-testing in laboratory, tent and field.

3. Correlations between the Stages of the Sequential Testing Scheme

The laboratory, tent and field studies are closely related to each other because the preceding level of the sequential testing scheme contains essential criteria for the decision to carry out a further study on the next level. It can be anticipated that in a tiered testing scheme the standardized laboratory test shall reflect a worst-case exposure situation. Higher levels of testing - tent, field - represent an increased approximation to an actual field situation with the according exposure scenarios. Therefore as a rule results obtained in the subsequent stage of the sequential testing scheme may corroborate or relativize data of the laboratory test. Table 2 summarizes the possible combinations (laboratory-tent, laboratory-field and tent-field) regarding classes I, II and III (see section 2).

Table 2
Combinations of Substance-Specific Assessments

a) Laboratory and Tent

		TENT		
		I	II	III
LAB	I	17	3 *	
	II	26	5	
	III	5	9	8

b) Laboratory and Field

		FIELD		
		I	II	III
LAB	I	9	1**	
	II	3		
	III	2	5	

c) Tent and Field

		FIELD		
		I	II	III
TENT	I	7	1**	
	II	3	9	
	III	1	4	1

Figures represent the number of test substances with the particular result combinations

I Results from all test institutes homogeneously "not hazardous to bees - yes"

II Non-uniform results from the test institutes

III Results from all test institutes homogeneously "not hazardous to bees - no"

According to the requirements to a sequential testing scheme, no substance must be lost at the laboratory testing level that constitutes a real potential risk for bees. (Reversely, it is very definitely permissible for substances to prove not hazardous to bees in the tent and/or field that did exhibit an effect in the laboratory study.) Against this background the three substances are of particular interest that did not have an effect in the laboratory but exhibited non-uniform results in the tent (Table 2 a, marked with *). Two of these are IGRs and the third is an acaricide where it was very difficult to interpret the individual results and which was classified later as "B 4".

Again, results of IGR tests are concerned at the two data combinations shown in Tab. 2 b, 2 c (marked with **) in which subsequent test stages generated a unfavourable data distribution comparing to the preceding one (field versus laboratory in Tab. 2 b, field versus tent in Tab.2 c).

It can be deduced as a whole from Table 2 that the subsequent level of testing - tent after laboratory and field after tent - has either confirmed the results of the preceding level or relativized them. Exceptions concern IGRs. This confirms extremely well - in spite of the considerable data variability that must be anticipated - the requirements that are provided to a sequential testing scheme.

4. Data Variability of Studies from Different Test Institutes

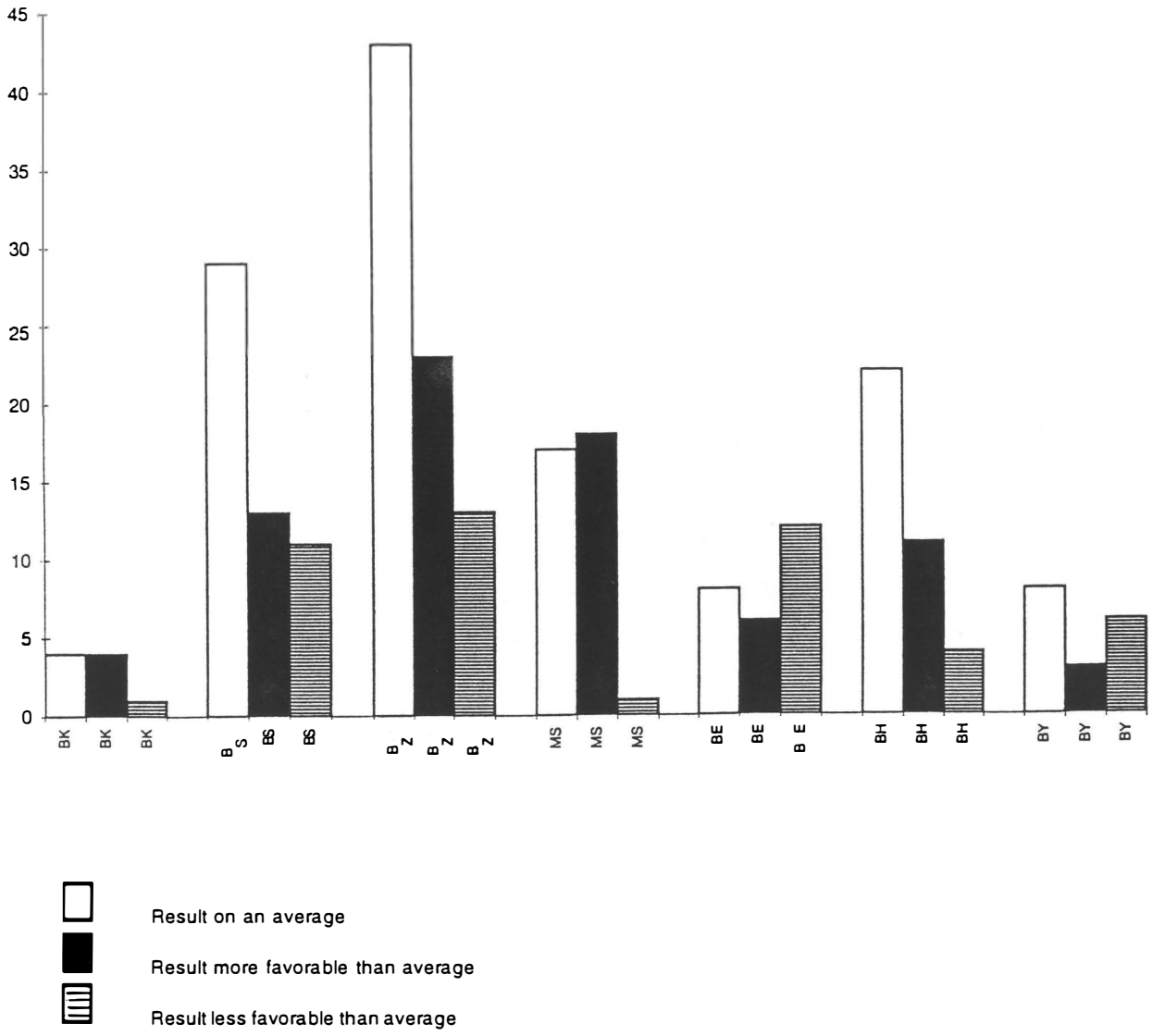
As repeated studies are carried out with each substance at different test institutes, the data analysis permits the evaluation whether an institute comes for a definite compound to a relatively favorable, a relatively unfavorable or an average result. As the test institutes did not examine identical compounds it must be emphasized that this kind of evaluation can only indiciate differences, the significance of which even in face of the high number of available results (Fig. 3) remains not finally clarified. Only laboratory data were used in this evaluation.

a) Substance with "B 4" Assessment

BASF AG's data pool was established with test substances that - on account of the absence of insecticides in this test program - led to substances with the "B 4" final compound assessment. Fig. 3 shows how often (7 different test institutes involved) the result of an individual study in the laboratory test was more favorable than the average of all tests with the same compound, less favorable than average or average.

Fig. 3

Mean of the Laboratory Test Assessments at Different Test Institutes with Compounds Classified "B 4".



The long-term assessment profiles of the testing institutes do indeed differ from one another on the laboratory studies level. For example, with a few exceptions the institute "MS" produced results that were favorable or average whereas in the case of "BE" the less favorable results clearly dominate.

The geographical distribution of the test institutes in Germany is important inasmuch as it cannot be ruled out that a geographical gradient of the sensitivity of the bees is responsible for the differing results. In fact no such connection is evident. If the differences were caused by the local sensitivity of the bees, a non-uniform distribution pattern of varyingly sensitive bees would have to be assumed. It seems more likely that test methods that are known to differ from each other caused the data heterogeneity (7, 9).

b) Substances with the "B 1" classification

The data pool of Bayer AG contains among others 19 substances that were classified as B"1" (IGRs were not taken into account because of the irregularity of results already described). In contrast to the "B 4" substances of BASF AG, this pattern of data variability of the laboratory results is completely different: the laboratory data agree well, they are assessed as "not hazardous to bees - no" (1 exception in 46 individual studies).

Thus, there is a harmonious pattern regarding the problem of the variability of the results, also against the background of institute-specific influence factors. Independently of the fact whether regional differences in the sensitivity of the bees or differences in the test procedures were responsible for the variability in data: substances hazardous to bees were recognized clearly at the laboratory level.

IV DISCUSSION

The summaries show that data which were obtained on bee toxicity over a period of more than a decade offer a high degree of consistency and reliability in assessment. The laboratory test constitutes a realistic worst-case scenario inasmuch as the tent and field studies supplement and relativize the data from the laboratory, as the summarizing structure of results shows. The exceptions are insect growth regulators, which may be considered to be an indication that new test methods are advisable here (10).

Whereas, in the case of fungicides and herbicides, laboratory effects were hardly found again in the tent and field studies, there appears a differentiated pattern in the case of insecticides at the subsequent test stages. This correlates with the fact that reports from various countries focus on insecticides as source of poisioning incidents to honey bees (5, 8, 12).

The laboratory test provides data of low variability from different test institutes in assessment for substances classified as "B 1";on the other hand, in the case of substances classified as " B 4" laboratory-specific differences may lead to further investigations. In retrospect it cannot be concluded with certainty what the reason for these laboratory-specific differences are. It is possible that regional differences of the bees play a role; however, what seems more likely is that differences in the test procedures had an effect on the study results. The data show again that the laboratory study detects substances that are hazardous to bees with a very high degree of reliability and thus corresponds to a realistic worst-case situation.

The honey bee testing procedure in Germany according to (11) has proved to be a scientifically valid sequential testing scheme that generates data for a realistic risk management. In spite for all that, from the industry's point of view, efforts should be made to harmonize the honey bee registration requirements in different countries (6). Undoubtely there are pros and cons concerning limit test versus dose range test that have to be considered from case to case. The principle of the LD_{50} test (1, 3) also requires further optimization steps; however, it has proved suitable in practice and should continue to be used in Germany in the future.

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Appendix 7

R. Hintzen, G. Vorwohl
Factors influential on the lethal dosis (LD_{50}) in the case of honeybees, *Apis mellifera* L.

Factors influential on the lethal dosis (LD_{50}) in the case of honeybees, Apis mellifera L.

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Summary

Between 1993 and 1996 about 26ooo test bees were used to study the influence of age, season, genetics (race) and the feeding technique on the oral and topical LD_{50}. In the case of oral LD_{50} single feeding and feeding in groups was tested. Topical application was done dorsally , tarsally and ventrally. The ventral application is the most appropriate. Jung bees (3 days) are more sensible than flight bees (pesticides tested: Pirimicarb and Thiodan EC, oral and topical application). There is no difference between the sensitivity of Apis mellifera mellifera and A.m. ligustica (Thiodan, oral and topical application). Single feeding leads to higher LD_{50} values than feeding in groups. Isolated bees ingest more food, and the LD_{50} is calculated on the amount of food ingested and not on the amount of active ingredient passing through the intestine into the body of the test bee. The LD_{50} values for Thiodan EC 35 for summer bees (July and August) oscillated between o,86o µg and 2,o41 µg/bee (A.m.ligustica). Tests during December , January and February resulted in LD_{50} values between 1,175 and 2,765 µg/bee. The influence of the season is negligible. The oral LD_{50} varies also with the time of deprivation (starvation time before feeding). The mortality after 2o min. of deprevation is much lower than after a hunger period of 8o min. (Thiodan EC 35; o,2 µg/µl). Mortality fluctuates considerably from cage to cage as shown by the following example: o,2 µg/µl Thiodan after a deprivation time of 4o min., 1o cages with 1o bees (A.m.ligustica) each. Mortality : 1, 3, 4, 5, 6, 6, 6, 7, 9, 9, average 5,6 dead bees/cage. 5 cages for every concentration step should be, therefore, the minimum. If LD_{50} values are determined in repeated

trials we can get variation quotients (highest LD_{50} value throug smallest value) of 3 - 5 even if we use uniform bee material, high numbers of cages, the same feeding technique, the same labo-ratory equipment and if the experiments are done by the same staff. The Hazard Quotient based on one good trial (EPPo-guideline) needs, therefore, further discussion. On the background of these results also a toxic standard seems to be of questionable use.

A copy of the full text in German is available from the authors.

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Appendix 8

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Test in bee flight room

Test in Bee Flight Room

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Bee flight room is a special research equipment which enables normal life of bees under fully controlled conditions. Bees fly in closed space, orient themselves on their hive, transport water, liquid sugar feed and pollen into their hive. The queen lays eggs and bees rear worker brood.

Description of bee flight room

The Bee Research Institute at Dol operates a bee flight room of the type Van Praagh-Velthuis (1972) from the year 1974. The structure is of a tunnel form, groundplane is 8 x 3 m and height 3 m. Walls are made of mull and are fixed to the simple tube construction. Walls are replaceable.The tunnel is in the distance 1 m from mull walls sorrounded by an exterior tunnel of the same form. Its walls are covered by black polyethylene foil which is in the inner side pasted with wrinkled aluminium plate. Indirect illumination of the inner tunnel is provided by 20 lamps directed against aluminium side of the exterior tunnel. The source of light are fluorescence tubes PHILLIPS TLA 05 with colour spectrum top 360 nm (ultraviolet zone).Each lamp has three 40 W tubes, each of them is connected to another phase.Bee flight room is fully climatized, temperature, as well as air humidity and day lenght may be regulated.

Use of bee flight room

Bee flight rooms may be used in the bee research universally.It is possible to prolong the season in queen rearing, to win sealed brood in winter, to evaluate feeds and to study behaviour of bees.

Of special advantage is the use of bee flight rooms for toxicological experiments. Bee flight room enables exact quantitative and time determination of the mortality of bees and a comfortable observation of knock down effects. It is

possible to simulate the field conditions in certain contamination of honeybees, to quantify doses of pesticides and to evaluate side effects on behavior of bees, brood rearing of bee colonies and brood damage.

Methods of determination of pesticides toxicity

Experiments may be started first 7 to 10 days after the transfer of the colony to bee flight room. This period is necessary for the adaptation of bee colony in bee flight room , for the winning of perfect space and time orientation and for finding the feed source. Utilization of a colony lasts maximally two months. After this period effects of Nosema disease may appear and distort the results. In the winter time it is necessary to wait for start of egg lying of the queen. Experiments are started first after the sealing of the first brood. During the whole stay of the colony in bee flight room a feeder with ground pollen loads is available ad libitum. 7 days before the colony treatment we observe the daily mortality of bees and the brood rearing of the colony.
The proper treatment is carried out as follows: One to three combs are taken from the colony, number of bees on these combs is estimated, bees are shaken off on the pad of defined area. Bees on the pad are sprayed with known solution/emulsion volume of known concentration of active substance. The pad with treated bees is put directly to the entrance or is located near to the entrance so that bees have to return into the hive by flying up. At the second method it is necessary to shake only forager bees from combs with stores. After the treatment the mortality is observed in daily intervals for further 7 to 10 days, and the first day mortality and knock down effect are follown continuously. The behaviour of bees and brood rearing are evaluated on subjective basis.

Evaluation

As non-toxic (relatively unharmful) evaluated product is that one which after the treatment does not show higher mortality than before the treatment. The mortality ranges from the

average in limits 2 s or it oversteps this limit only in one measurement and no changes in behaviour and brood rearing as well as no damage of brood are found.

As medium toxic (harmful) evaluated product is that one which after the treatment shows higher mortality above the limit $\bar{x} \pm 2$ s and this in two measurements at least, but the sum of dead bees is not to exceed the number of treated bees. No permanent changes in behaviour of bees, in brood rearing and no brood damage are to appear.

As very dangerous (toxic) product we evaluate such formulation which shows after the treatment very high mortality of bees. Number of dead bees above the limit $\bar{x} + 2$ s is higher than number of treated bees, or permanent changes in behaviour of bees and brood damage are found.

The mortality of higher number of bees than that one which was treated or the brood damage demonstrated a secondary toxicity of the product. This means that contaminated bees cause further losses after the return into the colony. Such products cause the largest injuries.

Bee flight rooms make possible toxicological experiments in many other fields, for instance, it is possible to give contaminated feed, to investigate the influence of the treatment on bees of different condition or to carry out experiments only with separate parts of the colony. The present contribution lists more in details only one of the methods which have come right in routine evaluation of the hazard of pesticides on bees.

Literature

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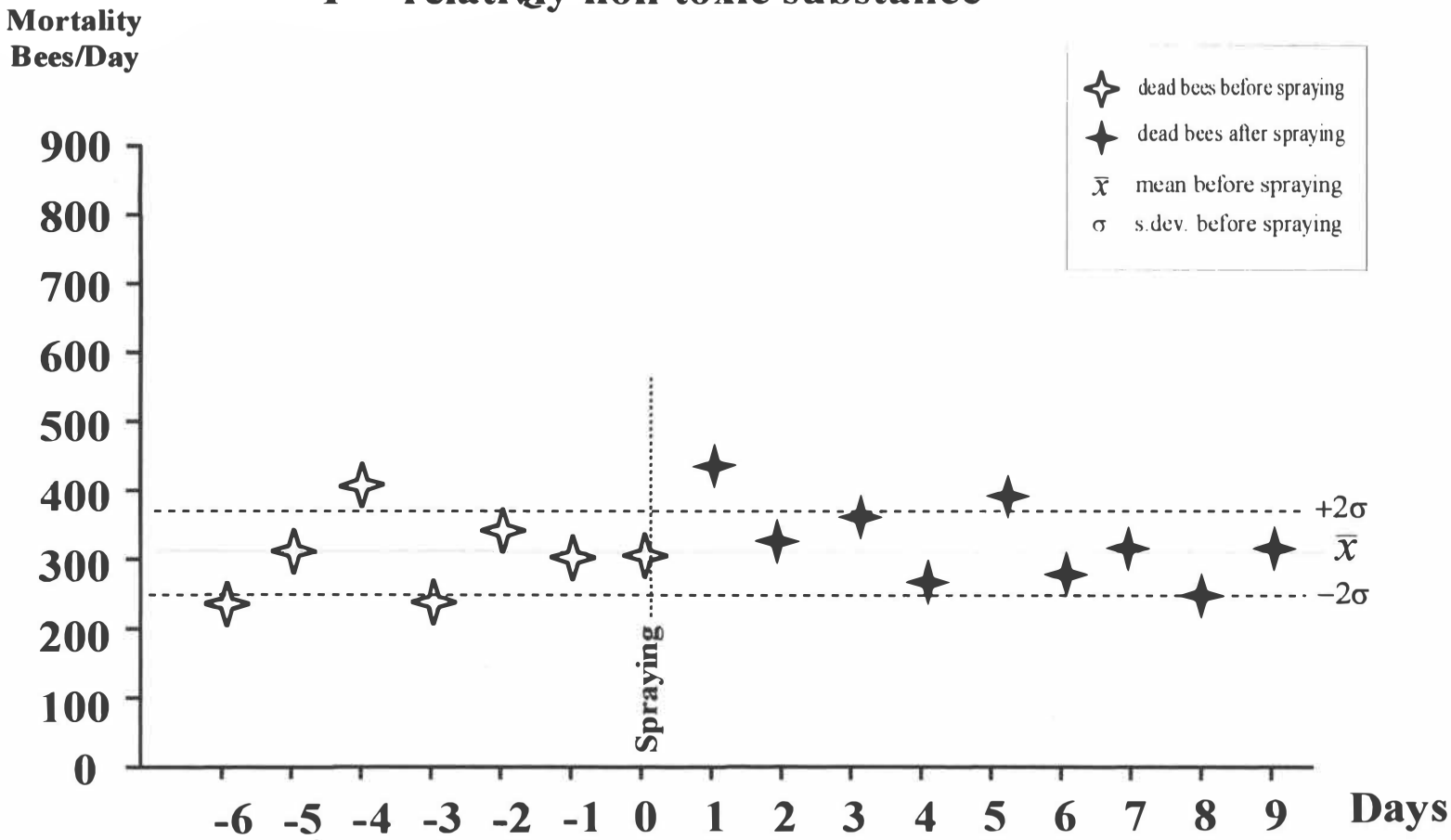
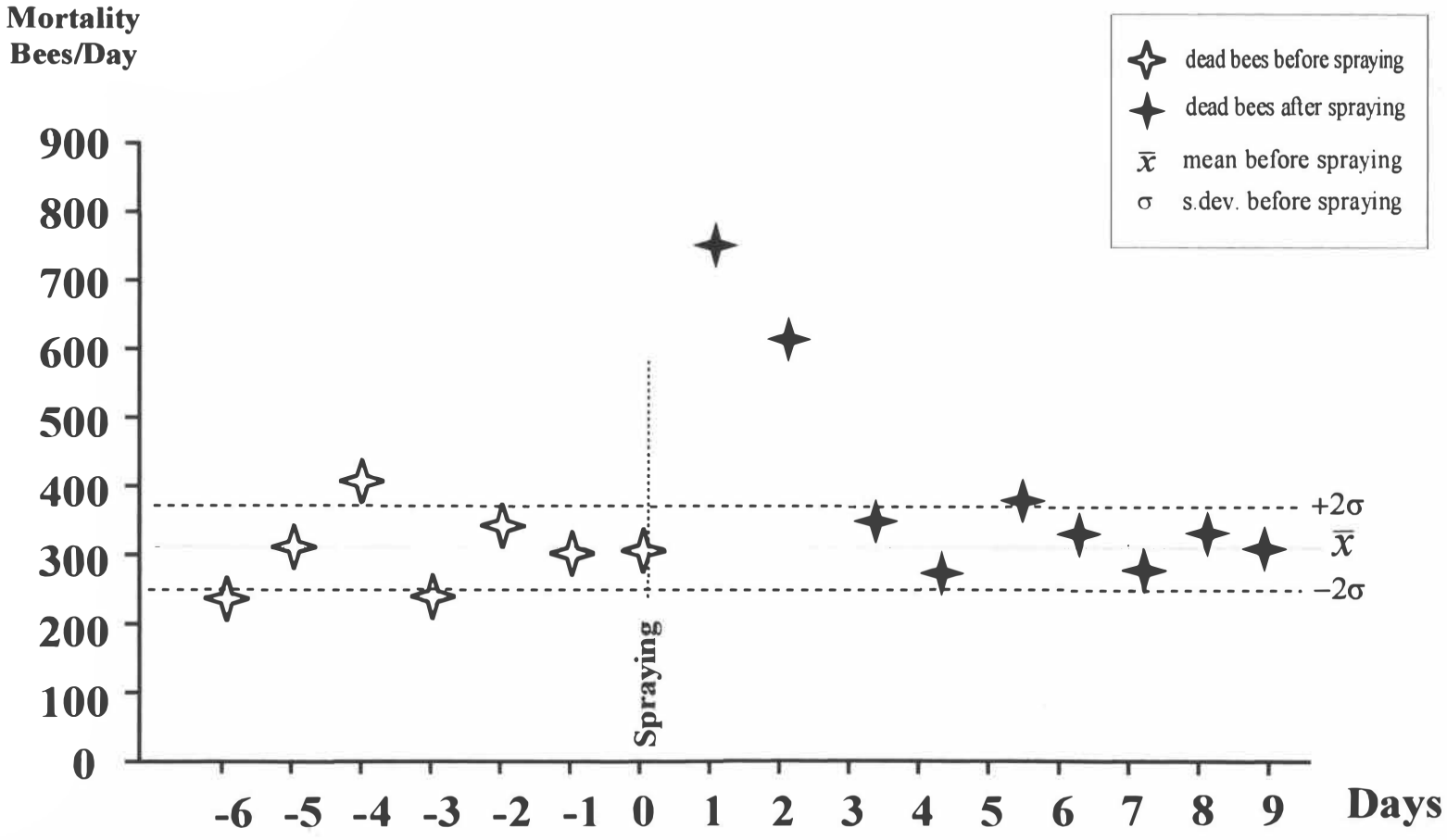
Mortality of Bees in the Bee Flight Room

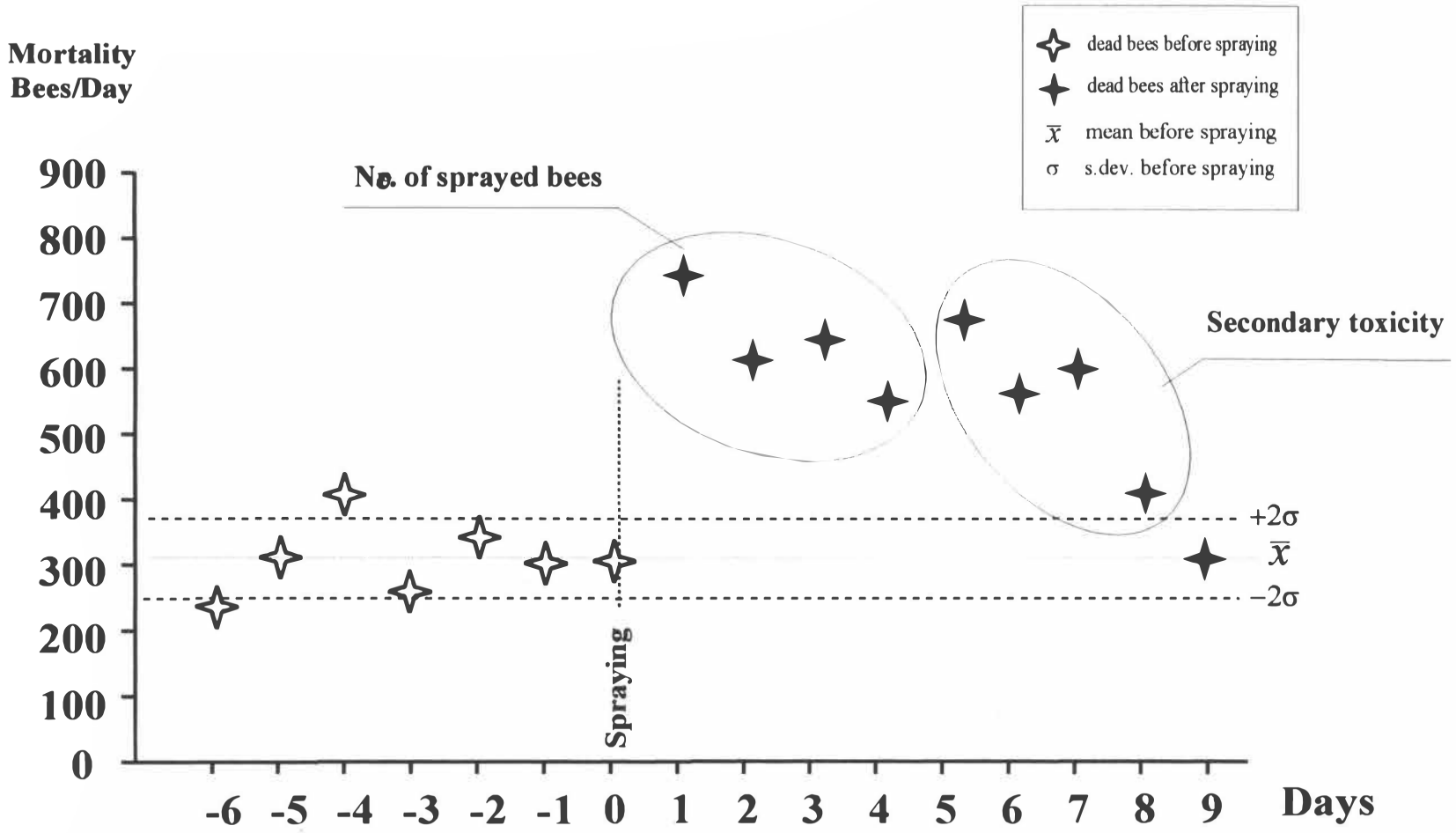
I - relatively non toxic substance



Mortality of Bees in the Bee Flight Room
II - medium hazard substance



Mortality of Bees in the Bee Flight Room
III - toxic substance



Appendix 9

G. Kovacs
Effect of the Fury 10 EC (Zeta-cypermethin) insecticide to honey bees in laboratory and field tests

Effect of the Fury 10 EC (zeta-cypermethrin) insecticide to honey bees in laboratory and field tests

G. Kovacs, Ecotoxicity Laboratory, Puszta u. 1, Facankert, Hungary

Many of the pesticides, especially insecticides present a hazard to pollinators in case of spraying attractive flowering crops. This hazard could be of different degree depending of the type of the insecticide, dosage, mode of application, residual toxicity, chemical properties etc. But the pollinators could be protected practicing an integrated pest management.

The bee toxicity of FURY 10 EC a pyrethroid insecticide, with 10 % zeta-cypermetrine active ingredient was studied in laboratory and field conditions. One of the main aim of this study was to extend the bee protecting spraying methods which in Hungary is exclusively restricted to spraying after the end of the daily bee flight. Althought is known that some insecticides could be applied early in the morning without producing any adverse effects to honey bees.
Spraying of large areas of flowering crops, after the end of daily bee flight is sometimes not completed on the same day and the remaining untreated area is sprayed on the following day in the morning. In these circumstances, bee toxicity may arise.

I.Acute contact and oral toxicity (LD_{50})

FURY 10 EC in contact test was diluated in acetone and was applied onto the thorax of the bees in 1 µl/bee volume. In oral test was offered to the bees in a 30 % sucrose solution, in 0.2 ml/10 bee volume at an exposure of 4 hours and LD_{50} value was calculated from the quantity of consumed test material.
Five doses were applied using wide dose range in both of the oral and contact tests according to range finding studies.

Oral

Concentration	**0.05**	**0.01**	**0.002**	**0.0004**	**0.00008 %**
Doses	**10**	**2**	**0.4**	**0.08**	**0.016 µg/bee**

Contact

Concentration	**0.1**	**0.02**	**0.004**	**0.0008**	**0.00016 %**
Doses	**1**	**0.2**	**0.04**	**0.008**	**0.0016 µg/bee**

RESULTS

Per os LD_{50} = 0.133 µg/bee

Contact LD_{50} = 0.013 µg/bee (Probit analysis - Finney, 1971)

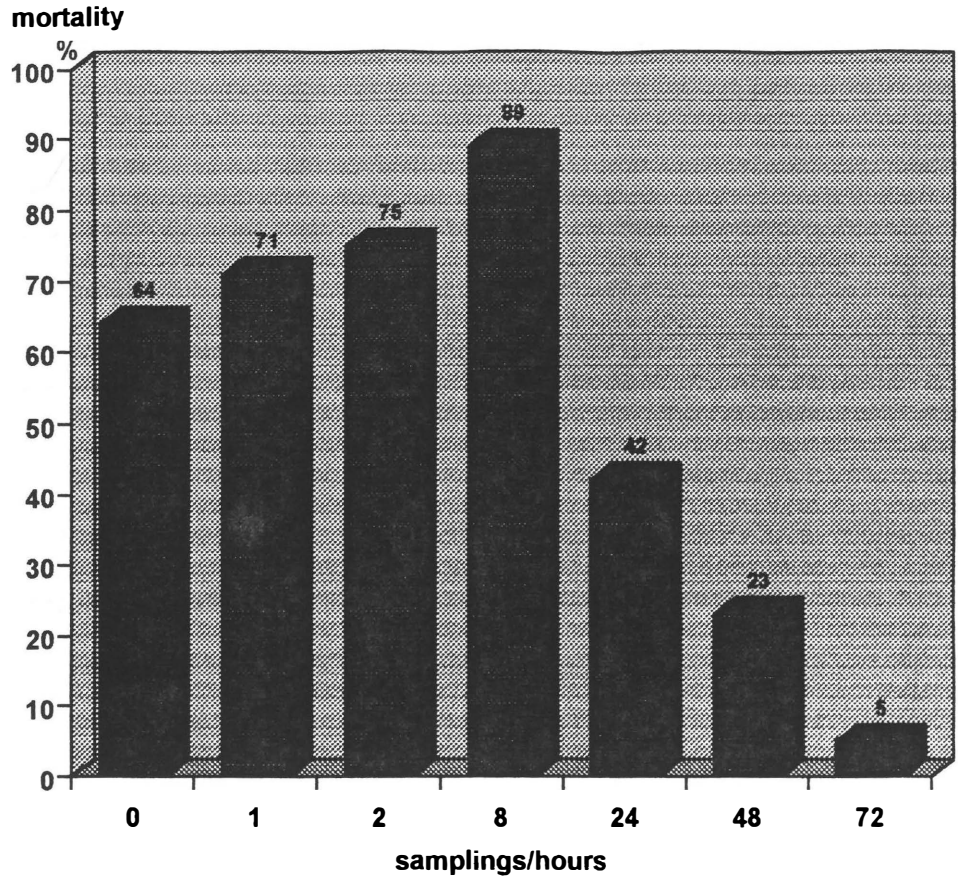
According to acute toxicity tests Fury 10 EC is highly toxic to honey bees.

In contact test bees showed complet paralysis even at 0.0016
µg/bee dose. But they recovered partialy after 4 hours and completely after 6 hours. This fact was also observed in experimental and field sprayings. These bees did not present watery aspect like the bees treated with higher doses.

II. Contact residual test

Bees were exposed in cages to Fury 10 EC treated alfalfa. The spraying of the alfalfa was carried out in the evening with a dose of 0.1 l/ha. Samles were taken at 0., 1., 2., 8., 24., 48. and 72 hours following spraying. The exposure was 24 hours when mortality was assessed.

Diagram of average mortality/samplings



Results - mortality

Bee mortality resulting from 8 hours sampling was higher than in case of samplings at 0., 1., 2. hours probably because of dew production during the night. The spraying liquid on leafs was disolved which resulted in more effective exposure of the bees.

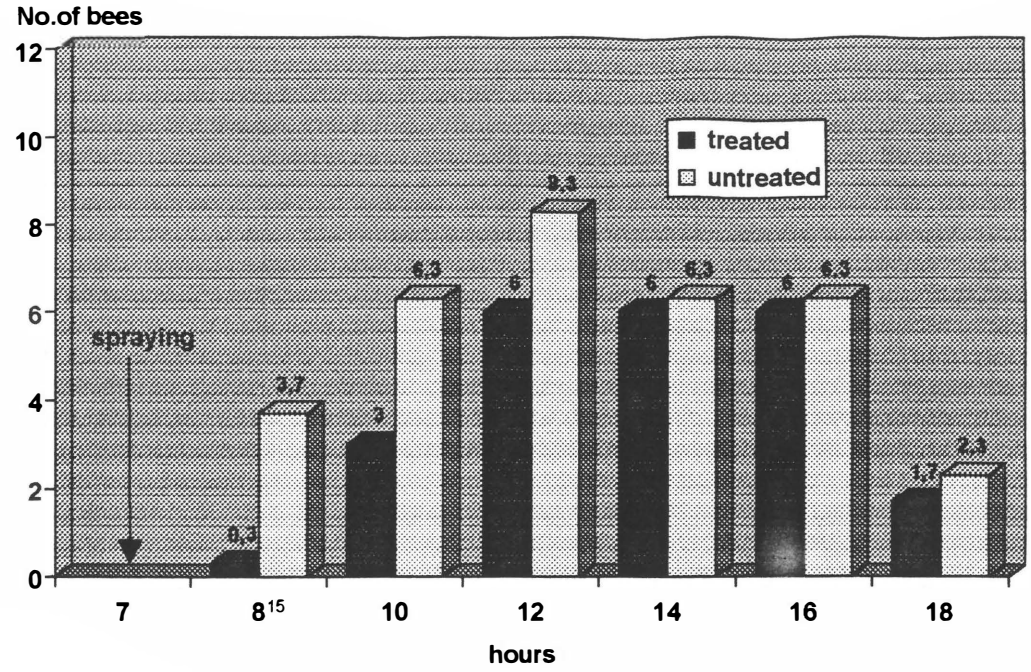
According to the results of the test Fury 10 EC exceeded 8 hours of the contact residual toxicity.

III. Cage test on Phacelia sp. - spraying in early morning -

Small bee colonies were placed into the field cages and the flowering Phacelia was sprayed in the early morning 7 5 minutes prior to the starting of the bee flight.

Chemical related effect was observed in the **Activity and behaviour of the bees.** The flower visit was normal on day before spraying, similar flower visit was observed in all cages. On the treatment day flower visit started at 7 5 minutes following spraying. But a strong repellent effect was observed in case of the treated colonies. This succeded 3 hours then the bee activity increased and reached the control level and kept this values to the the end of the test.

Flower visit on spraying day (day 0.) in cage test ($1m^2$)



Bee mortality, strength and behaviour, activity of the queens and brood status did not differ significantly compared to control colonies.

IV. Field tests

Five field tests were performed on flowering oilseed rape and sunflower where Fury 10 EC was intended to be used. In Hungary these crops are the major bee pasture according to season. So the exposure could be high.

The treatments were carried out by aerial spraying in a dose of 0.1 l/ha on fields sized from 17 to 86 ha.

Firstly timing of the spraying was accordingly to the authorized bee protecting method - after daily bee flight - then with early morning spraying, before the starting of the bee activity.

Finally - the day-time spraying, during full bee flight.

As records mortality, flower visit, colony strenght and behaviour and brood status were assessed.

Two tests were performed in the evening on oilseed rape and sunflower, using 3 treated and 3 control bee colonies.

In case of the rape due to weather conditions, windy and cool morning, bees flight started 14 hours following spraying. No adverse effect was observed.

In case of the sunflower the spraying was carried out after sunset but due to high temperature moderate bee flight was observed till total darkness.

Significant mortality did not appared. The increasing of the population in case of the treated colonies was slightly less then the untreated colonies.

Two test were performed in the early morning. First, on the sunflower field with 3 treated and 3 control bee colonies. The spraying was carried out 30 minutes before the commencement of the bee

flight. A strong repellent effect was observed, the flower visit fell off to 1/3 compared to the day before spraying.

The second test was performed on oilseed rape but in this case 20 treated and 20 control bee colonies were used. The spraying was carried out 80 minutes before the commencement of the bee flight.
No adverse effects were observed.

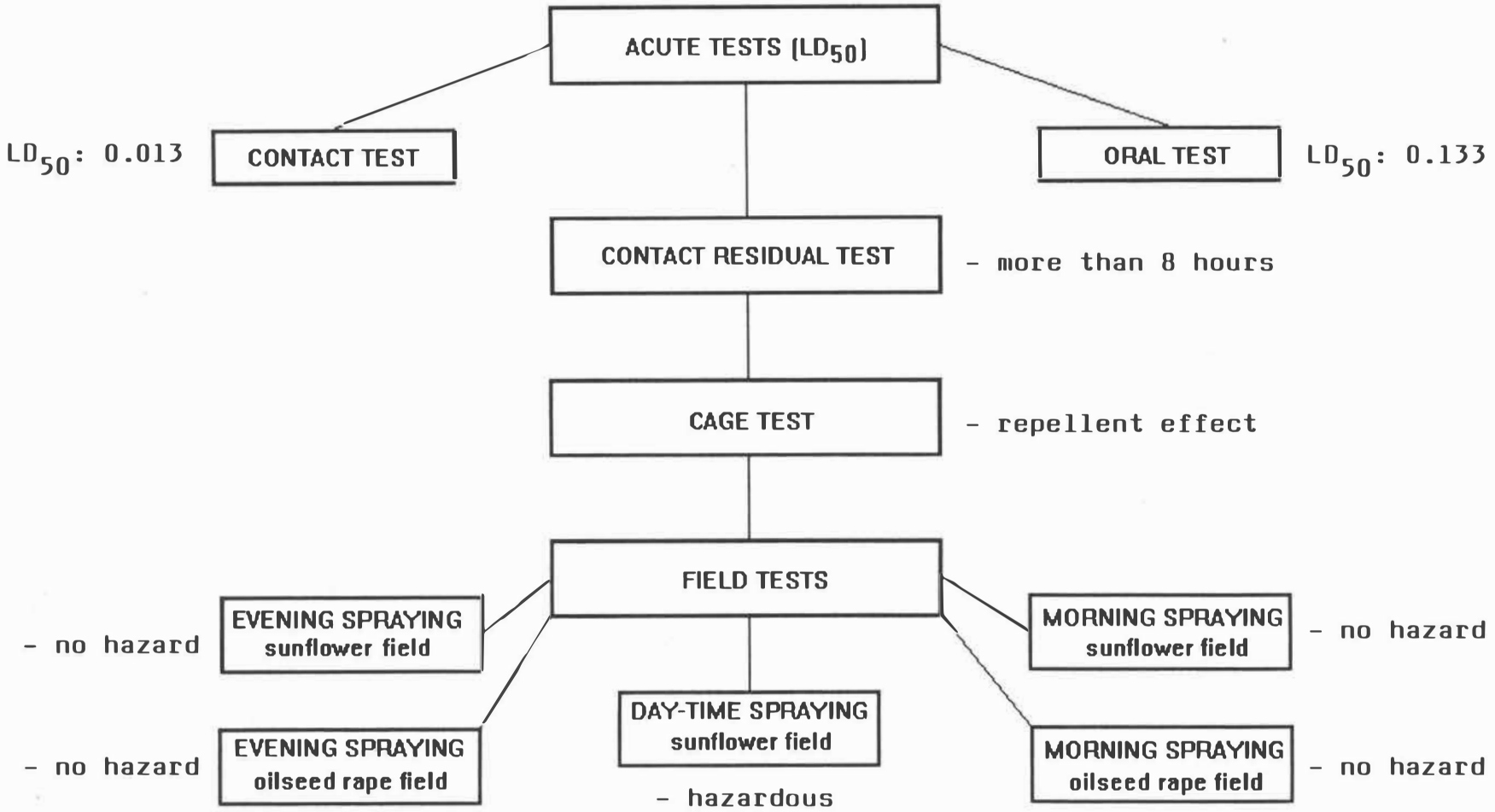
One test was performed on sunflower at day-time
The spraying was carried out in the late morning, durin intensive bee flight. Some paralised bees and mortality were observed in the dead bee traps. Further mortality appeared in the field. We concluded that foraging bees which were hit directly by the spraying liquid died also. This was demonstrated with caged bees placed at fly-over time in the field to measure the initial contact effect.
Supposly some of the bees which were contamined on flowers just after spraying died also. But all this mortality of the foraging bees remained bellow 1 0 %. This why the daytime spraying was classified as moderate harmfull to bees.

CONCLUSION

In the presented study Fury 10 EC shows high acute and relatively long residual toxicity to honey bees in laboratory condition. On the other hand, it causes no mortality and no other adverse effects in field conditions when applied outside flight hours. It appears that the relatively long residual toxicity of zeta-cypermetrin seen in the laboratory is not significant in practical field conditions. This was attributed to repellent-sublethal effect when bees are exposed to sublethal doses then they return to the hives and recover.
This repellent effect of the some insecticides many times is claimed that on entomophil (need pollinators for pollination) plants are very demaging because of low pollination rates, keeping the pollinators few days away. In case of Fury this effect is only few hours or maximum a day.

Finally we concluded that the bee protecting spraying methods could be extended to early morning spraying in case of FURY 10 EC.

BEE TOXICITY TESTS OF FURY 10 EC



Appendix 10

H. Koch, P. W eißer
Contamination of bees during application of pesticides

Contamination of Bees during Application of Pesticides

H. Koch and P. Weißer, Landesanstalt für Pflanzenbau und Pflanzenschutz, Mainz

As long as insecticides are used to control harmful insects in plant protection, beekeepers complain about effects of the chemicals on their bees. Consequently, legislation and registration introduced testing procedures to assess hazards of pesticides to bees prior to registration. Testing guidelines were developed and label recommendations specified.

Since insecticides originally were applicated in grapes and fruits by spraying until run off, dose recommendations have been expressed in terms of concentration without considering the actual quantity applied. Tests of effects on bees also focused on concentration rather than on the actual dose applied.
In Germany the BBA-Guideline 23.1 and a directive for the protection of bees exist both referring to product concentration.

Although the registration process includes testing of side effects of pesticides on bees, there is almost no information available about the real product quantities and time bees are exposed to when fields or orchards are sprayed. Our investigations were aimed to assess the chemical load of individual bees in field situations in order to understand the dose transfer which is the process of transformation label recommendations into deposits on the target. This deposit is a measurable quantity and may be used as a base for estimating effects of the chemicals on pests, diseases, beneficial organisms, etc.

Material and methods

To organize this type of investigation bee colonies provided by a professional bee keeper were placed next to an appropriate crop. At flowering stage the bee hives were placed at fields planted with Phacelia (*Phacelia tanacetifolia* Benth) and in apple orchards three days before treatment. This was aimed to ensure that bees were foraging inside the test plot.
Trials were carried out from 1992 to 1996. Because of the short flowering period especially of apples only a few test runs were possible per year.
Appropriate orchards should be in production and larger than 0,4 ha. Phacelia was grown in cereal growing areas to make it attractive for bees. The size of these plots should be 0,2 ha at least. Plot size varied between 0,4 and 1,2 ha (apples) and 0,2 and 1 ha (Phacelia).

As test substance a fluorescent tracer, Sodium-Fluorescin, was used. It was applicated with an axial fan sprayer (Sorarui) and a 12 m boom sprayer (Rau DX 2) respectively. Details are presented in table 1 .The dose rate was 20 g Sodium-Fluorescin in 200 to 500 l water per 10.000 m² sprayed area (fruit wall or ground area).

Immediately before application the number of bees per tree (apples) or per m² (Phacelia) were estimated. Then the entrance of the bee hive was closed for 20 minutes. During this time the arriving bees were collected in front of the bee hive in intervalls of five minutes (tab. 1), put each time into a box and cooled down in dry ice. The sampling in intervalls made possilble to interpret the different application situations in field and orchard spraying over time. The size

of each sample should be above 100 bees but could not be kept constant due to the sampling procedure. After transportation into the laboratory dead bees were individually rinsed in 20 ml aqua dest. A fluorimeter (Perkin Elmer LS 3B) at 484 nm excitation and 512 nm emmission wavenlengths was used to quantify the tracer concentration. Data were transferred on-line into a computer and calculated in ng/bee.

Results

Apple Orchards

Due to the difficult organisation of the trials, investigations have been possible only in one orchard per season with only two to three test runs. Weather conditions and duration of the blooming period limited the number of test runs as well as measuring capacity. Seven test runs were done in three years and are here reported.
Table 1 shows details on dates, spraying and sampling time. Sprayed area is the calculated size of the fruit wall and is different from the ground area (KOCH and WEIßER, 1995). Table 2 shows the frequency of deposit distribution for the samples taken in five minute inter-valls as well as accumulated for each trial. Because of the used technique to sample a „bulk" of bees at each time intervall into one box bees could contaminate each other to a certain ex-tent. It was not possible to separate individual bees at the hive entrance in the given time without irritating the bee colony to much. Inidiual handling of the bees began after freezing in the lab. Because of this flaw in methodology we decided to consider loads below 5 ng/bee as not contaminated. The portion of contaminated bees (more than 5ng tracer/bee) per sample varies between 0% and 100%. Per trial this value varies between 15% and 97% as indicated for samples in classes 0 to 5 ng/bee. Six out of seven trials show more than 57% of not contaminated bees. Only in one trial (No. 6) we found about 85% of contaminated bees. Mean deposit on the contaminated animals in the samples varies between 0 and 40 ng. The average deposit of all trials lies between 10 and 26 ng per contaminated bee.

Table 1: Technical information on seven trials (apple orchard)

Trial	Date	Spraying Time	Sampling Time	Sprayed area (ha)	Volume l/10 000m^2	Number of Bees/tree	Ground area (ha)
1	28.4.94	8.20-8.45	8.50-9.10	0,434	330	2-5	0,42
2	29.4.94	14.00-14.25	14.30-14.50	0,434	330	2-5	0,42
3	25.4.95	13.25-14.00	14.00-14.20	1,244	206	1-2	1,085
4	02.5.95	13.50-14.28	14.35-14.55	1.244	206	1-3	1,085
5	03.5.95	13.30-14.05	14.00-14.20	1,244	206	1	1,085
6	06.5.96	14.25-15.10	14.50-15.15	1.244	200	1	1,085
7	07.5.96	10.55-11.38	11.25-11.50	1,244	490	1	1,085

Table 2: Apple orchards: Percentage of bees (%) with deposits (ng/bee) in the samples taken in five minute intervalls. Bees with deposits below 5ng were defined to be not contaminated. The column Ø calculates only bees with deposits above 5ng/bee. Dose was 20 g Sodium-Fluorescein per 1 0 000m² sprayed area of fruit wall.

Trial	Sample	deposit ng/bee										No. of bees	Ø
		0-5	5-10	10-15	15-20	20-25	25-30	30-35		35-40	40-45	<45		
1	1	71.8	15.4	2.6	2,6	0	5,11	2,6	0	0	0	39	14.06
	2	86	8 14	2	2	0	0	0	2 0	0	0	50	12,1
	3	84	18	0	2	0	0	0	0 0	0	0	50	7.7
	4	78	10	2	2	0	0	0	0.4	0	0	50	7,7
	5	86	13.1	0	4	0	0	0	3 0	0	0	50	8.9
	Ø	81.2	0	1.3	2.5	0.0	1.0	0.5	0 0	0,0	0,0	239	10.1
2	1	94	4	0	0	0	0	0	0	0	3	33	53.4
	2	94	2.3	0	0	0	0	2	0,6	0	0	50	17.7
	3	93.2	2	0	4,5	0	0	0	1 0	0	0	44	13,1
	4	92	6	0	2	0	0	0	0 1	0	0	50	20.96
	5	90	2.9	4	0	0	0	0	0	0	0	50	9,24
	Ø	92.6	34,5	0.8	1.3	0.0	0.4	0,8	0,4	0.0	0,6	227	22.9
3	1	13,2	49	23.4	13,2	4,1	3	2	0 0	1	4,1	98	17.96
	2	11	10 4	20	9	5	2	0	0 0	2	2	100	13.8
	3	85	17	2	0	2	0	0	0	1	0	100	11.7
	4	92	22.9	1	1	0	0	0	0.0	0	1	100	17.9
	5	85	2,5	4	1	0	0	1	0 0	0	1	100	16,16
	Ø	57,2	1	10,1	4,8	2,2	1,0	0,6	0 0	0,8	1,6	498,0	15,5
4	1	95	2	1.25	0	0	0	1,25	0	0	0	80	14,6
	2	96	0	1	0	0	0	0	0,0	0	2	100	10.49
	3	94	0	1	0	0	0	0	1 0	0	3	100	35.42
	4	98		0	2	0	0	0	2 2	0	0	100	16.3
	5	100		0	0	0	0	0	4	0	0	100	-
	Ø	96.6	1.1	0.7	0.4	0.0	0.0	0.3	2.8	0.0	1.0	480.0	15.4
5	1	89	6	2	1	1	0	0	0 2	0	1	100	21.85
	2	85	6	3	0	2	0	1	1,1	0	3	100	29.85
	3	92,1	2.2	1. 1	0	1. 1	0	0	0 2	0	3.3	90	39.28
	4	96	2	0 1	2	0	0	0	1,0	0	0	100	11.96
	5	94	2	1.4	0	1	0	0		1	1	100	29.62
	Ø	91,2	3.6	15 6	0.6	1.0	0.0	0.2		0.2	1.7	490	26,5
6	1	2 65	24	20	23	15	5	2		1	12	100	23,9
	2		21	22	1	2 6	1	0		1	2	100	14.12
	3	5	44	11	11	4 5	6	2		1	5	100	17.66
	4	8	44	13,2	10	8,5	1	1		2	6	100	14.87
	5	14	44	1	3	1 3	3	3		1	12	100	33,12
	Ø	15,7	29,5		10.7		5.7	3,0		2,2	9,0	600	23.1
7	1	83	10		1		1	2		0	1	100	18.17
	2	70	12	5	4		0	2		1	1	100	18.05
	3	64,8	15.9	10,6	1. 1	0	0	2,2		0	5.3	94	21,26
	4	68	11	5	4 3	2	1	2		1	6	100	28.52
	5	68	18	5	2,7	1	0	0		0	3	100	16,76
	Ø	66,3	15,0	5,9		1.7	0.3	1,5		0.3	3,4	594	20,7

Fig. 1: Relation of contaminated bees to mean deposit (37 samples in seven trials)

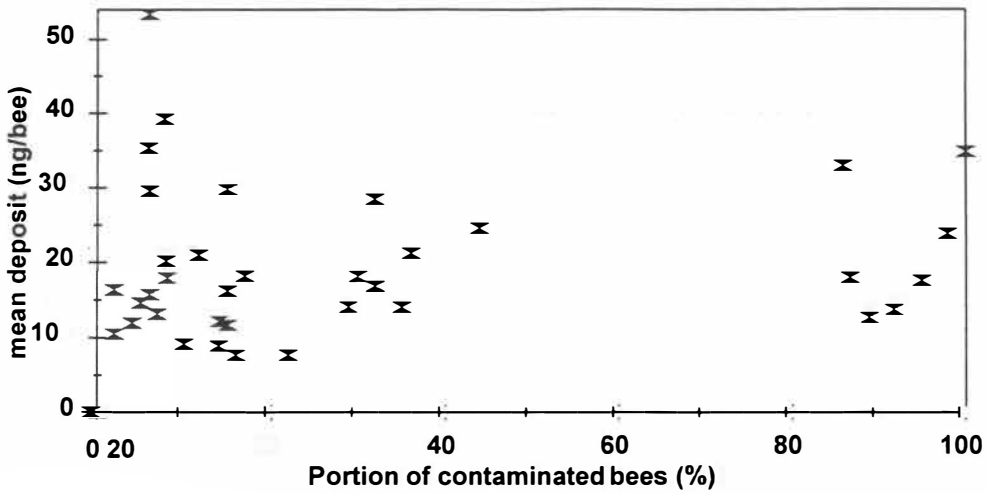
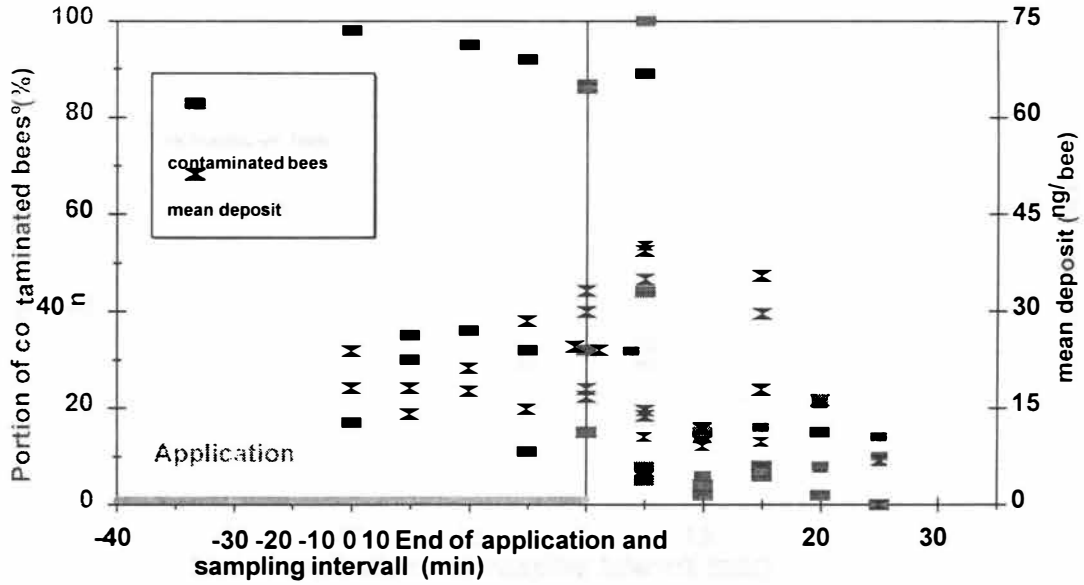


Fig. 1 shows for each sample the portion of contaminated bees (%) in relation to the mean load (ng/bee) of the contaminated group (deposit higher than 5 ng/bee). Mean deposit is about 20 ng/bee with a maximum of 55 ng. On the other hand the percentage of contaminated bees per sample is mostly below 20%. Only seven samples show contamination values above 80%, representing only two trials (No. 3 and No. 6).

Fig. 2: Mean deposit and portion of contaminated bees in relation to sampling time



The bottom line in fig. 2 shows the period of spraying and sampling as listed in tab. 1. In two trials sampling started 20 minutes before finishing the application, in two trials first samples

were taken five minutes after finishing the application. We assumed that bees usually return to their hive in about 20 minutes.
Squares mark the portion of contaminated bees as expressed in fig. 1. Mean deposits tend to increase until the end of application and decrease afterwards. Data prove the low correlation between portion of contaminated bees and mean deposit. The latter value might be a parameter for the amount of chemical substance transported into the hive.

Phacelia

Table 3: Technical information on five trials (Phacelia)

Trial	Date	Spraying Time	Sampling Time	Sprayed area (ha)	Volume l/10 000m²	Number of Bees/tree	Ground area (ha)
1	30.6.92	13.45-14.00	14.00-14.20	1	400	2	1
2	10.9.92	11.20	11.25-11.40	1	400	5	1
3	26.5.93	11.00	1 1.05-11.25	0,1	400	10-12	0,1
4	17.8.95	13.58-14.00	14.05-14.25	0,25	400	4-5	0,25
5	19.6.96	13.44-13.46	13.50-14.15	0,25	400	3-5	0,25

Table 4: Phacelia: Percentage of bees (%) with deposits (ng/bee) in the samples taken in five minute intervalls. Bees with deposits below 5ng were defined to be not contaminated. The column Ø calculates only bees with deposits above 5ng/bee. Dose was 20 g Sodium-Fluorescein per 10 000m² sprayed area.

Trial	Sample	deposit ng/bee										No. of	
		0-5	5-10	10-15	15-20	20-25	25-30	30-35	35-40	40-45	<45	bees	Ø
1	1	60.7	12	4.7	4	3.3	6.7 16	0.7	1.3	3.3	3,3	150	24.22
2	1	1.3	13.3	9.3 10	14.6	9.3	14 10	9.3 10	13.3	5.3 4	8	75	25.36
	2	22	2 10	2.5	8	12	17.1	10 5.7	10 7.5	5 2.9	8	50	29.22
	3	10	8.6	11.4	12.5	20	14.3	8.8 2	8.6	4.3 2	12.5	40	25.2
	4	17.1	8.5	8.3 6	8.8	8.6		2 5,3	9.9	4	11.4	35	26.84
	Ø	12.6	10 4	2 1,3	10.9	12.5				0	10	200	26.7
3	1	74	5,3	5.3	2	2	0		0	0 2,2	2	50	20.67
	2	80	8,4	5.6	4	2	0		2		0	50	23.8
	3	69,3	15,2	4.0	10.6	1.3	6.6		0		0	75	20,39
	4	76,8	8.6	7.1 12	1.1	3.2	4.2	1.1	0		0	95	15.7
	5	72.2		21 19	3.3	2.2	2.2	0	0		0	90	14.69
	Ø	74.5		3 12.4	4.2	2.1	2.6	2.1	0.4	1.6	0.4	360	19.1
4	1	0	2	3.1 2	9.1	10.1	3	5.1	5	7 4	50	99	49.27
	2	0	8		11	3	1	3 3	7	5 3	51	100	46.35
	3	0	25		1	3	2	10	3	2	37	100	35.87
	4	0	22		14	5	3	5	6	4.2	18	100	30.17
	5	45	22		2	1	3	5,2	0	1.5	17	100	29.03
	Ø	9.0	15.8		7.4	4.4	2.4	1.5	4.2	4 0	34.6	499	38.1
5	1	46.1	15.4		4.6	1.5	1.5	2	3.1	3 0	21.4	65	43.26
	2	57	8		2	9	4		2	4,8	10	100	33,96
	3	78,4	4,5	1. 1	4.5	0	0	1. 1	0	2.2	10.2	88	42,34
	4	22	37	15	7	4	2	41	3		3	100	16,38
	5	83	7	1	1	1	0	3,2	1		5	100	27.44
	6	75.8	3,2	1,6	0	4,8	1.6	2,1	1,6		3,2	62	31,08
	Ø	60,4	12,5	4,0	3,2	3,4	1,5		1.8		8,8	515	32.4

Figure 3: Relation of contaminated bees to mean deposit (21 samples in five trials)

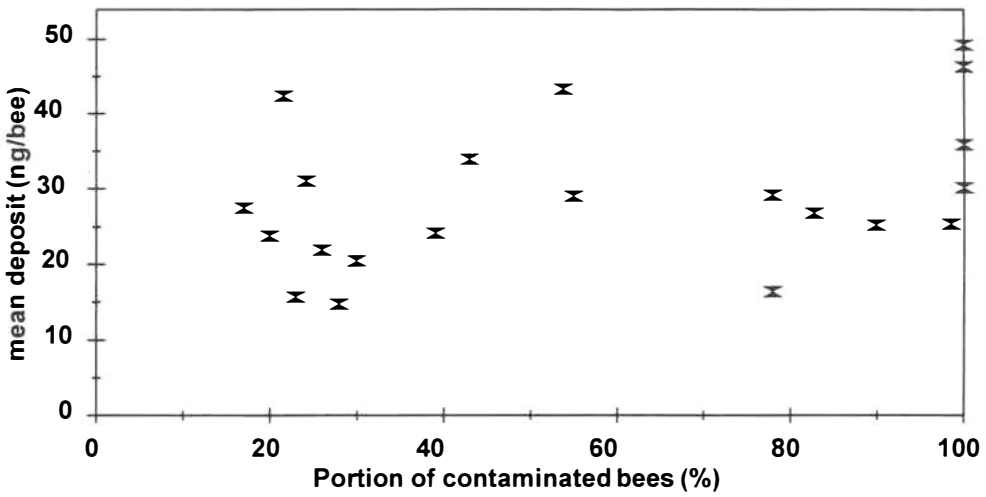


Fig. 4: Mean deposit and portion of contaminated bees in relation to sampling time

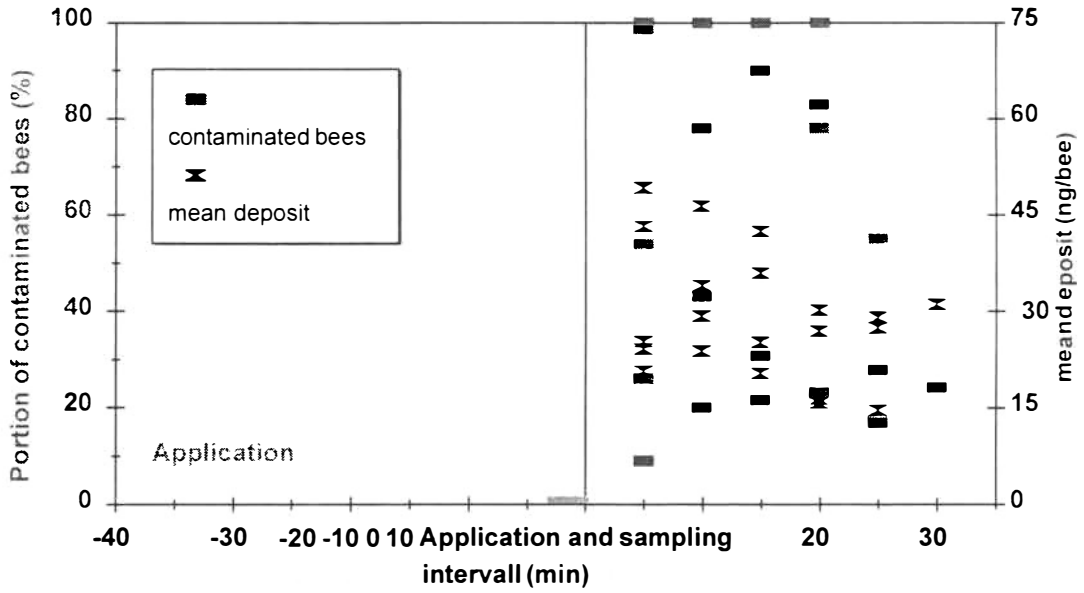


Fig. 3 and 4 show the results of the trials in Phacelia that is commonly used as a forage crop for tests on side effects of pesticides on bees (BBA, 1991; EPPO, 1992). Table 3 lists the technical details of the trials carried out in 1992, 1993, 1995 and 1996. In 1994 the test plot was not attractive to the bees due to severe drought. Although the test plot was isolated and surrounded by cereal fields bees did not enter the Phacelia field. Nevertheless the average portion of contaminated bees and mean deposits are in the same range as found in the trials in apples. Maximum mean deposit reaches 65 ng/bee.

Boom spraying in arable crops differs from the application technique used in fruit orchards with a shorter spraying period for the same size of sprayed area. Considering that bees are supposed to return within 20 minutes to their colony and that the hive can only be closed for 20 to 30 minutes we started sampling right after application. Comparable to the results found in apple orchards the range of contamination varies between 20% and 100%, but with higher mean deposits per bee. Values decrease over the sampling period.

Discussion

Investigations that prove the extent of side effects on bees have to be submitted to registration authorities. According to such data pesticides will be classified as harmless or harmful to bees. This must be indicated on the label and requires attention by users. Some of the products have to be applied on flowering crops what may result in high contamination of bees while they are foraging on crops during the application.
Chemicals may poison bees directly, can release changes in behaviour or can be transported into bee hives causing effects on the brood. Residues in honey may be another problem relating to pesticide contamination of bees. All these effects are influenced by the initial deposit per individual bee which totally represent a certain chemical dose.
Despite this facts no data are available about direct contamination of individual bees in field situations.

In our trials we tried to develop a sampling method that allows to investigate the exposition of individual bees and bee colonies under real life conditions. Although data are varying very much between different trials we can state that the application rate of 20g per 10 000m² sprayed area results in mean deposits of 20 to 40 ng/bee in Phacelia and only 10 to 25 ng/bee in apples. Compared to the application on dead bees under controlled conditions on a labora-tory spray track this is a much lower level. KOCH and SPIELES (1993) reported mean de-posits around 100 ng/bee after spraying 20 g fluorescent tracer per 10 000m² sprayed area. Mean deposits in the trials reported here should be seen critically and sample size was perhaps not sufficiently large. In somes cases individual loads are rather high and one or a few values can alter the statistics, e.g. in samples where deposits above 45ng/bee were found.
The figures give no evaluation of side effects of various pesticides. They just describe quantitative relations between delivered chemical amount (dose per sprayed area) and deposits on targets. To evaluate such effects one would have to take in account chemical characteristics and dose effect relations. The dose transfer ratio from 20g/1 0 000m² to 40 ng/bee as the highest mean found in our trials is tolerable as long as the water volume is below runoff. No runoff occured in the trials reported here.

One other major difference between the two tested spraying methods is that spraying time in apples is longer because of the smaller working width in orchard spraying. So the possible exposition period in orchards is longer. Because of the modified spraying technique in field crops the sprayed acreage is much higher and the possible exposition time is lower. A 15 m boom field sprayer covers at a speed of 6 km/h about 3 ha in 20 minutes. Spraying a 3 ha orchard would need 7 5 minutes, sufficient time for bees to return about three to four times to their hives.

The sampling technique was the most difficult problem in these trials. Bee colonies can not be irritated to much and some external effects could not be eliminated or even estimated. Whether or not the crop is attractive in comparision to other crops is most important for trials as well as for hazards to bees in agriculture.
Because of the "bulk sampling" technique some diffusion of test substance between collected bees in the sampling box could not be avoided. We defined deposits below 5ng/bee as not contaminated assuming that this is an artificial value.

Acknowledgements
The authors thank Bayer Pflanzenschutz, Biologische Entwicklung - Versuchswesen for supporting the organisation of the trials over the whole period.

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Appendix 11

D. F. Mayer
Effects of Pyriproxyfen insecticide on three bee pollinators

EFFECTS OF PYRIPROXYFEN INSECTICIDE ON THREE BEE POLLINATORS

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INTRODUCTION

Bee poisoning from pesticides is a serious problem worldwide (Johansen, 1977; Johansen and Mayer, 1990). Major concern exists for the safety of honey bees (Apis mellifera L.) as valuable pollinators of many crops and for alfalfa leafcutter bees (Megachile rotundata (F.) and alkali bees (Nomia melanderi Cockerell) that are used to pollinate alfalfa seed.

Pyriproxyfen, (4-phenoxyphenyl(RS)-2-(2-pyridyloxy)propyl ester), a pyriden compound, is an insect growth regulator (IGR), developed for insect control on agriculture crops and household pests throughout the world. Cozoppelt (1993) in an in vitro bioassay with honey bee larvae fed pyriproxyfen reported larval growth and pupation were not affected. However, toxic effects were found during metamorphosis with high mortality in the pupa state. De Wael et al., (1995) fed bumble bee colonies either 20 ppm a.i., 2 ppm a.i. or 0.2 ppm a.i. pyriproxifen and found that colonies developed normally.

This paper reports results of our research concerning the effects of pyriproxyfen on honey bees, alfalfa leafcutter bees, and alkali bees.

TOPICAL LD50 TOXICITY TO THREE SPECIES OF BEES

Materials and Methods

Pyriproxyfen 0.83EC (Valent USA Corporation) was dissolved in acetone to obtain concentrations of 50 gm (AI)/189 liters of water, 50 gm (AI)/379 liters and 50 gm (AI)/757 liters of water. Thirty female bees of each species were treated with each solution. Insecticide solutions were applied with a calibrated Eppendorf microsyringe and disposable tips. For each bee, 2 æl of solution was drawn into the tip and then gently dispensed onto the mesoscutum. For each test, a control group was treated with 2 æl of acetone only. After treatment, bees were kept in screen cages. Cages were made from plastic petri dishes (15 cm diameter) with tops and bottoms separated by a wire screen (6.7 meshes/cm) cylinder insert (45 cm long and 5 cm wide) large enough for bees to fly.

Worker honey bees were obtained from the top of colonies and anesthetized with CO2 to facilitate handling. Leafcutter bee prepupae in leaf-piece cells were incubated at 30 C and 50% RH. Emerging females were allowed to fly in the lab and collected off the windows. Alkali bee females were collected from nesting sites with an insect net and chilled to facilitate handling. All bees were held at 5 C until activity ceased, and then treated with the pyriproxyfen solutions.

After treatment, bees were maintained for 24 h mortality counts in cages at 26 to 29 C and 50%
RH. Bees were fed 50% sucrose solution in a cotton wad (5 x 5 cm) placed on the cage bottom.

Results and Discussion

There were no significant differences in mortality of adult honey bees, alkali bees or alfalfa leafcutter bees as compared to the untreated check with any of the rates. Pyriproxyfen is
not toxic to adult bees.

FIELD BIOASSA Y OF RESIDUES ON ALF ALF A

Material and Methods

Pyriproxyfen 0.83EC was applied to 0.004-hectare plots of alfalfa with a R&D CO2 pressurized sprayer at the rate of 234 liters of water/ha. Four samples of alfalfa foliage with field-weathered pyriproxyfen residues were collected from each of 12 sites in each treatment at 2 h and 8 h after application. Samples that consisted of about 500 cm2 of foliage taken from the upper 15-cm portions of plants were placed in cages. Bees were collected and caged as described previously. Residue exposures were replicated 4 times per treatment and time interval each using four groups of 50 worker honey bees, 20 female leafcutter bees or 20 female alkali bees caged on
a foliage sample. Mortality was assessed after 24 hours exposure.

Results and Discussion

There were no significant differences in mortality of adult honey bees, alkali bees or alfalfa leafcutter bees as compared to the untreated check with any of the rates Pyriproxyfen is
not toxic to adult bees.

FEEDING TESTS-HONEY BEES

Materials and Methods

A sugar syrup 1:1 volume was prepared with a concentration of pyriproxyfen equal to 124 gm (AI)/ha in 95 liters of water. On 30 August, 2 liters of the solution were fed to each of 4 different colonies and 4 colonies were fed only syrup. At 12 days after feeding, 300 randomly selected capped brood from each of the 8 colonies were opened to determine if any immatures showed abnormal development.

Results and Discussion

There was significantly more dead pupae in the colonies treated with pyriproxyfen as compared to the untreated check colonies (Table 1). There was significantly less live pupae in the colonies treated with pyriproxyfen as compared to the untreated check colonies (Table 1).

The mean percent mortality in the treated colonies was 35% and in the check colonies zero. Honey bee mortality occurred during the pupa stage.

Pyriproxyfen fed to honey bee colonies in a syrup solution resulted in high immature mortality. However, the dose used was much higher than that honey bees would likely pick up from field applications of pyriproxyfen.

FIELD TESTS-PEARS-HONEY BEES

Materials and Methods

One half of a 2.4 ha Anjou pear orchard was treated with pyriproxyfen at 124 gm (AI)/ha with an air-blast sprayer at a rate of 3 79 liters of water per acre at 0800 hr on 7 April when the trees were at 65% open bloom.

The number of honey bees per tree per 30 seconds (10 replications) were recorded on the treated and untreated trees at 173 0 hr on 7 April.

On 1 April, 4 strong honey bee colonies with Todd dead bee traps were established adjacent to the treated part of the orchard . On 6 April, each colony was opened and about 100 cells containing eggs or young larvae on one frame per colony marked using stick pins. The number of dead bees in the Todd traps were recorded daily following the application for 11 days.
At 11 days after application, the cells from each colony that were marked earlier were examined. In addition, at 12 days after application, 2 brood frames containing capped brood were taken from each colony and frozen. On 14 September, 150 randomly selected capped brood from each of these frames were opened to determine if any immatures showed abnormal development.

Results and Discussion

The mean number of foraging honey bees/tree/3 0 seconds was 4. 3 in the treated and 4. 5 in the untreated. Pyriproxyfen does not repel honey bees.

The number of dead bees in the Todd traps were from normal die-off (25-125 dead bees per day) and no immatures or deformed adult bees were found in the traps.

On 18 April, there were 325 capped cells of the 362 bees that were observed when they were eggs or young larvae and 10% of the cells were empty or contained honey. In the 604 randomly selected capped brood cells that were examined one dead adult and one dead pupa were found (Table 2).

Pyriproxyfen applied to blooming pears at the rate of 124 gm (AI)/ha is not hazardous to adult or immature honey bees.

FIELD TESTS-WHITE DUTCH CLOVER-HONEY BEES

Materials and Methods

One half of a 1. 6 ha white dutch clover field was treated with pyriproxyfen at 124 gm (AI)/ha with an tractor-drawn ground sprayer at a rate of 189 liters gallons of water per acre at

1 000 hr on 3 1 August when the clover was at full bloom.

The number of honey bees per 12.5 square meters per 30 seconds (10 replications) were recorded on the treated and untreated parts of the field at 1000 hr on 1 September.

On 27 August, 4 strong honey bee colonies with Todd dead bee traps were established adjacent to the treated part of the field. On 30 August, each colony was opened and about 1 00 cells containing eggs or young larvae on one frame per colony marked using stick pins. The number of dead bees in the Todd traps were recorded daily following the application for 10 days.

At 12 days after application, the cells from each colony that were marked earlier were examined.

In addition, at 12 days after application, 150 randomly selected capped brood from one frame per
colony were opened to determine if any immatures showed abnormal development.

Results and Discussion

The mean number of foraging honey bees/12.5 square meters/30 seconds was 2.2 in the treated and 2.4 in the untreated. Pyriproxyfen does not repel honey bees.

The number of dead bees in the Todd traps were from normal die-off (25-125 dead bees per day) and no immatures or deformed adult bees were found in the traps.

On 12 September, there were 333 capped cells of 355 cells that were observed when they were eggs or young larvae and 6% were empty or contained honey. In the 598 randomly selected
capped brood cells that were examined we found one dead adult and 2 dead larvae were found. (Table 3).

Pyriproxyfen applied to blooming white dutch clover at the rate of 124 gm (AI)/ha is not hazardous to adult or immature honey bees.

DISCUSSION

Pyriproxyfen was toxic to honey bees when fed directly to the colonies and our results confirm those of Cozoppelt (1993) who found high mortality in the pupa stage. Cozoppelt (1993)
reported an LC50 of 0.34 ug/ml of semiartifical diet for honey bee larvae; a dose similar to field
rate use. Cozoppelt (1993) suggested that honey bees would probably not pick this amount of dose from field applications of pyriproxyfen but field studies were necessary. Our field studies show that pyriproxyfen applied to blooming crops does not harm bees.

CONCLUSIONS

Pyriproxyfen can be applied to blooming crops without harming bees. Pyriproxyfen can be regarded as "non-hazardous" to bees.

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Table 1. Effects of feeding pyriproxyfen (124 gm (AI)/ha in 95 l of water) in a sugar syrup on 30 August on honey bee immature mortality (A= live adult, DA = dead adult, P = pupa, DP = dead pupa, L = live larva and DL = dead larva) when cells were examined on 12 September. Prosser, WA 1994.

	Mean No. Colony					
	A	DA	P	DP	L	DL
Treated Colonies	13a	2a	116a		94a	74a 7a
Untreated Colonies	1 1a	0a	265b		0b	25b 0a

Means within a column followed by the same letter are not significantly different at the P = 0.05 level, tableled
studentized range test.

Table 2. Effects of applying pyriproxyfen (124 gm (AI)/ha) to blooming pear on immature honey bees from randomly selected capped cells at 12 days after application (A= live adult, DA = dead adult, P = pupa, DP = dead pupa, L = live larva and DL = dead larva) larvae Prosser, WA.
1994.

A	DA	P	DP	L	DL
120	1	473	1	8	0

Table 3. Effects of applying pyriproxyfen (124 gm (AI)/ha) to blooming white dutch clover on immature honey bees from randomly selected capped cells at 12 days after application (A= live adult, DA = dead adult, P = pupa, DP = dead pupa, L = live larva and DL = dead larva) larvae Prosser, WA. 1994.

A	DA	P	DP	L	DL
190	1	395	0	2	1

Appendix 12

H. W. Schmidt
The reaction of bees under the influence of the insecticide Imadacloprid

Dr. H.W.Schmidt
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September 1996

THE REACTION OF BEES UNDER THE INFLUENCE OF THE INSECTICIDE IMIDACLOPRID

1. The new Insecticide

Imidacloprid is a new insecticide known under the trade names Confidor, Admire or Provado for the spray application and Gaucho for the seeddressing. It belongs to the new chemical class of the Chloronicotinyls. It was first synthesised 1985 in Japan by Nihon Bayer Agrochem.

Imidacloprid acts mainly against sucking insects, some beetles and their larvae, mining microlepidopterans and some phytophagous mining fly larvae. Imidacloprid acts sometimes slowly, but finally it causes the death of the pests. Besides mortality imidacloprid exhibits a very characteristic antifeedant effect, which enables the insecticide to prevent damage on the cultivated plants before the pests die.

Regarding bees imidacloprid causes specific reactions, which - in comparison to other insecticides - are different in some aspects. We have tested the reaction of bees towards Confidor and Gaucho in the laboratory, in the tent and in the field.

2. Laboratory tests

The results of the laboratory tests **(Fig. 2)** show, that imidacloprid acts mainly as a stomach poison. It acts quickly via oral toxicity and slowly via contact toxicity. The oral toxicity is much higher than the contact toxicity, by the factor of about 20 (except for the 200 SC). From these results can be concluded, that imidacloprid is toxic to bees, whereby the formulated products are substantially less toxic than the pure technical material by the factor of 10 - 30. The hazard quotient based on 0, 1 kg a.i./ha calculates higher than 20000, which indicates according to this method a highly hazardous compound.

It is interesting to know, that imidacloprid does not act via inhalation toxicity. The vapour pressure is too low **(Fig. 2)** and is distinctly lower than for other insecticides. This property of imidacloprid becomes important in the interpretation of the results from field experiments.

3. Spray treatment onto flowering plants

a) during the day, when bees are present

We sprayed Confidor at a rate of 100 g a.i./ha onto flowering Phacelia at noon in a tent and in the field and observed the mortality and foraging activity. On the day of treatment, but after the application, the mortality was significantly increased and remained so on the following two days. Then it came down to the range of natural mortality **(Fig. 3 and 4)**.

The evaluated mortality in front of the hives calculates to approximately 5% of all individuals in the tent test and less than 2% in the field test. We concluded that Imidacloprid is hazardous to bees and should not be applied onto flowering plants.

We looked to the foraging activity and we noticed in both tests (in the tent and in the field) a reduction in the number of flower visiting bees **(Fig. 5 and 6)**. 15 minutes after the spraying of Confidor the bees had left the treated flowering area. The intensity of gathering nectar was very high before treatment, but after treatment the flowering area was empty. The bees did not resume activity on the treated field for about four to five days. In the tent this effect was particular severe due to the fact that there was no alternative untreated area available for foraging. So the bees remained in the hive until the end of the experiment, when we removed the hive off the tent. From this moment, when we offered new untreated flowering fields, the bees behaved normally again.

The effect of refusing a treated area after application of Confidor appears in the field as well, but usually in a more mild degree. The bees remained in the hive for four days, provided they had no alternative untreated area for foraging. An alternative food source in this sense are a few flowering plants outside the treated area and further away, e.g. along a road or wayside or some flowering weeds in a field. Such flowering plants are always present in an agricultural landscape, particularly after the introduction of fallow land.

We confirmed the foraging activity of the bees on alternative plants by counting all bees entering the beehive with pollenloads **(Fig. 7)**. Before the application of Confidor the bees collected other pollen and a certain number of pollen from phacelia. After treatment the bees remained busy, but preferably on the alternative plants. We counted more „other pollen“ and no phacelia pollen. So we can state, that after the application the food supply was continued and the whole colony did not suffer. This is in fact important, that we not only observe the treated area and document the missing foraging bees and interpret this as a damage to the colony. This is not the case. Except the described limited mortality the colony is quite well and the bees forage somewhere else.

b) Remarks on the behaviour of the bees

We marked foraging bees on the flowers. When such bees returned from the Confidor-treated field, they were very nervous and performed the ***tremble dance.*** *This dance is considered to inform the bees about a dangerous substance outside. Such an information spreads very rapidly inside the colony. It is sufficient, that only few bees make experience with imidacloprid or ingest a very small amount. The tremble dance initiates, that the bees remain in the hive. On the following day the information is renewed, when in the early morning the first bees fly out, land on a treated flower, take up a very small amount of Confidor, which again leads them to perform the tremble dance. As long as no other information is transmitted, they remain in the hive. If however a bee comes from untreated plants and considers them as a good food source, such a bee will perform the wagging dance at the same time and in competition with another bee performing the tremble dance. Such a positive information will be taken up by the bees and they are stimulated to leave the hive and to exploit the alternative source. With the observation of both the tremble dance and the wagging dance we can explain the avoidance of the treated field and the continued activity on alternative untreated plants.*

c:) in the evening, during absence of the bees

We sprayed Confidor in the evening, when the bees have ceased foraging. By reducing the exposition, the mortality was less severe, but with roughly 50% of the midday treatment still too high for a non hazardous insecticide ***(Fig. 8 and 9)****. So we are careful and do neither recommend to use Confidor at noon nor in the evening during the flowering period.*

The foraging activity of the bees on the treated field was reduced even after the evening spray. The extent and the duration of this effect was more or less the same as after the treatment at noon ***(Fig. 10 and 11)****. The bees refused to forage on the treated area for 5 or 6 days. We came to the conclusion, that the application of Confidor in the evening brought only little advantages and should not be recommended.*

d) prior to flowering period

After having noticed the adverse effects of a treatment during flowering, we investigated, whether Confidor can be applied before the flowering period without harming the bees. This

is of particular interest in apples, where we applied Confidor at the Green tip stage (ES 54), which is 10-14 days before flowering. In this case the insecticide had no influence on the foraging activity of the bees **(Fig. 12)**. This application did not cause any mortality **(Fig. 13)** and did not interfere with the pollination by the bees. The application at the Green tip stage is a highly suitable stage for the control of Dysaphis plantaginea.

e) Effects of Confidor on the brood

We inspected in our field trials in 2 colonies all 2x20 combs and assessed in percent the area on each combside, which is filled with eggs, larvae or pupae **(Fig. 16 and 17)**. In no case we found any detrimental influence of Confidor on the broodstages. The brood developed very well as in the untreated colonies.

4. Systemic application

a) Seeddressing

One of the striking properties of imidacloprid is its systemic activity. After seeddressing with Gaucho aphids, white flies and jassids are controlled. Gaucho is mainly used in non-flowering crops like cereals, maize, sugarbeet, cabbage, onions or in flowering crops, which are not visited by bees like potatoes. Those crops are of minor importance regarding exposure of bees. As far as flowering crops are concerned, Gaucho is registered in sunflowers, oilseedrape and broadbeans. Oilseedrape is mainly a wintercrop and between planting, that is the application time of Gaucho, and flowering time the period is more than 200 days. This represents no exposure for bees. In summer crops the period between planting and flowering here is about 60 days or 9 weeks. This is just the time, when the efficacy of Gaucho against sucking pests breaks off. There remains the question whether there may be traces of imidacloprid in the nectar or in the pollen. Generally, systemically acting seeddressings are not considered as having a potential to harm bees. Due to its high systemic activity, imidacloprid appears to be an excellent model compound to proof this assumption.

We put 6 bee colonies in the centre of 1,5 ha isolated sunflowers, which were treated with 50 g a.i./ha Gaucho. The mortality in front of the 6 hives was slightly higher than on the untreated field, but was still considered as normal **(Fig. 18)**. We counted the number of bees foraging on 100 open sunflower heads. The flower visitation was unaffected **(Fig. 19)**. Compared with the untreated field no reduction in the number of bees was noticed. That means, that at the flowering stage residues of imidacloprid after seeddressing (if there are

any) must be so low, that they do not cause any reaction of the bees. We also checked the weight increase of all 6 beehives in the sunflower field. The weight increase of the colonies in the Gaucho-treated field was not different from the colonies in the untreated field **(Fig. 20)**. We could not detect any influence of the seeddressing with Gaucho.

5. Abstract

The toxicity of the insecticide imidacloprid towards bees was tested. In the laboratory the oral toxicity was distinctly higher than the contact toxicity. The formulated product was substantially less toxic than the technical material. Confidor was recognised as being hazardous to bees and should not be applied during the flowering period. We conducted however such experiments to describe the reaction of the bees.

When Confidor was sprayed onto flowering plants during full activity of the bees, it caused a high mortality and the bees avoided the treated area for some days. They continued, however, the collection of nectar and pollen on untreated plants. The colony remains vital and the food supply is ensured. The application prior to the flowering period is possible at the Green tip stage without any relevant effect to honey bees. The systemic property of imidacloprid after seeddressing did not influence the bees, because they behaved very normal in their foraging activity. The No effect level for the observed antifeedant effect was determined in a feeding test at 0, 1 ppm. Imidacloprid does not affect the brood in the hive. The juvenile stages develop undisturbed.

Imidacloprid triggers among the bees the tremble dance, which is a message to protect the colony from suffering damage. The hazard, which imidacloprid exercises on bees, can be avoided by the exclusive use outside the flowering period according to the registration and recommendation.

Fig. 2:

Bee toxicity: Laboratory results

	LD_{50} oral (48 h)	LD_{50} contact (48 h)	
active ingredient	0,0037 µg / bee	0,081 µg / bee	Huntington
Confidor 70 WG	0,0167 µg / bee	0,35 µg / bee	IBACON
Confidor 200 SC	0,103 µg / bee	0,29 µg / bee	IBACON

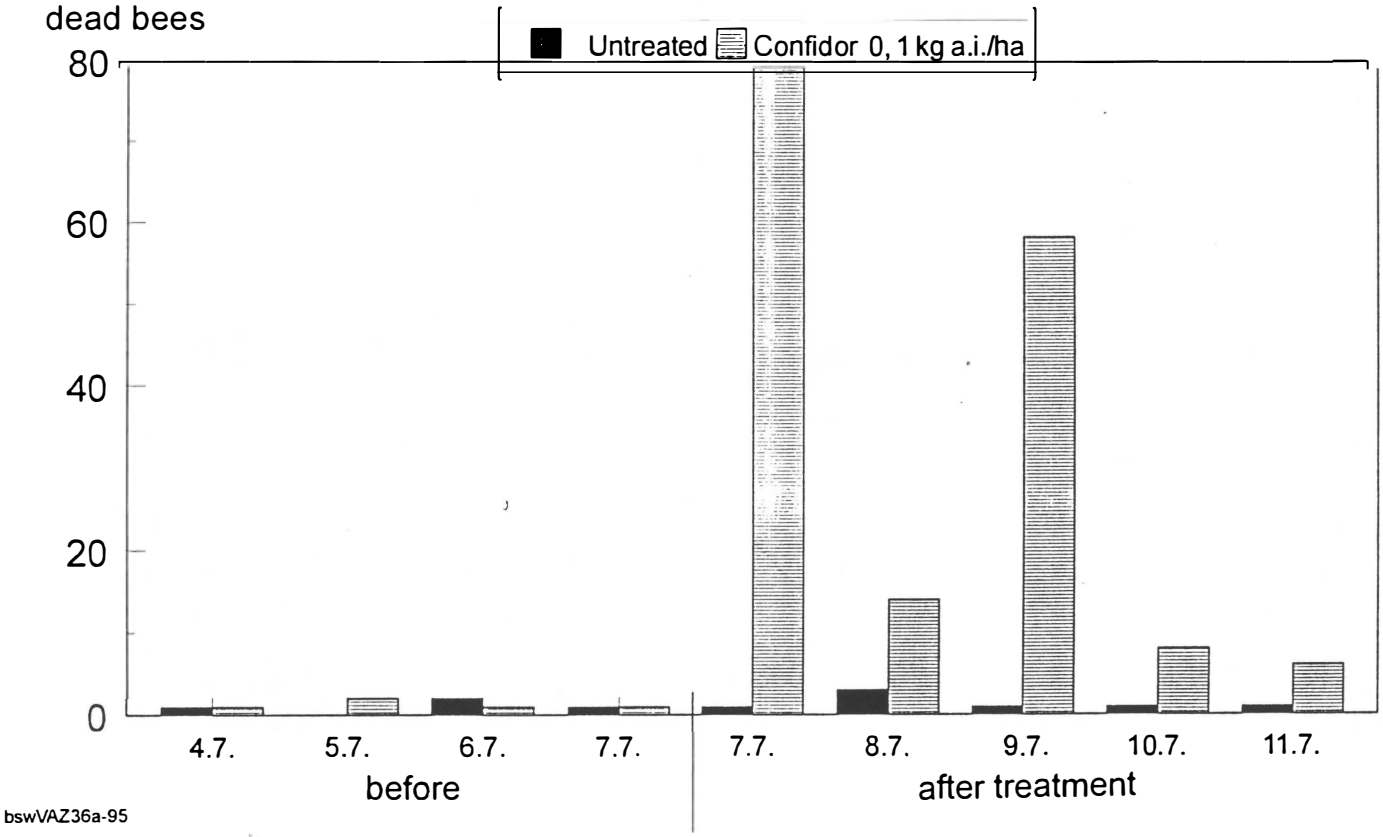
Confidor 70 WG tested as 0,036 % = 0,025 % a.i. Univ. Bonn

oral toxicity	100 %	mortality after 1 hour	
contact toxicity	60 %	„	48 hours
dusting toxicity	100 %	„	24 hours
inhalation toxicity	8 %	„	48 hours

(vapour pressure of a.i.: 2 x 1 0^{-9} h Pa at 20 ^{0} C)

3

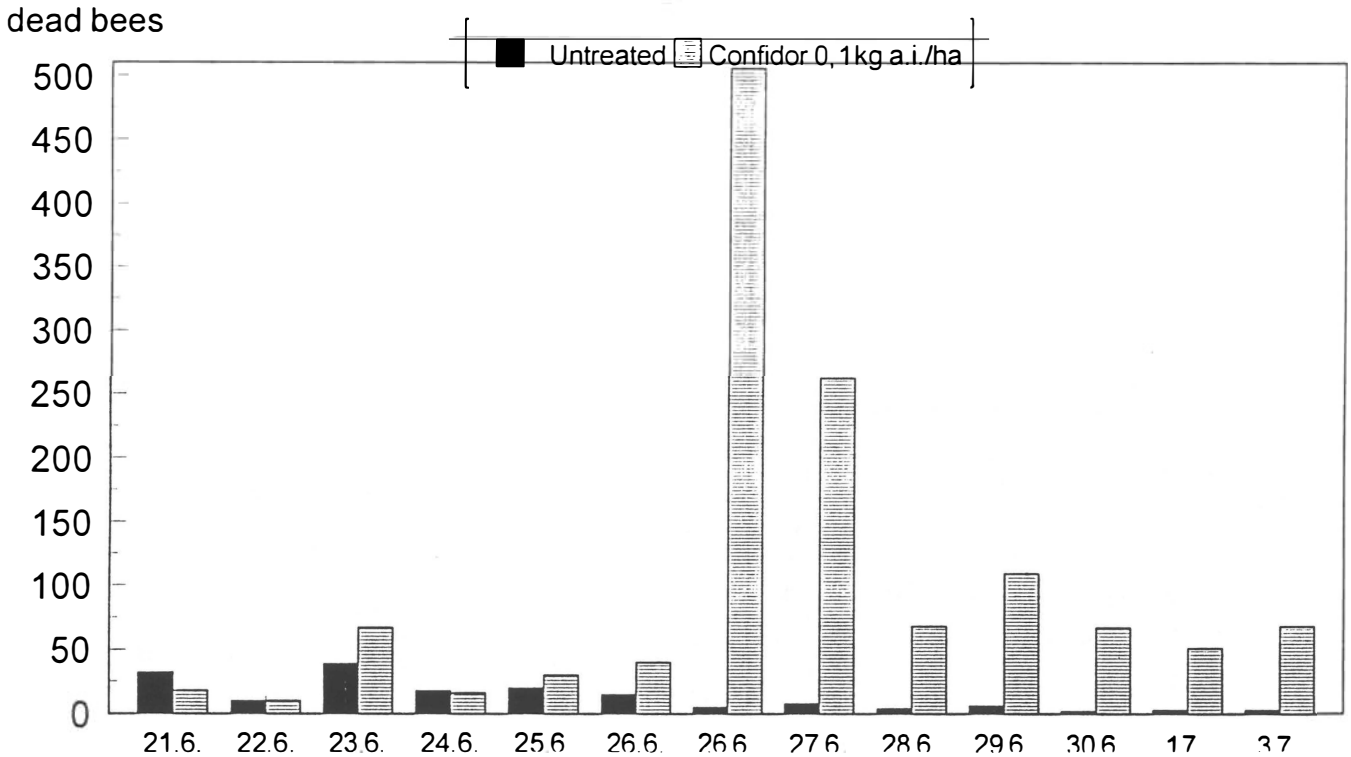
Tent: Mortality in front of beehive
treated 7.7.95, 12 sqm Phacelia



bswVAZ36a-95

4

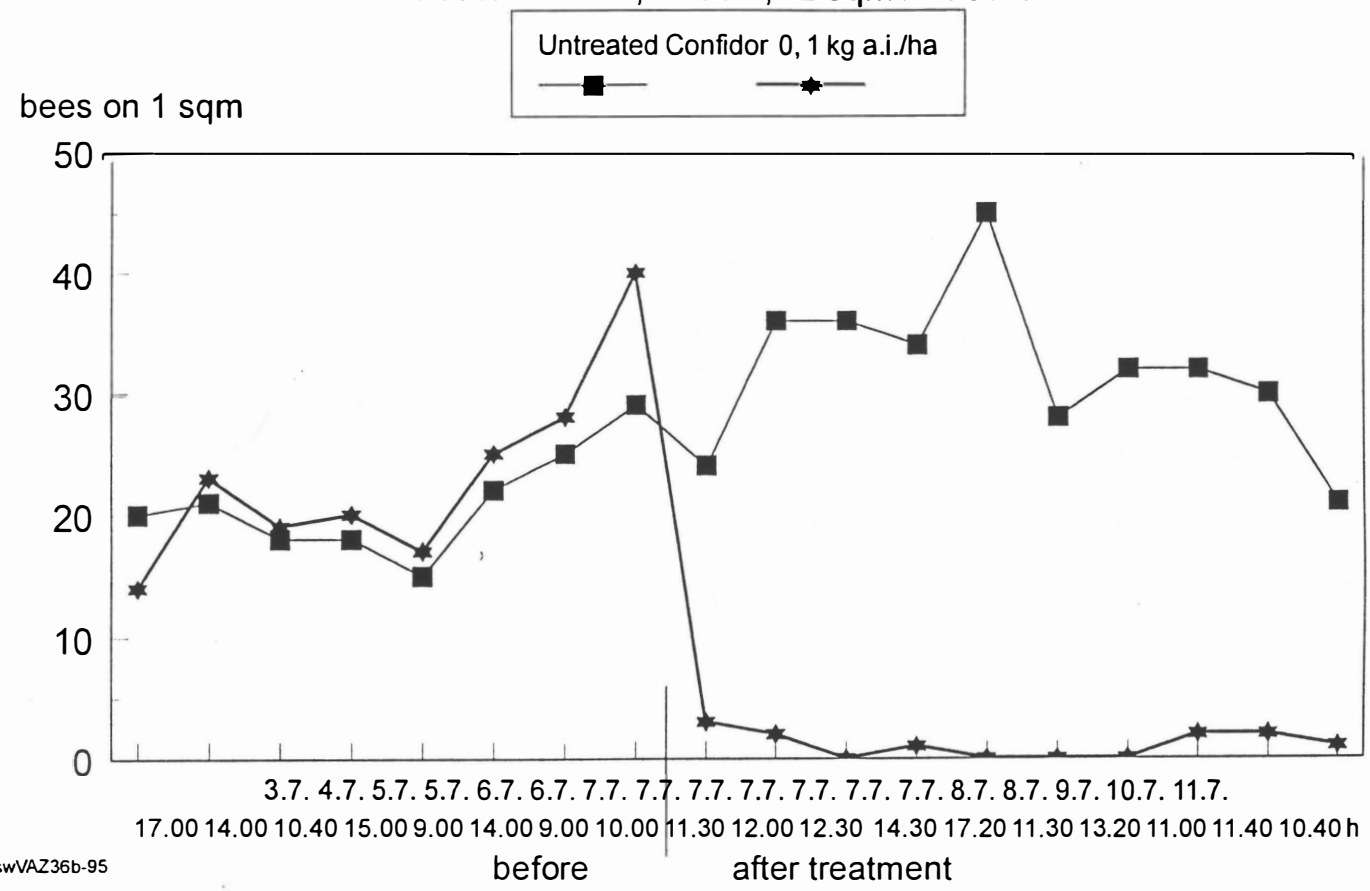
Field: Mortality in front of 4 beehives treated
26.6.95, 0,5 ha Phacelia



5

Tent: Foraging activity

treated 7.7.95, 11.00 h, 12 sqm Phacelia



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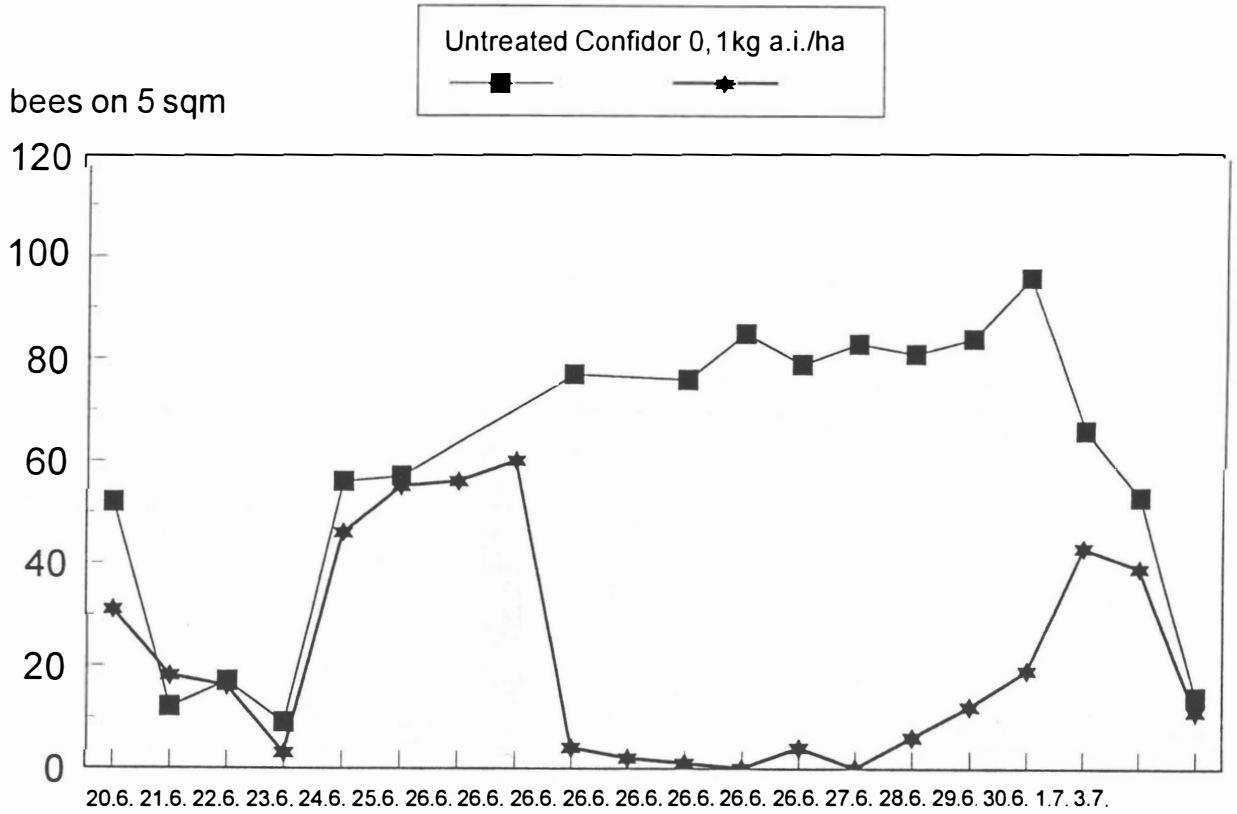
6

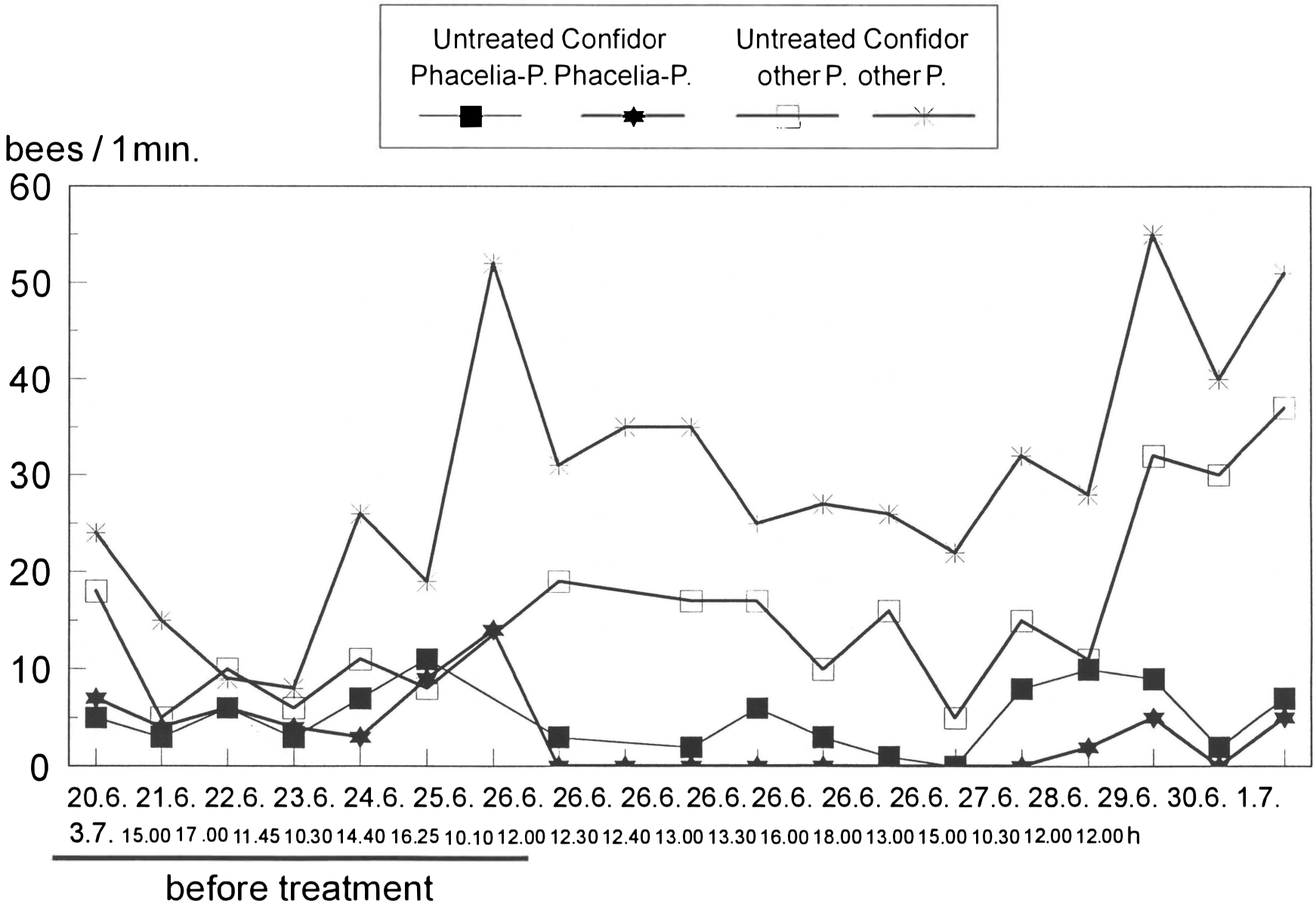
Field: Foraging activity

treated 26.6.95, 11.30, 0,5 ha Phacelia



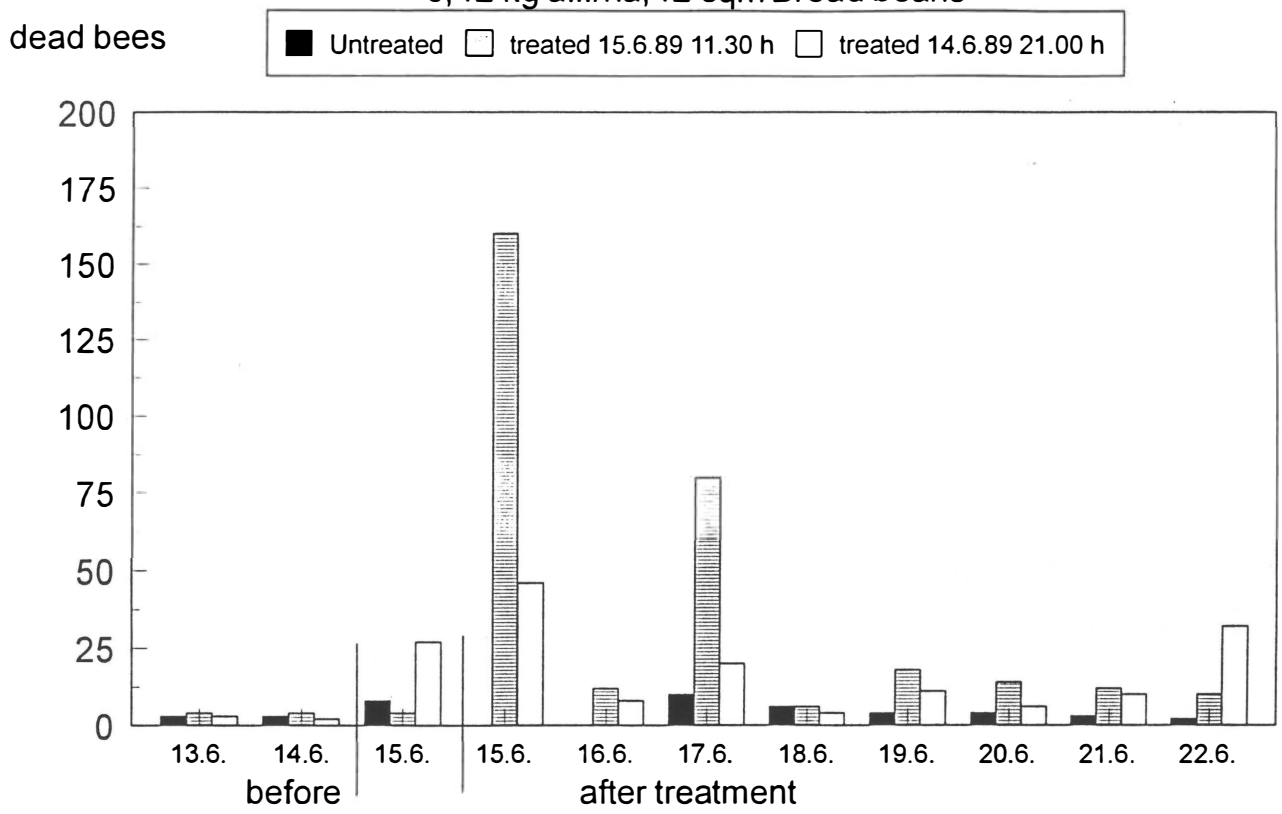
Field: Bees with pollen-loads entering the hive

treated 26.6.95, 0,5 ha Phacelia



8

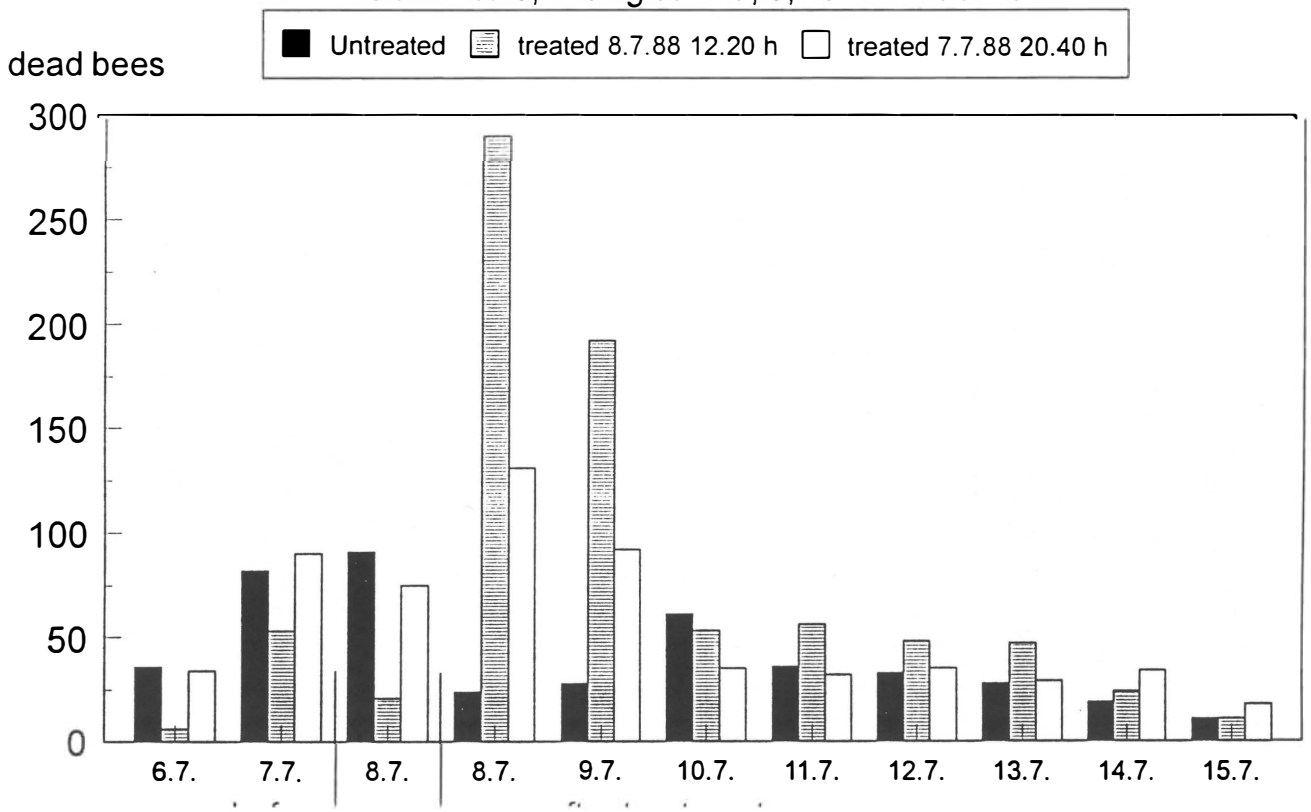
Tent: Mortality in front of beehive Confidor
0,12 kg a.i./ha, 12 sqm Broad beans



bswVAZ8a-89

9

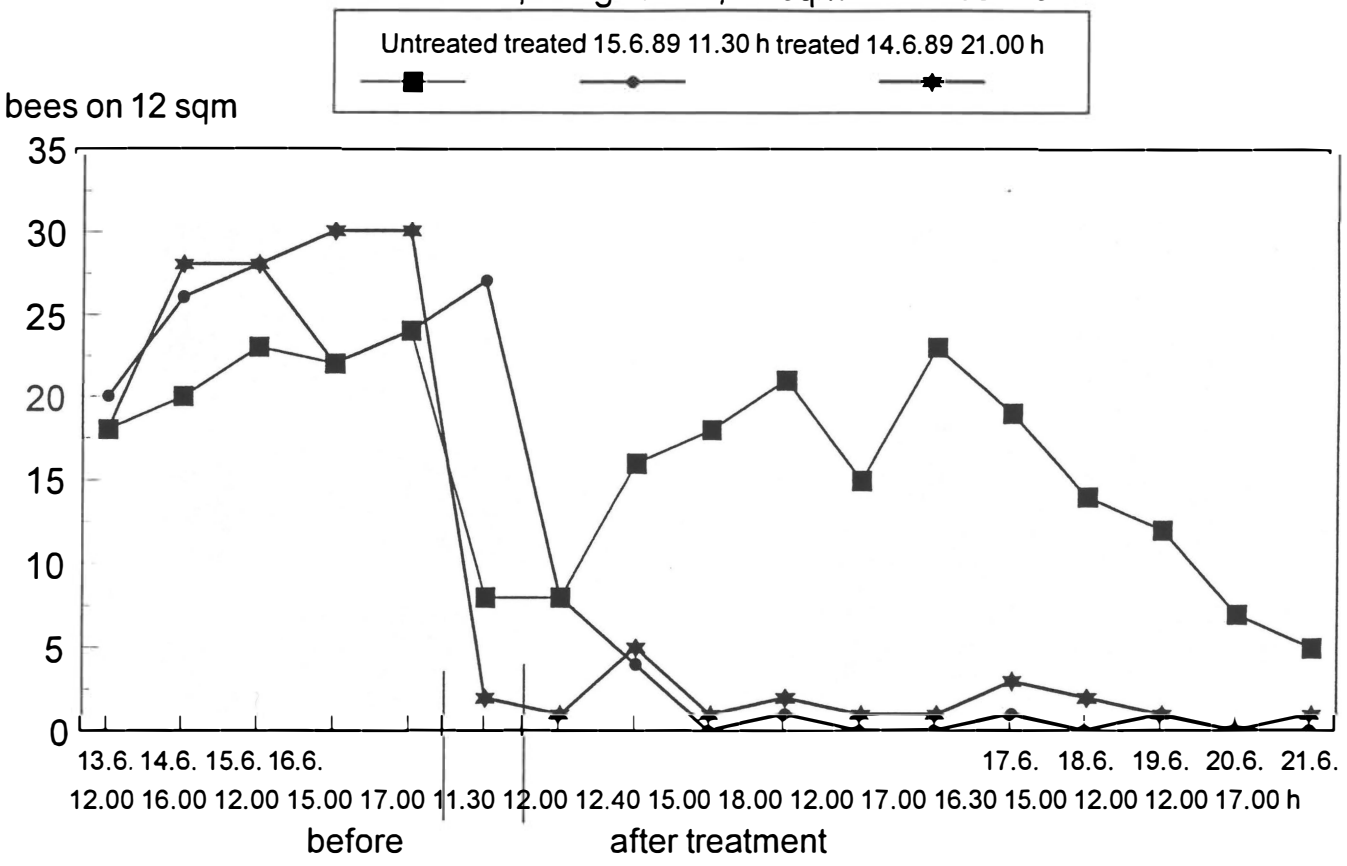
Field: Mortality in front of 4 beehives
Confidor 0,125 kg a.i./ha, 0,15 ha Phacelia



10

Tent: Foraging activity

Confidor 0,12 kg a.i./ha, 12 sqm Broad beans

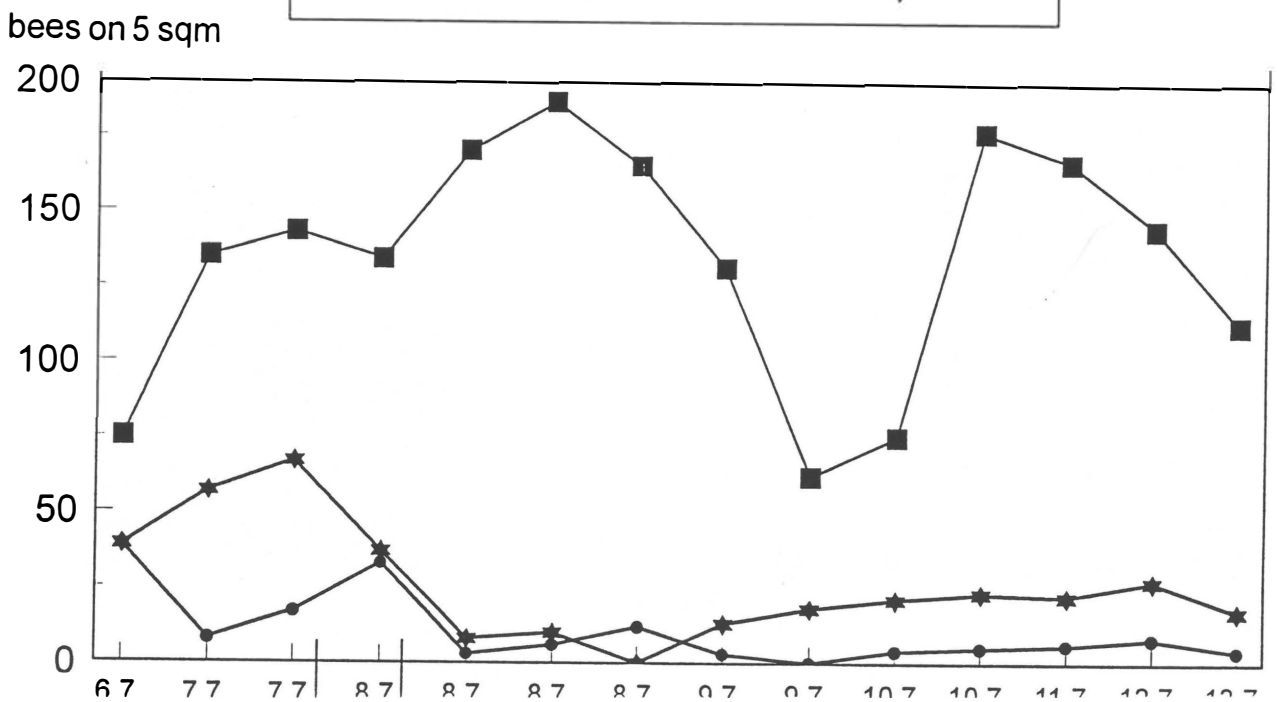


bswVAZ8b-89

11

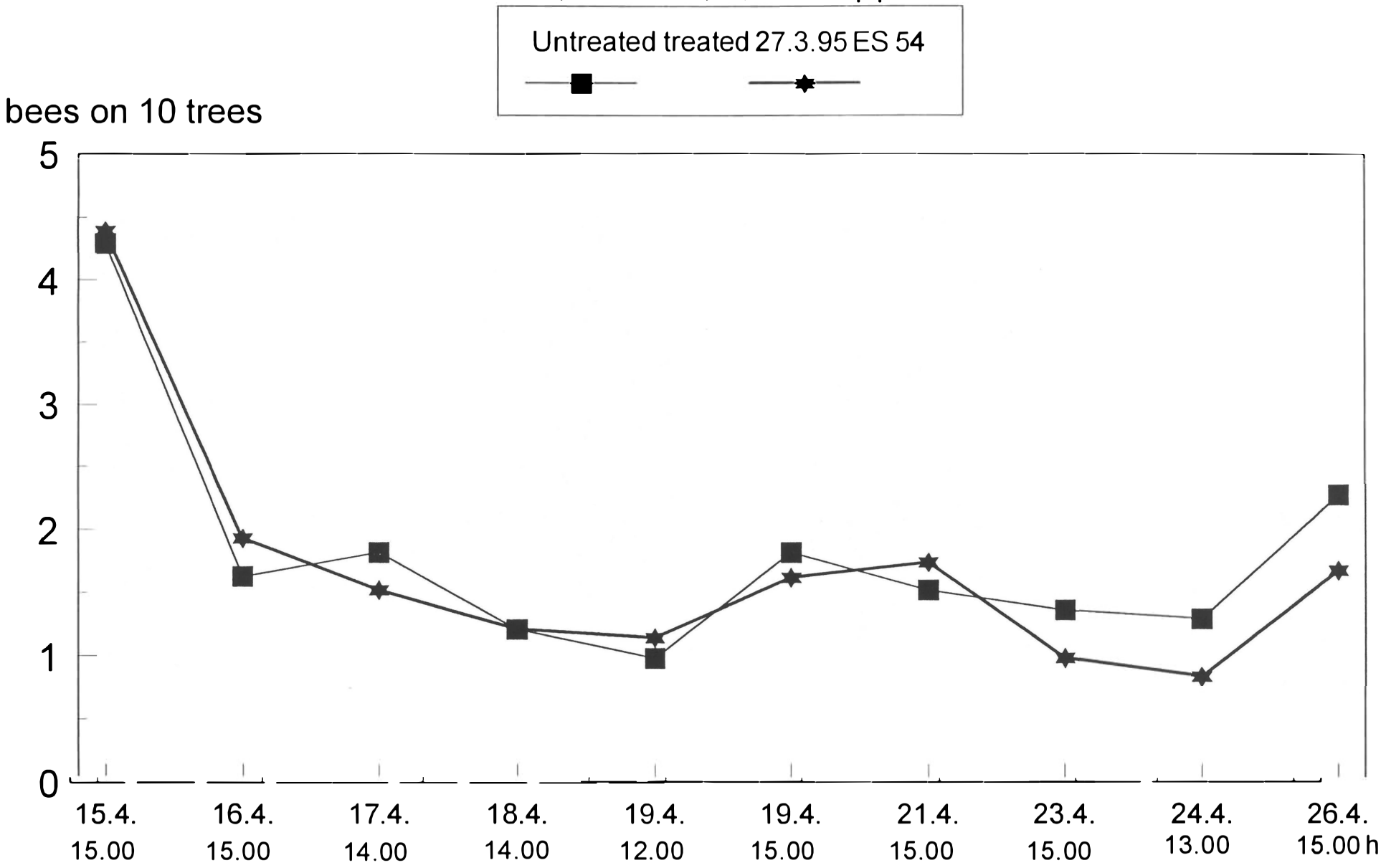
Field: Foraging activity

Confidor 0,125 kg a.i./ha, 0,15 ha Phacelia



Field: Foraging acitvity Confidor

0,01% a.i., 0,9 ha Apple

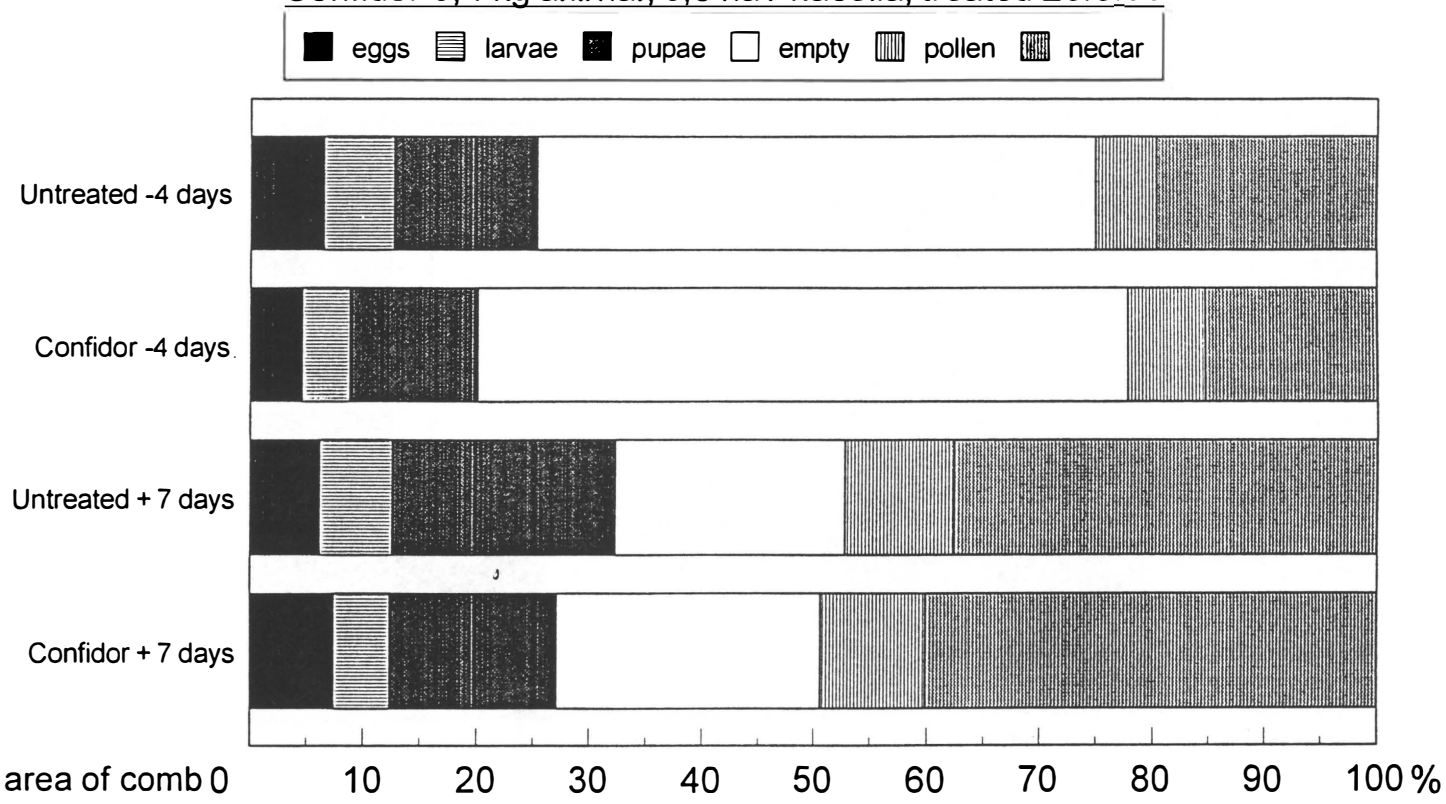


bswVAZ35b-95

16

Field: Brood development on 2x20 combs

Confidor 0,1 kg a.i./ha., 0,5 ha Phacelia, treated 26.6.95

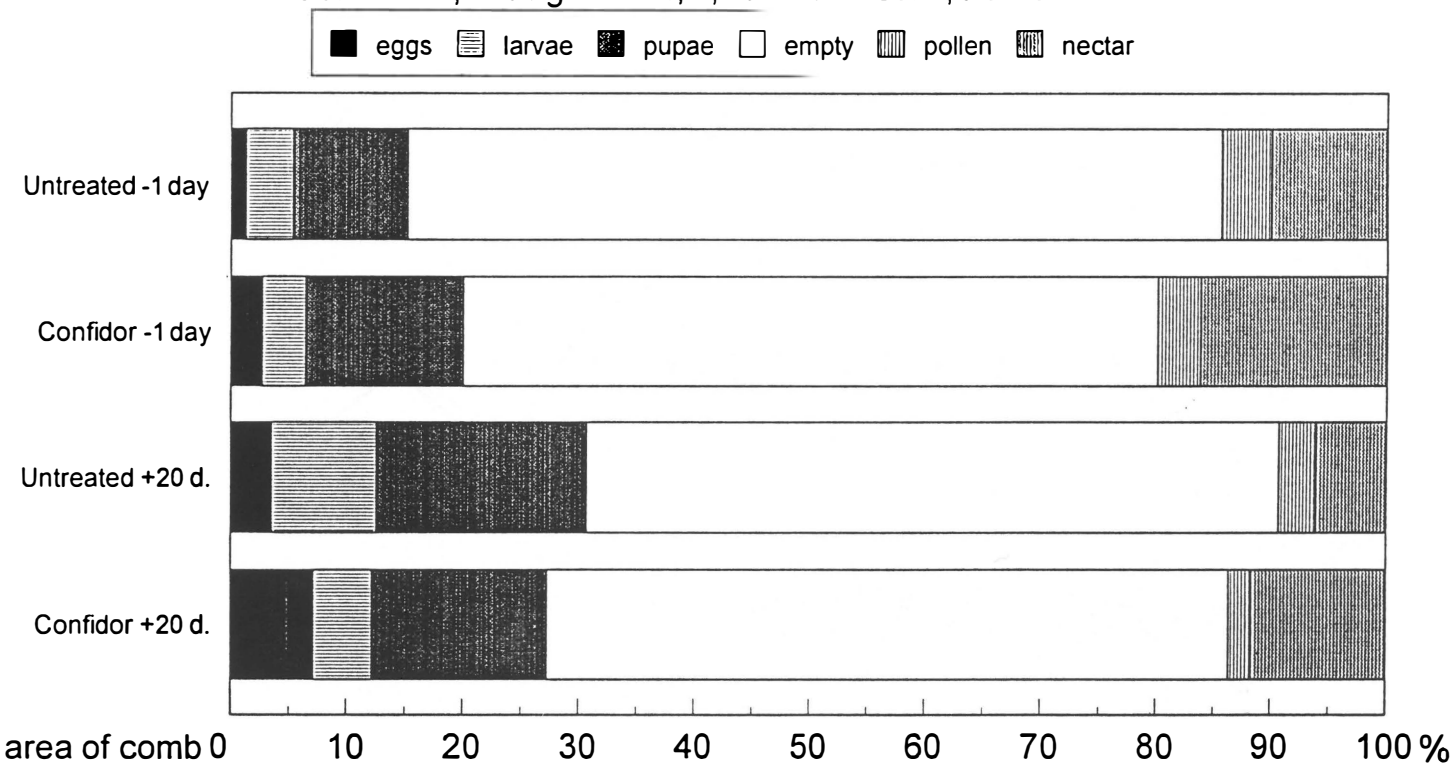


bswVAZ32e-95

17

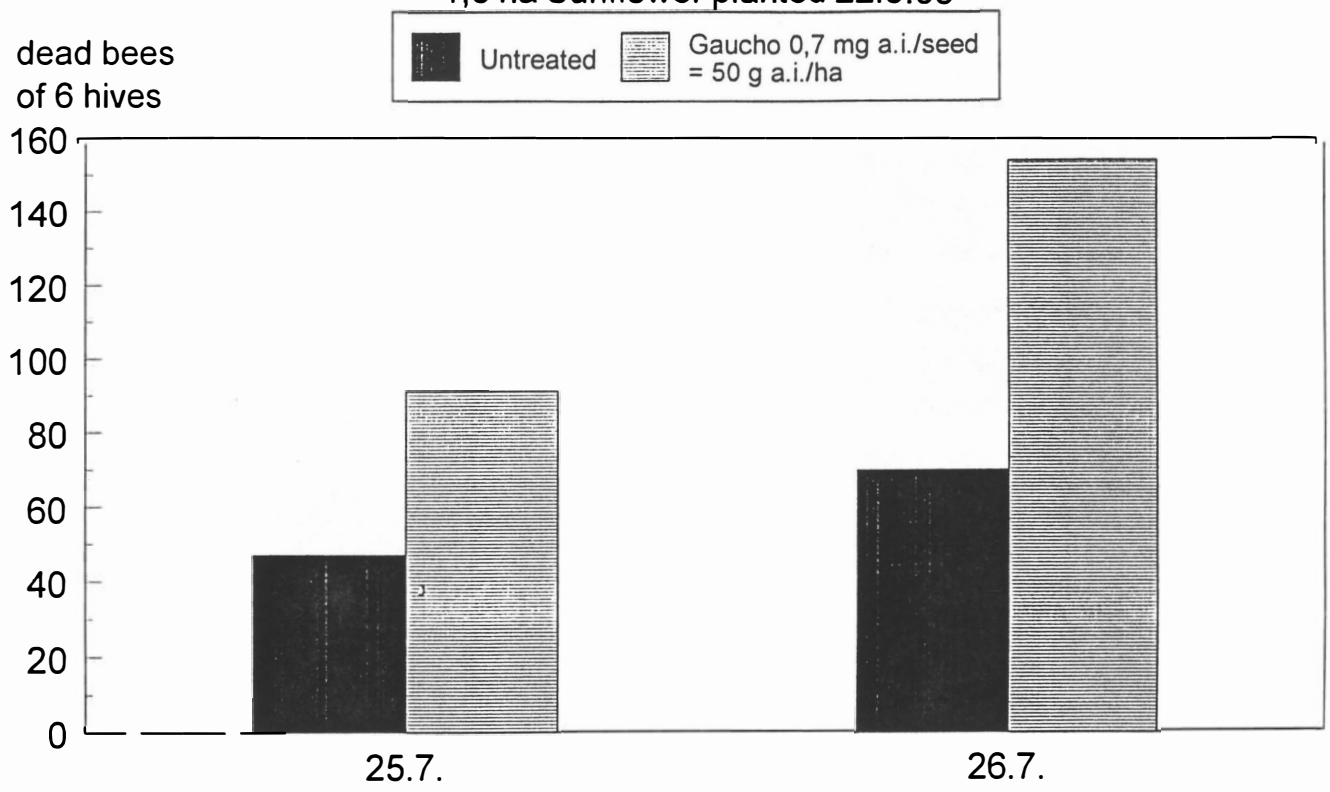
Field: Brood development on 2x30 combs

Confidor 0,125 kg a.i./ha., 0,15 ha Phacelia, treated 8.7.88



Field: Mortality in front of 6 beehives

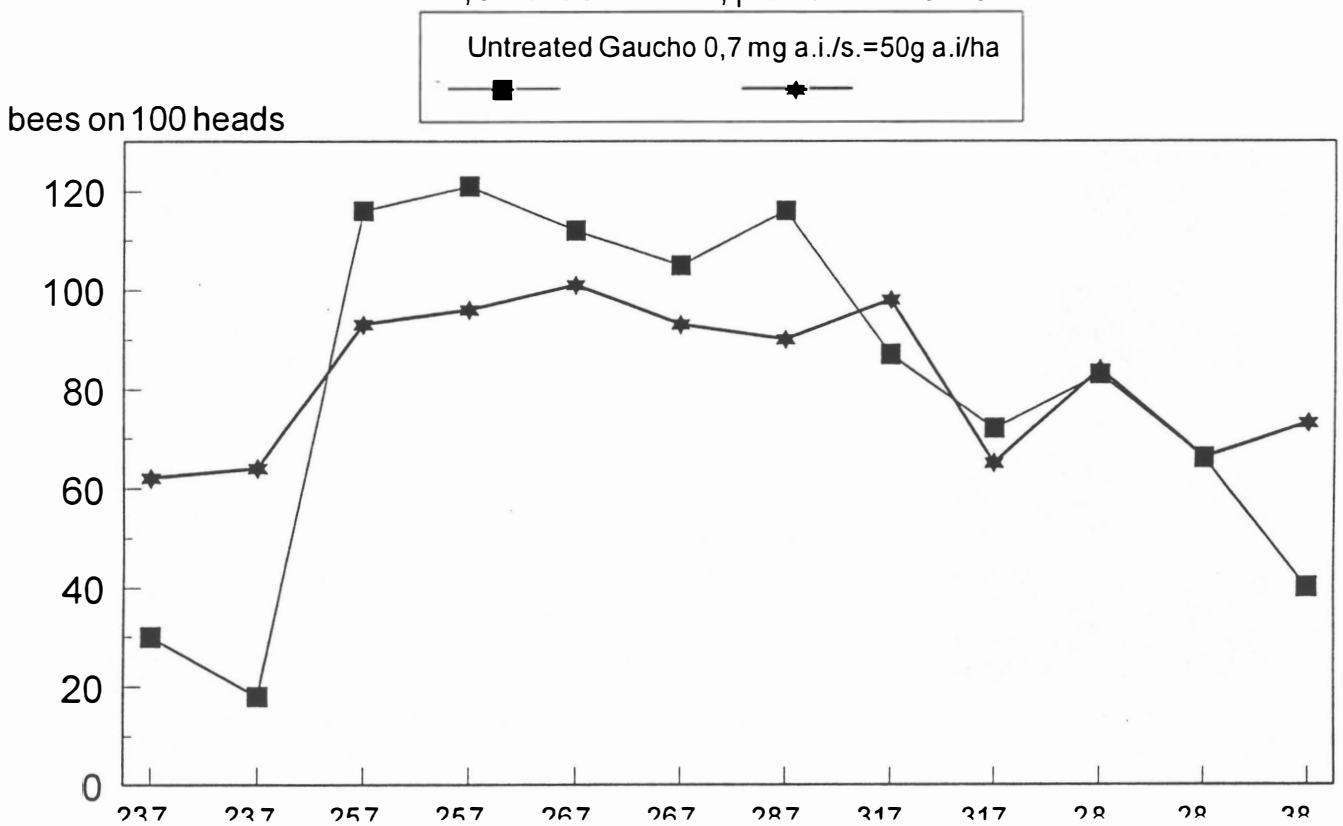
1,5 ha Sunflower planted 22.5.95



bswVAZ34a-95

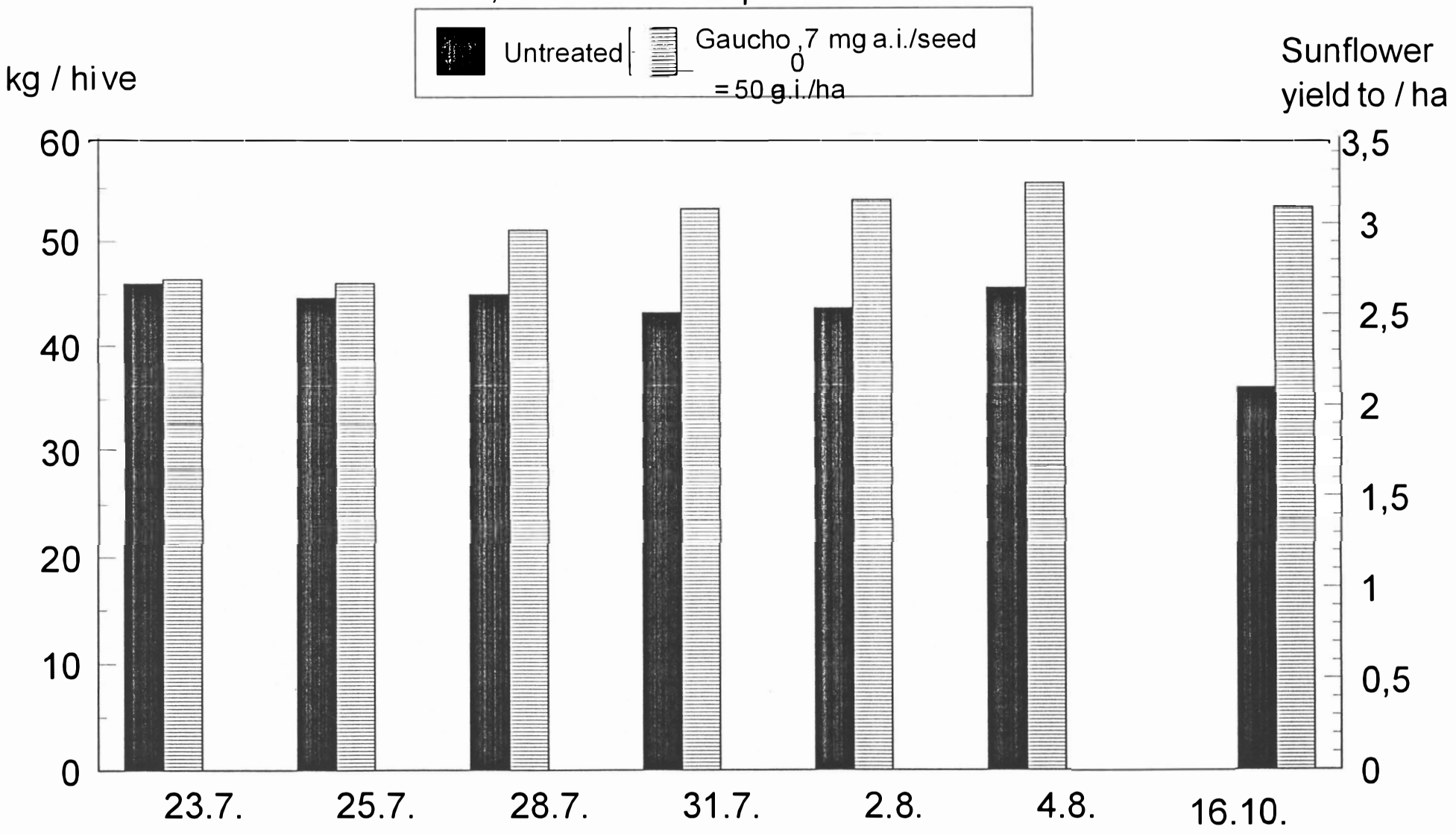
Field: Foraging activity

1,5 ha Sunflower, planted 22.5.95



Field: Average weight of 6 beehives

1,5 ha Sunflower planted 22.5.95



Appendix 13

P. A. Oomen
Report from the discussion group „Residue Testing“

ICPBR Residue Subgroup

Members: Brasse, Forster, Lewis, Oomen, Schmidt, Van der Steen

Report to ICPBR, Braunschweig
P.A. Oomen, coordinator, 16 August 1996

Background:
The ICPBR Symposium in 1993 in Wageningen discussed whether the ICPBR should consider residue testing. A subgroup was installed to discuss this question and to advice to the ICPBR Symposium in Braunschweig 1996. Herewith its report to the ICPBR Symposium:

The ICPBR subgroup considers residue testing important, because:
1 . Residues form an important route of exposure of bees to pesticides.
2. tiCertain risk management practices (like exclusive evening applica
   from treated areas including glasshouses) require information on the period that pesticide residues remain harmful to foraging bees. This information may be collected by residue testing.
3. The European Union proposes to require information from residue testing to honey bees, but methods for testing and for risk assessment are lacking yet. Technical advice will be welcomed by the EU.

Notes of the Residue Subgroup:
1 . The risks caused by exposure of bees to residues are already effectively covered by the EPPO/CoE risk assessment scheme, but not by way of specific residue testing. Specific testing would be helpful for the more specific risk management practices (e.g. evening applications), and for better understanding the risks of residues.
2. The risk assessment and the data requirements should not be made more complicated or larger. More attention to residues should have clear advantages to the applicant, such as faster access to classifications of low and medium risk.
3. Data requirements on residual effects should be ***optional.***
4. Development of an adequate testing method, of risk assessment including decision threshold values and of validation will require considerable work and time. We think nevertheless that it is worthwhile.

Steps to start this work and to carry it to a fruitful end:
1 . Find or develop a suitable test method.
2. Find or collect test results for a number of pesticides.
3. Find or collect information from practice about the risk from exposure to residues of different pesticides in the field, preferably 'how much time after application pesticide residues are harmless again?'.

Ad 1. Test method.
A test method should have as primary aim to obtain a realistic measure of residual toxicity which is as standardized as possible. Preferably a simple element of behavioural response to the residues should be included in the test, without the test becoming too complex. In contrast to the suggestion of the EPPO/CoE scheme, no test method is available yet. The publication 'Gerig & Oomen, 1993' does not exist, and will neither be written. ICPBR has asked the group to come up with a proposal. The group prefers adoption and where necessary adaptation of existing methods, rather than developing new ones. However, certain existing methods like the EPA-method seem unrealistically simplistic. The Gerig method at least requires considerable elaboration. Ideal would be an existing method from which a considerable number of test results already were available. Ed Pilling will propose a method at the symposium in Braunschweig. After a test is finally chosen, a ring test in as many laboratories as possible is wanted.

Ad 2. Test results
Test results are necessary to correlate practical risks with test results, and so develop threshold values. The value mentioned in the EPPO/CoE Scheme and in the EC proposals (LT50<8 hrs) is tentative only, and not scientifically founded. Collecting these could be the most time consuming part of the proposal. Experts aware of existing suitable test results are requested to make them available for analysis by the group.

Ad 3. Risk information from practice.
The same is true here: experts aware of suitable information on the duration that residues remain harmful (or become harmless again) in practice are requested to make them available. Possibly the German collegues have such data, on which the existing German prescriptions for evening applications are based. Otherwise such data should be collected.

Next
The work on residue testing has just started. I propose to ICPBR that the subgroup will continue its work and report to the next ICPBR meeting.

Appendix 14

E. D. Pilling, H. J. Gough, D. Jackson, J. D. Bembridge
Development and comparison of two laboratory methods to determine effects of pesticide residues on the honey bee *Apis mellifera*

Development and Comparison of Two Laboratory Methods to Determine Effects of Pesticide Residues on the Honey Bee *Apis mellifera*

E D Pilling, H J Gough, D Jackson and J D Bembridge

Zeneca Agrochemicals, Jealott's Hill Research Station, Bracknell, Berkshire, RG42 6ET, UK

ABSTRACT

Honey bees may be exposed to agrochemicals not only by direct application to bees foraging on flowering crops or aphid honeydew, but also to the residues remaining after application. At present there is no agreement in European guidelines for methodology to assess the risk of agrochemical residues to bees or when this type of study should be triggered. This investigation was therefore undertaken to compare existing and new methodology to assess risk from exposure to residues, and discusses how the method could indicate overnight reduction of hazard following evening applications.

Results indicate that, for different rates of triazophos and cypermethrin, the leaf disk approach is as sensitive as the box method when recording percentage mortality over set periods of time. Fresh residues were not surprisingly found to be more toxic to bees than residues left to age for 24 hours prior to exposure to bees. Both methods were found to be capable of detecting variations in toxicity of residues over time. Although there was slightly higher variability found in results from the leaf disk method, this approach is favoured as residues are picked up by bees from a leaf surface thus representing a more realistic exposure.

The implications of the variability, sometimes reported, of the toxicity of pesticide residues on foliage, according to species or significantly increasing when the residue is about a day old, are discussed in the context of interpreting the results of laboratory residual tests. The need for further method development to address this problem is indicated.

INTRODUCTION

The requirements of a laboratory bioassay to assess risk of agrochemical residues include: realistic exposure, residue pick up from a leaf surface, practical feasibility and repeatability in different laboratories and countries. Existing methods available include the US chopped leaf study, the Gerig flowering plant method, the BBA box method (Stute *et al.*, 1991) and a newly developed leaf disk method. Of these approaches, the US chopped leaf study has been shown to create an unrealistic high exposure of the residues to bees and is therefore inappropriate. The Gerig flowering plant method, although representing a realistic exposure scenario, produces highly variable results due to difficulty in maintaining a standard exposure surface area between replicates and studies. This investigation therefore compared results from the BBA box method, which has the advantages of being practical, repeatable and of low variability with a standard surface area, with the leaf disk approach that combines practicality with a standard surface area for exposure and realistic pick up of residues from a leaf surface.

METHODOLOGY

For comparison of toxicity of residues to bees, the agrochemicals chosen were triazophos (Hostathion™) and cypermethrin (Ambush C™). The two methods used are described separately. However the timings of spraying of substrates and of loading of bees at the beginning of exposure were strictly co-ordinated so as to not add any variables which might affect the response of the bees.

1 Box Method

The box was supplied (by U. Röhlig of Biochem Gmbh, Cunnersdorf, Germany) as a flat, cardboard cut-out which, when folded and assembled, made a box 95 x 65 x 50 mm (Plate 1). Each box had a window space 70 x 50 mm, sealed with an observation window of black cloth mesh with a hole size of approximately 1 mm, held in place by two rubber bands; two holes for feeding tubes in the top and twenty-one 3 mm diameter holes in the floor. A glass feeding tube containing 50% w/v aqueous sucrose solution as food for the bees was inserted in one of the holes and replaced by a clean tube containing fresh sucrose syrup, as necessary. The larger of the two pre-cut holes in lid of each box was not used and was blocked by inserting a rubber bung to prevent the escape of bees.

The internal sprayed surface available to the bees was 130 cm^2. The interior volume, at 309 cm^3, was similar to that of holding cages, used successfully for many years, for groups of 10 bees exposed in acute toxicity tests in the same laboratory. Therefore, 10 bees were used in each box.

The boxes were sprayed with insecticides by placing them, flat and unfolded, under the track sprayer. The boxes were folded and assembled after the spray deposits had dried (after a delay of 24 h for aged deposits). The double end wall of the box design caused the unsprayed surface to form the inner surface of each end wall of the boxes.

For each treatment and at each application rate there were 3 replicates with an additional 3 replicates sprayed with deionised water to serve as a control.

The flat box templates were sprayed as described above. Six boxes (three to be used for exposure of bees to fresh residues and three for aged residues) were sprayed for each treatment, including the control. Once the spray had dried (30 minutes) the templates for the exposure of bees to fresh deposits were assembled into boxes. The remaining boxes were left, flat and unfolded, with the treated surface uppermost, on a bench to allow the residue to age in the laboratory until 24 hours after spraying, when newly collected bees were placed in them.

2 Leaf Surface Method

A clear plastic container (14 x 24.5 x 11 cm) with a screw-on lid was adapted. The lid was removed and replaced with 1 mm mesh black netting, held on with a rubber band, to allow ventilation. An upturned plastic dish (4.5 cm high) with a hole the same size as the external diameter of the vial cut out of the bottom was attached, by adhesive tape, in the bottom of the container. A vial just taller than the dish was placed in the hole and absorbent cotton wool inserted to act as a wick when the vial was filled with sucrose solution.

On the morning of the test discs were cut from leaves of Dwarf French Bean plants (*Phaseolus vulgaris*) var. The Prince. Each leaf disk was attached, by ordinary office staples, to an 8.5 cm diameter filter paper. A hole of the same diameter as the vial was cut in the centre of the filter paper/leaf disk leaving approximately 54 cm^2 surface area available to the bees. The leaf disk was sprayed and placed on the platform with the vial containing sucrose solution just protruding from the centre. The bees therefore had to alight on the leaf disk in order to feed.

The base also had a door c. 5 x 1 cm through which dead bees could be removed from the test chamber, although this was not needed, as the bees could be assessed for effects without removing the dead ones. Two opposite faces had windows (9 x 8 cm) cut out and were covered with 1 mm mesh black netting for additional ventilation.

Twenty bees were held in each container. For each treatment rate there were 3 replicates with an additional 3 replicates sprayed with deionised water to serve as a control. This was done at the same time for discs for exposure of bees to fresh residues and for discs for exposure to aged residues, so that six discs were sprayed for each treatment and for controls.

Once the spray had dried, the leaf discs were placed on the platforms in the containers, and the bees introduced. The remaining leaf discs were placed in the containers and left on a bench in the laboratory until24 hours after spraying, when newly collected bees (handled in the same manner as described above) were placed in them.

3 Test Chemicals

The chemicals were applied to the boxes and leaf discs at the stated rates using a track sprayer fitted with a single fan jet (Teejet "8002E") travelling at a constant speed, spray pressure (2 bar) and jet height (20 cm above target) to produce a spray volume of200 ±20 l ha^{-1}. All test substances were prepared in deionised water.

The field rates simulated were as listed in Table1.

Table 1: Rates Tested

Spray Concentration	Half Field Rate	Field Rate	2 x Field Rate
"Hostathion" Triazophos 420 g l^{-1}	0.5 l ha^{-1} 210 g ha^{-1}	1 l ha^{-1} 420 g ha^{-1}	Not tested
"Ambush C" Cypermethrin 100 g l^{-1}	125 ml ha^{-1} 12.5 g ha^{-1}	250 ml ha^{-1} 25 g ha^{-1}	500 ml ha^{-1} 50 g ha^{-1}

RESULTS

The mean mortality of bees following exposure to triazophos and cypermethrin in the box and leaf disk experimental designs, is presented in Tables2 and 3, respectively.

Table 2: Mean % Accumulative Mortality of Bees in Box Method

Treatment	Mean % of Bees Dead Out of10							
	Fresh Deposits				Aged Deposits			
	1 h	24 h	48 h	72 h	1 h	24 h	48 h	72 h
Triazophos 210 g ha^{-1}	0	60	97	100	0	20	73	83
Triazophos 420 g ha^{-1}	0	93	100	100	0	87	100	100
Cypermethrin 12.5 g ha^{-1}	0	63	63	63	0	63	63	67
Cypermethrin 25 g ha^{-1}	0	77	97	100	0	87	100	100
Cypermethrin50 g ha^{-1}	0	63	97	100	0	97	100	100
Control	0	0	0	3	0	0	0	0

Table 3: Mean % Accumulative Mortality of Bees in Leaf Disk Method

Treatment	Mean % of Bees Dead Out of 20							
	Fresh Deposits				Aged Deposits			
	1 h	24 h	48 h	72 h	1 h	24 h	48 h	72 h
Triazophos 210 g ha^{-1}	0	100	100	100	2	82	95	98
Triazophos 420 g ha^{-1}	0	98	100	100	0	100	100	100
Cypermethrin 12.5 g ha^{-1}	0	48	53	55	0	17	27	27
Cypermethrin 25 g ha^{-1}	0	73	80	80	0	58	68	68
Cypermethrin 50 g ha^{-1}	0	58	75	75	0	47	62	72
Control	0	0	0	0	0	0	3	3

DISCUSSION

To investigate the risk to honey bees of agrochemical residues on plant surfaces after application, the toxicity of triazophos and cypermethrin residues was compared following the box and leaf disk methodologies. Results indicate that, for different rates of triazophos and cypermethrin, the leaf disk approach is as sensitive as the box method when recording percentage mortality over set periods of time. Fresh residues were not surprisingly found to be more toxic to bees than residues left to age for 24 hours prior to exposure to bees. Both methods were found to be capable of detecting variations in toxicity of residues over time. Although there was slightly higher variability found in results from the leaf disk method, this approach is favoured as residues are picked up by bees from a leaf surface thus representing a more realistic exposure.

One factor being investigated in the test design was the importance of the length of the exposure time. The results show that if the bees had been exposed to fresh deposits for only 24 h the disk method would have been much more sensitive for triazophos, with an opposite (weaker) trend for cypermethrin. However, after 48 or 72 h both methods gave 100 % mortality for triazophos. Using the box method, aged deposits of cypermethrin are more sensitive at 24 h: with the disk method they are less sensitive. This is lost at 48-72 h for the box but is still significant for the disk method.

Data from other studies indicate that some caution must be used in devising residual toxicity tests if they are to indicate potential risk to bees in commercial use of pesticides. In residual toxicity tests there have sometimes been differences in toxic effect depending on the substrate or delayed expression of toxicity, with deposits more toxic after about a day than when fresh. Similar effects to the latter, sometimes with even longer delays, have also been reported from field studies.

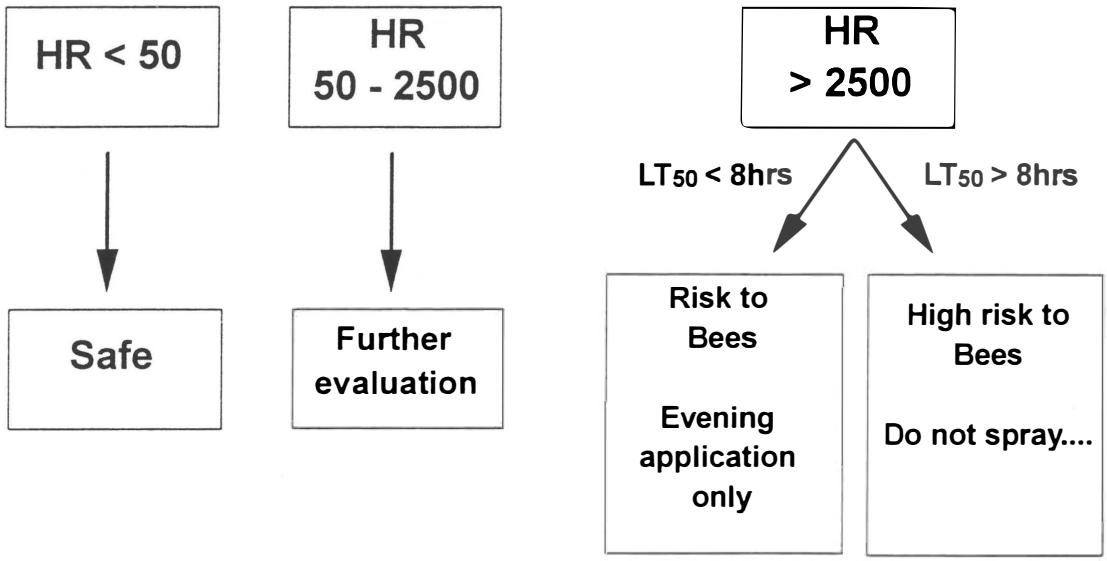
Buchler and Drescher (1989) found endosulfan to be nearly twice as toxic on bean (*Vicia faba*) leaves as on rape, *Phacelia* or sunflower. The authors warned that this should be taken into account in using such tests. Mayland and Bukhard (1970) found that DDT was highly toxic on rhubarb leaves but non-toxic on lucerne. Alternatively, with malathion about 50 % and carbaryl about 100 % more toxic on the former.

Increases in toxicity of aged deposits on cotton leaves in residual tests have been reported by El-Banby and Kansouth (1981) for fenvalerate and cypermethrin; Waller *et al.* (1988) for cypermethrin and tau-fluvalinate; Estesen *et al.* (1992) for cyfluthrin, tau-fluvalinate and acephate. Such an effect has been found with cypermethrin on lucerne by Benedek and Laubal (1989).

The work described has produced a worthwhile improvement in methodology in testing, using a given foliage substrate. However, the use of foliage substrates, especially if it is other than the target crop, and extrapolation of the results of such residual tests requires careful consideration and further development work.

An important aspect of this work is how, if necessary, it fits within the honey bee risk assessment scheme. It may provide valuable information for products that are highly toxic to bees but for a short period of time, allowing them to be applied in the evening when bees will not be exposed (Fig. 1), or be a requirement for products with a high hazard ratio and long duration of persistence on foliage.

Figure 1: Possible use of residual toxicity testing in the honey bee risk assessment scheme.



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Appendix 15

A. de Ruijter, J. van den Eijnde
Tests on honeybee larvae with insect growth-regulating insecticides

Tests on honeybee larvae with Insect Growth-Regulating Insecticides

A. de Ruijter and J. van den Eijnde
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At the fifth International Symposium on the Hazards of Pesticides to Bees, October 26-28 1993, Wageningen, the Netherlands, a working group was formed to prepare a proposal concerning tests on larval honeybees.

Up to the present day no international guidelines have been agreed upon to test insect growth regulators on honeybees. Different institutes have been using different methods to test the effects of these substances on honeybee brood.

Field tests with Insect Growth Regulators have to be designed individually, taking into consideration the specific characteristics of the substance and the circumstances under which it will be used in practise (crop, climate etc.), but it is important to have a relatively simple, standard screening test on larval honeybees to test the effect of Insect Growth Regulators.

Four types of tests have been used so far for this purpose.
First a laboratory test with larvae grown on an artificial diet in the incubator. This type of test was developed by Rembold & Czoppelt (1982) and by Wittmann (1982). Wittmann (1981) also developed a test where bee larvae are fed with a test-mixture, containing the test-sub-stance, sugar and royal-jelly. The comb with the treated larvae returns to its colony.

At the meeting in 1993, Calis proposed a third type of test using small honeybee colonies in which bees transfer the pesticide to the larvae (Calis, 1993).

A fourth type of test was described by Oomen et. al. In this test honeybee colonies are fed with a high dosage of the test substance and the development of the brood is monitored afterwards.

A letter was written to colleagues working in this field to discuss questions about these matters.

Laboratory test (Rembold & Czoppelt 1982, Czoppelt 1993, Wittmann 1982).
No further tests of this kind have been reported since 1993.
The general opinion about the in vitro test is that although this test can reveal important and detailed information on the sort of damage and the development stages that are affected, this type of test seems to be too complicated to make it a standard screening test. The royal jelly used in the diet for the larvae is not available as a standard product. It is a problem that the reproducibility of the test depends on the composition of royal jelly.

Laboratory/feeding test (Wittmann 1981).
One colleague suggests to use a kind of Wittmann's test as a standard test. He felt that the determination of an LD_{50} is the first step we cannot do without. Knowing the lethal doses through direct contact, the risks can be evaluated further by exposing bee colonies to the agrochemical in field or semi-field conditions. The genuine Wittmann's test was not done and no exact description or data were given of the performed test(s).
Chandel & Gupa (1992) compared the toxicity of IGR's to immature stages of Apis cerana and Apis mellifera. The test substance was dissolved in acetone and applied topically. Acetone proved lethal to egg's, I and II instar larvae. The LD_{50} values of Dimilin are different from the values in the Wittmann test (1981). One disadvantage of this test is that the toxicity is tested for older larvae only.
One colleague commented: "The Wittmann test is a good method to determine an LC_{50}, but a lot of skill and experience is needed to perform the test successfully. Extrapolation of the results to practise is impossible". Royal Jelly is used in the Wittmann test too. It is a problem

that the reproducibility of this test also depends on the composition of royal jelly.

Laboratory test (Calis, et al. 1993)

At the time the method needed to be developed further to overcome high control mortality and lack of reproducibility. No further work has been done to improve the method. In reaction the author believed the method is too complicated to make it a standard screening test.

Test with colony feeding and monitoring the development of the brood (Oomen, de Ruijter, van der Steen 1992).

This screening method was used several times in recent years.

The advantages of this method mentioned by working group members are:

- The method is easy to handle and could be done with ordinary lab facilities.
- All bee colonies can be fed at one moment and the larvae are nursed by the bees and not artificially in the laboratory, so the pesticide reaches the larvae via the bee.
- Minimal risk of artifacts due to beekeeping.
- The method allows for a fairly realistic situation with free flying bees that have access to natural honey and nectar stores.
- Minimal control mortality and reproducible results.
- Fairly quick and easy to perform.

Most people agreed that this test can be used as a standard screening on larval honeybees, provided that some adjustments are made to improve the method.

FURTHER COMMENT AND SUGGESTIONS TO IMPROVE THE COLONY FEEDING TEST

Extreme exposure, positive control

The test gives a very extreme exposure of honeybee brood. With this amount any IGR gener-ally result in bee brood toxicity, even if the compound has no negative effects under field conditions. The suggestion was made to drop the toxic control and include an additional lower concentra-tion (about 10% of field concentration).

Discussion

The screening test is meant as an extreme exposure. This makes sure that no possible negative effects are overlooked. The most important argument in favour of a toxic control is the cer-tainty of a good performed test. The possibility of additional treatments with lower concentra-tions are given in the method.

Mortality, robbing

Robbing is mentioned as a potential problem and on one occasion a significant control mortal-ity was noted. A high loss of eggs in the treated and control group was mentioned in a test performed in October. Bee colonies show variability, despite basic standardisation and using sister queens or queens of the same age.

Discussion

The feeding test with bee colonies should be done when the bees are actively nursing a broodnest. In the autumn many eggs disappear just before or just after hatching. Robbing is also related to the time of year, number of colonies in the apiary and time of the day when the feeding takes place.

The only way to decrease the effect of colony variability in the field is to increase the number of colonies, but that becomes a physical impossibility. The test is meant as a screening test to see whether there is an effect or not. Variability of colonies is not a major problem.

Mode of assessment

The guideline suggests that an assessment of the developing brood is made on a weekly basis. In practise it is important to assess the brood after 1 week but the second assessment will be immediately prior to the emergence of the bee.
The question was put: is it useful to take the pupae out of the hive a few days before they will emerge and place the comb in an incubator and check the young bees on malformations?
In one occasion the final assessment is 1 day prior to expected emergence to be certain that no test bees emerge before examination.
To assess 'old larvae' as mentioned in the method is a little ambiguous. If they are taken at the 4-5 day larval stage there is a chance that the 5 day larvae almost immediately become sealed over. These larvae therefore receive a minimal amount of test substance or no test substance at all. In this case larvae of the 3-4 day stage were used which ensures they have received some test substance.

Discussion

Brood development in the method is checked weekly until 3 weeks after application. It is difficult to do the second check prior to the emergence because it differs according to the initial age of the brood. In practise it will be very difficult to remove the combs because three different stages of brood are marked with three different times of emerging, stretching over a period of nine days.
Only samples of brood could be checked before emerging. It is an important issue that emerg-ing takes place inside the bee colonies. Checking on malformations is not enough. For example when fenoxycarb was tested young bees without any visible malformation died quickly after the bees had emerged. In the method of Oomen it is also mentioned that the colonies must be provided with a dead bee trap in front of the hive. Dead bees or pupae from this trap must be checked for abnormalities and these bees can be considered as a sample from the emerged young bees.
In the method larvae of all different ages are used, one day old larvae as well as three, four and five days old. A void to use larvae that will be capped very soon after. The reason is to study the possible effect on all different stages. If only one larval stage is studied, possible effects are maybe overlooked.

Varroa

Problems with Varroa could occur. Colonies could be treated previously but re-infestation could cause significant problems even in the short time scale of the study.

Discussion

Two proposals concerning V arroa and varoacides were made on the Fifth meeting. These proposals should also count for the feeding test.

We propose to accept the test by Oomen, de Ruijter and van der Steen as the standard screen-ing test for honeybee brood toxicity with the following improvements:

- The test must be performed in a time of the year that the brood is well nourished by the bees.
- It is necessary to wait for 4 weeks after the last treatment with varroacides, before using the colony in a test. Varroa is monitored after the trial and recorded in the study report, if appropriate.
- Avoid to mark cells with larvae shortly before capping.

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- D. Wittm ann Bonn, Germany

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Appendix 16

W. Mühlen
Implications of the IGR Alsystin on the development of honeybee colonies under field and semi-field conditions

Implications of the IGR Alsystin on the development of Honeybee Colonies under Field and Semi-Field Conditions

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1 Introduction

The purpose of this study was to make a contribution to the development of brood tests. To determine the side effects of insect growth regulators (IGR) on the development of honeybee brood.

Semifield and field-tests, as they are conducted in the testing facilities for assessing side effects of plant protection products on honeybees, are not appropriate to describe the implications these products do have on the development of honeybees' brood stages. The German Guideline BBA part VI 23/1 (STUTE ET AL. 1991) for fields- and semifield tests requires to check the condition of the brood three times:

1. shortly before application,
2. one week and
3. three weeks after application.

A check includes the estimation of brood areas differentiated in eggs, larvae and cupped brood (pupae) as well as the estimation of food supply, honey and pollen combs. We found these testing procedures not exactly enough to see side effects of plant protection products on the brood. The loss of eggs, larvae or pupae will be compensated by an increased breeding activity of the queen.

The decision-making scheme for the environmental risk assessment of plant protection products published by the EPPO (OEPP/EPPO 1992) requires a special brood test for those products which affect the honeybee brood.

At the last meeting in Wageningen several test methods were discussed: WITTMANN and ENGELS (1980), CZOPPELT (1991), OMEN ET AL. (1992) and CALIS ET AL. (1992). None of these methods allows to evaluate the hazardness of plant protection products on the development of brood stages under *natural conditions*. We still need a practical method taking in consideration the real exposition of bee colonies after application in the field. The 'German Working Group for Bee Protection' initiated a ringtest with the IGR Alsystin WP 25.

In 1995 we tested under semi field and field conditions the implications of Alsystin (active ingredient Triflumuron, producer Bayer, Leverkusen).

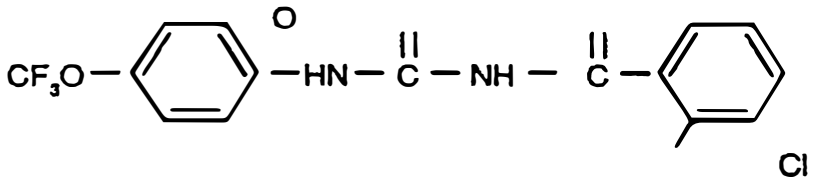
2 Method

2.1 Test Substance

We used the formulation of Trifumuron, an inhibitor of the chitin synthesis: Alsystin WP 25 is in Germany registered as harmful to honeybees (B 1) because of its IGR effect.

We used a high amount of Alsystin to produce a noticeable effect. The tests were not conducted to assess the hazardness of Alsystin. In accordance with Dr. Schmidt, Bayer Leverkusen, we applied 800 g Alsystin/ha (s. Table 1).

The field tests were conducted with two test variants in two repetitions: for testing the side effects of Alsystin a testfield were prepared. The control was set near the institute (no control field).



1-(2-chlorobenzoyl)-3-[4-trifluoromethoxyphenyl] urea (IUP AC)

Fig. 1: Alsystin: Active Ingredient: Triflumuron

Table 1: Characterization of Test Substance

Test Substance	
trade name:	Alsystin 25 WP
producer:	Bayer, Leverkusen, Germany
max. application rate:	orchard: 0,08 % (application twice) forest: 48 g/ha in 300 l water/ha
tested application rate:	800 g/ha in 300 l water/ha
Facts about Biological Effects	
purpose	insecticide in orchards and forest
mode of exposure:	contact and oral toxicity, insect growth regulator (IGR), inhibition of chitin synthesis
Physical-chemical Characteristics	
formulation:	WP: waterdispersible powder
aggregat conditions:	solid (crystal)
Identity of the Active Ingredient	
chemical group:	benzoyl-phenyl-urea
chemical-name IUP AC amount of ingredient:	1-(2-chlorobenzoyl)-3-[4-trifluoromethoxyphenyl] urea nominal: 250 g/kg

2.2 Performance of the Test

2.2.1 Field Test

In the field as test organism four queenright colonies of the honeybee were provided by the apiculture of the IPSAB. We used a breeding stream which was typical for the region (*A. m. carnica)*. The colonies covered 22 combs *(DNM: Deutsch-Normal-Maß ca. 35.4 cm x 20.7 cm; 7.3 dm²).*

The size of the colonies were about 30 000 foragers.

To prepare the bee colonies in the field the hives were set up next to the test field some days before the application. The foragers had time to get used to collect pollen and nectar from the flowering test field.

Dead-bee traps had been attached to the entrances of the bee colonies in order to register the number of foragers dying in the hives.

The dead-bee traps consisted of screens which cover the whole hive entrances forcing the bees to fly through the opening of the screen when leaving or entering the hives. The bottom in front of the traps was covered with a linen sheet (1.5 by 3.00 m). Here dead bees were collected, too.

Because of its flowering advantages for bees and its long flowering period *Phacelia tanacetifolia* Benth. was chosen as crop.

For every repetition a field of about 3 000 m² had been prepared in the following way:

Pathways, 2 m wide, had been made by removing the plants and levelling the ground. Subsequently, the pathways had been covered with a linen sheet, 1.5 m wide. The pathways, along which the tractor with the sprayer was moved, divided the test fields into several plots of 400 -800 m².

The size of the flower patch was 2 500 m² after deducting the area for the pathways covered by linen sheets.

2.2.2 Tent Test

The tent test were carried out in tunnels screened with plastic gauze, size 48 m²: width 4.0 m, length 12 m and height 1.8 m.

Phacelia tanacetifolia Benth. was chosen as test plant.

For every treatment three healthy, queenright nuclei in Kirchhainer boxes of about 1 000 bees were used. The nuclei were placed at the edges of the tunnels. In front of the bee hives dead-bee traps (GARY-Traps) were used to collect the dead bees at the entrance of the beehives.

At the uncropped border area of the gauze screened walls of the tent the vegetation was removed and the soil was covered by a 30 cm wide linen sheet, permeable for water. Here too, dead bees were collected.

One week after application the Kirchhainer nuclei were removed out of the tents to improve the providing of the nuclei with pollen and nectar.

2.3 Application Method

2.3.1 Field Test

For application in the field a standard tractor mounted sprayer was used. During application the bee hives were protected by a plastic cover to avoid contamination by spray liquid.

2.3.2 Tent Test

For application in the tunnels a portable hand sprayer operating with compressed air was used, constructed by the IPSAB.

2.4 Meteorological Data

The following meteorological data were recorded daily during the intire test period:
⇒ **Temperature** by a mini.-max. thermometer.
⇒ **Rainfall** by a pluviometer which was placed next to the test hives.
⇒ **The degree of cloud cover** was estimated in percent.

2.5 Mode of Assessment

2.5.1 Mortality

Mortality was assessed (1) on linen coverings of pathways (tent- and field tests) and (2) at dead-bee traps before the entrances of the colonies. Before application the dead bees were counted and removed once a day. At the day of application the dead bees were counted shortly before and one, two, four and six hours after application, the following 21 days once a day.

2.5.2 Flight Intensity

At each evaluation time the number of bees foraging, resting or simply flying on ca. 1 m² flowering Phacelia were counted at five different flowering plots characteristic for the test field.

The average of these data ("flight intensity") were calculated and were used to make sure, that enough bees had been foraging in the testplots during application. Further on, these data were used to describe the foraging behaviour of the bees after application.

In the tent test only three data were recorded.

2.5.3 State of Brood According BBA 23/1

Firstly, according BBA Guideline part VI 23/1 (STUTE ET AL. 1991), the condition of the colonies and the development of the bee brood were checked four times some days before and weekly after application. The following parameters were assessed:
⇒ Estimation of amount of pollen and nectar (honey) combs (dm²/ comb-side).
⇒ Estimation of amount of brood cells (eggs, larvae and capped brood (pupae)) (dm²/comb-side).

2.5.4 Brood Development According Omen et al.

Table 2: Evaluation of Mortality, Flight Intensity and Behaviour

	Evaluation scheme	
Time of the test	**Test field / test tent**	**Control apiary / tent**
Prior to application at least twice: (T_{-6}; T_{-1})	Once a day at the same time of day during bee flight activity	
Day of application (T_0)	1. Shortly before application 2. In the first hour after application continuously 3. ± 2 h after application 4. ± 4 h after application 5. ± 6 h after application	
2nd to 21 st day after application (T_2, T_3 ... T_{21})	once a day at the same time of day during bee flight activity	

The development of individual brood cells was further checked according the brood-feeding test of OMEN ET AL. (1992). One day before the application 100 eggs, 100 young larvae (1-3 days old) and 100 old larvae (four to six days old) were marked in each colony with the help of a transparent overhead sheet.

The development of these individual marked cells was checked weekly for a period of 22 days (the normal brood development period).

3 Results

3.1 Tent test

3.1.1 Mortality of Imagines

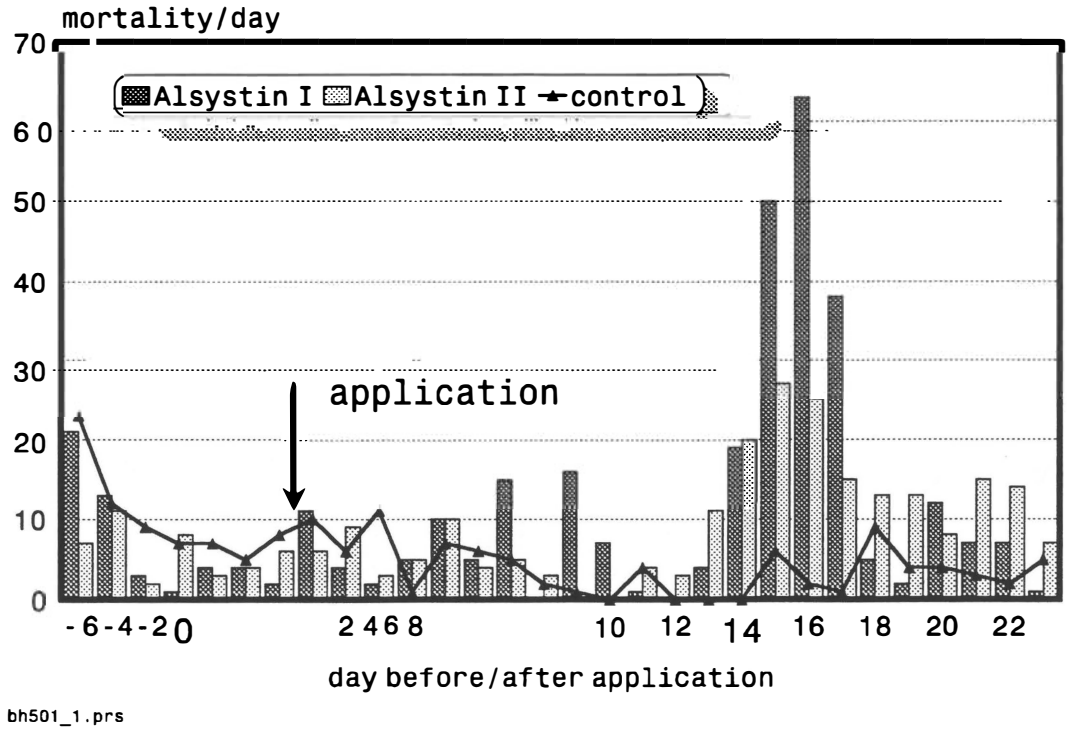


Fig. 2: Tent Test Alsystin: Mortality Border Area and Hive Entrance

Up to the 12th day after application of Alsystin less than 6.5 dead bees were found per day in the tunnels. In the control group only 5.3 dead bees/day were counted. 13 days after application the Mortality increased for four days up to 60 bees/day.

3.1.2 Condition of Dead Bees

The dead bees were very young, just emerged and looked bloated. Their chitin was very light coloured and thin. These damages are typical for Alsystin.

3.1.3 Brood Development after OMEN ET AL.

The development of Alsystin treated colonies is strongly reduced, but the control colonies also showed strong damages. In comparison with the water treated control group the reduction of normal development is less than 10 %. In the control group a lot of marked cells didn't develop normally. Eggs and young larvae showed strongest damages. Pupae developed normally. Interestingly Alsystin treated colonies have 50 % more empty cells as control colonies. The data spread over a wide range, so a statistical significance is not given.

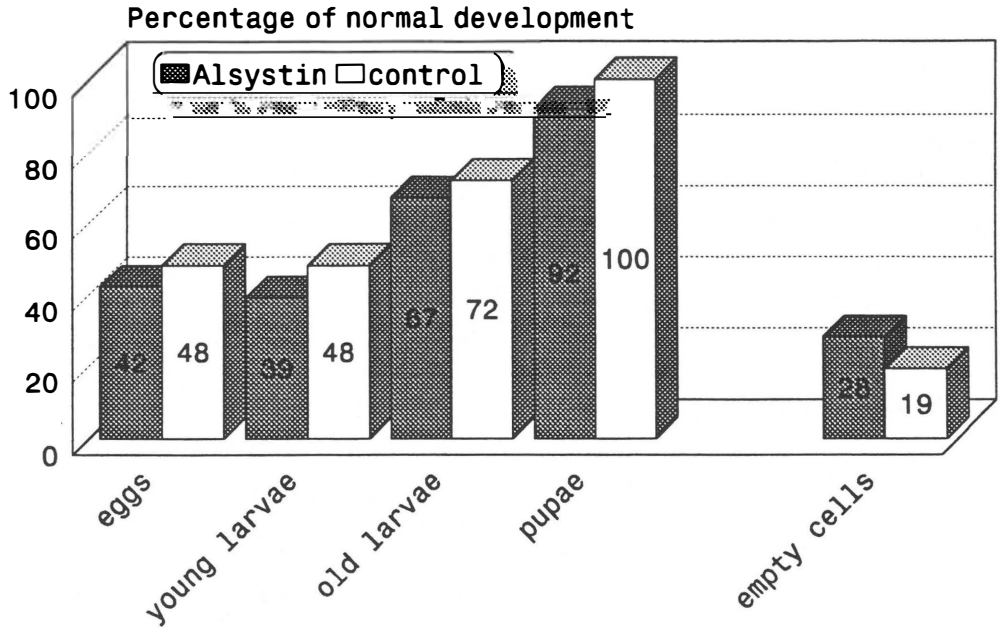


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Fig. 3: Tent Test Alsystin: Summary of Brood Development

3.2 Field Test

3.2.1 Mortality of Imagines

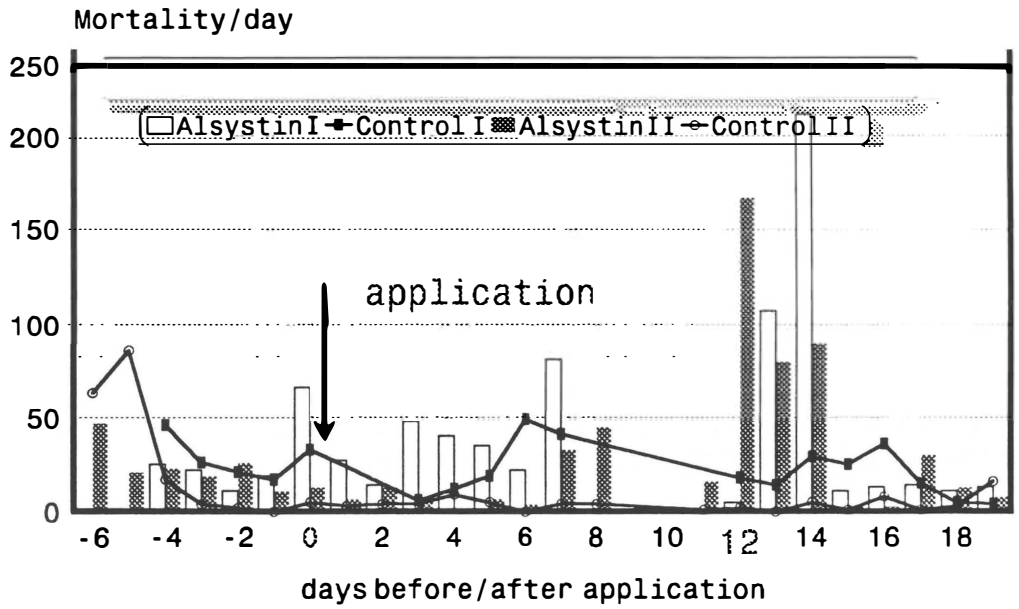

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Fig. 4: Field Test Alsystin: Mortality Field and Hive Entrance.
(Control only hive entrance)

After application of Alsystin the mortality of bees at hive entrance and in the field didn't increase. The bees collected pollen and nectar without any change in their foraging behaviour. At the 12th day after application of Alsystin the mortality increased up to 211 bees (I repetition day 14) and 167 dead bees (II repetition day 12). The increased mortality lasted 2 up to 3 days. At the control apiary on average 20.4 (I. rep) and 22.9 (II. rep) dead bees were found.

3.2.2 Condition of Dead Bees

The dead bees showed the same damages a in the tent tests. They were very young, just emerged, their chitin looked bloated and thin.

3.2.3 Brood Development after OMEN ET AL.

In the field test the effect of Alsystin is much stronger than in the tent tests. But here too 27 up to 40 % of the marked cells didn't develop normally. In comparison with the untreated control group the normal development is reduced in the range of 10 up to 14 %. Eggs and young larvae showed the strongest effect. Pupae developed normally. Of some interest is, that Alsystin treated colonies had 42 % empty cells control only 29 %. Here too, a statistical analysis was not possible.

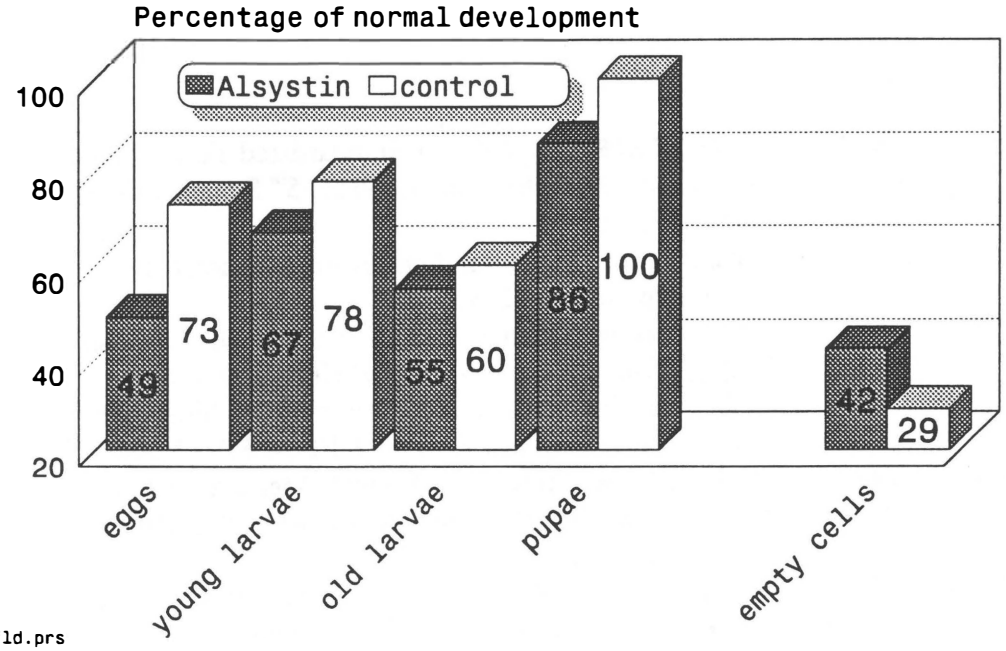

Fig. 5: Field Test Alsystin: Summary of Brood Development

3.2.4 State of Brood According BBA 23/1

Recording the state of brood after BBA Guideline (STUTE ET AL. 1991) no statistical signifcant differences between treated and untreated colonies could be documented neither in the tent nor in the field tests. The Alsystin colonies seemed to compensate the loss of brood cells by increasing breeding activity.

4 Discussion

These tests documented, Alsystin in the given dose do affect honeybee colonies. It was possible, to show the effect under semi and natural conditions by recording daily mortality rate. It was impossible to document the effects following BBA Guideline Part VI 23/1 (STUTE ET AL. 1991) or by Oomens' suggestion to follow the development of individual cells (OMEN ET AL. 1992).

The time interval of one week is too long, to find out any maldevelopment or loss of stages of brood. On the other hand the weekly disturbance of the colonies during a check (e.g. taking of combs, the cooling down of the brood stages during examination) are reason for the considerable

loss of brood in the control as well as in the Alsystin treated colonies. Therefore a shorter period of colony check is desirable but not possible.

The given test procedure supposed by OOMEN ET AL. (1992) is too sensitive and can not be used to evaluate the hazardness of a plant protection products on the brood development of honeybee colonies in the field and semi-field.

But we do believe, that following the development of individual cells after application of IGR in tunnel- or field-tests is the right way to evaluate the hazardness of these products.

Further on we do believe, that greater tents as we used or as they were common in France and the use of small colonies will contribute to successful test procedures. The flight and foraging behaviour of bees in these tunnels is very good, the tunnels are not overcrowded. Mortality at the uncropped border areas is low.

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Appendix 17

W. Von der Ohe, K. Schütze, F-W. Lienau
Sensitivity of *Paenibacillus larvae larvae* to Plantomycin

Sensitivity of *Paenibacillus larvae larvae* to Plantomycin

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Introduction

Plantomycin is used against fire blight in orchards. Depending on an tremendous increase of fire blight especially in south Germany it was recommended to spray antibiotics against *Erwinia amylovora*. Due to this alarming situation the BBA has allowed to use the not licensed antibiotic Plantomycin in those affected areas (Lange 1996). Reservations by apidologists against the use of Plantomycin are the possibility of residues in honey as well as unintended treatments with antibiotics. The use of antibiotics by beekeepers in several countrys of the world against brood diseases give evidence that antibiotics are non-toxic for adult bees (Matheson, Reid 1992, Ratnieks 1992). But it is well-known that pathogenes which are controlled by antibiotics often get resistant against these drugs (Bruns 1975). In Germany the use of antibiotics for controlling brood diseases is neither common nor allowed. In case of using antibiotics in orchards honey bees maybe gather nectar with antibiotics. Residues in honey can result in a camouflage of American Foulbrood by getting a low level infection without clear clinical symptomes, but with constant increase of spores of the pathogen *Paenibacillus larvae larvae*. Furthermore antibiotics can inhibit growing of antagonists of other bee pathogens.
Assignment for this study was to determine for different strains of *Paenibacillus larvae larvae* the minimum inhibition concentration of the active ingredient streptomycin-sulfate respectively the formulation Plantomycin. For this question a suitable method had to be developed.

Material and Methods

Agar-diffusion-tests (DIN 58940; DIN = German Institute of Standardization) suitable for testing sensitivity of bacteria against chemotherapeutics are the basis of the methods. The DIN-methods were adapted to specific cultivation terms of *Paenibacillus larvae larvae*. Culture medium is 20 ml of Columbia-agar (OXOID) per petri dish (94 mm diameter). It is a two layer medium. The first 15 ml serve as nutrient reservoir. After cooling down the second layer of 5 ml agar is piled up the first one. Shortly before this second layer is inoculated with a define spore-suspension of P. l. larvae. The spore-suspension is defined by optical density of 0.200-0.230 extinction (measured by 546nm). The optical density of spore-suspensions was identically per *P. l. larvae* strain and test of several antibiotica

concentrations. This procedure ensures a regular distribution of spores and growing of the bacteria.

Circular special test carriers (diameter of 6 mm, Schleicher & Schuell) were soaked with antibiotic solutions (20 µl of one concentration per carrier). 6 carriers with 3 different antibiotic concentrations and 1 control carrier only soaked with water were put on one *P. l. larvae*-petri-dish.

The bacteria were aerobic incubated by 37°C. The antibiotics diffuse from carriers into culture medium. There the active substance is able to inhibit growing of bacterias. The inhibition zone diameter depends on dose of antibiotic and sensitivity of bacterias. It is a degree for effectiveness of the antibiotic. The inhibition zone diameter was measured after 24 hours.

Dose effects were tested with five different *P. l. larvae* strains. The screening procedure covered 9 doses (0.04 µg to 200 µg/carrier) of streptomycin sulfate, the active ingredient of Plantomycin, and 13 doses (0.04 µg to 200 µg/carrier) Plantomycin. Each dose was repeated 60 times. The screening includes a stretching in sensitivity zone of Plantomycin (2 µg to 40 µg/carrier) with 30 repeats. The inhibition zone diameter was measured. Because the carriers have 6 mm in diameter, 6 mm means that there is no inhibition. Instead of inhibition zone diameter the radius minus 3mm and for further statistical calculations logarithm of dose were used.

Results

The new developed method was exceedingly satisfactory for this kind of question.

The results are presented in figure 1 to 2. Related to antibiotics and dose inhibition all *P. l. larvae* strains reacted very similar. Differences between doses are high significant ($p < 0.001$).

Streptomycin sulfate: The growing of *P. l. larvae* was inhibited down to 2 µg/carrier equivalent to 0.01 %. Streptomycin sulfate concentration in Plantomycin application is 0.01065%.

Plantomycin: The growing of *P. l. larvae* was inhibited down to 4 µg/carrier equivalent to 0.02%. Plantomycin concentration in application is 0.06%. One strain was inhibited down to 2,5 µg/carrier.

Discussion and Conclusion

Tests and data show obviously that *Paenibacillus larvae larvae* is sensitive against the plant protection antibiotic Plantomycin. The pathogen of the American Foulbrood was inhibited in these tests down to the concentration of 0.02%. This is lower than maximum

application concentration.

Depending on an intake of contaminated nectar or pollen there is a possibility of a camouflage of American Foulbrood. This unintended treatment with antibiotics in bee colonies can lead to low level infections without noticing clinical symptomes by beekeepers as well as cross resistance to antibiotics (Moosbeckhofer 1991, Ritter 1990). Lingering not diagnosed diseases can result in an increase of pathogens in those colonies. Well-known unpleasent consequences are spreading pathogens by robbing bees resulting in a contamination of other colonies in those areas (von der Ohe et al. in press).

Acknowledgement
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fig. 1

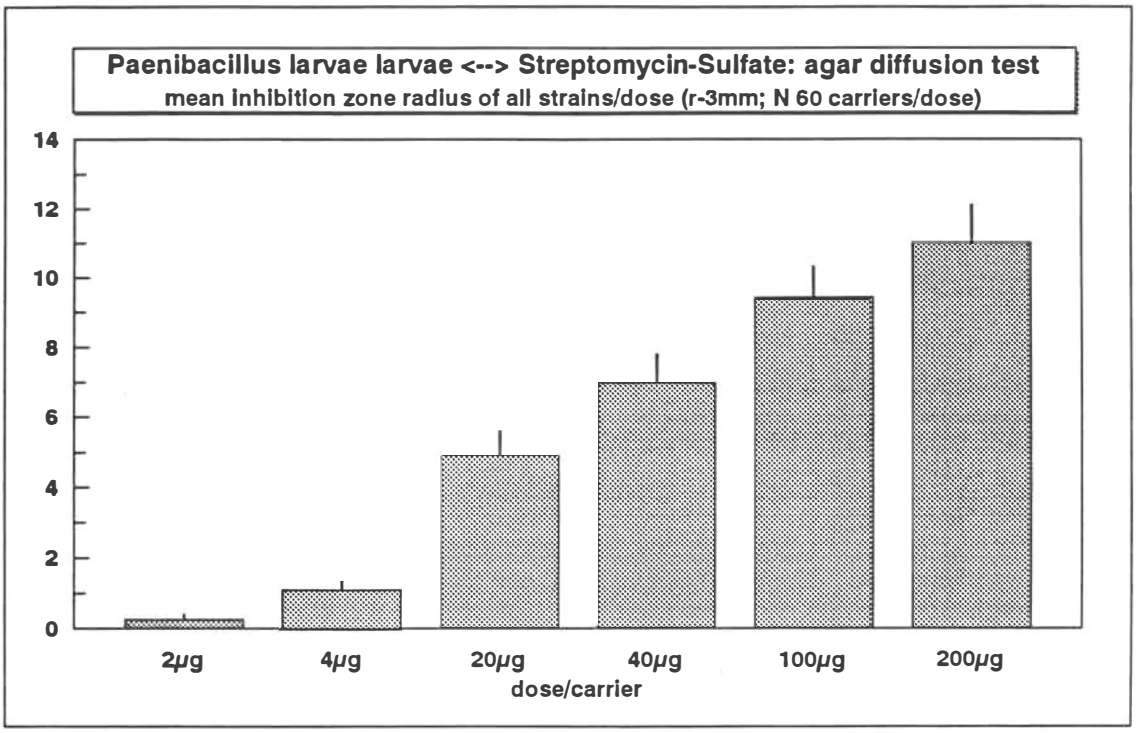
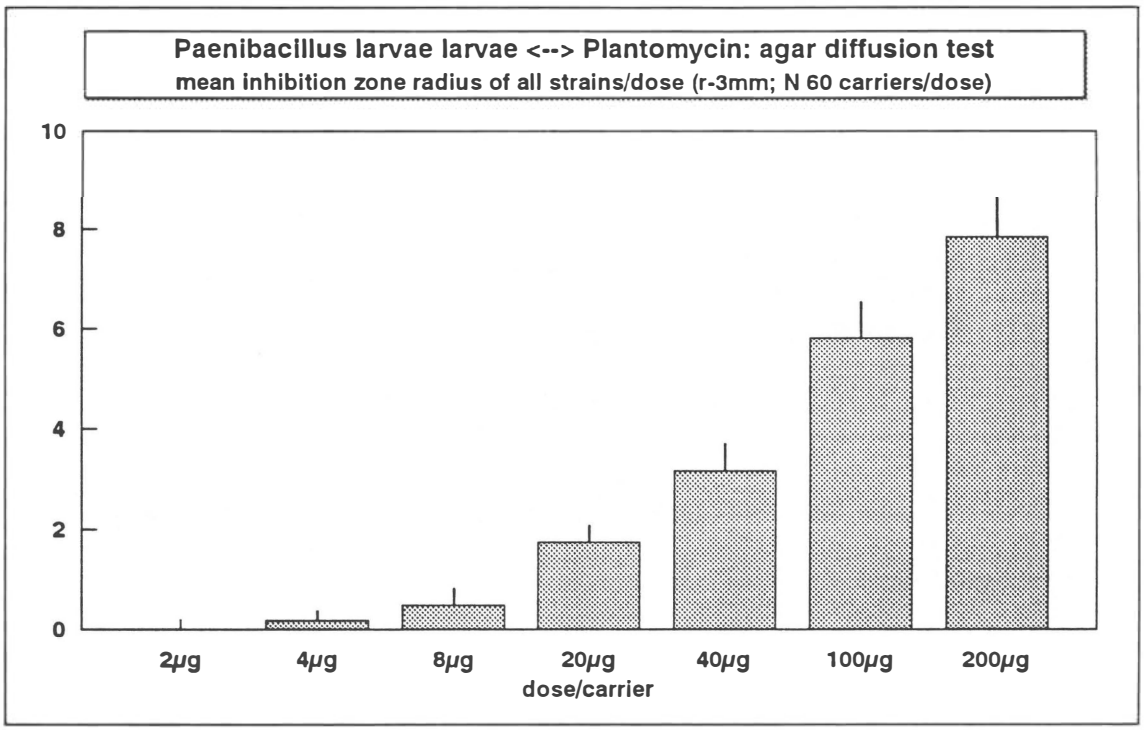

Paenibacillus larvae larvae <--> Streptomycin-Sulfate: agar diffusion test
mean inhibition zone radius of all strains/dose (r-3mm; N 60 carriers/dose)
14
12
10
8
6
4
2
0
2µg
4µg
20µg
40µg
100µg
200µg
dose/carrier

fig. 2


Paenibacillus larvae larvae <--> Plantomycin: agar diffusion test
mean inhibition zone radius of all strains/dose (r-3mm; N 60 carriers/dose)
10
8
6
4
2
0
2µg
4µg
8µg
20µg
40µg
100µg
200µg
dose/carrier

Appendix 18

K. Wallner, P. Rosenkranz, T. Held
Treatment of fireblight by use of Streptomycin: A problem for the honey quality?

Treatment of fireblight by use of Streptomycin: A problem for the honey quality?

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Abstract
During the last years, Streptomycin-sulfate (Plantomycin®) has been used increasingly to treat the fireblight (*Erwinia amylovora)* in orchards of Southern Germany. The recommended use of Plantomycin® during blossom may cause residue problems in fruit honey. We describe the actual problems for the beekeeper in fire blight regions which are treated by Plantomycin®. The methods for trace analysis of Streptomycin are discussed and first results of residues in honeys collected in treated orchards are presented.
In additional field experiments we collected foraging honey bees in distinct time intervals after a treatment and analyzed the honey sacs as well as the nectar from treated apple fruits. Our first results demonstrate a surprisingly high variation of the residue load between single foragers. The possibilities of this method for a general risc assessment concerning maximal residue levels are discussed.

1. Introduction
The fireblight, caused by the bacteria has become a serious problem for the cultivation of malaceous fruits like apple and pear in southern parts of Germany during the last years. Depending on climatic conditions the trees are treated several times with Streptomycin-sulfate (Plantomycin®) during blossom. Therefore, a contact of foraging honey bees with Plantomycin can hardly be avoided. As the toxicity of Streptomycin to honey bees is remarkable low, a poisoning of honey bees in orchards is not to be expected. On the other hand, a contamination of honey bees and/or nectar may lead to residues in the honey bee products. As Streptomycin is water-soluble, detectable residues in the honey are more likely compared to lipophile pesticides which accumulate in the wax (WALLNER 1996). Residues of antibiotics in honey will cause, independently from the direct toxicity to human health, enormous problems for the German beekeeping. The consumer in Germany, sensitized by the recent discussion on allergic effects and resistance of human pathogens, will not even accept traces of streptomycin in honey. By the actual use of Plantomycin, we are faced to three problems:

1. An analytical method for the trace detection of Streptomycin in honey was not available. Since last year we can analyze honey with a sufficiant detection limit. Therefore, the data basis for a general risk assessment is relatively small.
2. As Plantomycin is not registered in Germany the use has to be laid down yearly by special authorization. This means that a maximum residue level (MRL) for honey does not exist.
3. The timing of the treatments depend on weather condition. Therefore, the treatments are recommended only 24 - 48 hours in advance. Under this circumstances it is not practicable to remove honey bee colonies temporary.

2. Streptomycin residues in honey from apple orchards

Analytical methods: During the first Plantomycin treatments in 1994, no method for the detection of traces of Streptomycin in honey has been available. Therefore, the methods developed for the HPLC analysis of animal tissue (Gerhard et al. 1994) had to be adapted for honey. As this method depend on a derivatization of the aminogroup and detection by fluorescence, honey represents a problematic matrix for the analysis. Honey contains several aminogroups which also react with the fluorescent agent and cause problems with "background noise". Therefore, there are special requirements on sample extraction and „ clean up " steps. The successive steps of the method contain: Extraction of the honey (solved in perchloric acid) with solid phase extraction (SPE on C_{18} column), injection into the HPLC on an enrichment column, postcolumn derivatization with naphtochinone and detection with a fluorescence detector. A detailed description of this method with an additional clean up by cation exchange column is given by Kocher (1996). She reached a remarkable low detection limit of about 10 µg/kg (ppb), our samples were analyzed with a detection limit of 50 ppb.
An additional method using an enzyme immunoassay has been developed by Usleber et al. (1995). The detection limit were between 12 and 32 ppb (depending on sample preparation) and the specificity for Streptomycin were supposed to be sufficiant.
We analyzed 29 honey samples with HPLC method which were extracted by beekeepers in commercial orchards of the Bodensee region. The honeys were extracted in 1994, shortly after the end of the blossom of the apple trees. 3-4 treatments with Plantomycin had been carried out during blossom. Additionally, we analyzed some samples of freshly introduced fruit nectar.

Results & Discussion: With a detection limit of 50 ppb, none of the honey samples contained residues of Streptomycin. In freshly introduced nectar we found values of about 200 µg Streptomycin per kg honey (200 ppb). This indicates that the fruit nectar from treated orchards contains residue of Streptomycin. With our detection limit, this residues are not detectable in ripe honey, probably due to "dilution effects" by nectar of other plants (for example dandelion or rape) during the honey processing.
This is confirmed by the analysis of 38 commercial honey samples from Germany in 1995 by the CLUA, Sigmaringen (Jahresbericht 1995): two honeys contained residues of about 40 ppb. Usleber et al. (1995) found Streptomycin concentrations between 30 and 100 ppb in 50% of the commercial honey samples but no residues in honey from South Germany. Unfortunately, it was not possible to determine the origin of the commercial honeys exactly. In most cases, this honeys represent a mixture of honeys from different countries. Therefore, the source of the residues remain unknown. Nevertheless, recent analysis verify that also in original honey from South German traces of Streptomycin can be detected (Kocher, pers. comm.).

We can summarize that treatments with Plantomycin in orchards comprise a big risk that contaminated nectar will be collected by honey bees. The occurrence of residues in ripe honey may depend on the frequency of the treatments, foraging activity of the bees, "dilution" by nectar from other (not contaminated) sources and decomposition of Streptomycin in the honey. The latter effect is, obviously, lower than expected and not sufficiant to prevent the occurrence of positive honey samples on the market. A contamination of honey with antibiotics will cause enormous problems for the beekeeping in Germany. The German consumer is very sensitive concerning residues in food and honey as one of the last "natural products" has a special value. Residues of Streptomycin, independently from their toxicity to humans, will not be accepted and could destroy the economical basis of beekeeping in certain regions of Germany. The recent discussion on allergic effects and problems of resistance of human pathogens toward antibiotics (World health Report, Geneva 1996) has polarized the discussion. As Plantomycin is not registered, official regulations for the dealing with contaminated honey are lacking. Therefore, the central requirement remains that no detectable residues of Streptomycin in honey should occur. The actual situation may also have consequences for the beekeeping in orchards: A lot of beekeeper will leave this regions to avoid residues of Streptomycin in honey. An "exodus" of the beekeeper will cause problems for the pollination and will also be a bad publicity for the affected farmers. A strategy to prevent such a future development is urgently needed!

3. Intake of pesticides into the honey bee colony by foraging bees: An approach for a general risk assessment

The example of Streptomycin demonstrates the necessity of a risc assessment for residues in honey bee products before a pesticide is used extensively, even when the analysis of trace residues is difficult. The amount of residue coming into the beehive may depend mainly on the treatment(s) itself (amount of active ingredients) and on the foraging behaviour of the bees. Therefore, we tried to measure the intake of the residues by the individual bees.

Methods: 11 apple trees covered by a tent (60 m^2) and an apple orchard of 10 ha were treated with Plantomycin (Streptomycin) and Ronilan (Vinclozoline) together. Vinclozoline can be detected easily by GC methods with a detection limit of 0,5 ppb (Streptomycin 1 0-50 ppb, depending on the method, see above) and was therefore used as tracer. The same concentration of active ingredient (127 g/ha) were used for both pesticides. Treatments were performed as recommended. Flying honey bee colonies were present during the treatment. The treatment was performed in the morning before the flight activity of the honey bees had been started. Before, during and after the treatment the hive entrance were closed every hour for 5 minutes and the returning foraging bees were collected and deep frozen (-20°C) by CO_2 spray. Approximately 2.000 individual bees were collected. The honey sac of the bees were prepared, weighted, extracted by SPE and analyzed by capillary gaschromatographic methods. Because of the low amount of active ingredient per bee, only

data on Vinclozoline are presented. For the detection of Streptomycin we will have to pool several samples.

Results & Discussion: Not all of the collected foragers could be analyzed: In many cases the honey sacs were more or less empty, especially in the tent experiment. The preparation of empty honey sacs is difficult, so we only analyzed honey sacs with more than 10 mg. The few honey bees collected in the tent contained 0,2 - 30 ng Vinclozoline. This demonstrates again the problem of sample collection in tent experiments.

From the free flying honey bees obtained in the orchard we analyzed 150 individual bees which were collected over a time period of 30 hours (Fig 1). 27 of these bees contained residues of Vinclozoline between 0,2 and 2,5 ng. A single bee collected directly from a flower contained more than 100 ng! Surprisingly, the amount of residue per bee was not correlated with the weight of the honey sacs (Fig 2). Although the weight of the positive honey sacs varied from 10 to 50 mg, most of the residue level were equally low with values between 0,05 and 0,2 ng per bee (Fig 2).

From this preliminary results we can summarize that only a part of the foraging bees contained detectable residues during the first day after the treatment (about 20% in our experiment). Most positive bees had equally low residue levels, only a low number had a 5 to 1 0 fold higher contamination (Fig 1). The proportion of positive foraging bees remained stable during the day after the treatment (Fig 1: 30 hours). This was not expected and indicates a systemic and/or long lasting effect of the spraying.

To explain these results we need more data and exact information about the "history" of a single forager. This could be done by analyzing the pollen load of the individual bees to determine the plants which has been visited. We plan to repeat our experiment with other pesticides and under different environmental condition. The method seems promising for a better understanding of the conditions which are responsible for the formation of residues of honey bee products.

4. Conclusion

- The Plantomycin treatment in orchards causes a measurable contamination of the nectar with Streptomycin.
- The detection of Streptomycin residues in ripe honey may depend on the frequency of the treatment, foraging activity of the bees and dilution effects by non treated nectar.
- Because of the honey market and the emotional public discussion, no detectable residues of Streptomycin in honey can be accepted at the moment.
- The problem "Streptomycin" demonstrates that a general risk assessment for the maximal intake level of pesticides is urgently needed.
- The analyzing of individual foraging bees during and after the treatments seems to be a promising method for such a risc assessment. From our preliminary data, an explicit evaluation of our method is still not possible. For that purpose, we need more data with other pesticides and different ambient conditions.

5. Literature

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Legends

Fig 1: Content of Vinclozoline in the honey sacs of foraging honey bees which were collected before (control) and up to 30 hours after a treatment with Ronilan/Plantomycin in an apple orchard. Only a part of the 11-22 honey bees analyzed each time contained residues with values from 0,2 to 2,5 ng. Positive samples were obtained up to 30 hours after the treatment.

Fig 2: The weight of the honey sacs of the contaminated foraging honey bees and the amount of Vinclozoline per bee. There is no correlation between nectar load and amount of residue.

Fig. 1

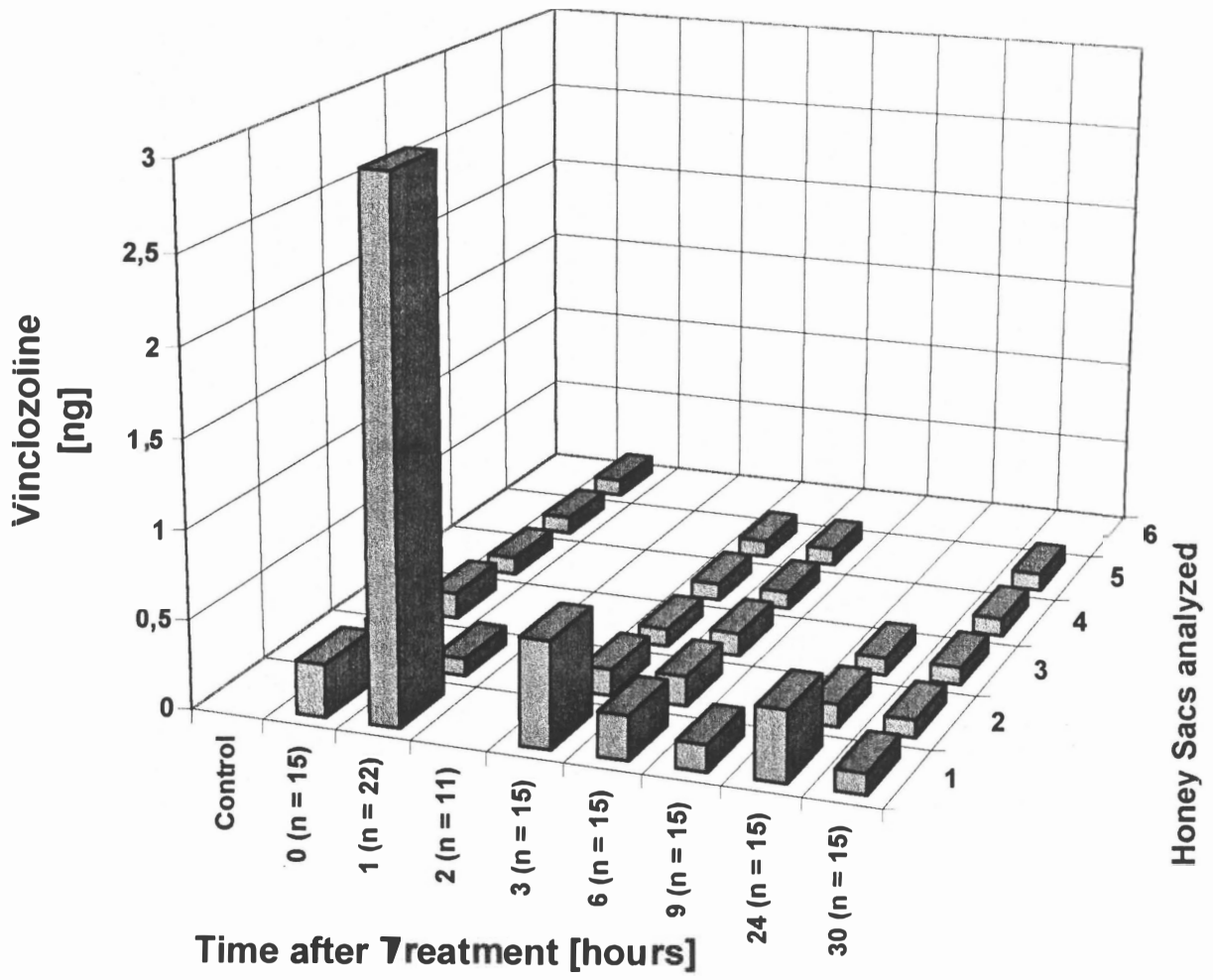
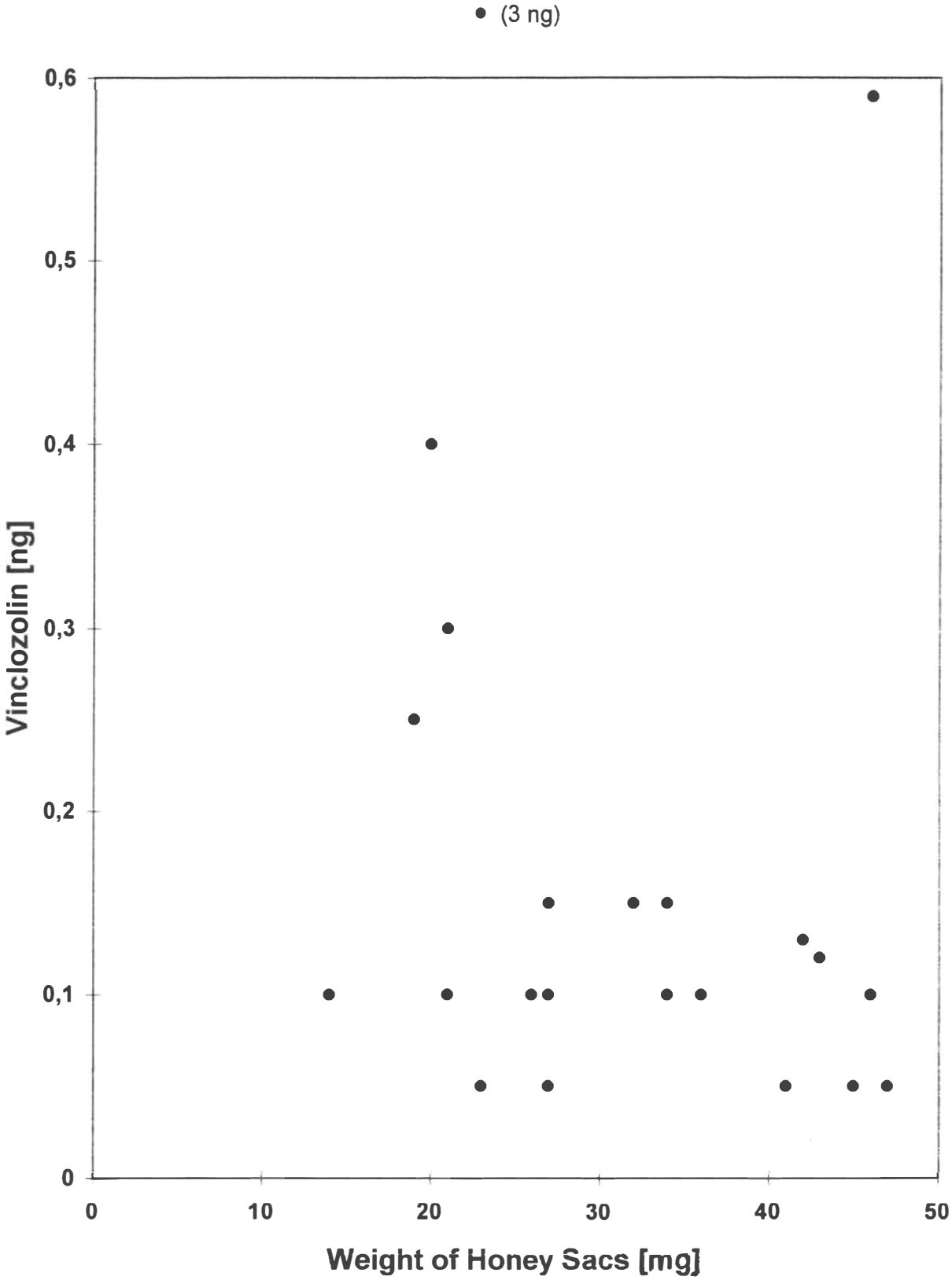

Vinciozoline [ng]
3
2,5
2
1,5
1
0,5
0
Control
0 (n = 15)
1 (n = 22)
2 (n = 11)
3 (n = 15)
6 (n = 15)
9 (n = 15)
24 (n = 15)
30 (n = 15)
Time after Treatment [hours]
1
2
3
4
5
6
Honey Sacs analyzed

Fig. 2


• (3 ng)
0,6
0,5
0,4
0,3
0,2
0,1
0
Vinclozolin [ng]
0
10
20
30
40
50
Weight of Honey Sacs [mg]

Appendix 19

D. Brasse
Development of a monitoring scheme for poisoning incidents of honey-bees by pesticides

Development of a Monitoring Scheme for Poisoning Incidents of Honey-Bees by Pesticides

Report of the Subgroup by D. Brasse, Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA), Braunschweig

Members of the group:

D. Brasse (organizer), BBA Braunschweig, Germany

M. Fletcher, MAFF York, U.K.

P. Oomen, Plant Protection Service, Wageningen, Netherlands

J. Stark, University of Uppsala, Sweden

The group had two meetings:

1. in the BBA Braunschweig, October 1994
2. in the University of Uppsala, May 1996

After having exchanged the experiences on clearing up the reasons of poisoning incidents of honey-bee populations in the different countries the group decided to develop a scheme for the performance of monitoring poisoning incidents. The scheme should be based especially on the experiences in U.K. and Germany, where such monitoring systems are already existing.

The procedure can be divided into four parts, regarding:

- all activities to be done at the place of the incident
- preparation of the samples and correct sending
- investigations of the samples
- evaluation and further use of the results.

Explanation of the scheme:

1. Assessment of the poisoning incident

This should include all circumstances which could have an effect on the origin of the incident and on the investigations.

- How many colonies are affected and in what extent? Therefore the hives should be opened for getting an overview, whether dead bees are laying inside or only foragers have been killed.

- Many factors as: weather (rain, temperature), stage of development of forage crops, stage of development of plant pests, diseases and weeds, stage of development of the colonies may influence the origin of a poisoning incident.
- All the details about the assessment of the damage and the additional factors, which could have influenced the origin of the incident, should be stated in a detailed form, as this will be the information basis of the investigators, who are normally not able to collect informations at the face. The better the informations by the form, the better is the chance for starting a specific investigation.

2. Sampling:

It is necessary that the samples fulfil standard requirements in order to guarantee a standardized basis for the investigations.

- A sample of dead bees should exist of about 1000 individuals (weight about 100 g).
- A sample of plant material should have a weight of about 100 g, preferably existing of flo-wers and leaves.
- Best information for clearing up the origin of the poisoning incident can give a sample of the spray liquid, which is thought to be involved.

The most important principle in sampling is, to keep the different materials well separated. When mixing the different materials with each other or with other substances this may influence the results of the investigations, as eventually existing residues can be transferred from one material to the other. It is also important to provide the minimum weight of 100 g/sample, to ensure that the investigations can produce representative results and that the investigations can be repeated if necessary.

3. Storage, packing and sending of samples:

- The different sample matarials must be packed separately to prevent that eventual existing residues can be transferred from one material to the other during transport. Mixed samples are unsuitable for investigations, as it is not possible - if residues of different active materials have been detected - to differ, which active material is originating from what sample mate-rial. All samples should be labelled dearly in order to prevent mistakes. Especially if a sample of the spray liquid was taken, the container should be packed carefully and in every case sent separated from all other samples. Reason: if the container is damaged during

transport, the liquid would contaminate all other sample materials and make them unsuitable for investigations.

- Suitable packing materials are non hermetically closing containers as cardboard or cigar boxes and paper bags. Plastic or tin boxes and all other hermetically closing containers are unqualified as within a short time the sample material is starting to mould and decay. By this breakdown of residues of active materials can be initiated and accellerated.
- If it is not possible to send the samples immediately after sampling - e.g. at the weekend -, the samples should be kept cool. In an ideal case the samples should be deep frozen immediately after sampling and sent in that status. In most of the cases this is unrealistic and it is better to keep the samples only cool and not frozen, as thawing of the frozen material is accellerating the process of decaying and the break down of eventual existing active materials. All samples and the filled form have to be sent by quickest way.

4. Biological investigations:

- Origin and extent of poisoning incidents can be influenced by honey-bee diseases and parasitism. Therefore be samples should be investigated on the most common diseases and parasites. Bees suffering from diseases or parasitism are much more susceptible to chemical agents than healthy bees. Moreover the bees should be investigated for starvation.
- By the determination of the pollen received from the bee bodies it is possible to get informations about the place(s) where and the plants on which the bees have resteted before they died. Best information is given by the analysis of pollen grains from the loads; but mostly the intoxicated bees loose their pollen loads by uncoordinated moving and it is necessary to wash out the grains from the hair on the bee bodies.
- Special determination of plant material is regarding mostly weeds, when crops and other cultivated plants (e.g. fruit trees) have not been attractive to the bees. Regarding the clarification of the incident there should be a correlation between the determination of the plant material and the pollen analysis by which it can be supposed that the bees have probably visited those plants from which the sampled material has bee taken.
- All cases which cannot be related to an intoxication, especially by the use of plant protection products should be eliminated from the further investigations. But nevertheless the sender of the samples should be informed by a report of the results so far.

5. Chemical investigations:

- The chemical investigations should be based on the most modern method for detecting resi-dues of chemical agents. The investigations should include all active materials of plant pro-tection products and pharmaceuticals for control of honey-bee diseases and parasites. This seems to be necessary from the following reason: In nearly all investigated cases of honey-bee intoxications there was found a number of different active materials, originating from compounds used for different purposes (e.g. plant protection, Varroa control, wood preservation). All active ingredients together may have a synergistic effect to the bees, also in that case if the different materials are classified as harmless for bees.
- In many cases the chemical investigations do not produce obvious results. This requires an interpretation of the significance of the results:
  - Is the poisoning incident caused by the use of plant protection products?
  - Is the incident possibly caused by misuse of pesticides?

  But also obvious results need an interpretation especially for the affected beekeeper.

6. Reporting and collation of results:

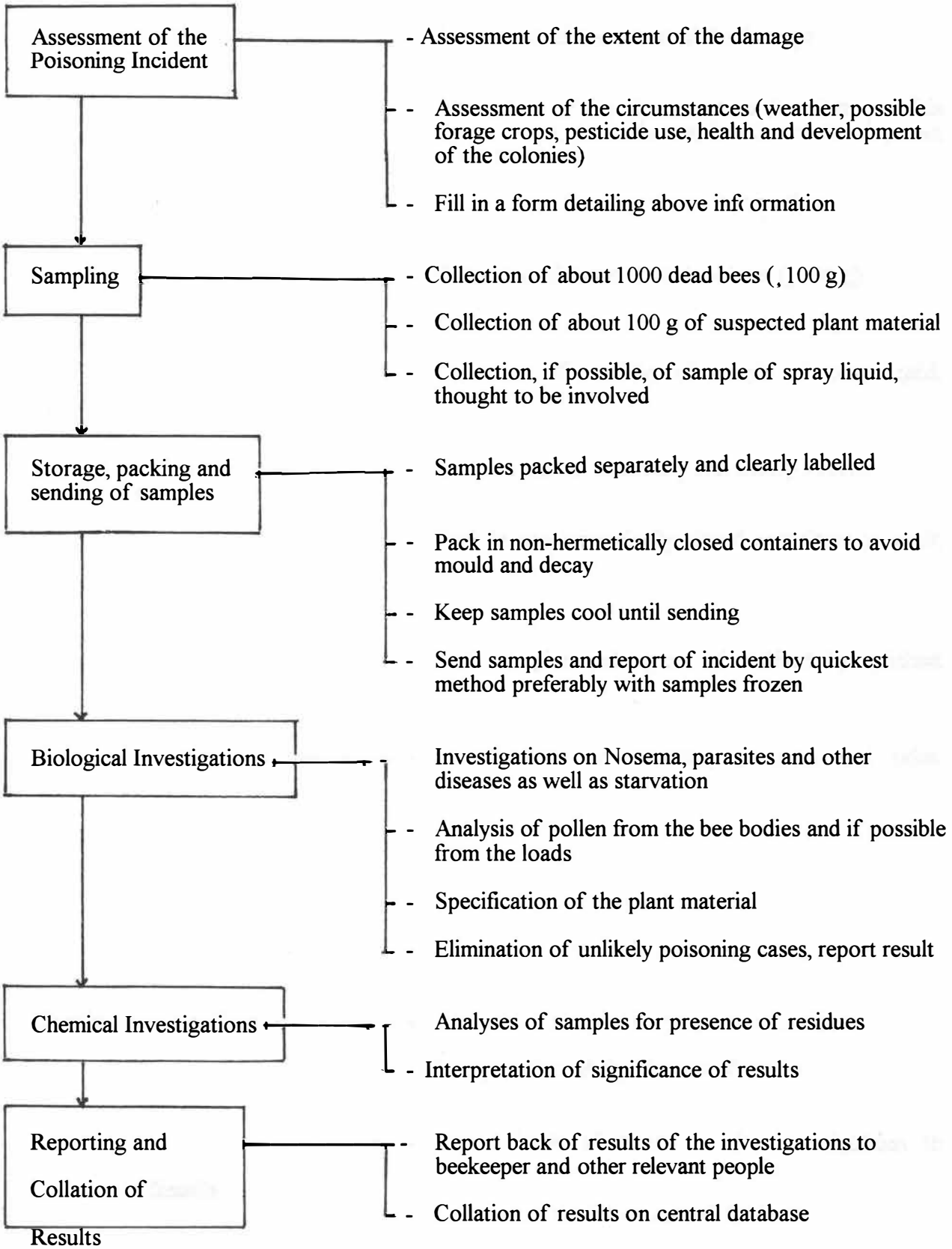
- All results of the investigations (biological and chemical) should be summanized in a report and sent to all people respectively institutions involved in the clarification of the incident, which may be
  - the beekeeper and beekeepers association
  - the local plant protection service
  - the registration authority.

  In the case of deliberate poisoning of the bees further institutions (e.g. police) may be informed.
- All results of the investigations should be collected on a central data base. This makes a collation of specific data of the results easier and could be used for a feed back to the registration authority.

Summarizing the explanations to the scheme and the experiences with clearing up of poisoning incidents two subjects for further work have been left:

1. The group believes that it could be useful to develop a harmonized questionaire for the assessment of the poisoning incident. (With respect to a possible adoption of the scheme by EPPO colleagues of more countries are invited to take part in the work of the subgroup).
2. The group did not find a reliable way to record poisoning incidents by IGR's. The reason is that the symptoms of an IGR-intoxication occur normally 10 - 14 days after the application.

Monitoring scheme for investigating suspected pesticide poisoning of honey-bees



Appendix 20

M. A. Clook
Label phrases regarding the risk to honeybees: A UK approach

LABEL PHRASES REGARDING THE RISK TO HONEYBEES: A UK APPROACH

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UK

Summary

As a result of a recent pesticide review, concern was raised regarding the existing classification scheme of pesticide products with respect to honeybee toxicity. The phrase was considered to be unclear, confusing to users and somewhat contradictory. The main problem with the classification scheme was that it was based on the toxicity of the active substance rather than the risk posed by the product. Therefore, it was proposed to change the basis upon which pesticides are classified to honeybees from a hazard based scheme to a risk based one.

If a product is seen to pose a high risk as a result of the assessment based on the EPPO honeybee risk assessment scheme, then the product will be labelled as:

'HIGH RISK TO BEES. Do not apply to crops in flower or to those in which bees are actively foraging. Do not apply when flowering weeds are present.'

If a low risk is predicted then the product will not labelled or classified.

Introduction - The problem

Products in the UK used to be classified regarding their toxicity to bees on the basis of the acute oral and contact toxicity test, as outlined in the The Registration Handbook (1995). Depending on the results of these standard toxicity studies (see table below) products would have been classified and hence labelled with the following phrase:

HARMFUL/DANGEROUS/EXTREMEL Y DANGEROUS TO BEES. Do not apply to crops in flower or to those in which bees are actively foraging. Do not apply when flowering weeds are present.

Classification of products with respect to toxicity to honeybees

Toxicity	Classification
<0.1 µg/bee	= extremely dangerous to
0.1-1 µg/bee	bees = dangerous to bees
1-10 µg/bee	= harmful to bees
>10 µg/bee	= unclassified

The data from these acute toxicity studies were then used together with application details to predict the risk to bees. If the assessment indicated a risk then further data, for example either a semi-field or field trial were generally requested. If this trial indicated that the use of the product should pose an acceptable risk to bees, then the above phrase was amended to include the phrase 'except as directed on *crop*' inserted.

An example of the above scenario is the use of certain synthetic pyrethroids, eg esfenvlaerate, on flowering oilseed rape. Laboratory data on the acute toxicity of the active substance or product together with application rate data indicate a high risk to bees. However extensive semi-field and field trial data indicate that the risk is not realised in the field. Therefore, the product would have been classified as:

'DANGEROUS TO BEES. Do not apply to crops in flower or to those in which bees are actively foraging except as directed on oilseed rape. Do not apply when flowering weeds are present.'

This phrase was considered to be unclear, confusing to users and somewhat contradictory. It was considered that the main problem with this honeybee classification scheme was the fact that it was based on the inherent hazard of the active substance rather than the risk posed by the use of the product. Changes in the wording of the label phrase would not have eliminated the possibility of confusing statements. Therefore it was proposed to change the basis upon which pesticides are classified with respect to bees from hazard or toxicity based labelling to risk based labelling.

The solution

It was decided that if exposure of bees to the product can be ruled out as outlined in Section 8. 3. 1. 1 of Annex II of 91 / 414/EEC, then no honeybee toxicity data, labelling and classification would be required. These uses include food storage in enclosed spaces, non-systemic seed treatments, non-systemic preparations for soil application, non-systemic dipping treatments for transplanted crops, wound sealing and healing treatments and rodenticide baits. It was also felt that no data were required if a product is to be used at a time of year when either the crop is not in flower or there are no flowering weeds present. However, if honeybees were considered likely to be exposed to the product from the correct use then the following scheme would be used :-

> If the hazard ratio (EPPO 1993), i.e. application rate in g as/ha ÷ LD50 µg as/bee, is calculated to be less than 50, then the product should not be labelled and hence the product can be used in crops that are flowering or where bees may be foraging.

If the hazard ratio is greater than 50 then the product should be labelled as:

'HIGH RISK TO BEES. DO NOT apply to crops in flower or to those in which bees are actively foraging. Do not apply when flowering weeds are present'.

The use of such a product would not be allowed on crops in flower or in situations where bees are likely to be exposed. This phrase could be removed and hence use of the product permitted, if data are supplied which indicates that the product does not pose a risk to bees, for example, field trial or semi-field trial data. Data from the Wildlife Incident Investigation Scheme (WIIS) may also be used in combination with other data to provide additional information on the likely risk of the product.

Insect growth regulators, will initially be labelled as 'HIGH RISK TO BEES .. ' with a requirement for further testing, for example brood tests, to indicate whether the use of the product poses a high risk to bees. Non-systemic soil drenches, seed treatments, pellets and granules are usually of low risk to foraging bees, therefore products will not normally be labelled. However, if the active substance is systemic and present in the plant at the 'bee activity stage' then honeybee toxicity data, labelling and classification may be required. It should be noted that the data are only required should the applicant wish to remove the phrase.

Worked examples

Outlined below are a selection of worked examples to demonstrate how the labelling scheme will work:-

a) The acute oral and contact toxicity of active substance A to honeybees is 3.2 and >54 μg as/bee. It is applied at the rate of 280 g as/ha, therefore the hazard ratio is 87.5 for the oral route and 5.1 via the contact route. This indicates that it should be labelled as 'High risk to bees..', with a requirement for semi-field/field trial data.

   Data evaluated indicate that mortalities did not occur following treatment of crops in flower where bees were foraging. Data also indicated that sub lethal effects were not observed. In addition to the trials data Wildlife Incident Investigation Scheme data indicates that mortalities do not occur when the product is used on a wide scale. Therefore, on the basis of these data the product should not be labelled regarding its risk to bees.

b) The acute oral and contact toxicity of active substance B is 0.15 and 0.12 μg as/bee. It is applied at the rate of 420 g as/ha, therefore the hazard ratio is 3500 and 2800 for the oral and contact route respectively. This indicates a high risk and the product should be labelled as 'High risk to bees. Do not apply to crops in flower or to those in which bees are actively foraging. Do not apply when flowering weeds are present'. In addition there are much data to indicate that active substance B can cause mortality in the field as a result of WIIS, field and semi-field trials. Therefore the phrase must remain and approval for use on flowering crops should not be permitted.

c) The contact toxicity of active substance C to honeybees was stated to be 0.06 µg as/bee. It is applied at 12.5 g as/ha, therefore the contact hazard ratio is 208. This indicates that the product should be initially classified as 'High risk to bees...' with further data required in order to remove the the phrase. The additional data indicate that the use of products containing active substance C does not pose a risk to bees, therefore the phrase should be removed.

d) An insecticide has a contact and oral toxicity of 0.05 and 0.13 µg as/bee, and its use as a spray would pose a high risk to bees and is labelled appropriately. However, use is recommended as a soil drench application on flowering plants. Data are submitted to indicate that the active substance is not systemic and does not pose a risk to bees when used in this manner. Therefore, the product should not be labelled regarding the risk to bees.

e) The contact and oral toxicity of compound X is 0.06 µg as/bee and 0.12 µg as/bee respectively. Approval has been requested for use on oilseed rape in flower at the rate of 36 g as/ha, and evening primrose in flower at 72 g as/ha. These result in hazard ratios of 300 and 600 for the oilseed rape use and 1200 and 600 for the evening primrose use. Field trial data indicate that the lower rate does not cause mortality in the field whilst the higher rate was found to cause high mortality. Approval would be granted for the oilseed rape use, but not for the use on evening primrose. The product would therefore be labelled as 'High risk to bees. Do not apply to crops in flower or to those in which bees are actively foraging except as directed on oilseed rape. Do not apply when flowering weeds are present.' It should be noted that the firm should be consulted to investigate the possibility of developing suitable use strategies, for example spraying early in the morning etc, prior to recommending a restrictions.

Products aimed at the home/ garden and amateur markets will be dealt with in exactly the manner as outlined above. However, the products will be labelled as:

'HIGH RISK TO BEES. DO NOT dust/spray plants in flower.'

As part of the development of this labelling scheme the Pesticides Safety Directorate consulted widely with the other government departments' representatives of the UK beekeeping industry, pesticides users as well as the agrochemical companies.

Conclusion

In conclusion, the hazard/toxicity based honeybee warning phrase previously used in the UK has been replace with a risk based labelling scheme. The risk posed to foraging bees by the use of products is assessed and the product classified as 'High risk to bees', if the hazard ratio is determined to be greater than 50 and data indicates that an effect on bees is expected from the correct use of the product. Products are labelled with the following phrase:

'HIGH RISK TO BEES. Do not apply to crops in flower or to those in which bees are actively foraging. Do not apply when flowering weeds are present'.

If as a result of the risk assessment process a hazard ratio of less than 50 is indicated, and there are no data to indicate an effect on bees from the correct use of the product, then the product will not be labelled.

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Annex II of Council Directive 91/414/EEC.

Appendix 21

E. A. Barnett, M. R. Fletcher, P. M. Brown, A. J. Charlton
Changing patterns of pesticide poisoning incidents of bees in England and Wales in recent years

Changing patterns of pesticide poisoning incidents of bees in England and Wales in recent years

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Introduction

The Wildlife Incident Investigation Scheme is operated by UK Agriculture Departments (Ministry of Agriculture Fisheries and Food, Welsh Office Agriculture Department, Scottish Office Agriculture Environment and Fisheries Department and Department of Agriculture Northern Ireland). It monitors the direct effects of agricultural pesticides on wildlife, including beneficial insects (honeybees and bumblebees) and companion animals. Suspected incidents are reported by beekeepers and the general public, so considerable efforts have been made to ensure public awareness of the Scheme. The Scheme is part of the pesticide regulatory process co-ordinated by the Pesticides Safety Directorate. The requirement to monitor unanticipated effects of pesticides on animals or post registration surveillance is funded by a levy on pesticide sales in the UK. If infringement of pesticide legislation is identified, further costs are paid for by Agriculture Departments.

The Scheme began about 35 years ago, but has operated more or less in its present form since 1980. There have been a number of review papers covering the results of the Scheme for monitoring bee deaths in England and Wales, (Stevenson *et al*, 1978; Fletcher *et al,* 1994) and Great Britain (Greig-Smith *et al* 1994). The results for the UK are also publ ished annually (eg. Fletcher *et al* 1996). In recent years (1993-1995) the pesticides frequently associated with bee mortality have not followed the pattern of pesticide poisoning incidents discussed in earlier reviews. Carbamate compounds, such as bendiocarb, have more frequently been identified in poisoning incidents, shifting the focus from crop spray related incidents to incidents involving the control of feral bee colonies. However, in 1995 there was again a large proportion of crop spray related incidents.

This paper will briefly outline the operation of the Scheme and the analytical methods used. It will also discuss the recent trends and circumstances of pesticide poisoning in bees in England and Wales.

Methods

Field investigations

These are undertaken as soon as possible following the report of bee mortality so information on the circumstances of the incident can be obtained. This information includes the number and condition of the bee colonies, how many colonies have been affected and any unusual symptoms or behaviours shown. An opinion on how far away the bees have been foraging and what flowers the bees have been working is also useful. Any recent pesticide use in the area is also looked for and details of these are recorded.

Analyses

Biological

A subsample of the bees submitted to the Scheme are screened by the Central Science Laboratory (CSL) National Bee Unit for common bee parasites, such as varroa, nosema, acarine and amoeba. Pollen present in corbicular loads or on body hair is identified to generic or species level where possible.

Chemical

The analytical procedures are carried out by CSL Wildlife Incident Unit. At least 60 bees are required for the five essential stages which are, extraction, clean up, analysis, measurement and confirmation. Soxhlet extraction with ether is currently used, but Super-critical Fluid Extraction will be used in the future. A clean up stage, for example using gel permeation chromatography, is necessary as bees produce many waxy co-extractives. The analyses must detect very low levels of the pesticides that are likely to cause bee poisoning incidents. Currently three multi-residue gas chromatographic (GC) methods are used.

GC with flame photometric detection can detect most organophosphate compounds, with detection limits ranging between 0.001-0.004 µg/bee.

GC with nitrogen phosphorous detection can detect many carbamate compounds, with detection limits ranging from 0.003-0.01 µg/bee.

GC with electron capture detection can detect many pyrethroid compounds and gamma-HCH, with detection limits ranging from 0.0005-0.006 µg/bee.

The residues identified by these methods are measured by comparison with a range of standard concentrations. Qualitative confirmation is achieved by using GC with an ion trap mass spectrometer (MS) detector. Analytical instrumentation and software developments may lead to the sole use of GC-MS to identify and confirm residues (for example using GC-MS-MS). More detailed information on the analytical methods is described in Brown *et al* 1996 (in press).

Other analyses may be undertaken if the field evidence indicates the use of a pesticide that is considered to be harmful to bees. Unless the field information indicates the use of a pesticide that has not been screened for, once a positive result is obtained further analyses are not undertaken.

Interpretation of results

To interpret the significance of the residues remaining on bees, contact LD_{50} values (Stevenson 1968 & Tomlin (ed) 1994) and where available, subsequent residue levels (SRL) are referred to (Greig-Sm ith *et al* 1994). The SRL is the residue that is determined from analyses of bees dying from an LD_{50} dose. Any bees with residues above the LD_{50} or SRL value have clearly died from pesticide poisoning. Residues below these values are assessed and are usually considered to be significant given the residue losses before and during analysis.

Sometimes the likely source of a pesticide in a bee poisoning incident has been identified during the initial field investigation. However, this may be more easily done following analysis results when the chemical involved is known. It is also important to establish whether the pesticide was used according to label instructions so the incident can be classified as; **approved use** of a pesticide according to the specified conditions of use, **misuse** of a pesticide by careless, accidental or wilful failure to adhere to conditions of use, **abuse** of a pesticide by its deliberate use to poison bees and **unspecified use** where despite extensive enquiries no information on the use of the pesticide involved has been found (Fletcher *et al* 1996).

Reporting of results

Bee incident reports are produced for every incident reported to the Scheme and these include a summary of the field information, the results of all the analyses and the interpretation of these results. This report is sent to beekeepers, pesticide regulatory departments and other parties involved in the Scheme about four weeks after receipt of the samples for chemical analyses.

An annual report is published (eg. Fletcher *et al* 1996) which covers all incidents in the UK. There is also an annual meeting on bees and pesticides with representative organisations, such as the Bee Farming Association, British Agrochemicals Association and pesticide regulatory departments.

A computer database has been maintained on all the incidents reported to the Scheme since 1984. This database produces the bee incident reports and enables searches on a variety of parameters of interest.

Results

Since the Scheme for monitoring bee poisoning incidents began there have been about 35 active ingredients identified. Some pesticides that have not been identified in the earlier reviews of the

Scheme include diazinon, propoxur, carbosulfan and tetramethrin. The diazinon incident was suspected to have been caused by an insecticidal lacquer containing this compound. It had been applied to the crown boards (in an unknown concentration) about six months prior to the re-use of these boards. A residue of propoxur was found on dead queen bees that were in transit from Tenerife and an incident that killed all the bees in two hives involved carbosulfan. The sources of the carbosulfan and propoxur were not established. Tetramethrin was identified in one incident of intentional poisoning that also involved bendiocarb (1 µg/bee) and cypermethrin (0.5 µg/bee). The residues for the above incidents are shown in Table 1, along with the range of residues for compounds involved in more than one incident.

Table 1: Compounds involved in bee poisoning incidents in England and Wales 1993-1995

Compound	Number of incidents*	Residue range µg/bee
bendiocarb	18	0.01 - 65
dimethoate	12	0.02 - 0.29
pirimiphos-methyl	7	0.002 - 0.28
chlorpyrifos	5	0.002 - 0.33
carbaryl	4	0.2 - 14
triazophos	4	0.2 - 0.69 0.03
fenitrothion	3	- 0.27 0.37 -
gamma-HCH	2	4.5 0.04 - 0.4
malathion	2	0.5
cypermethrin	1	0.13
permethrin	1	2.5
tetramethrin	1	0.012
diazinon	1	1.3
fluvalinate	1	1.2
carbosulfan	1	0.35
propoxur	1	

** More than one compound may be involved in an incident*

In 1993 there was an incident involving gamma-HCH and malathion
In 1994 there was an incident invo/vingfenitrothion and bendiocarb
In 1995 there was an incident invo/ving bendiocarb, cypermethrin and tetramethrin

During 1993-1995, 134 suspected bee poisoning incidents were submitted to the Scheme. Following chemical analyses, 60 of these incidents were found to involve pesticides, with 16 active ingredients

identified (see Table 1). All of these incidents involved honeybees, except one bumblebee incident where a residue of 0.29 µg/bee of dimethoate was found. It can be seen from Table 1 that just four compounds, bendiocarb, dimethoate, pirimiphos-methyl and chlorpyrifos account for 70% of the bee poisoning incidents, and bendiocarb alone accounts for nearly a third of the poisoning incidents.

Table 2: Uses identified in some of the bee poisoning incidents

Compound	Number of incidents	Number of incidents where use identified	Uses identified
bendiocarb	18	12	Feral bee treatment
		1	Intentional poisoning
dimethoate	12	5	Agricultural crop spraying
pirimiphos-methyl	7	2	Home and garden use
chlorpyrifos	5	3	Agricultural crop spraying

In nearly 70% of the incidents involving bendiocarb a feral bee treatment was identified as the source of the chemical (see Table 2). There were many incidents involving dimethoate where the source of the pesticide could not be found. However, agricultural crop sprays were often implicated, with four incidents involving applications to oil seed rape and one incident an application to a bean crop. Similarly with chlorpyrifos 60% of these incidents were agricultural sprays to apples and raspberries. With pirimiphos-methyl the source of the chemical was only found in about a third of incidents involving this compound. Home and garden use to control ants and pests on roses was noted.

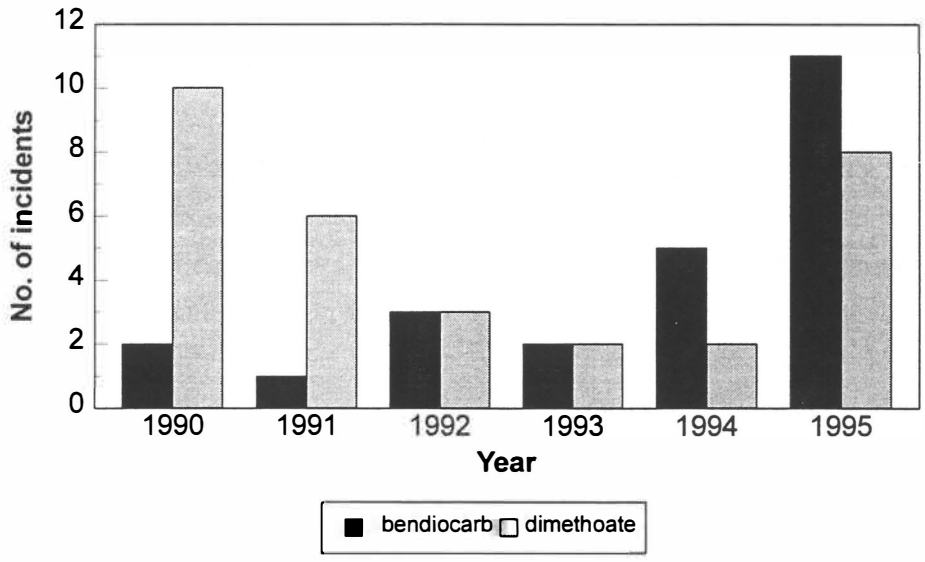

Figure 1: Trends in incidents involving bendiocarb and dimethoate

In 1994 and particularly in 1995 a dramatic increase in the number of bee poisoning incidents involving bendiocarb occurred (see Figure 1). Incidents involving dimethoate were common in late 1980, but since 1990 there had been a decline in the number of these incidents (see Figure 1). However, in 1995 a large increase in dimethoate bee poisoning incidents occurred. This is particularly significant as all the incidents in 1993 and 1994 involved a single beekeeper, whereas in 1995, four of the incidents involved eleven beekeepers.

Discussion

During 1993-1995, bendiocarb and dimethoate have clearly been the major cause of bee poisoning incidents and in 1995 dramatic increases in the number of incidents involving these compounds occurred. However, the actual effects on the number of bee colonies observed in these incidents are very different for the two compounds. Bendiocarb appears to be a significant problem, but in 18 incidents only 29 bee colonies were affected or about 2 colonies per incident. Whereas dimethoate in 11 honeybee incidents affected 107 colonies, which is nearly 10 colonies per incident.

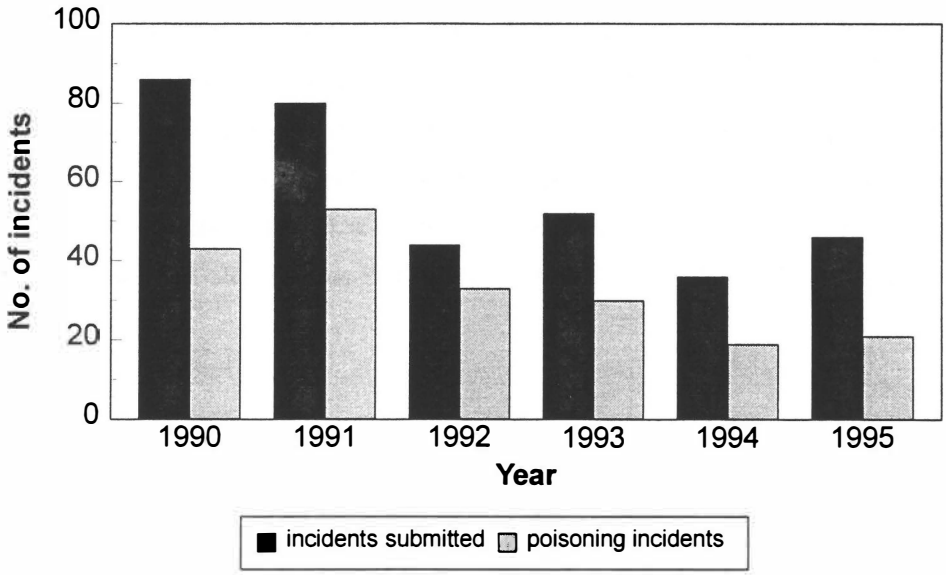

Figure 2: Incidents submitted to the Scheme

The increase noted in 1995 could be due to increases in the number of incidents submitted to the Scheme. However, Figure 2 shows that the number of incidents submitted has been more or less constant for the last four years, as has the number of these incidents that are found to involve pesticides. Therefore, there are real differences in the compounds associated with bee poisonings from year to year. There are a variety of factors which may account for these differences, some of these may be:-

- Weather conditions : in warm sunny weather there is greater foraging activity and certain weather patterns may also lead to increased robbing activity between colonies, both these circumstances will lead to more chance of exposure to a pesticide.

- Time of spraying : spray operations undertaken at times of peak bee activity are more likely to cause bee mortality than spraying at dawn or dusk.

- State of crop : increased pest populations on a crop will mean more spray treatments are undertaken. Non-uniformity of flowering and or flowering weeds in the crop can make it impossible to apply a pesticide to a field when no flowers are present.

- Proximity of bees : it is obvious that bees close to a treated area are more likely to be affected. However, bees will also travel several kilometres for an attractive crop such as oil seed rape.

- Reporting rate of suspected incidents : publicising the Scheme will increase the number of incidents reported. However, it is suspected that incidents may not always be reported by beekeepers, either because they assume the bees have died from disease or they prefer not to initiate a formal investigation as this may adversely affect their relationship with the landowner.

- Adhering to label conditions : the extent to which label conditions are followed will vary. The pesticide user must be aware of the correct practice and should apply these conditions to treatments in a wide variety of circumstances. Occasionally these conditions may be difficult to fulfil.

In 55% of the incidents involving pesticides the use of the chemical was identified. Agricultural crop spraying (nearly 20% of bee poisoning incidents) and feral bee treatments (nearly 30% of bee poisoning incidents) are clearly the major uses involved. In nearly all these incidents where the source of the pesticide was identified, the products had not been used according to label conditions. In feral bee control, treated combs were not removed and or access to these had not been prevented. These incidents usually occur towards the end of the bee season when bees are more prone to robbing from each other. It can be difficult when treating some areas, such as colonies behind airbricks, to fulfil the label conditions. However, feedback to pesticide users via the dissemination of bee incident reports and the annual bees and pesticides meetings has raised awareness of this problem so appropriate action can be taken. The spray related incidents mainly occur during June and July and the most common breach of the label instructions is spraying a crop in flower or when flowering weeds are present.

It is often difficult to identify the use of a pesticide, particularly as during the field investigation the pesticide involved may not be known. Follow-up visits to identify the source of the pesticide are very useful, but the results of analyses must be available within a reasonable time to make these visits worthwhile. Despite these measures there are a number of poisoning incidents where the source of the pesticide and therefore the use can not be definitively identified.

Conclusion

The cause of bee mortality in a large number of incidents has been organophosphate spray applications, particularly dimethoate to oil seed rape. This use affects a far greater number of bee colonies than the feral bee treatments. Incidents involving bumblebees are occasionally reported to the Scheme and in 1995 the first pesticide poisoning incident was identified.

A wareness of the Scheme amongst the beekeeping community is essential and beekeepers must be encouraged to report suspected incidents. Data must also be available to interpret the significance of the results obtained, such as LD_{50} values, subsequent residue levels, the use of the pesticide identified and where appropriate the crop involved. It is also important to have some knowledge of the pattern of beekeeping within the country and the extent of pesticide usage.

However, before valid conclusions can be drawn from the data produced, the methods of such a Scheme should be considered. Only the chemicals that have validated analytical methods to detect residues in bee samples will be found. The Scheme is reactive, bee poisoning incidents following a spray application are not actively looked for. Most bees submitted are those that have returned to the hive and so are likely to have lower residues, whereas the bees dying in the field may have higher residues and are unlikely to be found. Mainly honeybee incidents are reported as vigilant beekeepers will recognise a problem with their bees, but bees dying in the wild are unlikely to be noticed.

Despite the limited scope of such a scheme, post-registration surveillance monitoring is clearly essential. Extensive laboratory and field experiments can never cover the infinite variability that there is in the real use of pesticides. Problems with the use of certain pesticides, such as triazophos on winter oil seed rape have been identified by the Scheme and action taken. Bee poisoning incidents arising from the approved use of a pesticide are rare and is evidence for the success of the current pesticide regulatory process. The Scheme can also provide valuable data to ensure that correct risk assessments have been made and may contribute to improving future assessments.

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Appendix 22

K. Wallner
Pesticides in vineyards and unexpected bee intoxications

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Pesticides in vineyards and unexpected bee intoxications

Chemical plant protection, and especially the pest control, have always been a risk for bees and a number of other beneficial insects. On the other side this problem has been the basis for scientific researches since decades. In the meantime the extensive knowledge about the side ef-fects of pesticides, for example for bees, led to a reduction of toxic damages.

In our days most of the damages for bees either depend on the improper use of pesticides which are dangerous for bees - or on other exceptions. One of these exceptions will be examined in the following:

The damages for bees in the German viniculture area Ortenau around Baden-Baden have a long tradition. The first severe damages to a hundred of bee-colonies appeared in the early 70ies. At that time vine was still regarded as a plant which the bees do not contact and the use of insecticides during the blossoming period was allowed. The consequences were intoxicated vine pollen. This brought enormous brood damages and losses in the bee colonies. As soon as this problem had been detected, the use of pesticides that were dangerous to bees was forbidden on the blossoming vine. Thus the damages were reduced but they could not be avoided totally. Until today in this area bee intoxications can be found during the blossoming period.

The area we wanted to examine includes about 1000 hectares of vineyard going from the steep slope of the Rhine valley until the edges of the Black F orest. The reparcelling of the vineyards did remove most of the unused side areas.

The damages for bees did always appear in the beginning of the blossoming period. Mainly the foraging bees were concerned. They were lying dead on the flight boards and in front of the bee hives. The contaminated bees show typical symptoms of intoxication, which we know for example from the organophosphates. Usually there are no brood damages. Often we can find a non-uniform damage in the colonies of one apiary. The losses lead to a reduction of the bee colony, in the extreme to a total damage.
It attracted our attention that the damages appeared always at the beginning of the blossoming period, but independent from spraying, so there was no direct connection between the use of pesticides and the bee intoxication.

What is the reason for these damages?

To clarify this question we deposed bee colonies for several years in the affected area. With the colonies we made population measuring and pollen traps tests on 10 different places in the test area.
In the vineyards we observed the use of active ingredients and their dispersion.
For an identification of the used active ingredients and their behaviour on the plant surfaces we made laboratory tests with bees and gas chromatographic analysis.

One of the mayor pests in viniculture is the grape berry moth, a small butterfly whose larvae have two generations. The first generation eats the buds of the vine blossom, the second gen-eration eats the small grapes.
The best period for a pest control is determined with hormone traps. The summit of the flight activities of the butterflies and the climate determine it. Normally treatment starts around 10 days after the flight summit when the larvae start hatching out.

The control in the steep slope is effected with a wide jet range sprayer. These gadgets eject a spray amount approx. 1. 000-1. 600 litres per hectar. The advantage of this gadget lies in the fact that they must not drive through the vineyards, they can work from the vineyard roads that were built especially for these tractors with a distance of 40 meters. The spraying is made from the bottom to the top and reversed.
As the vineyards are close to the forests, the spray may disperse into the forests. Therefore the beekeepers of this region assume that intoxicated honey dew from the trees is the reason for bee damages.
Another disadvantage of the sprayer is an uneven distribution of the spray amount. With this procedure the edges of the parcels show overdoses, in the middle the spray layer is often not sufficient for an optimal pest control.

Graphic No. 1 shows the spray distribution in a vineyard under favourable conditions. We scanned the vineyard and took leaf samples to analyse them. The highest concentrations of spray cover, approx. 35 nanogrammes per cm^2 leaf surface, were found at the edges of the parcells near the roads. The more we got to the middle of the parcell the smaller became the spray amount.
The distribution of the active ingredients with a mounted atomisizer that drives through the vineyards (graphic No. 2) is much better.

Because it was very difficult to get some inf(ormation about the used pesticides, we developed a contact test, which shows in a very simple way if preparations which are dangerous to bees had been used or not. This test avoids difficult and costly analysis an can easily be used in the field. We caught the foraging bees in front of the entrance of the hive, subdued them shortly with carbone dioxide and put them on vine leafs to evaluate their spray cover. On toxic layers the test bees die within 2 hours or even earlier. With this test the persistence of various active in-gredients on the leaf surfaces can be observed. F or example, we found out that the surface of a vine leaf has a preserving effect on some active ingredients, compared with other plants. 20 days old layers of Gusathion MS were still active on vine leafs, but not on rape leafs (table 3). The higher the depot of active ingredients on the surface is, the longer is the persistence. This can be demonstrated on parcells which had been treated with the sprayer.

The contact test showed that this persistence can be found on the vine leafs as well as on the flower cluster of the vine, so vine leafs can be used to show the residue effect on the buds of the vine blossom.
The structure of a vine flower cluster is different to other bee plants. Buds and opened flowers are close to each other. When the blossoms emerge the bees work them intensively but this is hardly noticeable because the flowers are hidden below the leafs. If the working frequency is good, approximately 2.000 bees can be expected in one hectar at the same moment.
Often foragers also collect flower parts such as the caps and bring them into the hive. The pol-len productivity of the single flower is very low. The yield of a flower cluster is about 1 mg. A nectary secretion does not take place.
During the pollen collection the bees have contact to the buds of the whole flower. This is a speciality that cannot be found on any other bee plant. But this way the bees have contact with

persistent active ingredients, that had been used against the grape berry moth some days ago and thus they are exposed to contact poison effects.

To deduce the danger for the bee colonies we had to examine the bees' frequentation of vine flowers.
We examined the collecting strategy of bee colonies by pollen traps during the relevant period of time. Thus we were able to determine the importance of vine pollen for the bee colonies in in ten different places in this area.
The yield of a bee colony in one day may contain about 250 g of vine pollen. If we look at the 20 % effectivity of the pollen trap, this means more than 1 million of collected flower clusters. Obviously the vine blossom in this area is very attractive for bees. In the collected pollens you can also find see the green calyptras.

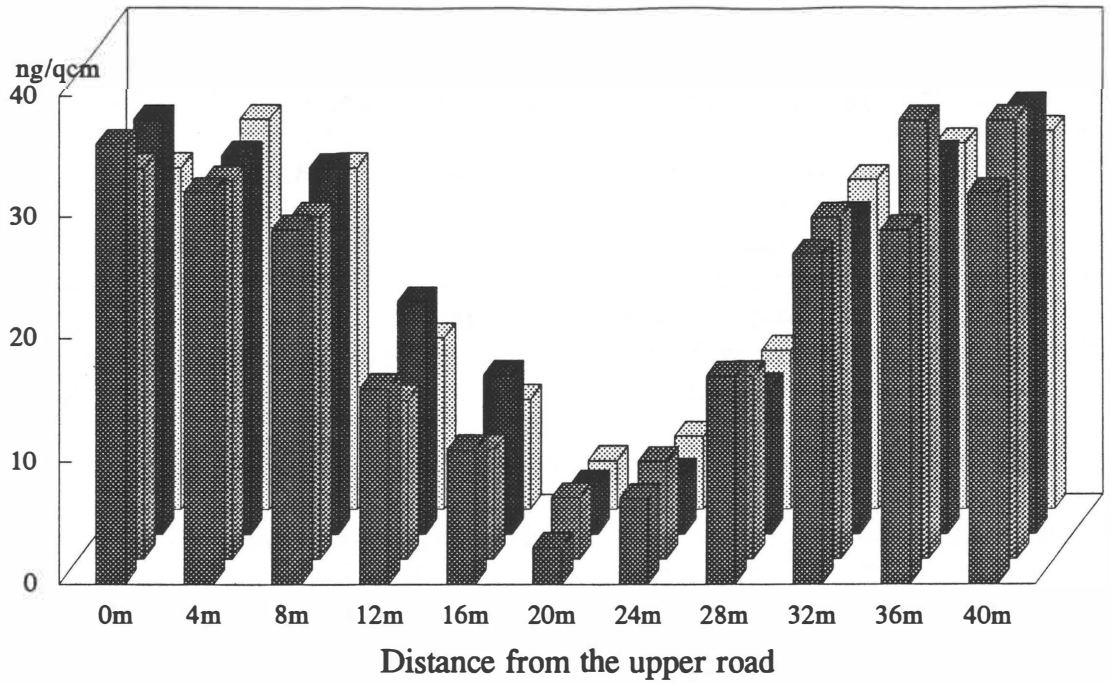
We have also tested this way of intoxication on isolated colonies. In a closed quarry we put up bee colonies and trained them to go to a round feeding place (ø 60 cm)
In the middle we offered a sugar solution and we arranged vine leafs with spray cover around it. By covering the feeding place the foraging bees were forced to walk on the leafs. Thus we had similar conditions as the pollen collection in the vineyards. Flying to a pollen or nectar source, contact with surface, and return to the hive.
The fact is that even 10 days old pesticide layers, for example by azinphos-methyl or medi-dathion, cause bee intoxications.
These intoxicated bees try to leave the hive and this is the reason why only 20 % of the con-taminated bees can be found close to the entrance of the hive. The other intoxicated bees can be found everywhere in the area. It is not possible to determ the exact quantity of damaged bees because an unknown percentage of bees left the controlled area.

With these examinations we could prove for the first time that even proper use of pesticides in the vineyards may couse bee damages when vine pollen is collected.
Because of the exceptional blossoming and the great preserving characteristics of the vine buds, persistent pesticides which are dangerous for bees should not be used in the pre-blooming stage.

Graphic 1

Spray distribution in a vineyard

Application with a wide jet range sprayer



Graphic 2

Application with a mounted atomisizer

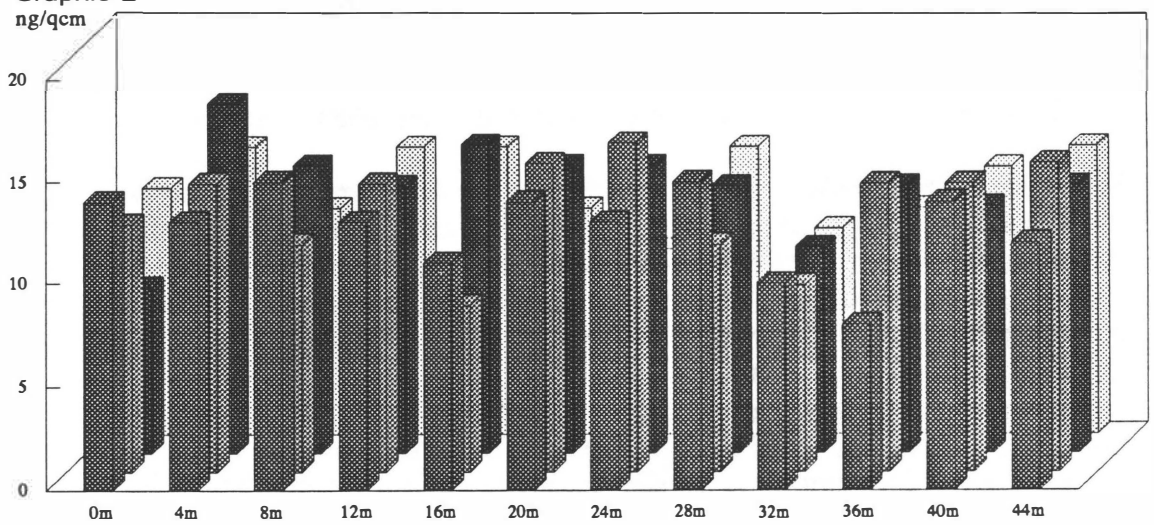


Table No: 3

Persistence of Gusathion MS on leafs of different plants
Contact test with bees
5 days old spray cover, 10 bees, colony No. 119

	time in hours						
	1	2	3	4	5	6	24
marple	0	0	0	1	3	4	10
Aegopodium pod.	0	0	0	0	0	0	0
ivy	4	7	10				
rape	0	0	0	0	0	0	0
vine	4	9	10				
alu foil	3	10					
paper	2	6	8	10			

Number of damaged bees — 10 — 100% mortality

Appendix 23

J. A. Stark, S. Bengtsson
Honeybees and chemicals

Honeybees and Chemicals

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ABSTRACT

Stark,J.A., Bengtsson,S. 1996. Swedish University of Agricultural Sciences

The accidens of beepoisoning under natural conditions was studied by monitoring apiaris established in different part of Sweden. Dead bees were collected in deathtraps, collection of pollen , live bees trapped to collection all wather data and folovinf brood development was used as basical sampels for differenet analyses.
As a result of their mobility, bees and other pollinating insects are exposed to a large number of chemical substances in the environment, and pesticides used in agriculture, forestry and horticulture.

Developments in the bee toxicity sector show a reduced number of incidences. Reports of bee toxicity, have been of greater extent and possibilities to explain causality by means of conventional analytical methods have decreased. No quantities of rest of pesticides used in Sweden today were found,but erlig spring 1994 and 1995 dead bees containing 0.01 ug per bee HCH and 0.02 ug/bee HCH were found . Us of HCH is forbidden in Sweden since several years and some weak correlation indicatet that HCH originated from rain. Chemical and statistical analys gawe indication on antioxydants in dead bees under season when bees are most active.The present complicated picture probably depends on bee toxicity interacting with the general chemical environment and use of pesticides.The results of statistical analysis of tha weather parameters contra beedeaths from the traps confirms the complicity of matter. Indications show there could be a connection between beedeaths and relative humidity and rain in the beeyard in Soutern Sweden. The regressin coefficients (r) however are very low, 0.14 and 0.20 respactively. As the correlation is rather weak, it may be concludet that much more needs to be done in the comming seasons of data collecting.

The key question is if this indication shows that HCH is comming long distance in concentration enough for killing bees?

BACKGROUND AND PRESENT DEVELOPMENT

The use of chemicals in the environment, mainly in agriculture, forestry and horticulture, has increased massively since the Second World War. New advances in chemistry have often resulted in new compounds which have been used without any particular testing for toxicity to pollinating insects.

During the late 1940´s, compounds developed for the military were used. The enthusiasm at that time to rebuild after the war, the need for production improvements, and belief in the positive effects of chemicals, overshadowed the negative secondary effects that occasionally occurred.

There is no doubt that the work of Ahlmark (1949) belongs to the early warning reports that contributed to increased demands for research into the pesticide sector. The use of pesticides has led to injury both to humans, animals and wildlife. We can observe that increasing demands placed on profitability, modern cultures, awareness about the need for control inputs, and the search for more effective compounds, has resulted in many new compounds being used during the 1950´s (Schwan, 1978).

At the same time as there were successful results in controlling weeds and parasites, there were increasing numbers of reports on damage to bees and other pollinators, mainly bumble-bees. It must be emphasized that follow-up studies of population swings for other pollinators were not made against the background of the use of pesticides.

The development of knowledge in the plant protection sector has resulted in increased use of chemicals for control according to need. This particularly refers to the case in agriculture where chemical control was earlier considered to be unprofitable in certain sectors.
The strong increase in oilseed crops would have been impossible without access to modern insecticides, which were used against the numerous pests that attack this crop.

The introduction of bee protection regulations in 1953 and other restrictions dealing with the handling of pesticides, together with successively improved knowledge of how the control work should be done and the risks associated with it, have made major contributions contributed in reducing the number of cases of bee toxicity. For example, mention can be made of the work by Åkerblom (1980), where 65 samples of dead bees were analysed for pesticides. The investigation covered the 1965-1979 period and represented most of the samples of poisoned bees submitted to the authorities.

Of these 65 samples, 51 contained pesticides in concentrations far below the LD50 values given for the herbicidal question. Thirty samples contained residues of the pesticide Fenitrotion, which was responsible for 95 % of all explained bee toxicities up to the early 1980´s. Annex 1 gives a list of the bee toxicities from 1950 until 1991, based on the bee-keepers´ own reports to the Bee-Keepers' Association.

A NEW PERIOD IN THE USE OF PESTICIDES

Between 1979 and 1983 the use of chemicals in Sweden changed. In forestry, there was a reduction in the use of phenoxyacetic acids and other pesticides as a result of strong protests. Further, we noticed a new awareness among farmers, that had been encouraged by the Board of Agriculture, the Swedish University of Agricultural Sciences, local agricultural committees, the National Federation of Farmers, the Lantmännen Cooperative Movement and the chemical companies. It is interesting to note that the amount of pesticides used by private people exceeded the amounts used in forestry.

During the 1980´s, a second generation of pyrethroids have been introduced (Stark, 1983). The substances in this group of pesticides are acutely toxic to pollinating insects. On the other hand, they demonstrate low field toxicity, mainly depending on the low dosing normally used and in some cases as a result of a repellent effect. Despite the change from the extremely field toxic organic phosphoric compounds to pyrethroids, there has still been a marginal increase in cases of bee toxicity during recent years.

TOXICITY OF PESTICIDES TO POLLINATING INSECTS

Insects exposed to pesticides absorb the compound through respiration air, feed, or directly through the body . Depending on the chemical structure of the substances, the use of solvents, and their solubility in water or fat, the uptake will take place along one of these routes. Regardless of the route in which the substance has entered the body, it causes deteriorated oxygen uptake with resulting respiratory problems.

The consequence of this is frequently an inability to fly. In some cases, the nerve system of the insects is attacked, leading to cramp and death.

Insecticides, herbicides and sometimes fungicides cause injury to bees and other pollinators. Guttation droplets on cereals may contain relatively large amounts of pesticide. During the early hours of the morning, the bees fetch water for the hive and during the dry part of the early summer the guttation droplets are frequently an easily available source of water.
The toxicity of the different pesticides to bees varies within wide limits. The effect on bees and other pollinating insects depends on factors such as spray time, temperature, distance (from the bee colony to the field), flowering biology, pesticide stability, etc. (Stark, 1992).

Risks and advantages when using pesticides should be weighed against each other. Obviously, the bee-keeping industry cannot itself be in the centre of interest in relation to the use of pesticides. By cooperation beween bee-keepers and users of pesticides it is possible to avoid damage to bees (Stark, 1992).

BEES AND BEE-KEEPING – A SURVEY

Apiculture in Sweden is conducted almost exclusively by amateurs and on a small scale. There are very few bee-keepers who entirely or mainly subsist on bee-keeping. This has resulted in the rationalization given by professional production only slightly affecting the management methods used by the amateurs, which is characterized by large variations between bee-keepers and by uncertainty as to the most suitable approach.

The result will be ineffective work and low profitability. To some extent, bee-keeper organizations have contributed to amateur bee-keeping coming to dominate this branch as a result of their efforts to attract as many members as possible with the justification that bee-keeping should be dispersed throughout the country in order to fulfil the need for pollinating insects (Stark, 1980).

On the whole, Sweden has good natural conditions for bee-keeping. Both the natural flora as well as cultivated plants offer good opportunities for yields of honey up to 40-60 kg honey per colony and year.

An increase in the size of the apiaries, towards a more professional form of management, will probably lead to the introduction of more rational working methods, which will have a stimulating effect on bee-keeping in all size categories. In order to utilize the bees for pollination by means of mobile bee-keeping, there is a decisive advantage in being able to recruit bee colonies from several different places, However, this involves an increased risk of spreading diseases between apiaries.

At present, there are about 15 000 organized bee-keepers within the Swedish Bee-Keepers Association (SBR). Together, these own 100 000 bee colonies. In addition, there about 200 professional bee-keepers with 6 000 colonies. Apart from these two groups, the number of unorganized bee-keepers is judged to be about 5 000, with 30 000 colonies. The domestic production of honey is about 3 000 tonnes which is about 40 % of the honey consumed in Sweden. The annual imports amount to about 2 500 tonnes of honey. Thus, Swedish production of honey could be doubled without the risk of over-establishment.

THE HONEY BEE

Bees belong to the Apoidea family group, of which 280 species are found in Sweden. The honey bee belongs to the species Apis mellifera. A characteristic of all bee species is that they visit flowers and exist on nectar and pollen, that they have licking, sucking mouth parts, and that the female is generally equipped with a sting. The nectar collected by the bees is stored during the journey back to the hive in a nectar sac, a widening of the oesophagus, and is re-gurgetated on arrival. The pollen is transported in special collecting devices on the outside of the body.

As regards mode of living, we usually distinguish between two groups of bees, solitary and social. Among species characterized as solitary there are, however, many who demonstrate different social features in their mode of life (Stark, 1984).

BEES AND PLANTS

It has long been known that flowers and pollinating insects benefit from each other. The bees receive protein and mineral substances from the pollen as well as energy from the nectar. Pollination is done either through self-pollination or cross-pollination. Bees play a decisive role for cross-fertilised insect-pollinated plants.

Seed-setting requires pollination in most plants. This implies that pollen must be transferred from the anthers to the stigmas of the pistil.

If anthers and pistil are from the same plant, then self-pollination takes place. In cross-pollinating plants, seed-setting is favoured if pollen is transferred from one plant to the pistil of another plant. Some cross-pollinators are self-sterile.

Pollen can be transferred, for example, by wind (e.g., rye) or by insects (e.g., clover species). As regards cross-fertilised insect-pollinated plant species, bees play an important role as pollinators. In order to inform the bee colony about plants in the neighbourhood that are attractive, scout-bees investigate the availability of suitable plants. These bees fly randomly in all directions to investigate. Some plants are visited for their pollen (brood production requires the protein found in pollen) whereas others are visited for nectar (e.g., black currants).

The composition of nectar is sometimes decisive for the attracting ability. Thus, rape and apple-trees in bloom compete for the bees. Rape has a higher sugar concentration in its nectar which means that bees prefer this crop, with reduced pollination of fruit trees as a result. However, a plant with low sugar concentration in the nectar but with many flowers per unit area and a long flowering period may be more valuable than a sparsely flowering species with higher sugar concentration in the nectar.

The shape, colour and smell of the flowers also have an effect on their degree of attractiveness. Some authors consider the smell to be the most important property

BEE FOOD

The diet of a bee colony consists of nectar, pollen, water and honey dew. The interest of the bees in the flowers on plants depends on their properties as a source of nectar and pollen. Bees also collect resins, but this does not contribute to pollination.

Nectar is a solution of different sugar species and is the main source of carbohydrate for the colony. Adult bees have no particular need for protein but live off the nectar and the honey processed from it. Pollen is just as important for the colony as nectar since pollen is the colony´s main source of protein, fat, vitamins and minerals. The need for protein varies with the development of the colony and in this respect most of it is required for the queen´s production of eggs, for brood growth and storage of protein reserves in the lipid body for winter hibernation. A bee colony requires about 20 kg of pollen per year for rearing brood. Pollen production and the biological value of the pollen depends on the species. Salix species (willow, sallow) have pollen with high biological value, whereas pollen from pine and spruce has a life-shortening effect

BEES AND THE ENVIRONMENT

The sources of air, water and soil pollution may be looked for in, e.g., industrial emissions, exhaust fumes from traffic, coal-fired power stations, the use of chemicals, and the use of chemicals by individuals (e.g. glycol).

The honey bee moves over a wide area, up to about 3 km radius from the hive, visiting numerous plants and many sources of water. Against the background of this behaviour, the bees exposed to numerous sources of pollution that imply the risk of toxicity. Tong et al. (1975) consider that contamination of the bee is possible when it flies in polluted air.

The same authors state that the bee occasionally confuses polluting substances in powder form with pollen and take it back to the hive. Atmospheric contamination of the flower´s nectar results in the same degree of pollution as if the bee colony had been directly contaminated. Plants absorb and concentrate specific elements from the soil (e.g., pesticides, heavy metals). Bees visiting these plants will thus become secondary transmittors of pollution. Pollution has also been found in the bee (body, mouth parts) and/or in collected pollen, nectar, water and propolis, as well as in honey (Stark, 1991).

MATERIAL AND METHODS

The experimental side os situated in five places around Sweden.
Two experimental fields in south, two in middle and one in north Sweden.
Area of monitoring around the apiaries was calculated to be 3 km in diameter. Main activity around these places is agriculture land, airport and highwayarea.
Experiments were carried out in five geografical replicants. In each place three replicants on each different samplingmehtod (pollen, dead traps, bee counter, live bees and developing of brood) were used.
Collection of pollen was carried out by using pollentraps. Collection of dead bees by using dead-traps and bee activity was recorded by using electronic bee-counter. Each experimental place was represented by another bee race.
Biological test of collected bees and pollen was made, *Drosophyla* and *Aedes egypty*.
Chemical anallysas were made by GLC and liquid chromatography.

RESULTS

Daily variation of dead bees was found in different experimental places. The difference depending on bee race, geographical posision, beekeeper and the variation in the environmental area at the specific apiary. The biggest difference was found when bees were infected by Varroa mites. Apiary located near to airport shows the highest amount of antioxidants and top of antioxidants was correlated to the highest number of dead bees in dead traps.

One of apiaries located in south Sweden gave indication of Lindan and chemical analyse gave the result of presence of Lindan.
Lindan was found in samples collected in early spring of 1994 and 1995. Amounts of Lindan in dead bees was 0.01 microgram HCH and 0.02 microgram HCH / bee.
This indication shows that HCH isomers found in dead bees were longlife isomers. Later in season Lindan only was indicated on detection level.
Statistical analyses indicated presence of antioxidants in dead bees.

SUMMARY AND CONCLUSIONS

Unfortunately, there is a conflict between bee-keeping and use of chemicals in environment. The use of chemicals results in numerous bee colonies being more or less damaged every year. Frequently this depends, however, on carelessness or lack of knowledge about the properties of the pesticide and the use of chemicals in general

This presented paper is showing that presence of chemical substances, as antioxidants, has a very strong effect on bees. The equestion is weather this effect is direct or results of additional effects through combination of other chemicals or degradation products of chemicals. The strongest correlation has bee found between number of dead bees in bee traps collected per day and antioxidants.

One case of acut bee death was caused be Lindan and the very day when HCH was deceted. A number of dead bees of several thausands were found.

The expanding use of chemicals in agriculture has not taken place in isolation from the total expansion in the use of chemicals in society. It may be stated that the environment of the bee has been changed from being fairly clean to an environment where the flying insect visiting flowers and collecting water is exposed to chemical pressure from the entire environment. Part of the difficulties in explaining the occurrence of bee toxicity may be sought in effects of this complex mixture of chemicals being added to effects of normal pesticides.

This additive effect of the use of chemicals may explain several per cent of the present winter losses of bees. The average winter mortality of bees is around 12 % and in many cases this cannot be explained by diseases or famine. Numerous bee colonies lose their orientation in the spring which in some cases depends on chronic toxicity.

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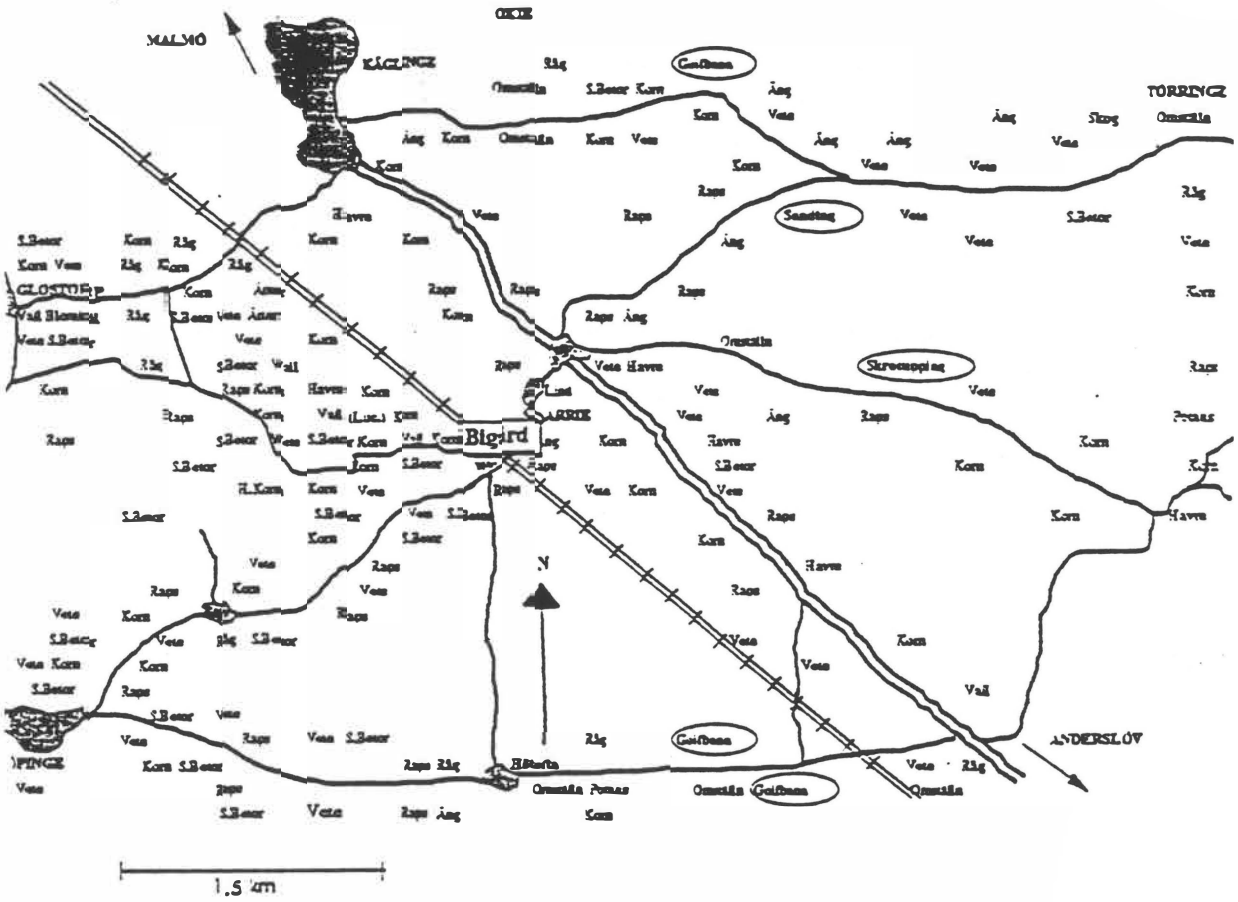
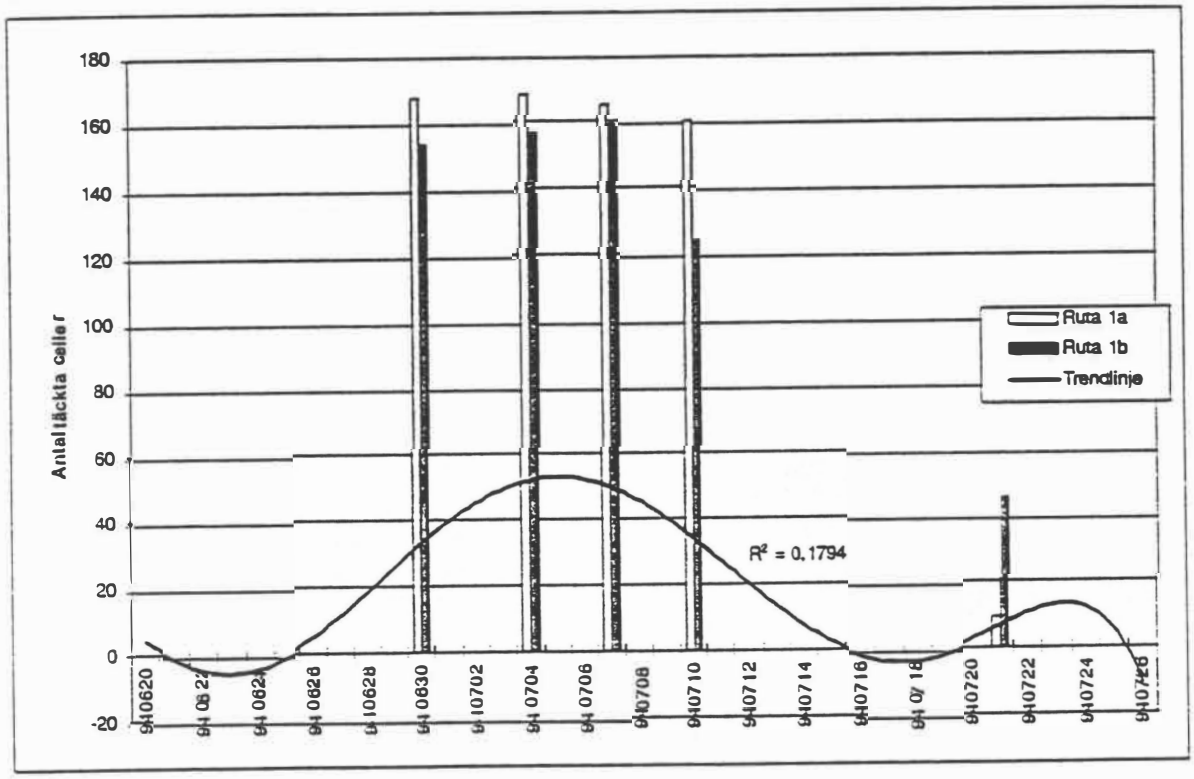
APPEDIX 1

General map of an apiaries surrandings



APPENDIX 2

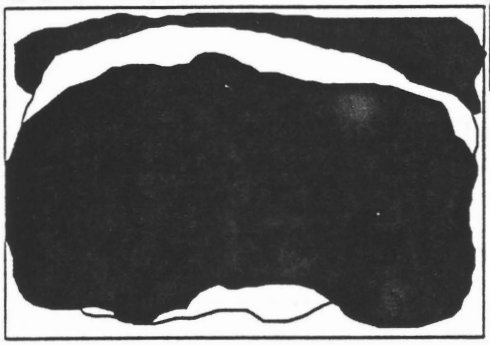
Bee-brood development in one hive



APPENDIX 3

Example of bee-brood development

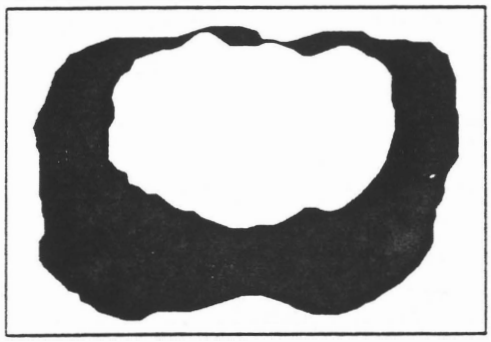
Datum 930714



Det äldre ynglet i kakans överkant håller på att krypa ut. Cellerna i kakans underkant är putsade och färdiga för äggläggning...

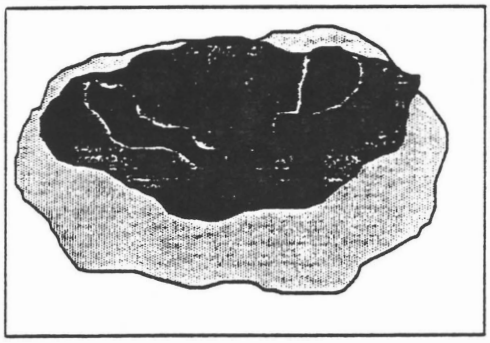
	Höjd	Bredd
Ägg	0	0
1-2:a larvstadiet	0	0
3-4:e larvstadiet	0	0
Täckning	22	34
Färdigt bi	20	34

Datum 930719



	Höjd	Bredd
Ägg	0	0
1-2:a larvstadiet	0	0
3-4:e larvstadiet	0	0
Täckning	21	35
Färdigt arbetsbi	14	15

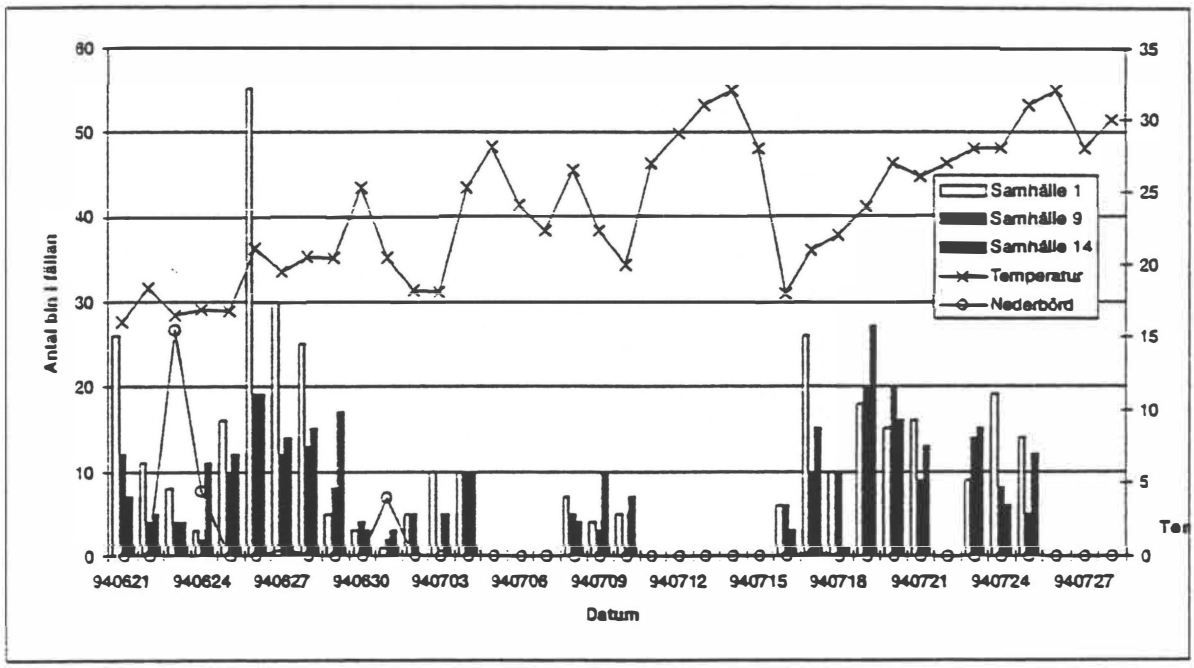
Datum



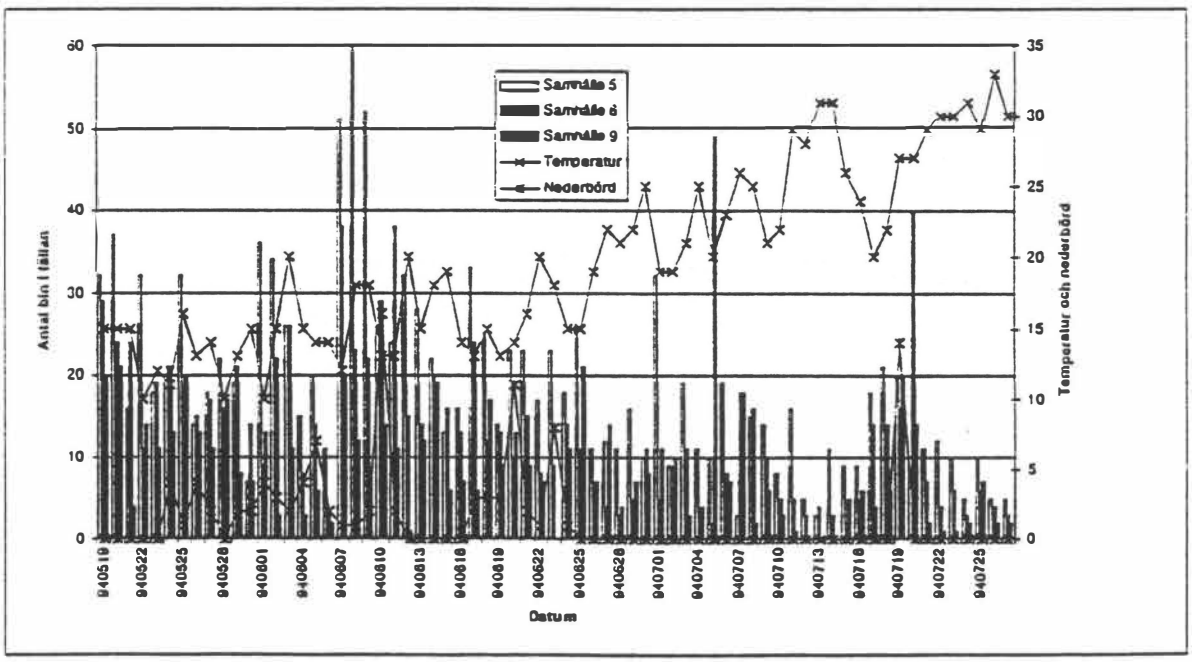
	Höjd	Bredd
Ägg	18	36
1-2:a larvstadiet	15	34
3-4:e larvstadiet	0	0
Täckning	0	0
Färdigt arbetsbi	0	0

APPENDIX 4

Examples of dead-trap and weather data collection



Figur 4. Avläsningar av Dead-Traps tillsammans med nederbörd och temperatur i Ågarp.



Figur 5. Avläsningar av Dead-Traps tillsammans med nederbörd och temperatur i Arrie.

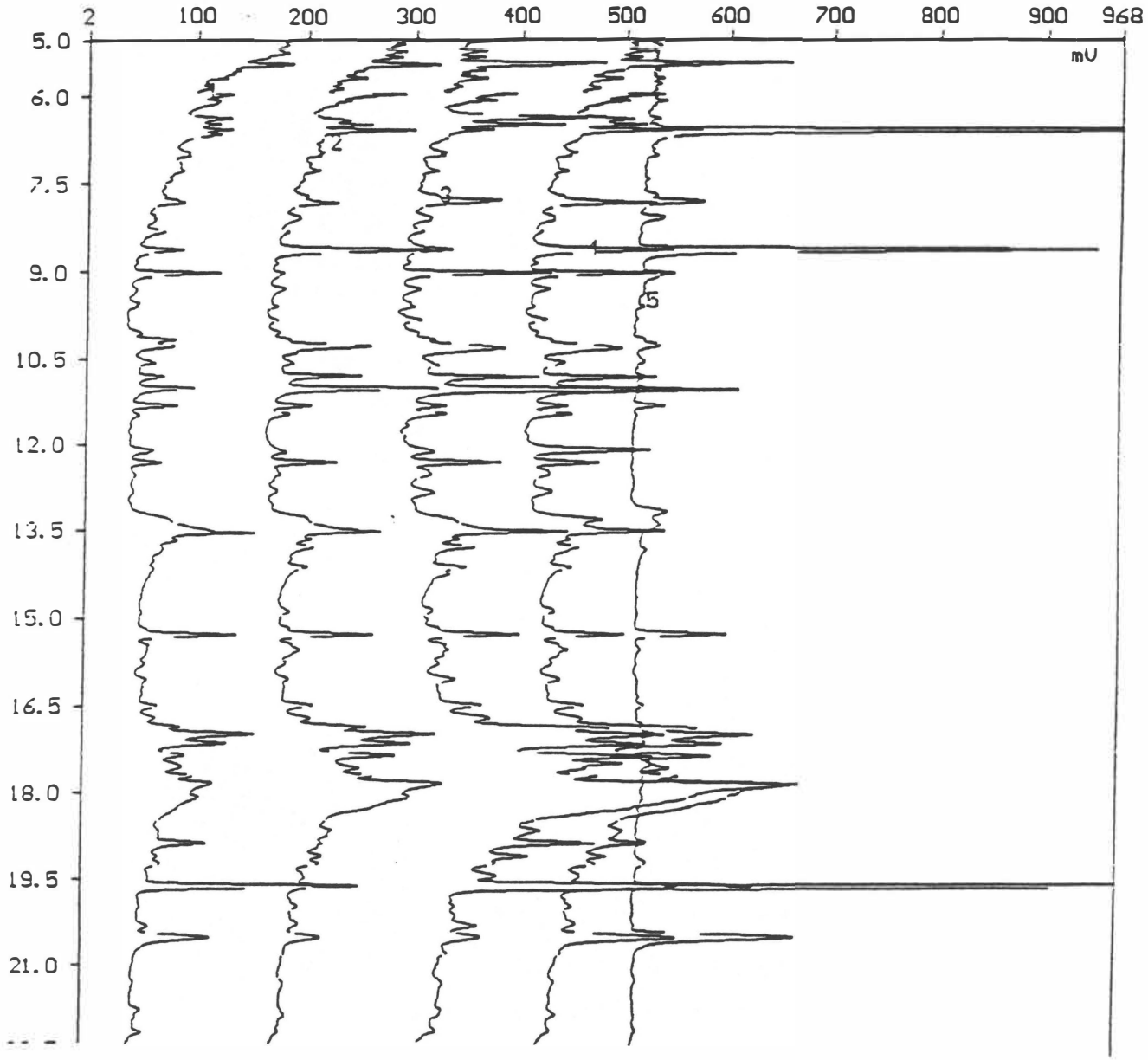
APPENDIX 5

Chromatogram comparison

DP.Chromatogram Comparison K% Page 2
GynkoSoft V5.21 1994-11-22/17:12

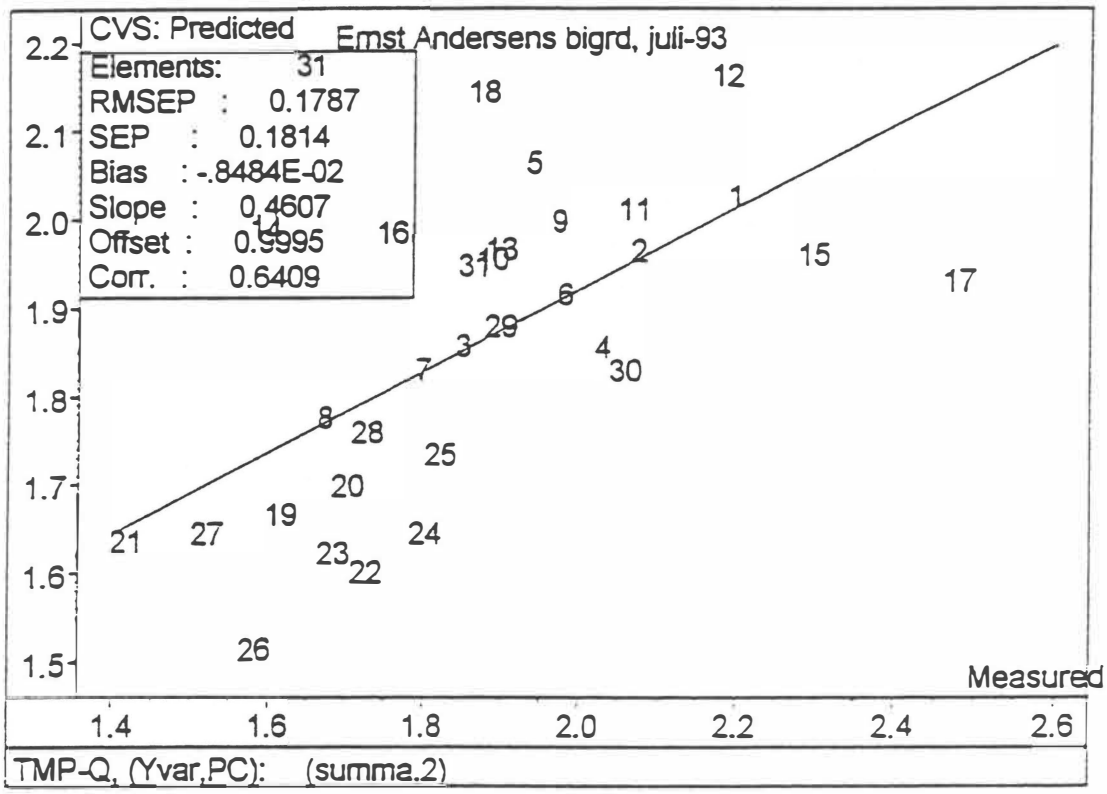
No Normalization.

1	F:<EC>AS940422-4	W 45:15-17 93/94	(SE-30) 1994-04-22/11:26	pollen 930630
2	F:<EC>AS940422-5	W 45:19-21 93/94	(SE-30) 1994-04-22/12:07	" 930708
3	F:<EC>AS940422-6	W 45:23-25 93/94	(SE-30) 1994-04-22/12:48	" 930709
4	F:<EC>AS940422-7	W 45:27-29 93/94	(SE-30) 1994-04-22/13:29	" 930719
5	F:<EC>AS940422-8	W 45:33-35 93/94	(SE-30) 1994-04-22/14:10	" 930729



APPENDIX 6

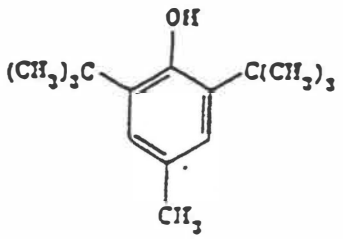
Example of the statistical model explains the 41% variance of number of dead bees in july 1993.



APPENDIX 7

One of the antioxydants correlated with beedeath.

Butylated Hydroxytoluene. BHT; DBPC; *2,6-di-tert.-butyl-p-cresol:* 2,6-di-*tert.*-butyl-4-methylphenol; Ionol; Impruvol; Vianol. $C_{15}H_{24}O$; mol. wt. 220.34. C 81.76%, H 10.98%, O 7.26%. Prepd. from *p*-cresol and isobutylene: Stillson, U.S. pat. 2,428,745 (1947 to Gulf).



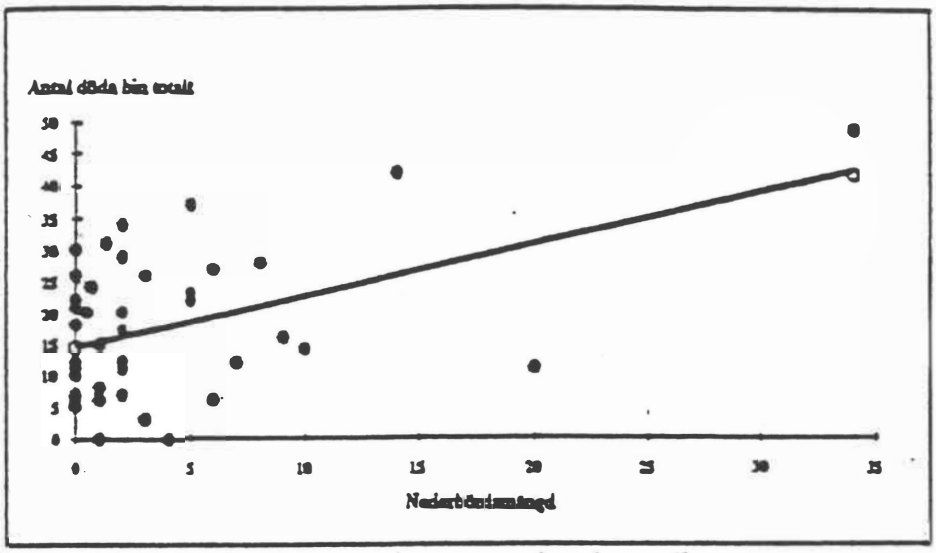
Crystals, d_4^{20} 1.048. m. 70°. b. 265°. Flash pt. open cup: 260°F (127°C). Insoluble in water. Freely soluble in toluene, soluble in methanol, ethanol, isopropanol, ethyl methyl ketone, acetone, Cellosolve, petr. ether, benzene, most other hydrocarbon solvents. Solubility in liquid petrolatum (white oil): 0.5% w/w. More soluble in food oils and fats than butylated hydroxyanisole. Good solubility in linseed oil.

Use: Antioxidant for food, petroleum products, syn. rubbers, plastics, animal and vegetable oils, soaps. Anti-skinning agent in paints and inks.

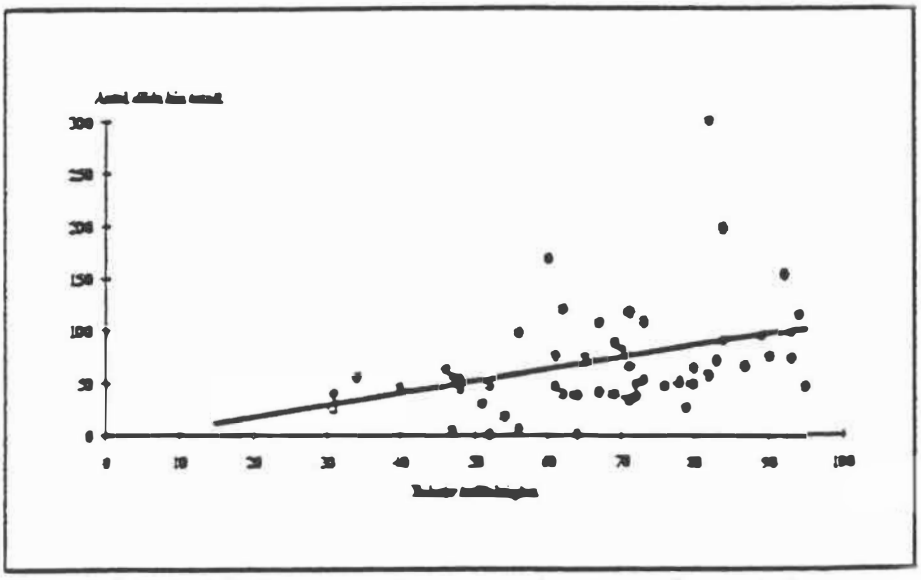
Human Toxicity: May cause sensitization type of dermatitis. Practically no systemic toxicity.

APPENDIX 8

Simple linear regression line between rainfall - relativ humidity and total number of dead bees.



Figur 14. Enkel linjär regression av sambandet mellan nederbördsmängd och totala bifångsten i Arrie.



Figur 15. Enkel linjär regression av sambandet mellan relativ luftfuktighet och totala bifångsten i Ågarp.

Appendix 24

O. Boecking, K. Wallner
Control of Varroosis - a necessity for beekeepers, why?, how?, difficulties!

ICP-BR Symposium on „Hazards of Pesticides to Bees", 17-19. Sept. 1996, BBA Braunschweig Germany

Control of Varroosis - a necessity for beekeepers, why?, how?, difficulties!

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W orld-wide beekeeping with *Apis mellifera* L. bees is endangered by the mite *V. arroa jacobsoni* Oud. since this mite, originally a parasite of *Apis cerana* Fabr. found a new host in *A. mellifera*. The excellent reproduction conditions for the mite in *A. mellifera* bees favours the mite population growth to the extend that infested colonies die from varroosis within a few years if the mite population growth is not regulated by the beekeeper. Obviously this impact changed beekeeping practices totally. But the control of varroosis is basic for successful beekeeping. Moreover, in Germany the treatment of colonies infested with V arroa is liable due to government regulations. If a beeyard is infested with V arroa all colonies have to be treated ones in a year by the beekeeper. For this the veterinarian board can force the beekeeper to treat his colonies at a particular time with a specific treatment („BIENENSEUCHEN-VERORDNUNG" [24.Nov.1995, BGBI.I. 1549, § 15] V. *Regulations concerning varroosis*).

The control of varroosis can be distinguished into 4 different methods; 1. chemical treatments (f.e. APIST AN, Perizin), 2. „gentle-chemical" treatments (f.e. Formic Acid, Lactic Acid), 3. physical methods (f. e. thermo-box) and 4. bio-technical methods (f.e. trap-comb).
A combination of these different methods can lead to a INTEGRATED PEST CONTROL SYSTEM. Using such a system as part of the beekeeping practice requires that on one hand the beekeeper is well informed about the different methods and on the other hand that he is willing to work a little more compared to the easiest method available. Each method mentioned above requires specific conditions for their use. For example Perizin and Folbex-VA-neu are only efficient in broodless colonies.

F or the control of varroosis today there are 6 different products approved by the German government, also obviously more products are used by the beekeepers. Those products with hydrophilic attributes are; APITOL (cymazol), Illertisser Milbenplatte (formic acid). And those with lipophilic attributes are BA YV AROL (flumethrin), Cekafix (coumaphos + synergism), FOLBEX-VA-neu (brompropylate) and Perizin (coumaphos).

Looking to the market statistics a clear trend can be seen that many beekeepers are using now the methods which can be easiest handled (plastic strips), irrespective from costs and possible disadvantages in using these methods.

As a fact the control of varroosis warranted the sustain of beekeeping till today.
Also most products and methods used by the beekeepers are helpful to reduce the mite population growth in the bee colonies till today, lipophilic chemicals might create new problems in future.
The use of lipophilic chemicals induces inevitably residues in bee wax and in honey. There are different mechanisms which can lead to residues in honey; the wrong use of medicaments, contaminated winter food which can penetrate the spring honey and contaminated bees wax. Small contaminated wax particles and the penetration of residues from wax into the honey can be responsible for residues in honey.
As a rule the higher the concentration of residues in wax is, the more residues can be detected in honey.
Some active substance, like Brompropylate, Coumaphos and to some extent Fluvalinate can be detected by analysis in the honey from a varroacide level at 1 ppm in the contaminated wax (WALLNER, 1995). This means that these pesticides have a weak binding strength in wax. In contrast the synthetic pyrethroid Flumethrin has a extremely high binding strength in wax, since in the investigation mentioned even at a Flumethrin level of 400 ppm in wax no residues could be detected in the honey (detection limit in honey Flumethrin 5 µg/kg).
But on the other hand those pesticides with a low tendency to migrate favour the accumulation in the beeswax and contaminated wax particles may lead to residues in honey later, as mentioned above.
The analysis of beeswax of more than 500 samples revealed that in all countries that uses Acaricides contaminated wax can be detected [1-10 mg/kg] (WALLNER, 1995). Often various pesticides can be detected in one sample.
Since wax is a recycling product in the beekeeping management system world-wide, the accumulation of residues can be expected . Presently many acaricides can be found in wax-foundation which are sold on the market. As a consequence even beekeepers who are using bio/technical methods to reduce the Varroa mite population growth in their colonies will have chemical residues in the bee products if they buy wax foundations on the market. This phenomenon has been proved with paradichlorobenzene too, which is used for wax moths

control (WALLNER, 1992). Till today there is any methods available which might allow to clean wax from the residues mentioned above.

As a consequences of subletal residues which are found in the beeswax the Varroa mite can develop resistance against the acaricides used. Since 1992, a reduction in the effectiveness of APISTAN against Varroa in a large, rapidly expanding area of northern Italy has been reported (LOGLIO & PLEBANI, 1992; LODESANI *et. al.*1995). The LC_{50} of mites from areas where treatments with fluvalinate are no longer effective was about 25 - 50 times higher than that of susceptible mites (MILANI, 1995).

Also the chemical control of varroosis warranted the sustain of beekeeping till today the negative side-effects are creating new problems, as pointed out above. Since bio/technical methods and „gentle-chemical" treatments (f.e. Formic Acid, Lactic Acid) are available and their effectiveness are comparable with those of chemical treatments their use is depending on the readiness of the beekeeper only.

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Appendix 25

M. E. Szalai, M E Molnar, Z S Pacs, L Lennert
New chemical control against Varroa mite in Hungary

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New Chemical Control Against Varroa Mite in Hungary

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New Chemical Control Against Varroa Mite in Hungary

Due to the density of apiaries and migratory beekeeping as an 'industrialized" form in the country, together with bee behaviour, we can't think of apiaries or regions without mites. The effect and damage of the Varroa mite in Hungary varies on a wide scale because of different external factors. Our aim is Varroa control with minimal use of chemicals, and introduction, propagation of a new chemical in the country.

At present biological methods and materials of natural origin themselves are not sufficient for proper protection of colonies against mite infestation and damage thus synthetic chemicals are also needed. These chemicals can get contact with honey and wax as well.

Timing and proper use of mite killers can minimize these contacts and accumulation in wax as well.

Production technology, reproduction cycle and status of the colony should be considered in timing control. To avoid harmful effects the best period for chemical control can be after the honey flow in late summer, early September. During this period the wintering population develops and its health condition has significant importance for the future of the colony.

Since honey harvest is finished it is good period to minimize honey contamination and at the same time the lesser brood, especially pupae, the more contact of chemicals and mites occur.

Putting mite killers on different carrying materials (like wood, plastic, etc.) between combs can be very effective.

The treatment of the colonies took place from August for 1 month.
In1994 the treatment was between 10 August and 10 September. After this period amitraz was used for three times to check the effect, results can be seen on Fig. 1.

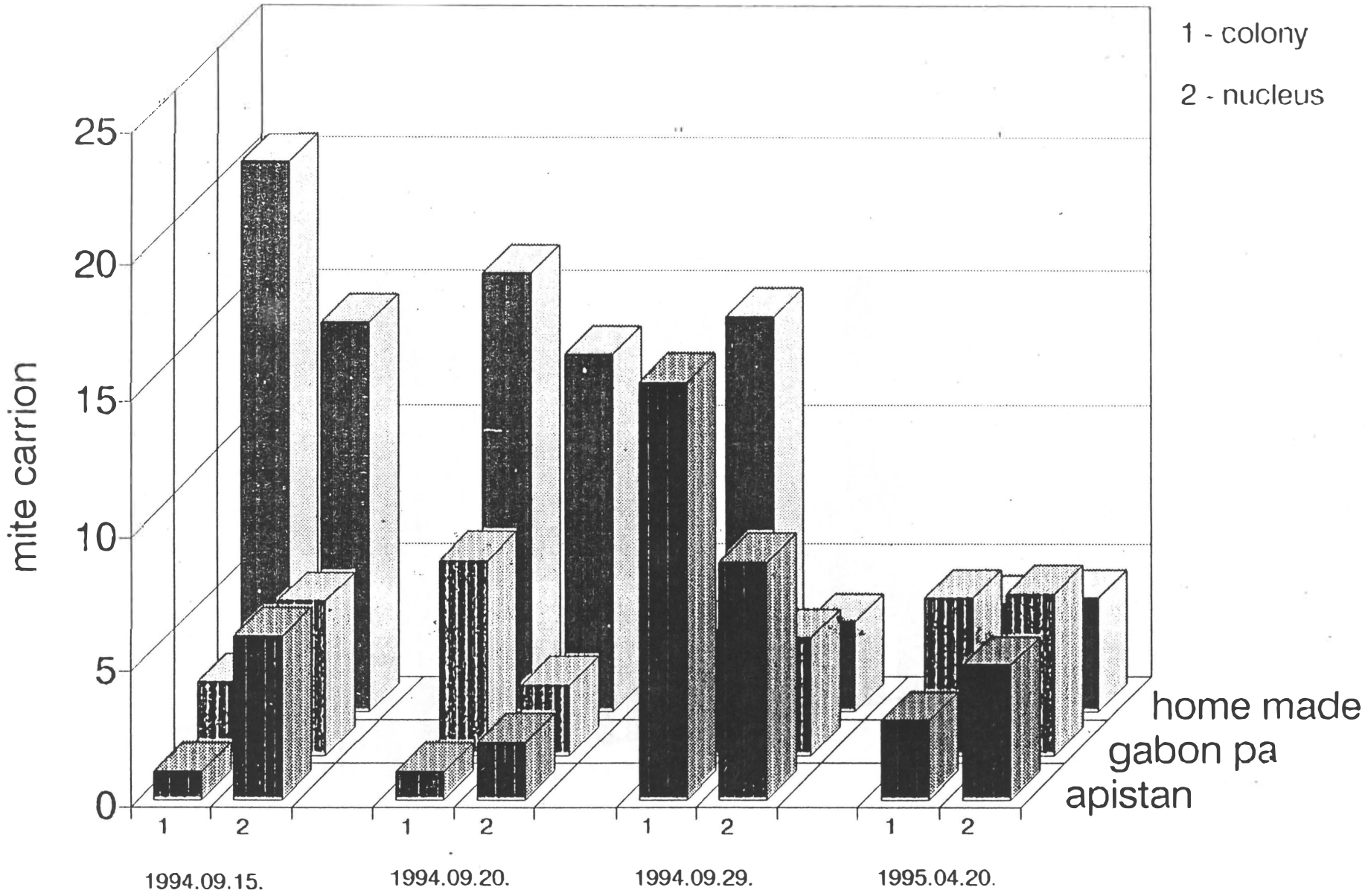
In our trial three different type of chemlcals have been used.
Two chemicals (Apistan and Gabon PA 92) have shown practically the same results with no significant difference between.
The third one - "home made" material - resulted in weaker effect, having significant differences comparing to the others.

In 1995 the treatment was between 22 August and 20 September in 50 colonies. The effect of Apistan and Gabon PA 92 can be seen on Fig. 2.
Fig. 3 and 4 show the mite infestation before the treatments, based on the results of the control (check) procedure with amitraz smoke. This diagnostic procedure was on 22 August. Four groups of colonies could be established bosed on mite infection. These groups were used in further treatments and analysis.
The next check (10 Okt.) shows the effectiveness of the different chemicals.

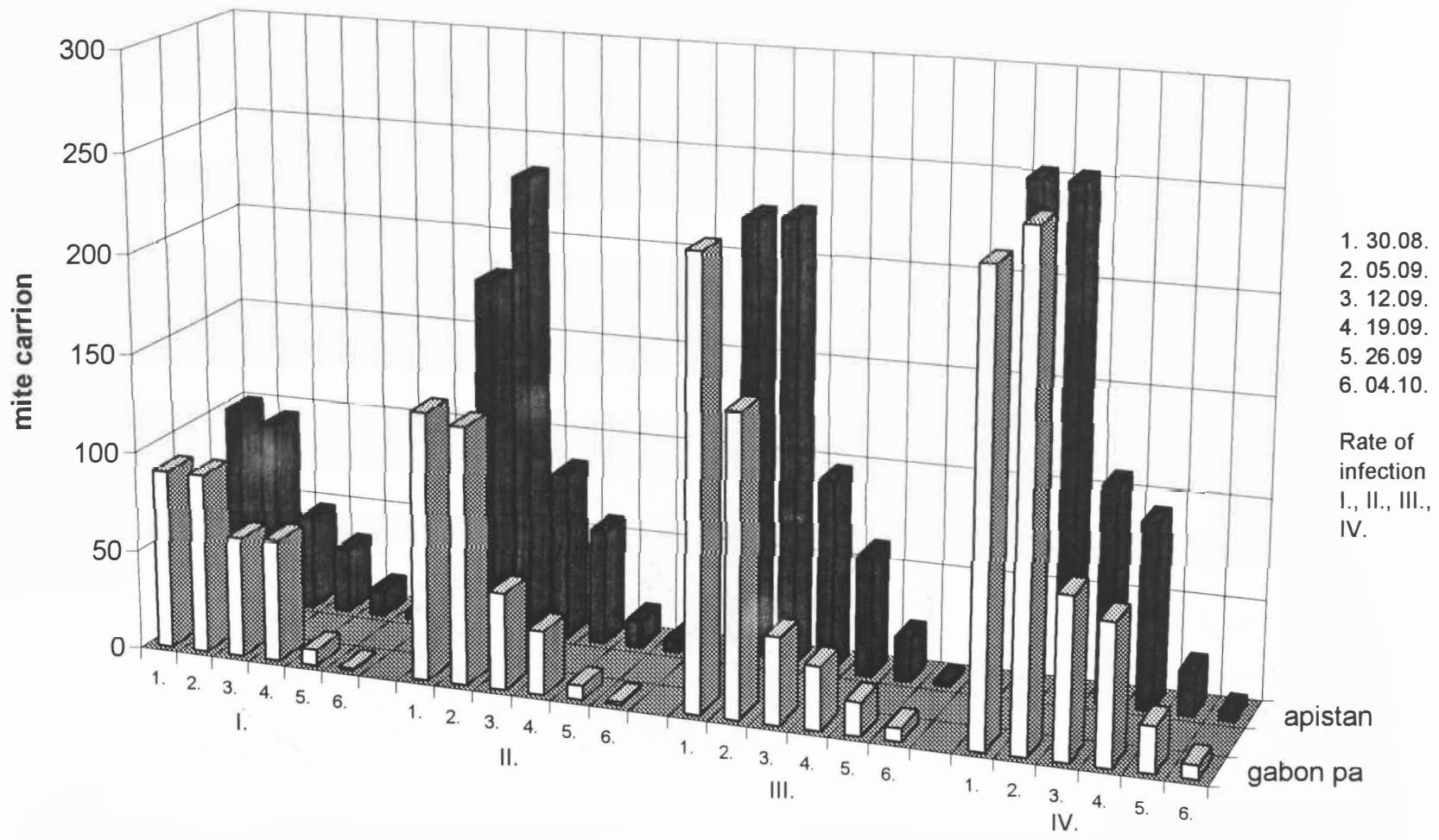
Conclusion

In our three- year experiment carried out on fifty and one - hundred colonies the acrinathrin based compaund significantly reduced the number of Varroa mites, while sid effects could not be observed.

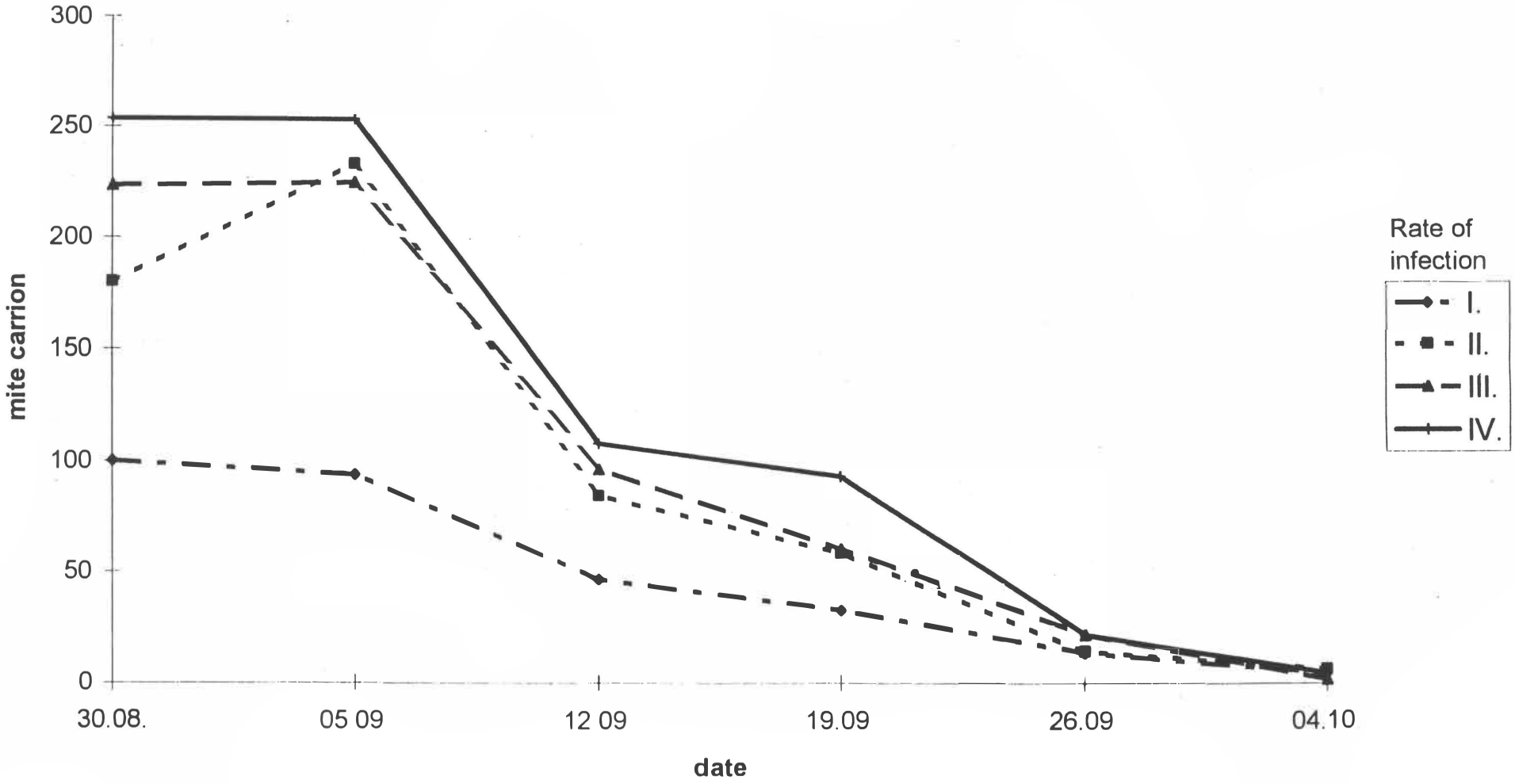
Several chemicals' effect on Varroa mite



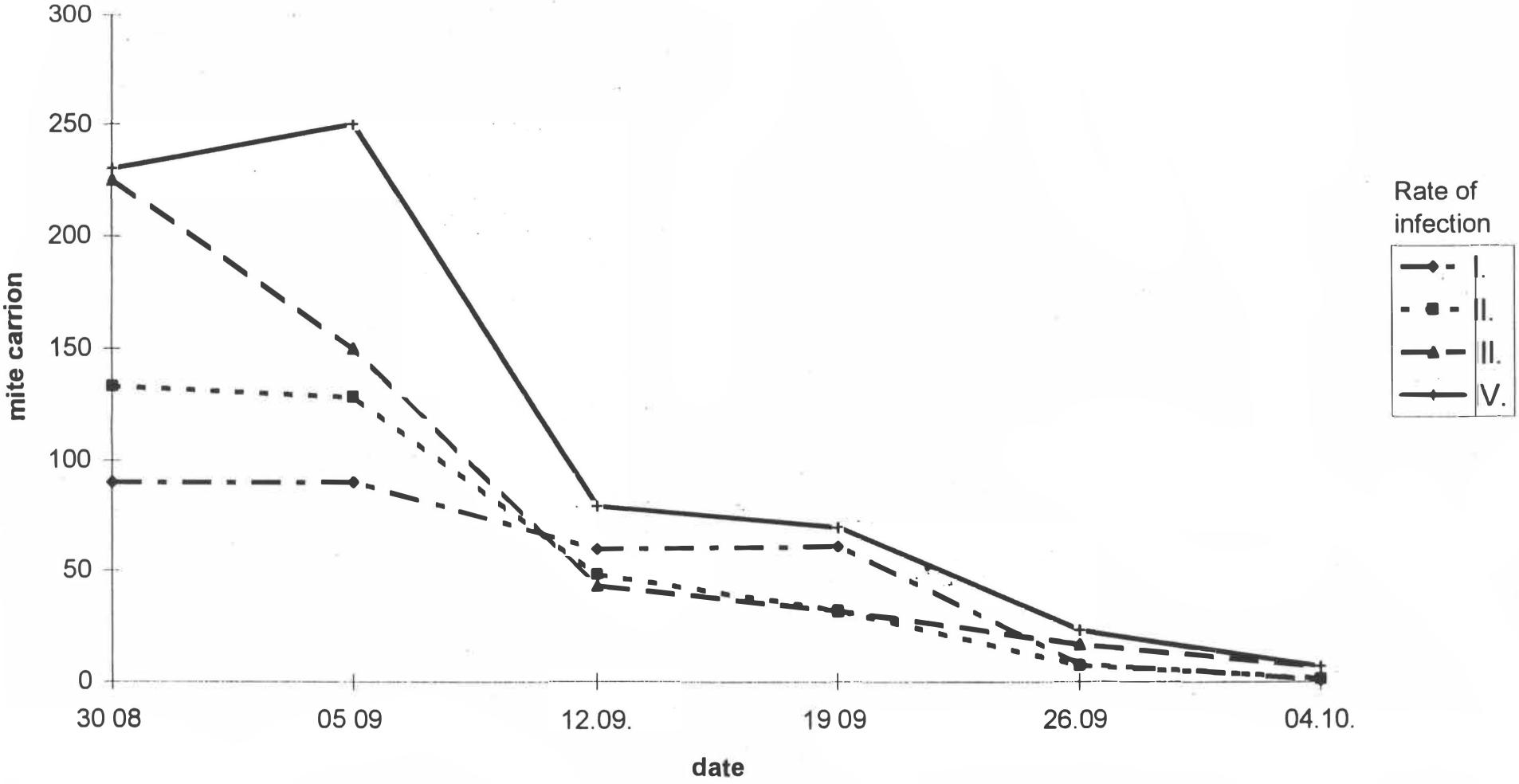
Several chemicals' effect on Varroa mite



Several chemicals' effect on Varroa mite
APISTAN, 1995



Several chemicals' effect on Varroa mite
GABON PA 92, 1995



Appendix 26

H. Geffcken
Mode of action and efficacy of APITOL ® against Varroa

Mode of action and efficacy of APITOL® against Varroa

Hermann Geffcken

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(Short version)

Description of the drug:

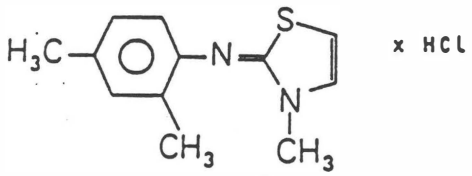
APITOL® was developed by CIBA-GEIGY, as a drug formulation for diagnosis and control of the honey bee pest *Varroa jacobsoni*. It is registered for use in Germany since 1991.

The original active substance - a basically reacting product with the common name "cymiazole" and the chemical name "2-(2,4-dimethylphenylimino)-3-methyl-4-thiazoline" - is a rather lipophilic compound with good solubilities in organic solvents (e. g. 80% in methanol and benzene), but with a remarkable solubility in water only under acid conditions (20% at pH 3). It was developed for the control of ticks and shows there a "detacher" effect: ticks drop down heavily damaged but not necessarily killed. CIBA describes the compound as a cyclic amidine and is deducting from this the interpretation of its neurotoxic action. From literature we know that related compounds, e. g. formamidines, inhibit the enzyme MAO (monoamide oxidase). This inhibition causes "k.o.-effects". Another effect of formamidines consists in the activation of octopamine receptors. This may cause hypermotility and paralysis. The biochemical mode of action being still unknown in detail there is no evidence of an inhibition of acetylcholine esterase and a synaptic block by this. The amidines therefore seem to be suitable for application against mites resistant to organo-phosphates. A change between APITOL® and the other drip-on formulations (e.g. Perizin, Cekafix) in Varroa control may thus minimize the hazard of setting up a resistance.

Dissolution of the original compound "cymiazol" in hydrochloric acid forms a saltlike derivative with a remarkable solubility in water of about 65% at 20 °C. This is the active ingredient "cymiazol hydrochloride" of the antivarroa drug "APITOL®" (table 1). The active ingredient of APITOL is a rather heat-stable compound, colourless, odourless, but harshly acid tasting. To increase the rapidity of dissolution CIBA recommends dissolving the needed quantity in a little portion of handwarm water previous to dilution to the accurate concentration.

For worldwide use CIBA has recommended the application of APITOL by the drip-on method as well as by the in-feed method with syrup, but in Germany only the drip-on method is licensed. We got to know both methods and observed some differences between several different Varroa control designs.

Tab. 1: Basic Data on APITOL®

Registered trademark:	**APITOL®**
Active ingredient (a.i.):	
Code number:	CGA-192'357 (CGA-50439 x HCl)
Common name:	cymiazole hydrochloride
Chemical name:	2-(2,4-dimethylphenylimino)-3-methyl-4-thiazoline hydrochloride
Structural formula:	 x HCl
Empirical formula:	$C_{12}H_{14}N_2S$ x HCl
Molecular weight:	254.78
Purity of technical material:	≥ 99 %
Melting point:	220 - 221 °C
Vapour pressure at 20 °C:	2.0 x 10^{-4} Pa (1.5 x 10^{-6} Torr)
Colour:	Colourless
Odour:	Odourless
Taste:	Harshly acid

Solubilities at 20 °C

		= g/litre
Water:	65 %	650
Isopropanol:	3.66 %	36.6
Acetone:	0.12 %	1.2
Toluene:	27.9 ppm	0.0279

Formulation:

17.5 % water soluble granules (code: A-7367 A) for bees.

Slightly changed from:
"Technical Information APITOL GRA 17.5" - CIBA-Geigy Ltd., 1992 - API/12.06(TI02) -

In this report we confirmed and completed data and comments already published in the cited literature by some observations noted along field trials performed by order of and sponsored by CIBA-GEIGY, Basel, and made some conclusions from this. The observations concerned some effects of different designs of Varroa control on their tolerability (by bees) and efficacy (against Varroa mites).

Mode of action:

Dropping a medicated liquid on the bees will lead to mutual cleaning and subsequent trophallactic dispersal of the honey sac contents as well as to brushing a general contamination of body surface and hairs from the directly wettened bees to their neighbours passing by in close contact. On the other hand presentation of a medicated syrup or candy forces ingestion as food and trophallactic dispersal if provisioning by the food chain is needed in the colony. If a drug acts only systemic you have to expect no differences in the effects of dropping and feeding it. But if there exists an effect due to contact toxicity less effectivity of application by feeding than by dropping may happen. During our field tests we could not observe significant differences in the effects of Varroa treatments by dropping an APITOL solution on the bees and by feeding it: APITOL acts truly systemic.
Bees are able to distinguish pure and APITOL medicated syrups. They prefer the former as may be concluded from the remarkable delay in the uptake of APITOL medicated syrup compared with the normal syrup feed given as last portion of winter feed in october from the hive bottom. Systemic action needs ingestion and ingestion brings the involved honey sac contents into competition with the attractive "normal" honey sac contents and the attractive food stores of the colony. Compared with tasteless drugs or drugs with a strong, activating or disturbing odour application of drugs with a specific taste may need some more efforts to make sure that ingestion (due to mutual cleaning or true feeding) and trophallactic dispersal is maintained at a high level of intensity without too much fluctuations or variations. The attractivity of the drug solution will become better if its taste is made as similar to honey (the honeybee's natural food) as possible. To increase its attractivity the drug solution should therefore not become sweetened only by pure sucrose, but better by a mixture of sucrose (or other sugars used for feeding bees) with honey or by pure honey. Since the instructions for use only refer to "to sweeten" or to "sugar" this recommendation does not hurt any license. Varying the sweet character of the medicated syrup by adding honey seemed to provide more attractivity than varying the acid taste by adding e. g. citric acid, as was tested by RADEMACHER (**9**).

Treatments should be preferably placed in the warm centre of the colony. For feeding syrup we used flat 500 ml polystyrene dishes (as designed for storage of food in refrigerators) filled with the syrup - the syrup covered with some straw or with polystyrene foam ("Styropor") chips - and put them in a flat space (2-3 cm) between the two hive storeys which we provided by simple wooden spacer frames set between the storeys. Thus prepared medicated syrup and normal syrup were both taken up within one week.

Perizin does not need sugar for good dispersal and shows a strong, perhaps irritating odour. Its

active ingredient is mainly lipophilic and dispersed in the drip-on solution by a detergent (solubilizer). The wet surface contamination may then be licked off by other bees or brushed away in the hairs of other bees e. g. in the close-up of the winter cluster. If water is dried, the remaining fat-soluble "smear" may be also easily distributed from bee to bee like the yellow pollen oils which make bees and wax "golden" during dandelion honey flow. A contact action of Perizin is probably the reason of its rapidity and depends on its lipophilic properties. These observations are confirmed by VAN BUREN **(2)**.

Efficacy against mites and tolerability by bees:

We compared the effects of different Varroa control methods by treating normal colonies in autumn. The colonies were of different origin and size and a seven days' diagnosis of the spontaneous drop down of dead mites showed remarkable differences in the infestation rate. The test groups were established therefore providing similar variations in the Varroa infestation rates by combining colonies of different mite fall levels during a selection week before the start of the trial. Colony strength was the second step criterion. If in a field test all colonies of a test group receive the same amount of the drug according to the informations of use but without individual regard to size and to degree of infestation, effects of colony size and infestation rate on the efficacy and tolerability of designs for Varroa control should become visible.

The comparison between the effects of fumigation (with Folbex VA), drip-on treatments (with Perizin and APITOL) and contact strips (Bayvarol) confirmed the idea, that there is a decrease in rapidity and inevitability of the spreading of drugs in a bee hive from an "external" inforced distribution (fumigation or fogging - evaporation does not produce longer lasting precipitations on exposed surfaces) over a "passive" distribution by the bees by brushing active substances from areas with impregnated surfaces (contaminated bees, prepared carriers) to the "active" distribution by trophallaxis after mutual cleaning or food ingestion. In the latter case there happens a race between food exchange between groups of bees and food consumption in the single bee: If regurgitation is not possible nearly the whole honey sac content has passed about 6 hours after ingestion into the intestine, and in the first hour after the ingestion the concentration of the active substance rises in the hemolymph to its maximum, decreases during the following hours and reaches about 12 hours after uptake a low, long lasting (ineffective) level. Because trophallaxis does not meet a colony as a uniform whole and because its intensity depends on the actual needs in the colony it is important to provide a good distribution - seen "spacially" (spreading the droplets on the bees in all bee ways) and/or "temporally" (e.g. pro-

longation of a treatment by feeding, repetition of drip-on).

Very remarkable is the high efficacy (in the range of 94 to 99%) of Folbex combined with its high tolerability by the bees (less than 0,5% of the bees died during the observation time). Tolerability was in the same range as in the colonies without treatment!

Compared with the figures from the Folbex-treated colonies the number of dead bees in the APITOL and Perizin groups were slightly elevated but within the same range - due to the systemic action of these substances.
The dropping of dead bees decreased also with increasing colony strengths in the APITOL and the Perizin groups like in the control colonies without treatment, while in the Folbex colonies the number of dead bees was independent from colony size - on a very low level.

In the APITOL and Perizin groups, too, the relative amount of dropped mites decreased with the total number of mites in the colonies. In the APITOL group we observed moreover an increasing variation in these figures with the decrease of the infestation rates - but the colony with the maximum mite fall in this group had one of the lowest mite infestations. Folbex treatment did not show this dependence from infestation rate.
In all groups the increase of colony strength brought more or less decrease in effectiveness - only a slight one in the colonies treated with Folbex and Perizin but a more obvious in the APITOL group. The variation of these figures increased with the increase of colony strength, too - slightly in the Perizin group, clearly in the APITOL group. The treatments had been performed with exactly the same quantity of the drug in each colony without respect to its strength. The observed effects show the influence and importance of a correct dosage and optimal distribution especially in the APITOL treatment, caused by its clear systemic action.

Variations in the dosage of APITOL® as well as the repetition of APITOL® dripping as only chemical control treatment (of nearly broodless colonies) for several years show that the amount of the drug and the administration method (drip-on method) licensed for use in Germany provide the optimal combination of high efficacy against the mites and tolerability by the bees and allow a living with Varroa on a low infestation level without any decrease in honey yield and colony strength.

If the infestation level is found to be low after the first, diagnostic, application of APITOL®, the repetition one week later to complete the full control treatment is not necessary for this year - as is already observed in the common practice of Perizin treatments, too.

Acknowledgements

I want to mention gratefully the support of CIBA-GEIGY, Basel.

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Appendix 27

J. J. M van der Steen
Report of the group "Toxicity Test For Bumblebees"

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REPORT OF THE GROUP "TOXICITY TEST FOR BUMBLEBEES"

The work of the group has been concentrated on the development of acute oral and acute contact LD_{50} tests for bumblebees. The members of the group have met and corresponded frequently and they have written a frame method for the determination of the acute oral and contact LD_{50}. During the "Arbeitstagungen der Institute für Bienenforschung e.V." in Lunz (1994) and Berlin (1996) presentations were given about the progress of the method development.

In Lunz results were presented of the tests about the link between the size of a bumblebee and the contact LD_{50}. The size of the bumblebees was determined by measuring the length of the forewing. The bumblebees were divided in "small" and "big" ones. Small bumblebees had forewings of maximal 10 mm and big bumblebees had forewings of minimal 11,5 mm. The test substance was Dimethoate 40%. The test bees were workers of *Bombus terrestris* L.. The LD_{50} of "small bumblebees, determined in two tests were 5,5 µg and 4.1 µg/bumblebees. The LD_{50} of "big" bumblebees determined in three tests was 10,5 µg, 8,7 µg and 13,0 µg/bumblebee. These results show the link between the size and the LD_{50} and, more important the need to take bumblebees of a certain size to perform the tests.

In Berlin the draft acute oral LD_{50} test was presented. The principle of the test is that:
1. the bumblebees must be fed individually,
2. workers of young colonies and of average size must be used and
3. per concentration at least 30 bees have to take in the test solution.

We presume that 30 bees per concentration is the minimum number of bees needed for a reliable test.

On 12/06/96 the frame methods for the acute oral and contact LD_{50} were discussed with research workers* from several institutes and firms who are working on the methods for testing the effects of pesticides on bumblebees. This meeting was meant to prevent the coexistence of different methods in the future, to discuss each others work and to find agreement on the test method. Agreements were made on the frame method for the acute oral and contact LD_{50} for bumblebees, based on the draft method produced by the working group. The methods of testing the effects on brood and the semi-field and field test are still in an experimental phase. So no agreements about these tests were made. The participants agreed upon: a) to keep each other informed and b) that these tests will be discussed and that the frame protocols will eventually be made when such an informal meeting will be organized again.

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Appendix 28

J. J. M van der Steen, C. Gretenkord, H. Schaefer
Method to determine the acute oral LD_{50} of pesticides for bumblebees (*Bombus terrestris* L.)

METHOD TO DETERMINE THE ACUTE CONTACT LD_{50} OF PESTICIDES FOR BUMBLEBEES (*Bombus terrestris* L.)

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Introduction

With respect to the method to determine the acute contact lethal dose 50% (LD_{50}) the significant relation between the size of a bumblebee and its sensibility for pesticides must be taken in account. So for the LD_{50} test, only bumblebees of an average size can be used.

Principle of the test

Individually adult bumblebee workers are exposed to a range of concentrations of the test substance dissolved in acetone.

Validity of the test

The test is valid if:

- the average mortality in the control of the test does not exceed 10%,
- the LD_{50} of the toxic standard meets the specific range.

Method

- Collection of the bumblebees

Worker bees of average size and age are taken from young colonies. The use of recently emerged bees, recognizable by their greyish fur, should be avoided.

- Preparation of the bees

The mean weight of the bees is determined.

The bees anaesthetized with CO_2 as short as possible.

- Preparation of the test solution (test substance solution)

The test substance is dissolved in acetone. For test substances with low acetone solubility, vehicles like water (max 10%) can be used. The test substance is first dissolved in the vehicle and this solution is later on diluted with acetone. In case the test substance is indissolvable in the vehicles mentioned, contact the manufacturer.

- Housing and keeping of the bumblebees

The bees are kept in the dark in a climate room at a temperature of 25 ± 2°C.

- Number of bees per concentration test solution

30 bumblebee workers.

- Number of concentrations

Per replicate the bees are treated with 5 concentrations of the test substance: two between the presumed LD_{100} and LD_{50}, one at the presumed LD_{50} and two between the presumed LD_{50} and LD_0.

- Number of replicates

An acute contact LD_{50} consists of two replicates in time, preceded by a range finding test.

- Administration of the test solution

After the bees are anaesthetized, 1 µl test solution is pipetted on the ventral part of the thorax between the base of the 2_{nd} and the 3_{td} pair of legs.

- Mortality

After treatment, the bumblebees are housed together and fed with sucrose solution ad libitum.

Mortality is recorded 24 hours, 48 hours and 72 hours after feeding and mortality rates are compared with control values.

- Positive and negative control

Simultaneously with the test solution, three concentrations of a pesticide of known toxicity (Dimethoate 40% or Parathion 25%) and acetone without the pesticide is administered. The LD_{50} of the positive control is calculated.

For the positive control Dimethoate 40% or Parathion 25% can be used.

- Data and reporting

The LD_{50} and if relevant the ED_{50} (effective dose 50%), is calculated with an appropriate statistical method.

The LD_{50} and the ED_{50} can be given as µg pesticide / bumblebee or as µg pesticide / gram bumblebee.

Appendix 29

C. Gretenkord, W. Drescher
Laboratory and cage test methods for the evaluation of the effects of insect growth regulators (Insegar®) on the brood of *Bombus terrestris* L.

Laboratory and cage test methods for the evaluation of the effects of insect growth regulators (Insegar®, Dimilin®) on the brood of *Bombus terrestris* L.

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Summary

A larval test and a cage test were developed in order to evaluate the effects of insect growth regulators on the brood of bumble bees. The larval test allows the calculation of the LC_{50} and LD_{50} of pesticides for bumble bee larvae of different ages. With the cage test method the effect of insect growth regulators on bumble bee colonies under semi-field conditions can be exactly monitored. Furthermore it allows to obtain pollen from the test colonies for the analysis of pesticide residues. Insegar, classified as hazardous to honeybees, proved to be non-hazardous to *B. terrestris*. Dimilin, classified as non-hazardous to honeybees, proved to be hazardous to *B. terrestris* under laboratory and cage conditions.

1 Introduction

The intention of our investigation was to find out whether bumble bees have a similar sensitivity to insect growth regulators (IGR´s) as honeybees. As test substances we used Insegar and Dimilin, two typical IGR´ s, which are widely used in agricultural practice.

The methods for the determination of the LC_{50} and LD_{50} of pesticides for honeybee larvae (e.g. WITTMANN *et al.* 1985, CZOPPELT 1990) could not be transfered to bumble bee larvae. Contrary to honey bees, it is so far not possible to rear isolated bumble bee larvae successfully *in vitro* and to apply the test substance directly to individual larvae. So we developed a new larval test for bumble bees.

The cage test method used so far (GRETENKORD & DRESCHER 1993) is appropriate to determine the effects of conventional pesticides on mortality, flight activity and behaviour of bumble bees under semi-field conditions. It is however difficult to monitor possible negative effects on the brood of the colonies, because (1) the 6 m^2 *Phacelia* plot does not provide enough nectar and pollen for stronger colonies with 80-100 workers. It is therefore necessary to give additional food, which causes an undesirable dilution effect. (2) The development of individual larvae cannot be traced, since the structure of the comb changes permanently and (3) the number of dead and removed larvae cannot be quantified exactly. Therefore this cage test method was modified.

2 Material and Methods

Larval Test

Test groups: First, we removed egg cups from colonies of our laboratory stock and kept them in the incubator at 32 °C and 55-60 % R.H. until hatching. Then the number of larvae

was standardized to 10 per egg cup by removing larvae or adding larvae from other egg cups. After that, each egg cup was kept together with three nurse bumble bees from the same colony in small rearing boxes (12,5*7*5 cm) in a climate room at 28 ± 1 °C and 50 ± 5 % R.H .. The test groups were fed with sucrose solution and pollen dough. On the 7th day, the first larvae started to pupate. After pupation of all larvae the workers were removed. The pupae were again kept in the incubator until the imagines emerged.

Test design: Larvae of three different ages were tested, namely 1, 4 and 6 day old larvae. The test substances were dissolved in pollen dough or 50 % sucrose solution, which were fed to the test groups for 24 hours. The test series were started with the recommended concentration for field use. If negative effects on the brood could be observed, the test series were continued with lower concentrations. For each larval age, application medium and test substance 3 replicates as well as one untreated control were carried out.

With the mortality rates for the different test concentrations the LC_{50} was calculated, using probit analysis. For the calculation of the LD_{50}, the amount of food had to be determined, which a single larva consumes during the application period. For this, the consumption of contaminated pollen and sugar solution for each test group during the 24 h application period was measured. At the same time, the food consumption of three workers without larvae kept under the same conditions was determined. With the help of these values the average food consumption of a single larva during the application period could be calculated.

Cage Tests

For the cage tests, colonies with 50-70 workers were used. The nest boxes were placed in the ground outside the cages to protect the colonies from overheating. After few days, when a sufficient flight activity was reached - about 10 foragers on the *Phacelia* plot during the whole day - the colonies were moved to the laboratory in the morning. The foraging workers were left in the cage. In order to reduce the colony size, the queen together with 5 nurse workers and a defined amount of brood was transfered to a new nest box. The brood included all brood stages in a small number: 4-6 egg cups, a brood clump with 1-2 day old larvae, a brood clump with 3-4 day old larvae and a brood clump with 5-6 day old larvae. After the colonies had been experimentally reduced, they were again connected with the tube to the cages. The foragers, which were left in the cage, were now able to go back to the colony.

So we had standardized colonies with a defined number of nurse bees, foragers and brood. With colonies of this size, no additional feeding of pollen was necessary (only sugar solution was provided). The effect of the contaminated pollen on the brood could now be exactly monitored.

One day after the experimental reduction of the colonies the test substances were sprayed on the *Phacelia* plot. Before this, all the pollen collected so far was removed. The test colonies remained in the cages for 2-3 weeks after application. Afterwards they were kept in the laboratory for 2 more weeks in order to look for possible malformations of the emerged imagines.

3 Results and Discussion

Larval Test

Insegar was tested with the recommended concentration, which is 100 ppm a.i .. In all tests, there was no higher mortality of the larvae or pupae compared to the control groups, where mortality was low, 7 % on average. All of the emerged imagines had a normal appearence and showed no malformations. So the LC_{50} of Insegar for *B. terrestris* larvae must be higher than 100 ppm a.i .. This is confirmed by the investigation of WAEL *et al.* (1995): They fed 100 ppm a.i. Insegar in 50 % sucrose solution over 24 h to whole *B. terrestris* colonies and observed no negative effects on the brood.

With Dimilin we got different results. Depending on the test concentration and the larval age, the test larvae died 2-7 days after application. The dead larvae were removed from the larval clump by the workers or remained in the wax covering, where they dried out. F or Dimilin, 9 different concentrations in the range of 0.3 to 375 ppm a.i. were tested. The calculated LC_{50} was independent on the application medium, pollen dough or sucrose solution, but different for the 3 larval ages: for 1 day old larvae about 1 ppm a.i., for 4 day old larvae 3-4 ppm a.i. and for 6 day old larvae about 100 ppm a.i. (table 1). Although there is a considerable difference between the two values for 6 day old larvae, it is statistically not significant. According to WITTMANN (1982), the LC_{50} for honey bee larvae is 3.7 ppm a.i. for the larval stages L2 to L4, which is comparable to the value for 4 day old bumble bee larvae.

The LD_{50} of Insegar is in a µg-scale (table 1). Since there was no higher mortality with the concentration tested, the actual LD_{50} must be higher then the dosis the larvae consumed during the experiment. For honeybee larvae, NITSCH & VORWOHL (1992) determined a LD_{50} of 17 ng a.i .. This means that *B. terrestris* larvae would be more than a hundred times less sensitive to Insegar. The reason for the big difference between the sensitivity of honeybee and bumble bee larvae to Insegar can not yet be explained. It could be due to different juvenile hormones.

Table 1 LC_{50} and LD_{50} of Insegar and Dimilin for larvae of *B. terrestris*

application medium	larval age	average food consumption / larva / 24 h (	Insegar		Dimilin	
			LC_{50} (ppm a.i.)	LD_{50} (ng a.i.)	LC_{50} (ppm a.i.)	LD_{50} (ng a.i.)
pollen dough	1	mg) 6.5	> 100	> 650	1.18 3.04	7.7
	4	17.4	> 100	> 17	137.79	52.9
	6	37.1	> 100	40	0.82 4.10	5112.0
sucrose solution	1	7.4	> 100	> 37	77.43	6.1
	4	13.6	> 100	10		55.8
	6	45.8	> 100	> 740		3546.3
				> 136		
				0		
				> 45		
				80		

The calculated LD_{50} for Dimilin for 1 day old larvae was 6-8 ng, for 4 day old larvae about 50 ng and for 6 day old larvae 3.5-5 µg. There are no significant differences between the values for the two different application mediums. But the difference between the LD_{50}´s for 4 and for 6 day old larvae is striking.

Cage Tests

We carried out five experiments with Insegar, one with the recommended dose (600 g/ha; 100 ppm a.i.) and four with the double recommended dose (1,200 g/ha; 200 ppm a.i.). In all these tests, no brood damage and no malformed imagines were observed.

Altogether, eight experiments with Dimilin were conducted, three with the normal (300 g/ ha; 250 ppm a.i.) and five with the double recommended dose (600 g/ha; 500 ppm a.i.). In all of these cage tests, almost the whole brood died two days after application, with the exception of some older larvae, which were shortly before pupation at the time of application. This supports the results from the laboratory tests, where the older larvae proved to be much less sensitive to Dimilin.

During the 14-21 days in the cages the colonies were not able to rear new brood, although the queens kept on laying eggs. Dimilin may have an ovicidal effect on the ovaries of bumble bee queens. This effect has already been proved for some insect species from different taxonomic groups (GROSSCURT 1978). The test colonies only recovered when they were transfered to the laboratory, where the contaminated pollen was removed and fresh pollen was fed.

Dimilin caused malformed cocoons in the cage tests, which we also observed in the larval test. These cocoons were spherical and the whole surface was speckled with brown dots. Normally the cocoons of bumblebees are egg shaped and the upper half has a light yellow colour.

Residue analyses: In our experimentally reduced test colonies, the proportion of foragers is higher than in a normal colony. More pollen is collected and stored than can be consumed. This offered the possibility to remove 1-2 gram of pollen each day, which is enough for an analysis of pesticide residues.

The analysis of Insegar residues in pollen was carried out for cage tests with the double recommended dose (1,200 g/ha; 200 ppm a.i.). The Insegar residues found for the 1st day after application was 217 ppm a.i. (average of 2 replicates), which correspond to the applied concentration. On the 2nd day it was 22 ppm a.i. and on the 7th day 7.5 ppm a.i ..

F or the analysis of Dimilin residues we used pollen from cage tests where the normal recommended concentration (300g/ha; 250 ppm a.i.) had been applied, that is one fourth of the dose used for Insegar. The Dimilin concentration in pollen on the 1 st day after the treatment was 62 ppm a.i., which is one fourth of the applied concentration. Considering the different doses applied, the residues for both IGR´ s were on a similar level. The Dimilin residue on the 2nd day was 8 ppm a.i. and on the 7th day 2 ppm a.i ..

The residue analyses revealed, that the toxic concentrations determined in the larval test were also reached under semi-field conditions. The results of the cage tests correspond to the results found in the laboratory. With Insegar, no brood damages were observed in the cage tests, although the Insegar concentration in the pollen of the first day after application was more than twice the concentration fed in the larval test.

The residues of Dimilin in pollen, even from the 7th day after application, were in the range of the LC_{50} values for 1-4 day old larvae. But the LC_{50} for 6 day old larvae was not even reached on the first day after application. This supports the observations from the cage tests, that older larvae, already 1-2 days before pupation at the time of application, were not affected by the Dimilin treatment.

Insegar, classified as hazardous to honey bees, proved to be non-hazardous to bumble bees under the conditions we tested. From these results, we can assume that it will not be hazardous to bumble bee colonies under field conditions. This is at least true for *B. terrestris*.

Dimilin, classified as non-hazardous to honey bees, proved to have very negative effects on bumble bee brood. It is, therefore, likely that it might also have hazardous effects in the field.

This summer (1996), field tests were carried out by a coworker of our institut, Ms. M. PLATEN. The analysis of this data still has to be finished. If these field experiments will prove Dimilin to have sustainable damaging effects on bumble bee colonies, the following fundamental questions arise: (1) Should Dimilin then be generally classified as "hazardous to bees"? (2) Or should it be classified as "hazardous to bumblebees" additional to "non-hazardous to honeybees"? And if so, will the use of Dimilin be restricted?

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Appendix 30

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First experiences to test side-effect of Alsystin on bumblebees (*Bombus terrestris* L.) in the field

First Experiences to Test Side-Effect of Alsystin on Bumblebees (*Bombus terrestris* L.) in the Field

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Until now methods according to the Guidelines of BBA part VI 23/1 and EPPO Guideline 170 were developed to test side effects of pesticides on bumblebees in the laboratory and under semi-field conditions. However there is nothing known about the effects of pesticides on bumblebees in the field. A field test would complete the testing repertoire.

Preliminary tests on larvae of *Bombus terrestris* in the laboratory showed a lethal effect of Alsystin, an IGR - Insect Growth Regulator. It would be interesting to see if it has the same effect on bumblebee colonies in the field.

In July 1995 six small bumblebee colonies (max. 47 workers) which were reared in the laboratory were placed near a Phacelia field (2400 m²).

Analogous to field tests with honeybees the following parameters were determined:

- **Density of flying insects in the field**

On five randomly chosen spots of 1 m² all *Bombus terrestris*, honeybees and other bumblebees were counted for 1 minute. The average number of bees per 5 m² was used to calculate the density over the 2400 m² of Phacelia.

- **Flight activity of each colony**

At the flight hole of each colony all movements were registered for 10 minutes daily.

- **The origin of collected pollen and an estimation of the amount of pollen in the colonies**

With the help of a microscope the origin of the collected pollen was determined. The different coloured pollen was looked at daily and the amount was estimated.

- **The number of workers**

Every day all workers of one colony were counted three times (in short intervals).

- **The mortality of larvae**

The number of dead larvae in and in front of a colony was counted. From pictures taken daily of the colony the number of larvae, eggcups and cocoons were counted.

On the 6th of July, three days after the colonies were placed at the field, Alsystin (800g/ha in 400 l water) was applied around 2:00 p.m.

The density of flying bumblebees did not change during two days after the application (s. Fig. 1).

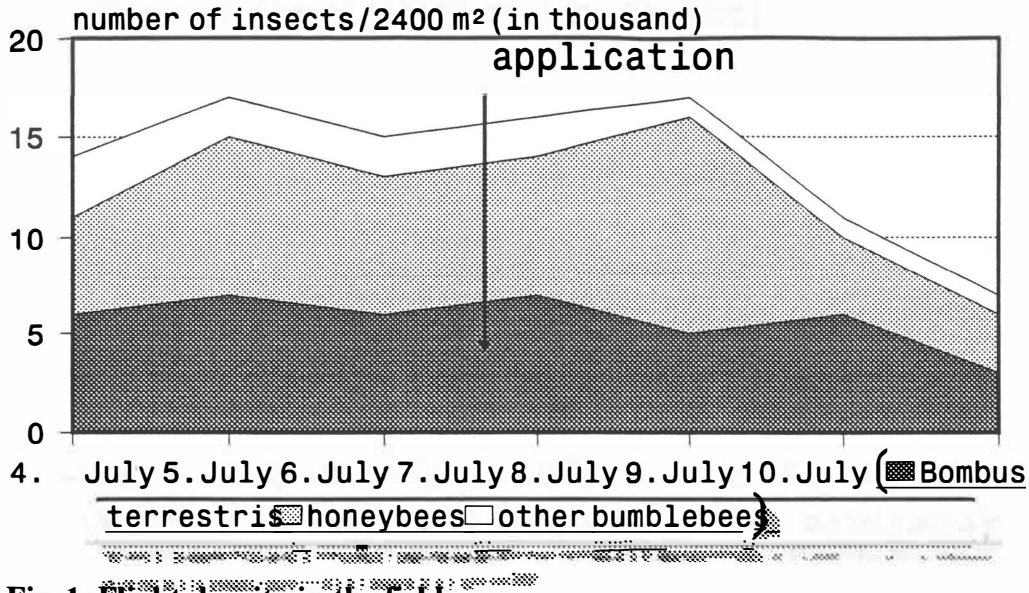

Fig. 1: Flight density in the field.

Almost the same number of honeybees and bumblebees were found in the field. Honeybees had the highest number of bees (10-7-95: 115 20) . The highest number of registered bumblebees were 8 160 (5-7-95). When there are 8 000 honeybees it suggests 8 colonies as 1 000 workers forage, but 8 000 bumblebees may come from 80 colonies considering 100 workers fly of an averaged sized colony. Other bumblebees like e.g. *Bombus pratorum*, *Bombus lapidarius* were found less often on Phacelia.

Every colony showed flight activity during the experiment (s. Fig. 2).

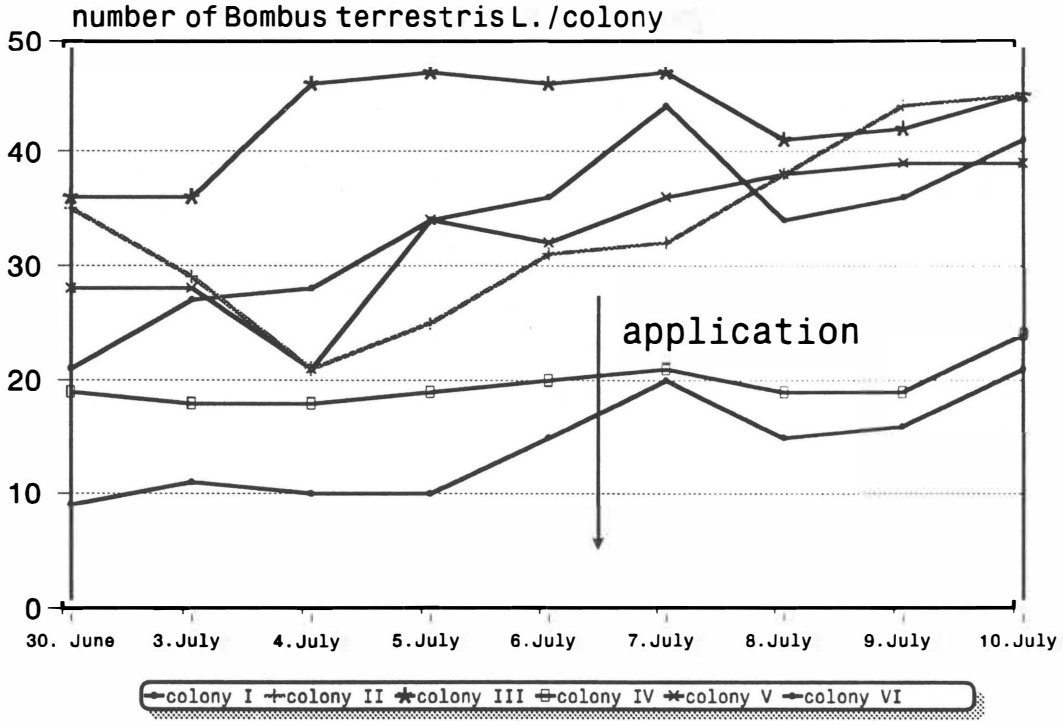

Fig. 2: Flight activity of the colonies.

The number of flying bees counted in 10 minutes varied between 0 (4-7-95 Colony I) and 20 (7-7-95 Colony II). Application of Alsystin did not influence flight activity of the colonies.

Analyzing pollen samples showed the variation in bumblebee foraging behaviour. One day there was a lot of Phacelia- and Asparagus-Pollen in the colonies. Another day most of the pollen came from clover or different species of rosacea. Over the course of the experiment every colony collected pollen from the Phacelia field.

The total number of workers in each colony did increase, but not more than 20 workers during the experiment (s. Table 1).

Table 1: Average Number of Workers in Each Colony during the Experiment

Colony no.	I	II	III	IV	V	VI
average number of workers	14	33	43	18	33	33

Counting dead larvae in or in front of a colony was almost impossible because the workers carried them away or wasps or birds ate them. Therefore only 2 to 9 dead larvae could be found a day (s. Table 2).

Table 2: Mortality of Larvae

	4. July	5. July	6. July	Application	7. July	8. July	9. July	10. July
n = 6 colonies	0	0	0	–	2	9	5	4

Looking at the photos of one colony showed the loss of larvae. After application one colony lost 10 larvae without increasing the number of cocoons. This colony also lost 4 eggcups without increasing the number of larvae. Therefore one may conclude that Alsystin is damaging larvae in free flying bumblebee colonies outside in the field.

This first experiment illustrates how difficult it is to test the side effects of pesticides on bumblebees in the field. It is unpredictable how a bumblebee colony will develop. Therefore it is almost impractical to interpret the results. To work out a standardized method for field tests is also very difficult.

For testing the impact of IGR on a bumblebee colony it is necessary to find all dead larvae. Therefore a trap for dead larvae needs to be constructed. The duration of this first field test was too short to say anything about long term effects on the colonies e.g. producing queens and males or size of colony. Testing bumblebee colonies takes more time than testing honeybee colonies. In following field tests these factors need to be considered.

The preliminary results of this field test gave clear evidence that bumblebee colonies are affected by this high dosage of Alsystin in the field. Even more colonies are affected than expected. This field test also out lined the differences between honeybees and bumblebees. Honeybees are effected by Alsystin, too, but in another way. Testing IGR only with honeybees makes it impossible to say anything about the damage to bumblebees. To find out if a pesticide is harmful to bumblebees in nature more field tests are necessary.