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INTERNATIONAL COMMISSION FOR PLANT - BEE RELATIONSHIPS

PROCEEDINGS
OF THE FOURTH INTERNATIONAL SYMPOSIUM
ON THE HARMONIZATION OF METHODS FOR TESTING
THE TOXICITY OF PESTICIDES TO BEES

MAY 15 - 18 , 1990
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ICI - Agrochemicals, England

ATTENDANCE:

Appendix 1 lists participants. The meeting expressed its regret that Drs. GERIG and VAN DER STEEN were unable to attend.

OPENING SESSION

Dr.J.N. TASEI, Secretary of the International Commission for Plant Bee Relationships, opened the meeting.

Dr. V. VESELY (Chairman of the Organising Committee) welcomed all participants on behalf of both the Organising Committee and the Bee Research Institute at Dol. Thanks were expressed to the Director of the Institute for Atomic Energy at Rez for the provision of accommodation and symposium facilities in the Vltava Congress Centre. Thanks were also expressed to those organisations which had provided financial and material support for the symposium. Dr. Vesely then briefly reviewed the programme of the meeting and drew particular attention to the urgent need for the continuing development of methods for combating Varroa, and for continuing efforts to investigate the presence and fate of pesticide (varroacide) residues following chemical control programmes.

Dr. BACILEK (Secretary of the Organising Committee) addressed organisational aspects of the symposium, and reiterated thanks to those organisations which had provided financial and material support for the symposium.

Dr. STEVENSON (Symposium Chairman) welcomed participants, thanked Drs. Vesely and Bacilek for their role in organising the symposium and wished all present a rewarding and productive symposium. Dr. Stevenson then briefly recalled the history of the Working Group, from its inaugural meeting in Wageningen in 1980, with subsequent meetings in Hohenheim (1982) and Rothamsted (1985), and commented on the excellent progress made during this time towards harmonisation of methods for assessing the hazard of pesticides to bees. He also noted that the principles followed during the assessment procedure are increasingly being applied to other areas of research, such as assessment of the hazard of pesticides to predatory and parasitic arthropods.

Dr Stevenson then drew the attention of participants to the difference between the terms "Harmonisation" and "Standardisation", as they relate to the development of methodology. Harmonisation was defined as the comparison of methodology to determine the degree to which results obtained by different methods can be judged acceptable and comparable. In contrast, Standardisation was defined as the strict definition of methodology with the aim of obtaining reproducible results. It was emphasised that the objective of the Working Group is the Harmonisation of methodology. Dr. Stevenson also reminded participants of the key difference between Toxicity and Hazard. Toxicity is an inherent property to cause harm to an organism, whereas hazard is a function of the toxicity of a substance and the exposure of an organism to that substance. It was noted that assessment of hazard should remain the major focus of attention of the Working Group.

Dr. Stevenson summed up the primary objective of the Working Group as the harmonisation of methodology for assessing the hazard of pesticides to bees, and identified this as the key theme underlying the activities of the Working Group since its inception in 1980.

SUMMARY OF THE MEETING (Prepared by Dr. C. Inglesfield)

Abstracts/texts of the papers are included in these proceedings as Appendices 2-25.

The subject matter of papers presented at the meeting can be classified into five broad areas:

1) Progress towards harmonisation of guidelines for assessing the hazard of pesticides to bees.

2) National schemes for assessing hazard to bees from reports by beekeepers.

3) Further development/experience with laboratory and field methods for assessing hazard to bees.

4) The use of pesticides in Varroa control.

5) Others.

Taking these areas in turn:

1) Progress towards harmonisation of guidelines for assessing the hazard of pesticides to bees

Dr. OOMEN described progress made to date in developing the recommendations of the ICPBR as a draft EPPO Guideline for evaluating the hazard of pesticides to Honey bees (Appendix 2). This document was prepared by Dr. Oomen following the third ICPBR Symposium (Rothamsted, 1985) and sought to incorporate all relevant recommendations from ICPBR members. Following standard EPPO procedures, the draft has been circulated to competent authorities in member states for comments. In common with other EPPO Guidelines, it is intended that the final version should be suitable for inclusion in national legislation, if desired. However, EPPO member states will be free to adapt the Guideline to their own requirements.

It was noted that further method development work will be required to enable the proposed Guideline to recommend procedures for fully evaluating the hazard which could be presented by compounds such as insect growth regulants, systemic insecticides and compounds which could modify bee behaviour. However, it was noted that the proposed Guideline draws attention to the need to establish whether compounds can be properly evaluated using recommended procedures, and that it could be revised as and when appropriate procedures are fully developed.

Dr. BRASSE expressed his concerns about the potential publication of the Working Group's recommendations as an EPPO Guideline. These concerns are summarised in Appendix 3, and stem from the possibility that such a Guideline could be incorporated within European regulatory legislation. While accepting the value of a stepwise testing programme, reservations were expressed concerning application of some of the recommended methods

to the situation in W. Germany. Some members of the Working Group also noted that national representatives to EPPO in certain countries had not discussed the draft document with their national experts, and that the expertise available within the EPPO Panel for Biological Evaluation of Plant Protection Products might not be appropriate for evaluating such a document.

Considerable discussion followed the presentations by Drs. Oomen and Brasse. Three salient points emerged:

1) The emphasis of the ICPBR recommendations is firmly placed on harmonisation of methodology rather than standardisation. As noted earlier, EPPO Guidelines are intended to provide procedures which can be incorporated into national regulatory legislation, if desired. However, Guidelines can also be adapted to fulfil local requirements. In this spirit, the draft document submitted to EPPO is intended to provide guidelines rather than inflexible, definitive protocols.

2) Although the draft document submitted to EPPO had not been reviewed by all participants in the meeting, it was clear that it is not unanimously supported by all members of the Working Group.

3) The ICPBR is an advisory group. Some members of the Working Group expressed concern that EPPO appears to be placing excessive emphasis on input from the ICPBR in developing this Guideline.

The following actions were agreed:

a) Drs. Brasse, Oomen, Greig-Smith and Stevenson to agree a revised draft of the proposed guideline.

b) Dr. Greig-Smith to present this document to EPPO and draw attention to concerns expressed by members of the Working Group (points 1-3, above).

While it was generally agreed that test guidelines should contain a degree of flexibility, the meeting also noted that impending regulatory developments within the EEC (implementation of the European Registration Directive) may result in some loss of flexibility due to standardisation of methodology.

Since the meeting in Rez, Dr. Greig-Smith has provided the following update on progress with this guideline:

The draft guideline on testing the hazard of pesticides to honeybees has been submitted to EPPO for consideration as an officially approved standard Guideline. It has been examined by the Insecticides and Fungicides Panel of EPPO, following the customary procedures. However, the context in which it is being considered has now changed, by virtue of the establishment of a new EPPO Panel, jointly with the Council of Europe. This panel is concerned with environmental risk assessment of plant protection products, and will address a wide range of environmental issues, including the hazard of pesticides to honeybees. It has been agreed that from now on, the honeybee guideline will be

developed by a working group of this new panel. This will entail some changes, to conform to a consistent style being adopted for all the approaches to risk assessment that are being produced. EPPO and the Council of Europe have now also agreed that the initiative for this working group should best come from those involved drafting the original guideline for ICPBR. This arrangement offers an excellent opportunity to complete the work already done on the production of a scientifically-based, yet practical scheme for assessing the risks of pesticide uses to bees.

2) National schemes for assessing hazard to bees from reports by beekeepers

Dr. GREIG-SMITH described the results of investigations into incidents of Honey bee poisoning by pesticides in the UK between 1981 and 1989 (Appendix 4). Attention was drawn to the success achieved by regulation of the use of triazophos on oilseed rape, and to increasing numbers of incidents during 1988/89 involving dimethoate following applications to arable crops. In the latter case, mortalities are thought to have resulted from bees foraging aphid honeydew in cereals. It was also noted that there may be an association between the incidence of Nosema disease and the presence of triazophos and carbaryl residues in bees. Further investigations will be required to determine whether exposure to pesticides can increase the susceptibility of bees to pathogens. It was noted that the interpretation of residue data relies on information being available concerning diagnostic concentrations i.e. tissue concentrations which are known to result from lethal exposure.

The meeting acknowledged the value of the UK scheme in the regulation of pesticide use. A comparable scheme exists in W.Germany, where it was reported that analyses frequently detect residues of several compounds in dead bees, including compounds used for Varroa control. Incidents of suspected pesticide poisoning are also investigated in France, although difficulties exist in implementing legal aspects. In the USA, incidents are investigated on a state-by-state basis. There is a desire to implement an incident investigation scheme in The Netherlands, but funding remains a problem.

3) Further developments/experience with laboratory and field methods for assessing hazard to bees

The scope of this section is not restricted to the Honey bee, and includes papers describing work with Alfalfa leaf-cutting bees and the Alkali bee. It should also be noted that this section includes several papers describing methods for investigating the toxicity and hazard of compounds to bee larvae. This section is therefore divided into two sub-sections, the first describing work with compounds with a "conventional" mode of action, and the second describing studies to specifically investigate toxicity and hazard to pre-adult stages of bees.

3.1 General

Prof. ARZONE described the results of laboratory and field experiments with pesticides and bees (Appendix 5), drawing attention to the need for test methods to investigate possible longer-term adverse effects.

Dr. HELLER described a method for investigating the hazard of pesticides to the Alfalfa leafcutting bee, illustrating the talk with results for deltamethrin (Appendix 6). The methodology used represents a further development of "tunnel test" procedures which have been increasingly used, particularly in France, to investigate the hazard of pesticides to Honey bees.

Dr. INGLESFIELD described a large-scale monitoring trial to investigate the hazard of an insecticide/fungicide mixture to Honey bees (Appendix 7). This was followed by a general discussion of practical aspects of field trial methodology, including the use of statistical procedures to analyse results, and the interpretation of results of field experiments against a background of natural variation.

Dr. LEWIS described experiments carried out to investigate the relative value of a laboratory residual toxicity test and a semi-field "tunnel test" as methods for assessing the hazard of a pyrethroid insecticide to bees (Appendix 8). Results obtained using these methods were compared with results from large-scale field trials. It was concluded that the "tunnel test" method was more useful than the laboratory residual toxicity test in evaluating the hazard of test compound to bees.

Dr. MEYER described methods used to assess the hazard of tralomethrin to the Honey bee, the Alfalfa leafcutting bee and the Alkali bee, (Appendix 9). These methods have been used to assess the hazard of a large number of compounds to these species, enabling a large database to be developed concerning the relative susceptibility of these species. Taken together, results of such tests indicate that the relative susceptibilities of these species can be ranked as follows: Alfalfa leafcutting bee > Alkali bee > Honey bee.

Dr. SCHMIDT described the advantages and disadvantages of the various semi-field and field methods used to assess the hazard of pesticides to Honey bees in W.Germany (Appendix 10). Attention was drawn to some of the limitations inherent to small-scale "tent" studies, such as difficulties in investigating repellent effects. He suggested a number of ways in which additional valuable information could be obtained from larger-scale trials, including detailed observations on foraging behaviour and repellency, and detailed investigations into possible effects on brood. During discussion of this paper, it was noted that difficulties have been experienced in France with carrying out longer-term observations on the small colonies used in "tunnel test" experiments, due to problems in keeping such small hives healthy for extended periods under natural conditions. Further work is in hand to try to overcome this problem. The meeting noted this shortcoming of the "tunnel test" approach.

Drs. SIHAG and KUMAR were unable to attend the meeting. A summary of their work on laboratory toxicity tests is included in the Proceedings as Appendix 11.

3.2 Effects on pre-adult stages

Dr. BOGDANOV presented a paper (Appendix 12) on behalf of Dr. Gerig describing work in Switzerland to assess the hazard of fenoxycarb to bees. Field experiments have demonstrated the potential for inadvertent contamination of wild flowers (including weeds) and flowering wild trees to result in brood mortality. Current recommendations for orchard use in Switzerland require that the compound is only applied outside flowering of the fruit trees, that grass in the orchard is mown prior to application, and that herbicides are used to kill any flowering plants prior to application.

Dr. CZOPPELT described a laboratory test method for investigating the effects of pesticides on bee larvae (Appendix 13). The presentation was followed by considerable discussion of the relative merits of this method compared with those developed at Hohenheim University. While such methods have been used to investigate possible effects on brood of compounds which interfere with larval development (eg insect growth regulants), it was suggested that they could also be used to bioassay samples of pollen collected from field trials in order to directly investigate the toxicity of residues to brood.

Dr. ENGELS further discussed the need for investigating the toxicity of pesticides to bee larvae (Appendix 14). Particular attention was drawn to the increasing use of pesticides to protect hives against Varroa, and to the further development of insecticides (eg chitin inhibitors, juvenile hormone analogues) with modes of action which could present a hazard to brood. It was suggested that pesticide registration schemes should take full account of the potential for adverse effects on brood.

Dr. VAN DER EIJNDE presented a paper (Appendix 15) on behalf of Drs. van der Steen and de Ruijter describing detailed large-scale field experiments to investigate the hazard of fenoxycarb to Honey bees when applied in orchards before flowering, during flowering (full bloom), at 80% petal fall and at around 100% petal fall. It was reported that application during full flower and at 80% petal fall resulted in significant pupal mortality. This hazard could be significantly reduced, but not completely eliminated by application either prior to flowering or at around 100% petal fall. It was suggest that mortalities following application at these timings may have resulted from bees foraging on flowering weeds which may have been inadvertently contaminated by the test compound, or from the presence of a very limited number of flowers remaining on the trees at the time of treatment.

4) The use of pesticides in Varroa control

The number of papers concerned with aspects of Varroa control reflects the increasing importance of this issue in continental Europe. All the papers summarised here relate to the use of pesticides to control Varroa, with emphasis on the detection and quantification of varroacide residues in hive products and components.

Dr. BACILEK described laboratory experiments to investigate the oral and contact toxicity to bees of several pesticides which are used to control Varroa (Appendix 16), relating results to concentrations of the compounds used under practical conditions.

Dr. BACILEK then described the development of a paper which can be used for fumigating hives in Varroa control (Appendix 17). It was noted that this could also have potential in domestic and glasshouse pest control.

Dr. BACILEK described an ongoing programme designed to investigate and monitor the presence of coumafos in honey and beeswax (Appendix 18). This compound is widely used in Czechoslovakia as a varroacide. This was the first of a number of papers which described the investigation of varroacide residues in honey and wax. The meeting noted that repeated treatment of hives gives the potential for relatively high residues of lipophilic compounds to accumulate in wax.

Dr. BOGDANOV described the results of studies to investigate the occurrence and distribution of residues of compounds which are currently used in Switzerland to control Varroa (Appendix 19). Results were presented for residues in wax, winter sugar stores and honey. The attention of the meeting was again drawn to the potential for relatively high residues of lipophilic compounds to accumulate in wax.

Dr. DRESCHER described a bioassay test using waxmoth larvae to investigate the presence of pesticide residues in wax (Appendix 20). This approach has the drawback that toxicants cannot be identified. However, it was considered that this method could be particularly useful for screening commercial foundation for the presence of compounds which could have adverse effects on bees, and that the method could be very useful in areas where facilities for residue analysis are limited. This paper drew attention to the need to investigate the bioavailability of residues.

Dr. KRIEG was unable to attend the meeting, but an abstract (Appendix 21) is included in these Proceedings summarising the use of waxmoth larvae in bioassay tests for pesticide residues in wax.

Dr. LENICEK further developed this theme by describing methods used in Czechoslovakia to analyse for residues of fluvalinate in wax and honey (Appendix 22). The potential for improving the sensitivity of the methods was noted.

5) Others

Dr. PTACEK presented a paper (Appendix 23) describing the use of the solitary bee Rhophitoides canus in the broad context of implementing Integrated Pest Management procedures in commercial lucerne (alfalfa) production. Particular attention was drawn to use of environmental criteria, including hazard to bees, in the selection of pesticides, and on the use of pest monitoring to determine spray programmes.

Dr. TORNIER extended the scope of the discussions by describing a "semi-field" method for assessing the hazard of pesticides to the syrphid, Episyrphus balteatus (Appendix 24). Adults of this species are thought to be important pollinators of certain crops, while larvae are voracious predators of some pest species, such as aphids.

Appendix 25 is a paper by Drs. IONESCU, MIRCEA and BURSUC describing studies to investigate the toxicity of nitrates and nitrites to honeybees.

CLOSURE

Dr. TASEI addressed the meeting, reviewing the history of the International Commission for Plant Bee Relationships (ICPBR), and drawing attention to links with the International Union of Biological Sciences (IUBS), the International Bee Research Association (IBRA) and Apimondia. He reminded participants that the primary objective of the ICPBR is promotion of international cooperation in research and information exchange. The Working Groups of the ICPBR provide the mechanism by which this objective is pursued. Relating to this, the attention of the meeting was drawn to the forthcoming symposium on Pollination, to be held at the Ambrosiushoeve Research Institute (NL) during August 1990.

Dr. Tasei expressed his thanks to Dr. Stevenson (Chairman) Dr. Inglesfield (Secretary), Dr. Vesely, Dr. Bacilek, Dr. Pagac and all others involved in organisational aspects of the symposium.

Dr. STEVENSON opened a General Discussion by asking the meeting to consider the need/timing for future symposia. It was agreed that another symposium should be held in around two years time (1992). It was provisionally agreed that this meeting will be held in Wageningen (NL), with France as an alternative venue. It was agreed that this meeting will be entitled "Hazard of Pesticides to Bees." The following topics were suggested for discussion at this symposium:

1) The use of pesticides to control Varroa, with emphasis on possible side effects on bees including the presence and bioavailability of varroacide residues in hive components.

2) Methods for assessing the hazard of pesticides to bee larvae.

3) Methods for assessing the hazard of systemic pesticides to bees, including information concerning the occurrence of residues of such compounds in pollen and nectar.

4) Methods for assessing the hazard of pesticides to species other than the Honey bee, including relevant data/information concerning hazard.

5) Developments towards the harmonisation of methodology for assessing the hazard of pesticides to bees, including Regulatory Aspects.

6) Developments with National Incident Reporting Schemes.

Dr. Stevenson closed the meeting by expressing his thanks to all participants for ensuring that the meeting was both stimulating and rewarding, and in particular praised all those involved in hosting the meeting for their major contribution to ensuring the success of the meeting.

APPENDIX 1

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APPENDIX 2

P.A. Oomen, Plant Protection Service, Wageningen, the Netherlands

GUIDELINE FOR EVALUATING THE HAZARDS OF PESTICIDES TO HONEY BEES, APIS MELLIFERA +

For registration of pesticides and regulation of their use, hazards to honey bees have to be taken into account. A guideline for evaluating these hazards is described. The guideline makes use of testing methods that result from the harmonization efforts of the International Commission for Bee Botany. In the form of a sequential decision-making scheme the guideline provides a framework for classifyng the hazards of pesticides to bees. Two laboratory test methods (oral LD50 and contact LD50), a cage test method, and a field test method described in detail provide the test data required for evaluating the hazards to bees. The laboratory test data may serve to classify many pesticides without having recourse to cage or field tests. The scheme makes special allowance for differences in dose rates between pesticides and the possible occurrence of unusual effects in honey bees. Criteria for evaluating test data are suggested. Several examples of the use of the sequential scheme are elaborated.

Introduction

The hazard of pesticides to honey bees has to be taken into account when regulating pesticide use. Most countries classify this hazard to bees into three categories: toxic or dangerous, non-toxic or non-dangerous and intermediate. Data and evaluation methods used for classification of pesticides seem to vary between countries. The Council of Europe (Pesticides, 6th Edition, Strasbourg 1984) and FAO (Second Expert Consultation on Environmental Criteria for Registration of Pesticides, Rome 1981) recommend pesticide registration authorities to use laboratory toxicity data, cage trial data and field trial data for evaluation of the hazard to honey bees. The Symposia on the harmonization of methods for testing the toxicity of pesticides to bees, organised by the International Commission for Bee Botany (ICBB) (Third Symposium, Rothamsted Experimental Station, March 18-21. 1985) recommend on methods for collecting data. The recommendations by ICBB are included in this guideline as "Methods for testing the effects of pesticides on bees."

In the form of a sequential decision-making scheme, this guideline provides a framework for classifying the hazard of pesticides to bees. Classification in terms of toxic and non-toxic is avoided since toxicity is a property inherent to the material and the test organism only, while a classification in terms of hazard takes other important factors (e.g. dosage rate, mode of application, repellency) into account as well. The sequential approach is chosen as very suitable for standardizing the evaluation procedure and for reducing to a minimum the amount of test and trial data required for classification, while being in line with the recommendations by the Council of Europe, FAC and the ICBB Symposia. The scheme makes special allowance for differences in dose rates between

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+ Drafted for EPPO by Dr P.A. Oomen, Plant Protection Service, P.O. Box 9102, 6700 HC Wageningen (Netherlands), from whom additional information is available.

pesticides and the possible occurrence of unusual effects in honey bees. Criteria for evaluating test data are suggested in the scheme, which was approved by the EPPO Working Party on Pesticides for Plant Protection - October 1987. The hope was expressed that official service of EPPO Member Countries would encourage testing following this guideline.

Sequential decision-making

For classification of a pesticide, one should proceed step by step through the levels of the sequential decision-making scheme (Fig.1). Depending on the answer (alongside/arrows) to the questions or test (left) at each level, either a hazard classification (right) can be decided upon, or one should proceed to the next level. Use and special aspects of each level are commented on below. Methods suitable for collecting the necessary laboratory, cage and field data are described in this guideline.

Level I

Evaluation of hazard to bees is necessary only for compounds with a use pattern likely to put bees at risk. In other cases, no data will be required.

Level II

Information about unusual use patterns, mode of application or characteristics of the compound (e.g. systemic properties possibly resulting in toxic nectar, pesticide particles physically resembling pollen, insect growth regulators) may render the routine evaluating scheme inadequate. In this case, the test scheme will have to be adapted in consultation with bee research specialists. Proceed to field test when other than acute effects are expected.

Level III

The laboratory tests (determination of contact LD50 and oral LD50) may serve to classify many pesticides for hazard without having recourse to cage or costly and laborious field tests. A hazard ratio (or factor) is determined for each pesticide by dividing the recommended dose (in g a.i. per ha) by the laboratory LD50 (in µg a.i. for one bee). For example deltamethrin the hazard ratio would be 7.5/0.051 = 147. (To obtain the actual number of LD50 doses applied per hectare, this value would have to be multiplied by 10^6). When the ratio between highest dosage recommended per hectare and the LD50 (i.e. lowest of contact LD50 and oral LD50) is greater or lower than appropriately defined threshold values, as indicated in the scheme, classification of the pesticide is possible and further testing is not necessary. Examples of calculations are given in the Appendix.

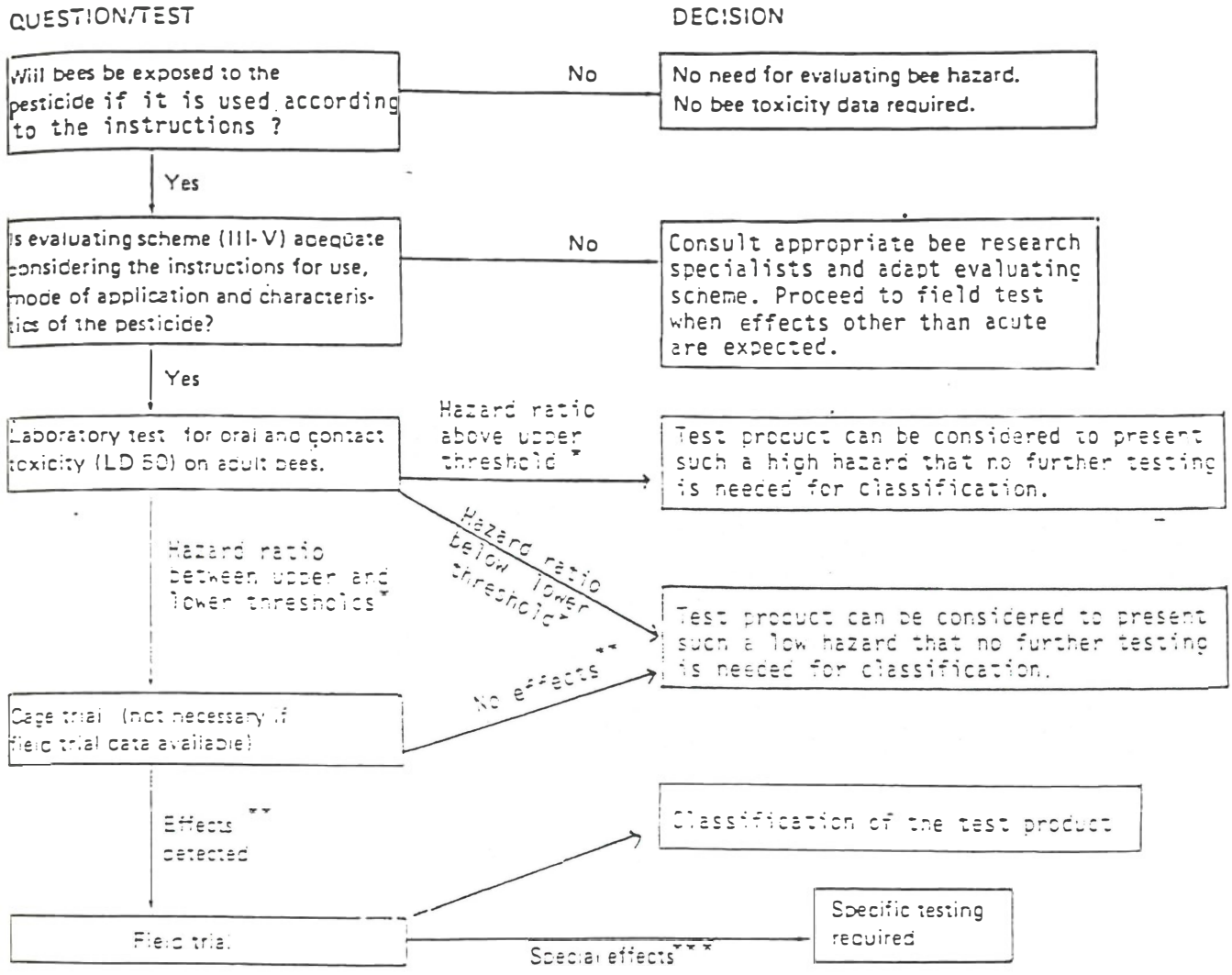
Pesticides containing mixtures of active ingredients may be evaluated by expressing both dosage rate and LD50 in formulated product instead of active ingredient. This method for evaluation of the hazard ratio allows for the large variation in dosage rates between different pesticides.

Level IV

A facultative cage or tunnel trial may serve to classify a number of non-dangerous pesticides. Pesticide exposure in a cage or tunnel is more intensive than in the field. The pesticide tested is therefore classified as presenting a low hazard if the effects on colony survival and development are similar to those in a non-pesticide control, provided that enviromental conditions are suitable for expression of bee hazard (see under field test). Cage or tunnel trials are not required if field-trial data are available.

Level V

Field trials serve to classify all remaining pesticides. Both cage and field trials should include a reference product known to present a high hazard to bees, to demonstrate that test bees were at risk under the enviromental conditions (especially weather) of the trial. A reference product known to present a low hazard is necessary in field trials in order to enable evaluation of the effects of the test product on colony survival and development and arrive at an appropriate hazard classification. Special effects (larval toxicity, long residual effect, disorienting effects on bees etc.) identified by the field test may require further investigation by specific methods. If field trials are virtually impossible (e.g. for evaluating the hazard to bees foraging the honeydew secreted by cereal aphids), tunnel trials may replace field trials.



Notes : * Hazard ratio = $LD50^{-1}$ x dose/ha. Dose/ha = highest dose rate (in g a.i./ha) recommended for practice. LD50 = lowest of oral LD50 and contact LD50 (in µg a.i./bee). The upper and lower thresholds are fixed on the basis of bee toxicity, dosage rate and an independent hazard classification of pesticides verified by extensive practical experience. Suggested figures are hazard ratios of 2500 for the upper threshold and 50 for the lower threshold.

** Effects on colony survival and development. For parameters to be estimated see under "Methods for testing the effects of pesticides to bees" (section IV).

*** Special effects : e.g. larval toxicity, long residual effect, disorienting effects on bees.

Fig. 1 Sequential scheme for evaluating the hazard of pesticides to bees. See text on sequential decision-making for detailed explanation.
Schéma séquentiel pour l'évaluation des risques occasionnés par les pesticides vis-à-vis des abeilles. Voir le texte correspondant pour plus de détails.

APPENDIX

Examples of the use of the sequential scheme for classifying hazard to bees.

Example product A

Level I: yes
Level II: scheme not adequate. Juvenile hormone analogues are likely to affect juveniles only. Field test required.
Level V: Field test in progress.

Example product B

Level I: yes
Level II: yes
Level III: Recommended dose 5-7.5 g a.i./ha
oral LD50 0.079 µg a.i./bee
contact LD50 0.051 µg a.i./bee
For calculation select highest recommended dose rate (7.5 g/ha) and lowest of both LD50's (0.051 µg/bee).
Hazard ratio = $LD50^{-1}$ x dose/ha = 7.5/0.051 = 147
The value of this ratio is between the suggested lower and upper thresholds (50 and 2500) ; hence classification is not yet possible and further testing is needed (cage or field trials).
Level IV : No cage trial data available.
Level V : In field trials B is compared with a low-hazard reference product and classified accordingly.

Example product C

Level I: yes
Level II: yes
Level III: Recommended dose 375 g a.i./ha
oral LD50 > 10 µg a.i./bee
contact LD50 > 8 µg a.i./bee
Hazard ratio = $LD50^{-1}$ x dose/ha = 375/8 = 47
The value of this ratio is less than the suggested lower threshold (50). Hence, no further testing is needed and C can be classified on the basis of the data already available.

Example product D

Level I: yes
Level II: yes
Level III: Recommended dose 750 g a.i./ha
oral LD50 : 0.09 µg a.i./bee
contact LD50 : 0.08 µg a.i./bee
Hazard ratio = $LD50^{-1}$ x dose/ha = 750/0.08 = 9375
The value of this ratio is more than the suggested upper threshold (2500). Hence, no further testing is needed and D can be classified on the basis of the data already available.

More examples on the use of the sequential scheme, including recommended dose rates, contact LD50 and oral LD50 of eighty pesticides are given by Oomen, P.A. (1986) : A sequential scheme for evaluating the hazard of pesticides to bees, Apis mellifera . Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 51/3b, 1205-1213, 1986.

Methods for testing the effects of pesticides on bees

The description of these methods is based upon the "Recommendations for harmonization of methods for testing hazards of pesticides to honey bees", decided by the International Commission for Bee Botany at the Third Symposium on the Harmonization of Methods for testing the Toxicity of Pesticides to Bees, Rothamsted Experimental Station, Harpenden (UK), 18-21 March, 1985.

I. Laboratory tests

1. Experimental conditions

1.1 Principle of the trial

Oral and contact toxicity of test compounds to adult worker honey bees are assessed in the laboratory. Bees are exposed to different doses of the compound by way of feeding or topical application. Mortality values are used to provide a regression line and LD50.

1.2 Trial conditions

Keep bees in holding cages that are well ventilated and easily cleaned. Do not use plastic cages unless disposed of after use, because of possible contamination. Avoid reuse of wooden cages unless very well cleaned and sterilized. Cages should not cause control mortality. Store bees after treatment at a temperature of 25 ± 2 °C and a high relative humidity (about 60-70 % RH).

1.3 Preparation of the bees

Use preferably uniform, young adult worker bees. Bees should be adequately fed and from a healthy and queen-right colony. Collect bees in a standardized way. Avoid collection in early spring or late autumn. Bees collected from frames without brood or from the flight board at the hive entrance are suitable. Bees may also be reared in an incubator, fed with fresh or well preserved pollen and sucrose solution. The method of collection used, age of bees, race of bees (if known) and date of the experiment should be reported.

Bees may be anaesthetized with carbon dioxide. Keep the amount used and times of exposure to a minimum, but ensure anaesthesia is complete. Ensure that application does not lower the temperature of the holding cage and the bees.

1.4 Design of the trial

Treatments : either formulated products or active ingredients are tested. Include a control treated with the solvent and an appropriate reference product to check consistency of results (e.g. parathion, dimethoate).

Test units : dose bees individually or in groups of at least 10. Bees must not be confined individually for more than 1 h.

Replicates : use at each concentration at least three groups of 10 (or more) bees.

Concentrations : use a suitable range and number of concentrations to provide a regression line and LD50.

2. Application of treatments

2.1 Oral toxicity test

2.1.1 Products to be tested

Use 20-50 % sucrose solution. Dissolve formulations without additional solvents if possible. Use the minimum amount of any solvent to aid the dispersion of active ingredients and technical materials. Use solvents other than acetone only if the compound is insoluble in acetone.

2.1.2 Mode of application

Starve bees for up to 2 h before tests. Dose at 10 or 20 µl of test solution per bee through glass tubes. By group feeding bees will share the test solution between themselves and so receive similar doses. Supply fresh sucrose solution after dose has been taken and change daily if test period exceeds 48 h.

2.2 Contact toxicity test

2.2.1 Products to be tested

Dissolve the compound in acetone where possible. Use other solvents only if the compound is insoluble in acetone.

2.2.2 Mode of application

Treat anaesthetized bees individually by topical application. Dose a measured amount of product to the thorax of the bee. Supply fresh sucrose solution after application and change daily if the test period exceeds 48 h.

3. Mode of assessment

The treated bees are returned to the cages. Count the number of dead and affected bees at 24 h intervals for up to 48 h, or longer if mortality is still increasing.

4. Results

Repeat tests where control mortality is considerable (generally above 15 %). Calculate mortality after correction for control mortality. Analyze by appropriate statistical methods and calculate the mediant lethal dose value (LD50), expressed in µg of active ingredient per bee.

II. Cage tests

1. Experimental conditions

1.1 Principle of the trial

Bees from small colonies are made to forage on a flowering crop in field cages. The test product and a reference product known to present a high hazard to bees are sprayed in separate cages during bee flight, while other cages are left as untreated controls. The reference product is necessary to confirm that bees are at risk. The effects of the treatments on bees are assessed immediately before and several times after treatment.

1.2 Trial conditions

Use cages with a minimal size of 2 x 2 x 3 m. The cage should have a maximal mesh size of 3 mm. Plastic coating on the roof may be used to prevent trapping of the bees.

Suitable test crops are Borago, Phacelia, Sinapis, and other flowering crops attractive to bees on which use of the test product is proposed.

1.3 Preparation of the bees

Use one small healthy colony per cage, preferably queen-right, of at least three full frames, or a nucleus . Avoid where possible the introduction of field bees into the cage to reduce trapping on the ceiling. Feeding of the colonies during the trial may be necessary.

1.4 Design of the trial

Treatments : product(s) to be tested, reference product known to present a high hazard to bees (e.g. parathion, dimethoate) and non-pesticide control. The control may or may not receive a water spray. Arrange treatments where possible in a randomized plot design.

Test units : cages with one colony.

Replicates : sufficient to enable appropriate statistical analysis.

2. Application of treatments

2.1 Products to be tested.

Use formulated products only.

2.2 Mode of application

Apply the pesticide during the day when bees are flying. Avoid spraying the cage walls.

2.3 Dosage

The product should normally be applied at the highest dosage recommended on the (proposed) label for use in flowering crops.

3. Mode of assessment

Record effects immediately before and at several intervals, preferably 0, 1,2,4 and 7 days, after treatment. Record foraging activity and the behaviour of bees on the crop and around the hive. Count the bees in dead bee traps and those dying in the rest of the cage. Record temperature and humidity. Other assessments, e.g. effects on brood, should be made as appropriate to the type of product under test.

4. Results

Repeat tests where control mortality is considerable (generally above 15%) and also where mortality in the reference treatment is low. The results should preferably be analyzed by appropriate statistical methods. Raw data should,however, also be included and any statistical method used should always be indicated.

III. Field test

1. Experimental conditions

1.1 Principle of the trial

Bee colonies are placed in or on the edge of large test fields. Bees are made to forage on a flowering crop in the adjacent test field only. Test fields should be well separated. The test product and reference products known to present high and low hazards to bees are sprayed in separate test fields during bee flight. The effects of the treatments on bees are assessed shortly before and several times after treatment.

1.2 Selection of the crop

Carry out the tests on the crop on which use of the test product is proposed. The crop should be in full flower and attractive to bees.

1.3 Trial conditions

Place the colonies in or on the edge of the flowering crop to be sprayed. To ensure that bees are foraging only the adjacent plot on the day of treatment, place colonies only a few days before the trial as bees tend to begin foraging in areas immediately adjacent to their hives.

1.4 Preparation of the bees

Use healthy, well fed, queen-right colonies in normal condition that contain at least 10,000 to 15,000 bees, according to the season. Each colony should cover at least 10 to 12 frames, including at least 5 to 6 brood frames. If colonies differ in size, ensure equitable distribution.

1.5 Design and lay-out of the trial

Treatments: product(s) to be tested, reference product known to present a high hazard to bees (e.g. parathion, dimethoate), reference product known to present a low hazard to bees. Arrange treatments where possible in a randomized plot design.

Plot size: at least 1500 m^2. Full strength colonies require larger areas. Plots should be well separated (by at least 500 to 1000 m) to avoid bees foraging on the wrong plot. The plots should not be close to other flowering crops which are attractive to bees.

Replicates : although very desirable, replication is often not feasible because of requirements of separation. Use at least 3 colonies per treatment.

2. Application of treatments

2.1 Products to be tested

Use formulated products only.

2.2 Reference products

Choose products registered for a use similar to the (proposed) use of the test product.

2.3 Mode of application

Apply the products during the day when bees are actively foraging. Apply treatments simultaneously, i.e. within at most 2 h. Follow the recommendations for application on the (proposed) label.

2.4 Dosage

The product should normally be applied at the highest dosage recommended on the (proposed) label for the crop/pest situation under test ; if desired an additional higher rate may also be tested. Volume of application and droplet size should be as recommended and should be recorded.

3. Mode of assessment and recording

3.1 Meteorological data

Record temperature and humidity during the entire period of the trial.

3.2 Type, time and frequency of assessment

3.2.1 Type

Estimate or record the following parameters : number of foraging bees in the crop, behaviour of bees on crop and around hives, mortality of bees (using dead bee traps).

It is desirable to estimate also : pollen collection (using pollen traps), pollen in collected honey, number of bees on frames, brood status in frames, and residues in dead bees, pollen, wax and honey.

3.2.2 Time and frequency

Pre-treatment assessment one day or immediately before treatment.

Post treatment: at several intervals, preferably 0,1,2,4,7 and 14 days after treatment.

Assessment may be continued at larger intervals for up to 3 months after treatment.

4. Results

Repeat trials if mortality in the non-hazardous reference treatment is considerable (generaly above 15%) and also if mortality in the hazardous reference treatment is low. The results should preferably be analyzed by appropriate statistical methods. Raw data should, however, also be included and any statistical method should always be indicated.

IV. Tunnel tests

Certain hazards to honey bees are virtually impossible to study by field tests, for example the evaluation of the hazard of pesticide application to honey bees foraging the honey dew secreted by cereal aphids. In such cases field tunnel tests are suitable alternatives.

1. Design of the trial

Plots of cereal growing in the field are covered by nylon mesh tunnels. Honey dew is simulated by applying sucrose as a high volume spray. Bees from a small colony inside are made to forage on the sucrose. The test product and reference products known to present high and low hazards to bees are sprayed in separate tunnels during bee flight. The effects of the treatments on bees are assessed shortly before and several times after treatment.

2. Trial conditions and application of treatments

Trial conditions and methods described by S.W. Shires, J. Le Blanc, P. Debray, S. Forbes , J. Louveaux (1984), Pesticide Science 15, 543-552, are suitable.

3. Mode of assessment and recording, results

As in field test.

RESUME

Dans le cadre de l'homologation des pesticides et de la réglementation de leur usage il faut tenir compte des risques encourus par les abeilles. Nous présentons une directive destinée à évaluer ces risques. Elle fait état des méthodes issues des travaux d'harmonisation de la Commission Internationale pour la Botanie des Abeilles. Elle présente, sous forme d'un schéma séquentiel un moyen de classer les risques occasionnés par les pesticides - dangereux ou non - vis-à-vis des abeilles. Quatre trests visant à fournir les données nécessaires à l'évaluation de ces risques sont décrits en détail : deux tests de laboratoire (DL50 orale et DL50 de contact), un test en cage et un test au champ. Les résultats des tests de laboratoire peuvent permettre de classer définitivement de nombreux pesticides comme dangereux ou non dangereux sans avoir recours aux tests en cage ou au champ.

Le schéma prend particulièrement en compte les différences entre pesticides (exprimées en doses), et la manifestation possible d'effets non usuels sur les abeilles. On propose des critères d'évaluation des résultats. On présente plusieurs exemples d'utilisation du schéma séquentiel.

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APPENDIX 3

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SUMMARY OF CONCERNS WITH THE DRAFT EPPO GUIDELINE FOR EVALUATING THE HAZARDS OF PESTICIDES TO HONEY BEES

From the point of view of the german participants, who have taken part also in the previous meetings, there is a lot critical points in the guideline itself and in the way of promotion by the EPPO. The main points, which are not compatible with the German testing principles, are the following ones:

1. All tests (laboratory, semi-field and field) should be done with the formulated product and not with the active ingredient. This is necessary in order to catch all possible effects, which may arise from the formulation, and also in order to avoid all undesirable effects, which may arise from an unusual solvent.

2. The anaesthezation of the test animals should be avoided because also the aneasthezation with CO_2 may cause uncalculable effects.

3. The proposed test design for semi-field- and especially field-tests can not be performed in Germany for practical reasons.

On the former meetings the ICBB-working group has defined the terms "harmonization" and "standardization" and agreed to establish harmonized principles for the testing procedure. A standardized testing method has not been taken into acount. Therefore it was of some surprise, that EPPO planned to publish a honey bee guideline. The draft had been discussed in the EPPO-panel for the evaluation of insecticides and fungicides. Most of

the members of this panel did not discuss the draft with their national experts. The English word guideline is translated into German as "Richtlinie". Richtlinien = guidelines do have a rather binding character in Germany. Therefore it is necessary to make them acceptable for those experts, who have to work with them, especially with the view to the expected adoption of the EPPO-guidelines by the European Comunity. For this reason the honey bee guideline should be discussed again in an EPPO-panel including experts for this subject. The draft should be worded in such a way that it will be possible to deviate from the proposed test method. It should be mentioned that there are existing some well experienced testing methods in several european countries and that these methods can also be used in future.

APPENDIX 4

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INVESTIGATIONS OF HONEYBEE POISONING BY PESTICIDES IN THE U.K., 1981-89

SUMMARY

Investigations of suspected poisoning of honeybees by agricultural pesticides in England & Wales have been carried out by ADAS since 1981. Samples of dead bees are examined for disease and analysed for pesticide residues, and field evidence is gathered. Results for 1981-89 revealed pesticides in 58% of 938 cases. Many incidents arose from use of triazophos on oilseed rape, but the scale of this problem has declined to a consistent level. Poisoning by dimethoate use on a variety of arable crops is increasing. Results suggest that synthetic pyrethroids are responsible for very little mortality. Other pesticides implicated occasionally in incidents included a variety of insecticides, and the herbicides paraquat and diquat. This post-registration monitoring scheme is an important part of UK pesticide regulation, complementing pre-registration testing for safety to bees.

INTRODUCTION

Before approval is granted for use of pesticides in the UK, a series of laboratory and, where appropriate, field tests is required, including trials to assess potential hazards to bees (MAFF 1986, Felton *et al* 1986). In addition, a system of post-registration monitoring is operated to allow any problems that may arise during commercial use to be detected. If necessary, regulatory action can then be taken to prevent recurrence of serious bee mortality. Since 1981, the Agricultural Development and Advisory Service (ADAS) of the Ministry of Agriculture, Fisheries and Food has investigated cases of suspected poisoning by pesticides in England and Wales. This paper describes these investigations, and summarises some of the main results obtained over a nine-year period. Detailed accounts of incidents are published in annual reports (e.g. Greig-Smith *et al* 1990) and will be reviewed in a forthcoming article (Greig-Smith, Hardy & Stevenson in preparation).

INVESTIGATION PROCEDURES

Incidents are discovered and reported by beekeepers who find unusually high numbers of dead bees at their hives. In England and Wales, they are invited to inform ADAS and to send a sample of dead bees to the National Beekeeping Unit at Luddington in Warwickshire. An investigation in three parts is then initiated. The bees submitted to Luddington are examined for disease (Amoeba, Nosema and Acarine). A biologist from the ADAS Wildlife & Storage Biology department visits the site of the incident, to gather evidence about local pesticide use that may have been responsible, and any other relevant information. A sample of dead bees is also sent to the ADAS Central Science Laboratory at Tolworth, for chemical analysis to detect the presence of residues of pesticides. Similar procedures are operated in Scotland by the Department of Agriculture & Fisheries for Scotland (DAFS).

Deaths of bees are attributed to pesticide poisoning only if residues are detected, and confirmed by an alternative method of chemical analysis. Levels of residues can be interpreted in the light of laboratory dosing experiments

that have established how much pesticide remains detectable in bees after they have received a lethal dose of a particular chemical. It is then possible to compare the levels of residues detected in bees to the LD_{50} and to the lower levels expected in bees that have received a LD_{50} dose. In theory, this calibration should allow sublethal exposure to be distinguished from that which is the cause of death. However, delays in the discovery of incidents, variable storage conditions, and the possibility that toxicity may be affected by other stresses on bees in the field, mean that these residue thresholds can only be used as approximate aids in interpretation.

The findings of these various parts of the investigations are collated into a report, in which the cause of death is categorised as due to the approved use of a pesticide, to misuse (i.e. careless or wilful failure to adhere to the conditions of approval), abuse (deliberate malicious poisoning of bees) or to other causes, not connected with pesticide poisoning (see Greig-Smith 1988).

Action may then be taken against farmers or contractors who have failed to observe proper care in the use of pesticides where bees might have been affected.

Each year, several of the cases reported independently to the scheme are identified as the result of a single pesticide application, affecting bees from a number of apiaries. These spray events are the 'incidents' reviewed below.

RESULTS FOR 1981-89

Table 1 summarises the results of investigations in three periods, showing the numbers of cases reported, the numbers in which pesticide residues were detected, and the active ingredients involved. Overall, pesticides were confirmed in 58% of cases, many of the remainder being attributable to starvation under adverse weather conditions. The majority of the pesticide-related incidents occurred in early summer (May to June) when spraying of agricultural crops coincided with bees' foraging activities in the crops themselves or at flowering weeds in and around fields.

A very small number of incidents were the result of deliberate malicious contamination of hives. Most, however, could be attributable to crop protection, which in some cases was carried out according to the approved use, but in others involved mistiming or failure to liaise with local beekeepers before spraying.

Table 1 shows that a few chemicals accounted for a large proportion of the incidents. There are suggestions of some changes during the nine-year period, which are best examined for each chemical separately.

Incidents involving triazophos

In the late 1970s, triazophos ('Hostathion') was provisionally approved for use to control seed weevil (*Ceutorhynchys assimilis*) and brassica pod midge (*Dasineura brassicae*) on oilseed rape. Early attempts at aerial application resulted in heavy bee mortality (Stevenson & Smart 1979). Thereafter, approval was restricted to ground applications, and was made conditional on satisfactory results from ADAS monitoring from 1981 onwards. During the 1980s, the incidence of poisoning by triazophos has gradually declined, despite a large increase in the area of oilseed rape grown, and in the area treated with triazophos (Fig. 1). This decrease can be attributed to the imposition of a restriction on the permissable period for spraying triazophos to the time after 90% of petals have fallen from the crop, when few bees should be foraging. This measure, combined with an extensive education campaign directed at users by the manufacturer of the product has successfully reduced mortality to a level which corresponds to

less than 0.5% of the honeybee colonies in the country. This is considered sufficiently low to permit the continued use of triazophos, although it may produce heavy local mortality. In practice, many of the incidents that now arise are caused by failure to observe the conditions of approval, by spraying winter crops in full flower, or spring-sown crops (on which the product has no approval for use).

Incidents involving dimethoate

In contrast to the decline in triazophos as a cause of bee mortality, poisoning by the insecticide dimethoate has increased, particularly in 1988 and 1989. This chemical is used for aphid control on a wide variety of arable crops, and many of the incidents could be identified as a result of spraying field beans, peas, oilseed rape, or cereals, in some cases by aerial application. The recent heavy losses of bees can probably be attributed to high use of dimethoate in response to serious aphid infestations, and the scarcity of less damaging alternative products such as pirimicarb. A minimum of 236 colonies of honeybees was affected in 15 incidents in 1989, compared to 78 colonies affected in 15 incidents of triazophos poisoning.

Incidents involving gamma-HCH

Although less frequently implicated than triazophos or dimethoate, gamma-HCH regularly causes a few incidents each year. Many of these are associated with crop sprays in mid-summer, although there have also been cases of poisoning by persistent timber-treatment formulations, affecting swarms of bees in roof voids.

Incidents involving carbaryl

The use of carbaryl as a blossom thinner in orchards in the spring presents a potential threat to bees visiting adjacent pollinator trees that may be in full flower at the time. A total of 26 incidents involving carbaryl has been recorded (Table 1), most of them in orchards.

Incidents involving synthetic pyrethroids

The recent introduction of synthetic pyrethroid insecticides for summer use was not expected to lead to any significant honeybee problems, because of the low rates of application of the active ingredients in these products, and their reputed repellency to bees. In order to provide reassurance, all the samples of bees received in 1988, and some of those in 1989, were screened for residues of the principal pyrethroids in use. Low residues of cypermethrin were detected in only two of the 123 samples, and in one case were accompanied by much larger residues of triazophos that were likely to have been the cause of death. Deltamethrin was identified in one incident in 1985, but these results support the overall view of pyrethroids as relatively safe to bees.

Incidents involving other insecticides

A variety of other chemicals have been detected in incidents, usually in isolated cases that have affected only small numbers of bee colonies. They include pirimiphos-methyl, azinphos-methyl, fenitrothion, chlorpyrifos, bendiocarb and malathion. Residues of phosalone, which is comparatively harmless to bees (Oomen 1986) have been detected on eight occasions, although in most of these triazophos was also present, suggesting that exposure to phosalone was sublethal, a view supported by the low residue levels identified.

Incidents involving herbicides

Although most incidents are attributable to insecticides, residues of the

herbicides paraquat and diquat have been found in three cases. One of these was the result of weed control with paraquat on a school playing field where bees were foraging, and another resulted from the use of diquat as a haulm dessicant on a potato crop.

CONCLUSIONS

This approach to investigation of reported honeybee mortality provides the UK regulatory authority with a valuable 'safety-net' to identify any problems that are not fully anticipated on the basis of pre-registration testing of pesticides. It has allowed the success of steps taken to reduce the adverse effects of triazophos, for example, to be monitored, permitting a judgement on approval for the product's continued use on oilseed rape. Currently, the results are providing evidence to support the acceptability of pyrethroid insecticide use on summer crops, and are raising a concern over the apparently growing scale of poisoning by dimethoate.

Overall, it appears that rather less than half of the suspected cases of poisoning are probably due to other causes. However, only a proportion of the incidents that occur will get reported to the scheme, so that the total number of honeybee colonies affected by pesticides is likely to be substantially higher than those revealed by the investigations. Although it does not provide an exact measure of the numbers of poisoning cases that occur, investigation of reported incidents is the best way of assessing the poisoning of bees by pesticides.

The scale of losses (less than 350 colonies affected in 1989 incidents, for example) is small compared to the numbers of registered colonies in Great Britain (over 150,000). One contributor to the problem is the inadequacy of liaison between beekeepers and those applying pesticides. Improved communication, and enforcement of the regulations governing safe use of pesticides, should help to reduce the incidence of poisoning.

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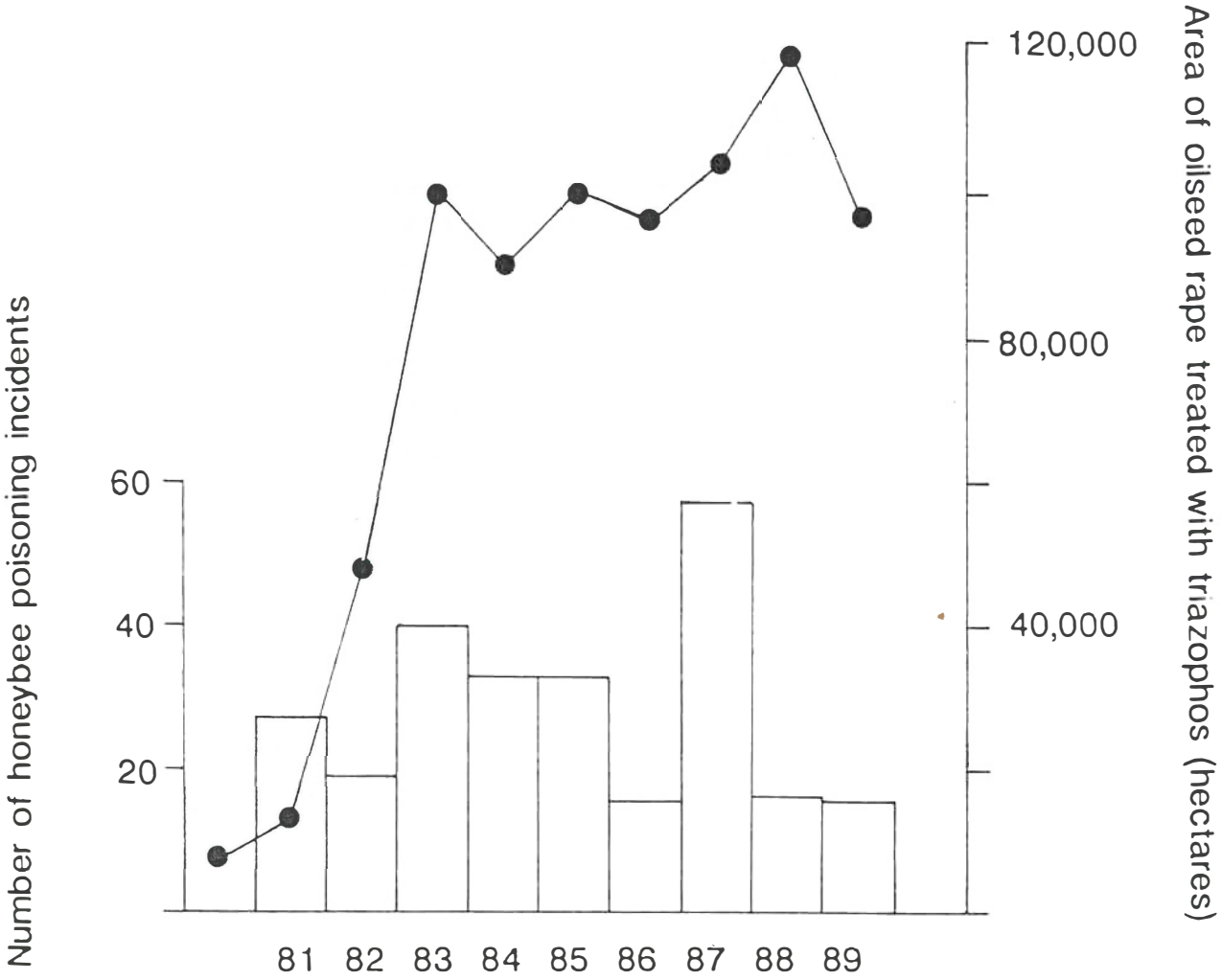
TABLE 1 Results of investigations into suspected pesticide poisoning of honeybees in England and Wales, 1981-89.

	1981-83	1984-86	1987-89
Number of cases investigated:	296	327	315
Number in which pesticides were detected:	177 (60%)	180 (55%)	191 (61%)
Number of incidents * :	132	140	166
Organophosphorus insecticides			
triazophos	88	81	88
dimethoate	13	32	45
other compounds	6	11	20
Organochlorine insecticides			
gamma HCH	12	9	16
Carbamate insecticides			
carbaryl	16	5	5
bendiocarb	0	0	3
Pyrethroid insecticides		1	2
Herbicides			
paraquat/diquat	0	2	1

NOTE More than one pesticide may be detected in a single incident.
In 1981, only limited monitoring was carried out, for two months in summer.

Figure 1. Changes in the occurrence of incidents of poisoning of honeybees by triazophos in England & Wales, in relation to the area of oilseed rape treated with triazophos.



APPENDIX 5

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TWENTY YEARS OF RESEARCHES ON THE ACTION OF PESTICIDES ON HONEYBEES

SUMMARY

The action on honeybees of 28 insecticides, 8 insecticide-acaricides, 6 acaricides, 11 fungicides, 3 fungicide-acaricides, 3 herbicides, 1 food integrator, and 1 attractive compound is reported. The 61 products were tested by ingestion and by indirect contact under laboratory conditions; 9 were tested also in the field; for 6 of them the LD_{100} was ascertained. The results showed the high toxicity of both insecticides and acaricide-insecticides besides the dangerousness of several acaricides and fungicides, that are generally considered as selective towards insects. In any case the check of the label reliability and the prohibition to spray hazardous chemicals during the blossoming period must be absolutely recommended with the aim to safeguard the foraging and pollinating honeybee.

TWENTY YEARS OF RESEARCHES ON THE ACTION OF PESTICIDES ON HONEYBEES

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In the last 20 years in the Institute of Agricultural Entomology and Apiculture of the University of Turin several pesticides were submitted to analysis in order to clarify separately the action by ingestion and by contact of their active ingredients on the honeybee. At first the aim was to detect the causes of the recurrent havocs of honeybees above all during spring and summertimes, then to ascertain the real risk in using either new chemical products or those labelled as not dangerous to useful and pollinating insects. The pesticides were tested with an average of 3 per year and chosen within the wide range of insecticides, acaricides, fungicides, and herbicides.

MATERIALS AND METHODS

Altogether 28 insecticides, 8 acaricide-insecticides, 6 acaricides, 11 fungicides, 3 fungicide-acaricides, 3 herbicides, 1 food integrator, 1 compound with attractive action were tested.

The tests were carried out by ingestion and by indirect contact under laboratory conditions. In few cases, also field tests were made and the LD_{100} was ascertained. The investigation were accomplished following the method described in detail in a previous paper (Arzone and Vidano, 1980). The active compounds are classified as highly, markedly, moderately and slightly toxic when they led to a mortality percentage ranging from 100 to 76, 75 to 51, 50 to 26, 25 to 1, respectively (Arzone, 1975).

RESULTS

The active compounds showed the toxicities as reported below.

Among the insecticides: high both by ingestion and contact for the organophosphorus acephate, azinphos-methyl, chlorpyrifos-methyl, diazinon, dimethoate, fenitrothion, fenthion, heptenophos, isofenphos, malathion, parathion-methyl, pyridafenthion, quinalphos, trichlorfon, the carbamates butocarboxim and carbaryl, the pyrethroids cypermethrin, cypermethrin (S 1R-cis- and R 1S-cis-isomers) and permethrin, and the organonitrogen methomyl; high by ingestion and marked by contact for the pyrethroid deltamethrin; high by ingestion and moderate by contact for the thiocarbamate cartap; high

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Studies of the C.N.R. coordinate research Unit for Integrated Control of Plant pests: 285.

Table 1 - Relative toxicity of active ingredients to honeybees as determined by laboratory and field tests at the dose suggested for crop treatments or at decreasing doses*

Active ingredient	Action	Dose ‰	Laboratory ingestion	Laboratory contact	LD_{100} µg/bee	Field
ACEPHATE	I	0.6375	high	high		
		0.0049*	high			
AMITRAZ	AI	0.432	marked	slight		
ATRAZINE	H	1.32	insign.	insign.		
AZINPHOS-METHYL	I	0.50	high	high		
		0.0157*	high	high		
BENOMYL	F	0.30	slight	insign.		
BUTOCARBOXIM	I	0.727	high	high		moderate
		0.181*	high			
CAPTAN	F	1.50	insign.	insign.		
CARBARYL	I	1.00	high	high		marked
		0.007*	high			
CARTAP	I	0.90	high	moderate		
		0.0281*	high			
CHLORPYRIFOS-METHYL	I	0.442	high	high		
		0.0069*	high			
CYHEXATIN	A	0.30	slight	insign.		
CYPERMETHRIN	I	0.20	high	high		
		0.0031*	high			
CYPERMETHRIN	I	0.095	high	high		
(S1R-cis- and R1S-cis-isomers)		0.0475*	high	high		
DELTAMETHRIN	I	0.0125	high	marked		
DEMETON-S-METHYL	IA	0.21	high	high		high
		0.003*	high			
DIALIFOS	AI	0.90	high	slight		
		0.225*	high			
DIAZINON	I	0.60	high	high		
		0.0188*	high			
DICOFOL	A	0.37	marked	slight	16.33	slight
DIMETHOATE	I	0.57	high	high		
		0.0022*	high			
DINOCAP	FA	0.30	slight	marked	29.43	slight
DITALIMFOS	F	0.40	insign.	insign.		
DITHIANON	F	1.125	insign.	high		
		0.5625*		high		
DODINE	F	0.975	insign.	insign.		
ENDOSULFAN	IA	0.70	high	marked	20.60	moderate
ETHIOFENCARB	I	0.69	high	slight		
		0.0862*	high			
FENBUTATIN OXIDE	A	0.50	insign.	slight		
FENITROTHION	I	0.95	high	high		
		0.0074*	high			
FENPROPATHRIN	AI	0.25	high	high		
		0.002*	high			
FENTHION	I	0.485	high	high		
		0.0152*	high			
FENVALERATE	I	0.055	high	insign.		

Table 1 - continued

Active ingredient	Action	Dose ‰	Laboratory ingestion	Laboratory contact	LD_{100} µg/bee	Field
FLUCYTHRINATE	I	0.06	moderate	moderate		
HEPTENOPHOS	I	0.50	high	high		
		0.0039*	high			
HEXYTHIAZOX	A	0.05	insign.	insign.		
HYDROLYZED PROTEINS	AC	2.97	insign.	insign.		
ISOFENPHOS	I	0.75	high	high		
		0.375*	high			
MALATHION	I	0.75	high	high		
		0.0117*	high			
MANCOZEB	F	2.40	insign.	insign.		
METHOMYL	I	0.51	high	high		
		0.004*	high			
OMETHOATE	IA	0.50	high	high		
		0.0039*	high			
PARAQUAT	H	0.893	slight	slight		
PARATHION-METHYL	I	0.48	high	high		
microcapsulate		0.03*	high	high		
Pb ARSENATE	I	0.84	high			
PERMETHRIN	I	0.2104	high	high		
		0.0066*	high			
PHOSALONE	IA	0.70	high	slight	4.12	slight
		0.08*	high	insign.		
PIRIMICARB	I	0.37	high	slight	2.94	slight
		0.25*	high			
PROPARGITE	A	0.57	insign.	insign.		
PROPICONAZOLE	F	0.25	moderate	insign.		
PYRAZOPHOS	F	0.30	high	high		
		0.0047*	high			
PYRIDAFENTHION	I	1.20	high	high		
		0.0012*	high			
QUINALPHOS	I	0.50	high	high		
		0.0039*	high			
QUINOMETHIONATE	FA	0.125	insign.	insign.		
Sb and K TARTRATE	I	2.00	high	slight		
		0.50*	high			
SEAWEEDS	FI	0.80	insign.	insign.		
SIMAZINE	H	6.65	slight	slight		
SULPHUR	FA	1.38	slight	slight		
TETRADIFON	A	0.17	high	slight	1.01	slight
		0.04*	high			
TRIAZOPHOS	IA	1.00	high	high		
		0.001*	high			
TRICHLORFON	I	2.00	high	high		
		0.0078*	high			
TRIFORINE	F	0.27	insign.	slight		
ZINEB	F	3.185	slight	slight		
ZIRAM	F	1.80	slight	slight		

A acaricide, AC attractive compound, AI acaricide-insecticide, F fungicide, FA fungicide-acaricide, FI food integrator, H herbicide, I insecticide, IA insecticide-acaricide.

by ingestion and slight by contact for the carbamates ethiofencarb and pirimicarb, the pyrethroid fenvalerate and the organometallic Sb and K tartrate; high by ingestion and insignificant by contact for the inorganic Pb arsenate; moderate by ingestion and by contact for the pyrethroid flucythrinate.

Among the acaricide-insecticides: high both by ingestion and contact for the organophosphorus demeton-S-methyl, omethoate, triazophos, and the pyrethroid fenpropathrin; high by ingestion and marked by contact for the organochlorine endosulfan; high by ingestion and slight by contact for the organophosphorus dialifos and phosalone; moderate by ingestion and slight by contact for the organonitrogen amitraz.

Among the acaricides: high by ingestion and insignificant by contact for the organochlorine tetradifon; marked by ingestion and slight by contact for the organochlorine dicofol; slight by ingestion and insignificant by contact for the organotin cyhexatin; insignificant by ingestion and slight by contact for the organotin fenbutatin oxide; insignificant both by ingestion and contact for the organonitrogensulphur hexythiazox and the organosulphur propargite.

Among the fungicides: high both by ingestion and contact for the organophosphorus pyrazophos; slight by ingestion and high by contact for the organosulphur dithianon; moderate by ingestion and insignificant by contact for the organonitrogen propiconazole; slight both by ingestion and contact for the dithiocarbamates zineb and ziram, insignificant by ingestion and slight by contact for the organonitrogen triforine; insignificant both by ingestion and contact for the organonitrogen benomyl and the organonitrogensulphur captan, the organophosphorus ditalimphos, the guanidine dodine, and the dithiocarbamate mancozeb.

Among the fungicide-acaricides: slight by ingestion and marked by contact for the organonitrogen dinocap; insignificant both by ingestion and contact for the organonitrogen quinomethionate and the inorganic sulphur.

Among the herbicides, all organonitrogens: moderate by ingestion and slight by contact for paraquat; slight by ingestion and moderate by contact for simazine; insignificant both by ingestion and contact for atrazine.

Seaweeds, an organic complex integrating the nutrition, and hydrolyzed proteins, a compound with attractive action, were insignificant both by ingestion and contact (table 1).

DISCUSSION

All the products having insecticide or insecticide-acaricide action appeared to be dangerous for honeybees. Several resulted to be highly toxic also at concentrations many times lower than those suggested for crop treatments (e.g. triazophos and pyridafenthion until 1024 times by ingestion) or very persistent (e.g. cypermethrin until 42 days after spraying: Arzone and Patetta, 1986). Nevertheless, besides

the action also the chemical composition needs to be considered: for example, the organophosphorus pyrazophos is highly toxic both by ingestion and contact even if labelled as a fungicide. Obviously, it is necessary not only to consider the pesticides provoking acute toxicity (e.g. acephate, azinphos-methyl, butocarboxim, chlorpyrifos-methyl, diazinon, dimethoate etc.) but also those provoking slow and progressive poisoning (e.g. amitraz, deltamethrin, dialifos, endosulfan, paraquat, simazine, zineb, ziram), those exerting prolonged toxic action when stored in the hive (e.g. carbaryl associated with pollen: Vidano et al., 1976), and those whose metabolites induce a gradual plant pollution (e.g. the endometotoxic systemic demeton-S-methyl in the nectar: Arzone and Vidano, 1978).

From the practical point of view, these acquisitions show that in order to safeguard foraging and pollinating honeybees all the compounds having insecticide and acaricide action, although not exerting acute toxicity, are not to be used on melliferous and polliniferous sources.

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APPENDIX 6

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A NEW METHOD TO TEST THE HAZARDS OF INSECTICIDE SPRAYING ON *Megachile rotundata* F: TRIALS IN TUNNELS

SUMMARY

The aim of the method is to test the hazards of insecticide sprayings to *Megachile rotundata* F. The compounds are applied on a flowering crop of white mustard in the presence of pollinators and are compared to water spraying. The experimental design has 3 replications. The elementary plot has a 33 m2 area and is a part of a polyethylen tunnel. In the trial, deltamethrin 6,25 g/ha and fenvalerate 50 g/ha show a short-time repulsive effect but do not induce any pollinator mortality.

INTRODUCTION

Hazards encoutered by pollinator insects during insecticide treatments on flowering crops may be evaluated either by laboratory or by field tests. Honey-bees are the best known pollinators in terms of toxicology and hazards.

To a lesser extent, the Alfalfa leaf cutting bee has also been included in such experiments (JOHANSEN and al. 1963, WALLER 1969, TORCHIO 1983, GEORGE and RINCKER 1985).

In France the experiments realized in Alfalfa field provided a number of data on the effects of some insecticide applications (TASEI and CARRE 1985, TASEI and al. 1987).

This method implied an important number of fields in order to have enough replications. Thus, testing a new experimental method under netted tunnels appeared to be interesting, in order to make a simultaneous replication of treatments in the same site possible.

MATERIALS AND METHODS

1) Insects obtaining :

The Alfalfa leaf cutting bees were issued from a breeding site in Manitoba (Canada). The larvae containing cocoons (prepupae) had been stored for 15 days at 5°C. Prior to incubation, the cocoons have been dipped into an insecticide solution containing 1 g/l SEVIN L 85, in order to preventively treat against *Melittobia*. The following day, incubation started in a chamber regulated at 30°C temperature and 60-70 % relative humidity. This incubator contained a U.V. trap used to eliminate *Pteromalus sp.*
3 weeks later, the first males emerged, and the cocoons were transplanted in equal portions under the shelters in the tunnels. The emergence of females started 8 days later.

2) Experimental design :

The experimental plots were set in PROCIDA/RU's experimental station under 4 netted polyethylene tunnels (17 x 6 x 2,7 m); each tunnel was divided into 3 sections. Each section was covered by 20 m2 of *Sinapis alba* and enclosed a nesting shelter and a watering place. As soon as the females emerged, Alfalfa pots were introduced in order to provide the leaves used by the insects to build their nests.
At the end of the *Sinapis* flowering, the experimentalists introduced pots from later blooming *Sinapis* plants, which had undergone the same treatments as the tunnel plants.

3) Treatments :

Treatments were applied only when the bees had adapted to the tunnel conditions, and when the number of females visiting the nests had stabilized. The compounds were applied on 25/07/89 between 14h30 and 15h20, when the bees were fully foraging (temperature under tunnel : 32°C). Each compound was sprayed in 3 sections of the tunnels, i.e. 3 replications : deltamethrin at 6,25 g a.i./ha (DECIS® EC 25 g/l), fenvalerate at 50 g a.i./ha (SUMICIDIN 10® EC 100 g/l), and phosalone at 600 g a.i./ha (ZOLONE FLO® SC 500 g/l). 3 sections were used as control and treated with water. Applications were carried out with 500 l/ha water, under a pressure of 3 bars, using an individual AZO backsprayer with a 6 hollowcone nozzles spraying boom.

4) Observations :

- each day, at 7 H , males and females were counted in the nests, and at 14 H, on mustard flowers.
- twice a week, colored spots were painted on the plugs closing the completed nests.

- at the end of the trial, the nests were placed at 5°C temperature for 2 months and then were incubated at 30°C in order to evaluate the % of surviving larvae, by counting the adults emerging. Non hatched cocoons were opened in order to determine the reason for death.

RESULTS

1) Foraging activity of bees : (table 1)

After water treatment, the leaf cutting bees fly away behind the spraying boom and then come back rapidly. The number of foraging insects remains constant during the next 4 days.
After deltamethrin or fenvalerate treatments, the bees fly away behind the spraying boom, and then gradually come back into the crop. This repelling effect is very clear the day following the treatment but decreases afterwards.
The day of treatment, phosalone induces effects similar to the 2 other compounds. However, during the 4 following days, the number of foraging bees steadily decreases, because of the death of a part of the population.

2) Number of females in the nests : (table 2)

No difference were noted between water treated tunnels and deltamethrin or fenvalerate treated tunnels, which confirms a good selectivity of both compounds. With phosalone, the number of females in the nests is sharply reduced the day following the application and remains low afterwards (statistically significantly different from deltamethrin, fenvalerate, and water 4 and 5 days after treatment).

3) State of larvae :

The number of larvae cells obtained in each treatment is relatively low : 66, 78, 57 and 100 respectively for deltamethrin, fenvalerate, phosalone and water.
The adults producing cells were respectively 52, 44, 42 and 58 % of larvae population. Mortality occured mostly at "pupae" and "prepupae" stages. Because of the size of the sample, no interpretation can be made as far as larvae are concerned.

CONCLUSION

The proposed trials method shows several benefits : possibility to use the material (tunnels) commonly used in France to study the action of compounds on honey-bees (FLORELLI and al. 1986); possibility of using replications; acurate observations of bee behaviour during and after the treatment; possibility of drawing valid conclusions on short term toxicity of products, even though the number of leaf cutting bees per section is rather low.

When applied on leaf cutting bees foraging on white mustard flowers, deltamethrin at 6,25 g a.i./ha and fenvalerate at 50 g a.i./ha, induce the flight of the insects from the crops, this repelling effect decreasing gradually during the 3 following days. However, no abnormal adult mortality, nor change in the female nesting behaviour was noted after the treatment. In the same conditions, phosalone at 600 g a.i./ha, didn't appear to be selective, eliminating 3/4 of the population. These results may be used in order to choose compounds to be applied on leaf cutting bees visiting crops (Alfalfa, white clover, cruciferae ...) during flowering.

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TABLE I - NUMBER OF GATHERING LEAF CUTTING BEES ON 10 POTS OF FLOWERING WHITE MUSTARD IN THE MIDDLE OF THE DAY

- results expressed as total number of bees (males + females) on 3 replications

Products	dosages in g a.i./ha	prior to treatment	After treatment					
		25/07 T - 15'	25/07 T + 30'	25/07 T + 60'	26/07 T + 1 day	27/07 T + 2 days	28/07 T + 3 days	29/07 T + 4 days
deltamethrin	6,25	20	10	8	8 b	17	9 b	17 a
fenvalerate	50	24	9	8	12 b	10	12 ab	13 ab
phosalone	600	18	10	10	6 b	8	5 b	3 b
water check	-	14	16	13	21 a	22	22 a	21 a
Variance analysis : F. calculated		2,63	1,41	1,56	8,43	0,84	6,26	6,34
conclusion		NS	NS	NS	S	NS	S	S

TABLE II - NUMBER OF FEMALE LEAFCUTTING BEES IN THE NESTS AT 7 A.M.

- results expressed as total number on 3 replications

Products	dosages in g a.i./ha	Prior to treatment				After treatment				
		22/07	23/07	24/07	25/07	26/07	27/07	28/07	29/07	30/07
deltamethrin	6,25	27	15	20	15	12	17	21	20 a	21 a
fenvalerate	50	19	17	21	20	16	13	16	17 a	19 a
phosalone	600	16	13	16	18	5	7	4	3 b	4 b
water check	-	20	14	11	9	17	18	14	19 a	16 a
Variance analysis : F. calculated		2,03	0,15	0,61	1,30	1,86	1,25	2,49	5,76	8,26
conclusion		NS	NS	NS	NS	NS	NS	NS	S	S

S (NS) = significant (non significant) trial according to Fisher test at 5 % level

- a, b = groups of non statistically different values according to Newman-Keuls test at 5% level

APPENDIX 7

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DEMONSTRATING THE SAFETY OF ALPHACYPERMETHRIN TO BEES: RESULTS OF A LARGE-SCALE FIELD MONITORING STUDY IN THE U.K.

The sequential testing system used by Shell to investigate the hazard of pesticides to honey bees was described at the symposium held at Rothamsted in 1985. This system was used to investigate the hazard of the pyrethroid insecticide, alphacypermethrin (Fastac), to bees. The results of the laboratory toxicity tests showed that technical grade alphacypermethrin is highly toxic to bees by contact and oral dosing (Murray, 1985). However, the results of cage tests (Ibid.) and small-scale field studies (Shires et al., 1984a,b) indicated that the compound was of low hazard to bees under field conditions. This was confirmed by the results of the large-scale field trial (Shires et al., 1984c), which demonstrated that commercial-scale applications of alphacypermethrin at recommended field rates had no adverse effects on bee survival or on the longer-term health of the experimental colonies.

On the basis of this work, alphacypermethrin has been registered for use on flowering crops in a number of European countries, and no reports have been received concerning adverse effects on bees. During the process of registering the product for use on oilseed rape in the UK, we also carried a large-scale field trial to monitor possible effects on bees of alphacypermethrin when applied as an in-tank mixture with the fungicide vinclozolin. The latter compound is known to be of very low toxicity to bees. The study was carried out between April 24 and May 5, 1989, in a 206 ha block of flowering oilseed rape in Kent, UK. Five beehives were positioned at each of two sites adjacent to the crop on April 23. These hives were fitted with either pollen traps or dead bee traps. Alphacypermethrin was applied as an in-tank mixture with vinclozolin at dose rates of 20 and 100 g ai/ha, respectively, using a tractor-driven boom-and-nozzle sprayer at a volume of 500 l/ha. Spraying began on April 26 and was continued and completed on April 28. The weather on April 27 was unsuitable for spraying. Biological observations were carried out prior to, and up to 7 days after the end of spraying. Dead bees at the hives were counted and collected daily, and observations on levels of hive activity were carried out at intervals during each day of the study. On days when significant numbers of bees were actively foraging in the crop, pollen traps were used to collect samples of pollen, which were taken to the laboratory to determine their composition. During June, 1989 (two months after the end of the study), a professional beekeeper inspected all the hives used in the study to investigate possible longer-term effects on the colonies. In addition to the experimental hives, a local beekeeper had placed 30 hives at two locations at the site prior to spraying. These hives remained in position for the duration of the trial. Their condition was closely monitored by the beekeeper during and after the study.

The weather during the trial was somewhat variable. However, field observations showed that the bees actively foraged the treated crop. Indeed, analysis of the pollen samples showed that at least 90% of the pollen collected following treatment was oilseed rape. Since no other rape fields were within flying range of the colonies, it was concluded that this pollen had been collected from the treated crop. Table 1 shows that numbers of dead bees collected during the study remained low throughout the experiment, and did not increase following treatment. The hives were found to be in good condition after the study was completed, and subsequently yielded a good crop of honey. Similarly, the hives which had been placed in the crop by the local beekeeper were not affected by the treatment. It was therefore concluded that the alphacypermethrin/vinclozolin treatment had no adverse effects on honey bee survival or longer-term colony development.

Table 1: Number of dead bees collected from hives fitted with dead bee traps

	Collection date										
Hive no.	25/4	26/4[1]	27/4	28/4[2]	29/4	30/4	1/5	2/5	3/5	4/5	5/5
1	78	17	21	9	10	20	0	4	0	0	0
2	15	16	11	4	5	3	1	0	6	0	1
3	185	146	56	17	22	36	3	5	15	21	2
4	36	21	33	19	21	11	0	0	0	0	2
5	18	22	7	5	5	0	0	0	0	0	0
Mean	66	44	26	11	13	14	0.8	1.8	4	4	1

Note: [1] - the treatment was first applied on 26/4 after the dead bee traps had been emptied. The area closest to hives 1 & 2 was treated on this date.

[2] - spraying was completed on 28/4, commencing after the traps had been emptied. Hives 3 - 5 were adjacent to the area sprayed on this date.

The results of this study confirm the safety of alphacypermethrin to bees under field conditions, and demonstrate the safety of the product when applied in tank mixture with vinclozolin.

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APPENDIX 8

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A COMPARISON OF A LABORATORY RESIDUAL TOXICITY TEST WITH A SEMI-FIELD 'TUNNEL' TRIAL TO ASSESS THE EFFECTS OF PESTICIDE RESIDUES ON HONEY BEES

INTRODUCTION

The 24-hour acute contact LD50 value of the pyrethroid insecticide, lambda-cyhalothrin ("KARATE") to honey bees (Apis mellifera) is 0.051 ug ai bee^{-1} (Gough & Wilkinson, 1984). It is thus classed as 'highly toxic' according to the toxicity classification of the International Commission for Plant-Bee Relationships. However, a series of field and semi-field or 'tunnel' trials (Gough & Wilkinson, 1984; Wilkinson et al, 1986) have shown that lambda-cyhalothrin is of low hazard to honey bees at rates of up to 15 g ai ha^{-1} when applied to flowering oilseed rape or to cereals sprayed with simulated honeydew (sucrose solution), even when the bees are actively foraging.

The effects on honey bees of lambda-cyhalothrin on lucerne (Medicago sativa) were assessed in a procedure analagous to that of Lagier et al (1974). The purpose of this test is to measure the toxicity of field weathered residues to honey bees in the laboratory. However, the results suggested a level of residual toxicity inconsistent with the results of the field and semi-field trials.

The study described here was designed to assess the effects of field-weathered lambda-cyhalothrin residues on honey bees using the laboratory residual toxicity test and a semi-field 'tunnel' trial. This was so that the results of the two tests could be directly compared and in particular to account for any differences observed, so aiding the hazard assessment process.

LABORATORY RESIDUAL TOXICITY TEST

Materials and Methods

Two plant species, oilseed rape (Brassica napus) and lucerne were used in the laboratory residual toxicity test. Oilseed rape is a flowering crop highly attractive to honey bees (which is necessary for the 'tunnel' trial) and on which most field data regarding lambda-cyhalothrin is available. Lucerne was also included to allow comparison with the data from the previous laboratory residual toxicity test which used this crop.

Two tests were conducted based on the method of Lagier et al (1974). In each, three strips of oilseed rape and two of alfalfa, each measuring 10 x 2.5 m were treated using a one-man hand-held plot sprayer. The plants were mature but not in full flower. With both crops, one strip was sprayed with lambda-cyhalothrin at a rate of 35 g ai ha^{-1} and another left untreated as a control. The third oilseed rape strip was treated at a rate of 15 g ai ha^{-1}. After residue ageing periods of 3, 8, 24, 48 and 96 hours, foliage samples were collected for bioassay: only the top halves of the plants were sampled so as to ensure no dilution effect from the lower, sheltered leaves. The oilseed rape samples were cut into pieces approximately 65 x 30 mm, while the alfalfa samples were cut into approximately 50 mm lengths. Half litre portions of

the chopped foliage were put into ventilated cages: these were constructed of a wire-mesh cylinder, 50 mm high, 140 mm diameter, with the top and bottom formed by 150 mm petri dishes. Thirty bees were added to each cage, there being three replicate cages for each treatment. The cages were supplied with sucrose solution in cotton wool, as food for the bees. This was located at the bottom of the cage so that the bees had to crawl through the foliage to reach it. Any effects on the bees were assessed after 24 hours of exposure, in darkness at 25 °C, to the residues.

Results

The change in percentage mortality of the bees, exposed to the various treatments, (corrected for control mortality) were plotted against time. From these curves, regression analysis was used to estimate the age of residue which was lethal to 50% (LT50) and 25% (LT25) of the bees in the test system (a linear regression model was found give the best fit). These estimates together with their 95% confidence limits are presented in Table 1.

Table 1: Estimates of the residue age (hours) lethal to x% (LTx) of bees in the residual toxicity test.

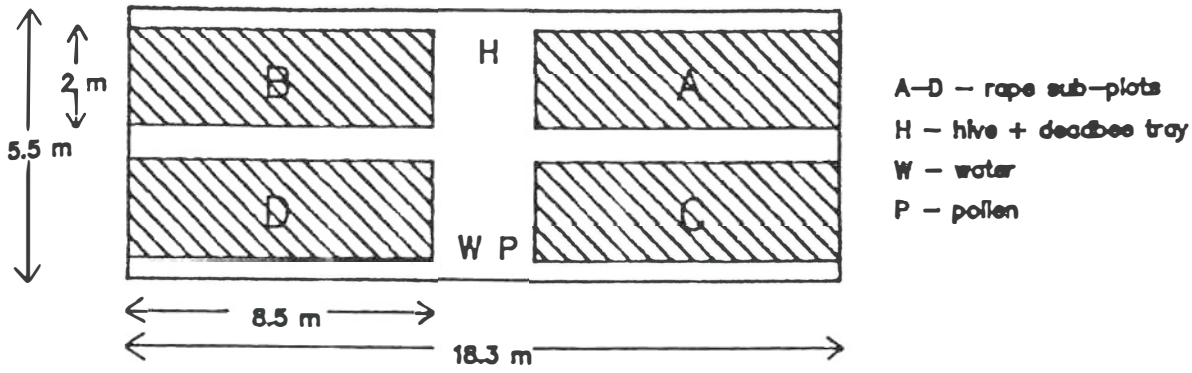
Treatment	Test	LT50 (95% confidence interval) / hours	LT25 (95% confidence interval) / hours
Rape / 35 g ai ha^{-1}	1	11.5 (0.02-7640)	154 (0.02-1.42×10^{6})
	2	0.2 (0.04-1.03)	30.4 (0.1-9650)
Rape / 15 g ai ha^{-1}	1	0.13 (0.04-0.41)	3.9 (0.12-123)
	2	0.0 (0.0-1.43×10^{46})	0.0 (0.0-1.18×10^{14})
Lucerne / 35 g ai ha^{-1}	1	56.1 (0.002-1.72×10^{6})	542 (0.001-4.35×10^{8})
	2	44.7 (0.002-1.1×10^{6})	4756 (0.0002-1.05×10^{11})

SEMI-FIELD 'TUNNEL' TRIAL

Materials and Methods

The trial was based on a method developed in France by INRA (Delabie J, 1984). Oilseed rape only was used: a plot 50 x 40 m was grown, according to normal agricultural practices. One half was planted about six weeks later than the other in order to ensure an adequate supply of flowering plants for two successive replicate runs. Plots of the rape were enclosed by tunnel greenhouse frames, covered with a plastic mesh sufficiently fine to keep bees inside whilst still permitting weathering. The layout of each tunnel is shown in Fig 1.

Fig. 1 Tunnel layout



A single, queenright colony of honey bees was placed inside each tunnel, as shown, and left for up to one week to acclimatise to the new surroundings. Due to the limited foraging area available, supplementary food was supplied throughout the bees' confinement within the tunnel (sucrose solution in a contact feeder on the top board of the hive and dried pollen in a dish in front of the hive).

Treatment took place in the early morning, using a one-man hand-held plot sprayer, about one hour before the bees emerged, c. 07.30 h (this was ensured by closing up the hives the night before and then opening them up one hour after application). There were four tunnels each receiving a seperate treatment: control (water only), lambda-cyhalothrin at rates of 35 and 15 g ai ha^{-1} over all four sub-plots (A-D) in a tunnel and lambda-cyhalothrin at a rate of 35 g ai ha^{-1} over two sub-plots (B an D) only. The last treatment was designed to look at any differences in foraging between the treated and untreated ends. There were two successive replicate runs.

Assessments of the bees were carried out pre and post treatment. Mortality and behavioural assessments of the bees were carried out at the end of the first week (the acclimatisation period), for several days prior to treatment and were continued for up to a week after treatment. Hive inspections were carried out in the week prior to treatment and at about two and five weeks after.

Mortality in the hive and the tunnel was monitored by daily counts of dead bees from "dead bee trays" placed at the entrance to each hive and from polythene sheets placed along the longitudinal and transverse paths in each tunnel respectively. Foraging activity was assessed by counting the bees along a 1 m wide strip of each 8.5 m sub-plot: the observer walked alongside each sub-plot holding a 1 m cane horizontally to define the strip and to provide a reference point for counting, taking 90 seconds to cover the 8.5 m. All 4 sub-plots were counted twice, the first time counting bees in the air (flying), the second time counting bees settled on the flowers (and also noting any in contact with the rest of the plant). Activity was also monitored at the hive with observations, lasting 5 minutes, of bees at the hive entrance and foragers departing and returning. The foraging and hive observations were made hourly on treatment day and every two hours on all other days. The state of the colony was monitored by a full inspection of the hive recording the approximate numbers of the different brood stages and the amount of stored food by visual counts, with the back-up of photography should more detailed analysis have proved necessary.

Results

Results are shown for one of the runs only, the results for the two runs being similar. The results of the mortality assessments are shown in Fig. 2 (the dead bee tray and path counts). These show no significant difference between mortality in the control and treated tunnels. The foraging results for treatment day and the day after are presented in Fig. 3 (total bees present - flying and on the plants) and Fig. 4 (percentage of total on the flowers). There is a reduction in the total number of bees recorded for about half a day after spraying as well as a reduction in the percentage of the foragers settling on the plants. Very few bees were seen on the foliage as distinct from the flowers. No harmful effects were seen in the hives from the lambda-cyhalothrin treatments when compared with the controls.

DISCUSSION

The laboratory residual-toxicity test is undoubtedly extremely severe and gives a highly variable result. The LT50 and LT25 values indicate that there is a high level of toxicity on the day following treatment and marked toxicity for several days. The residue levels in this test are field derived and are therefore of the magnitude that foraging bees might normally encounter. However, in the laboratory cages the bees experience a very high degree of exposure throughout the test period, having to crawl through the chopped foliage for 24 hours. Also, they are unable to respond behaviourally as they might normally do in the field e.g. show repellancy, thereby reducing their exposure to the residues. In addition, the results themselves show a large amount of variability such that it is not possible to make any predictions from them with any degree of confidence. Thus the 95% confidence limits for the estimated LT50 and LT25 values vary in some cases from a few minutes to several years. This variability comes into the system at several stages, notably in the field (pesticide application, weathering and sampling of the residues) and in the laboratory (exposure, stress and response of the bees). Consequently, because of this high variability in the results, the ability of the test to predict field hazard is limited.

The results of the laboratory test were not manifested in the 'tunnel' trial, there being no increase in mortality. In this semi-field environment exposure is more realistic, with free flying bees operating from a hive and so subjected to minimal stress. It is still more severe than a full field experiment as they are confined to an area containing only treated plants. However, compared to the laboratory residual toxicity test, exposure of the bees to the residues will be much less. As the foraging results show the bees only foraged during the day with the intensity varying during this time (possibly related to such factors as nectar flow and the weather). Even during the periods of foraging a proportion of a forager's time will have been spent in the hive or in transit to and from it. While active around the plants only some of a bee's time was actually in contact with the plants as indicated by the percentage of the total foraging population actually on the plants. The foraging observations also showed that the bees only came into contact with the flowers, not the foliage (except in a few isolated instances). This contact with the residues will have been further reduced due to the fairly rapid turnover of flowers - those opening after treatment not being contaminated. The limited contact with the residues on the plants may be further restricted by repellancy although this was not marked in this study,

possibly because the residues were dry most of the time. This repellancy may be manifested both by a reduction in the numbers of foragers present and also in the proportion of foragers settling on the flowers.

It is clear then that whereas the laboratory residual toxicity test indicates high toxicity over a significant period of time the hazard measured in the 'tunnel' trial is low. Similar contrasting results have been found in other studies e.g. Atkins et al (1977). This discrepancy is probably due to the excessively high levels of exposure experienced in the laboratory test compared to the field environment where the bees' contact with the residues is limited. The laboratory residual toxicity test is therefore potentially misleading if used in isolation to assess hazard and not as part of a sequential testing scheme, as has been realised with other laboratory derived toxicity data. However, the test may have improved value if the sprayed foliage is not chopped up but placed in a flight cage where exposure will be more realistic e.g. Murray (1985). This could give an indication of behavioural effects such as repellancy. Where laboratory studies indicate toxicity though, any realistic assessment of hazard must involve studies under field or semi-field conditions.

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FIG. 2 TEST 2 DEAD BEE COUNTS

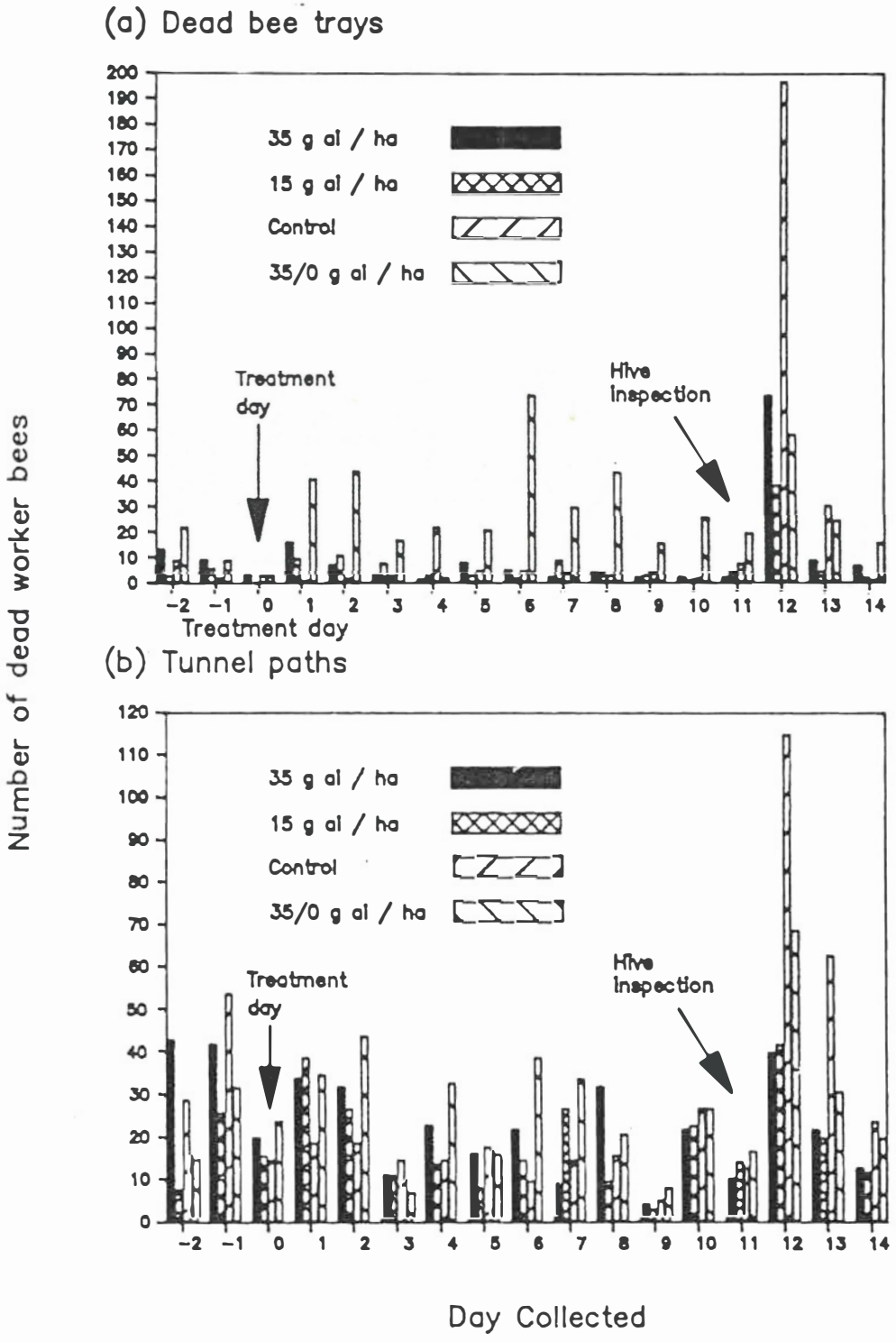


FIG. 3 TEST 2 FORAGING ASSESSMENTS – TOTAL (BEES ON PLANTS + BEES FLYING)

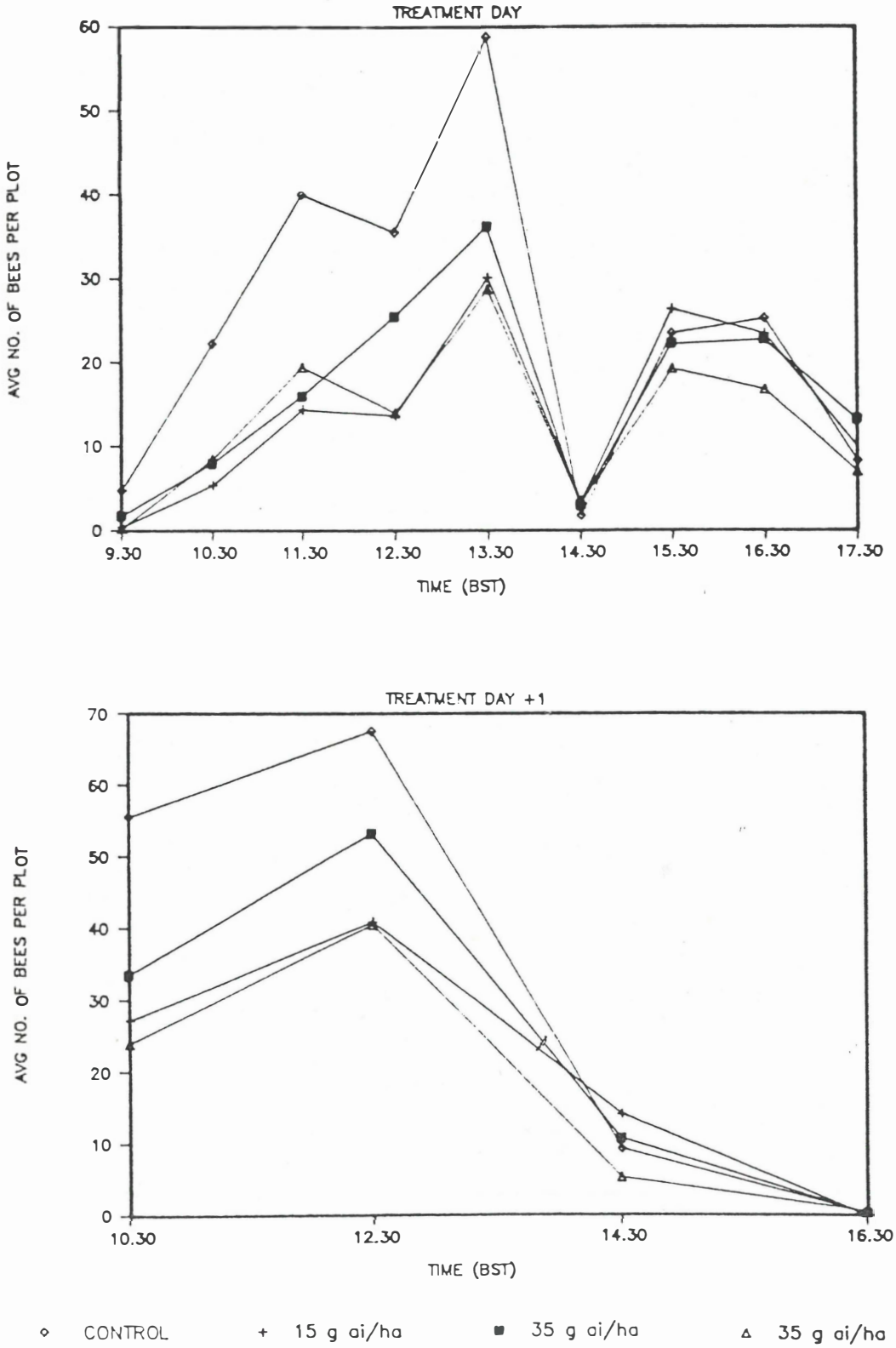
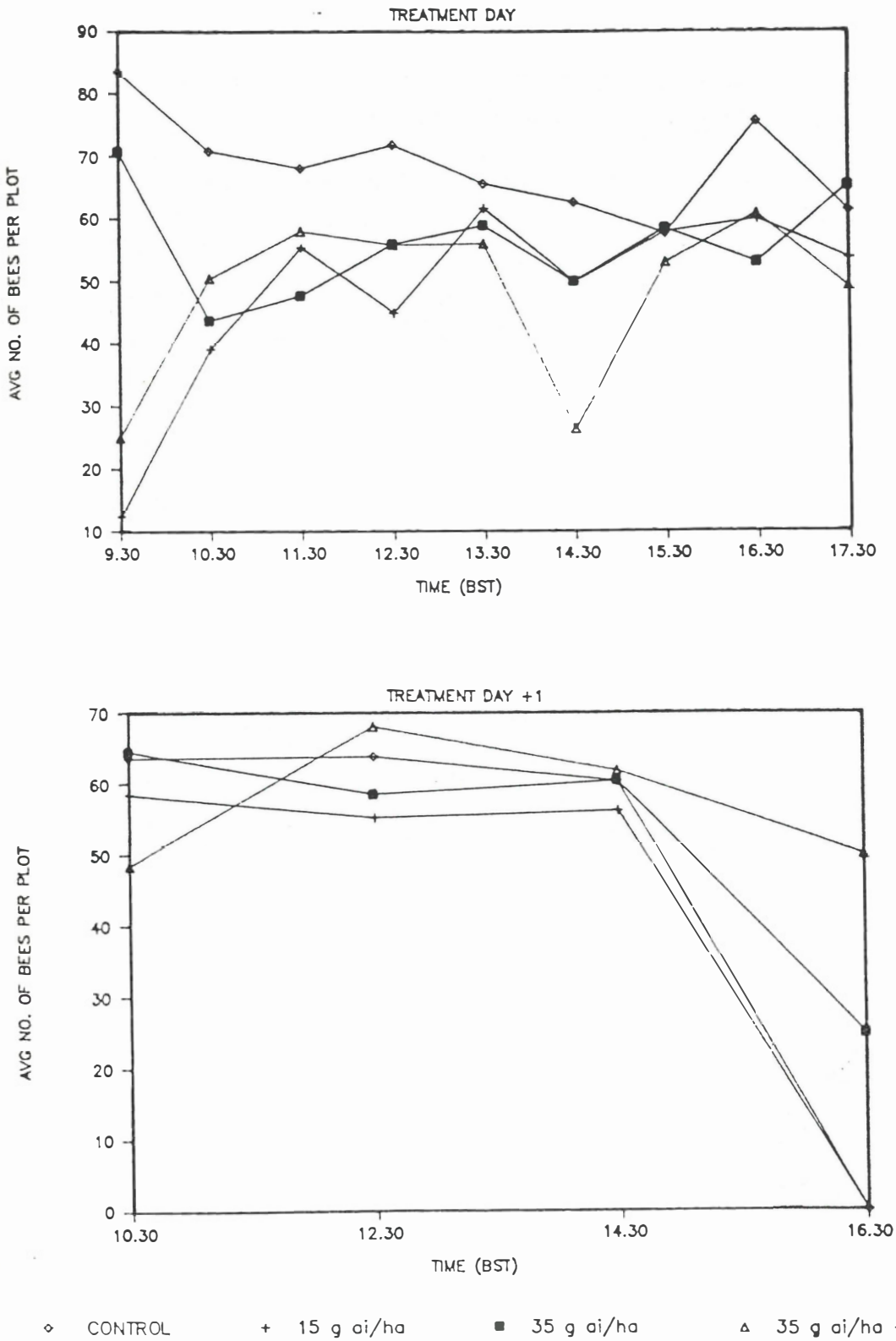


FIG. 4 TEST 2 FORAGING ASSESSMENTS – PERCENTAGE (BEES ON PLANTS / TOTAL BEES)



APPENDIX 9

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TRALOMETHRIN INSECTICIDE AND DOMESTICATED POLLINATORS

SUMMARY

Susceptibility to tralomethrin sprays was greatest for alfalfa leafcutting bees, Megachile rountundata (Fabr.); least for honey bees, Apis mellifera L.; and intermediate for alkali bees, Nomia melanderi Cockrell in residue bioassay studies. In LD_{50} studies alkali bees were less susceptible to tralomethrin than honey bees. Adding tralomethrin to sugar syrup caused a reduction in honey bee visitation, and higher rates caused more reduction than lower rates. Field tests of tralomethrin on blooming alfalfa resulted in no adult alfalfa leafcutting bee mortality. Tralomethrin is not hazardous to the bee species studied if applied in late evening when bees are not foraging.

INTRODUCTION

Tralomethrin a new synthetic third-generation pyrethroid emulsifiable concentrate kills as a contact or stomach poison, and is being registered for insect control on corn, alfalfa and other agricultural crops. Tralomethrin controls pea aphid, Egyptian alfalfa weevil, alfalfa weevil, European corn borer, fall armyworm, black cutworm, and lygus bugs (Larson 1989; Mayer et. al., 1990).

Bee poisoning or killing of honey bees from applications of pesticides is a serious problem worldwide (Johansen, 1977; Mayer and Johansen, 1983). Most bee kills are accidental and result from applications of insecticides for pest control on agricultural crops. Also, more than 120 crops require bees for pollination and often pesticides must be applied for pest control on these crops without harming the bees. For the past 35 years we have evaluated the bee hazard of many pesticides and determined methods to use pesticides without harming bees (Mayer and Johansen, 1988; Johansen and Mayer, 1990).

This paper reports results of our research concerning the effects of tralomethrin insecticide on honey bees (Apis mellifera L.), alfalfa leafcutting bees (Megachile rotundata (Fabr.) and alkali bees (Nomia melanderi Cockrell).

RESIDUE BIOASSAY

Materials and Methods. Tralomethrin was applied to 0.004-hectare plots of alfalfa with a R&D pressurized sprayer at 234 liters of water/ha. Field-weathered residual test exposures were replicated 4 times with 4 foliage samples per treatment and time interval. Alfalfa foliage samples (upper 15-cm portions of plants) were clipped into 2.5- to 5-cm lengths, and 500-cm3 placed in each cage. Cages were prepared from a 15-cm plastic petri dish. A strip of metal screen (6.7 meshes per cm) 45 cm long and 5 cm wide was stapled to form a circular insert which provided ample room in the cage for bees to fly.

Worker honey bees were obtained from the top of colonies and anesthetized with CO^2 to facilitate handling. Leafcutting bee prepupae in leaf piece cells were incubated and emerging adults allowed to fly in the lab and collected off the windows. Alkali bees were collected from nesting sites and chilled to facilitate handling. Residual test exposures were replicated 4 times by caging 40 worker honey bees or 20 leaf cutting bees or 20 alkali bees with each of 4 foliage samples per treatment and time interval. Bees were maintained in cages at 29 C., 60% RH, and fed 50% sucrose solution (1:1 ratio) in a cotton wad (5 by 5 cm) for 24-hr mortality counts.

Abbott's (1925) formula was used to correct for the natural mortality.

Results: The honey bee was more tolerant of tralomethrin than the other species (Table 1). The mortality sequence was typical, in that alfalfa leafcutting bees were most susceptible, alkali bees were intermediate in susceptibility and honey bee least susceptible to tralomethrin. For many insecticides the leafcutting bee is more susceptible which is a function of size or surface/volume ratio which is related to chance adherence of residues to the body of a foraging bee (Johansen et al., 1983).

The mortality of bees in 24 hr continuous contact with treated foliage samples did not decrease as the age of residues increased, which is atypical.

LD_{50} Toxicity

Materials and Methods. Tralomethrin was dissolved in acetone to obtain 100, 10, 1, 0.1, 0.01 and 0.001 active ingredient per 2.5 ul of solution. Thirty female bees were treated with each solution to determine between which two the LD_{50} occurred. A second series of 6 tralomethrin solutions whose concentrations fell between the 2 previously determined values was then used to treat 30 bees with each solution. Solutions were applied to the dorsal surface of each bee's mesoscutum with 5 ul capacity VWR micropipets that had been calibrated at 2.5 ul. For each test a control group was treated with 2.5 ul of acetone only. After treatment bees were placed in screen cages as described above and held for 24-hr mortality counts.

Bees were collected as described above and held at 5 C. until activity had ceased to facilitate handling and treatment with the tralomethrin solutions.

To obtain a closer fit the LD_{50} ug/bee data were analyzed with a computerized probit analysis program.

Results: Alkali bees (LD_{50} 0.11) were less susceptible to tralomethrin than honey bees (LD_{50} .007). Using the classification of Johansen and Mayer (1990) insecticides with an LD_{50} of < 2.0 are highly toxic to bees.

Syrup Feeding Studies

Materials and Methods. Feeding stations were prepared as follows. A piece of cotton (9 x 18 cm) was doubled over and placed in each plastic 15-cm petri dish, and a piece of wood placed on top of the cotton to serve as a landing board. A 50:50 sugar water solution was mixed with honey at 3 parts sugar solution to one part honey. The feeders containing 150 ml syrup with known ppm of tralomethrin were set out at the bee yard on 19 September at 0830 on top of 6 hive boxes set 3 m apart in a line in front of 12 honey bee colonies. Free

flying honey bees were allowed to choose between the feeders. Feeders were arranged randomly and rotated at noon. Evaluations were done by recording the number of bees per 5-second scan count visiting each syrup feeder at various times after setting the feeders on the hive boxes. The test was repeated on 21 September and in addition to bee visitation counts the feeders containing the syrup were weighed before and at the conclusion of the test to determine percent syrup consumed.

Results: Adding tralomethrin to the syrup caused a reduction in bee visitation and higher rates caused more reduction than lower rates (Table 2). Rates of 2 and 10 ppm did cause reduced bee visitation but the effects were not near as great as with higher rates. Furthermore, with the low rates of tralomethrin, the number of honey bees visiting the feeders was nearly the same as the untreated check after a few hours.

Field Tests -- Alfalfa

Materials and Methods. Tralomethrin was evaluated for alfalfa leafcutting bee toxicity in 3 tests. In 1988, 3 separate 0.4-hectare plots each directly in front of a large leafcutting bee shelter, were established in a commercial field of blooming alfalfa. The bees were removed from incubation and placed in the field on 5 July. On 19 July one plot received an application of tralomethrin 0.3 EC at 0.015 kg AI/ha, one plot received an application of tralomethrin 0.3 EC at 0.017 kg AI/ha, and one plot was left untreated. Applications were at 2100 by helicopter using 94 liters of water per hectare. In 1989, 2 separate one-hectare fields were used. The bees were removed from incubation and placed in the fields on 5 June. On 17 July, tralomethrin 0.9 EC was applied by airplane at 0.021 kg AI/ha in 94 liters of water/ha at 2100 to an isolated 0.4-hectare field of blooming alfalfa directly in front of an alfalfa leafcutting bee shelter and one field was left untreated.

Evaluations for alfalfa leafcutting bee hazard of the treatments were done by recording the number of leafcutting bees per 5 second scan per nest block (10 replications) pre-application and several days after application, and by recording the number of female bees in 13 nest holes (25 replications) at 2200 pre-application, and at 2200 one and two days after application.

Results: There were no significant differences in the number of bees/5 second/scan nor the number of females in the holes at night as compared to the pre-application counts, (Tables 3 and 4). Tralomethrin applied in the evening does not appear to be hazardous to alfalfa leafcutting bees.

Conclusion

It appears from these studies that tralomethrin is not hazardous to bees at the rates used and if applied judiciously. In our studies, the residual degradation time in hours (RT) required to bring bee mortality down to 25% in cage test exposures to field-weathered spray deposits was not greater than 2 hr. Materials or rates with an RT 25 of 8 hr or less are useful in terms of bee safety, if applied judiciously, i.e. if applied during the late evening or night. Those with an RT 25 of 2 hr can often be applied early morning before bees are foraging and cause bee mortality. The field data support the conclusion that tralomethrin will not harm bees if applied late evening. However, the LD_{50} data indicate that direct toxicity of tralomethrin is high.

Acknowledgements

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Table 1. Mortalities of alkali bees (AB), alfalfa leafcutting bees (LB), and honey bees (HB) exposed to different age and rates of tralomethrin applied to 0.01-hectare plots of alfalfa. Bees caged with treated alfalfa for bioassay mortalities. Prosser, WA. 1988-89.

Treatment	kg(AI)/ha	24 hr % mortalities of bees caged with treated foliage, age of residues							
		AB		LB			HB		
		2 hr	8 hr	2 hr	8 hr	12 hr	2 hr	8 hr	12 hr
Tralomethrin 0.3 EC	0.015	1a	0a	10a	4a	--	1a	0a	--
Tralomethrin 0.3 EC	0.017	0a	2a	16ab	15b	13	0a	0a	0
Tralomethrin 0.3 EC	0.021	15b	16b	18bc	17b	--	4b	2ab	--
Tralomethrin 0.9 EC	0.021	14b	19b	20c	21b	--	5b	5b	--

Means within a column followed by the same letter are not significantly different P=0.05, Duncan's [1951] multiple range test.

Table 2. Effect of tralomethrin on honey bee (HB) visitation to honey-sugar syrup. Number of HB/dish/5 second scan, percent reduction of HB's from untreated check, and percent syrup consumed. Prosser, WA. 1989.

Test #1 tralomethrin (ppm) (in syrup)	1000*	1130	1330	1530	Total	% red
0	75	75	36	33	219	--
2	6	8	46	75	135	38
10	13	10	53	65	141	36
50	2	1	7	2	12	95
100	7	8	3	6	24	89
500	0	0	0	0	0	100

Test #2 tralomethrin (ppm) (in syrup)	0902*	0915	0928	1035	1241	1505	Total	% syrup % red	% syrup consumed
0	22	4	18	100	100	50	299	--	73
2	75	14	16	100	100	50	365	0	72
10	75	8	2	75	100	50	330	0	63
50	14	2	2	3	2	50	74	75	32
100	6	0	1	1	0	3	17	94	8
500	2	1	0	0	0	0	3	99	4

* Syrup stations set out at 0830.

Table 3. Effect of tralomethrin 0.3 EC applied on 19 July at 2100 to 0.4-hectare isolated plots of blooming 'Cal West BNZ 27' alfalfa on alfalfa leafcutting bees (LB). Pasco, WA. 1988.

Treatment	kg(AI)/ha	Mean No. LBs/ 5 second scan			Mean No. LBs/ 13 holes	
		19 Jul	20 Jul	21 Jul	19 Jul	20 Jul
Tralomethrin 0.3 EC	0.015	50a	49a	40a	8.1a	9.1a
Tralomethrin 0.3 EC	0.017	45a	44a	33a	7.6a	7.0a
Untreated check	--	32a	35a	34a	6.8a	7.5a

Mean followed by the same letter in a line and counting method did not differ significantly (ANOVA), Fisher's LSC, P > 0.1).

Table 4. Effect of tralomethrin 0.9 EC applied on 17 July at 2100 to a 0.4-hectare isolated plot of blooming 'Washoe' alfalfa on alfalfa leafcutting bees (LB). Prosser, WA. 1989.

Treatment	kg(AI)/ha	Mean No. LBs/ 5 second scan				Mean No. LBs/ 13 holes		
		17 Jul	18 Jul	19 Jul	20 Jul	17 Jul	18 Jul	19 Jul
Tralomethrin 0.9 EC	0.021	65a	60a	65a	60a	9.1a	8.5a	9.0a
Untreated check	--	20a	20a	30a	20a	6.6a	6.5a	6.6a

Mean followed by the same letter in a line and counting method did not differ significantly (ANOVA), Fisher's LSC, P > 0.1).

APPENDIX 10

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EXPERIENCES IN TENT TRIALS AND FIELD TESTS WITH BEES

Summary

In the many tent studies and field trials which we conducted during the last ten years, we obtained a very good impression about the advantages and limitations of the Germann guideline. Due to the fact that a 12 m2 tent is rather small, the mortality on the borders of the tent is not a good indicator for the toxicity of a test product. But the tent studies reveal a repellent effect and disturbances of the brood.

For the interpretation of the repellent effect in tent and field trials we propose to use indexes which compare the foraging activities before and after treatment. A further means for the interpretation of the repellent effect is the quality and quantity of the pollen load.

Influences on the brood are detectable by a detailed assessment of each comb. Our method is described and an example is given on a graph.

In accordance with the detailed German guideline it is possible to recognize quite a large range of effects which an agrochemical can potentially cause.

1. Sequential Tests

The toxicity of agrochemical products to honey bees -a very important useful insect- is determined in a three-step
foil 1 testing scheme stipulated by German authorities. Those three steps are: laboratory tests, tent studies and field trials.

Laboratory tests with our experimental products are carried out at university or independent institutes.

Our experiences with tent studies and field trials date back to 1980. Since then they have been carried out every year.

foil 2 As you know, it is caracteristical for laboratory tests that groups of adult bees are directly exposed to the test product.

2. Advantages and Limitations of Tent Studies

In tent studies, contrary to laboratory tests, the
foil 3 agrochemical is applied on flowering plants. This enables us to observe not only the mortality but also the foraging behavior of bees. As a small bee colony is used, it is possible to recognize influences on the brood. Because of the short duration of the tent studies, only acute effects can be detected.

Even a small colony on three combs consists of too many bees for a flowering crop of 12 square meters. Especially during a period of good weather with ideal flight conditions, the nectar and pollen sources are quickly exhausted. For this reason, the bees intensify their search for new nectar and pollen sources and fly against the walls of the tent. This leads to the curious situation, that at times with
foil 4 particularly ideal flight conditions, the mortality increases. This observation can be made in untreated as well as in treated tents. A tent study always represents an aggravated, kind of prison situation, which has consequences on the behavior of the bees and has to be taken into account at the interpretation of the results.

3. Interactions between Repellent Effect and Mortality in Tent Studies

An increased mortality can also be observed in the tent,

if agrochemical products cause a repellent effect. Bees are repelled by many insecticides (Pyrethroids, Carbamates, P-esters). This is actually a protective behavior of the bees. The high sensory perception keeps the bees away from the treated plants. Oral intake and contact with treated blossoms or plants are so avoided and mortality is prevented. This is the course of proceedings in an open system as for instance in the field. But in a tent, the bees cannot fly away. They crash against the walls of the tent
foil 5 and fly themselves to death. This kind of mortality is not a direct consequence of the agrochemical application but is caused by the prison situation in the tent. We come to the conclusion, that the mortality at the borders of the tent is not a suitable criterion for the judgement of the toxic effects of an agrochemical.

4. The Individuality of the Bee Colonies

In tent tests, great importance is laid on the foraging activities which do not only depend on the flowering
foil 6 behavior and the weather but also on the vitality of the bee colony. In one test series we observed quite different flight activities between the individual colonies before treatment. In order to harmonize the bee material, we collected all adult bees in a kind of bee-pool from which they were again distributed to the different colonies under test. In this way, the individuality of the single colonies was reduced and the vitality was on the same level. So far, our experiences with this procedure have been encouraging.

5. Plots and Plot Size in Field Trials

Focal points of the German Guideline for Field Trials are mortality and influences on the brood. It is probably for this reason, that the usual untreated control plot is abandoned in field trials.

But in trials where a comparison of the foraging activities and the repellent effect are of particular importance, a flowering control field is indispensable. We regularly use an untreated control field in our trials for an easier and better assessment of the repellent effect.

The guideline provides that the test area should consist of a flowering crop of at least 2500 square meters. The trial is not impaired if this area is smaller. 1000 square meters are sufficient and have the advantage that the number of collecting bees per square meter increases.

6. Evaluation of the Mortality

In a field trial, the mortality is assessed only by dead bee counts. Bees that die in the treated crop are not taken into

foil 7

account. In order to detect dead bees on the ground, we cut narrow lanes into the crop and covered them with foils or gauze. This method turned out to be inappropriate and too expensive, as nearly no dead bees were found in those lanes. I assume, that the bees chiefly die on the plants and falll to the ground after getting in contact with the spraying residue.

Usually, the bee mortality in front of the hive is sufficient for an indication. Additional dead bee traps are unnecessary. They do not give additional information and are only a hindrance to the bees. In case the mortality should be very high and casualties occur within the test crop, the reduction of the foraging bees will be noticed during the evaluation of the colony strength (see chapter 9).

7. Interpretation of the Repellent Effect

Many insecticides induce a repellent effect, that means, they repel the bees from visiting the flowering crop. The limited duration of the repellent effect depends firstly on the product and secondly on the temperature. If the temperature declines on the day after the treatment, the foraging activities decline as well and can so be mistaken for a repellent effect. Therefore, a comparison with the foraging activities at the untreated control field is imperative. This comparison enables us to differentiate whether the repellent effect is caused by the test product or due to the temperature.

foil 8 with formula

The introduction of indexes like in France, could be an additional possibility. In France, the flying activities prior to and after treatment are compared. This index can be calculated independent from the number of observations. It is possible to indicate the specific intensity of the repellent effect for any period of time (hours and days) after treatment.

example

An indication for a repellent effect is given, if the index is lower than 1 or if it is considerably lower than that at the untreated control field. The same index can be applied for the mortality. This index is not common practice in Germany, but it is very helpful for the interpretation of the results, particularly at changing weather conditions. We use it for both, tent studies and field trials.

8. The Pollen Problem

I should like to make some comments on pollen. In Germany, pollen serves as an evidence that the offered nectar or pollen source has been accepted. But this is unnecessary. The same information can be obtained by a count of the foraging bees. Furtheron, a pollen analysis is supposed to make sure that only pollen from the offered nectar or pollen

source has been collected. But this observation is overvalued. During our trials we always observe bees carrying pollen loads not originating from the trial field. It is unavoidable, that the bees collect pollen from the wayside, dead furrows, etc. A few plants will be sufficient to attract the bees. Besides, not all the bees of a colony collect pollen from the same source. A certain percentage will ignore the flowering Phacelia field and fly away. The reason is not yet known. The German guideline demands that the pollen has to originate solely from the trial field. According to our experiences, this is an illusion and does not correspond with practice.

We suggest to count the bees returning with a pollen load at the alightning board (entrance of the hive). We regard this as additional information to the collecting activities. The blue pollen of the Phacelia blossoms is easily
foil 9 distinguishable from others. In case a repellent effect occurs, the number of blue pollen loads will decrease. An increase of their numbers is an indication that the repellent effect is receding and that the foraging bees returned to the treated Phacelia field.

With this test it is easy to observe whether the repellent effect can bring the foraging activities to a standstill or whether it diverts the bees to other sources.

It is also possible to collect the pollen yield of a day by means of a pollen trap. A repellent effect can be identified by the weight of the yield.

The pollen collected on the day of the treatment is fed to young bees in a separate test. This is a contradiction. What could be the use of this test, as no pollen has been collected by the bees from the treated field because of the repellent effect. I never understood, why special emphasis is given to the evaluation of the oral toxicity of the pollen. What would result, if this particular test would turn out negative and, on the other hand, no negative influences on the bee colony and in the beehive could be detected? In my opinion, this test should be discarded.

9. Assessment within the Hive

For each agrochemical product it has to be guaranteed, that the brood is not influenced. A particular test is neither designated nor necessary. It is sufficient to observe the test colonies at the treated field in great detail and to compare these observations with those from the control colonies

We proceed as follows: On each comb we estimate the percent comb area covered with eggs, larvae, pupae, pollen and nectar. We receive five values for four colonies with thirty combs each: 5 x 30 x 4 from which we calculate the average value. Inspections of the combs prior to treatment, six days after treatment and once more after four weeks are of great

importance. Besides a quantitative check, we also note qualitative changes like the state of the larvae, perfect oviposition, incompleteness of the brood nest etc.

If an agrochemical product should have influence on the brood, it would be noticed at this detailed evaluation. Later defects of development inhibitors can be recognized,
2 foils if the observation time is prolonged. The average values of
10 + 11 the comb evaluation are then entered on a beam and compared with the control colonies. This graphic method enables us to discover whether the recorded changes are caused by the test product or whether they are due to seasonal successions. For example, so far, not once has a repellent effect been detected in the pollen and nectar stores. This shows quite clearly, that a repellent effect is insignificant for a bee colony.

10. Concluding Remarks

In the confinement of a tent, only short-time effects are discernible. For this reason, it is necessary to carry out field trials under practice conditions. The detailed evaluations of field trials allow a thorough estimation of the agrochemical under test. Those viewpoints mentioned by me, are not thought as a criticism of a good guideline, but I wanted to show other possibilities to obtain more information in accordance with the stipulations of the guideline.

APPENDIX 11

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DETERMINING THE DOSE-CONCENTRATION RELATIONSHIP FOR THE BEE TOXICITY EXPERIMENTS

Summary

Toxicity of pesticides to bees can be determined by topical application or feeding tests. In each case, determination of LC_{50} is easier. However, LD_{50} also has equal importance in toxicity tests and safety measures. Therefore, it is desirable to determine an emperical relationship between LC_{50} and LD_{50}. This paper is aimed to meet this objective and gives a mathematical formula from which LD_{50} from LC_{50} and vice-versa can be determined.

Measuring the toxicity of a pesticide in the laboratory or in the field involves determination of both LD50 and LC50 values (Kumar 1989). At present there exists great confusion regarding their derivation and use. In this paper, our aim is to clear such confusions about the two toxicaty measurements. The LD50 is useful in comparing the relative toxicity of different pesticides to the test bees where actual amount of the pesticide (the active ingredient or e.I) causing 50 per cent mortality becomes known. On the other hand, LC50 broadly gives the concentration of the pesticide without exactly giving the amount of pesticide actually coming in contact with or entering the body of the test bees. However knowledge on LC50 is important as it is useful for making comparison of this concentration with the normal recommended concentration for the field applications (Hamsed et al. 1973). Therefore, both the toxicity measurements are important. But, in the present test methods, at most of the occasions, only LC50 can be determined. Then for quicker determination of LD50 from LC50 or vice-versa, devising of a mathematical formula is important which can explain a numerical relationship between these two toxicity measurements. The present paper aims to meet this objective.

Existing experimental method of determination of LC50

To determine the toxicity of a pesticide, normally two kinds of tests are performed viz. topical application and gustatory feeding. In the former test, the given volume of a pesticide solution is applied on the body of the organism (e.g. 1 µl on the test bees - Kumar 1989) where as in the latter test given volume of the pesticide solution is fed to the organism. In the laboratory and field tests too, over 80 per cent of the field honeybees were found to consume a fixed volume of the pesticidal solution e.g. 20 µl pesticidal solution in sugar-water was consumed by the honeybee Apis mellifera L. and 35 µl by Apis dorsata F. (Kumar 1989). With these respective test, the concentration which caused 50 per cent mortality was determined, after usual correction of the observed mortality by Abbott's formula (Abbot 1925, 1987) and subsequent probit analysis of the range concentrations causing mortality from 20 to 90 per cent following Finney (1952). This, then, gave the LC50 valus for a pesticide.

Relationship between LC50 and LD50

Then from the LC50 so worked out, LD50 could be determined with the help of the following mathematical relationship:

$$LD50 = 10V \times LC50$$

where

V = volume of pesticidal solution applied to or ingested by the individuals of a species. In the topical application test V=1 µl and in gustatory feeding experiments, V= the volumed by the test bees e.g. 20 µl by A. Mellifera or 35 ul by A. dorsata.

Validation of the dose-concentration toxicity relationship

By definitions:

LD50= Actual dose of the pesticide which gives 50 per cent mortality of the test organisms.

LC50= Actuel concentration of the pesticides in the solution which gives 50 per cent mortality of the test organisms.

If LC50 is the concentration of the pesticides in the solution and expressed in grams, then

In 100 ml of the pesticical solution, the amount of a.I.

$$a.I = LC50 \text{ in grams or } LC50g$$

In 1 ml (i.s. $1x10^3$ ul) of the pesticidal solution, the amount of

$$a.I = \frac{LC50}{100} \ g$$

In 1 ul of the pesticidal solution, the amount of

$$a.I = \frac{LC50}{100} \times \frac{1}{10^3} \ g$$

$$= LC50 \times 10^{-5} \ g$$

When a volume 'V' is topicaly applied or fed to the test bees, the amount of

$$a.I = VxLC50x10^{-5} \ g$$
$$= VxLC50x10^{-5}x10^6 \ ug$$
$$= VxLC50x10 \ ug$$
$$= 10VxLC50 \ ug$$

But the amount of a.I. in the 'V' volume of the pesticide solution causing 50 per cent mortality is the actual dose of pesticide applied or entering of the test bees, and this is simply nothing but LD50.

Therefore, LD50 = 10VxLC50 µg

For bees, the unit of LC50 is in grams where as the unit of LD50 is in micrograms.

In this way LD50 can be worked out from the derived values of LC50 and vica-versa.

Acknowledgaments

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APPENDIX 12

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NEWS ABOUT THE USE OF INSEGAR (FENOXYCARB) IN SWITZERLAND

Our previous measurements of population density and counting the amount of dead bee-brood stages showed no measurable detrimental effects after treatment of Insegar (IGR = Insect Growth Regulator). We classified 1985 Insegar temporary as non hazardous to bees. After further field tests in 1987: we classify it as hazardous to bees. (A. de Ruijter & J. van der Steen, Hilvarenbeek, found in 1986 for the first time pupae damages after Insegar treatment). In our following field tests with treatments after the main flowering period (petal fall) we observed for the first time different distincly damaged pupae and young bees from the 10th till about the 30th day after treatment too.
The characteristic symptoms are:

abnormal habits with underdeveloped wings
shrinked and squashed abdomens
compound eyes with white, redish or brounish rims on the inner border of the eyes. Bees stages with white eye-rims are the most characteristic feature to discribe Insegar induced bee damages.

In Switzerland the use of Insegar for the Integrated Fruit Production (IP) is still necessary. To minimalise the loss of bee-brood we ordered this year a specific instruction of use:

"Insegar should be used only when there are no open blossoms on the fruit trees and no flowers beyond the trees. So the treatments must be done before or after bloom. *Immediately before treatment the grass in the orchards have to be cut and the rest of the flowering weeds have to be burned down by a herbici* "

For a good control of the leafroller pest (*Adoxophyes orana* F.v. R.) the timing of IGR-fenoxycarb for orchards in the north of the Alps should be just before bloom and in orchards in the south of the Alps: just after bloom.

Current treatments by the company in different parts of Switzer-land at the end of March just before bloom showed in "Gary's dead bee traps" mounted on bee-hives at the border of the treated orchard the following results:

a) no damaged pupae or young bees, if the instruction of use was followed;

b) a limited damage of pupae or young bees if the air-dispersed product contaminated wild flowering cherry trees or Taraxacum flowers in the neighbourhood. - Between the 10th and the 26th day after treatment the mean value of pupae per day and colony rises to a maximum of 15 individuals and 12 individuals with eye-rims. (The number of damaged young bees by Insegar is not yet analysed).

At the end of this year we will have to decide which amount of damaged bee-brood can be tolerated.

In connection with the loss of bee-brood after the feeding of contaminated pollen the following question might be asked:

Should we introduce for the harmonisation in the groups of IGR and Chitin Synthesis Inhibitors the new term **"bee-brood damaging"** instead of "hazardous for bees"?

APPENDIX 13

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EFFECT OF INSECT GROWTH INHIBITORS AND PESTICIDES ON HONEY BEE LARVAE (Apis mellifera L.) IN CONTACT POISON AND FEEDING POISON TESTS IN VITRO

Introduction

Evaluation of pesticide toxicity to honey bees is usually carried out in a bioassay with adults. The application of a standard larval bioassay is hardly found in practice. Usual screening tests for larvicidal effects of insecticides have been described (Wittmann & Engels, 1980; Atkins & Kellum, 1986). A standard procedure for evaluation of toxic insecticide effects as well as of insect growth inhibitors on honey bee larvae has also been presented (Rembold & Czoppelt, 1982). The test is based on the artificial rearing of first instar larvae up to adults in an incubator under controlled conditions (Rembold et al., 1974; Rembold & Lackner, 1981).

In this study, the influence of two chemicals (Diflubenzuron, Parathion) on growth, development and vitality of bee larvae is shown in an in vitro-assay by an analysis of larvae which were grown either in contact and in feeding poison tests. The larvicidal effects are as well a consequence of growth inhibition as of interference with metamorphosis.

Material and Methods

First instar worker larvae with an individual body-weight of 0.1 - 0.3 mg were collected from colonies of Apis mellifera. They were continuously reared throughout their whole development from first (L1) to fifth (L5) instar on a semiartificial diet (RJ) which consisted of a mixture of royal jelly, sugar solution and yeast extract (Rembold et al., 1974;1981). The larvae were kept in an incubator with controlled climatic conditions (35 °C; 55 % RH).
Both the chemicals were either diluted in acetone for topical application or mixed in the diet for oral administration.
Diflubenzuron or Dimilin is a derivative of uric acid. It inhibits chitin synthesis in insects and is therefore applied as an insect growth regulator (IGR).
Parathion, a conventional insecticide, belongs to the group of organophosphates which act as contact, feeding or breathing poisons.

In the contact poison test, acetone, containing 0 - 500 ppm of the chemical, was topically applied to third instar larvae (1 ul/L3; 5 - 8 mg body-weight). The weight gain was estimated 48 h later. Control larvae, treated with the solvent only, were kept in a second incubator separate from the chemically treated larvae.

In the feeding poison test, L1 were transferred into artificial cells which contained RJ (0.2 ml/thimble/L1). 24 h later larvae (L2) again were transposed into new thimbles with RJ to which various amounts of the chemical (0 - 10 ug/ml RJ) had been added. The control larvae were kept separate from the treated ones. Larval weight gain was measured during a 48 h period in the most intense growth phase between L4 and L5.

Results and Discussion

Topical application of 0 - 20 ppm Dimilin to L3 in the contact poison test did not show clear effects on larval development (Fig. 1a). Lethality during larval growth was low showing no remarkable differences between treated and untreated larvae (L3 - L5). Low mortality rates of 1 - 6 % elucidate that these toxic effects came from the applied substance and not from the solvent itself. However, increasing amounts of the IGR from 30 up to 100 ppm caused high mortality rates (between 15 and 60 %) in all three larval stages.

Topical application of Parathion to L3 (0 - 50 ppm) induced acute toxicity in larvae up to a death rate of about 10 % (Fig. 1c). By comparing the larvicidal effects of this chemical in the succeeding stages, both L4 and L5 showed only mean losses. The rates of total mortality remained on a constant level due to the mixture of solvent and low concentrated insecticide. Treatment of 100 - 500 ppm of the pesticide, however, gave a clear response to L4- and L5-larvae; more than 20 % of them died. L3 did not show any reaction to treatments with high amounts of Parathion. Development to the next stage took an undisturbed course.

With regard to the high mortality rates after topical treatment of both of the chemicals, only Dimilin induced decreasing survival rates of L5 to pupal and adult stage corresponding to increasing concentrations (Fig. 1b).

On the other hand, influence of Parathion on larvae caused relatively high survival rates in L5-stage (Fig. 1d). The vitality of L5 was reduced during metamorphosis to pupae and adults, resp. In consequence of a further decrease of the mortality rates a level of 50 % survival was obtained within a concentration range from 0 - 100 ppm. Only the high dose of 500 ppm caused high mortality in all developmental stages up to adults.

Permanent rearing on a Dimilin treated diet in the feeding poison test likewise induced strong effects on growth and development of bee larvae (Fig. 2a). Increasing amounts of Dimilin (0 - 1.2 ug/ml RJ) were correspondingly followed by an increase in larval mortality and a decrease in larval vitality with the consequence of a complete drop in survival rate from 95 to 0 % (Fig. 2a,b).

Continuous feeding of a Parathion treated diet (0 - 0.1 ug/ml RJ) in the feeding poison test resulted in poor effects on development of larvae. Only the last larval instar showed mortality of less than 10 % (Fig. 2c). Oral administration of high amounts of Parathion (0.5 - 10.0 ug/ml RJ) led to strong effects on all three instars. Feeding of Parathion doses of more than 0.1 ug/ml RJ to L3 caused an abrupt increase of brood mortality which was high in all the four moulting steps from L3 to L4, from L4 to L5, from L5 to pupa, and from pupa to adult (Fig. 2c,d). Acute toxicity was found only after feeding of 1.0 and 10.0 ug/ml RJ.

With the contact poison as well with the feeding poison test two standard procedures for screening pesticides and IGR toxicity in honey bee larvae were successful under laboratory conditions. By use of this larval in vitro bioassay in an artificial environment is it possible to simulate the brood nest and to exclude all the influences which arise from the nurse bees (Czoppelt & Rembold, 1988).

The half lethal dose of Parathion in field as well as in laboratory tests was found in the range of 0.18 ug/adult (Knight, 1982; Czoppelt & Rembold, 1988). It is therefore supposed that toxic amounts for adults by regular application conditions in field trials will not harm larvae in the colony. Uptake and sharing of an active agent by adult bees result in a dilution effect before incorporation into larvae is starting (Czoppelt & Rembold, 1988). The extent of insecticide incorporation into a larva depends on its developmental stage. It increases from 2 in L3 and from 12 in L4 up to 86 % in L5 with regard to the weight gain (Rembold et al., 1980). The increased incorporation of both the chemicals into larvae is clearly shown in the respective percentage of mortality of larval instars (Figs. 1a,c; 2a,c).
In the contact poison test bee larvae show a faster response to both the tested substances than in the feeding poison test because larvae are able to incorporate the total amount of the insecticide at once. In the feeding poison test sufficient of the insecticide has accumulated in larvae at the end of their feeding period in L5-stage to display its toxic effect completely (Czoppelt & Rembold, 1981).

This in vitro standard method for screening toxicity of insecticides to bee larvae allows to follow larval growth and larvicidal effects of the agent as well as to observe completion of larval and pupal development, morphogenesis and adult emergence.

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Summary

Larvicidal effects of Dimilin and Parathion on honey bee larvae were tested *in vitro*. 1st instar larvae were reared in an incubator on semisynthetic diet (RJ) under controlled conditions. Both of the agents were either topically applied to 3rd instar larvae (*contact poison test*) or dissolved in the diet (*feeding poison test*). Increasing doses of topically applied Dimilin and Parathion up to 100 and 500 ng/L3, resp., induced high mortality in 4th and 5th instar larvae. Oral administration of increasing doses (0.1 - 10.0 ug/ml RJ) of both the agents caused severe growth disruptions and interference with metamorphosis. Larval growth and vitality are controlled by weight, moulting, pupation and adult emergence. The utility of rearing bee larvae *in vitro* for evaluation of insecticide toxicity in *contact* as well as in *feeding poison test* is particularly shown.

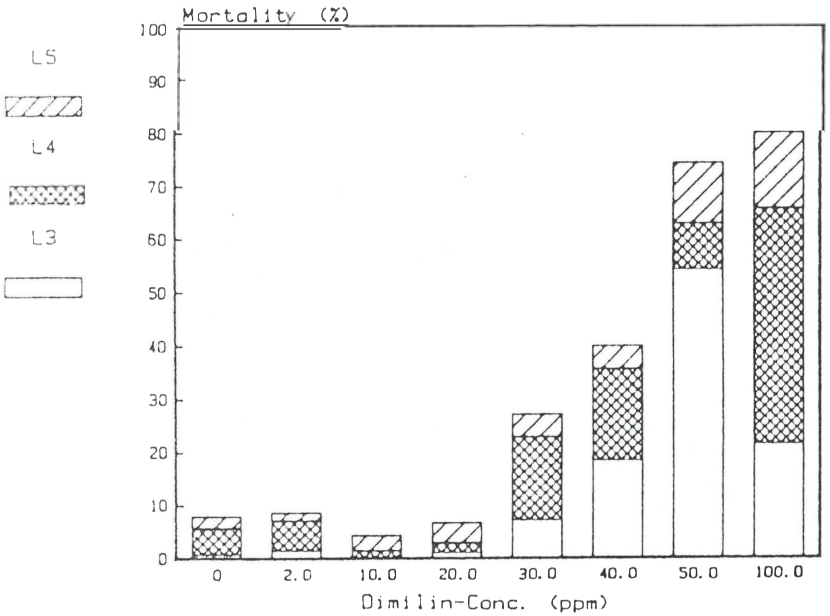
Legends

Fig. 1 Toxicity of Dimilin and Parathion on honey bee larvae in the contact poison test.
Effect of Dimilin on larval development (a) and metamorphosis (b). Effect of Parathion on larval development (c) and metamorphosis (d).

Fig. 2 Toxicity of Dimilin and Parathion on honey bee larvae in the feeding poison test.
Effect of Dimilin on larval development (a) and metamorphosis (b). Effect of Parathion on larval development (c) and metamorphosis (d).

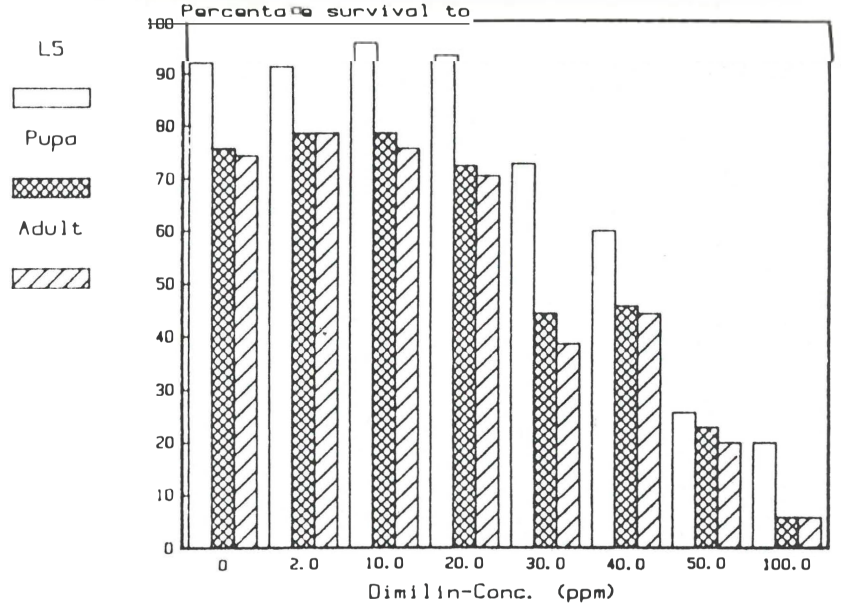
DIMILIN IN CONTACT-POISON-TEST

Effect on larval development



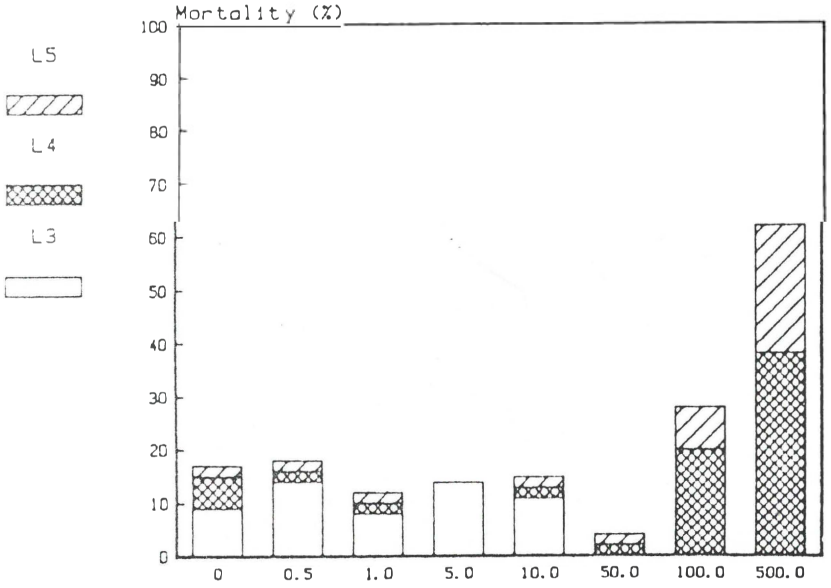
DIMILIN IN CONTACT-POISON-TEST

Effect on metamorphosis



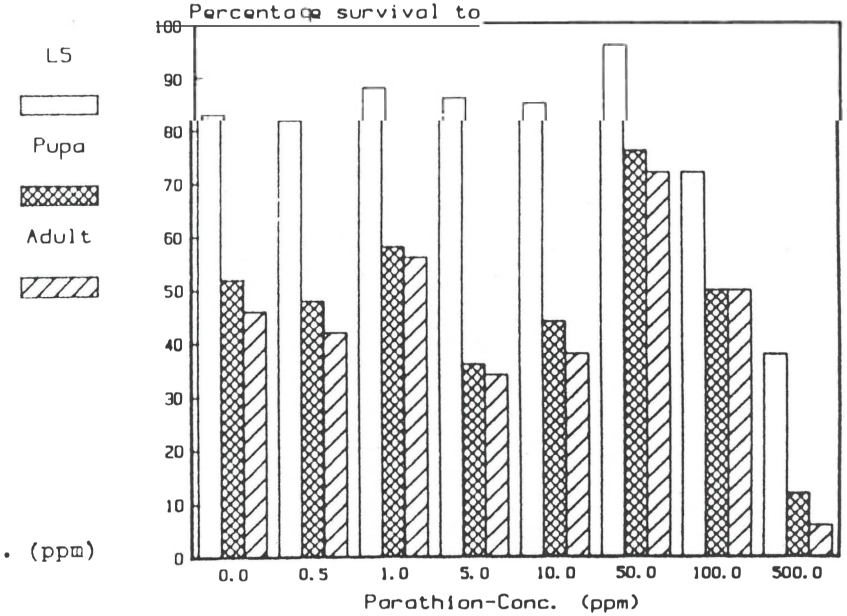
PARATHION IN CONTACT-POISON-TEST

Effect on larval development



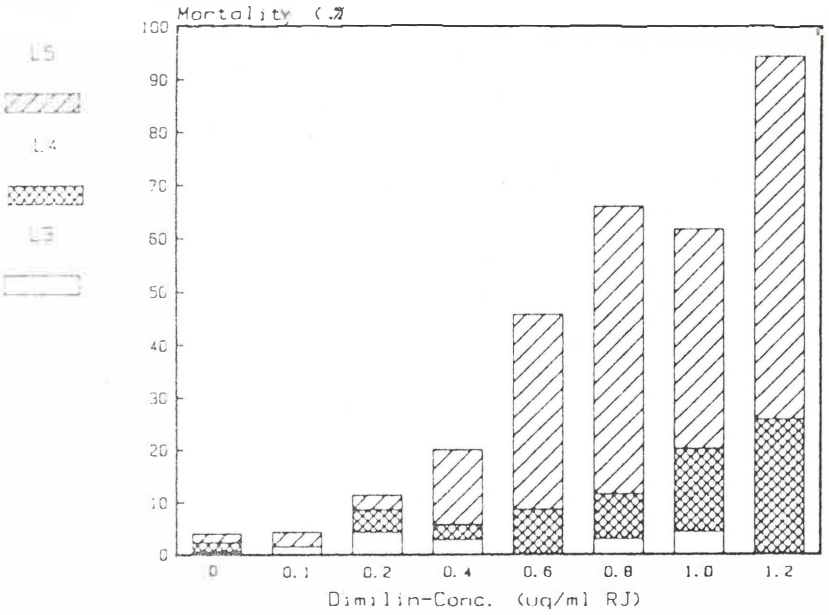
PARATHION IN CONTACT-POISON-TEST

Effect on metamorphosis



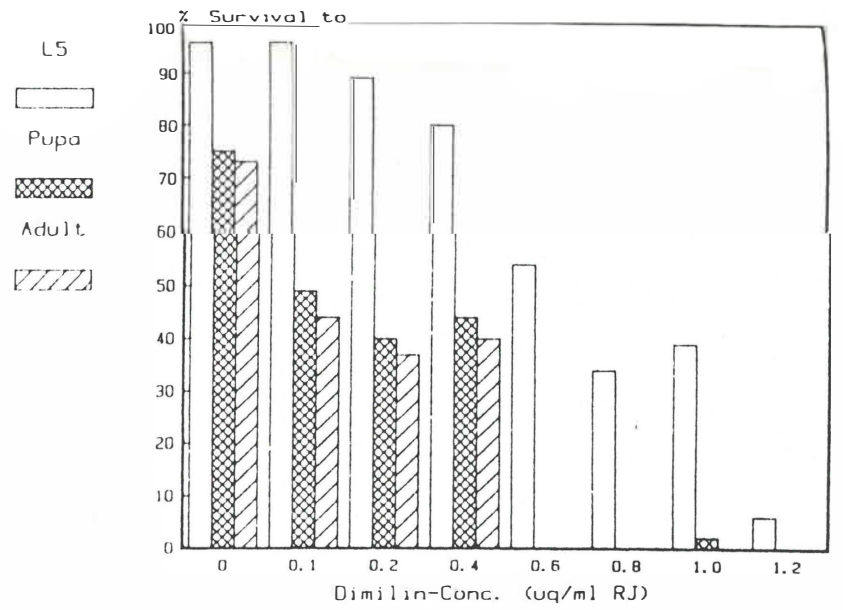
DIMILIN IN FEEDING-POISON-TEST

Effect on larval development



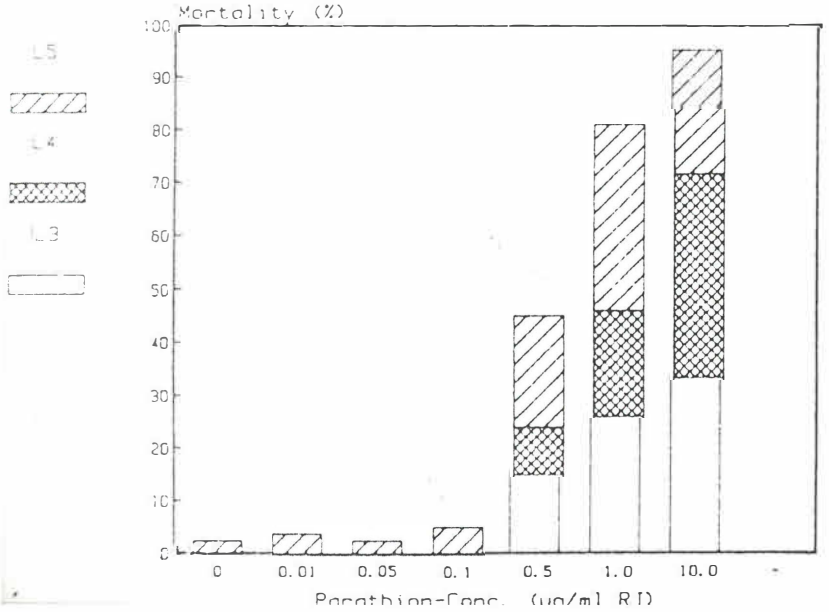
DIMILIN IN FEEDING-POISON-TEST

Effect on metamorphosis



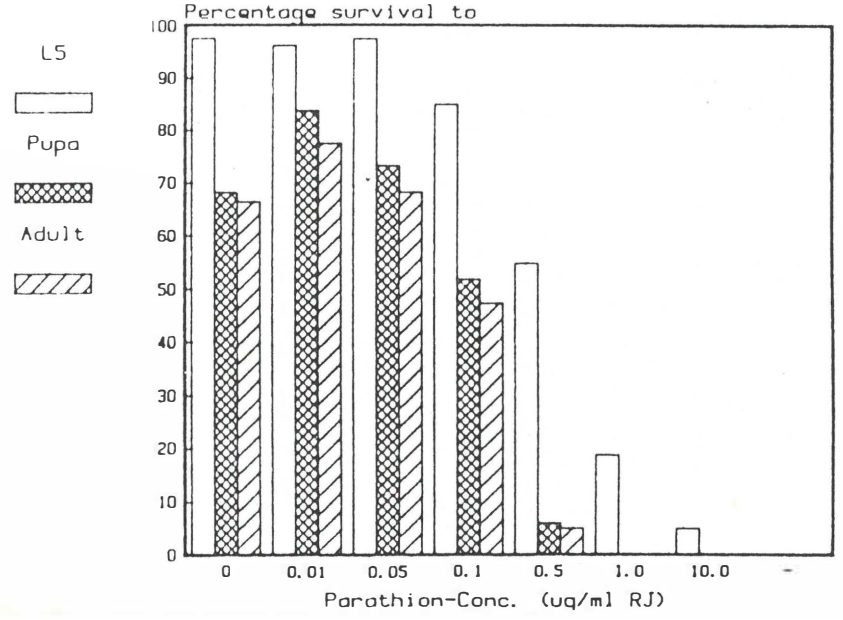
PARATHION IN FEEDING-POISON-TEST

Effect on larval development



PARATHION IN FEEDING-POISON-TEST

Effect on metamorphosis



APPENDIX 14

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TESTING OF INSECT GROWTH REGULATORS AND OF VARROACIDES BY THE APIS-LARVAE-TEST

The **Apis-Larvae-Test** was developed in our laboratory 10 years ago in order to determine the LC_{50} of insecticides for honey bee brood. In the original version (Wittmann 1981), the Apis-Larvae-Test was designed as an in-hive-test for measuring the acute toxicity of pesticides after oral application of know concentrations of the active ingredient. The advantage of a direct supply of the test compound by feeding a contaminated semiartificial diet to individual larvae resulted in highly reproducible data on mortality. Later on, further test systems were described in which the compounds were applied to crops, fed to colonies or nursing bees of experimental colonies, or added to the diet of larvae reared *in vitro* (Wittmann and Engels 1981). The latter technique turned out to be very useful for an accurate determination of the LD_{50}. For this purpose series of staged larvae are grafted to 0.5 ml depressions of titration trays, allowing the testing of several dosages at the same time under identical incubator conditions (Wittmann et al 1985). Both the in-hive and the advanced *in-vitro*-version of the Apis-Larvae-Test were also used to measure increased toxicity of two or more simultaneously applied pesticides, herbicides and fungicides as commonly sprayed from tank mixtures (Kuhn 1987). In the meantime, the Apis-Larvae-Test has become a routine method (Davis 1989). Numerous preparations, formulations and pure compounds have been tested in our laboratory since, most of them on request of governmental authorities or in cooperation with companies.

The Apis-Larvae-Test can be performed with any feeding stage, but for most purposes L3 or L4 worker larvae are ideal because these instars are easy to handle and consume food amounts ranging between 10 and 20 ul within less than 24 h. The test substance has to be mixed into the diet which sometimes causes problems if non water soluble compounds are formulated as wettable powder or suspension. Evaluation of hazardous effects or mortality is made 1, 2, 3, 4 or 7 days after application and eventually at emergence of the adult bees.

In a normal Apis-Larvae-Test, 3 or 4 series of about 100 larvae each are evaluated per concentration or dosis, respectively. The data on mortality have to be corrected regarding blank and untreated controls. With a computer program the relationship between concentration or dosage of the test substance and mortality probits is calculated and plotted out. Any mortality can be determined, besides LC_{50} and LD_{50}, the LC_{10} and LC_{90} values are often of special interest. Since the procedure of the Apis-Larvae-Test gives highly reproducible data, the mean mortality values normally fit well onto the computed regression line. Consequently, test series with a coefficient of correlation < 0.9 are discarded and have to be repeated. Usually the reason for such deviations is found in an insufficient mixture of the test substance within the diet samples which results in varying individual dosages.

Although the Apis-Larvae-Test is principally a laboratory test, the data of field trials can be used to calculate the concentration of active ingredient which reached the bee larvae. If larval mortality in the field is monitored at certain intervals after spraying, and if the toxicity of the compound is known, having been determined by the Apis-Larvae-Test, then the possible damage to honey bee brood through field application of the compound can be exactly estimated.

As it turns out, particularly the modern insecticides which act as insect growth regulators (IGR) are very dangerous for bee brood. This was shown for inhibitors of chitin formation, juvenile hormone analoga (JHA), juvenoids, antiallatines and others. Since most of the IGRs interfer with moulting events, only subimaginal instars are target stages. Normally IGRs cause little or no hazard in adult insects including honey bees.

All insect growth regulators without exception effectively disturb honey bee development. Through safety studies the correct conditions of field applications must be determined in order to ensure that no contamination of bee colonies occurs. In most cases this can be avoided if not only blooming crops but also weed blossoms are considered. All pesticide contamination of pollen, nectar or water collected by foraging honey bees is normally also hazardous for the brood. Since most of the insect growth regulators are not toxic for adult bees, colony damage after spraying is often not recognized because no dead bees are found in front of the hive entrance. In contrast to traditional insecticides with a high acute toxicity by which foragers are killed before they can deposit much of the noxious material within the hive, the insect growth regulators do not affect the field bees which therefore return home with the contaminated forage. Brood losses are often overlooked

under such circumstances and become obvious only if after heavy contamination of the larvae over a long period, the colony population rapidly dwindles. Since such effects occur only 10 days or more after spraying, the causal relations are not always easy to reconstruct. On the other hand, minor brood losses are normally tolerated and compensated by strong colonies.

A new situation resulted through the appearance of the *Varroa* mite in western countries. Since varroatosis is mainly controlled by chemical measures, pesticides are introduced directly into the bee hive. The question is whether the applied acaricides are dangerous for the bee brood. Of course this depends also on the technique of application and the timing of treatment within the seasonal colony cycle. For chemical treatment of *Varroa* infested colonies, periods in which the hive is free of brood are recommended. This condition, however, cannot always be observed strictly, particularly in countries with a subtropical or tropical climate. Consequently, the acaricide may reach the bee larvae. Varroacides are applied as fumigants, vapours, systemics or on carrier strips. Most of the active ingredients are lipophilic and accumulate in the beeswax, thereby possibly also reaching the larvae in the brood cells. Systemic formulations are fed to the adult bees and distributed within the colony by trophallaxis. This interindividual food exchange includes feeding the brood which can lead to a direct contamination with the varroacide. Most of the acaricides which are used as varroacides today, exhibit a relatively low toxicity for adult bees. The data collected on compounds which have been screened for larval mortality by the Apis-Larvae-Test so far did not signal much danger for the bee brood if over-dosage is avoided.

According to the experience gathered by our determinations of larval toxicity of IGRs and varroacides by means of the Apis-Larvae-Test, this procedure can be strongly recommended for use in safety studies. Particularly for these two types of pesticides is an evaluation of possible hazard or damage regarding the honey bee brood by a routine test like the Apis-Larvae-Test important and should be incorporated into the registration requirements. The data on larval toxicity, especially LC_{50}, LC_{10}, LC_{90} and LD_{50} values, could be made available through a European Informational Network which reports pesticide effects on non-target organisms.

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APPENDIX 15

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TEST METHODS TO DETERMINE THE HAZARDS OF IGR's (INSECT GROWTH REGULATORS) ON HONEY BROOD, USING PHENOXYCARB AS AN EXAMPLE

Abstract.

Two methods, a feeding test and a field test are described. In the feeding test the pesticide is added to sugar solution and fed to a honey bee colony directly. Brood development is studied by marking brood cells on a translucent sheet and checking these cells weekly. In the field test brood development is studied in detail. Before pesticide application, cells containing brood in different stages, are marked. The same cells are checked 3 times a week. By comparing the sequential sheets, the oviposition date can be calculated. Development of brood of known oviposition dates is studied until the cells are empty. In this way brood mortality can be determined. As it was found that, in practise, mortality due to phenoxycarb shows only in the pupal stage, the field test was modified when repeated the second year. In this test only pupae of a known age are checked for malformations and mortality.

The feeding test determines the toxicity of an IGR to bee brood. In a field test the effects in practice can be recorded.

Test methods to determine the hazard of IGR's (Insect Growth Regulators) on honey bee brood, using phenoxycarb as an example.

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IGR's are used to disturb the normal development of insect brood. Sprayed in crops on which bees forage, these pesticides are taken into the hive with the collected nectar and pollen. Nectar and pollen are fed to larvae during the second half of the larval stage and pollen is consumed by young bees. As IGR's are harmless to adult bees, the usual test methods to determine the hazard for bees, can not be used. Test. methods to determine the toxicity of pesticides on honey bee larvae have been developed (Wittmann 1980, Czoppelt 1985). These tests have not yet become an integrated part of pesticide toxicity tests. This means that the toxicity of several IGR's is still unknown.

The Ambrosiushoeve developed a simple feeding test to determine the effect if IGR's on honey bee larvae, when fed directly to honey bee colonies. In order to establish the effects of IGR's in practise, a field test was developed.

Feeding test.

In this test the pesticide is dissolved in sugar solution 50 % and fed to bee colonies. About 200 cells, containing brood in the egg stage, larval stage and pupal (capped) stage, are marked by means of a translucent sheet. Every stage has its own colour on the sheet. The larval stage is divided into the young larval stage in which the larva floats in the royal jelly and the older larval stage in which the larva fills the bottom of the cell completely. As the normal duration of the brood stages is known, brood mortality can be determined by checking the same cells weekly.

Phenoxycarb was fed in two concentrations: 100 mg/litre (recommended dose for field application) and 200 mg/litre. The feeding had an adverse effect on brood development. The brood in the larval stage died almost completely within one week. Within the observation period of three weeks, in the colonies fed with 200 mg/litre no larvae reached the pupal stage. In colonies, fed with 100 mg/litre most larvae died. Larvae reaching the pupal stage, died in this stage, showing malformations: a white rim in the compound eye which is considered to be a juvenile character (Beetsma, J. Houten ten, A. 1975).

Field tests

Two field tests were done, test 1 in 1986 and test 2 in 1987. For test 1 bee colonies were placed in an orchard in which phenoxycarb was sprayed during full bloom and in an orchard where no pesticides were used during full bloom. In front of the hives, traps were placed to collect dead pupae. When flowering began, within a rectangle of 80 cm^2 brood cells were marked as decribed in the feeding test. Three times a week the same cells were checked. The oviposition dates could be calculated by comparing the sequential sheets of the same brood area. Brood development of successive oviposition dates was checked until the

brood cells were empty.

Results: Contrary to the feeding test larvae developed normally into pupae. Seven days after application the first dead pupae with white eye rims were found. At that time brood samples were taken. The age of the pupae was determined by the method, described by Rembold (Rembold et al. 1980). It was found that all brood that reached the second half of the larval stage within 5 days after application, died when it became seven days old pupae. Mortality was observed for a period of 12 days. During the first ten days dead pupae had malformed eyes.

Field test 2 was done to find out whether a phenoxycarb application before blooming, when 80 % of blooming was over or after the petals had fallen, was also hazardous to honey bee brood. The expression of the poisoning was known so in this test only mortality and malformation of pupae were checked. Bee brood, in three different stages at the moment of phenoxycarb application was studied:

A. brood just capped at the day the pesticide was applied.
B. three days old larvae at the moment of application.
C. larvae, hatched the day of application.

To study A, two days before spraying fullgrown larvae were marked as described previously. The cells capped the next day were used for this study. To study B and C the same method was used, starting with fullgrown larvae three days and six days after application respectively. Brood development of A, B and C was studied in a similar way. Six days after capping, the cells were checked and subsequently 40 % was removed and fixed to be studied for eye rims. Eleven days after capping the remaining cells were checked and another 40 % was removed to be studied for malformations. Finally 14 days after capping the emergence was assessed.

Results after application just before flowering: A small part of the brood died (about 200 pupae and young bees / colony). As a result of the low dose, part of the brood died at the end of the pupal stage at about the moment of emergence. Few of the pupae and dead young bees had eye rims.

Application when 80 % of flowering was over, caused serious damage to bee brood comparable with the results of field test 1.

Application after petal fall had an effect, comparable with mortality due to application just before flowering. A small part of the brood, that was in the second half of the larval stage on the day application, died.

The successive tests demonstrated clearly the effects of phenoxycarb to honey bee brood both in the feeding tests and after application in apple blossom on which bees forage.

To determine the necessity of a field test, a preliminary feeding test can be done. If, in the feeding test, no effects are found, a field test can be omitted. In case effects are recorded a field test should be done to determine the possible effects of IGR's on honey bee brood in practice.

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APPENDIX 16

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PERORAL AND CONTACT TOXICITY OF COUMAPHOS, FLUVALINATE AND AMITRAZ

Introduction

Varroa control in Czechoslovakia is at the present time effectuated by amitraz applied to bee colonies. The registration is also made for coumaphos which is not used but the registration trials for fluvalinate are nearly at the end. Varroa control is done in our country only by the Veterinary Service which is consequently responsible for the misuse of chemicals. That is the reason for comparative tests which enable to evaluate possible hazard of applied chemicals on bees.

Material and Methods

In all experiments author used winter bees from frames and bees kept in the flying room at Dol.

Peroral tests were effectuated in wooden cages 75x50x140 mm provided by two glass feeders, ventilation holes and observation glass on the front side. Tested material was placed in volumetric flask, 96% ethanol (1ml) was added and volume was adjusted to 50 ml by sucrose solution (1+1 parts by weight). The consumption of the solution was evaluated volumetrically. Mortality was observed mostly daily, and dead bees were discarded from the cages.

Contact tests were peformed on Petri dishes (diameter 10,9 cm). Aceton solution of the tested material (2ml) was evaporated on the bottom of Petri dishes. Glass tubes (diameter 2-4 mm, lenght 12 mm) were sticked by beeswax to the bottom of dishes and filled with sugar solution (1+1) to feed the honeybees during the experiment of 24 hours. The mortality was corrected according to Abbott to control.

Results

Peroral test was made for two concentrations of coumaphos and for one concentration of fluvalinate and amitraz. The bees were fed not only by intoxicated solution, but also by sugar solution as to assess the palability-repellence of substances during the whole experiment. The consumption of administred solutions was measured for first 4 days of experiment. The bees were fed by toxic solutions only for first four days of experiment. Results are in Tab. I.

The tested concentration is compared to theoretical maximal concentration of the substance. This concentration in bee hive can be judged from the fact that in the time of inproper use bees can have minimal sugar stores or honey (about 10 kg). As the dose of amitraz for the Varroa control is 6-7 mg, then bees can eventually come in contact with the concentration of 0,6 - 0,8 ppm in stores. For coumaphos the concentration can reach 4 ppm and for the application of fluvalinate 0,1 ppm, except the use of Apistan strips. Results show that the danger of overdosage is largest for amitraz and minimal for fluvalinate. But differences between toxic and usually used concentration of all tested substances are enough for the safe use of these substances in Varroa control. Palability-repellence was observed only for coumaphos.

For contact test we compared the experimental concentration with theoretical maximal concentration supposing that the surface of frames and the walls of bee hive is about 5 m^2. For these test we used not only winter bees from the hive but also the bees taken from the colony living in flying room for several monts. It was clearly shown that the stressed bees were more sensible than those ones from the hive. Tab. II shows that in absolute values the most tolerant substance to bees is coumaphos.

Conclusions

Peroral hazard for the bees treated by tested acaricides is obviously not so dangerous in comparison with contact ones. Peroraly, the bees are most tolerant to fluvalinate. Palability-repelence was observed only for coumaphos.

Absolute contact toxicity for the bees from bee hive is minimal for coumaphos. Contact hazard for the bees treated against Varroa is important for amitraz and coumaphos, where the toxic and in the practice used concentrations are close each other.

Results show that in case of suspected damage to bee colonies by acaricides, the stress or disease of bees can increase the toxicity significantly. It is recommended mainly for amitraz to keep the right concentration for Varroa control carefully.

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Tab. I Peroral toxicity and repellence-palatability of coumaphos, fluvalinate and amitraz to bees

Mortality (%)	Coumaphos		Mavrik	Tactic	Control
	(active substance concentration in sugar solution)				
	160 ppm	800 ppm	250 ppm	125 ppm	
in 24 hours	0,0	1,8	1,1	2,2	0,9
10 days	10,1	18,8	9,4	50,7	4,1
16 days	24,5	27,5	10,6	57,2	8,5
Coefficient of repellence - palatability	0,48	0,11	0,63	0,94	0,72
possible maximal concentration of substances in stores in hive during Varroa control	4 ppm		0,1 ppm	0,6-0,8 ppm	

Tab. II Contact action coumaphos, fluvalinate and amitraz to bees

concentration of a. i. µg/1000 cm^2	mortality in 24 hours (%) amitraz bee yard	fluvalinate bee yard	coumaphos bee yard	coumaphos flying room
4280	100			100
3429			0,0	
1329			3,3	100
1072	100	80		
1000			3,3	93
800			6,6	97
686			0,0	
428	20	23	0,0	10
343			0,0	
214	0	16		
170			0,0	
107		20		
non effect level (µg/1000 cm^2)	214 less than	107	3429	319
possible maximal concentration of substance on the surface in bee hives	125	20	800	

APPENDIX 17

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FUMIGATION TAPE

The cooperation of the IRAPA Research Institute and the Bee Research Institute at Dol has resulted in a new product for fumigation purposes.

The task indicated conditions from the point view of the tape function and from the point of view of the production technology. The principal request concerns the quality of the raw material, mainly the pulp component.This component at the burning must not form pollutants which endanger the life of bees. For that reason we selected the pulp with high content of alphacellulose produced on the magnesium basis and bleached by oxygen. The grinding process in the paper equipments prepares the pulp for the processing in the paper machine. The special type of the grinding process ensures the quality of the pulp component that the requested properties of the paper-card-board with the requested areal weight and thickness may be achieved. The requested volume weights of the paper are given by the ratio of the parametrs. The following operation of the chemical procession by means of inorganic salts of nitrate types is necessary for the suitable process of burning. The process occurs from the salt solution in the solvent, in the given case in the diluted solution of ethylalcohol on the impregnation machine. This machine is to be equipped with the coating roller with regulation of revolutions.

The setting up of the weight increment on the leaf of the paper of inorganic salt may adjust the burning speed. The burning of the type develops the maximal temperature 350^{o}C. This parameter is very important that no decomposition of the acaricide component occurs.

The dropping of the active component in liquid form on tapes closely before the application makes the type more universal. This component may be changed in the case of the occurred resistence. The quantity of the active substance is used in the minimal sufficiently active quantity

and the applier who applies the acaricide comes in contact with this substance only for a minimal time.

The producer of the fumigation type is at the present time State Corporation IRAPA, enterprise Štětí, Litoměřická 272 , Zip Code 411 08. The corporation ensures all production steps inclusive the final works, i.e., all ordered dimensions, cutting and packaging. The final arrangement may be done according the requests of the customer.

IRAPA is to produce the fumigation tapes also for other customers.

The product is protected by the CS Pat, Appl. 264 506 and 254 412.

APPENDIX 18

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THE FATE OF COUMAPHOS RESIDUES IN HONEY AND BEESWAX

Organophosphate Coumaphos (Asuntol, Perizin etc.) is one of active acaricides against Varroa jacobsoni. The work includes results of the monitoring the dynamics of coumaphos residues in honey and beeswax in dependence on various application forms of Coumaphos on honeybee colonies, its concentration and the interval between the application and the analysis. The Bee Research Institute at Dol carried out experimental application of coumaphos on bee colonies, evaluated the effectiveness of coumaphos against Varroa jacobsoni and studied the toxical effects on bees. The Research Institute of Veterinary Medicine at Brno prepared a rapid method for the determination of coumaphos in honey and beeswax based on gas chromatography with the use of alcalic flame-ionization detector on the filling column with the phase OV-101. Coumaphos residues are extracted from the water solution of honey into the dichlormethane. The yield for honey at this method is 97 %, detection limit 0,005 $mg.kg^{-1}$, for beeswax above 70 % and limit detection 0,050 $mg.kg^{-1}$. This method monitored coumaphos residues in winter stores, honey and wax.

Coumaphos Residues in Winter Stores. Honey and Wax 1987

Coumaphos was applied in hives mostly in the form of candy with Perizin. Coumaphos quantity ranged from 83 to 250 mg per one hive (Tab.1). Consumption time of honey sugar candy by bees was in individual hives considerably different (27 to 140 days). The sampling of winter stores started 14 or 30 days after the end of candy feeding. In the analyzed samples of winter stores coumaphos residues were found in concentrations 0 to 0,143 $mg.kg^{-1}$. In the same period, there were analyzed three beeswax samples after the application of 250 mg coumaphos in the hive. Coumaphos

Coumaphos Residues in Honey - 1988, 1989

In the years 1988 and 1989 the application of coumaphos was done (pouring, fumigation, warm aerosol) in concentrations 30 - 40 mg active substance per one bee colony. Honey samples from treated bee colonies, not only from the harvest 1988 but also from the harvest 1989 did not contain coumaphos in concentrations above 0,005 $mg.kg^{-1}$ (detection limit of the method).

Results show that the used forms of application (aerosol, fumigations, pouring) and concentrations of the active substance of coumaphos 30 - 40 mg per a bee colony did not contain coumaphos residues in honey above the concentration 0,005 $mg.kg^{-1}$ and the honey complies fully with the strict hygienic requirements in all western countries.

Tab. 1 Coumaphos content in winter stores - $mg.kg^{-1}$

Sample No.	Coumaphos applied (mg)	Consumption time (days)	Sampling day after the consumption	Coumaphos residues $mg.kg^{-1}$
1	0			0,005
2	0			0,005
3	166	40	14	0,043
4	83	23	14	0,072
5	83	27	14	0,042
6	166	27	14	0,143
7	166	34	14	0,005
8	83	34	14	0,005
9	250	140	30	0,050
10	250	140	30	0,060
11	250	140	30	0,005

Tab. 2 Coumaphos content in the first honey - $mg.kg^{-1}$

Sample No.	Coumaphos applied (mg)	Consumption time (days)	Sampling day after the consumption	Coumaphos residues $mg.kg^{-1}$
1	250	140	244	0,019
2	250		240	0,033
3	32		7	0,235[x]
4	83		77	0,013
5	166		77	0,053
6	250	131	233	0,006
7	250	131	233	0,017
8	83	9	83	0,052
9	83	10	106	0,018
10	83		120	0,005
11	83		120	0,051

Tab. 3 Coumaphos dynamics in honey - $mg.kg^{-1}$

Analysis date	Storage (days)	Sample 1	Sample 2
2.2.88	0	0,24	0,25
16.3.88	42	0,21	0,16
14.4.88	71	0,26	0,17
19.5.88	107	0,23	0,23
24.5.88	112	0,24	0,30
21.6.88	140	0,20	0,20

Tab. 4 Coumaphos dynamics in beeswax - $mg.kg^{-1}$

Analysis date	Storage (days)	Sample 78	80	81	82
10.11.87	0	3,32	17,79	12,80	1,72
19.11.87	9	3,00		10,27	
8.2.88	82		15,45	3,17	1,23
26.2.88	107	2,52	12,18		
4. 5.88	174		8,08		
22.6.88	216	2,48	9,75	3,58	0,66

Sample: 78 - Asuntol aerosol; 80 - poured by Asuntol
81 - Asuntol in water, aerosol; 82 fumigated by Asuntol

APPENDIX 19

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RESIDUES IN BEESWAX, WINTER SUGAR STORES AND HONEY AFTER TREATMENT WITH APISTAN AND FOLBEX VA

INTRODUCTION

When acaricides are often used for varroa control there is a danger for contamination of the bee products. Up to now there are very few studies dealing with the residues of acaricides in the bee products after a long term varroa control. As most of the antivarroa drugs are lipophilic, they can contaminate predominantly the beeswax. Folbex VA has been un use for antivarroa treatment in Switzerland since 5 years and is also widely used in Europe. Apistan is used in some contries like France, Italy and Austria. We conducted this study in order to measure the extent of contamination of beeswax, winter sugar and honey in dependance of the time of use of these two acaricides for varroa control. Extensive reports of these trials have being published elsewhere (Bogdanov et al., 1990).

MATERIALS AND METHODS

Acaricide treatment

One Folbex treatment was with 4 strips per year in "Swiss bee hives" (one Folbex strip contains 0,4 g bromopropylate). Each hive was treated for 1 to 5 years.

Bee colonies in Dadant hives were treated with 3 Apistan strips each (one strip contains 900 mg fluvalinate). The treatment was initiated on the 7th of September 1988.

- one hive was treated for 4 weeks (control)
- in the other hive the strips were left for a 13 month continuous treatment.

Sampling

Beeswax and winter sugar stores from the brood chamber

From each comb about 10 cm^2 were scratched off with a spoon down to the foundations. The winter sugar stores and the wax were then separated for analysis.

The Folbex samples were taken early spring 1989.

The Apistan samples were taken periokecally from bee colonies at the indicated times (see Fig.1).

Honey combs and honey

Honey was harvested from individual colonies (Apistan trials) and from the whole (Folbex trial). The honeycomb wax samples were taken as described above for the broodcomb wax.

Tab. 3 Coumaphos dynamics in honey - $mg.kg^{-1}$

Analysis date	Storage (days)	Sample 1	Sample 2
2.2.88	0	0,24	0,25
16.3.88	42	0,21	0,16
14.4.88	71	0,26	0,17
19.5.88	107	0,23	0,23
24.5.88	112	0,24	0,30
21.6.88	140	0,20	0,20

Tab. 4 Coumaphos dynamics in beeswax - $mg.kg^{-1}$

Analysis date	Storage (days)	Sample 78	80	81	82
10.11.87	0	3,32	17,79	12,80	1,72
19.11.87	9	3,00		10,27	
8.2.88	82		15,45	3,17	1,23
26.2.88	107	2,52	12,18		
4. 5.88	174		8,08		
22.6.88	216	2,48	9,75	3,58	0,66

Sample: 78 - Asuntol aerosol; 80 - poured by Asuntol
81 - Asuntol in water, aerosol; 82 fumigated by Asuntol

APPENDIX 19

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RESIDUES IN BEESWAX, WINTER SUGAR STORES AND HONEY AFTER TREATMENT WITH APISTAN AND FOLBEX VA

INTRODUCTION

When acaricides are often used for varroa control there is a danger for contamination of the bee products. Up to now there are very few studies dealing with the residues of acaricides in the bee products after a long term varroa control. As most of the antivarroa drugs are lipophilic, they can contaminate predominantly the beeswax. Folbex VA has been un use for antivarroa treatment in Switzerland since 5 years and is also widely used in Europe. Apistan is used in some contries like France, Italy and Austria. We conducted this study in order to measure the extent of contamination of beeswax, winter sugar and honey in dependance of the time of use of these two acaricides for varroa control. Extensive reports of these trials have being published elsewhere (Bogdanov et al., 1990).

MATERIALS AND METHODS

Acaricide treatment

One Folbex treatment was with 4 strips per year in "Swiss bee hives" (one Folbex strip contains 0,4 g bromopropylate). Each hive was treated for 1 to 5 years.

Bee colonies in Dadant hives were treated with 3 Apistan strips each (one strip contains 900 mg fluvalinate). The treatment was initiated on the 7th of September 1988.

- one hive was treated for 4 weeks (control)
- in the other hive the strips were left for a 13 month continuous treatment.

Sampling

Beeswax and winter sugar stores from the brood chamber

From each comb about 10 cm^2 were scratched off with a spoon down to the foundations. The winter sugar stores and the wax were then separated for analysis.

The Folbex samples were taken early spring 1989.

The Apistan samples were taken periokecally from bee colonies at the indicated times (see Fig.1).

Honey combs and honey

Honey was harvested from individual colonies (Apistan trials) and from the whole (Folbex trial). The honeycomb wax samples were taken as described above for the broodcomb wax.

Residue analysis

For wax analysis a method of the Zoecon (Sandoz) company was modified in Liebefeld. For the winter sugar stores and honey analysis we used solid phase extraction (SPE). Fluvalinate, bromopropylate (BP) and dibromobenzophenone (DBP) could be determined simultaneously. They were analyzed with cappilary gas chromatography and EDC detection.

RESULTS

1. Accumulation in beeswax

Apistan

In Fig. 1 the wax residues of fluvalinate after one treatment (control) and after a permanent Apistan treatment for the whole 18 month trial are given.

If the Apistan strips were taken away after 4 weeks (normal) treatment relatively small residues of fluvalinate were found in the wax of the brood combs. The values during the following year varied from 0.2 and 7.3 mg/kg, the mean value being 1.9 mg/kg. If the same strips were kept during one year in the colony, the fluvalinate residues increased with increased duration of the experiment. During the months May, June, July and August the residues remained the same, may be due to dilution by new wax synthesis.

The fluvalinate residues in the honeycomb wax were half as big, those in the honeycomb uncapping about the same as those in the broodcomb wax (results not shown).

Folbex

Fig 2 shows the accumulation of residues in wax in dependance of the number of Folbex treatments. The total Folbex residues were the combined residues of bromopropylate (BP) and its metabolite dibromobenzophenone (BBP). The residues increased with increasing number of treatments ($2P = 0.026$). These results are from colonies, where 2 foundation per year and colony were used for comb construction. The residues were 2 to 5 times smaller if 3-4 foundations per colony were used (results not shown). 75 to 84 % of the residues were bromopropylate, the rest being its metabolite dibromobenzophenone. The Folbex residues in the wax of the honey combs was about 15 times lower than those of the wax of the brood combs (results not shown).

2. Winter sugar stores and honey

Table 1 gives an overview of the residues in the sugar stores and in the honey after a permanent Apistan treatment and after increasing number of Folbex treatments.

The residues in the beefeedings increased slightly with increased duration of Apistan treatment, the highest value being 0.016 mg/kg after 54 weeks treatment ($2P$ 0.05). The residues in 2 honey samples, harvested after 44 weeks of treatment were below the detection limit. The Folbex residues in the beefeedings increased also slightly ($2P < 0.05$), the highest value detected was 0,16 mg/kg in a colony, treated during 3 years with Folbex. The residues in 3 honey samples increased also slightly with increasing number of treatments, the highest value being after 5 years of Folbex with 0.07 mg/kg.

DISCUSSION AND CONCLUSION

With these results we show that the residues of the acaricides Apistan and Folbex VA accumulate in a time dependent manner in the wax of the broodcombs: the longer or the more often the acaricide treatment, the higher the residues in the beeswax. The residues of the acaricides increased also slightly in the winter sugar stores and in honey with increasing number of treatments, but they remeined very low and did not exceed the tolerated limits.

Our results confirm the results of other investigations with lipophilic acaricides, where much higher residues were found in beeswax than in honey or in the winter sugar stores (Binder et al. 1988, Faucon and Flamini, 1988; Hansen and Petersen 1988, Klein et al. 1986, Thrasyvoulou and Pappas, 1988). As beeswax is recycled the residues of persistent, lipophylic acaricides might increase with time also in the beeswax foundations. This possible accumulation has not been investigated up to now and attention to this topic should be payed in the near future.

The toxic effects of high levels of acaricides in beeswax have not been well investigated. Chmielewski (1984) found that extremely contaminated brood combs, containing 10g/kg bromopropylate had no effect on 15 day-old pupae or bees. However, the eventual effect of bromopropylate-contaminated wax on eggs and younger larvae stages has not been investigated. In our knowledge the effect of high levels of fluvalinate on bee brood has not be conclusively elucidated. In wax, there are also many persistent chlorinated hydrocarbons and pesticides (Davies, 1989, Bogdanow 1988). The different pesticides mifht act together in a synergistic manner. The results of toxicologic laboratory tests on single acaricides cannot necessiraly mean that these acaricides have had bad effects in the beehive.

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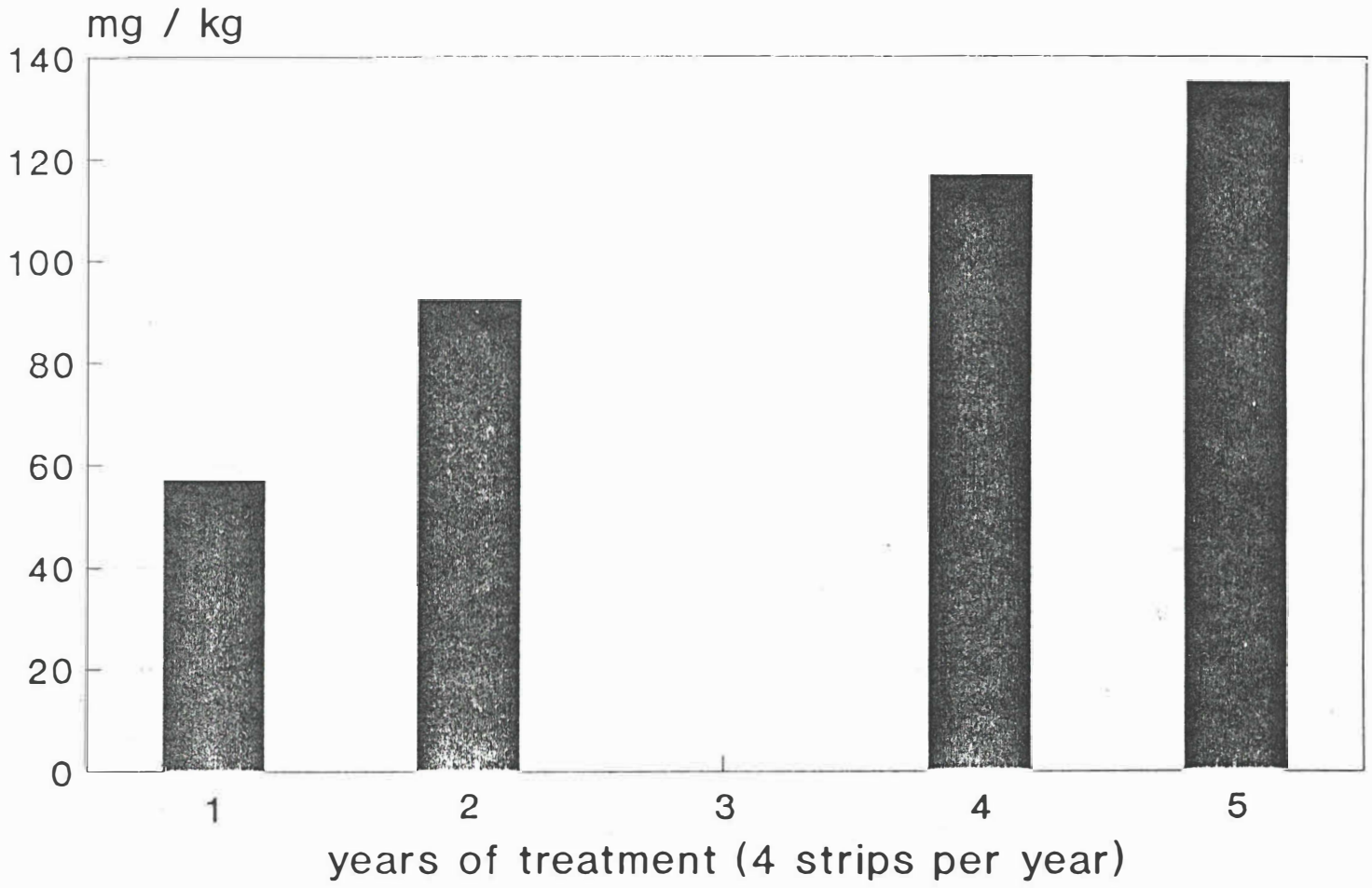
	APISTAN		FOLBEX	
	sugar stores	honey	sugar stores	honey
treatment	from 4 to 54 weeks		1 to 5 years	
extreme values (mg/kg)	0.003-0.016	≤ 0.003	0.04-0.016	0.015-0.070
a	9	2	12	3
2P	0.05	-----	0.05	-----

Table 1. Residues in winter sugar stores and honey in dependance of the duration of treatment with Apistan and Folbex. The residues in mg/kg were from fluvalinate (Apistan treatment) and the total of bromopropylate and dibromobenzophenone (Folbex treatment). The dependance of the residue amount on the duration time of treatment (Apistan) and on the number of treatments (Folbex) was expressed as a 2P value.

FIG. 2 BROMOPROPYLATE: RESIDUES IN BEESWAX
(BBP+BP) after treatment with Folbex
mean values from 3 colonies



2 foundations per year

APPENDIX 20

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BIOTEST FOR PESTICIDE DETECTION IN BEESWAX BY THE USE OF THE GREATER WAXMOTH GALLERIA MELLONELLA L.

Several substances for the treatment of bee colonies against Varroa are highly lipophil. Due to this their accumulation in beeswax after the recycling of old combs has to be considered. Comb building bees show little sensitivity against adulterated and contaminated foundations. So larvae of the greater waxmoth, Galleria mellonella, have been used for the development of a biotest where their survival and growth-inhibition were determined as parameters. Results with different concentrations of Malathion, Lindan, Perizin, Folbex and Paradichlorbenzene will be reported.

Abstract:

Repeated application of varroacides create a hazard for the quality of beeswax used for foundations. Since honeybees proved little sensitive in the detection of these contaminations, Galleria mellonella L. larvae have been used for a bioassey, monitoring survival and growth. Results with Lindan, Paradichlorobenzene, Coumaphos (Perizin), Folbex VA neu and Malathion are reported.

Introduction:

Medical treatment of Varroatosis brought a steep increase in the application of chemical substances in bee colonies. Since most of these varroacides are of highly lipophil character, the contamination of beeswax is more likely than the accumulation of the drugs in honey.
Residue analysis of beeswax can be a highly complicated and expensive method for the evaluation of a potential hazard of contaminated wax to bee colonies. A chemical value of contamination within the ppm range may tell nothing about its biological importance.
So we tried to use small bee groups, including a queen, as units for a bioassey of the quality of beeswax. The bees proved to be very insensitive in their inclination to accept foundations of different levels of contamination with varroacides for the construction of new combs.
So we tried to use the greater waxmoth, Galleria mellonella L. as test organisms. This insect feeds on beeswax, so necessarily contaminated wax samples have to pass through the whole digestive system of the growing larve. During this passage potential harmful substances, varroacides or pesticides, can act as contact or stomach poisons.

Material and Method:

Waxmoths can be easily raised in laboratory cultures. If eggs of known age are required, filter paper is pressed on the screen cover (18 mesh/cm) of the glass container. The female moth deposits by its extruding egg depositor groups of eggs through the wire screen on the filter paper. The deposited eggs can be collected through storage in a refrigerator (about 8°C) for several days. When sufficient eggs are available these are transferred to an incubator with 31°C. About 6 days later the larvae hatch and move around very actively; they receive little pellets of synthetic food so they will not starve.
Samples of about 20 1-2 day old larvae are distributed to the glass container filled with the test samples of beeswax. It was found out that the inocculation of the testfood with larvae is more reliable than using egg samples, since the active larvae are mixing very thoroughly, thereby randomizing the available genotypes; In that way steril eggs can not upset the inocculation with similar numbers of test organisms.
Shortly before subdividing the population larvae are stored for about 1-3 min in the refrigerator to facilitate the counting and separation of the subpopulations. Since waxmoth can not persist on pure wax foundations, this material is very precisely enriched by the following synthetic diet:

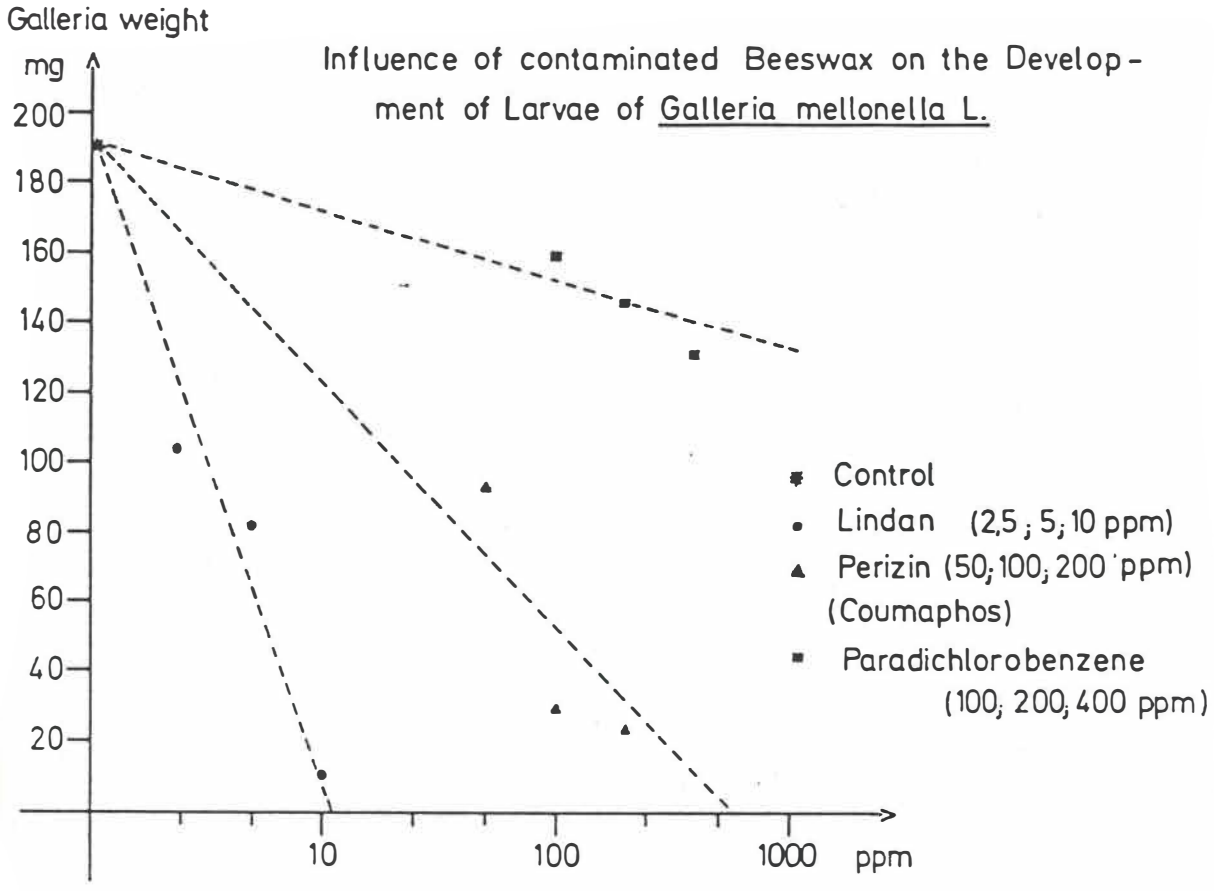
wheat flour	160 g	mixed deepfrozen pollen	53 g
wheat bran	200 g	brewers yeast	40 g
skimmed powdered milk	80 g		

60% test wax (20 g) are molten and thoroughly mixed with 40% dry powdered synthetic food (13,3 g). If new substances shall be tested in several concentrations they are dissolved in aceton and added to the molten beeswax. This mixture is quickly poured on a plastic foil, covered by a second foil and pressed to a thin cake between these two plastic layers. These thin cakes are immediately transfered to the deep-freezer where they form thin hard layers, which can be

broken into small pieces and ground to a flour - like well mixed food substrate for the waxmoths.
After the inocculation of this testsubstance in glass containers of a diameter of 8 cm and 5 cm hight it is advisable to cover the top by a well fitting glass lid to prevent the very active tiny waxmoth larvae to escape; 2-3 days later these glass lids should be replaced by plastic covers with tiny holes to permit ventilation of the culture containers. These are kept in incubators with 30°C and about 50% rel. humidity. Each sample has 3 repetitions. A control made from pure uncontaminated beeswax is added.
In that temperature after 28 days the larvae have reached their final size and will pupate within the next 2-3 days They are collected from the culture medium which is more than sufficient to maintain 15-20 larvae, and their weight is afterwards immediately determined with a laboratory scale.

Results:

Each waxsample is tested by 3 waxmoth subpopulations of 13 - 20 animals each.
Fig. 1 shows the contamination of wax samples by the substances Lindan, Paradichlorobenzene and Perizin (a.i. Coumaphos) of different concentrations.
The values demonstrate that Galleria reacts fairly sensitive to Lindan, where a contamination of 2,5 ppm already reduced the growth of the larvae. Also 50 ppm Perizin gave a significant weight reduction of the treated larvae; an increase in the replicate number might be a suitable way for the detection of even smaller amounts of Perizin in beeswax.
Surprisingly the effect of Paradichlorobenzene, normaly used as antiwaxmoth chemical, is fairly low, even when concentrations of 100 ppm or 200 ppm are applied.



Beeswax contaminated with Malathion gave the following results:

20 ppm	no larvae developed;
10 ppm	only very few larvae with reduced growth survived;
5 ppm	no significant weight reduction;

"Folbex VA neu" applied only once as smoke on empty combs caused a significant weight reduction in the larvae.

Discussion:

If we compare the sensitivity of honeybees constructing combs on foundations contaminated with varroacides or pesticides with the reaction of waxmoth larvae we have to recognize that the later ones already indicate lower doses. For instance reacted honeybees on foundations with 100 ppm Mala-

thion by a 50% reduction of wax production, waxmoth larvae showed a severe block already with 10 ppm contamination. KNAPPWOST (1986) published residue values of more than 100 ppm after 4 "Folbes VA neu"-treatments; and WEISS, MAUTZ and SCHAPER (1986) could not observe any adverse effects on bees transferred to foundations where the beeswax had been treated 20 times with this varroacide. Galleria showed even after one treatment a significant weight reduction.
Till now the qualification for bioasseys of Galleria mellonella has been used only once. EISCHEN and DIETZ (1987) used this insect to test the influence of different amounts of propolis from several origins mixed with beeswax. In these experiments they also detected a very sensitive reaction, changes in growth and survival, to different propolis fractions.
One severe drawback connected with the Galleria-test is the lack of specific information about the nature of the contamination. Reduced survival or growth only signals a harmful component. These samples can be singled out and submitted to a chemical residue analysis. The lower efford in equipment and labor involved in the Galleria-test at least permits a screening of wax samples which show negative biological effects.

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APPENDIX 21

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TESTING OF THE CONTAMINATED COMBS BY MEANS OF WAX MONTH GALLERIA MELLONELLA LARVAE

The feeding test proved the possible use of Galleria mellonella L. larvae for the determination of the residues of pyrethroid fluvalinate in beeswax. As for instance, Drosophila melanogaster is applied for the determination of residues (Bacílek 1976) author used the experimental material, larvae of Galleria mellonella. Larvae were tested for a long time mainly on the resistance to various strains of Bacillus thuringiensis aimed at the protection of combs. We tried also other antibiotics mainly as well as other substances. Burgess (1977) found a certain toxicity for larvae only at chloramphenicol and dipikolin acid.

Trials tested combs in vicinity of which two fluvalinate carriers with long term trial effects occur. These carriers are applied by hanging. Each type contained approximately 100 g active substance - fluvalinate. After gaining negative results author tested combs exponed for 35 to 41 days and finally the chemical on the filtration paper in various quantity. The experiment used wax moth larvae in the age from the 3rd to the 6th instar. The original material cultivated on Haydak´s medium is transformed to natural food. We started from our previous results about the development and weight of larvae in dependence on the given diet (Krieg 1972). The rearing and trials in Petri dishes (diameter 150 mm) were carried in the thermostat at 30^{0}C, relative moisture 50 - 70 % in the darkness. Each container contained 250 cm^2 comb of brown colour with about the same sugar and pollen content,further 10 larvae of the 6th instar and 20 larvae in the age of the 3rd to the 5th instar. This made possible the receive of food during the experiment within the range of 8 to 14 days.

The contamination of combs after 24 days of exposition does not offer residues which may be proved by the feeding test by means of wax moth larvae. These larvae finished the development and there develop normal moths. But author found that after the establishment of experiments larvae are repelled and omit the natural reaction, the boring into the combs. We observed 20 to 46 % within the range three to ten days which moved out of the comb (no larvae occurred in the control test). The artificial increase of the fluvalinate concentration in trial dishes achieved the stop of the development and later also the mortality of larvae. The addition of 1,7 mg fluvalinate on the filtration paper to the comb exposed for 41 days (for one test) was still without influence. The growth delay was achieved by the addition of 3,5 mg fluvalinate to the given comb. After the addition of the whole used carrier - tape, the weight of larvae after 10 days in comparison with control, was a third part (97,may be 260 mg) and after 20 days the mortality of larvae was 80 % from the experimental tests.

The obtained results show that larvae of Galleria mellonella are not suitable for the testing of the contamination of combs at the normal dose for the treatment of the bee colonies against Varroa jacobsoni. The found repelling activity could be done also by other substances and cannot be therefore used as the decisive indicator of the test. The evidence of the developed residues by means of the biological method is to be based on the use of more sensitive organisms.

APPENDIX 22

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DETERMINATION OF FLUVALINATE IN HONEY AND WAX

Fluvalinate is the active ingredient in Sandoz´s accaricide Mavric 2E. This material was used to control Varroa jacobsoni on bees. The residue method applied to the analysis of honey and wax was based on the determination of the intact compound by GLC and on the confirmation of the identity after derivatization.

Honey

For the extraction of fluvalinate in honey Celite 545 was used. The sample was dissolved in water and let pass trough a column containing 1,5 g of the above material. After that the column was washed with water and fluvalinate collected on the column was eluted with acetone. Water was added to the eluate and fluvalinate was extracted in a separatory funnel with n-hexane. N-hexane layer was dried and further cleaned-up by column chromatography.

Wax

The sample was dissolved in mixture of cyclohexane /50/, etylacetate /40/ and dichlormethane /10/ and cooled in a refrigerator at 5° C. Two layers were separated after several hours and the upper one was let pass through a column with Bio-Beads SX-3. The above mentioned mixture (of cyclohexane, etylacetate, dichloromethane) was used as a mobile phase. The clean-up by gel permeation chromatography was automated and the system consisted of a stainless steal column (500 x 10 mm Supelco) a Rheodyne valve (No.7125) with 2 ml sample loop and the solvent was pumped through the column with a micro pump (LCP 3001).

The "pesticide fraction" (22-30 ml) was concentrated and cleaned-up by column chromatography.

Column chromatography clean-up

A glass column (i.d. = 1 cm) was packed with Florisil deactivated with 1 % of water. Fluvalinate was eluted with 2 % aceton in hexane. The solvent was evaporated to a small volume (just to dryness) and a known volume of hexane was added for enjection into the gas chromatograph.

CC detrmination

The measurements were performed by means of gas chromatography and both capillary and packed columns were used.

In Fig.1 there is a chromatogram of extract of honey and wax on glass column packed with 5 % DOW 200 on Varaport and at the Fig.2. is the sume extract analysed on a capillary column.

In pesticide residue analysis, GLC with an appropriate detector is still the most widely used technique for routine analysis.For the analysis of pyrethroids the almost universally used detector is the selective ECD because it provides the necessary sensivity for low level determination of pyrethroids in many applications. Unlike a specific detector, ECD is selective in response to certain types of compounds. The other weaker electron-capturing compounds mainly derived from natural sources are often present in the sample as co-extractives and thay are usually in thousand fold excess of the pyrethroid in question. Therefore their ECD responses can be significant. Consequently, identification based on a single set of consitions is not valid.

Therefore, additional identification techniques based on a chemical confirmatory test was used.

The derivation was achieved by means of transesterification carried out as follows: The eluate from the Florisil column was evaporated just to dryness and 0,5 ml of toluene and 0,1 ml of (c = 0,2 M) potassium hydroxide in methanol was added. This solution was heated at 60°C for 10 minutes. After cooling was the mixture neutralized by adding (8,1 ml) sulfuric acid in methanol (c = 1,0) and the volume adjusted to 2 ml with hexane. Typical gas chromatogram of honey and wax after derivatization are shown in fig.3.

Recovery

The samples of untreated material were fortified with known quatities of fluvalinate. The limit of determination of the whole procedure was estimated 0,01 ppm for honey and 0,2 ppm for wax. Mean recoveries are shown in Table 1.

Sample	Added (ppm)	Recovery (%)
honey	0,1	73 ± 4
wax	1,0	81 ± 10

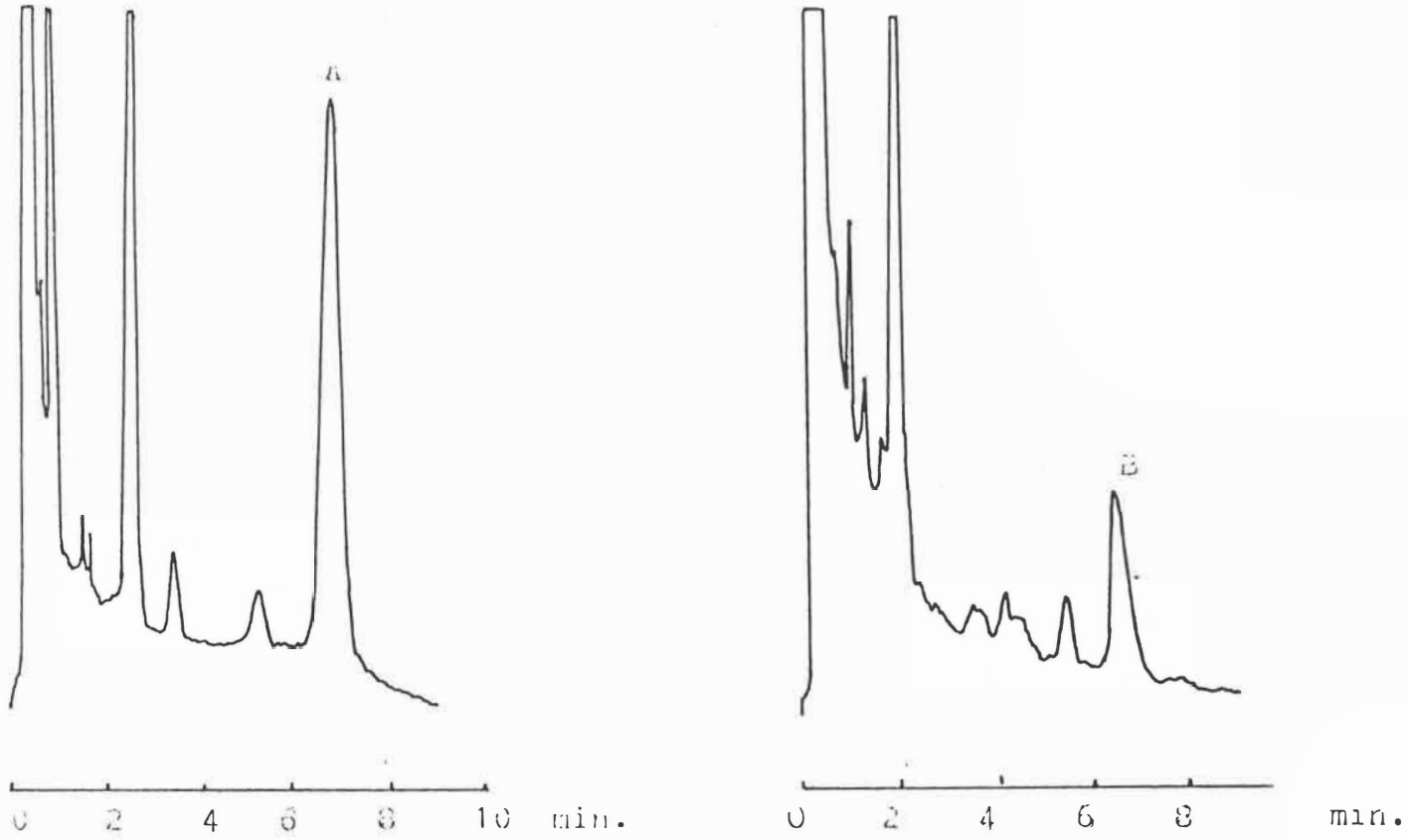

Fig. 1. Typical chromatogram on packed column

Chromatographic conditions: HP-5750, 5 % DOW 200 (Varaport/1200 x 3 mm), 240° C, ECD 250° C, injector 250° C, carrier N_2 - 40 ml/min.

A - extract of honey (injected 25 mg), fluvalinate c = 0,04 mg/kg

B - extract of wax (injected 0,5 mg), fluvalinate c = 0,5 mg/kg

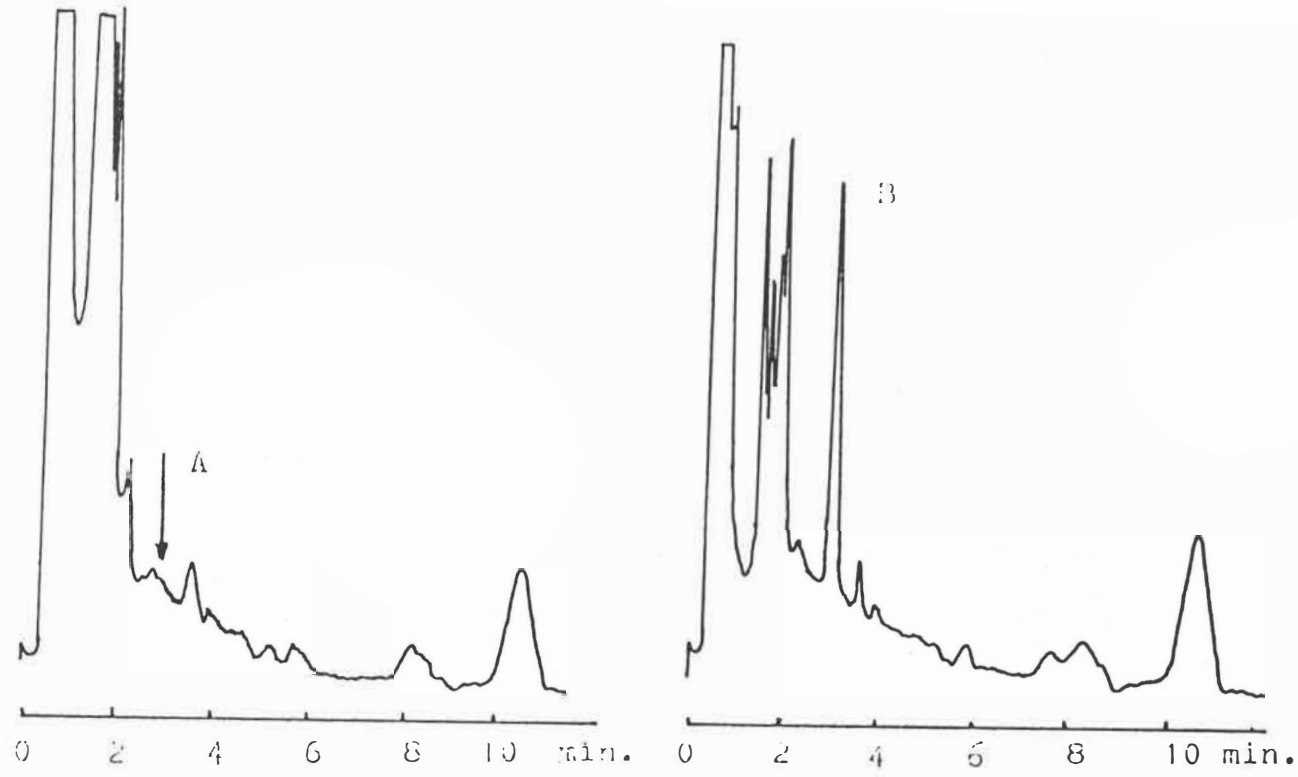

Fig. 3. Chromatogram of fluvalinate after derivatization

Chromatographic conditions: HP-5750, 5 % DOW 200 (Varaport/1200x 3 mm), 190° C, ECD 250° C, injector 200° C carrier N_2 - 40 ml/min.

A - extract of honey (injected 12,5 mg), blank

B - extract of honey (injected 12,5 mg), spiked level 0,2 mg/kg

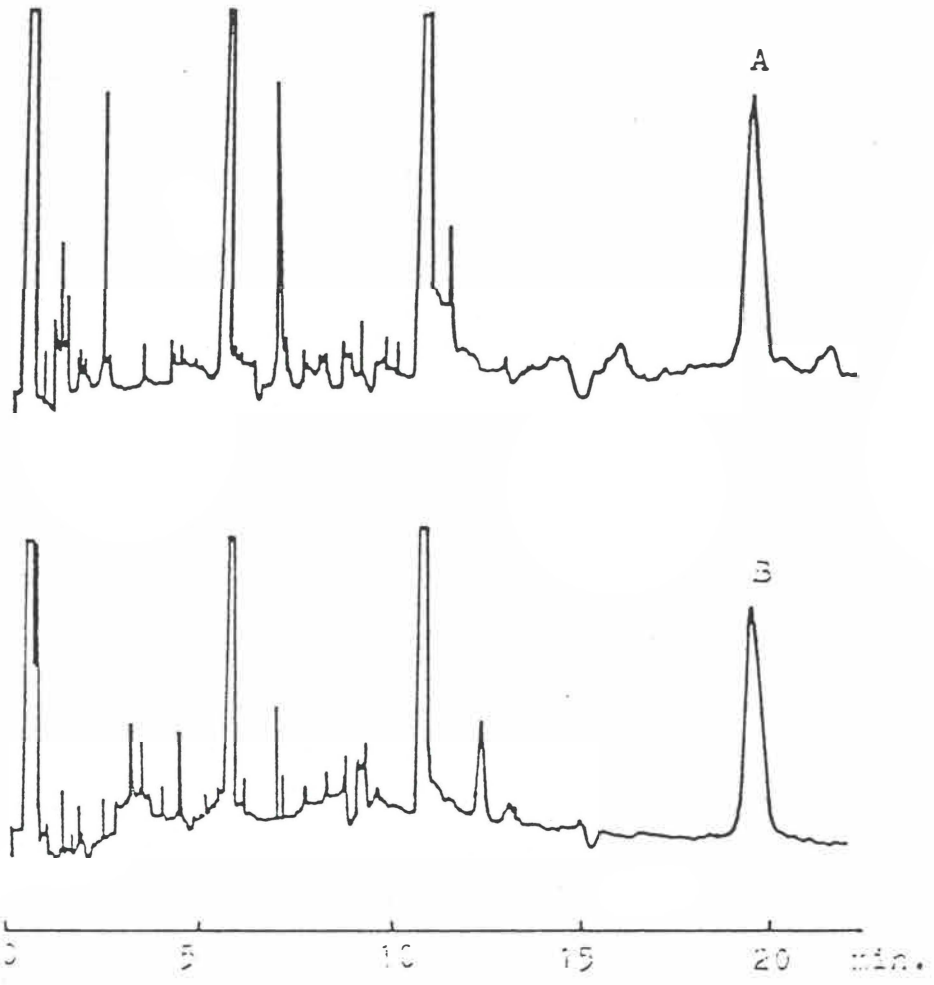

Fig. 2. Typical chromatogram on capillary column

Chromatographic conditions: HP-5890, HP-5 (10 m x 0,53 mm), ECD 300° C, on column, carrier N_2 - 8,50 ml/min. Temperature programming 80° c - rate 30° C/min. - 230° C - rate 10° C/min. - 270° C - hold

A - extract of wax (injected 0,1 mg), fluvalinate c = 1,90 mg/kg

B - extract of honey (injected 5 mg), fluvalinate c = 0,04 mg/kg

APPENDIX 23

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POLLINATION AND PLANT PROTECTION IN SEED PRODUCTION OF LUCERNE (Medicago sativa L.)

Due to the concentration of seed production of lucerne (Medicago sativa L.) in the climatically most favourable regions large complexes of stands for seed production are established the acreage of which may be as much as several hundreds of hectars. An integral part of this technology became the propagation of the solitary bee, Rhophitoides canus Ev.. This method consists of securing food for emering bees (food stripes of the lst cut of lucerne), equal distribution of their nests throughout the field (nesting stripes), and timing the flowering of lucerne with the flying activity of the bee population.

The system of protection against pests was adapted with regard to the needs of increasing numbers of pollinating ensects. Organophosphates and chlorinated hydrocarbons had been gradually replaced by such preparations which were relatively harmless to bees (e.g. pyrethroids). Each chemical treatment of stands must be based on reliable data about the occurence of pests.

Key words: Lucerne (Medicago sativa L.); pollination; seed production, Rhophitoides canus Ev.; plant protection

In the first half of 1980, the seed production of lucerne had been gradually concentrated into the climatically most favourable regions of Czechoslovakia. Some agricultural enterprises were specialized in production of lucerne seed and cultivated crop on field blocks larger than hundreds of hectars. From the technological point of view such a specialization had a number of advantages; on the other hand, however, the pollination of these huge blocks of fields became a problem.

It was not possible to rely on pollination activities of honey bees because of their low individual effectiveness. A replacement of this lack of individual bees through the increase of their numbers within the corp, which was recommended earlier by same authors, was not possible due to the lack of migratory colonies necessary for such great concentration of lucerne. The situation was further complicated by a prohibition of colony migration due to the prophylactic measures associated with the fight against mites Varroa jacobsonii Oud.

Since 1983, We have tried to use leaf cutting bees as pollinators of lucerne. This method showed to be effective as far as the yield of seeds was concerned; however, bee populations remained on the same level and did not propagate so that it would be necessary to import them from the Western countries and to pay them in hard currencies. Further, the rearing of leaf cutting bees requires a lot of labour and facilities so that it is relatively complicated.

The third possibility to solve this problem was to use solitary bees of the species Rhophitoides canus Evers. which nests in soil in the close neighbourhood of lucerne fields or even directly in sites with a thin vegetation cover and sufficient amounts of solar

radiation. Trying to propagate gradually these small pollinators directly on fields of farms producing lucerne seed we have elaborated a method considering above all the requirements of bees for nutrition in the proper moment and nesting in the proper site (Ptáček 1984, 1989). This means that although the seed material is produced from the second cut, the nutrition for a part of population of solitary bees R. canus (before the onset of flowering of the second cut) is ensured in the so-called food stripes remaining after the first cut. The period of flowering of the second, i. e. seed-producing cut is then timed in such a way that it coincides with the period of maximum flying activity of solitary bees. In Czechoslovakia, this peak occurs in July. Sites for nesting as well as a uniform distribution of bee population throughout the whole seed stand are provided in the so-called nesting stripes which cross the stand and remain to be relatively without the vegetation cover. In this context it is possible to say that the whole technology of lucerne seed production was principially changed due to our efforts to propagate and protect solitary bees.

These changes showed also a significant effect on the protection of stands against pests. In the past, organophosphates were commonly used in seed stands of lucerne; Metation E 50 was the most frequent preparation which should replace older preparations Intration E 50 and Fosfotion E 50. Metation was mostly used in the dose of 2 $l.ha^{-1}$ in the period prior to flowering of the second (seed-producing) cut, i.e. approximately in the decade of June and the first decade of July. It is well-known that organophosphates are toxic both for bees and warm-blooded animals. Melipax (effective agent camphechlor) was the first preparation which was registred according to our rules as only

harmful for bees; later on it was Thiodan 35 EC with the effective agent endosulfane. Both belonged to the group of chloranated carbohydrates and especially the former was a dangerous poison. These preparations were recommended for the treatment of lucerne stands to the end of flowering as a protective measure against bugs.

Regarding the necessity of protection of pollinating insects living directly in lucerne stands it was necessary to start gradually with the application of preparations showing a lower toxicity as well as to change our approach to chemicals in general. For the time bieng, the system of protection of lucerne seed stands involves the following stages:

(1) Before the flowering of the first cut)i.e. of food stripes)
In this period it is possible to use any preparations because are no pollinating insects in the stand. Solitary bees of the species R. canus are still in their nests. A general ecological point of view may be applied.

(2) Before the flowering of the second cut (i.e. of seed stands)
This is the most problematic period! Solitary bees emerge massively from their soil nests and an improper application of toxic preparations may significantly reduce their numbers within the locality. In this period it is of crucial importance to known the abundance of pests within the stand and to apply the preparations only in such cases when the numbers of some pests (e.g. thrips, bugs, lucerne flower midges) are above the economic threshold. If necessary, only such preparations may be used which are relatively harmless for bees; they should be applied in periods of low activity of pollinating insects (i.e in the morning or in the evening and at lower temperatures).

(3) To the end of flowering of the second cut (i.e. of seed stands)
Int this period, the solitary bees have already their progeny for the next year in their soil nests and their activity in stands decreases very quickly. Although the lucerne stand still flowers, the economically important proportion of seeds is already in the stage of green pods. After the harvest of cereals, pests migrate into lucerne stands. For that reason it is necessary th take away the migratory apiaries and the treatment of stands even with preparations harmful for bees may be performed in the periods of low activity of pollinating insects (evening, early morning, cold weather) to control overpopulated pests. However, from the hygienic points of view and with regard to stationary apiaries existing in the neighbourhood it is more suitable to use preparations relatively harmless for bees also in this period. Regarding the possibility to implement this protective measure in the proper time interval, i.e. immediately after the harvest of cereals, it is necessary to consider the right date of green matter harvest from the first cut!

Trying to reduce as much as possible the negative effects of protection of sead stands against pests, both on pollinating insects and on environment in general, a number of preparations were tested in our institute. In the first place it is possible to mention some other organophosphates which were less harmful for bees, i.e. Actellic 50 EC (active agent pirimiphosmethyl) and Zolone 35 EC (active agent phosalone). The discovery and refinement of pyrethroids provided an opportunity to use their positive properties also for protection of lucerne. They have above all the following advantages: a re-

latively low toxicity for warm-blooded animals, low doses per unit area and (in the case of some preparations) a low toxicity for bees.

At present, only preparations relatively harmless for bees are used in lucerne seed stands; the treatment is always performed after a check of abundance of pests. The following preparations are tested: Vaztak 10 EC (alfa-cypermethrin), Decis 2,5 EC (deltamethrin), Karate 5 EC (lambda-cyhalothrin) and, lately, also Mavrik 2 E (fluvalinate).

During the last decade, we have started to use, in association with technological changes in lucerne seed production, relatively harmless preparations instead of these toxic for bees; also their actual application has been minimalized.

APPENDIX 24

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DEVELOPMENT OF A SEMIFIELD TESTING METHOD FOR THE EVALUATION OF PESTICIDES WITH HOVERFLIES (DIPT., SYRPHIDAE)

Abstract.
While information concerning the effect of pesticides on honeybee is abundant, experiments dealing with the influence of pesticides upon other beneficial insects, especially pollinators, are scarce. After several beneficial insects species, among these also syrphids, in the GFR where included into the official evaluation procedure of toxic effects of pesticides a semifield method for the testing of syrphids has been developed in our laboratory.
It permits the evaluation of the following parameters: hasards to imaginal flies by contact with sprayed leaves, by consumption of contaminated nectar several days after spraying of systemic insecticides, residual effects after 8 days, and influence upon the fecundity of females. About 9 insecticides already tested by this method, the results of "Pirimor", "Tamaron" and "Ripcord 10" are represented.

1. Introduction

Up to now toxicological investigations have preferably been carried out with the honeybee, Apis mellifera, the most important pollinator. After amendment of the plant protection law of the GFR in the thereafter issued Pflanzenschutzmittelverordnung 28.7.1989 § 1,2,2, the obligatory testing of the impact of pesticides has been extended to other beneficial insects. To strengthen the basis for integrated pest control comparable to the testing of the bee-toxicity of pesticides the testing of beneficial insects proceeds in three steps: laboratory - semi-field - field. Concerning syrphids a laboratory test method has been developed for the larvae as well as for the adult stages of the syrphids (RIECKMANN,1989).
A semifield testing method for the verification and completion of the laboratory data has been elaborated at the "Institut für landwirtschaftliche Zoologie und Bienenkunde".
This method is concerned with the evaluation of the oral and contact toxicity-effects on imaginal syrphid flies of Episyrphus balteatus Deg. and also on their fecundity.

2. Habits of test organismen

Next to hymenoptera, syrphids are the most important pollinator group of endemic insects. Adults of all known species feed on pollen, nectar and honeydew of aphids. The preferred plants belong to species of the Umbelliferae. About 40% of the 800 syrphid species known in Central Europe are zoophagous. Consequently, hoverflies are one factor in the integrated pest control. Episyrphus balteatus, one of the syrphid species reared in our institute, as well as Metasyrphus corollae, Syrphus vitripennis, S. ribesii and Sphaerophoria sripta are the species mostly found in the agricultural areas of Central Europe.

2.1. Mass Rearing of syrphids

For testing the side-effects of pesticides on beneficial insects, an efficient mass-rearing method of the predator is required in qualitative and quantitative respect.
The adults of Episyrphus balteatus are kept in gaze- covered wooden cages in environmentally controlled greenhouse-cabins with a photoperiod of L:D = 16:8. The animals are feed with:

- cubes of cristallized sugar
- solution of 80% honey and 20% water, offered on yellow sponges
- water in petridishes with cotton-swaps
- freshly ground pollen, collected by honeybees

For oviposition potted broad beans (Vicia faba) infested with aphids are placed into the rearing-cages. Shortly before hatching of the larvae the eggs (200-250) are distributed to trays with freshly germinated beans under laboratory conditions (20^0 C and rel. humidity of 50-60 %). The larvea are fed with aphids. In our rearing system a mixed population of Aphis fabae, Acyrtosiphon pisum and Megoura viceae proved to be the most useful. Under favourable conditions the development requires 14 to 16 days on the average.

2.2. Requirements for the experiments

2.2.1. Test-plant

It must meet the following requirements:

- it should belong to cultivated plant species
- the anthers and nectar must be easily accessible for syrphids
- the plants should be easy to cultivate, with a relatively short vegetative phase but a long flowering phase
- the leaf area should be sufficiently large for pesticide application

The experiments proved that buckwheat (Fagopyrum esculentum) is especially suitable. Buckwheat is quite easy to grow in crates. The plant will reach the budding stage about 6-8 weeks after planting. It is also very frequently visited by hoverflies in the field. For the pesticide application the size of the leaves is sufficient.

2.2.2. Experimental tents

Each experimental phase requires two tents measuring 1x1x1m. The wodden tent-frames are completely covered with white plastic screen. To facilitate the daily control the tents are placed on tables (50cm high) which are covered with white plastic foil. Due to a zipper at the front side, each tent can easily be managed during experiments. To prevent the escape of syrphids while working in the tents a transportable gaze-tunnel as a sluise is used. Through this the controlling person can enter the tent with head and arms.

2.2.3. Pesticide application

A newly designed device facilitates the simulation of spraying under field conditions (BÜCHLER & DRESCHER, 1989) This apparatus proved to be suitable for spraying buckwheat too.
A spraying nozzle, which can be adjusted in height and drop size, is fastened above guiding rails. An electrically driven sledge, which can be moved back and forth with two different speeds, is placed on these rails underneath the spraying nozzle. Crates, containing plants in the budding stage are put on the sledge and sprayed with concentrations used under normal agri-

cultural conditions. The plants in the two control tents are treated with water. If there are a few open blossoms these are removed first.

2.3. Execution of the pesticide screening

2.3.1. Initial toxicity test

As soon as the sprayed pesticide film has dried up, the buckwheat plants are placed in the middle of the experimental tents (one crate containing 12-15 plants). A container with aphid infested beans is added at about blossom level . Then 16 about four to six dayold imaginal hoverflies (8 females,8 males) are released. In order to guarantee uniform animal material required for the evaluation these animals should be reared under laboratory conditions (same age, similar fitness).
The following parameters are registered daily:

- Number of dead animals (The dead animals are removed from the tents with forceps and the sex is determined)
- The behaviour of the animals (abnormal behaviour, flight-activity, flower visits, visit of the aphid infested beans, recording of oviposition

2.3.2. Residual effects of the pesticide

Eight days after pesticide application the buckwheat plants are in full bloom. At this stage another 16 adult hoverflies per tent of known age and sex are released. Animals which survived the initial toxicity test are first removed from the tents; the aphid infested beans are replaced. The determined parameters of this test corresponds with that of the initial toxicity test of the pesticide. If any eggs have been deposited on the beans, these are replaced by new ones. Bean leaves on which eggs have been laid, are removed from the plant and kept in petri dishes, so the larvae can hatch under laboratory conditions. The number of the hatched larvae is counted after two days.

3.Number of replication

For statistical reasons the experiment is repeated (at least two experimental tents and two control tents) within the same vegetation period. Analogous to the bee-toxicity test a parallel investigation at other laboratories would be desirable.

3.1. Results of the experiments with "Pirimor", "Tamaron" and "Ripcord"

The number of dead animals is registered daily and summed up at the end of the experiment. For calculation of the effect of a pesticide the SCHNEIDER-ORELLI formula is used:

$$WG\% = \frac{b - u}{100 - u} \times 100$$

b= treated
u= untreated

Tab.1: Results of initial toxicity test
Efficiency (WG%) after 24 and 96 hours

Pesticide	active ingredient	Conc.	n	WG% 24	WG% 96
Pirimor	Pirimicarb	0.075	2x2	50.87	35.55
Tamaron	Methamidophos	0.2	2x4	62.92	96.00
Ripcord10	Cypermethrin	0.1	1x6	1.04	38.89

Tab.2: Results of residual toxicity test
eight days after pesticide application

Pesticide	active ingredient	Conc.	n	WG% 24	WG% 96
Pirimor	Pirimicarb	0.075	2x2	18.75	-9.31
Tamaron	Methamidophos	0.2	1x3,1x2	31.25	93.75
Ripcord10	Cypermethrin	0.1	1x6	58.88	78.88

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APPENDIX 25

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RESEARCHES REGARDING THE TOXICITY OF NITRATES AND NITRITES TO HONEYBEES

The toxic action of both nitrates and nitrites is being long known, however, as yet, the matter became significantly actual especially ever since greater quantities were noticed to be present in the vegetal origin food and drinkable water, fact which lead to chronical intoxications. The toxicological implications of these chemical substances became more complex following the pointed cumulative effect of the nitrates and the possibility of nitrozemines formation with cancerigenous effect.

The average content in the vegetal origin products varies between 40 mg NO_3/kg - 3,320 mg/kg. The quantity of 6.700 mg/kg was also present.

The daily admitted dose was fixed to be of OMS to 5 mg/kg body.

The nitrates from food and water while reaching the digestive tube suffer a series of transformations under the action of bacterian enzymes.

Nitrate Nitrite Nitrogen oxid Hydroxilamine Ammonia

The nitrites, compared to the nitrates, have a higher toxicity, the established admitted dose by OMS being of 0.2 mg/kg body.

The toxical effects are also produced through the water consumption that contains a large quantity of nitrates. Water admitted limits in our country are the following ones: nitrates = 40 mg/l; nitrites = 0.40 mg/l.

The studies carried out on various animals have shown that the nitrites diminish the biological effectiveness of food vitamines (vitamine A and B7), as well as the absorption of proteins and lipids.

At the same time, the nitrites may also inhibit the cellulary respiration, and the effect is stronger with a lower pH.

The experiments in animals have also shown mutagenous and teratogeneous actions.

The nitrates and nitrites impurification of nectar, pollen and the water gathered and stored by the honeybees, most frequently lead to the occurence of intoxication states in many honeybee colonies.

To this aim preliminary studies were carried out on the toxicity of nitrates and nitrites to the honeybees, within laboratory conditions.

Working method

- The testing of each nitrate and nitrite was done on groups of 4 cages with honeybees, having 50-70 honeybees/cage, simultaneously with other 4 cages with control honeybees.

- The study was carried out by administrating, through ingestion, sugar syrup in concetration of 1:1, containing either nitrate or nitrite in decreasing dilutions, starting from the concentration of 1 g to 100 ml of sugar syrup.

- The toxical effect on the honeybees was pointed out by counting the dead honeybees at an interval of 24 hours, for a period of 10 days.

- Working temperatures were in average of 26° C.

Results

NO_2^- (nitrite)

- Dilution I (1g to 100 ml syrup)

Total number of honeybees in the experiment = 155 (100%)
Dead and intoxicated honeybees within 240 hours = 155 (100%)
Surviving honeyvees by the end of the experiment = 0

- Dilution II (0.8 g to 100 ml syrup)

Total number of honeybees in the experiment = 236 (100%)
Dead and intoxicated honeybees within 240 hours = 236 (100%)
Surviving honeybees by the end of the experiment = 0

- Dilution III (0.6 g to 100 ml syrup)

Total number of honeybees in the experiment = 182 (100%)
Dead and intoxicated honeybees within 240 hours = 168 (92.8%)
Surviving honeybees by the end of the experiment = 14 (7.69%)

- Dilution IV (0.4 g to 100 ml syrup)
Total number of honeybees in the experiment = 238 (100%)
Dead and intoxicated honeybees within 240 hours = 185 (77.73%)
Surviving honeybees by the end of the experiment = 53 (22.27%)
- Dilution V (0.2 g to 100 ml syrup)
Total number of honeybees in the experiment = 186 (100%)
Dead and intoxicated honeybees within 240 hours = 54 (29.03%)
Surviving honeybees by the end of the experiment = 132 (70.97%)
- Dilution VI (0.1 g to 100 ml syrup)
Total number of honeybees in the experiment = 195 (100%)
Dead and intoxicated honeybees within 240 hours = 24 (13.4%)
Surviving honeybees by the end of the experiment = 162 (83.08%)
- Dilution VII (0.075 g to 100 ml syrup)
Total honeybees in the experiment = 179 (100%)
Dead and intoxicated honeybees within 240 hours = 24 (13.41%)
Surviving honeybees by the end of the experiment = 155 (96.59%)
- Dilution VIII (0.050 g to 100 ml syrup)
Total number of honeybees in the experiment = 149 (100%)
Dead and intoxicated honeybees within 240 hours = 17 (11.41%)
Surviving honeybees by the end of the experiment = 132 (86.59%)
- Dilution IX (0.025 g to 100 ml syrup)
Total number of honeybees in the experiment = 108 (100%)
Dead and intoxicated honeybees within 240 hours = 11 (10.19%)
Surviving honeybees by the end of the experiment = 97 (89.81%)
- Dilution X (0.01 g to 100 ml syrup)
Total number of honeybees in the experiment = 193 (100%)
Dead and intoxicated honeybees within 240 hours = 10 (5.18%)
Surviving honeybees by the end of the experiment = 183 (94.82%)
- Dilution XI (0.001 g to 100 ml syrup)
Total number of honeybees in the experiment = 216 (100%)
Dead and intoxicated honeybees within 240 hours = 7 (3.24%)
Surviving honeybees by the end of the experiment = 209 (96.76%)
- Dilution XII (0.0801 g to 100 ml syrup)
Total number of honeybees in the experiment = 215 (100%)

Dead and intoxicated honeybees within 240 hours = 2 (0.93%)
Surviving honeybees by the end of the experiment = 213 (99.07%)
The control - throughout those 240 hours it showed a mortality of 3 honeybees (1.34%) out of total of 223 (100%)

NO_3^- (nitrate)

- Dilution I (1 g to 100 ml of syrup)
Total number of honeybees in the experiment = 186 (100%)
Dead and intoxicated honeybees within 240 hours=186 (100%)
Surviving honeybees by the end of the experiment = 0
- Dilution II (0.8 g to 100 ml syrup)
Total number of honeybees in the experiment = 217 (100%)
Dead and intoxicated honeybees within 240 hours = 217 (100%)
Surviving honeybees by the end of the experiment = 0
- Dilution III (0.6 g to 100 ml syrup)
Total number of honeybees in the experiment = 112 (100%)
Dead and intoxicated honeybees within 250 hours = 58 (51.8%)
Surviving honeybees by the end of the experiment = 54 (48.2%)
- Dilution IV (0.4 g to 100 ml syrup)
Total number of honeybees in the experiment = 142 (100%)
Dead and intoxicated honeybees within 240 hours = 38 (26.76%)
Surviving honeybees by the end of the experiment=104 (73.24%)
- Dilution V (0.2 g to 100 ml syrup)
Total number of honeybees in the experiment = 163 (100%)
Dead and intoxicated honeybees within 240 hours = 40 (24.24%)
Surviving honeybees by the end of the experiment = 125 (75.76%)
- Dilution VI (0.1 g to 100 ml syrup)
Total number of honeybees in the experiment = 196 (100%)
Dead and intoxicated honeybees within 240 hours = 42 (21.43%)
Surviving honeybees by the end of the experiment = 154 (78.57%)
- Dilution VII (0.075 g to 100 ml syrup)
Total number of honeybees in the experiment = 152 (100%)
Dead and intoxicated honeybees within 240 hours = 18 (11.84%)
Surviving honeybees by the end of the expeiment = 134 (88,16%)

- Dilution VIII (0.050 g to 100 ml syrup)
Total number of honeybees in the experiment = 96 (100%)
Dead and intoxicated honeybees within 240 hours = 10 (10.42%)
Surviving honeybees by the end of the experiment = 86 (89.58%)
- Dilution IX (0.025 g to 100 ml syrup)
Total number of honeybees in the experiment = 177 (100%)
Dead and intoxicated honeybees within 240 hours = 17 (9.60%)
Surviving honeybees by the end of the experiment = 160 (90.40%)
- Dilution X (0.01 g to 100 ml syrup)
Total number of honeybees in the experiment = 188 (100%)
Dead and intoxicated honeybees within 240 hours = 16 (8.51%)
Surviving honeybees by the end of the experiment = 172 (91.49%)
- Dilution XI (0.001 g to 100 ml syrup)
Total number of honeybees in the experiment = 133 (100%)
Dead and intoxicated honeybees within 240 hours 3 (2.25%)
Surviving honeybees by the end of the experiment 130 (97.75%)
The control throug the 240 hours registered a mortality of 3 honeybees (1.34%) out of total of 223 (100%).

We here below present the synthesis of the results obtained:
- The Nitrites - concentration of 1 % causes a mortality of 100 %
DL 50 = 0.286 g%
Maximal tolerable concentration was 0.0001 % = 1 mg/l
- The Nitrates - concentration of 1 % causes a mortality of 100 %
DL 50 = 0.6 g%
Maximal tolerable concentration was 0.001 % = 10 mg/l
The toxicological laboratory researches were carried out and continued with the identification of nitrites and nitrates in the water resources used by honeybees, sugar and honey.

Method

The drawn samples were analysed within laboratory conditions, using the following methods:

- For a qualitative determination of the nitrates in the water samples, we have employed the method with salicylic acid, which gives a yellow colour in the presence of nitrates.

- To point out the presence of nitrates in the sugar and honey samples, we have used the sulphuric solution of difenilamine which renders a blue colour with the nitrates. The method of brucinazation renders a red colour turning to orange, yellow and finally green.

- To point out the nitrites identified in the water, sugar and honey samples, we have employed the method with Griess reactive (sol.1) = sulphanic sold + (sol.2) = alfanaftilamine which gives a red colour.

We have obtained the following results: Nitrates

In the water collected from padles:

Ist apiary - 138 mg/l

IInd apiary - 11.7 mg/l

IIIrd apiary - 1370 mg/l

In the water collected from well:

Ist apiary - 37 mg/l

IInd apiary - 190 mg/l

IIIrd apiary - 98 mg/l

In the sugar administrated as syrup:

Ist apiry - absent

IInd apiary - absent

IIIrd apiary - absent

In the honey from the comb collected from the honeybee colony:

Ist apiary - absent

IInd apiary - absent

IIIrd apiary - absent

The Nitrites

In the water collected from padles:

Ist apiary - absent

IInd apiary - 0.93 mg/l

IIIrd apiary - 3.60 mg/l

In the water collected from well:

Ist apiary - 0.12 mg/l

IInd apiary - 0.14 mg/l

IIIrd apiary - 0.16 mg/l

In the sugar administered as syrup
Ist apiary - 0.17 mg/l
IInd apiary - 0.16 mg/l
IIIrd apiary - 0.25 mg/l
In the honey from the comb collected from the honeybee colony:
Ist apiary - 0.16 mg/l
IInd apiary - 0.17 mg/l
III rd apiary - 0.12 mg/l

GENERAL REMARKS

Making a synthesis of the obtained results, through laboratory experiments, compared to the results of the determinations from the water sources used by the honeybees, results a hight content in nitrates: 3.7 mg - 1370 mg/l, as compared to 10 mg/l, maximal tolerable concentration and in nitrites: 0.93 mg - 3.60 mg/l, as compared to 1 mg/l, maximal tolerable concentration, fact which leads to the occurence of frequent states of severe and chronical intoxications, in honeybees.

SUGGESTIONS

In order to avoid the above mentioned situations, the following suggestions should be considered:

- a depollution of the environment, which is to be done through a general action of rational and limited use of nitrogenous fertilizers, until now used in excess.

- the tolerable industrial noxis should be within the admissible limits.

- a periodical control of the water source used by honeybees and the water used in making the syrup administered in the honeybees food.

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