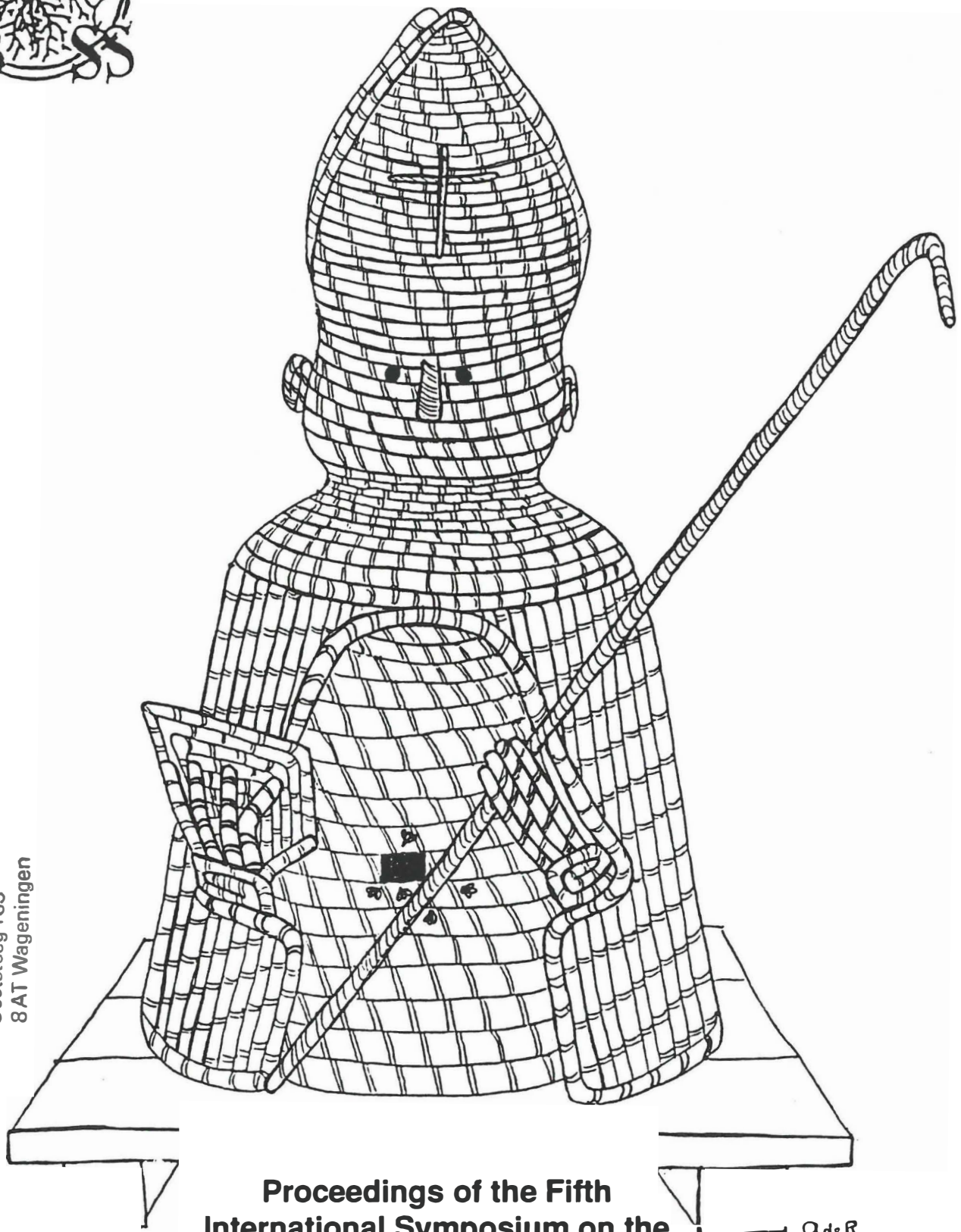

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Proceedings of the Fifth International Symposium on the Hazards of Pesticides to Bees

October 26 - 28, 1993
Plant Protection Service,
Wageningen, The Netherlands

Proceedings of the Fifth International Symposium on the Hazards of Pesticides to Bees October 26 - 28, 1993 Plant Protection Service, Wageningen, The Netherlands

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INTERNATIONAL COMMISSION FOR PLANT-BEE RELATIONSHIPS

AFRC Institute of Arable Crops Research
Rothamsted Experimental Station
Harpenden
Herts, AL5 2JQ
England

I welcome the publication of this report on the Fifth ICPBR Symposium on Hazards of Pesticides to Bees, and I congratulate everyone concerned with the organisation of the meeting and preparation of the report. In particular we are greatly indebted to the Director and staff of the Plant Protection Service in Wageningen for providing all facilities for the meeting and to the following companies for generous financial support:

FMC Europe NV
Sandoz Agro AG
Schering Agrochemicals Ltd
Shell Internationale Mij
Zeneca Agrochemicals Ltd

These meetings provide a forum for detailed discussion of laboratory and field assessment of the hazards to bees of crop protection operations. In doing so they serve to identify problems requiring attention, and most importantly have pioneered the principles of sequential assessment of hazards which are being adopted by the European Plant Protection Organisation and the Council of Europe for pesticide registration. Indeed these principles are likely to be used not only for honeybees but also for other beneficial organisms.

Ingrid H. Williams

Ingrid H Williams PhD
Chairman ICPBR

1. Opening Session

Dr. Stevenson, Chairman of the Symposium opened the meeting expressing thanks to the Plant Protection Service (PPS), Wageningen for hosting the fifth meeting of ICPBR Symposium on pesticides and bees, a return to the venue of the first meeting of this group held in 1980.

Dr. Stevenson was pleased that Dr. Tasei, Secretary of ICPBR, was present, and thanked Dr. Oomen and his colleagues for the excellent organisation. He also thanked Dr. Harrison for agreeing to act as secretary to the meeting.

Dr. Duringhof, Director of PPS Wageningen then welcomed all the "honey bee experts" of ICPBR to the meeting and to the PPS. He noted that there were many people at this meeting who were present at the inaugural meeting in 1980, also held in Wageningen. In particular he mentioned Prof. Besemer who despite retiring some time ago was still making important contributions to discussions on pesticides and honeybees; also Drs. Beetsma and Mr Van der Steen were mentioned , and Ing. Pettinga, who played such a large part in organizing the first meeting was also now enjoying retirement, and the meeting sent greetings to him.

He drew our attention to the new EC registration directive and Uniform Principals which rely on harmonised and Internationally developed guidelines such as the one of EPPO/CoE for the environmental risk assessment of plant protection products to honeybees. In turn EPPO/CoE relies on international groups of experts such as ICPBR in order to formulate and test these methods and guidelines. He recognised the work of the members of ICPBR in contributing to the development of the EPPO/CoE guideline. Through meetings such as this Symposium a harmonisation of ideas occurs which is important. He believes that this group has set a very good example to other such groups developing risk assessment guidelines in other fields.

2. Summary of the meeting by Dr. E.G. Harrison, Shell Internationale Petroleum Maatschappij Den Haag.

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

2.1 The European Plant Protection Organisation and Council of Europe (EPPO/CoE) Risk Assessment Scheme.

2.2 Special tests and methodology:

- - Growth regulators
- - Honeybees in cereals
- - Synergism
- - Varroacides

2.3 Guideline development.

2.4 National Schemes to investigate reported poisoning incidents.

2.5 Work with other bee/pollinator species.

2.1 The EPPO/Council of Europe Risk Assessment Scheme.

Dr. Oomen gave a paper on the EPPO/CoE Risk Assessment Scheme its history, need for implementation and improvement of the guidelines (**Appendix 2**).

The Honeybee Risk Assessment guideline is one of 11 guidelines under development by the EPPO/CoE joint panel on Environmental Risk Assessment and was the first to be finished as part of this scheme. It has therefore been used as an example for the development of guidelines in other areas. The aim of these guidelines is to have reliable, scientifically sound and internationally applicable risk assessment procedures acceptable to all European Countries.

The guidelines are designed for use by Government Regulatory authorities and Industry alike.

Dr. Oomen described the complicated development process of an EPPO/CoE guideline. The guideline must pass through numerous working parties, committees etc. before reaching acceptance. However this process ensures that a truly harmonised and mutually acceptable guideline results.

Copies of the Honey Bees guidelines;

- Guideline on test methods for evaluating the side-effects of plant protection products on honeybees.
- Decision-making scheme for the environmental risk assessment of plant protection products.
- Method for honeybee brood feeding tests with insect growth-regulating insecticides.

were given to delegates and are reproduced in **Appendix 22**.

The ICPBR group on pesticides and bees has provided much of the detailed methodology referred to in the EPPO/CoE honeybee guideline, through discussions and in symposiums such as this one decisions have been made and mutually acceptable methods developed.

Dr. Oomen drew our attention to some areas where the guideline needed further development or improvements. These included a bee brood feeding test, tests for residual activity, and repetition of tests.

Considerable discussion followed this presentation particularly on the relevance of a persistence test. This discussion focused on whether foliage was appropriate for such a test since bees spend very little time on foliage; the need for bees in the test method or could the chemically measured persistence of residues on foliage/flowers be enough to predict effects. This subject was discussed in more detail later on in the Symposium and is summarised in Section 3.

Other comments made were that the group should follow developments in the United States. The European Government National Regulation Authorities already have links with the U.S. and this should be the route for monitoring developments.

Dr. Oomen asked for comments on the EPPO/CoE guideline to be sent to him.

Dr. Lewis' presentation was on the use of dimethoate as a toxic standard in laboratory acute toxicity tests with honeybees from 1981-1992 (**Appendix 3**).

The EPPO guideline suggests that a a toxic standard should be used. Dimethoate is one of the chemicals recommended. The Zeneca Research Laboratory at Jealotts Hill, England has over 12 years of experience with dimethoate as the toxic standard for this test. Dimethoate was chosen because of its ready solubility in both water and organic solvents, its high toxicity, similar LD_{50} results for both contact and oral tests, low variation and constancy between strains.

At Jealotts Hill there is a very good data base of test results (over 62 contact and oral toxicity tests) with dimethoate as the toxic standard. Over the years, many different bee colonies from different commercial suppliers have been used. The test methods are those described in EPPO guideline 170. Results from tests were analysed for variability of response and constancy of results.

No significant trends were identified between or within years and it was concluded that the results with dimethoate are consistent and in good agreement with published data from other sources.

These results confirm that dimethoate is a suitable toxic standard.

Dr. Lewis made two concluding remarks, he first suggested that these results could form part of a much larger data base including the results from other laboratories in order to further validate the EPPO guideline.

He also stressed the importance of the use of a toxic standard to verify the methodology and the "health" of the bees used. In doing this he drew our attention to the difference between a toxic standard (used to validate the test) and a reference compound (which is used to compare the effects of the test chemical with a chemical of known or similar properties).

A question was asked about the number of dose rates used for the toxic standard in these tests. Dr. Lewis replied that with such a good and consistent data base they were now able to use only 3 dose rates; the predicted LD_{50} and two others one a factor of 2 higher and one a factor of 2 lower than the predicted LD_{50}. However he stressed that it is important to have validated the toxic standard before it is possible to do this.

Mr Van der Steen gave a presentation on the implementation of the EPPO guidelines in an oral LD_{50} study in honeybees (**Appendix 4**). During this presentation he drew our attention to areas of the guideline where the instructions are vague and where decisions are needed to ensure consistency of methodology between laboratories. He highlighted the problem of humidity; the EPPO guideline suggests a humidity range of 60-70%. In his experience this range is too limiting and should be extended.

The preparation of the bees for tests was also discussed especially the issue of the use of a starvation period. The amount of food that a bee has consumed just before testing could affect the amount of dose taken during an oral test. It was proposed that bees should be kept in a flight cage without food for a period of 1-2 hours before testing in order to allow the bees to share food (tropholactic feeding) the bees will then be more equal in terms of the food contents of their guts and should therefore give less variable results in terms of food acceptance.

He also presented some proposals for the trial design;

(i) 30 bees should be used divided equally into 3 test cages.

(ii) 6 concentrations of test substance should be used, 3 above and 3 below the predicted LC_{50}.

(iii) There should be 3 replicates of each test.

Some of these issues and proposals were discussed in more detail during the concluding session of the meeting and are summarised in Section 3, below.

Dr Lewis asked whether variation between different hives was observed? Mr Van der Steen confirmed that little variation was seen.

Prof. Mrs Arzone proposed the use of candy as an alternative to sucrose solution.

Ms. Aldridge made a presentation on the validation in the U.K. of the EPPO/CoE risk assessment guideline for honeybees (**Appendix 5**). This work was a collaboration between the U.K. Pesticide Safety Directorate (PSD) and the Central Science Laboratory of the U.K. Ministry of Agriculture Fisheries and Food (MAFF). The analysis used data from laboratory tests submitted as part of regulatory packages to PSD and data from the MAFF Pesticide Monitoring Scheme. A total of 20 pesticide/crop combinations were analysed using the EPPO/CoE risk assessment guideline. The results of the risk assessments were then compared with the same pesticide/crop combinations and the number of bee incidents recorded.

Her conclusions were;

(i) that the EPPO scheme accurately identifies high risk products.

(ii) that the hazard ratio alone provides a good indicator of risk with a good margin of safety - it may even be possible to refine this ratio; a proposal was made to increase the low risk hazard ratio from > 50 to > 100 or even > 500 and to reduce the high risk-hazard ratio to < 1000.

(iii) some potential improvements were suggested including a need for better information on residual toxicity and information on the attractiveness of different crops to honeybees.

Finally it was concluded that monitoring data is a very useful tool for validating risk assessment.

It is intended to publish this paper in full in the SETAC journal.

2.2 Special tests and methodology

Developments in test methodology since the last meeting of the ICPBR were presented.

- Growth regulators

Prof. Mrs Arzone described methods developed for testing and evaluating the action of insect growth regulators on honeybees (**Appendix 6**). She showed us a comprehensive series of slides illustrating the effects of fenoxycarb on honeybee pupae and adults and warned us of confusion with the effects seen after Varroa infestations.

Dr. Czoppelt described the results of a comparative study examining the effects of fenoxycarb and pyriproxyfen on post-embryonic development of honeybees (**Appendix 7**). A standard larval bioassay dose not exist. Historically larvicidal activity is tested in acute tests for insecticidal activity, in this paper Dr. Czoppelt presented a bioassay which examines effects on honeybee development.

Mr. Calis described the development of a new test method for evaluating the effects of pesticides on honeybee larvae (**Appendix 8**). This test was designed so that the worker honeybees bring the dosed food to the larvae in order to ensure exposure. The method is still not established because of high control mortality and lack of reproducibility. However, once these problems have been overcome this test method should be useful for examining the effects of pesticides, particularly IGRs, on honeybee broods.

- Honeybees in cereals

Dr. Lewis presented a paper comparing the effects of two formulations of a pyrethroid insecticide, lambda-cyhalothrin (Karate), on honeybees foraging on simulated honeydew on winter wheat (**Appendix 9**).

He described some of the hypotheses of pyrethroid repellency and hoped to solve some of the outstanding questions on this subject using this experiment. It was concluded that lambdacyhalothrin inhibited foraging for up to 3 days. No treatment-related effects were seen on the bee broods. The two formulations of lambdacyhalothrin both had similar effects on the honeybees. The data supports the theory of contact repellancy "a sub-lethal toxic insult" of the bees prevents foraging. This effect is reversible and the bees recover in the hives. The rate of recovery will be dependent on the level of exposure and other conditions such as weather, temperature etc. Dr. Lewis commented that under certain environmental conditions Phosalone is not an effective reference product. The inclusion of a toxic standard such as dimethoate or azinphos-methyl would be more useful.

- Synergism

Dr. Tasei presented a paper on behalf of Dr. Belzunces on a biological method for demonstrating synergism between pesticides in bees. Effects of pyrethroid insecticides and azole fungicides applied at sub lethal doses were presented (**Appendix 10**). A laboratory bioassay was described where groups of anaesthetised bees were treated using a spray tower. Different combinations of the insecticides and fungicides were used in combination or sequentially. The control treatments were water, the insecticide alone and the fungicide alone. The conclusions were that synergism was demonstrated and that honeybees may be at risk when mixtures of certain pesticides are used or if they are sprayed sequentially within a short period of time.

A question was asked about recommendations to growers on how to deal with this problem. In France a number of companies have carried out experiments and informed the farmers not to mix particular products. In the U.K. and Germany there is no regulation of tank mixtures.

Unfortunately Dr. Mayer could not attend the meeting. A summary of his paper on the effects on the adjuvant Sylgard on the hazard of selected insecticides to honeybees is given in **Appendix 11**.

Mr. Pilling gave a presentation on the synergistic effects of EBI fungicides and a pyrethroid insecticide (lambda-cyhalothrin) in the honeybee **(Appendix 12)**. Initially laboratory studies were carried out followed by residue studies using flight cages. Synergism was demonstrated under both conditions. Using the flight cages the time scale of the effects could be examined, and was found to last up to 80 hours.

Field tests were carried out using a tunnel design. It was demonstrated that only the bees foraging at the time of application were affected, since the pyrethroid reduces the foraging activity the mortality is reduced.

The mechanism for the synergistic effect was elegantly described using computer aided modelling techniques. The Cytochrome P450 mixed function oxidase system was implicated in the expression of this synergistic effect, suggesting that the fungicide inhibits detoxification of the pyrethroid.

- Varroacides

Prof. Dr. Dustmann presented a paper on the synergistic action of the varroacide Perizin with other organophosphorous pesticides **(Appendix 13)**. Laboratory studies demonstrated that bees pre-treated with Perizin are more sensitive to organophosphorous insecticides such as dimethoate. This sensitivity lasted for up to 17 days after the Perizin treatment. This effect was also demonstrated in semi-field trials. Some hypotheses were presented on why this increased effect occurs.

This paper raised some interesting questions on how to describe a healthy colony in terms of the requirements for toxicity testing by Registration Authorities. Nowadays many colonies are treated for Varroa and in some cases this treatment may be prolonged using slow release chemical treatments.

2.3 Guideline Development.

Ir. Brouwer described the EC requirements for risk assessment and the EPPO scheme, a summary of his presentation and copies of the OHP slides are given in **Appendix 14**.

Ir. Zweep initiated a discussion on the EPPO guideline challenging the group to advise her on replication of laboratory, cage field and tunnel tests. In her job at the PPS, Wageningen she has to advice chemical Companies and Regulatory Authorities and she doesn't feel as though she has enough information to do this.

The questions posed by Ir. Zweep and some further questions that arose during the meeting will be documented in the summary of the final discussion Section 3.

• Discussion

Drs Stevenson and Oomen conducted a discussion session on recommendations for new or improved testing methods for use in the risk assessment scheme.

Dr Mühlen suggested that for field trials the plots must be separated by at least 4 km otherwise the bees will be foraging over more than one of the treated areas.

He also raised a concern over the use of toxic reference compounds in these trials since other non-target insects such as bumble bees could be affected. Dr. Besemer reiterated this concern and suggested that good consideration should go into the need for a toxic reference compound during the design phase of a field trial.

Drs Lewis and Stevenson both defended the field trial guideline saying that there was data to demonstrate that the minimum distances stated were adequate and that the guideline had been designed to accommodate the experiences of many different workers. These distances should be regarded as the minimum requirement.

Dr. Brasse suggested that part of the problem is one of linguistics, that the German word for guideline is "Richtlinie" and that this implies a much stricter interpretation than the English word "guideline".

Further discussions in this session were led by Ir. Brouwer and based on some questions that arose out of his presentation on the new EC requirements for testing plant protection products.

Clarification was needed on whether testing of bees refers to all bees or to honeybees alone. In the group there was a general feeling of concern for other species of bee and in particular bumble bees. However the group agreed that the EC document refers to honeybees and the section on beneficial organisms refers to natural enemies of insect pests.

Methods are under development to test the effects of chemicals on bumble bees. It was proposed that guidelines are needed for risk assessment of bees other than honeybees.

The question of whether dimethoate is the most appropriate toxic standard was discussed in the final session, Section 3 below.

Dr. Lewis reiterated his earlier comments that it is important to distinguish between "toxic standard" and "toxic reference". A toxic standard is used to validate the exposure of the test organisms during a test. A toxic reference chemical is used to compare the effects of the test chemical with one of known toxicity. Dr. Lewis recommended that the phrasing in the EC documents should read e.g. dimethoate as a toxic standard.

The bee brood feeding test was discussed - a proposal was made to form a sub-committee within the ICPBR to further develop this test and to discuss a laboratory larval feeding test. See Section 3 below.

2.4 National Schemes to investigate reported poisoning incidents.

In this session four papers were presented illustrating the different European National Governments incident reporting schemes.

Dr. Brasse presented investigations on poisoning incidents on honeybee colonies in Germany (**Appendix 15**). These investigations are funded by the German Government. The monitoring of bee poisoning has helped to identify a number of issues including;

- The use of carbaryl in vineyards was identified as a hazard to bees. It had been assumed that honeybees do not forage in vineyards because of the low availability of pollen. However, when bees have no other choice they may forage in orchards and can be affected by the carbaryl treatments. Thus in 1981 the use of carbaryl products in German orchards was banned and the number of bee incidents subsequently reduced.
- Deliberate poisonings comprise up to 30% of the incidents recorded.
- Prosecutions can be made but they need to be backed up by good chemical analysis.

Dr. Stark reported work on monitoring bee mortality in Sweden (**Appendix 16**). This work included the monitoring of bee incidents since 1946 and a prolonged experiment (5 years) monitoring 5 apiaries in different parts of Sweden. The understanding of bee poisoning incidents is very complicated and can be affected by many factors including the use of varroacides, local land-use such as the military and industrial activity and also local weather conditions.

In Sweden the use of pesticides has reduced by 70% since 1985 and this long-term monitoring scheme has shown that it is not only pesticides that are implicated in the poisoning of bees in Sweden.

Dr. Oomen reported a signalling scheme for poisoning incidents in the Netherlands (**Appendix 17**). This is not a true monitoring scheme but voluntary reports by bee keepers that may "signal" a problem. It was set up in 1990. The reports are not verified by chemical analysis. The scheme is not quantitative but may help to identify errors in the registration of chemical or suggest areas where risk management is needed e.g.;

In 1990, 2 out of 19 reported cases suggested that MCPA (registered as safe for bees) was the cause of the poisoning incidents.

In 1991, 13 out of 40 cases signalled problems for bees after aphid control in potatoes. The cause of these problems was due to a large presence of the flowering weed *Polygonum persicaria* in the crop which was attracting foraging honeybees.

These signalling data are useful but the quality of the data is not as good as a true monitoring scheme.

Dr. Stevenson presented a paper by Dr. Fletcher on a scheme to investigate the suspected poisoning of honeybees by agricultural chemicals in England and Wales (**Appendix 18**).

This scheme is run by the U.K. Ministry for Agriculture Fisheries and Foods and is run alongside similar schemes for domestic animals, wild birds and mammals. The scheme has three purposes;

- post-registration surveillance
- enforcement of legislation
- validation/improvement of risk assessment schemes.

This scheme is supported by chemical analysis. If a chemical is implicated it normally falls into one of the following categories;

- deliberate abuse
- misuse
- approved use
- unspecified use

This monitoring scheme is regarded as a very valuable exercise by all who use it, and Dr. Stevenson hoped that some way would be found to incorporate evidence from it into the U.K. Pesticide Registration Scheme.

Ms. Aldridge pointed out that there have been suspected problems of under reporting of incidents in the UK. The threat of prosecution of farmers for misuse/abuse of chemicals and the fact that the bee keepers do not want to sour their relationship with the farmers - they need to use the crops and are worried that the farmers may prevent them- was thought to be a significant deterrent.

A paper supplied by Dr. Tasei is reproduced in French in **Appendix 19** giving details of the French Scheme monitoring for bee poisoning incidents.

The main conclusions from this session were that National Monitoring Schemes are extremely useful. The reporting of incidents is important in the understanding of the real exposure of bees to pesticides. This can depend on many factors including the timing and use of the chemical and the behaviour of the bees as well as many factors that may be unique to the local environment.

2.5 Work with other bee/pollinator species.

Mr Gretenkord described the development of a cage-trial method for assessing pesticide effects on laboratory reared bumble bees, *Bombus terrestris* (**Appendix 20**). Foraging activity and mortality can be assessed in the cages. The natural mortality in these cages is low. Metasystox was used as a test chemical. Mortality was high for a number of days after treatment. Effects on the brood were also measured using a photographic record. Further work is ongoing to develop methods for examining effects on brood and also on larvae.

Dr. Tasei described work carried out to examine the sub lethal effects of deltamethrin on bumble bees (**Appendix 21**). One of his conclusions was that the sub-lethal exposure of workers modifies their food consumption, this may have implications especially for the queens.

3. Discussion and Recommendations of the Fifth Meeting

Dr. Stevenson made a number of proposals about the future of the ICPBR Working Group on the Hazards of Pesticides to Bees. It was decided, unanimously, that the group should continue with its discussion and work. The next meeting will be held in about 2 years time in Braunschweig at the BBA, hosted by Dr. Brasse. Dr. Stevenson will continue as Chairman of the group, however as he is due to retire from Rothamsted later this year he suggested that Dr. Oomen could support him as Vice Chairman. This was agreed.

Action Drs Stevenson, Oomen and Brasse.

The final session was a discussion led by Dr. Harrison. A number of unanswered questions were identified from previous discussions;

1. Should we study the relation between the effects of pesticides on Bumble bees and honeybees?

It was proposed that a group should meet to discuss this question and would develop a laboratory method including larval/brood feeding. This group will be;

Mrs. Schaefer
Mr. Van der Steen
Mr. Gretenkord
Dr. Tasei

This group will be co-ordinated by Mr Van der Steen who will report their findings at the next meeting.
Action Mr Van der Steen

2. Tests on larval honeybees.

This was discussed earlier see section 2.3 - Guideline Development. A group was proposed to discuss the development of both a larval test with honeybees and a bee brood feeding test. The group is;

Al wat briefjes geschreven
Ziek geweest, longontsteking

Mr de Ruijter
Dr Czoppelt
Mr Calis
Mr Tornier
Dr Dustmann
Mr Colin (contact via Mr Tasei)

This group will be co-ordinated by Mr de Ruijter who will report their findings at the next meeting.
Action Mr de Ruijter

3. Should the group be considering residue tests?

There was much discussion throughout the meeting about the value of residue tests. Questions to consider include; will exposure of the bees occur on the plant? if so then where?, flowers or foliage? Also the residue pattern and fate could be different on different parts of the plant due to growth factors.

Another consideration should be the value of the information on residues in terms of risk management. For example if the chemical in question is known to be very toxic e.g. if the Hazard Quotient, HQ > 2500 and the residual effect is < 8hrs then to reduce the risk to foraging honeybees the chemical could be applied in the late evening.

It was proposed that a group should correspond on the value of residue testing and how to test it.. This group will comprise;

Dr Oomen
Dr. Lewis
Dr. Schmidt
Dr. Forster
Dr. Brasse
Mr. Van der Steen

Mr Oomen will co-ordinate this group which will report back at the next meeting.
Action Dr Oomen

4. A proposal was made to discuss the different National Monitoring Schemes. A group was proposed consisting of ;

Dr. Oomen (NL)
Dr. Stark (S)
Dr. Fletcher (U.K.)
Brasse (D)

This group will be co-ordinated by Dr. Brasse and will report their findings at the next meeting.
Action Dr. Brasse

A number of questions remained unanswered from the presentation of Mr Van der Steen;

1. What should be the relative humidity range in the EPPO laboratory test? The working group decided that it is not necessary to specify this range, but that the relative humidity during the test should be recorded.
Action Dr. Oomen. This change should be incorporated into the next update of the EPPO method.

2. In field tests which chemical should be used as a toxic standard? A proposal was made and agreed by the group that the same wording used in the EPPO laboratory test method should be used i.e. "..reference product (toxic standard) known to present a high hazard to bees (e.g. parathion, dimethoate)." It was then mentioned that a positive control in field tests is sometimes not possible, or has nearly prohibitive consequences because of risk to operators and non-target arthropods. For those circumstances it was agreed that a toxic standard was not necessary, provided that exposure was convincingly demonstrated, e.g. by evidence based on observations such as:

- counting foraging bees before and after application
- collecting pollen
- marking bees in the field etc.

Action Dr. Oomen and Ir. Brouwer to convey information to EPPO/CoE and EEC respectively.

3. Nowadays many bee colonies are treated with varroacides. This raises the question of how "healthy" these bees are for use in laboratory toxicity tests? A proposal was made and agreed by the group that if a varroacide has been used then the treatment should be identified and the timing recorded. A second proposal agreed by the group was that a minimum time lag of 4 weeks after the end of treatment should be left before the bees are used for tests.
Action Dr. Oomen. This change should be incorporated into the next update of the EPPO/CoE method.

There was some further discussion of the questions arising from the presentation of Ir. Brouwer. on the EC documents Annexes II and III (the sections of these annexes relevant to honeybees are reproduced in **Appendix 25**).

Is Dimethoate the most appropriate toxic standard? The group agreed that Ir. Brouwer should recommend to the commission the wording "e.g. Dimethoate as a toxic standard".
Action Ir. Brouwer.

Is it relevant to record the highest dose causing no mortality? It was agreed that Ir. Brouwer should convey the decision of this group to the EC that it is not appropriate to measure this parameter in the EPPO laboratory test method.
Action Ir. Brouwer.

In risk assessment guidelines for other chemicals e.g. the European Community Commission Directive 93/67/EEC of 20 July 1993, "laying down the principles for assessment of risks to man and environment of substances notified in accordance with Council Directive 67/548/EEC", the "actual" exposure is estimated to calculate the hazard ratio and is called the Predicted Environmental Concentration (PEC). In the honeybee scheme the maximum recommended application rate of the active ingredient (or dose rate) is used.

Is there a need for adoption of the EEC 93/67/EEC Directive at this point? The group agreed that the use of maximum recommended application rate (dose) is relevant and appropriate to the honeybee risk assessment scheme.

The group agreed that in the laboratory tests proposed for Annexes II and III control mortality should not exceed 15%.

The group agreed that in the cage, tunnel and field tests the minimum number of pre-treatment assessments should be 2.

These Recommendations were presented by Ir. Brouwer to the EEC after the meeting (**Appendix 23**).

Finally the group returned to the presentation of Ir. Zweep on the number of tests that should be carried out. There was much discussion on both the number of laboratory and field tests required.

Experience of researchers using the EPPO methodology is that there is very little variation, over seasons, years or even races of honeybees (c.f. Dr. Lewis' paper **Appendix 3**), and that LD_{50} values for contact and oral laboratory tests are remarkably constant.

Some of the concern was over the degree of variability experienced in the German laboratories. Dr. Mühlen agreed to collect evidence on the level of variation in LD_{50} values in laboratory tests and whether this could be related to variation due to different races of honeybees.

A paper prepared by Dr. Mühlen after the symposium is given in **Appendix 24**.

Three proposals were made;

1. A single "good" laboratory test should be sufficient. The test method must include a toxic standard to verify exposure and "quality" of the colony.

In general the group agreed with this proposal however there were some reservations particularly from Dr. Forster and members of the Ambrosiushoeve Institute. So a second proposal was made;

2. Two laboratory tests should be carried out if the subsequent HQ is close to 50 then a third test should be performed.

3. A third proposal was also made. At least one laboratory test should be carried out, if the HQ is > 25 <100 then a second test should be performed.

These proposals need to be resolved and it was agreed that the EPPO/CoE sub-group on honeybees would do so.
Action Dr Oomen and EPPO/CoE honeybee sub-group.

During the discussion on the number of laboratory tests the group also discussed replication in laboratory tests. There was some discussion about what actually represented a "true replicate". A proposal was made that unless the test solutions for each of the three replicates at each dose rate in the oral test are prepared separately then there is not true replication. Drs Harrison and Lewis agreed to discuss this issue with Statisticians at Shell Research Ltd. and Zeneca's Jealotts Hill Research Station respectively.

They reported after the meeting that the Statisticians from both Shell Research and Zeneca agreed that if the test solution was made up separately three times then an extra degree of variation is introduced to the test design. Each of the three solutions will vary slightly from one another and so true replication of the test can not be claimed. It is much better to **make up the test solution once** and then divide it between the three cages, this would not be regarded as pseudo-replication. Both statisticians were happy with the current test design as described in EPPO guideline 170.

The number of field tests required was also debated. A proposal was put forward that one field trial per country/climatic region, if the test design is good, should be sufficient. For tunnel trials and cage tests then a minimum of 2 tests was proposed. These proposals will be put forward to the EPPO/CoE sub group on honeybees.
Action Dr Oomen and EPPO/CoE honeybee sub-group.

Finally the meeting was closed by Dr. Stevenson, he thanked Mr Oomen and Mrs Ackerman for all their hard work in hosting the meeting, Dr. Harrison for her role as secretary and the participants of the meeting for their excellent presentations and lively discussions.

The next meeting will be hosted by Dr. Brasse at the BBA in Braunschweig. The date of this meeting will be announced to the participants of the Wageningen Symposium, and anyone else who informs Dr. Oomen of his interest.
Action All.

Appendix 1

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Appendix 2

P.A. Oomen: The EPPO/CoE Risk Assessment Scheme: history, need for implementation, and improvements of the guidelines.

ICPBR - Symposium
Hazards of Pesticides to Bees
Wageningen, 26-28 October 1993

Pieter A. Oomen
Plant Protection Service, Wageningen, The Netherlands
version 24/10/93

The EPPO/CoE Risk Assessment Scheme: history, need for implementation, and improvements of the guidelines.

Decision Making Scheme

The European and Mediterranean Plant Protection Organisation (EPPO) has developed jointly with the Council of Europe (CoE) a 'Scheme for Environmental Risk Assessment'. The scheme includes a 'Decision Making Scheme on Honey Bees', besides several environmental issues. The scheme has been developed by the EPPO/CoE Panel on Environmental Risk Assessment with an eye on offering the European Community (EC) a feasible and scientifically sound approach for environmental risk assessment for pesticide registration purposes. The joint Panel has developed the assessment scheme for use by chemical producers and by regulatory authorities that aims to:

1. guide assessors on the questions that should be addressed and on the data that may need to be requested from registrants;
2. provide information on test methods and approaches that are suitable in each case;
3. indicate how the data should be interpreted in a consistent manner, involving expert judgement where appropriate;
4. produce a reliable assessment of environmental risk that is suitable to aid risk management, although it will not provide all the information necessary for decisions about the acceptability of plant protection products.

Official publication

The first scheme finished and accepted by the Panel was the one on honey bees - setting an example to all other schemes. It was published as an official EPPO/CoE scheme, after extensive consultations in the (1) Honey Bee Subgroup, (2) the Panel, (3) the EPPO Working Party, (4) the EPPO countries, and (5) the EPPO Council. A comparable procedure was followed within the CoE. The official honey bee scheme was officially published as chapter 10 of the scheme (OEPP/EPPO, 1993).

Information/test requirements

The scheme requires well specified information to evaluate. For collecting suitable information it refers to published test methods that are preferrably internationally well accepted. So reference is made to EPPO Guideline 170 for the methodology for testing the LD50 in the laboratory, and for cage, field and tunnel tests. It further refers to some specific other test methods, bee brood feeding test and duration of residue activity on foliage.

ICPBR recommendations

The EPPO guideline 170 is mainly based on recommendations of the ICPBR working group, made in its previous symposia (Wageningen 1980, Hohenheim 1982, Harpenden 1985 and Rez 1990). The EPPO/CoE honey bee Subgroup and the Panel are eager to receive further recommendations by the ICPBR Symposium for:

1. improvement and completion of existing test guidelines, and
2. development of some new tests to further implement the scheme.

Recommendations for improving and completing test methods may come in particular from experts with experience with these tests, from assessors who met practical problems in interpreting and decision making, and from experts involved in validation exercises with the scheme. New tests need to be developed by scientists involved in honey bee research. All these are represented at this symposium. The EPPO/CoE Subgroup will take up the recommendations and newly developed guidelines in future updates of the scheme.

Problems identified

At this stage several specific problems can be identified which can be addressed by this meeting:

1. The bee brood feeding test (Oomen, De Ruijter, Van der Steen, 1992) has been published provisionally but needs a review by ICPBR. One of the questions: what is a proper dosage for the reference substance (fenoxycarb, diflubenzuron)?
2. The test on duration of residue activity on foliage (announced as Gerig & Oomen, 1993 but non-existing) needs to be developed.
3. The LD50 laboratory tests uses a reference substance (e.g. dimethoate) in parallel. Needs the reference be applied in several concentrations in order to determine the LD50, or would a single toxic concentration be sufficient? If so, what concentration of dimethoate or parathion would do? [Questions from testing institute]
4. The numbers of each test to be required for decision making reliable enough for registration and risk management (cf. contribution by mrs Annet Zweep).

Suggestions for research

In the area of risk assessment of Insect Growth Regulators to honey bee brood, laboratory screening for IGR-effects possibly can be considerably improved. In order to find threshold values for laboratory toxicity data it is suggested to carry out the following research on the relations between:

1. Field dosage of IGR's in different crops
2. Toxicity of IGR's to bee brood (e.g. as oral LD50 for brood)
3. Level of harmful effects of pesticides used in these crops
4. Modifying factors, e.g. weather, brood development, stage of flowering

Bumble bees and other pollinators are now coming into picture for risk assessment and risk management. In place of developing a new approach for testing, assessing and evaluating bumble bees it seems much more efficient to develop a 'translation' from honey bees to bumble bees. Data enabling development of such 'translation' are scarce. Most needed are LD50 data of bumble bees, and recordings of (absence of) hazard after well defined exposure to pesticides in the field.

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Appendix 3

G.B. Lewis, H.J. Gough and E.C. McIndoe: The use of dimethoate as a reference compound in laboratory acute toxicity tests in honeybees (*Apis mellifera* L.) 1981-92.

THE USE OF DIMETHOATE AS A REFERENCE COMPOUND IN LABORATORY ACUTE TOXICITY TESTS IN HONEY BEES (*APIS MELLIFERA* L.) 1981 - 92

G B Lewis, H J Gough and E C McIndoe.
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SUMMARY

At the 1st International Commission for Plant-Bee Relationships (ICPBR - formerly the International Commission for Bee Botany) Symposium at Wageningen in 1980 it was agreed that a reference compound should be used in laboratory acute toxicity tests on honey bees (*Apis mellifera L.*), the choice of compound being left to individual laboratories. Compounds suggested, and already in use by some workers, included lindane (γ-HCH), parathion and dimethoate.

Technical dimethoate was considered for use by Zeneca Agrochemicals at Jealott's Hill as a representative organosphosphate insecticide, having the advantage of being readily soluble in water and organic solvents (Worthing, 1979). It is highly toxic to bees, and has similar oral and contact LD_{50} values (Stevenson *et al*, 1978). In a series of tests carried out at Rothampsted Experimental Station over three years the mean LD_{50} showed less than 10% variation and its order of toxicity to honey bees relative to other pesticides did not change (Stevenson, 1968a). Also, five different strains of honey bees were affected to a similar extent by the compound (Stevenson, 1968b).

Initial tests at Jealott's Hill showed dimethoate to give consistent results with no obvious seasonal pattern. In cooperation with the International Commission for Plant-Bee Relationships the toxicity of dimethoate and of ethyl- parathion to worker honey bees were compared during the 1983 season. Dimethoate was found to be more consistent than parathion and was confirmed as the reference material for use at Jealott's Hill (Wilkinson *et al,* 1985).

Dimethoate has thus been used as a reference compound at Jealott's Hill since 1981 in all laboratory toxicity tests with honey bees. Results are presented for contact and oral acute toxicity tests determined between May and October for twelve consecutive years, 1981-1992 (63 contact tests and 62 oral tests), using standard laboratory methods. Twenty-four hour LD_{50} values ranged from contact 0.11 to 0.26 with a mean value of 0.16 µg ai bee (active ingredient) bee^{-1}; oral - 0.11 to 0.33 with a mean value of 0.18 µg ai bee^{-1}. There were no obvious seasonal trends in contact or oral toxicity nor were there any marked trends over the twelve year period. The 48 hour LD_{50} values were similar to the 24 hour ones indicating that there were no delayed toxic effects.

This low variability is confirmed by the published LD_{50} values for dimethoate from studies carried out elsewhere in the UK and also in Germany. Stevenson *et al* (1978), using the method on which the Jealott's Hill version is based, obtained slightly lower values (contact 0.12 and oral 0.15 µg ai bee^{-1}). Beran (1969, 1970), quoted by Glofke with additional comments (1976), and by Fiedler (1987), obtained a contact LD_{50} of 0.16 and an oral value of 0.08 µg ai bee^{-1}. His method differs in that for the contact test he applied 1 µl drops of dimethoate to the tarsi, rather than to the thorax, and for the oral tests bees were fed individually. Ali *et al* (1973), obtained a 24 hour contact LD_{50} of 0.07 µg bee^{-1}. Their method differs in that they used technical dimethoate in 1 µl drops of acetone applied to the thorax of 1 - 2 day old bees and kept them at 30°C. A summary in graphical form only, of the Jealott's Hill data for 1981-1984 was given in Wilkinson *et* al (1985). Fiedler (1987), gives the range for the 25 hour acute oral LD_{50} value for bee as approximately 0.08-0.17 µg bee^{-1}. Drescher (1991), obtained an oral LD_{50} value of 0.17 µg bee^{-1}. These results differ from the Jealott's Hill mean values by up to a factor of about 2 only.

The results presented confirm the suitability of dimethoate as a toxic standard in these laboratory toxicity tests. Additionally, 0.5% and 99.5% percentiles are estimated, representing 99% confidence limits for an individual test result. These can be used as the criteria for what is an acceptable

result: an individual laboratory toxicity test conducted with Jealott's Hill colonies can be validated if the LD_{50} value obtained with dimethoate falls within the range defined by the 0.5% and 99.5% percentiles. Results outside this range would indicate that the bees were not responding normally and that the test should be repeated. This validation could be extended if comparable results were pooled from a number of different laboratories/countries. It is envisaged that the acceptance criteria (0.5% and 99.5% percentiles) produced in this way would be similar to those obtained with the Jealott's Hill data presented here.

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Appendix 4

J.J.M. Van der Steen The implementation of the EPPO guideline in an oral LD_{50} study on honeybees.

ICPBR BEE PROTECTION GROUP

Hazards of Pesticides to Bees

Wageningen, The Netherlands
26-28 October 1993

Title of the paper

The implementation of the EPPO guideline in an oral LD_{50} study on honey bees.

Name of author, Institute and country

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Summary

The implementation of the EPPO guideline in an oral LD_{50} study on honey bees is described. The relative air humidity in the trial conditions, the collecting of the bees, the way the starvation period before feeding is arranged, the number of bees per test group, the number of concentrations to obtain a suitable range to provide a regression line and a LD_{50} and the number of replicates are discussed.

Introduction

In the "Guideline on test methods for evaluating the side-effects of plant protection products on honeybees", EPPO bulletin 22 (1992), the principles of an oral LD_{50} test is described. The oral LD_{50} is an important value for the decision making scheme and must therefore be determined precisely. During the preparation and carrying out of this test choices must be made: like how many bees are taken per test group, how many replicates must be done and how many concentrations must be fed to the bees to have a suitable range to provide a regression line and a LD_{50}. Besides that, some prescriptions are not practical to be carried out, like the relative humidity during the test period and the starvation period. In this paper, our implementation of this EPPO guideline will be discussed and summarized.

Trial conditions The relative humidity in a bee colony is, under normal conditions, 40% to 50%. This percentage can rise up to 70% depending on the season and the activities of the bees (Simpson 1961). The guideline prescribes storage conditions with a relative humidity of 60% to 70%. These values are hard to maintain in a

climate room. We believe the prescribed range is too limited and suggest to prescribe 40% to 80%.

Preparation of the bees The guideline prescribes the use of preferably uniform young adult worker bees. The term young bees is not defined. Newly born bees are grey and easy to collect but not suitable for test purposes. In practice it is hardly possible to collect uniform young bees (up to 2 - 3 weeks) directly from a colony because these bees are found in the broodnest doing activities generally correlated to their age and are therefore not uniform. A certain way to have uniform young adult bees is to let bees emerge in an incubator and rear them in there for one week with pollen and sucrose-solution. A practical way to collect uniform bees is to take them from the frames without brood. These bees however are not young.

Starvation period We have the experience that, when groups of 10 bees are put in a test cage directly after collection from the frame, the period in which the bees are willing to take the sucrose-solution with or without test substance is very variable. This is probably due to the fact that bees are put together with different amounts of honey in their stomach. To obtain groups of 10 bees per cage, that are about equally willing to take the sucrose-solution, we place all collected bees together (at least 300 individuals) in a flight cage for 1 - 2 hours. The bees share the available food by trophallaxis. After this starvation period the bees are uniformly hungry and are put in the test cages (10 bees per test cage) and fed by group feeding. Our experience is that these bees take all the 100 mm^3 per test cage within the same period of some hours.

Design of the trial According to the guideline, groups of at least 10 bees must be used. We use groups of 30 bees, equally divided over 3 test cages. Each group is fed with a certain concentration of the test substance. Each concentration is fed to 3 groups (3 replicates). For each replicate, bees from different hives are taken. According to the guideline a number of concentrations should be used to provide a regression line and LD_{50}. Per replicate we feed 6 concentrations of the test substance to the bees after having determined the presumable LD_{50} in a range finding test (4 concentrations of the test substance). For the suitable range to provide a regression line and LD_{50} we feed 3 concentrations between the presumable LD_{100} and LD_{50} and 3 concentrations between the presumable LD_{50} and LD_0.

Our implementation of the EPPO guideline in an oral LD_{50} study in honey bees is summarized as follows:

- At least 300 bees are taken from the outer frames without brood from the hive and put directly into a flight cage (50 x 40 x 40 cm) deprived of food for a maximum of 2 hours.
- After the starvation period test cages are filled with 10 bees each. The test cages are placed in a climate room at a temperature of 25 $\pm$ 2°C and a relative humidity between 40% and 80%. The test cages are provided with the test solution (test substance in sucrose solution 50%). During feeding, the test solution bees are kept in the light. After the bees have taken the test solutions, they are kept

in the dark. The controls are done in red light to prevent disturbance.
- One test group consists of 3 test cages.
- Each test cage is provided with 100 mm^3 test solution for a maximum of 3 hours.
- The test solution is offered via a glass pipette. The amount of test solution taken is determind by reweighing the pipettes.
- As soon as the bees have taken the test substance or after 3 hours they are pro vided with sucrose solution.
- Mortality is recorded after 24 hours and after 48 hours or longer if mortality is still increasing.
- Mortality percentages per group are corrected for control mortality with the formula of Schneider Orelli: % mortality = $\{(b-k)/(100-k)\} \times 100$.
  b = % of individuals dead in treatment
  (concentration test substance or Parathion).
  k = % of individuals dead in negative control.
- Per replicate 6 concentrations of the test substance, 3 concentrations of Parathion (positive control) and sucrose solution 50% (negative control) are provided.
- Each study consists of 3 replicates with bees from different hives.
- The mortality values are used to provide a regression line and LD_{50}.

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<u>RELATIVE HUMIDITY</u>	
EPPO GUIDELINE	60% to 70%
PROPOSAL	40% to 80%

<u>PREPARATION OF THE BEES</u>	
EPPO GUIDELINE	PREFERABLY UNIFORM YOUNG ADULT WORKER BEES
AMBROSIUSHOEVE IMPLEMENTATION	BEES FROM FRAMES WITHOUT BROOD OR BEES EMERGED IN AN INCUBATOR AND REARED FOR ONE WEEK WITH POLLEN AND SUCROSE-SOLUTION

<u>STARVATION PERIOD</u>	
EPPO GUIDELINE	UP TO 2 HOURS
AMBROSIUSHOEVE IMPLEMENTATION	ALL COLLECTED BEES IN A FLIGHT CAGE FOR 1 TO 2 HOURS AFTER STARVATION PERIOD BEES IN TEST-CAGES (10 BEES/TESTCAGE)

DESIGN OF THE TRIAL

EPPO GUIDELINE	
TEST UNIT	AT LEAST 10 BEES
AMBROSIUSHOEVE IMPLEMENTATION	
TEST UNIT	30 BEES DIVIDED EQUALLY OVER 3 TESTCAGES
EPPO GUIDELINE	
CONCENTRATIONS	A NUMBER OF CONCENTRATIONS
AMBROSIUSHOEVE IMPLEMENTATION	
CONCENTRATIONS	6 CONCENTRATIONS OF THE TEST SUBSTANCE
EPPO GUIDELINE	
REPLICATES	3 REPLICATES OF EACH CONCENTRATION
AMBROSIUSHOEVE IMPLEMENTATION	
REPLICATES	3 REPLICATES WITH BEES FROM DIFFERENT HIVES

SUMMARY OF AN LD_{50} STUDY

- AT LEAST 300 BEES ARE TAKEN FROM FRAMES WITHOUT BROOD AND PLACED IN A FLIGHT CAGE DEPRIVED FROM FOOD FOR A MAXIMUM OF 2 HOURS
  AFTER THE STARVATION PERIOD TEST CAGES ARE FILLED WITH 10 BEES EACH AND PLACED IN A CLIMATE ROOM (25 $\pm$ 2°C AND RH 40% to 80%)
  ONE TEST GROUP CONSISTS OF 3 TEST CAGES
  EACH TEST CAGE IS PROVIDED DIRECTLY WITH 100 µl TEST SOLUTION (TEST SUBSTANCE IN SUCROSE-SOLUTION 50%) VIA A GLASS PIPETTE
  DURING FEEDING BEES ARE KEPT IN THE LIGHT
  THE FEEDING PERIOD IS MAXIMUM 3 HOURS
  THE AMOUNT TAKEN IS DETERMINED BY REWEIGHING THE PIPETTES
  AFTER THE BEES HAVE TAKEN THE TEST SOLUTION, BEES ARE KEPT IN THE DARK. OBSERVATION ARE DONE WITH RED LIGHT
  MORTALITY IS RECORDED AFTER 24 HOURS AND 48 HOURS OR LONGER IF MORTALITY IS INCREASING
  MORTALITY PERCENTAGES ARE CORRECTED FOR CONTROL MORTALITY
  PER REPLICATE 6 TEST GROUPS ARE FED WITH 6 CONCENTRATIONS OF THE TEST SUBSTANCE, 3 TEST GROUPS ARE PROVIDED WITH 3 CONCENTRATIONS OF PARATHION AND 1 TEST GROUP IS PROVIDED WITH SUCROSE-SOLUTION
  EACH STUDY CONSISTS OF 3 REPLICATES WITH BEES FROM DIFFERENT HIVES
  THE MORTALITY VALUES ARE USED TO PROVIDE A REGRESSION LINE AND LD_{50}

Appendix 5

C.A. Aldridge and A.D.M. Hart: Validation of the EPPO/CoE Risk Assessment scheme for honeybees.

VALIDATION OF THE EPPO/CoE RISK ASSESSMENT SCHEME FOR HONEYBEES

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Summary

A scheme for assessing pesticide risks to honeybees, described by P Oomen elsewhere in this volume, has recently been published by the European and Mediterranean Plant Protection Organisation (EPPO) and the Council of Europe (CoE). This paper presents the results of a study aimed at validating the scheme, by comparing predicted risks with data on actual effects.

The scheme was used to assess the risks to honeybees from a selection of pesticide sprays currently approved in the UK, using data from laboratory and field studies. The risk outcomes were then compared with independent data on the actual frequency of poisoning incidents.

The scheme successfully identified as 'High Risk' those pesticides which have most frequently been implicated in poisoning incidents. It was found that attractiveness of the crop to bees was an important factor in determining risk. It was also shown that by adjusting the criteria used for the initial classification of high and low risk pesticides the amount of data required could be reduced. The study thus confirmed the reliability of the scheme, and identified ways in which it might be further improved.

Objectives of the study

The first objective was to apply the EPPO/CoE risk assessment scheme (see P Oomen) for honeybees to pesticide/crop combinations that have been approved in the UK for several years and to determine a risk outcome for each of these. Then to validate these results by comparison with monitoring data so as to test the accuracy of the risk classifications.

As a result of this study it was hoped that we could optimise the assessment criteria to see if accurate predictions could be obtained more efficiently. Also it was possible that we would identify additional risk factors to further improve accuracy.

Application of the scheme to approved products

The risk assessment scheme produces a risk classification of either 'Low Risk', 'Medium Risk' or 'High Risk'. A key step in the scheme is the estimation of the 'Hazard Ratio', which gives an estimate of millions of LD50s/ha:

$$\text{Hazard Ratio} = \frac{\text{Application rate (g/ha)}}{\text{LD50 (}\mu\text{g/bee)}}$$

It is used as a preliminary screening to decide which pesticides need further testing. Where the Hazard Ratio is greater than 2,500 a pesticide use can be classified as of High Risk to honeybees, where it is less than 50 the pesticide use will be classified as Low Risk, without further testing, unless the product is an insect growth regulator. Where the Hazard Ratio is between 50 and 2,500, further testing will be needed to determine the risk.

The scheme was applied to 20 pesticide/crop combinations currently approved in the UK, using data previously submitted by companies on PSD files. Table 1 shows what type of data was critical in determining the risk outcome.

Table 1: Determinants of the results of assessments

Assessment results	Data leading to result	Number of products
High Risk	Hazard Ratio	7
High Risk	Special tests	6
Low/Medium Risk	Field trials	4
Low Risk	Hazard Ratio	1
Unclassified	Insufficient data	2

There are several uses where the Hazard Ratio alone was insufficient to classify the risk, either because the ratio was between 50 and 2500, or because the product was systemic and required special tests. In 2 cases there were no data beyond laboratory tests and therefore the risk could not be conclusively classified.

Using data submitted in the past to apply the scheme we found that data were not always of sufficient qualilty as they did not always conform to current guidelines. Residual toxicity was rarely measured, field trials were often inappropriate or lacked a toxic standard treatment. Satisfactory operation of the scheme in future will require data to modern standards.

The risk outcomes for the pesticide/crop combinations were as follows:-

HIGH RISK:	Oilseed rape:	triazophos, dimethoate, gamma-HCH
	Field bean:	triazophos, dimethoate
	Cereals:	dimethoate
	Pea:	triazophos, dimethoate
	Carrot:	triazophos
	Orchards:	chlorpyrifos, dimethoate
	Blackcurrant:	dimethoate
	Raspberry	fenitrothion
MEDIUM RISK:	Oilseed rape:	cypermethrin
LOW RISK:	Oilseed rape:	phosalone, endosulfan, deltamethrin, prochloraz
INSUFFICIENT DATA:	Field bean:	demeton-S-methyl
	Potato:	demeton-S-methyl

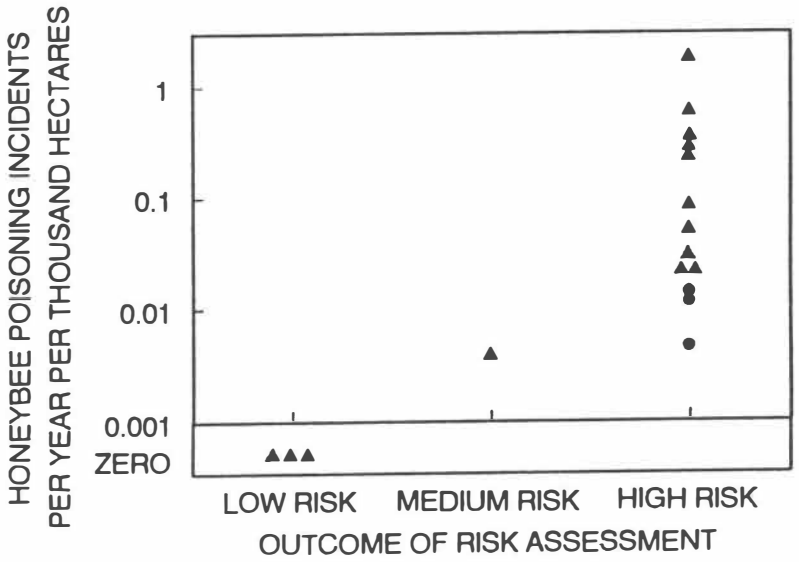
The next stage was to test the accuracy of these predictions using independent data on actual risk/effects.

Validation of risk outcomes

The scheme for reporting bee poisoning incidents in the UK is described in this volume by J Stevenson. For the validation, bee incidents in England and Wales from the period 1983 - 1990 were used. A total of 753 incidents were reported in this period of which 403 could be attributed to pesticide. Those incidents (24) where residues of more than one pesticide were detected were discounted as it could not be certain which pesticide caused the incident. A further 235 incidents were discounted as the crop was not identified and the risk assessments had been crop specific. This left 144 incidents involving one pesticide and where the crop treated was identified for use in validation.

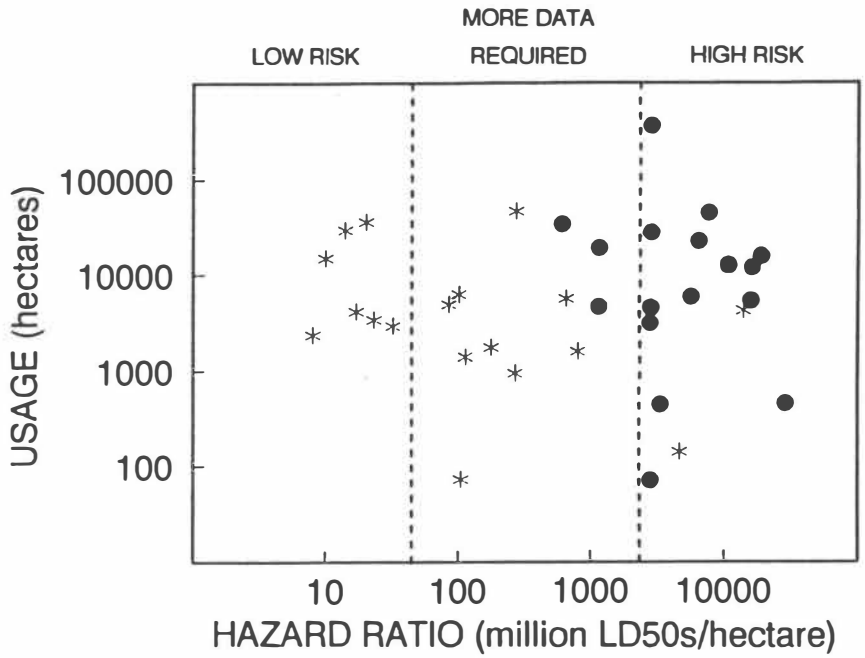
Figure 1 shows honeybee incidents relative to risk outcomes, with incidents expressed as incidents/year/million ha to allow comparison between pesticides used widely and those with a lower spray hectarage. It is clear that some pesticide/crop combinations classified as High Risk have a similar level of incidents to the Medium Risk pesticide. These are for crops which are less attractive to bees, such as wheat, peas and potatoes, marked with a circle in Figure 1. Crop attractiveness should perhaps be a factor in determining risk to differentiate between High Risk uses which could cause many incidents, and Medium Risk uses causing fewer due to the lower attractiveness of the crop.

Figure 1: Honeybee poisoning incidents versus risk outcomes.



The risk asssessment scheme currently uses Hazard Ratios between 50 and 2,500 to trigger a requirement for further data, such as cage/tunnel tests or field studies. Figure 2 shows that based on the experience of validation it may be possible to refine these triggers, such that data are required on fewer occasions. A change to Hazard Ratios of 100 - 1000 to trigger further data would not alter the accuracy of the predictions here, but efficiency is improved because extra testing is required for fewer products. The lower trigger could possibly be raised to 500, depending on how conservatively the scheme was to be applied. It would be necessary to take account of experience with the scheme in other countries before making such changes.

Figure 2: Hazard Ratio in relation to honeybee poisoning incidents.



Conclusions

The scheme successfully identified as 'High Risk' those pesticides which have most frequently been implicated in poisoning incidents. Pesticides identified as Low Risk have not been implicated in poisoning incidents. The Hazard Ratio alone provided a good indicator in many cases of the risk outcome. Detailed analysis showed that within the High Risk category, the frequency of poisoning incidents was lower for pesticides used on crops which are less attractive to honeybees. The scheme could be modified to take more account of this factor, to provide a more graded classification of risk. It was also shown that by adjusting the criteria used for the initial classification of high and low risk pesticides the number of cases in which cage and field studies were required could be reduced, without reducing the accuracy of the risk classifications. The study thus confirmed the reliability of the scheme, and identified ways in which it might be further improved. It also demonstrated the usefulness of monitoring data in validation of risk assessment schemes.

A fuller account of this validation study is to be submitted in the near future to the journal 'Ecotoxicology' for publication.

Appendix 6

A. Arzone, M. Dolci and F. Marletto: Methods for testing and evaluating the action of growth regulators on honeybees.

METHODS FOR TESTING AND EVALUATING THE ACTION OF GROWTH REGULATORS ON HONEY BEES[1]

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Summary

After the introduction of Insegar (AI fenoxycarb) in the agricultural practice, hives were treated in different times with different methods and doses of the new IGR, with the aim to ascertain its action on honey bees: by spraying the water solution of Insegar on the comb, by immission of mixtures of fenoxycarb with pollen or candy inside the hive, by putting fenoxycarb inside cells with pollen. Anomalous specimens were thrown out from all treated colonies. Expulsion began one-two weeks after treatments and continued during two-three months. The particular anomalies of pupae, adultoids and adults of workers and drones are described. Fenoxycarb showed a slow and prolonged action and predisposed the hives to the attacks of European foul brood and sac brood.

Introduction

Our studies on the action of JHA (Juvenile Hormone Analogues) began in 1978 within an *ad hoc* programme of the Italian National Researches Council on new phytodrugs and new phytoregulators. According with the methods adopted by the above mentioned programme, new molecules were synthetized and tested by contact on worker pupae of *Apis mellifera ligustica* Spin., not older than 48 hours, in separate comb portions. After uncapping the cells, 1 µl of acetonic solution containing either 200 µg, 20 µg, 2 µg or 0.2 µg of each active ingredient (AI) was put on the head of pupae. Sesamex, 5-{1-[2-(2-ethoxyethoxy)ethoxy]ethoxy}-1,3-benzodioxole, was used in comparison. The control was composed of two groups of pupae: one was treated with 1 µl of acetone and the other was only uncapped. The experiment was carried out in a conditioned chamber with a temperature of 35°C and a relative humidity of 75%.

The juvenilizing action was evaluated adopting the following activity classes:

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[1] Studies of the C.N.R. coordinate research Unit for Integrated control of Plant pests: 319.

0 - normal adult
1 - adultoid not completely free from pupal exuvia
2 - adultoid with wings completely enclosed in the pupal exuvia
3 - adultoid with badly-formed and sometimes not well distended antennae
4 - not emerged pupa.

The activity degree of the products at the different doses was calculated multiplying the number of specimens by the activity class and dividing the total by the number of treated pupae. The products were classified highly, markedly, moderately, and slightly active when their activity degree was between 3.1 and 4, 2.1 and 3, 1.1 and 2, 0.1 and 1, respectively. The effect of the treatments was checked two days after the emergence of the controls (Marletto & Dolci, 1980).

The introduction in the agricultural practice of Insegar, AI fenoxycarb (ethyl 2-(4-phenoxyphenoxy)ethylcarbamate), besides the missed cocooning of silkworms (Cappellozza *et al.*, 1990), caused worrying pathologic manifestations on the bee brood. This phenomenon induced us to test the action of this IGR (Insect Growth Regulator) directly on the brood with the aim to point out the morphological alterations in honey bees, because such alterations may be confused with those caused by infestations of *Varroa jacobsoni* Oud. (Marcangeli *et al.*, 1992).

Materials and methods

Preliminary tests to check the symptoms of the action of fenoxycarb were carried out in September employing a special hive with five combs: three brood combs and two honey and pollen combs on the sides. One side of a brood comb bearing, besides pollen and honey, all the preimaginal instars was treated with a manual sprayer containing 10 ml of a water solution with 200 mg of the commercial product Insegar, corresponding to 50 mg of fenoxycarb.

In order to find out the best administration modes simulating the natural introduction of IGR into the hive, the subsequent tests were made in different periods of the year with very strong families that were managed for honey production.

At mid July, 10 g of fresh pollen, in which 1 mg of fenoxycarb dissolved in acetone was dispersed in the most homogenous way, were pressed inside empty cells of a brood comb after evaporation of the solvent. In another hive, 150 g of candy, in which 1 mg of fenoxycarb dissolved in acetone was dispersed accurately, were introduced with a feeder after evaporation of the solvent.

Further tests were made in autumn, spring and summer introducing 10 µg of fenoxycarb contained in 1 µl of

acetonic solution inside pollen cells by means of a Hamilton microsyringe.

In autumn 10 mg of fenoxycarb were introduced into 1,000 cells of a hive; in spring 2.5 mg, 5.0 mg, 7.5 mg were introduced into 250, 500, 750 cells, respectively, of three hives; in summer 2.5 mg, 5.0 mg, 7.5 mg and 10.0 mg of fenoxycarb were introduced into 250, 500, 750, 1,000 cells, respectively, of 12 hives, three for each dose.

Treated and control hives were provided with Gary traps to collect anomalous and dead specimens expelled from the colony.

The experimentation started four years ago and is still in progress.

Results

The anomalous specimens collected in the Gary traps showed the following symptoms:

- worker and drone adults with poorly sclerified integuments and wings loose on the abdomen or with badly-formed or not completely distended wings, sometimes with a smaller body size
- worker adultoids with pigmented head and thorax, brown eyes, sometimes with a white or pinkish semilunar stripe, poorly pigmented legs, variously pigmented abdomen, wings enclosed in the pupal exuvia, abdomen shortened and dorso-ventrally depressed; drone adultoids with pigmented head and thorax, reddish eyes with or without whitish stripe or brown, poorly pigmented legs, variously pigmented abdomen, wings enclosed in the pupal exuvia, abdomen rarely depressed, sometimes smaller
- worker pupae with non pigmented integuments, eyes not pigmented, reddish or brown with a white semilunar stripe of a variable width on the inner margin; drone pupae with partially pigmented head and thorax, non pigmented abdomen, reddish eyes with or without a white semilunar stripe on the inner margin.

In the hive in which a comb side was treated in September with a water solution of Insegar, the first anomalous specimens appeared after one week; mortality continued until the exhaustion of the brood. In February, when egg laying started again, the anomalies re-appeared and the colony decreased progressively. In March only one thousand workers remained with evident symptoms of European foul brood and sac brood and the colony was eliminated.

About 1/3 of the pollen was thrown out by workers within six hours from the hive in which 10 g of pollen containing 1 mg of fenoxycarb were pressed into the cells. The expulsion of worker pupae with non pigmented integuments and of anomalous adults began nine days later and continued until the end of October.

The expulsion of anomalous specimens from the hive, in which 1 mg of fenoxycarb was mixed with candy, took place in the first half of October. These two colonies, weakened by European foul brood and sac brood, were eliminated in May the year after the treatment.

Expulsions began 13 days later in the hive treated in autumn and continued until the exhaustion of the brood at the end of October. The colony was eliminated in the following May for the attacks of European foul brood and sac brood.

The hives, which received 2.5 mg and 5.0 mg of fenoxycarb introduced in 250 and 500 cells in spring, had dead specimens in the capped cells ten days later and showed clear symptoms of European foul brood and sac brood 23 days later; so they were immediately eliminated. From the hive treated with 7.5 mg of fenoxycarb, expulsions of anomalous specimens began eight days later and continued for over three months. This hive did not show further anomalies.

Concerning the hives having cells treated with fenoxycarb in summer, expulsions of badly-formed specimens began ten days after treatments and continued until mid October, when the brood exhausted normally. The expulsions of badly-formed specimens, among which a non negligible number of pupae with a white semilunar stripe on the eyes, appeared again in spring and continued until end June.

In control hives the expulsion of dead specimens always remained normal and no specimens showed the anomalies caused by the treatment with fenoxycarb.

Conclusions

With the methods employed to check the effects of IGR on honey bees it was possible to put in evidence that fenoxycarb is active on the preimaginal instars of honey bees at all tested doses independently of application modalities.

In many cases honey bee mortality was ascertained in different phases of the pupal instar, often with the peculiar white or pinkish semilunar stripes in connection with the eyes, besides a smaller development of the abdomen.

Anomalies involved a high number of adults which showed unexpanded wings or poorly sclerified integument. Generally the expulsion of anomalous specimens began in the second week after the treatment and continued intensively for two-three months.

With the exception of one hive, all the hives that were treated in autumn and spring, *i.e.* in critical periods for the life of the family, underwent the attacks of opportunistic pathogenic agents, especially the European

foul brood bacteria and the sac brood virus, and had to be eliminated.

Also the summer treated hives appeared weakened by the action of fenoxycarb at the beginning, but subsequently they could recover owing to the autumn broods, overwinter and develop regularly in the following spring. However, the expulsions of anomalous specimens continued until the end of June even if at a lower rate.

Although the juvenilizing activity of fenoxycarb is markedly higher than that of other IGR, the number of beekeepers who report damages caused to hives by this AI is very low. The lack of such reports may be justified by the convergence of the symptoms produced by fenoxycarb with those of heavy attacks of the mite *V. jacobsoni*.

Also the development of opportunistic brood diseases and the fact that strong colonies may be only partially enfeebled by the initial mortalities help to hide the serious damages caused to hives by the slow but prolonged action of fenoxycarb (de Ruijter & van der Steen, 1987; Gerig, 1991).

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Appendix 7

Ch. Czoppelt: Effect of fenoxycarb and pyriproxifen on post-embryonic development of honeybees, *Apis mellifera* L. Evaluation of toxicity by an in vitro test.

EFFECT OF FENOXYCARB AND PYRIPROXYFEN ON POST-EMBRYONIC DEVELOPMENT OF HONEY BEES, APIS MELLIFERA L. EVALUATION OF TOXICITY BY AN IN VITRO TEST

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SUMMARY

Growth regulating effects of fenoxycarb (FEN) and pyriproxyfen (PYR) during honey bee development were studied with an in vitro bioassay. First instar larvae were continuously reared up to 5th instar in artificial cells on a semisynthetic diet (SF) into which the chemicals FEN and PYR were mixed. Larval growth and pupation were not affected at all. Toxic effects of both juvenoids were found during metamorphosis only. They strongly interfered with morphogenesis resulting in specific symptoms of malformations and high mortality in late pupae. The LC_{50} of FEN was with 0.03 µg/ml SF lower by one order of magnitude than that of PYR with 0.34 µg/ml SF.

INTRODUCTION

Evaluation of pesticide toxicity to honey bees is usually carried out in a bioassay with adults (ICPBR, 1982). 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 regulators on honey bee larvae has also been presented (Rembold & Czoppelt, 1982).
Within the scope of studies on the effect of growth regulating agents on honey bee development, two synthetic compounds, the juvenoids fenoxycarb (FEN) and pyriproxyfen (PYR), were studied. FEN is mainly used as an insecticide in integrated plant protection. It showed high toxicity to honey bee metamorphosis (Czoppelt, 1991) and affected the juvenile hormone III titer in bee larvae if fed in vitro (Czoppelt & Rembold, 1992). PYR is mostly applied as novel control agent against diverse pest insects. The effect on honey bee larvae is unknown.
For evaluation of larvicidal and growth disrupting effects, a standardized bioassay was used (Czoppelt & Rembold, 1988). It is based on the in vitro rearing of first instar larvae under controlled conditions (Rembold & Lackner, 1981).

MATERIAL AND METHODS

First instar larvae (L1, body weight 0.2 - 0.4 mg) were collected from colonies of Apis mellifera on the area of the Max-Planck-Institute and continuously reared up to fifth instar (L5) on a semiartificial diet (SF) which consisted of a mixture of royal jelly (Seip, 1990/91), sugar solution (mixture of fructose and

glucose) and yeast extract (DIFCO). The larvae were kept in an incubator with controlled climatic conditions (35 °C; 90 ± 5 % RH)(Rembold et al., 1974, 1981).
The chemicals fenoxycarb (Maag) and pyriproxyfen (Sumitomo) were diluted in methanol and mixed into the diet for oral administration (feeding poison test).
L1 were transferred into artificial cells (thimbles) which contained SF (0.2 ml/thimble/L1). 24 h later the same larvae, thirty per test and dilution, now as L2, again were transposed but into new thimbles with SF to which various amounts of the chemical (0 - 1 µg/ml SF) had been added. Control larvae were kept separately from the treated larvae to prevent contamination by fumigation. Larval weight gain was measured every 24 h up to L5. Larval stages were characterized by individual weight and measurement of head-capsule width. Eye color and changing of body color were used for characterization of pupal stage (Rembold et al., 1980).

RESULTS AND CONCLUSIONS

Permanent feeding of larvae with various amounts of FEN (0.001 - 1.0 µg/ml SF) and PYR (0.01 - 1.0 µg/ml SF) did not display any dissimilarities in growth if compared with control larvae. The growth-rates of untreated and treated larvae were almost identical (Fig. 1 and 2). Larval fitnes to moult and complete development was not affected at all: pupation was successful in both treatments without any visible indication of a possible contamination.

However, toxic effects of both juvenoids were found during metamorphosis only (Table 1).
Mortality of pupae and pharate adults clearly depended on the applied dose of both juvenoids. Even feeding of 0.5 µg FEN/ml SF and 1.0 µg PYR/ml SF, resp., resulted in a pupal death rate of 100 %.
Both juvenoids strongly interfered with mophogenesis (Table 2). Increasing amounts of FEN and PYR created some specific symptoms of malformations in pupae which appeared either as severe alterations of the compact eye tissue (sickle-shape anomaly), as stunted wings in newly hatched adults or as pupal death.

The sublethal concentration (LC_{50}) of FEN compared with that of PYR was with 0.03 µg/ml SF lower by one order of magnitude than that of 0.34 µg/ml SF for PYR.

With the feeding poison test a standard procedure for screening toxicity of pesticides to honey bee larvae is now available. By use of this <u>in vitro</u> bioassay it is possible to simulate the brood nest and to exclude all the influences which arise from the nurse bees (Czoppelt & Rembold, 1988). This standardized bioassay allows to follow larval growth and larvicidal effects of chemical agents as well as to observe completion of larval and pupal development, morphogenesis and adult emergence.

The doses of FEN and PYR applied to pest insects in field trials were comparatively in almost the same and lower range, resp., than those in the bioassay. Nevertheless, it is supposed that toxic amounts for adult bees at regular application conditions in field trials will not harm brood 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).

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FIG. 1

FENOXYCARB - FEEDING-POISON-TEST

EFFECT ON LARVAL GROWTH

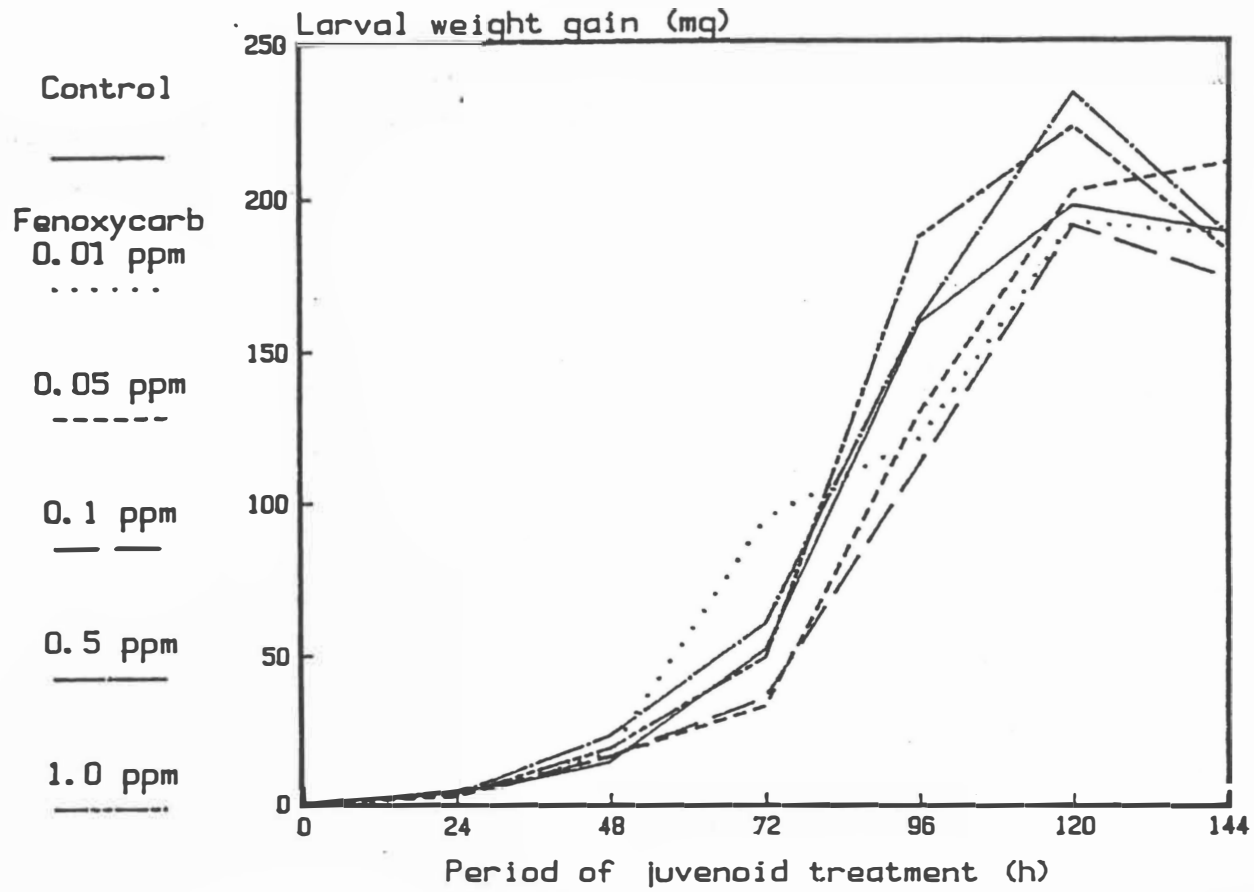

FIG. 2

PYRIPROXYFEN/FEEDING-POISON-TEST

EFFECT ON LARVAL GROWTH

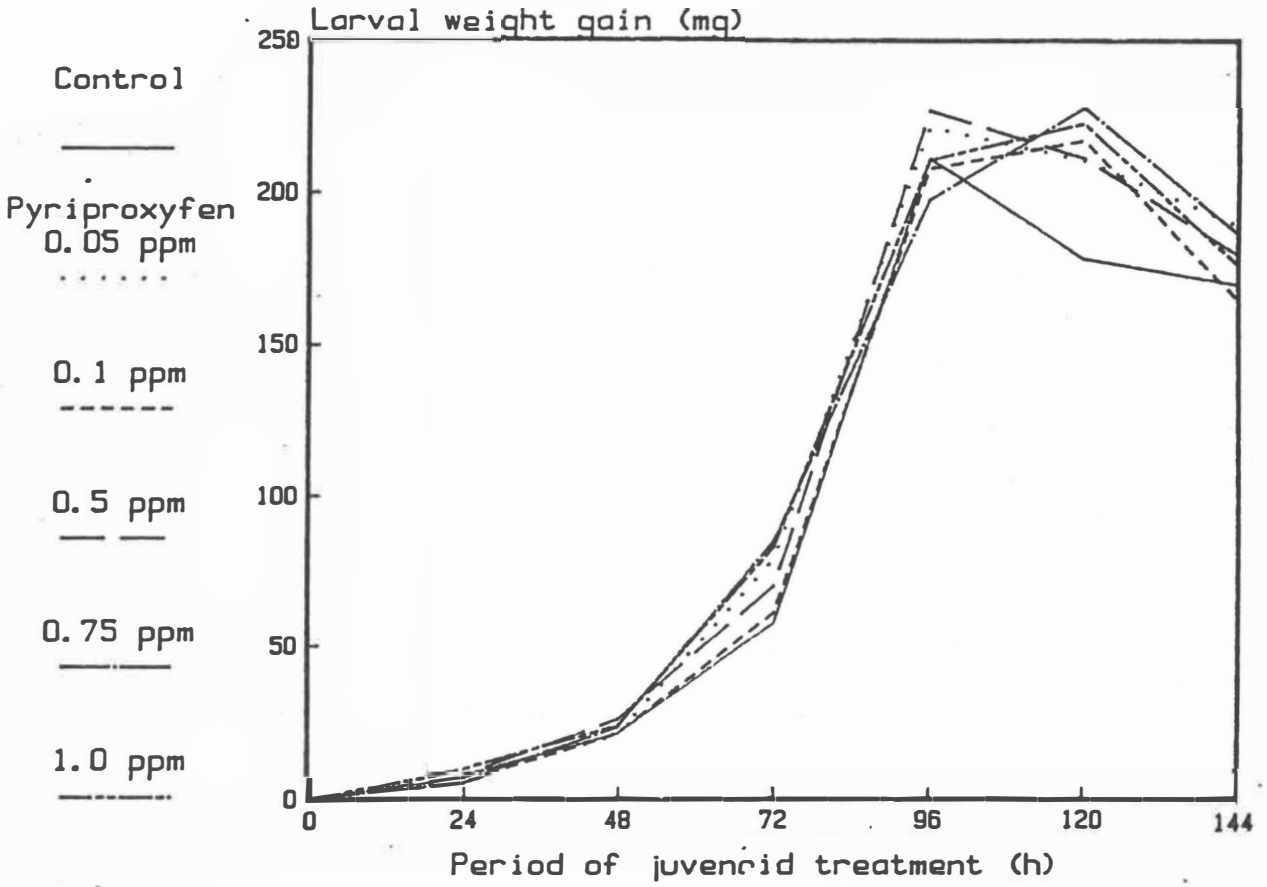

TABLE 1

EFFECT OF JUVENOIDS ON METAMORPHOSIS OF HONEY BEE LARVAE GROWN IN VITRO

Fenoxycarb			Pyriproxyfen		
Treatment µg/ml SF	Number of treated larvae (n)	Mortality of P and PA (%)	Treatment µg/ml SF	Number of treated larvae (n)	Mortality of P and PA (%)
0	240	5.4	0	210	1.1
0.001	90	1.7	0.01	120	2.8
0.005	180	7.1	0.05	120	3.2
0.01	180	9.5	0.1	90	3.5
0.05	180	31.9	0.25	120	5.3
0.1	180	71.6	0.5	120	11.5
0.5	180	100.0	0.75	120	69.1
1.0	180	100.0	1.0	120	100.0

P = pupa; PA = pharate adult

TABLE 2

EFFECT OF JUVENOIDS ON HONEY BEE MORPHOGENESIS

Fenoxycarb				Pyriproxyfen			
Treatment (µg/ml SF)	% Malformation			Treatment (µg/ml SF)	% Malformation		
	E	Px	W		E	Px	W
0	0	1.2	0	0	0	0	0
0.001	0	1.7	0	0.01	0	0	0
0.005	0	7.1	0	0.05	0	5.8	6.5
0.01	0	8.0	3.2	0.1	0	2.4	0
0.05	21.3	30.5	13.5	0.25	0	4.2	22.2
0.1	44.5	71.6	29.5	0.5	0	9.7	43.2
0.5	100.0	75.5	-	0.75	1.0	68.0	80.0
1.0	100.0	74.4	-	1.0	100.0	100.0	-

E = Eye with sickle-shape anomaly; Px = Death at late pupal stage (Pd1-Pdd; Rembold et al., 1980); W = Adult with stunted wings.

Appendix 8

J.N.M. Calis, W.J. Boot and J. Beetsma: A standardized test method to evaluate effects of pesticides on honeybee larvae.

A standardized test method to evaluate effects of pesticides on honeybee larvae

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Abstract

No standardized toxicity test on honeybee larvae, in which bees transfer a pesticide from the source to the larvae and in which the results allow an accurate dose-response analysis is yet available. Such a method would particularly be useful for the evaluation of effects of products with insect growth-regulating properties. Therefore, we designed a test using small test units, standardized according to number and age of the bees and larvae, and the environmental conditions. Up to now units of maximally 500 bees and 100 larvae kept in an incubator were tested. However, the mortality of the larvae was variable and too high, probably due to keeping the units in an incubator. A new design is proposed in which a test unit consisting of 0.2 kg of honey bees and 100 larvae is used.

Introduction

Test units used to evaluate effects of pesticides on honeybee larvae, *Apis mellifera* L., range from large free flying colonies (e.g. van der Steen & de Ruijter, 1990) via small caged colonies (e.g. Barker and Taber, 1977) to larvae that are reared in vitro (e.g. Czoppelt, 1990).
When large colonies are exposed to pesticide treatment often variable results are obtained due to varying conditions. Tests with large colonies can demonstrate that a pesticide causes pre-adult mortality. However, when no effect on mortality is found it is not conclusively shown that the tested pesticide is non-toxic. Changes in the size of the test colonies or differences a pesticide diluting factor, such as honeyflow, may lead to variable results.
In the laboratory tests the pesticide is applied to the larvae directly and not via the bees. In field situations the pesticide reaches the larvae via the bees, however. The bees may function as a filter between the pesticide source and the larvae, thus precluding effects on pre-adult mortality. Reproducible results can be obtained with laboratory tests, but a demonstrated effect of a pesticide does not predict effects in the field.
In tests with small caged colonies, experimental conditions are kept constant, but control mortality is either not accurately measurable (Barker & Waller, 1978) or too high to allow accurate dose-response analysis (Barker & Taber, 1977). Moreover, differences in colony size and the number of larvae complicate comparisons between tests.
Thus, a test in which bees function as a filter between the pesticide source and the larvae, and in which standardized test units give reproducible results that can be used for dose-response analysis, is not available yet. Such a test method would particularly be useful to evaluate the effects of products with insect growth-regulating properties. Therefore, we designed a new test with small units, standardized according to number and age of the bees and larvae, and to environmental conditions.

Materials and Methods

Outline of the test

Test units:
The test units consisted of small wooden boxes containing one comb with excess of honey and pollen, 100 eggs/larvae and 300 or 500 bees both of known age, and an egg-laying queen. A queen was either caged (experiment 2 and 3) or free walking (1 and 4).
Environmental conditions:
The test units were kept in an incubator at 30° or 34.5°C (in experiments 1-3, and experiment 4, respectively) and 80%+10% R.H.. When the cells containing the larvae were capped, the bees were removed and the comb with capped cells remained in the incubator at 34.5°+1°C and 80%±10% R.H. until the young bees emerged.
Application:
The application volume of 3-5 ml saccharose solution (50%) was based on the amount of 10 microliter per adult bee as commonly used in oral toxicity studies. This saccharose solution was consumed by the bees within two hours, which seems sufficiently fast for a standardized test. The moment of application was chosen at different moments during larval development.

Bees

The age of the nurse bees was standardized as follows. Combs with emerging bees were placed in an incubator at 34.5°+1°C and 80%+10% R.H. for 1 day. After this day the emerged bees were collected in a hive which contained combs with honey and pollen and an egg-laying queen. After 4 to 6 days 300 or 500 of these bees were individualy counted and used per test unit.

Eggs/Larvae

The age of the eggs/larvae was standardized by obtaining 0-1 day-old eggs, according to Boot and Calis (1991). Subsequently, this dated brood was tranferred to large colonies in which the queen was confined to one frame to prevent egg laying on the combs with dated brood. In these colonies the brood was nursed until use in the test units. At that moment the number of eggs/larvae was reduced to 100 per unit.

Data analysis

In experiment 1 the mortality during the capped stage was calculated. In the other experiments (2, 3 & 4) mortality of the pre-capping development stages was calculated.

Results & Discussion

The results of our experiments are summarized in Table 1. In experiment 1 we followed the development of the brood nest in small caged colonies to determine the age at which bees are able to nurse larvae. Larvae in these test units (n=2) hatched from eggs laid by a free walking queen. When the nurse bees were 6-7 days old 2-3 day-old larvae were present indicating that already the first eggs of the queen had been nursed successfully. Experiment 2 and 3 were carried out according to the above summarized protocol. The difference between these experiments is that in experiment 3 both the bees and introduced brood was

younger at the moment the test units were composed. In both experiments mortality of the pre-capping stages was about 67%. In experiment 4 the number of bees was increased from 300 to 500, 0-1 day-old larvae were introduced instead of eggs, a free walking queen was introduced, and the units were kept at 34.5°±1°C instaed of 30°±1°C in experiment 2 & 3. Mortality was lower when compared to experiments 2 and 3, namely 46%.

Table 1. Experimental setup and results of experiments.

	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Queen	free	caged	caged	free
Bees				
-number used	ca. 400	300	300	500
-age at introduction of eggs/larvae	-	5-6 days	3-4 days	4-5 days
-Dead bees after cell capping	-	2.0±0.9	9.4+5.2	11.0+4.1
Eggs/Larvae				
-number used	-	100	100	100
-age at introduction in test unit	-	2-3 days (eggs)	1-2 days (eggs)	3-4 days (0-1 day-old larvae)
-number of cells capped	136±49 (n=2)	33.2±25.4 (n=5)	32.4±12.6 (n=5)	54.0±7.4 (n=5)
-mortality before capping(%)	-	66.8±25.4	67.6±12.6	46.0±7.4
-number of emerged bees	134±48.5	-	-	-
-mortality of capped brood (%)	1.1±0.03	-	-	-

Although the bees in the test units were able to nurse the larvae the experiments did not give reproducible results and low larval mortality. This may be due to the use of an incubator and the relatively small number of bees. Therefore we propose another design using larger amounts of bees that are allowed to fly freely until application of the pesticide. Preliminary experiences with colonies of the proposed (see below) size showed that the colonies nursed an introduced patch of brood well as desired.

Outline of proposed test

Test units:
The test units used are Apidea mating boxes. They contain 0.20 kg newly emerged bees, an egg-laying queen and an excess of food (sugar candy and pollen). After 7 days the brood produced in the test unit is removed and the standardized brood is introduced.
Enivironmental conditions:
The test units are kept outdoors and free flying. Before application the test units will be

covered with a small flight cage. When the cells containing the test larvae are capped by the bees, the combs will be placed in the incubator until the young bees have emerged.
Application:
Before application the test units will be covered with a small flight cage. The pesticide will be applied in 20 ml saccharrose solution. The number of bees in the test unit will approximate 2000, again 20 ml reflects the normally used quantity of 10 microliter pesticide solution per adult bee in oral toxicity studies.

Bees

The age of the bees will be standardized as before. The number of bees will be standardized by using 0.20 kg bees per test unit into which they are introduced directly after their emergence. Since the weight of bees is variable, the number of bees per test unit will be estimated by weighing a counted number of bees. After 7 days the parts of the combs with eggs and larvae are cut from the combs of the test unit and one comb is replaced by a comb with dated brood.

Eggs/Larvae

The age of the eggs/larvae will be standardized as before. When dated brood is present parts of the combs containing at least 100 eggs are attached to the Apidea frames. These combs are than transferred to nursing colonies. The combs are stored there for two days until they are used in the test units. At that moment the number of eggs is reduced to 100 per test unit.

Data assessment

Capped stage: At the end of larval development when all the cells are capped the bees are removed from the test unit. The number of capped cells is used to calculate the pre-capping mortality. To check for an unexpected loss of bees, the weight of the bees is determined by weighing the test unit with and without bees. The number of bees can be estimated by weighing a counted number of bees. The combs with capped cells are kept in the incubator at 34.5°+1°C and 80%±10% R.H..

Adult bees: Nineteen days after the dated brood has been obtained the first adults will emerge from the combs. From this day emerging bees can be collected and counted twice a day, as long as capped cells are present in the comb. Five days after the last bee emerged, the remaining capped cells can be considered to contain dead bees. Mortality during the capped phase and during the total development can be calculated. Emerging bees can be examined on their weight, time of emergence, and possible malformations to e.g. wings and eyes. Also the contents of the remaining capped cells can be examined.

Data of mortality are proposed to be analysed with probit analysis (Finney, 1971).

Risk assessment

This test could assess the risk of a pesticide when the relation between field application of a pesticide and colony contamination is known. This relation will depend on and vary with field and colony conditions, however. Therefore, we chose an application volume that will cause a higher colony contamination than in a field application with the same concentration.

The application volume is of the same order of magnitude as that of the 'honeybee brood feeding test' (Oomen *et al*, 1992) when the colony size is considered. When compared to this 'honeybee brood feeding test' the proposed test, provided that reproducible results can be obtained, allows a more accurate use of a toxic standard and comparison of toxicity of different compounds and concentrations. Moreover, only small experimental units are contaminated, providing an economical use and prevention of the contamination of colonies, combs and hives.

Conclusion

The proposed test method would particularly be useful to evaluate the effects of products with insect growth-regulating properties on honeybee brood. However, the utility of the proposed test, depending on a low control mortality and on reproducible results, still has to be demonstrated.

Acknowledgement

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Appendix 9

G.B. Lewis: A comparison of the effect of two formulations of a pyrethroid insecticide, lambda-cyhalothrin, on honeybees, *Apis mellifera* L. foraging on simulated honeydew on winter wheat.

A COMPARISON OF THE EFFECT OF TWO FORMULATIONS OF A PYRETHROID INSECTICIDE, LAMBDA-CYHALOTHRIN, ON HONEY BEES, *APIS MELLIFERA* L. FORAGING ON SIMULATED HONEYDEW ON WINTER WHEAT.

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Summary

Colonies of honey bees were confined in mesh-covered tunnels erected over plots of winter wheat sprayed with sucrose solution to simulate aphid honeydew. There were five tunnels in each of two consecutive tests: a water-sprayed control; lambda-cyhalothrin emulsifiable concentrate (EC) at 6.25 g active ingredient (ai) ha^{-1}; lambda-cyhalothrin wettable granules (WG) at 6.25 g ai ha^{-1}; lambda-cyhalothrin WG at 12.5 g ai ha^{-1}; phosalone at 600 g ai ha^{-1} as a reference standard. The insecticide treatments were applied to half of the crop in each tunnel at a time when the bees were actively foraging. The bees were observed before and after treatment for foraging activity and other behavioural aspects, mortality and the state of the brood. Lambda-cyhalothrin had an inhibitory effect on foraging on treatment day and for up to three days after: phosalone had a more severe effect for at least four or five days after. Mortality was generally low following all treatments, the most marked effect occurring with phosalone which gave a small (non-significant), short-term increase. There were no treatment-related effects on the brood. It is concluded that the repellant effect on foraging bees of WG and EC formulations of lambda-cyhalothrin are similar and that, at the rates tested, neither formulation will cause significant mortality.

Introduction

Following the sequential testing approach (Oomen, 1990), a consistent picture is found with regard to the hazard of pyrethroid insecticides, such as lambda-cyhalothrin ("Karate"), to honey bees (*Apis mellifera*). Laboratory acute toxicity tests show that they are nearly all 'highly toxic' to bees, following the International Commission for Bee Botany classification (LD_{50} < 1 µg ai bee^{-1}). However, under conditions of use in the field they are found to have a low hazard, with little or no additional mortality, relatively short-term reversible behavioral effects and no long-term consequences for the health of the colony e.g. brood development (e.g. Wilkinson & Gough, 1984).

The reason for this apparent anomaly is that, although toxicity is high, the other main factor determining field hazard, exposure, is low. There are three main reasons for this. (1) The rates of application of pyrethroids tend to be low as reflected by the ratio of the field application rate to the LD_{50} value, the 'hazard ratio' (Smart & Stevenson, 1982). Thus, lambda-cyhalothrin has values typically in the range 50-350 which are at the lower end of the range requiring further testing in the sequential testing schemes (*ibid*). (2) The residual activity of pyrethroids is short-lived due to breakdown, adsorption onto the plants and turnover of plant tissues. For example, in a field trial in which lambda-cyhalothrin was applied to flowering oilseed rape on which bees were actively foraging, residue levels on bee-collected pollen had declined to below the limits of detection (0.002 µg ai g^{-1}) within ten days of application (Zeneca Agrochemicals, unpublished data). (3) Repellancy, a property characteristic of most pyrethroids, which is manifested as a reduction in the numbers of bees foraging (Inglesfield, 1989). This limits the contact between foraging bees and the residues which are present on the crop.

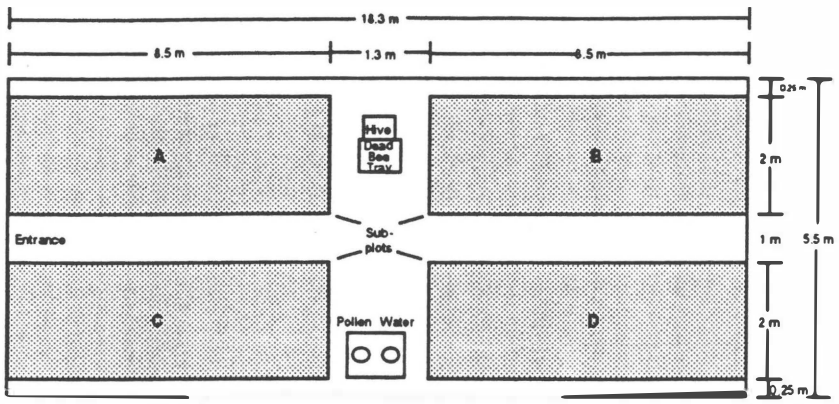
The repellant property of pyrethroids has been widely reported and a number of mechanisms have been proposed. Atkins (1981) originally proposed that they were classical repellants acting in an olfactory manner. Bos & Masson (1982) attributed the repellancy not to the pyrethroids themselves but to the formulation adjuvents. However, in their work Rieth & Levin (1987) found the opposite situation with the repellancy occurring only with the active ingredient and not the adjuvents.

Accordingly, it was decided to investigate the effects of two different formulations of lambda-cyhalothrin to see if there was any difference in their effects on honey bees. A trial was conducted under semi-field conditions, to assess and compare the effects on honey bees foraging on simulated aphid honeydew applied to winter wheat.

Materials and Methods

The experimental design and methodology was based on Method No. 129 of the ANPP, Paris, France (Debray, 1989). Tunnel greenhouse frames, as used by horticulturalists, covered with a mesh sufficient to confine honey bees but allowing field weathering, were erected over plots of winter wheat. The layout of each tunnel was as shown in Fig. 1.

Fig. 1 Tunnel layout



The crop inside each tunnel was cut into four equal sub-plots, separated by cross-paths which were covered with polythene sheeting. A single colony of honey bees was introduced into each tunnel. The wheat was sprayed with sucrose solution to simulate aphid honeydew and was kept attractive to the bees by moistening with water or replenishing as necessary. There was a separate tunnel for each of five treatments:

(1) lambda-cyhalothrin, formulated as an emulsifiable concentrate (EC), applied at 6.25 g ai ha^{-1} (recommended field rate on cereals in France).
(2) lambda-cyhalothrin, formulated as a wettable granule (WG), applied at 6.25 g ai ha-1
(3) lambda-cyhalothrin, formulated as a WG, applied at 12.5 g ai ha^{-1}
(4) a reference compound, phosalone ("Zolone-Flo"), applied at a rate of 600 g ai ha^{-1}
(5) a water-treated control.

The insecticide treatments were applied to two diagonally opposite sub-plots in each tunnel. To give the bees a choice the other two sub-plots were sprayed with water as were all four sub-plots in the control tunnel. Application was carried out in the late morning when the bees were actively foraging.

The foraging activity of the bees on the four sub-plots in each tunnel was monitored for several days before and after treatment. At each assessment time the bees were counted in two 90-second passes on a one-metre strip along the sub-plots. There were four assessments at intervals of two hours except on treatment day when they were hourly. The behaviour of the bees at each hive entrance was also assessed at the same time. The numbers of bees entering or leaving each hive were counted over a 60-second period and additionally observations were made on any changes in the nature of the behaviour exhibited at the hive entrance. Dead bees were collected and counted early each morning, from dead bee trays fitted to each hive and from the paths between the sub-plots. The brood of each colony were assessed for quantity and quality about one week before and after treatment and additionally about six weeks after.

There were two consecutive, replicate tests. The tunnels were moved onto fresh plots of crop after the first test and new colonies of bees placed inside. The trials were conducted from June to July.

Results

A number of biologically significant effects were observed on mortality and foraging activity although only some of these were statistically significant due to the low replication and relatively high variability inherent in the trial design.

The majority of dead individuals found were adult workers although a few larvae killed by the fungal disease, *Ascosphaera apis* (chalk brood), worker pupae and drone pupae and adults were also found. The results are presented in Table 1, showing the total numbers of dead bees (all castes) collected in the days following treatment. The mortality in Test 1 was generally low throughout with no increase attributable to any of the treatments. In Test 2 the mortality was again generally low throughout, but there was a marked increase in the mortality from the phosalone treatment. This occurred in the day +2 and +3 samples and was most noticeable on the paths but was also evident in the dead bee trays. The slight delay in the emergence of this effect may have been due to the pattern of exposure of the bees, the spray initially drying quickly but subsequent rain increasing the availability of the residues. The effect was also seen to a lesser extent with the lambda-cyhalothrin EC treatment but not with either rate of the WG.

Table 1 Total dead bees collected from dead bee trays (DBT) and paths following treatment.

Test 1

Day (1)	Control		Phosalone 600 g ai ha^{-1}		L-C (1) EC 6.25 g ai ha^{-1}		L-C WG 6.25 g ai ha^{-1}		L-C WG 12.5 g ai ha^{-1}	
	DBT	Path	DBT	Path	DBT	Path	DBT	Path	DBT	Path
-0	29	30	0	32	8	58	9	32	4	33
+0	6	14	2	5	8	18	3	19	9	12
+1	14	9	0	7	4	21	7	8	2	10
+2	0	15	2	40	5	46	7	30	2	47
+3	1	21	0	12	5	28	30	20	1	27
+4	13	18	4	20	5	34	34	39	3	23

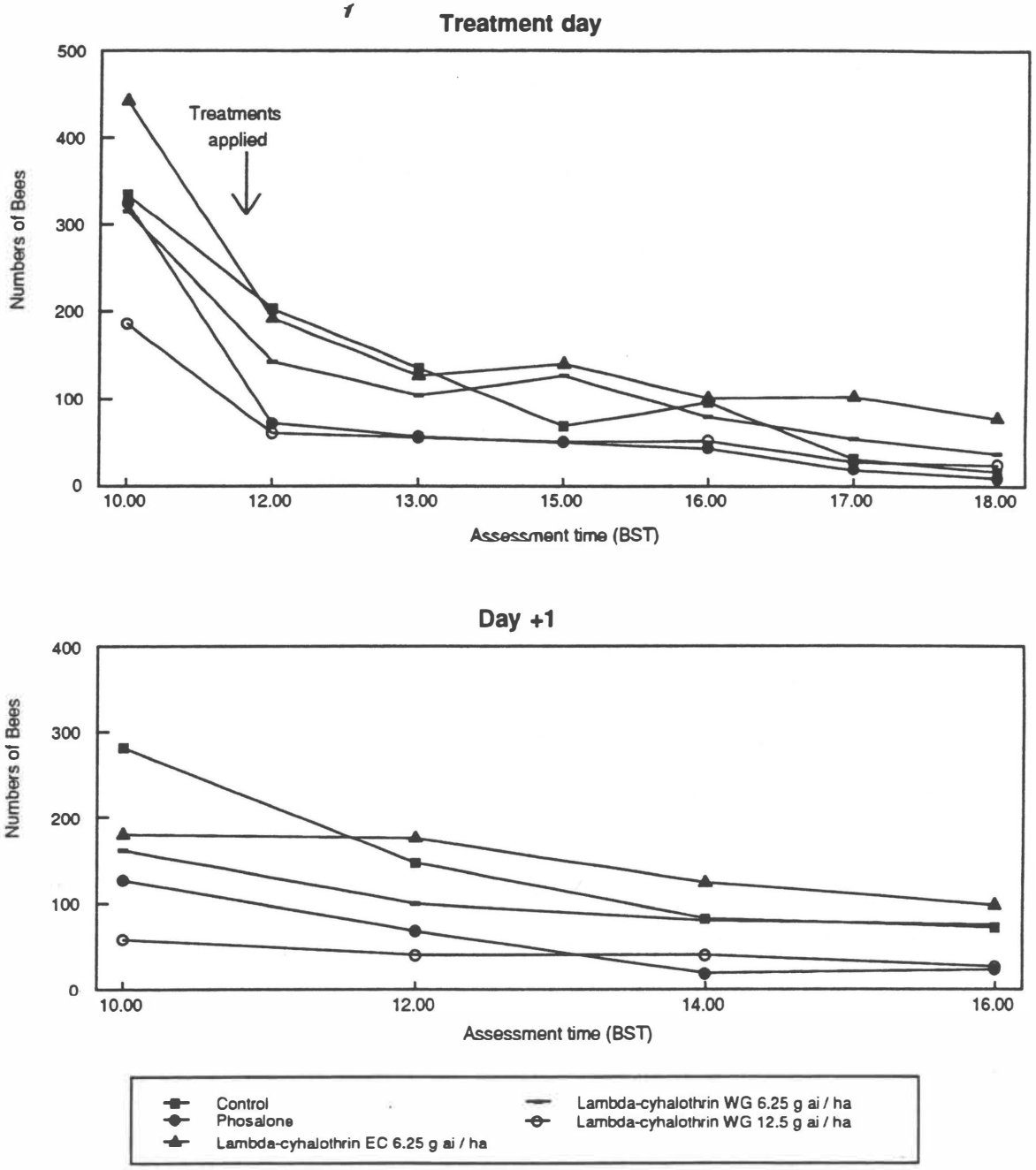
Test 2

Day (1)	Control		Toxic standard 600 g ai ha^{-1}		L-C EC 6.25 g ai ha^{-1}		L-C WG 6.25 g ai ha^{-1}		L-C WG 12.5 g ai ha^{-1}	
	DBT	Paths	DBT	Paths	DBT	Paths	DBT	Paths	DBT	Paths
-0	3	14	7	17	17	57	4	35	16	5
+0	2	4	2	12	7	2	1	6	5	4
+1	6	2	16	13	16	13	0	6	14	5
+2	3	6	27	113	2	37	2	17	8	5
+3	6	11	30	78	9	81	5	24	14	12
+4	1	6	1	7	9	31	5	23	12	8

(1) 0 - treatment day (- pre, + post)
(2) L-C - lambda-cyhalothrin

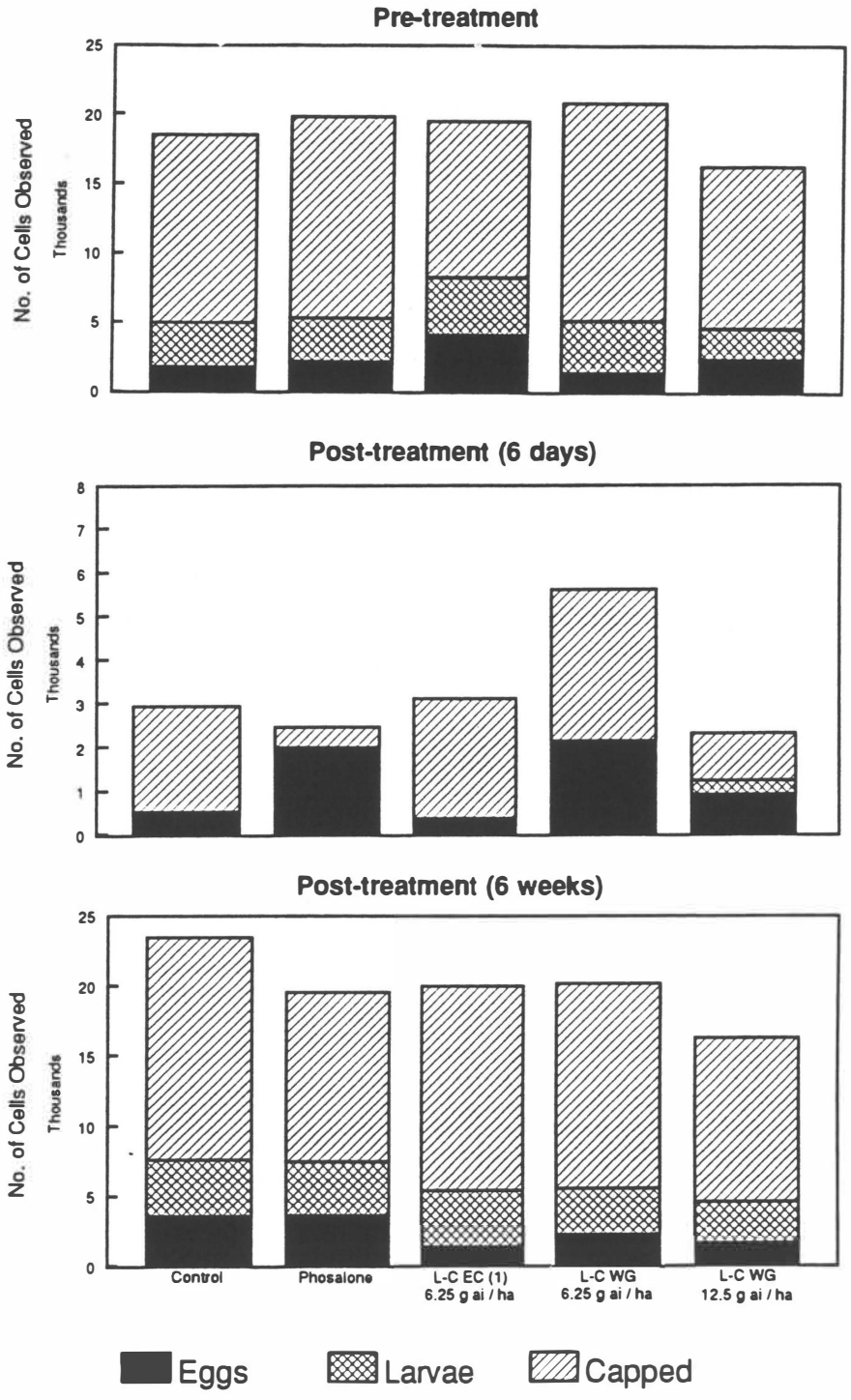
Lambda-cyhalothrin had an inhibitory effect on foraging on treatment day and for up to three days after. With the WG formulation the magnitude of the effect was rate-related but there was little difference between the EC and WG formulations at the same rate. Phosalone had the most severe effect on foraging activity, reducing it for at least four or five days after treatment. The results of the foraging assessment for treatment day and day +1 in Trial 1, as an example, are shown in Fig. 2.. On treatment day the pre-treatment assessment shows the consistent variation that was found between colonies, the lambda-cyhalothrin EC colony in particular showing a higher level of activity than the others and the lambda-cyhalothrin WG high rate colony having a lower level of activity. Any effects are obscured on treatment day by the normal pattern of activity, declining from an early morning peak, but by the following day the reduction in foraging could be clearly seen.

Fig. 2 Total numbers of foraging bees on treatment day and day +1



The amount of the different brood stages (eggs, larvae and capped brood) present at intervals throughout Test 1 are shown in Fig. 3. In both tests all the colonies, including the untreated control, showed a marked reduction in the levels of brood present following their move into the tunnels. This undoubtedly reflected the limited availability of forage within the confined area of the tunnel, particularly the protein-rich pollen required for brood production. The brood returned to pre-treatment levels once the colonies had been removed from the tunnels and allowed to fly freely again. There were no effects on the brood attributable to any of the insecticide treatments.

Fig. 3 Test 1 brood assessment



(1) L-C - Lambda-cyhalothrin

Conclusions

The results of this tunnel trial confirm the results of earlier studies (e.g. Wilkinson et al, 1986) in showing that the hazard of lambda-cyhalothrin to honey bees under field conditions is low and, additionally, that the WG and EC formulations have similar effects on foraging bees. Thus, at the rates tested, both formulations cause a similar short-term reduction in foraging and neither causes significant mortality nor has any effect on the long-term health of the colony. This result is consistent with a field trial conducted by Atkins in California (reported in Hearn, 1985) in which he found that a wettable powder formulation of permethrin had the same effects as EC ones tested previously.

The results of this study, indicating no difference between pyrethroid formulation types in their effects on bees, is supported by the work of Rieth and Levin (1987). They proposed a mechanism for pyrethroid repellancy resulting from contact with the active ingredient. This contact is very brief, owing to a high irritancy effect of pyrethroids, but allows a sub-lethal dose to be picked up which renders the bees inactive in the hive i.e. 'repels' them by stopping them foraging on the treated crop. This effect is reversible, with the bees recovering to resume normal activity. However, the effect is only produced by the active ingredient not by the formulation adjuvents. Any effect of the latter seems to be confined to the oral route of exposure, as found by Bos and Masson (1982). This will be less important because contact, either by direct overspray or by settling on treated plants, will be the first and main route of exposure in the field.

The repellancy effect observed with honey bees on contact with lambda-cyhalothrin is consistent with their known ability to cause repetative firing in neurons. This repellancy effect may be considered to be related to the reversible effects sometimes experienced by humans, known as paraethesia, which is manifested as a tingling sensation in the skin.

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Appendix 10

L.P. Belzunces: A convenient biological method for evidencing synergies between pesticides in bees : effect of pyrethroid insecticides and azole fungicides applied at sublethal doses.

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A convenient biological method for evidencing synergies between pesticides in bees : effects of pyrethroid insecticides and azole fungicides applied at sublethal doses

SUMMARY

In laboratory experiments, we have demonstrated the synergistic action of prochloraz and deltamethrin in bee using sublethal doses cooresponding to 0,125 g of deltamethrin.ha^{-1} and 25 g of prochloraz.ha^{-1}. Simultaneous treatments gave a maximum synergistic effect whereas sequential treatments produced lower mortalities. Studies were extended to the insecticide formulation Decis® (active ingredient, deltamethrin), sprayed at doses 10 times and 20 times lesser than the dose registered in France, and to the fungicide formulations Eria® (active ingredients, difenoconazole and carbendazim) and Calidan® (active ingredients, iprodione and carbendazim) used at the registered doses. A marked synergy was observed with Decis-Eria association. No significant lethal effect was observed when the treatments of Decis and Eria were spaced by 24 h.

INTRODUCTION

The number of agrochemical associations has increased considerably during the past few years. These associations are used both to control pests by simultaneous action of different active ingredients and to treat several plant diseases at the same time. If the agronomical efficacy is often enhanced, the risk of observing a synergistic effect on beneficial insects is more probable.

Deltamethrin, a pyrethroid insecticide, and prochloraz, an imidazole fungicide are two agrochemicals considered as non dangerous for bees in field conditions (Fell and Rajotte, 1983 ; Mayer and Lunden, 1986). However, bloom spraying with these molecules have been reported to produce dramatic mortalities of bees in the Northeast of France. A synergy phenomenon seemed to be responsible for the noxious effect of the mixture in bees. To study a possible occurrence of synergy between prochloraz and deltamethrin we based ourselves on Macht's definition of synergy (1929) :"the phenomenon exhibited by the combination of two or more drugs in which the pharmacodynamic effect produced by the mixture is not a simple summation of the effects produced by two or more individual components." Thus, we propose using sublethal doses of agrochemicals to demonstrate unambiguously a synergy between two different products. Studies began with associations of active ingredients and were extended to formulations containing other actives matters belonging to the pyrethroid family and triazole and dicarboximide families.

MATERIALS AND METHODS

Bees were captured from honey and pollen combs of the hive and corresponded mainly to foragers. Immediately before treatment, bees were anaesthetized with carbon dioxide and put on the spraying plate by groups of 50. During the experiments, they were then stored in 9x8x11 cm cages and placed in a thermostated chamber at 28±1°C and 60±10% relative humidity. They were fed with a solid mixture of glucose and honey (4/1, w/w) and water *ad libitum*. Deltamethrin and prochloraz were purchased from CLUZEAU INFO-LABO (France). For studies with formulations, we used the fungicide Eria® (62.5 g of difenoconazole.l^{-1} and 125 g of carbendazime.l^{-1}), the fungicide Calidan® (175 g of iprodione.l^{-1} and 87.5 g of carbendazime.l^{-1}) and the insecticide Decis® (25 g of deltamethrin.l^{-1})

To approximate field treatment conditions, bees were sprayed in a Potter-Burgerjon-type spraying tower (Burgerjon, 1956) to obtain doses of active ingredient in g.ha^{-1}. All deltamethrin and prochloraz solutions were prepared in distilled water containing final concentrations of 1% (v/v) acetone and 5% (w/v) isostearylisostearate. Formulations were directly prepared in distilled water. According to the International Organization of Biological Control (IOBC) recommendations, deposits of agrochemicals were 1.79±0.10 mg of solution.cm^{-2} at 7240 Pa pressure (Hassan *et al.*, 1985).

Bees were treated with sublethal doses of deltamethrin and prochloraz in either a simultaneous or a sequential fashion. In the sequential fashion, the treatments of deltamethrin and prochloraz were spaced by a 0.8 day interval. The 0.8 day duration was chosen considering the effect of prochloraz on the time-course of cytochrome P-450 induction observed in quail (Rivière *et al.*, 1985) and the possibility of a vesperal insecticide treatment in the absence of foraging activity. Each experiment corresponded to 12 determinations.

RESULTS

Treatments with active ingredients

In a first series of experiments, we used the association of an imidazole fungicide, prochloraz, and a pyrethroid insecticide, deltamethrin (Colin and Belzunces, 1992). Doses were progressively reduced to obtain doses of 0.125 g of deltamethrin.ha^{-1} and 25 g of prochloraz.ha^{-1} that did not produce an effect different from that of the control. These doses were referred as sublethal doses of prochloraz and deltamethrin.

In the synergy experiments, deltamethrin and prochloraz were sprayed separately, simultaneously, or sequentially. Deltamethrin (0.125 g.ha^{-1}) and prochloraz (25 g.ha^{-1}) used alone did not produce a mortality significantly different from that of the control during 96 hours of observation (Fig. 1). Analysis of variance (ANOVA) of the mortalities at 50 hours showed a strong effect of treatment ($F=33.73$; $DF=95$; $p<0.001$) (Fig. 1). The comparison of means showed three significantly different groups ($p=0.01$) : a group including control, prochloraz and deltamethrin treatments, a group corresponding to the sequential treatments (deltamethrin followed by prochloraz and prochloraz followed by deltamethrin) and a group corresponding to the treatment

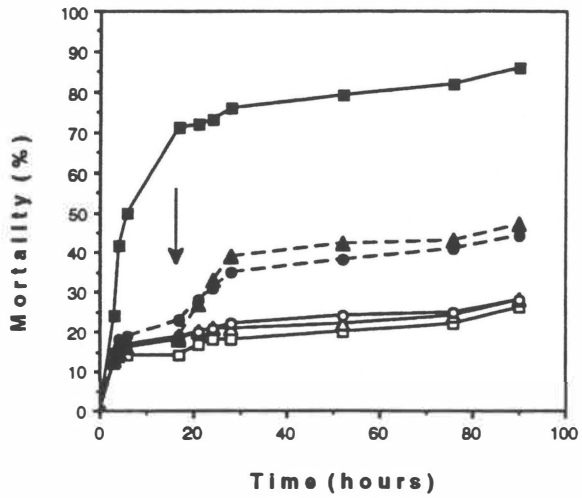

Figure 1. Synergy between deltamethrin (0.125 g.ha^{-1}) and prochloraz (25 g.ha^{-1}). (■), Deltamethrin and prochloraz sprayed simultaneously as a mixture. In the sequential sprayings, the second treatment was spaced by a 0.8-day interval and is indicated by an arrow. The controls were sprayed with solvent and were also submitted to carbon dioxide anaesthesia before each treatment. This induced a slight increase in mortality after 0.8 day. (❐), Control; (Δ), deltamethrin; (O), prochloraz; (▲), deltamethrin followed by prochloraz; (●), prochloraz followed by deltamethrin. Mortalities were expressed as percentages of the initial population. Each point represented mean of 12 values corresponding to three experiments made in quadruplicate. Standard deviations were not represented for clarity.

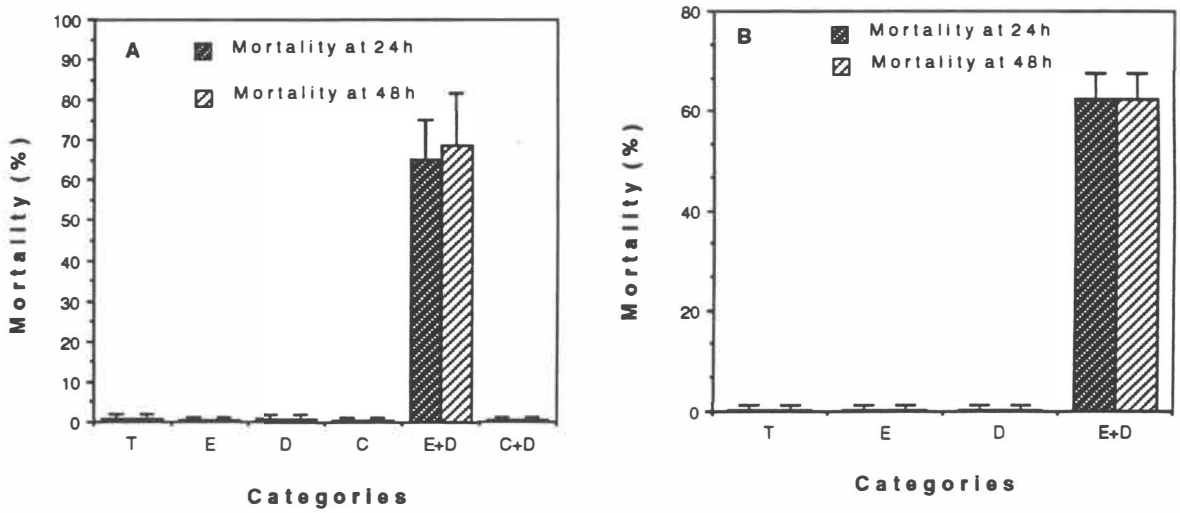

Figure 2. Synergy between formulations of a pyrethroid insecticide and a triazole fungicide. Associations with the pyrethroid insecticide at 1/10th (A) et 1/20th (B) of the registered dose. C, control sprayed with water; E, Eria at 2 l.ha^{-1}; D, Decis at 0.03 l.ha^{-1}; C, Calidan at 3 l.ha^{-1}. D+E, association of Decis and Eria at the doses indicated above. D+C, association of Decis and Calidan. Mortalities were expressed as percentages of the initial population and were recorded 24 h and 48 h after treatments. Values represented means of 12 determinations ± S.D corresponding to three experiments made in quadruplicate.

with deltamethrin-prochloraz mixture. Sprayed as a mixture, deltamethrin and prochloraz produced 79.3% mortality (74.1%, corrected according to Abbott, 1925) within 50 hours. At 50 hours, the sequential treatments of the fungicide and the insecticide spaced by a 0.8 day interval produced 42% mortality (27.5%, corrected) for deltamethrin followed by prochloraz and 39.1 (23.8%, corrected) for prochloraz followed by deltamethrin.

Treatments with formulations of agrochemicals

In a preliminary study, we determined that doses of 2 l of Eria ha^{-1}, 3 l of Calidan ha^{-1} and 0,03 l of Decis ha^{-1} (1/10th of registered dose in Francc) did not produce lethal effects different from that of the control 24 h after treatments (ANOVA : F=1.1 ; p=0.40 ; df=9) and 48 h (ANOVA : F=1.0 ; p=0.44 ; df=9). These doses were further used in synergy experiments with water as control. In these conditions, the average mortalities induced by the fungicides and the insecticide sprayed separately were less than 1% after 48 h of observations (Fig. 2A). Conversely, a very marked synergy of toxicity was observed when Decis was associated with Eria. In the same conditions, the association of Decis and Calidan did not produce any significant lethal effect. Mortalities observed at 48 h (mean: 68.5±13.3%) were slightly higher than those observed at 24 h (mean: 65±10.4%) with a maximum effect at 93%. It is noteworthy that the major part of the lethal effect of the Decis-Eria association was obtained within 10 min following treatments.

In a second series of experiments, the dose of Decis was lowered to 0,015 $l.ha^{-1}$ (1/20th of the registered dose) as the treatment with the fungicide Eria was not modified. Results showed that Decis, at this dose, still interacted with Eria (Fig. 2B). The mortalities at 24 and 48 h were identical (mean: 62.5±5.2%). In this case too, the major part of the lethal effect was observed within 10 min following the treatments.

Sequential treatments were performed to provide information on the security lag time between fungicide and insecticide treatments (figure 3). In all cases, independently of the treatment order, no synergistic effect was observed when treatments were achieved at 24 h interval. Same results were obtained with a lag time of 48 h.

DISCUSSION

The experimental approach based on the use of sublethal doses unambiguously demonstrates synergy between two agrochemicals considered as non toxic for bees. With sublethal doses, the classical quantification of co-toxicity and synergy factors (Sun and Johnson, 1960 ; Welling and de Vries, 1985 ; Horowitz *et al.*, 1988) based on LD50 or LD90 (Lee and Brindley, 1974) is impossible as the expression of synergy is incontestable. The LD50 is an important tool for classifying the effectiveness of an active ingredient or a formulation. However, it is less convenient in toxicology for assessing insidious or chronic toxic effects or for demonstrating a synergistic action of several compounds at very low doses. In our experiments, doses of deltamethrin able to produce mortality by synergy is about 34 to 68 times less than the LD50 reported by Atkins and coworkers (1981). Thus, pesticide residues in bees killed by a synergistic

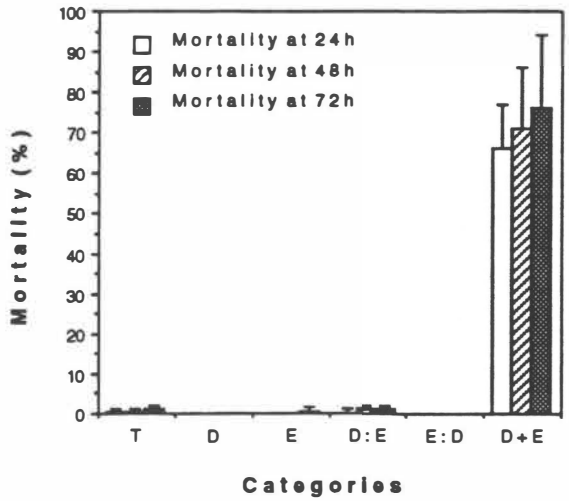

Figure 3. Effects of sequential treatments of a triazole fungicide and a pyrethroid insecticide on bee longevity. The sequential treatments were spaced by a 24-hours interval of time. C, control sprayed with water; E, Eria at 2 $l.ha^{-1}$; D, Decis at 0.03 $l.ha^{-1}$; D+E, association of Decis and Eria at the doses indicated above. D:E, Decis followed by Eria; E:D, Eria followed by Decis. Mortalities were expressed as percentages of the initial population and were recorded 24 h, 48 h and 72 h after treatments. Values represented means of 12 determinations ± S.D corresponding to three experiments made in quadruplicate.

effect may be lower than the detection limit of analytical methods involving gas chromatography or, at least, do not allow unquestionable intoxication diagnosis.

In our experiments, we started from the report of mortalities elicited by field treatments to study the synergistic effect of both active ingredients involved in the formulations. The intermediate stage has been experiments on formulations to explore a synergy between a pyrethroid insecticide and a triazole fungicide. If we consider that synergy observed in field with formulations is confirmed and demonstrated by laboratory experiments on active ingredients, we can assume that laboratory experiments with associations of formulations may reflect a field toxicity. Taking into account the repetitive or simultaneous pesticide treatments, which can occur at close interval of time, the frequency and the seriousness of bee intoxications in the agrosystem are very probably underestimated. This may be accentuated by the fact that bees can be contaminated either by direct spraying or by contact with treated surfaces and by storage of contaminated food.

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Appendix 11

D.F. Mayer and J.D. Lunden: Effects of the adjuvant Sylgard on hazard of selected insecticides to honeybees.

EFFECTS OF THE ADJUVANT SYLGARD ON HAZARD OF SELECTED INSECTICIDES TO HONEY BEES

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INTRODUCTION

Bee poisoning, the accidental killing of bees from insecticides, is a major problem for beekeepers worldwide. Chemical residues play a major role in the relative safety of pesticides to bees. Sizable differences in toxicity to bees occur with various pesticides (Waller et al. 1988) and formulations (Atkins et al. 1981). Under equivalent conditions of application and dosage, dusts are typically most toxic, wettable powders less toxic and emulsifiable concentrates least toxic. This sequence of toxicity is probably due to differential pick-up of toxic residues by bees (Pradhan 1949). Formulations that stick or otherwise bind insecticides to plant surfaces generally reduce bee hazard. Thus, adjuvants added to insecticides to improve effectiveness of a pesticide may greatly decrease their hazard to bees (Erickson and Erickson 1984; Johansen and Mayer 1990). Sylgard is a fairly new organosilicone surfactant from Dow Corning for use in tank-mixes with insecticides for improved efficacy.

This paper reports results of concerning the effects of adding Sylgard to insecticide tank mixes on honey bee (Apis mellifera L.) mortality in residue bioassays tests.

MATERIALS AND METHODS

Thirty-eight insecticides alone and in combination with Sylgard (3.5 L/940 L) were applied to 0.004-hectare plots of alfalfa with a R&D CO_2 pressurized sprayer at 234 liters of water/ha. Treatments of field-weathered insecticide residues were replicated four times with four foliage samples per treatment and time interval. Samples consisting of approximately 500 cm^2 of foliage taken from the upper 15 cm portions of plants were placed in cages.

Cages were made from plastic petri dishes (15 cm diameter) with tops and bottoms separated by a wire screen (6.7 meshes/cm) cylinder insert (45 cm long and 5 cm wide). The metal screen was stapled to form a circular insert which provided 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. Residual test exposures were replicated 4 times by caging 40-50 worker honey bees with each of 4 foliage samples per treatment and time interval. Bees in cages were maintained in cages at 26 to 29^0 C. and 50% RH and fed 50% sucrose solution in a cotton wad (5 x 5 cm) placed on the cage

bottom and mortality was assessed after 24 hours exposure. Data were analyzed using ANOV techniques with means separation by LSD (Student's T) (Lund 1989).

RESULTS AND DISCUSSION

Adding Sylgard reduced the bee mortality of some, but not all insecticides. Using the 8 hour mortality data, honey bee mortality was significantly reduced by adding Sylgard to 14 insecticides, carbofuran (Furadan), phosmet (Imidan), methidathion (Supracide), trichlorfon (Dylox), cymermethrin (Ammo, Cymbush), deltamethrin (Decis), esfenfalerate (Asana), fluvalinate (Spur), tralomethrin (Scout), zetamethrin (Fury), carbaryl (Sevin), methomyl (Lannate), endosulfan (Thiodan) and imidacloprid (Confidor) out of the 38 tested (Table 1). In addition, honey bee mortality was significantly increased by adding Sylgard to fipronil, idszophos (Triumph) and pyridaben.

Johansen (1972) and Lagier et al. (1974) showed powered plastics, latex-resin materials and menthenes combined with carbaryl and methomyl safened these materials to honey bees. Mayer et al. (1987) showed adding Bond, Sur-Stix or Bio-Film combined with some, but not all insecticides, safened these materials to honey bees.

Adding Sylgard to pyrethroids showed more promise for reducing bee hazard than adding Sylgard to other groups of insecticides (Table 2.). Sixty percent of the pyrethroid insecticides showed reduced bee mortality by adding Sylgard, compared to 31% of the organophosphates, 29% of the carbamates and 25% of the miscellaneous insecticides.

Plastic adjuvants apparently make insecticidal sprays safer to bees because of a locking-in effect reducing the contact between bees and the insecticidal residues. We suspect Sylgard performs in much the same manner. It remains unclear why this effect occurs with some, but not all insecticides. The effect does not appear to be related to formulation or chemical class. We were surprised that Sylgard increased the hazard of 3 insecticides. We are not aware of other reports where an adjuvant increased the bee hazard of an insecticide.

CONCLUSION

We conclude that the synergistic action of Sylgard depends on the insecticide. Also, generalized statements concerning the bee hazard effect of adding adjuvants to insecticides are not possible. The effect depends on the particular adjuvant and insecticide. Further work is necessary with different adjuvants and insecticides.

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Appendix 12

E. Pilling: Synergism between EBI fungicides and a pyrethroid insecticide in the honeybee, *Apis mellifera* L.

SYNERGISM BETWEEN EBI FUNGICIDES AND A PYRETHROID INSECTICIDE IN THE HONEYBEE, *APIS MELLIFERA* L.

Edward Pilling

Zeneca Agrochemicals, Jealott's Hill Research Station, Bracknell,
Berkshire, RH12 6EY, UK.

Summary

The synergistic interaction between ergosterol biosynthesis inhibiting (EBI) fungicides and a pyrethroid insecticide was studied in the honeybee, *Apis mellifera* L. The investigation results from reports by farmers that tank-mixing of these particular pesticides was causing a higher than expected honeybee mortality. The aim of the study was to demonstrate and quantify the synergistic effect with laboratory and field experiments, and to elucidate the underlying biochemical mechanism causing the enhanced toxicity.

In the laboratory, the pyrethroid insecticide lambda-cyhalothrin was combined with a range of EBI fungicides, at ratios according to their recommended application rates, and dosed topically onto the thorax of honeybees. Mortality assessments 24h after dosing were used to generate LD_{50} values and synergistic ratios. All the fungicides tested increased the toxicity of the pyrethroid to bees. The fungicide propiconazole was found to have the strongest synergistic effect, decreasing the LD_{50} of lambda-cyhalothrin from 68.0ng ai bee^{-1} to 4.2ng, thus having a synergistic ratio of 16.2 (Pilling and Jepson, 1993).

Cage studies showed that honeybees were susceptible to residues of a mixture of the fungicide prochloraz and lambda-cyhalothrin. Consequently, semi-field experiments were conducted to determine the hazard of tank-mixing lambda-cyhalothrin with prochloraz to honeybees foraging on simulated aphid honeydew on winter wheat. Results indicated a large honeybee mortality directly after spraying the tank-mixed pesticides, however the repellency or reduced foraging effect of pyrethroids reduced subsequent exposure of foragers to the pesticide mix, and thus lowered the hazard.

The mechanism by which the fungicide prochloraz enhances the toxicity of lambda-cyhalothrin was investigated. *In vitro* incubations with honeybee midguts were used to study the metabolism of [^{14}C]lambda-cyhalothrin alone, and in combination with prochloraz. Pre-incubation with the fungicide revealed an inhibition of microsomal monooxygenase activity. *In vivo* studies indicated that prochloraz delayed the metabolism, detoxication and excretion of lambda-cyhalothrin for upto 16h by inhibition of microsomal oxidation, effectively enhancing the toxicity of the pyrethroid to the honeybee. Computer molecular modelling studies have provided further evidence of enzyme inhibition, indicating the sterically unhindered nitrogen on the imidazole ring of EBI fungicides directly interacts with the haem active site of cytochrome *P*-450_{III}.

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Appendix 13

J.H. Dustmann and F.W. Lienau: Synergistic action of the varroacide Perizin to other organophosphorus pesticides.

Dustmann, J.H, Lienau, F.W.:

Nieders. Landesinstitut für Bienenkunde, D-29221 Celle

Synergistic action of the varroacide Perizin to other organophosphorus pesticides.

There are several reports, that various pesticides which are not hazardous to honeybees as single preparations show toxic effects when the preparations are applied in combination. This synergistic effect, usually not expected by the farmer, is especially known from tank-mixtures. Combinations like Decis (deltamethrin) and Sportac (prochloraz), Ronilan (vinclozolin) and Dithane Ultra (mancozeb) are some examples for this phenomenon.
If the varroa disease of honeybees is chemically treated with acaricides, which in their nature are similar to specific plant protection pesticides, applied by the farmer, the question arises, if there is any additional danger for the bees. Does the mortality of bees increase by an eventually synergistic effect from compounds of medical treatment and plant protection treatment?

We tried to give an answer to this question using organophosphorus pesticides and the varroacide "Perizin" (coumaphos).

Usually application of varroacide and pesticides take place at different times of the year: Varroa treatment in fall or winter (no brood in the colonies or as less as possible, not much honey flow).

In laboratory tests we took care of these conditions, using young bees in queenless artificial swarms (4.000 bees per swarm). These swarms were treated two times in special swarmboxes with Perizin and water respectively. Usually the bees were fed by chlorella-sugar-paste and water.

2 days after the last varroa-treatment the bees from the swarmboxes were transfered into so called Liebefeld cages, 50 bees each. Another two days later they were fed by 50 % saccharose containing the pesticide. To improve the uptake of pesticide containing food the bees had to starve for one night. The mortality had been calculated by the Abbott-formula ($M = \frac{K - T}{K} \times 100$).

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Results

The first figure shows that Perizin treated bees react more sensitive to other organophoshorus compounds. The mortality does increase in comparison to those bees not treated with Perizin (Fig. 1).

In a second experiment the time region of observation was extended: 4, 8, 12, 17 days after the last Perizin treatment the bees received the organophosphorus pesticide. The increased toxicity did show up over the whole period, that means even 17 days after the last Perizin treatment, additional toxicity could be proved.

To be sure, that the increase of toxicity was due to the treatment with Perizin the conditions of the experiment were changed in some important factors:

a) the bees were fed with pollen instead of chlorella
b) the bees were fed with saccharose only
c) the bees were enabled to fly out from the opened swarmbox in order to get rid of their feces by defecating outside.

These modified conditions demonstrated again that the increase of toxicity was indeed caused by the Perizin treatment.

These results from laboratory tests are they also valid in bee colonies kept under free semifield conditions?

We tried to answer this question by using two methods:

1. Queenright artificial swarms were treated twice with water (W) or twice with Perizin (P). Some days later these bees were distributed into Kirchhainer mating nuclei boxes, each of them having a mated queen. Respectively two Kirchhainer boxes were transfered into a gaze cage of 2 x 4 meter, containing blooming phacelia or mustard plants. These plants were sprayed with water or organophosphorus pesticides like Rubitox (6 g in 360 ml water), Torak (1,2 ml in 360 ml water), Afugan (0,2 ml in 200 ml water). Also in this semifield test with organophosphorus pesticides the effect of Perizin was very significant, especially in the case of Rubitox (Fig. 2). Those bees which had been treated first with Perizin and later with Rubitox showed a much higher mortality than those treated with water and Rubitox (Fig. 2).

...

2. In a further experiment we followed another way: After the time of overwintering, in spring the queens of each colony were caged. About 6 weeks before the rape was expected to be in bloom, the meanwhile broodless colonies were treated with Perizin or with water. After the second Perizin treatment bees were collected from the top of the combs and transfered to Liebefeld cages in definite intervals (3, 7, 14, 21 days) and then fed by organophosphorus pesticides solved in saccharose like in the laboratory test (parathion: 40 ng/bee; dimethoate: 80 ng/bee). The results - received in three years during spring season - confirm the experiments mentioned above (Fig. 3).

By which physiological factors the increase of toxicity is caused?

For this reason we analyzed the activity of several potentially detoxicating enzymes of the midgut and one enzyme of the head, the well known acetylcholinesterase.

We found that the only enzyme activity effectively inhibited by Perizin were those carboxylesterases, which are not inhibited by para-hydroxymercuri-benzoate (inhibitor in fig. 4a + 4b).

Acetylcholinesterase of bee heads - main target of the toxicating effect of organophosphate compounds - usually was not inhibited by the Perizin-treatment though a slight inhibiting effect could be measured in some cases.

If we summarize the results, we can say: the tolerance for organophosporus pesticides is reduced in those bees which have been treated with Perizin. The biochemical reason seems to be an inhibition of certain esterases.

FIG. 1

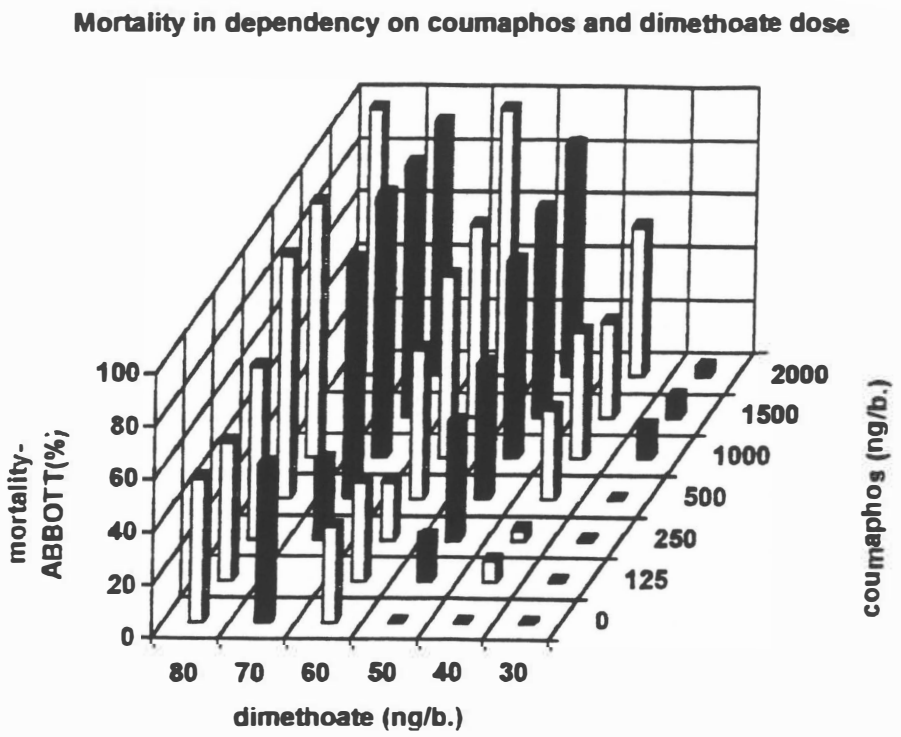
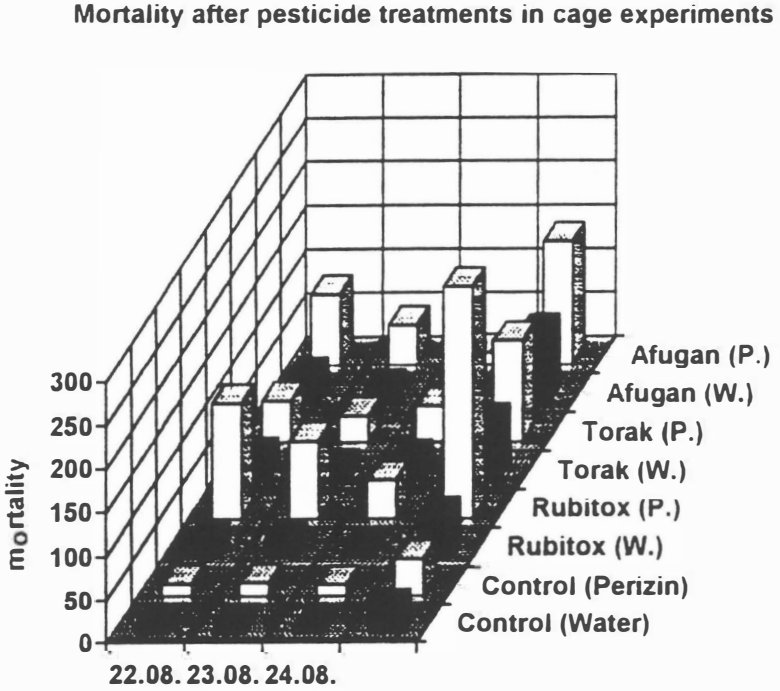

Mortality in dependency on coumaphos and dimethoate dose
mortality-
ABBOTT(%)
100
80
60
40
20
0
80 70 60 50 40 30
dimethoate (ng/b.)
2000
1500
1000
500
250
125
0
coumaphos (ng/b.)

FIG. 2


Mortality after pesticide treatments in cage experiments
mortality
300
250
200
150
100
50
0
22.08. 23.08. 24.08.
Afugan (P.)
Afugan (W.)
Torak (P.)
Torak (W.)
Rubitox (P.)
Rubitox (W.)
Control (Perizin)
Control (Water)

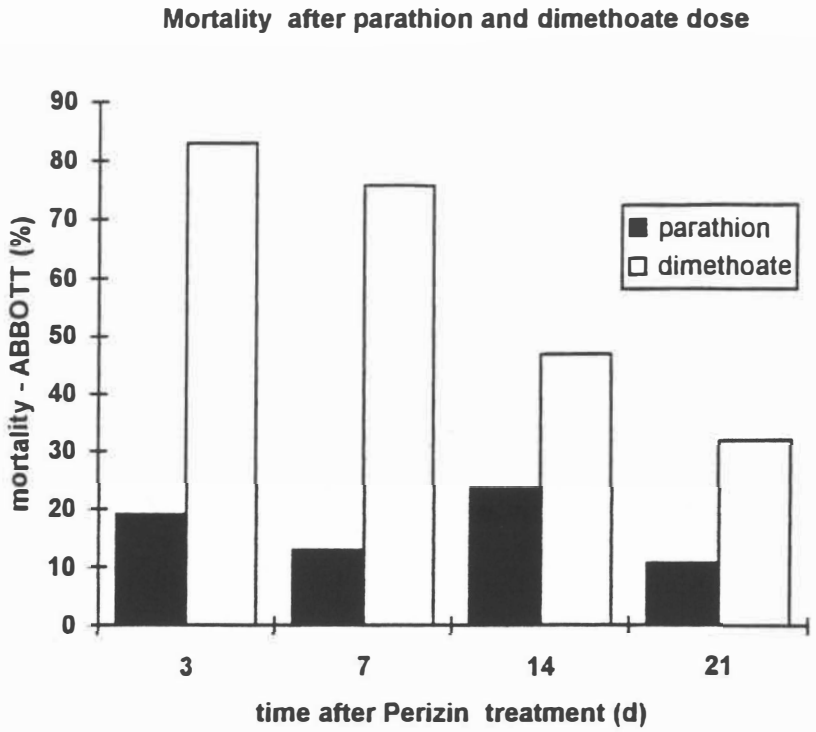

Mortality after parathion and dimethoate dose
mortality - ABBOTT (%)
90
80
70
60
50
40
30
20
10
0
parathion
dimethoate
3
7
14
21
time after Perizin treatment (d)

FIG. 4a

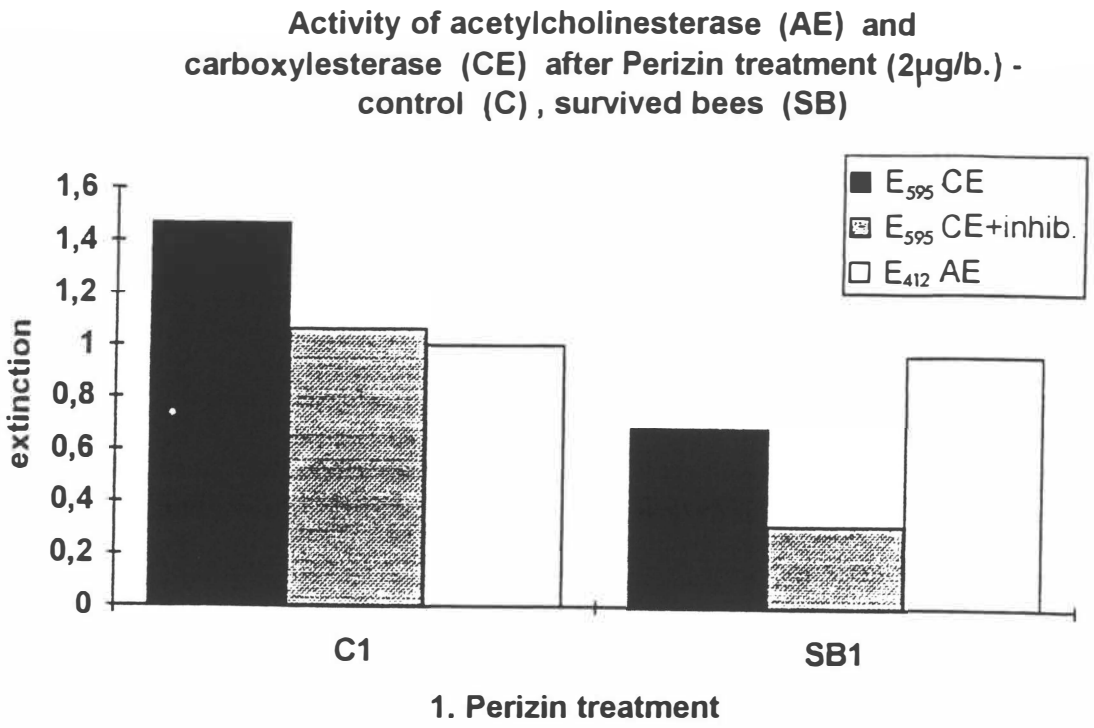
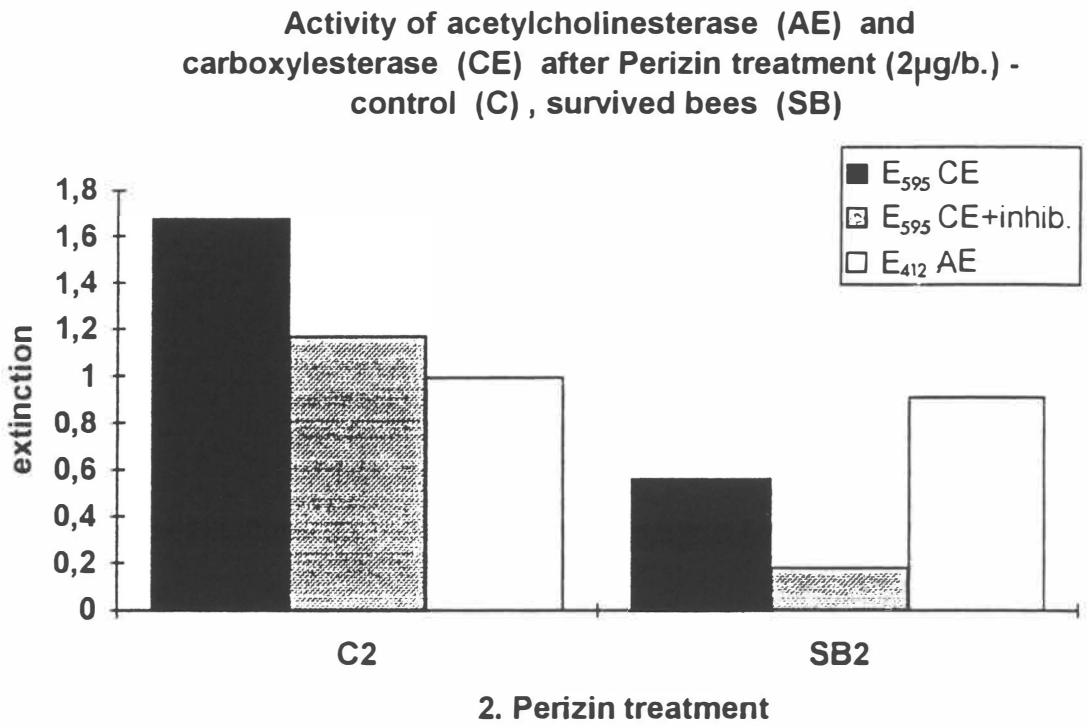

Activity of acetylcholinesterase (AE) and carboxylesterase (CE) after Perizin treatment (2µg/b.) - control (C) , survived bees (SB)
E595 CE
E595 CE+inhib.
E412 AE
extinction
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
C1
SB1
1. Perizin treatment

FIG. 4b


Activity of acetylcholinesterase (AE) and carboxylesterase (CE) after Perizin treatment (2µg/b.) - control (C) , survived bees (SB)
E595 CE
E595 CE+inhib.
E412 AE
extinction
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
C2
SB2
2. Perizin treatment

Appendix 14

W.W.M. Brouwer: European Community proposals for Evaluation and Decision making with respect to Pesticides.

European Community proposals for Evaluation and Decision making with respect to Pesticides

Ir. W.W.M. Brouwer
Plant Protection Service, Wageningen, The Netherlands.

July 25, 1991 the EC-Directive on registration of Pesticides was adopted. Within the EC, a positive list of active ingredients will be set up by the EC. This positive list of active ingredients (maximum of 700) will be Annex 1 to the EC-Directive. Data requirements for possible inclusion of active ingredients in Annex I, are given in Annex II.
Member states can registrate plant protection products, based on active ingredients included in Annex I. Data requirements for plant protection products are presented in Annex III.
Registration in one Member State implies that it has to be registrated in other Member States of the EC, when applicants apply to approval. Agreement on the content of Annex VI, the so called Uniform Principles (UP) - being rules for evaluation and decision making - and adoption of it allows for this mutual recognition.
Member States can only reject the mutual recognition of specific (applications) of plant protection products if the are able to demonstrate that it is reasonable for them to refer to one of the possible derogations. These derogations deal with (i) a specific ecological situation, (ii) deviating climatological conditions and (iii) deviating plant health conditions.
The UP consist of an introductionary, an evaluation and a decision making part. Text elements of each part, relevant to honeybees, are presented. No registration of a specific of a plant protection product is possible if the hazard ratio (defined as the ratio of dose (g a.i./ha) and LD50 (acute oral and contact)) is above 50, unless it will be shown by means of an appropriate risk assessment, that under field conditions there will be no unacceptable effects on honeybee larvae, honeybee behaviour, colony survival and development.
The Netherlands assume, that the UP will be adopted no later than the second half of 1994.
The parts of Annex II and Annex III, dealing with data requirements for honeybees, are presented. They appear to a large extent, to be based on the EPPO Risk Assessment Scheme for honeybees as was presented by Dr. Oomen.
In Annex II (active ingredients), an acute oral and contact toxicity test (LD50) and, when an active ingredient is an IGR, a bee brood feeding test are required.
In Annex III (plant protection products), none of the 4 tests is obligate. The need for these tests depends entirely on the results of the experiments with the a.i. and the results of former tests with the plant protection product. The sequence of the tests is the following: acute oral and contact toxicity tests, residue tests, cage tests and field (including tunnel) tests, indicating the need for more field related testing when applying risk assessment.
On specific aspects, the ICPBR is asked for her advise. These advises are submitted to the EC for possible inclusion in the EC-documents mentioned above.

EUROPEAN COMMUNITY PROPOSALS

for

EVALUATION and DECISION MAKING

with respect to

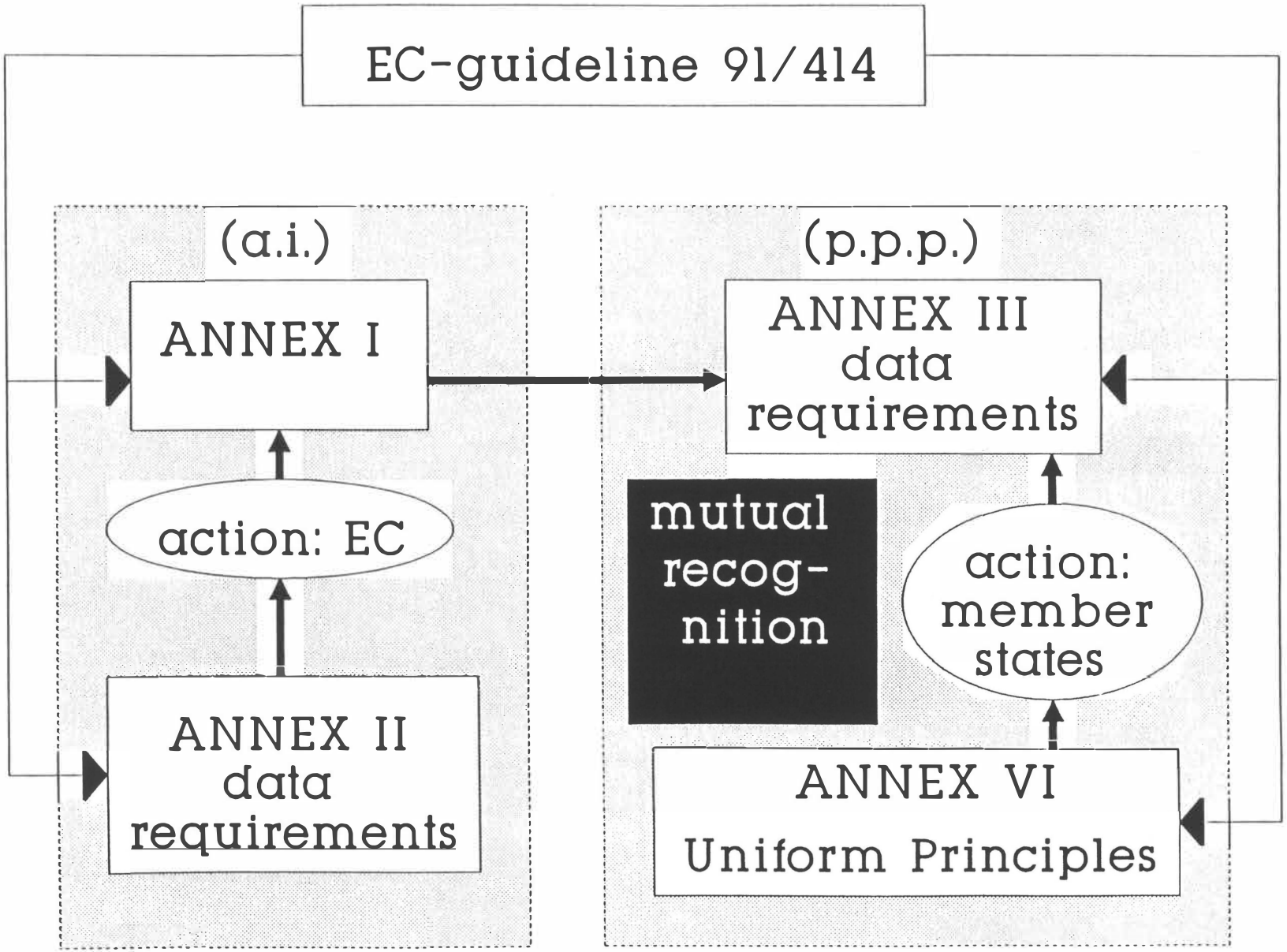
REGISTRATION OF PESTICIDES

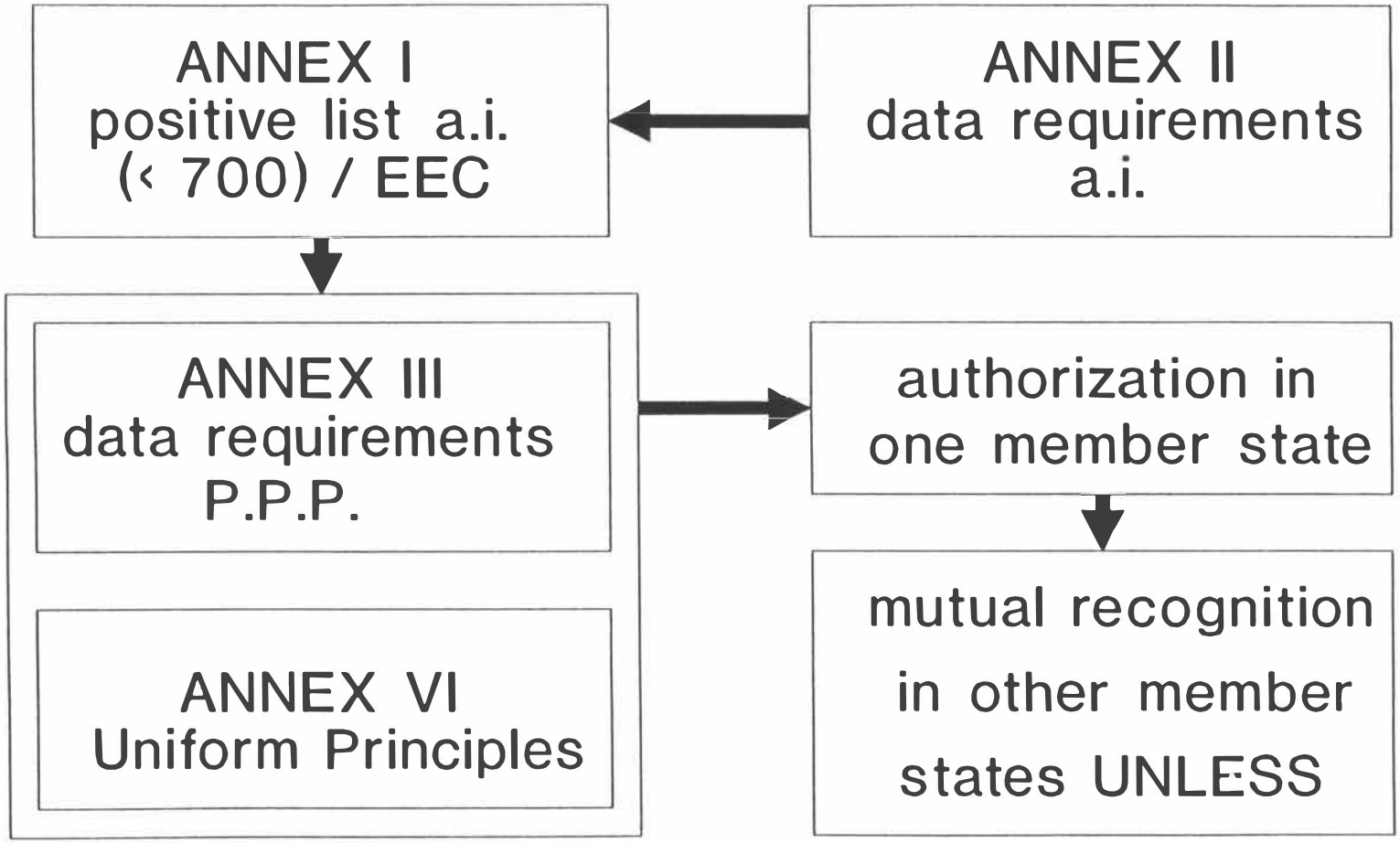
= =

EC-DIRECTIVE 91/414 and ANNEXES

Ir. Willem W.M. Brouwer/Plant Protection Service/Nl

presentation at ICPBR-workshop 26-28/10/1993, Wageningen


EC-guideline 91/414
(a.i.)
ANNEX I
action: EC
ANNEX II
data
requirements
(p.p.p.)
ANNEX III
data
requirements
mutual
recog-
nition
action:
member
states
ANNEX VI
Uniform Principles



DEROGATIONS:
1. environmental
2. climatological
3. plant health conditions

UNIFORM PRINCIPLES (ANNEX VI)

- PART A: Introduction
- PART B: Evaluation
  - general principles
  - specific principles (*)
- PART C: Decision making
  - general principles
  - specific principles (*)

(*) on: efficacy
worker protection
environment
public health
physical-chemical properties

environmental parts of UP

contains i) rules for Evaluation
ii) rules for decision making

with respect to:

(honey) bees

also to be used for Efficacy

INTRODUCTION

1. Uniform Principles aim to ensure that evaluations and decisions results in a high level of protection of man, animals and the environment

2. in evaluating applications and granting authorizations Member States shall:
   * take into account the data of Annex II
   * take into account the data of Annex III

EVALUATION - GENERAL PRINCIPLES

1 b. Member States shall:

* identify hazards arising,
* assess their significance
* judge on likely risks to man, animals and environment

2. Member states shall ensure that evaluations carried out have regard to proposed practical conditions of use

EVALUATION - SPECIFIC PRINCIPLES

Member States shall evaluate:

* possibility of exposure of honeybees: if yes ①
* short term risk to be expected
* long term risk to be expected

after use of P.P.P. according to the proposed conditions of use

information to be taken into consideration

(i) toxicity data from Annex II

(ii) relevant information on a.i., such as:

- Sw, Kow, P, DT50(photodegradation)
- mode of action (e.g. IGR activity)

(iii) relevant info, including tox data from Annex III

(iv) other authorized uses of P.P.P.

②

this evaluation will include:

(i) hazard quotients:

$$\text{ratio} \quad \frac{\text{maximum dose (g a.i./ha)}}{\text{contact and oral LD50 (µg a.i./bee)}}$$

and, where necessary, persistence of residues on or in treated plants

(ii) where relevant, effects on honeybee larvae, honeybee behaviour colony survival and development

DECISION MAKING

no authorization granted if:

$$\frac{\text{maximum dose}}{\text{oral/contact LD50}} > 50$$

UNLESS

- appropriate risk assessment
- under field conditions

there are no unacceptable effects
on honeybee larvae,
honeybee behaviour,
colony survival and development


Commission
of the
European Communities
expert meeting
5 member states

ANNEX II - Active ingredient

1. Acute toxicity
2. Bee brood feeding test

re 1. Acute toxicity

Aim of test:
- acute oral and contact LD50
- highest dose causing no mortality

When required: always, except when use of P.P.P. excludes exposure

Test conditions:
- a.i. not possible (precipitation in test medium, application problems) then with formulation
- mortality control: < 15% (48 h)
- reference compound ③

Test guideline: EPPO guideline 170

ANNEX II - Active ingredient - 3

re 2. Bee brood feeding test

Aim of test:	evaluation risks P.P.P. to honey bee larvae
When required	a.i. is IGR
Test guideline:	ICPBR method

④

ANNEX III - Plant Protection Product

1. Acute oral and contact toxicity

2. Residue test

3. Cage tests

4. Field tests

none of these tests is required obligatory

ANNEX III - Plant Protection Products

re 1. Acute oral and contact toxicity

Aim of test:
- LD50
- highest dose with no mortality

When required:
- only if exposure is possible
- more than 1 a.i. in P.P.P.
- if co-formulants may enhance toxicity of P.P.P. compared to a.i. ⑤
- prolonged exposure due to multiple applications
- 50 < Q(oral, contact) a.i. < 2500
  (Q = hazard quotient)

Test conditions:
- mortality control < 10% ④
- toxic reference substance

Test guideline: EPPO guideline 170

ANNEX III - Plant Protection Product

re 2. Residue test

Aim of test: ▶ evaluation risks of residual traces of P.P.P. on crops

When required: ▶ only when significant residual traces remain on crops

▶ Q(contact) › 2500

⑥

Except when: cage, tunnel or field are available

Test conditions: ▶ ageing of residues on leaves for 8 h, then 24 h of exposure ⟶ LT50 (h)

▶ if LT50 › 8 h, no further testing

Test guideline: not stated ----› subsidarity

re 3. Cage tests

Aim of test: ▶ evaluation of risks of P.P.P. on behaviour, colony survival and development

⑦

When required: ▶ significant effects in bee brood feeding test

▶ Q(oral, contact) > 50

Except when: field tests are available

Test conditions: ▶ control and toxic reference must be included

④

▶ mortality, behaviour changes developmental changes colonies

Test guideline: ▶ EPPO guideline 170

ANNEX III - Plant Protection Products

re 4. Field tests

Aim of test:

- evaluation of risks of P.P.P. on behaviour, colony survival, colony development

⑧

When required:

- significant effects in bee brood feeding tests
- significant effects in cage tests

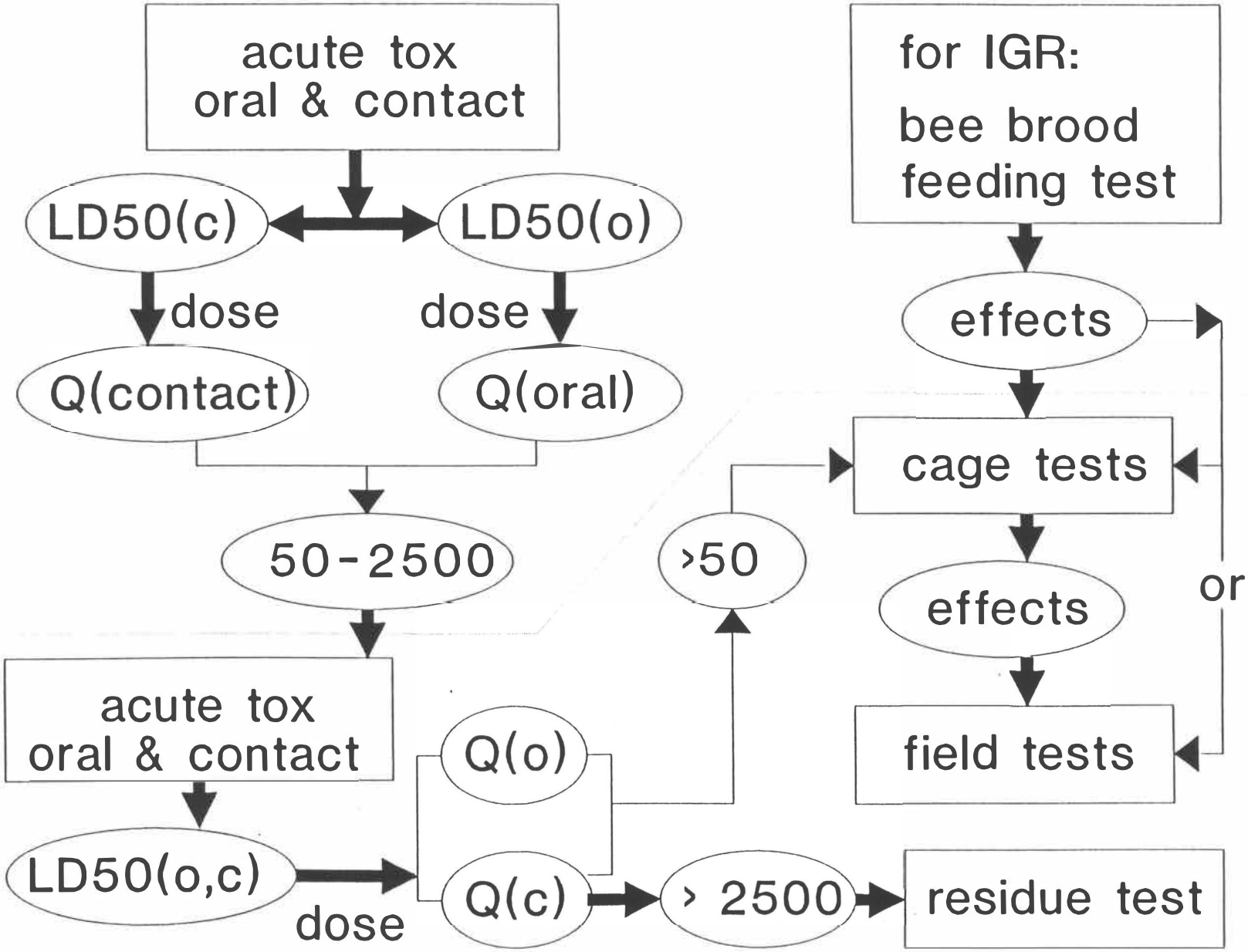
Test conditions:

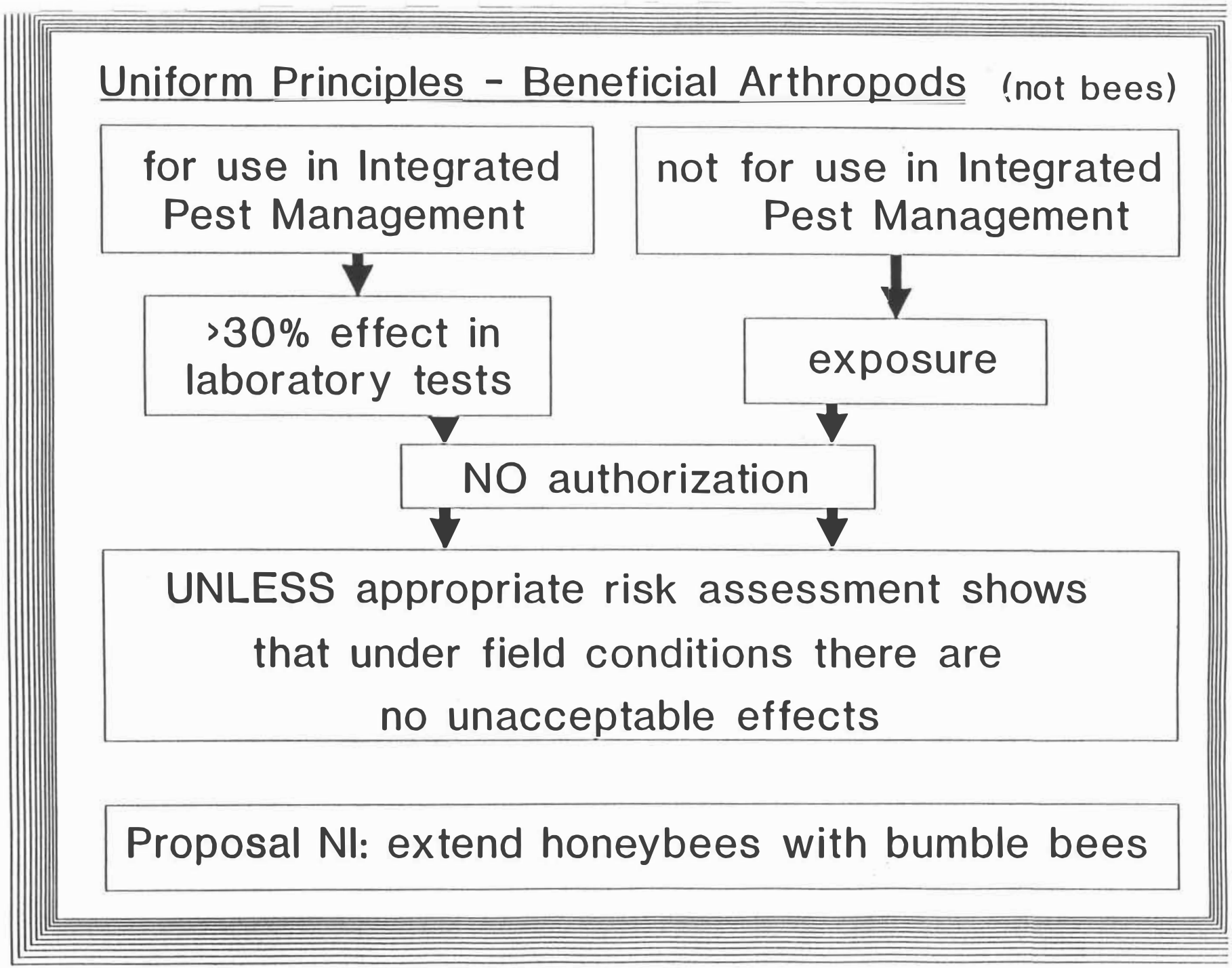
- strong, well developed hives
- control and toxic reference
- conditions representative of proposed use
- tunnel test when field test is not possible
- Special effects (larval tox, long residual effect, disorientation) require further specific testing

④

Test guideline:

- EPPO guideline 170


acute tox
oral & contact
LD50(c)
LD50(o)
dose
dose
Q(contact)
Q(oral)
50-2500
acute tox
oral & contact
LD50(o,c)
dose
Q(o)
Q(c)
>50
for IGR:
bee brood
feeding test
effects
cage tests
effects
field tests
or
> 2500
residue test


Uniform Principles - Beneficial Arthropods (not bees)
for use in Integrated Pest Management
not for use in Integrated Pest Management
>30% effect in laboratory tests
exposure
NO authorization
UNLESS appropriate risk assessment shows that under field conditions there are no unacceptable effects
Proposal NI: extend honeybees with bumble bees

①

25 July 1991 : 91/414 adopted

Uniform Principles

Annex II and III (environment)

april 93:

Definitive Commission proposal

august 1993

most recent version

21-10-93

discussions Working group Pesticides finalized

29-10-93

deadline comments Member States

? Raadsattaches

? Corps Representants Permanent

adoption by Standing Committee ?

? Council of Ministers

Appendix 15

D. Brasse: Investigations on reported poisoning incidents of honey-bee colonies in Germany.

Investigations on reported poisoning incidents of honey-bee colonies in Germany

Dietrich Brasse

Honey-bees are an important producing factor in fruit- and seed-growing. An increase of yield of some hundred percent can be reached by pollination with honey-bees. Therefore honey-bees have been protected since pesticides have been used for plant-protection. But nevertheless there are still existing some fields of conflict between beekeeping and plant-protection, which cause a lot of poisoning incidents every year. Since 1951 in (western) Germany nearly 11.000 poisoning incidents have been reported to the Federal Biological Research Center (BBA) in Braunschweig. In connexion with these incidents about 22.000 samples of dead bees, plants, wax and other materials have been investigated.

As it is not possible to take the samples by ourselves, there has been developed a guide-line for this purpose. In this guide-line are put together prescriptions for taking samples and prerequisites for the performance of investigations. The main points are:

- A bee sample must contain at least 1000 individuals, weight about 100 g.
- The minimum weight of a plant sample has to be at least 100 g.
- Samples of dead bees and plants must be packed separately to prevent a contamination of these materials to each other. Mixed samples are excluded from investigations, because they don't give the chance to clarify the cause of the incident.
- Samples must be packed thus that they do not start to decay. Decayed samples are also excluded from investigations.
- The beekeeper has to sent in an application form for the investigations which gives information about the circumstances of the poisoning incident. This application form must be sent by the beekeeper, to
  - the BBA
  - the beekeepers assotiation
  - the local plant-protection service
  - the beekeepers insurance.

Because of the high number of samples which are sent in every year to the BBA it was necessary to develop a practical running off for the investigations, as it is not possible to perform a com-

plete chemical investigation with all samples. The way out choosen was to divide the whole complex of investigations into a biological and a chemical part (Fig. 1).

The main part of the incoming samples exists of dead bees or contaminated plants. Samples of dead bees are running through three investigations:

1. Bioassay with larvae of Aedes aegypti L. (yellow fever mosquito). The test result gives an unspecific information, whether the bees are contaminated with a toxic substance or not.

2. Investigation on attack by Nosema apis Zander. Nosema is a wide spread disease of honey-bees, which is present in all populations at least in a latent stage. It is well known, that bees, which are attacked by the disease, are much more susceptible for the influence of pesticides. So the result of this investigation gives information, whether the incident might be influenced by an attack of Nosema. If indicated the bees are also investigated for other diseases and parasites.

3. Pollen-analysis. Mainly the pollen, which is attached to the hair of the bees gives information, where the bees have rested or collected food finally before they died.

Samples of contaminated plants have to be determined by flowers or leaves, if possible. They are tested in the bioassay with Aedes larvae.

The results of all biological investigations are combined in a diagnosis, which is according to the application form sent to the beekeeper, the beekeepers association, the local plant-protection service and the insurance. It is depending on this diagnosis whether investigations are continued by a chemical analysis on the basis of a combined method of gas-chromatography and mass-spectrometry (GC/MS).

The chemical investigations are performed, if

- results of the bioassay with bee and plant sample is positive, this means that in the bee and plant sample may existe residues of one or more substances toxic to bees,

- the pollen washed from the hair of the bees may come from that plant species which were collected for the plant sample.

- Chemical investigations are usually not performed, if the beekeeper sent in only a sample of dead bees. Usually in such a case it will not be possible to clarify the reason of the incident and to find out the causer of it.

Also the diagnosis of the chemical investigations is sent to the above mentioned institutions. By the involvation of responsible representatives of the beekeepers assotiation and the local plant-protection service there is given a good chance to clarify that poisoning incident, which has just taken place and to prevent those in future.

By the German plant protection act it is task of the BBA to investigate poisoning incidents to honey-bee populations. All investigations are done free of charge for the beekeepers.

Figure 2 shows the development of

- the stock of honey-bee populations in Germany
- the number of reported poisoning incidents all over Germany
- the number of investigated samples from all over Germany
- the number of reported poisoning incidents from Baden-Württemberg (south-west Germany)
- the number of investigated samples from Baden-Württemberg.

The number of poisoning incidents went down in the last 15 years continually. The highest number of incidents in the last 30 years was caused in the vine-growing areas in Germany between 1970 and 1980 by the application of Carbaryl-products. Nearly 50 % of all incidents annualley happend in the upper Rhine valley. The reason for this calamity was the common use Carbaryl-products during the flowering time of grape. This was only possible because it was unknown up to this time, that bees do fly into vineyards for pollen collection. During the establishment of the former version of the German honey-bee protection decree it was planned to allow the use of pesticides hazardous to bees also during the flowering period in vineyards. But Vorwohl described 1977 that honey-bees collect grape pollen. In the samples which had been investigated afterwards, grape pollen was found rarely and by the chemical analysis residues of Carbaryl could not be proved for a long time. But in the mainly used product Carbaryl was combined with Tetradifon, which is much more persistent than Carbaryl. So residues of this activ ingredient could be taken as proof for the use of Carbaryl-products and the cause of the poisoning incident. In 1981 the registration of Carbaryl-products was cancelled and promptly the number of poisoning incidents decreased.

In the period between 1980 and 1985 there was an increase of poisoning incidents in cereals, caused by the contamination of honeydew. The main reason for these damages was found in the wide application of Parathion to cereals.

In 1972 Kees and Obst found out, that it was possible to control leaf spot (Septoria nodorum) in wheat with Parathion and that Parathion had a side-effect also to other cereal diseases. Consequently farmers used Parathion-products for insect- **and** disease control. The presence of flo-

wering weeds and of honeydew was neglected. Up to this time the honey-bee protection decree prevented only the application of pesticides declared as hazardous to honey-bees to flowering plants. Stimulated by these poisoning incidents it was tried to complete the honey-bee protection decree regarding the protection of honeydew in cereals. By an intensive information campaign of the plant-protection services in connection with BBA the poisoning incidents in cereals decreased. But nevertheless in 1992 there was published a new version of the honey-bee protection decree which is now preventing the application of hazardous pesticides to plants with honey-dew, if these are visited by bees.

In the last few years there had not happend other eminent poisoning incidents. But together with a change in agriculture the fields of conflict between plant-protection and bee-keeping are changing too. This is the case with the increase of green farming or cultivation of crops for alternative energy. Some of these crops have been common in former times and are very attractive for bees as sunflower, buckwheat and broad bean. Especialley with the cultivation of broad beans there are arising again problems between plant-protection and beekeeping, because broad beans have a long flowering period, they are very attractive for aphids, which produce a lot of honeydew, and they have extrafloral nectaries. Further there are registrated only 2 insecticides for this purpose. All this makes aphid control in broad beans with most of the insecticides nearly impossible. As consequence there happened between 1988 and 1991 a lot of poisoning incidents in bread beans. But the number of these incidents decreased again so that it can be stated that there is a decrease of the reported poisoning incidents in total. Figure 3 gives a survey of the distribution of honey-bee poisoning incidents to different cultivations. The most remarkable changes in the portions of poisoning incidents since 1973 have happened with those in bread beans and vineyards. Damages of honey-bee populations by the use of pesticides in vineyards went back from 12 % to 3 % and damages in bread beans went up from 3 % to 14 % in the period between 1988 and 1991. Moreover it is unsatisfying that there can be recorded during the whole period a great portion of poisoning incidents (27 % - 42 %) of which the circumstances of their origin remain unknown. Likewise it is depressing that there are happening many deliberate poisonings of honey-bee populations every year: 13 % - 30 % of all recorded incidents.

Though there are applied always new methods of investigation in the chemical analysis it is not possible in every case to clarify the real cause of the poisoning incident. Partly there have been found different active ingredients of hazardous pesticides in bees and plants, partly the spectrum of detected residues in the samples do not harmonize with each other, partly the number of detected compounds is too high to make one special responsible for the poisoning incident. A special problem in clearing up the real causes of the poisoning incidents is the influence of honey-bee diseases and their control, especially Varroa control, and the influence of wood preservation of the beehives. In many of the investigated samples of dead bees and wax residues of agents for Varroa control and wood preservation can be found. It is possible to prove the active ingredients

of all registrated and not registrated products for Varroa control. As the influence of these compounds to bees is long lasting, there are various situations in the life of a bee, in which it may intensity the influence of other chemicals. It seems to be necessary to investigate this problem in future.

Figure 4 shows the geographical distribution of the poisoning incidents, which have happened in 1993. The map shows a concentration of incidents in south-west Germany (Baden-Württemberg). Most of the incidents are supposed to be caused by the use of Fenoxycarb in orchards. Because of this concentration of poisoning incidents the BBA has started a monitoring programme for the proof of Fenoxycarb in samples of dead bees and pollenloads.

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Investigations on poisoning incidents of honey-bee colonies

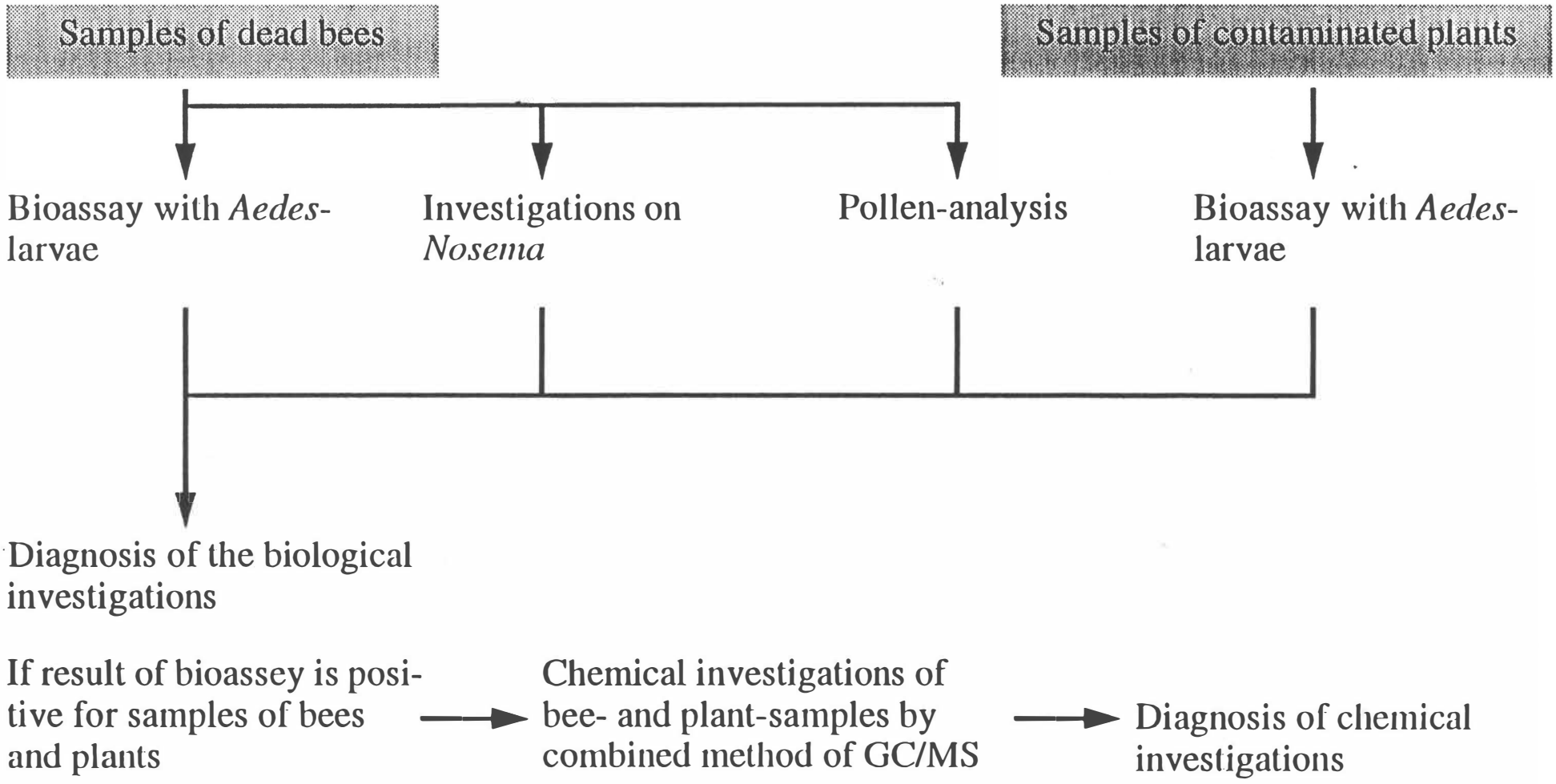


Fig. 1

Poisoning Incidents in Germany 1960-1992

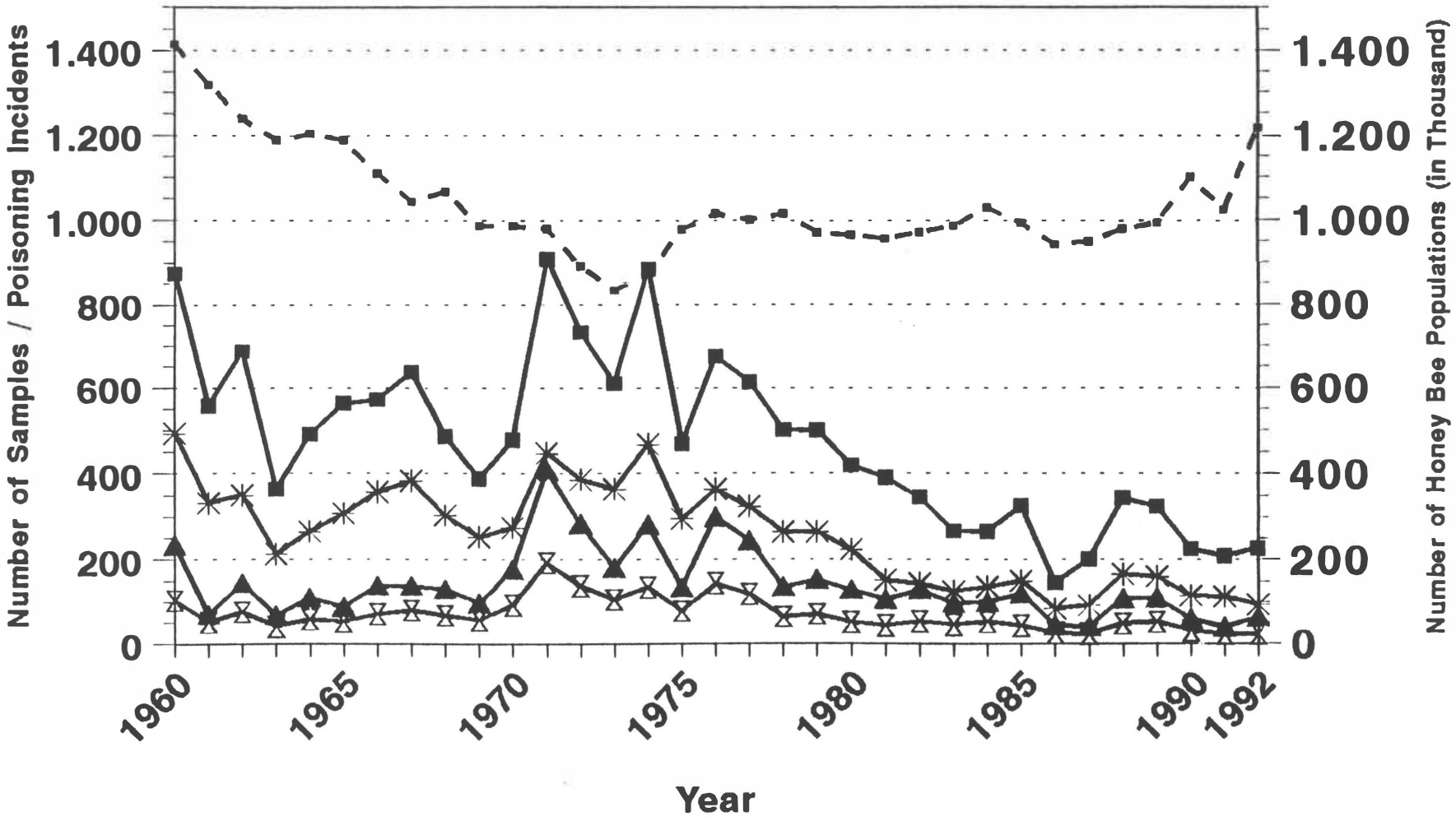
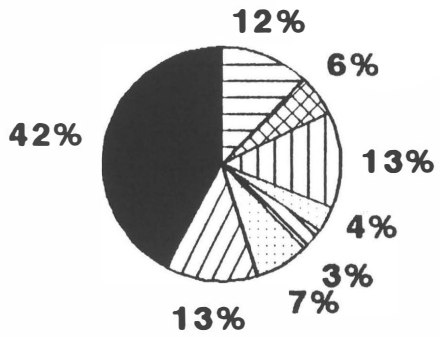
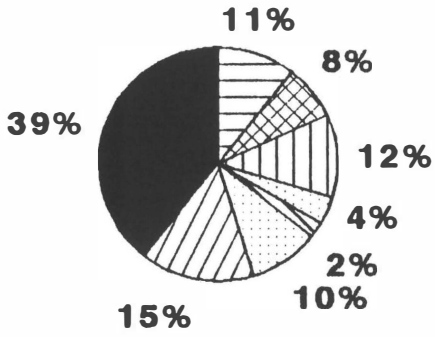


Fig. 2

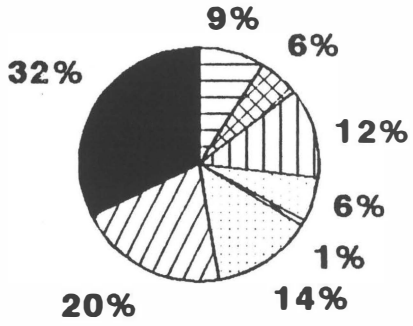
Distribution of Poisoning Incidents to Different Cultivations
1973-1992



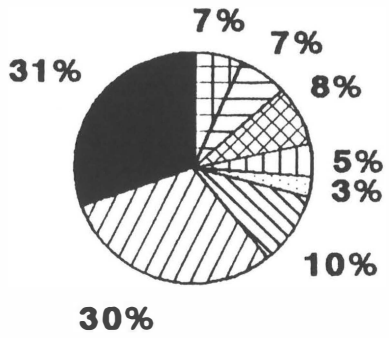
1973-1976 (N=1514)



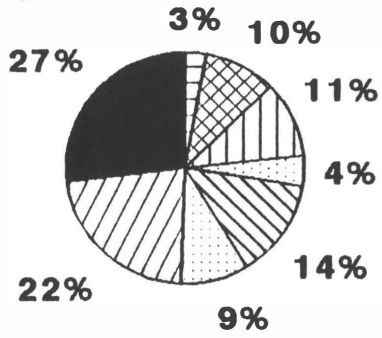
1977-1979 (N=852)



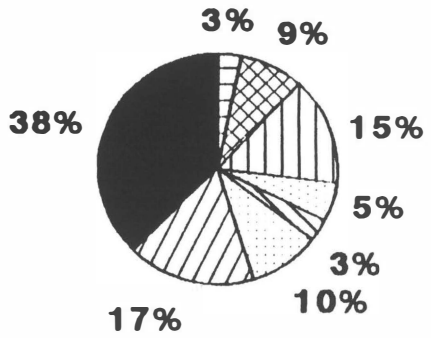
1980-1983 (N=638)



1984-1987 (N=459)



1988-1991 (N=552)



1992 (N= 93)

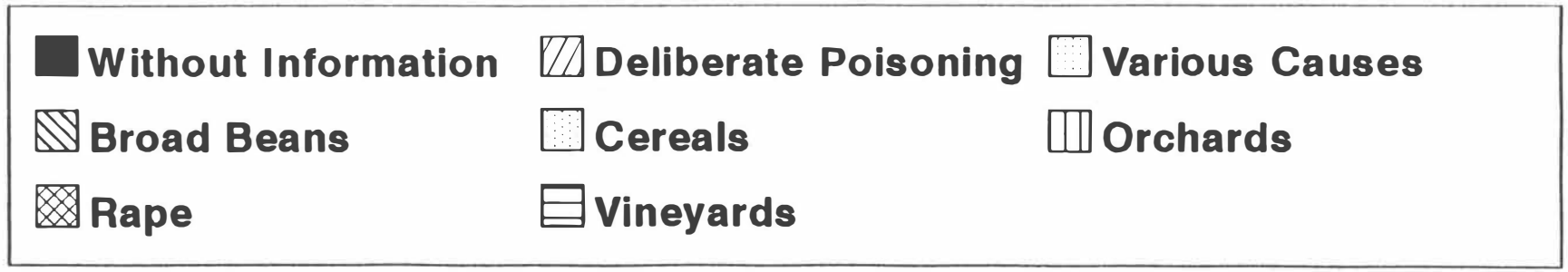


Fig. 3

Geographical Distribution of Poisoning
Incidents all over Germany in 1993

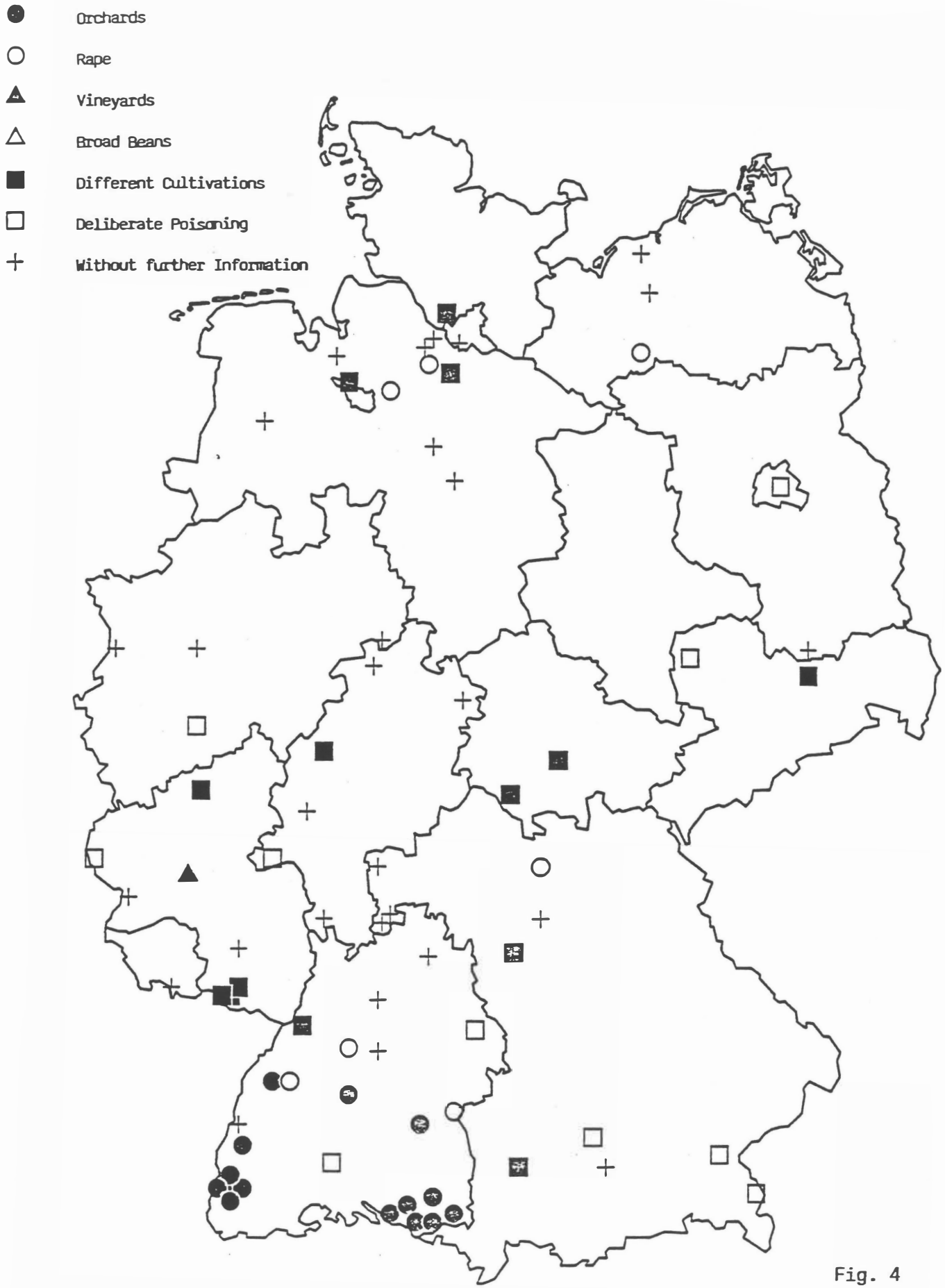


Fig. 4

Appendix 16

J.A. Stark: Bee toxicity

BEE TOXICITY

by
Josef A. Stark

Swedish University of Agricultural Sciences
S-750 07 Uppsala (Sweden)

SUMMARY

As a result of their mobility, bees and other pollinating insects are exposed to a large number of chemical substances in the environment, including pesticides used in agriculture, forestry and horticulture. Developments in the bee toxicity sector show a reduced number of incidences. Reports of bee toxicity, however, have been of greater extent and possibilities to explain causality by means of conventional analytical methods have decreased. The present complicated picture probably depends on bee toxicity interacting with the general chemical environment and use of pesticides.

SWEDISH APICULTURE

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

On the whole, Sweden has good natural conditions for bee-keeping. Both the natural flora as well as cultivated plants offer good opportunities for yields of honey up to 40-60 kg honey per colony and year. An increase in the size of the apiaries, towards a more professional form of management, will probably lead to the introduction of more rational working methods, which will have a stimulating effect on bee-keeping in all size categories. In order to utilize the bees for pollination by means of mobile bee-keeping, there is a decisive advantage in being able to recruit bee colonies from several different places, However, this involves an increased risk of spreading diseases between apiaries.

At present, there are about 15 000 organized bee-keepers within the Swedish Bee-Keepers Association (SBR). Together, these own 100 000 bee colonies. In

addition, there about 200 professional bee-keepers with 6 000 colonies. Apart from these two groups, the number of unorganized bee-keepers is judged to be about 5 000, with 30 000 colonies. The domestic production of honey is about 3 000 tonnes which is about 40 % of the honey consumed in Sweden. The annual imports amount to about 2 500 tonnes of honey. Thus, Swedish production of honey could be doubled without the risk of over-establishment.

BACKGROUND AND PRESENT DEVELOPMENT

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

During the late 1940´s, compounds developed for the military were used. The enthusiasm at that time to rebuild after the war, the need for production improvements, and belief in the positive effects of chemicals, overshadowed the negative secondary effects that occasionally occurred. There is no doubt that the work of Ahlmark (1949) belongs to the early warning reports that contributed to increased demands for research into the pesticide sector. The use of pesticides has led to injury both to humans, animals and wildlife. We can observe that increasing demands placed on profitability, modern cultures, awareness about the need for control inputs, and the search for more effective compounds, has resulted in many new compounds being used during the 1950´s (Schwan, 1978).

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

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

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

Of these 65 samples, 51 contained pesticides in concentrations far below the LD50 values given for the herbicidal question. Thirty samples contained residues of

the pesticide Fenitrotion, which was responsible for 95 % of all explained bee toxicities up to the early 1980´s.

A NEW PERIOD IN THE USE OF PESTICIDES

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

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

PROBLEM IDENTIFICATION

The use of chemicals in agriculture has noticeably changed character since the early 1980´s, mainly as a result of the introduction of pyrethroids and later as a result of the decrease in use of chemicals in agriculture. An increased area of fallow has temporarily brought the relationship between herbicides and bees into focus.

During recent years, there have been a number of comprehensive cases of poisoned bees which have been unexplainable. Large-scale bee poisoning was earlier mainly caused by insecticides, but herbicides and occasionally fungicides and synergism may also be possible reasons. The situation was complicated by the introduction of pyrethroids. The substances have been demonstrated in the laboratory to be 100 times more toxic than the phosphoric substances used earlier. Under some conditions, pyrethroids show relatively low toxicity if recommendations on time of application, dose, crop development, etc., are followed. A number of studies, including those at the Bee Division, have demonstrated that despite repellence the bees continue to collect contaminated pollen which is packed and/or mixed with other pollen from cultivated or wild plants. A situation of this kind may lead to gradual toxicity with decreased colony strength, loss of queens and sometimes winter losses that cannot be explained by the normal reasons for such losses.

Another problem is the reliability of the established LD50 values. Among 65

samples of bees analysed for insecticides, concentrations found in all cases were below the established LD50 value. The reason might be that some degradation of insecticides had taken place before the samples reached the laboratory or that under field conditions, the LD50 values might be lower than those found in the laboratory studies. A third explanation might be that some LD50 values have been taken from winter bees that have a physiology different to that in summer bees.

The number of bee toxicities reported decreased in the early 1980´s but a number of difficult cases have occurred. It has been reported that glyphosate caused bee toxicity, or that rain following application of pyrethroids resulted in toxicity. In the neighbourhood of the apiary concerned, there was a workshop where worn-out car tyres were burnt.

In addition to the cases of bee death mentioned above, there have also reports on losses in apiaries located near major industrial complexes. The problem is complicated further since only one of 25 reported cases of bee mortality contained a sample demonstrating the presence of pesticides following laboratory analysis. Thus, many cases of toxicity occur where the traditional chemical analysis is not capable of explaining the cause.

In discussions with bee-keepers, experts on pesticide analysis, and scientists working in the pesticide sector, the following questions have been seen as possible hypotheses in coming closer to solving the bee toxicity problem.

PROBLEMS TO STUDY

1) Chemical analyses of submitted samples must include not only the original substance but also possible products that have developed after chemical, photochemical or biological degradation of the pesticide concerned. This may also concern products formed during detoxification of the pesticide in the bee´s alimentary tract.

2) The occurrence of isomers should be identified and details on such isomers obtained.

3) The stress situation of the crop when pesticides are used. Stress may result in secretion of toxic substances, which are largely found in the flower. These may also be a combination of the pesticide´s degradation products and the plant´s own toxins.

4) Occurrence of mycotoxins formed under field conditions.

5) Use of fungicides in connection with full-bloom. The effect on the lipid body of bees in connection with the use of fungicides and they synergistic effects with pyretroids.

6) Herbicides and their effect (new low-dose herbicides).

7) Industrial discharges, exhaust fumes from traffic (glycol).

8) Reductions of bee immunity and susceptibility to chemicals resistans and generaly immunobiology related to chemical and pesticides.

BEES AND THE ENVIRONMENT

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

The honey bee moves over a wide area, up to about 3 km radius from the hive, visiting numerous plants and many sources of water. Against the background of this behaviour, the bees exposed to numerous sources of pollution that imply the risk of toxicity. Tong *et al.* (1975) consider that contamination of the bee is possible when it flies in polluted air. The same authors state that the bee occasionally confuses polluting substances in powder form with pollen and take it back to the hive. Atmospheric contamination of the flower´s nectar results in the same degree of pollution as if the bee colony had been directly contaminated. Plants absorb and concentrate specific elements from the soil (e.g., pesticides, heavy metals). Bees visiting these plants will thus become secondary transmittors of pollution. Pollution has also been found in the bee (body, mouth parts) and/or in collected pollen, nectar, water and propolis, as well as in honey (Stark, 1991).

AGRICULTURE — BEE-KEEPING

Unfortunately, there is a conflict between bee-keeping and use of chemicals in agriculture. However, at present it is unrealistic to expect Swedish agriculture to operate entirely without pesticides. The use of chemicals results in numerous bee colonies being more or less damaged every year. Frequently this depends, however, on carelessness or lack of knowledge about the properties of the pesticide and the use of pesticides in general (Stark, 1992).

PYRETHROIDS

The increase of bee toxicity following the introduction of pyrethroids expected by bee-keepers did not occur, probably depending on a number of factors that can be ranked as follows: low-dose pesticides, repellents, time of application, better education, length of the flowering process, temperature, crystallization, and spray drift (Stark, 1992). We may state that adult bees are rarely injured by pyrethroids

(Sumicidin-alpha, Karate, Decis, Fastac) under field conditions (Fries & Stark, 1984). In some cases, there is no effect on bee brood on account of the dilution effect (admixture of pollen from unsprayed crops or wild plants).

PESTICIDE RESISTANCE

The economic importance of the bee in agriculture has led to studies of the relationship between the honey bee and pesticides, where it has been demonstrated that the bee tolerates or demonstrates resistance to pesticides. This can be seen within and between bee colony resistances to certain pesticides. It appears that the within-colony resistance may be greater than the resistance between colonies as a result of individual drones contributing to this property.

Several examples in the literature suggest that the reaction of bees to pesticides can be influenced by artificial and natural selection, which should be able to result in pesticide resistance (Hoy, 1992). As results of resistance , some cuses of beepoisning never be solved. Bees probably degradated pesticides befor they died.

SUMMARY AND CONCLUSIONS

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

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

Against the background presented in this paper, together with earlier papers published on pesticides a suitable start to the mapping of the bee toxicity problem would be:

1. Compilation of data on bee toxicity and causality published in scientific, popular scientific and extension literature.

2. A review of the National Chemical Inspectorate´s data on pesticides used today with regard to the occurrence of any degradation products of their isomers and their effect

on beneficial insects in general and pollinating insects in particular.

3. Survey of collected material hitherto (bees and pollen) using biological tests (banana flies). If possible, a parallel development of an enzymatic detection system (benzopyrenemonoxidase) should be conducted for determination of the extent of the effect of the chemical environment on the honey bee.

4. Monitoring of the two areas in southern Sweden mentioned earlier where the most difficultly explainable cases of bee toxicity have occurred.

5. Establishment of a small number (5) of monitor apiaries where activities and losses of bees can be studied.

During summer 1993 a number of monitor apiaries were established with design as follows: Every monitoring apiay consisted of 9 colonies. Three of these used to monitor dead bees using dead-trapmethod. In three colonies sampling and collecting pollen was performed every day when collecting was possible. Three colonies were controlled by beecounter, registrating out and ingoing bees. In the experiment two different methods were used to follow the development of beebrood. Samples of bees were collected once a week. Wheater data were collected everyday using small meteorological station and maping of the environment was done in diameter of 3 km from the apiary. Biological and chemical analyses on above colected material were started during the autumn of 1993.

In addition to studying the above problems, ongoing research in apiculture should be directed at identifying the total risk area related to toxification of bees and other pollinators.

Known technology should be used to develop systems of following-up cases of bee toxicity.

Studies should be made to map the interaction of pesticides with the total chemical load on the environment where pollinating insects are active.

Recommendations should be prepared for authorities, pesticide users, and bee-keepers giving information that can lead to the minimization of injury to pollinating insects. Results and methods in the research programme must be continuously revised and, if possible, coordinated with corresponding activities in other countries.

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Appendix 17

P.A. Oomen: Signalling scheme for poisoning incidents in the Netherlands as reported by bee keepers: an overview 1990-1992.

ICPBR - Symposium
Hazards of Pesticides to Bees
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Plant Protection Service, Wageningen, The Netherlands
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Signalling scheme for poisoning incidents in the Netherlands as reported by bee keepers: an overview 1990-1992

1. Approach of honey bee protection

The approach of protecting honey bees from side effects of pesticides in the Netherlands is based on five different steps:
(1) well defined pesticide uses allowed for crop protection
(2) risk assessment for each use
(3) risk management by eliminating high risks by legal prescriptions on the label, prohibiting pesticide use on specific crops in flower
(4) information about good agricultural practice on pesticide use distributed by extension service among users
(5) legal enforcement of label prescriptions by AID (Agricultural Inspection Service).
The Plant Protection Service (PPS) advices the Board for Registration of Pesticides (CTB) on all matters concerning 1, 2 and 3.

2. Possible errors

The effectivity of honey bee protection depends mainly of the:
quality of risk assessment
effectivity of risk management
In risk assessment two errors are possible: incorrect classifications as high risk (false positives) and incorrect classifications as low risk (false negatives). In risk management also two comparable errors are possible: unnecessary prohibitions and omitted prohibitions. All these errors should be traced by feed back from practice for correction and optimization. In short, a sixth *evaluation* step is necessary.

3. Need for a monitoring scheme

For this purpose of evaluation a monitoring scheme for investigating all suspected poisoning incidents would be ideal: a scheme that would enable to identify the main errors, i.e. false negatives and omitted prohibitions. Such a scheme is operational in some European countries but lacking in Netherlands. It has up to now not been possible to start such a formal monitoring scheme. Data on side effects of pesticide use in practice has been formally available only from the enforcement inspections by the Agricultural Inspection Service. These data however are good to confirm the effectivity of the risk management but not to evaluate the (errors in) risk assessment or risk management.

4. Development of a signalling scheme

Therefore an approach was developed in the Netherlands for signalling suspected cases of bee poisoning. Bee keepers are well organized in bee keeper societies. These societies provide bee keepers with standard forms for registering cases of suspected bee poisoning. Simultaneously they urge bee keepers to inform the societies by way of the form about all suspected poisoning incidents. Every year these forms are collected by the societies and analyzed by the PPS, and discussed in a meeting of all parties concerned (Bee Keepers Societies, Agricultural Union, PPS, Extension Service, Agricultural Inspection Service). In this meeting appointments are made for avoiding the signalled problems in future. The tasks of the different institutions are:

PPS: improving risk assessment and risk management.

Extension Service: informing farmers how to apply pesticides more safely.

Agricultural Inspection Service: improving enforcement of prescribed uses.

Bee Keepers Societies: Stimulate and facilitate registration by bee keepers.

5. Results of 1990-1992

Since the scheme started functioning in 1990 only a limited number of responses have been registered (1990: 19; 1991: 40; 1992: 17).
The registered cases are not verified or validated in any way: they just signal potential problems. There is a certain overlap with cases from the Inspection Service. This overlap gives some clues to the completeness and the reliability of the signals from the bee keepers. The limited number of signalled cases and the overlap with the AID-cases suggest that honey bee protection from pesticide effects is relatively well functioning.

The experience with the scheme is that information in many cases is insufficiently complete to be used as a signal. Nevertheless in most years there are some remarkable cases that can be used to improve the risk assessment and/or management.

In 1990 2 out of 19 cases suggested the use of MCPA as cause of bee poisonings. MCPA is registered as not harmful to honey bees in the Netherlands. Indications how MCPA may be harmful to honey bees are completely lacking.

In 1991 13 out of 40 cases signalled serious poisoning of bees after aphid control in potatoes with different insecticides. The problem was the massive presence of flowering *Polygonum persicaria* in the potato fields.

In 1992 there was only one useful case out of 17 registered. Bees appeared to be poisoned as a consequence of aphid control in lime trees by injection of the trunc with the systemic insecticide acephate. This is not a registered use in the Netherlands but occurred during an experiment for efficacy evaluation of the method.

6. Preferred way of feed back

The preferred way to use the registered signals is principally in complete accordance with the normal procedure for new pesticides (see par. 1).

The signalled problem is analyzed. When an error in risk assessment (false negative) is likely, then the pesticide producer is required to send information for specific risk assessment in the crop in question, usually at the moment of reregistration. Then a specific risk assessment is done, and a new risk management instruction in the label of pesticide may be prescribed. When an error in risk management (omitted prohibition) is likely, the company may be required to adapt the label at the first moment of reregistration. When the risk management appears insufficiently effective the extension service is asked to inform the farmers about the preferred way to avoid risks.

7. Conclusion

The scheme has a very useful signal function that enables to improve the methods to protect honey bees in the Netherlands from side effects of pesticides. Very serious drawbacks however are the incompleteness of the information received and the total lack of verification. The scheme therefore has little quantitative value other than in general terms. So it appears from the signalling scheme that the number of registered problems as experienced by the bee keepers is relatively low. This suggests that risk assessment, risk management and legal enforcement are effective in the Netherlands. For true evaluation of the effectivety of bee protection from side effects of pesticides however a real monitoring scheme would be necessary.

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Appendix 18

MR. Fletcher, P.W. Greig-Smith and J.H. Stevenson: The scheme to investigate the suspected poisoning of honeybees by agricultural chemicals in England and Wales.

THE SCHEME TO INVESTIGATE THE SUSPECTED POISONING OF HONEYBEES BY AGRICULTURAL CHEMICALS IN ENGLAND AND WALES.

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INTRODUCTION

In England and Wales, incidents involving honeybee deaths are investigated as part of the Wildlife Incident Investigation Scheme (WIIS). Before approval is granted for the use of pesticides, any side-effects on wildlife, including honeybees, has to be assessed. This post-registration monitoring carried out by the Scheme is a component of the regulation system. It acts as a long-stop to detect honeybee mortality problems that were not identified in the risk assessment procedure carried out prior to approval. The findings of the Scheme are reported to the regulatory bodies and if an unacceptable risk is found to occur then the conditions of approval of the compound involved may be withdrawn or modified under the Control of Pesticides Regulations 1986. A similar scheme is in operation in Scotland and Northern Ireland.

The Scheme also provides evidence in those cases where there has been an infringement of the legislation. This may have arisen as a deliberate abuse of an agricultural compound or the misuse of a product, by careless, accidental or wilful failure to adhere to the correct use.

The data generated by the Scheme shows actual risks to honeybees and this can be used to validate and improve the risk assessment methods used in the registration of products (Hart and Greig-Smith, 1992). A report collating the results of the Scheme from England, Scotland, Northern Ireland and Wales, is published annually (eg Fletcher and Hunter, 1993).

There has been a monitoring of honeybee poisoning incidents for several years (Needham *et al.*, 1966; Stevenson *et al.*, 1978). The provisional approval to allow aerial applications of the organophosphorus compound triazophos for control of pests in oilseed rape in 1977, resulted in a large number of incidents of honeybee mortality during the following two years (Stevenson *et al.*, 1979). As a result of these incidents a more formalised system was set up to investigate honeybee deaths, which has developed into the present scheme.

This paper outlines the operation of the Scheme and presents some of the results from the last ten years (1983-1992) updating the previous paper of Greig-Smith (1990).

METHODS

The present scheme has been in operation since 1980, although in the first two years the emphasis was placed on the detection of organophosphorus compounds, particularly triazophos.

Mortality amongst honeybees is usually detected at the hives by the beekeeper. In England and Wales, samples of dead bees are sent to the Central Science Laboratory's (CSL) National Bee Unit. Here the bees are screened, on the day of arrival, for the presence of disease. In particular, examination for the presence of diseases such as Acarine, Nosema, Amoeba and Varroa are carried out on a sample of about ten bees. The identification of pollen loads is also carried out so that the crops on which the bees have recently been foraging can be identified. The bees are then deep frozen and batches are sent overnight to the CSL's Wildlife Incident Unit for pesticide analysis.

Field inquiries are carried out by trained field staff in the area near to where the bees were reported dead. Information is collected to gather relevant information as to the possible source of the poisoning. Information is gathered from the beekeeper on symptoms and behaviour of the dying bees; the condition of the colonies and weather conditions at the time of the incident. An opinion as to where, and on what, the bees had been foraging is also sought and if any

spraying had occurred locally, by whom, with what and on what. Local farmers with fields likely to be visited by bees may be similarly questioned.

The samples of bees, usually in excess of 200 bees, are dispatched to the Wildlife Incident Unit of the CSL in England and Wales. The results of the disease assessment and the field inquiry report are also forwarded. After extracts are taken through a clean-up process, analyses for residues of organophosphorus, organochlorine, carbamate and pyrethroid compounds or their metabolites are carried out using multi-residue, chromatographic, detection techniques. If there is evidence to suggest the involvement of certain herbicides or fungicides, then, if a method of analysis from bees is available, this may be carried out. All residues detected are confirmed using an alternative method. Additionally, samples of foliage, honey or comb may be analysed to help identify the source or cause of an incident.

In the earlier years of the scheme (up to 1984), bees were routinely screened to assess if there was significant acetyl-cholinesterase inhibition from the heads of the casualties. The mode of action of organophosphorus and carbamate compounds is to inhibit this enzyme. However, work carried out by Westlake *et al.*, (1985) showed that this could be unreliable as natural inhibition of activity occurs when there is a time factor between the time of death of the bees and when they are found. The conditions under which the bees are subsequently kept may also affect inhibition. This biochemical measurement is no longer applied to bee samples.

The levels of residues detected are closely examined, so that their significance can be reported. Using figures giving the acute oral and contact LD50 of various compounds (Stevenson, 1968; Oomen, 1986), and with some idea as to the potential losses in the time prior to and during analysis a judgement can be made as to whether the residues represent lethal levels to the bees or merely exposure.

RESULTS

During the years, 1983 to 1992, some 859 incidents occurring in England and Wales were investigated. There have been 458 incidents (53%) where poisoning by agricultural chemicals was identified. Each incident may involve several beekeepers around the area where it occurred, and each beekeeper may lose one or several colonies as a result of the poisoning.

The number of incidents investigated varies from year to year, and since 1987 there has been a decline in those investigated and also in those found to involve pesticides (see Table 1).

Table 1: Number of incidents involving honeybee in England and Wales 1983-1992

Year	Number of incidents investigated	Number (%) in which pesticides was detected
1983	103	74 (72%)
1984	127	60 (47%)
1985	96	47 (49%)
1986	52	35 (67%)
1987	106	57 (54%)
1988	99	52 (53%)
1989	87	49 (56%)
1990	81	43 (53%)
1991	74	29 (39%)
1992	34	12 (35%)
TOTAL	**859**	**458 (53%)**

Organophosphorus insecticides are the compounds most likely to be involved in honeybee deaths. Table 2 shows the number of these compounds that have been detected in incidents over the ten year period. Two compounds dominate the results over these ten years, triazophos and dimethoate. These two compounds accounted for over three quarters of all pesticide poisoning incidents.

The only organochlorine compound to be detected was gamma-HCH; there were 40 incidents over the ten years (see Table 3).

Table 2: Number of organophosphorus compounds detected in honeybee incidents from England and Wales 1983-1992

Year	Triazophos	Dimethoate	Others #	Total incidents*
1983	44	8	1	53
1984	33	17	6	56
1985	34	7	2	42
1986	17	6	4	27
1987	57	6	12	65
1988	16	22	3	41
1989	15	16	5	34
1990	20	10	7	33
1991	8	8	11	20
1992	2	3	5	7
Total	**246**	**103**	**56**	**378**

* more than one compound may be found in a single incident

Other compounds

1983	Malathion	1
1984	Chlorpyrifos	2
	Pirimiphos-methyl	2
	Demeton-S-methyl	1
	Oxydemeton-S-methyl	1
1985	Chlopyrifos	1
	Disulfoton	1
1986	Pirimiphos-methyl	1
	Demeton-S-methyl	3
1987	Demeton-S-methyl	2
	Phosalone	8
	Fenitrothion	2
1988	Azinphos-methyl	1
	Pirimiphos-methyl	2
1989	Azinphos-methyl	1
	Chlorpyrifos	1
	Pirimiphos-methyl	2
	Fenitrothion	1
1990	Demeton-S-methyl	3
	Quinalphos	1
	Omethoate	3
1991	Chlorpyrifos	2
	Chlorpyrifos-methyl	1
	Pirimiphos-methyl	1
	Demeton-S-methyl	1
	Omethoate	6
1992	Malathion	2
	Fenitrothion	1
	Omethoate	2

Table 3: Number organochlorine and carbamate compounds detected in honeybee incidents from England and Wales 1983-1992

Year	Organochlorine compound	Carbamate compounds		
	gamma-HCH	Carbaryl	Bendiocarb	Pirimicarb
1983	6	5	-	-
1984	4	-	-	-
1985	2	1	-	-
1986	4	4	-	-
1987	-	6	-	-
1988	8	-	1	-
1989	8	5	2	-
1990	7	3	2	-
1991	1	-	4	1
1992	-	-	4	-
Total	**40**	**24**	**13**	**1**

Residues of carbamate pesticides have also been detected. Three compounds were identified, carbaryl, bendiocarb and pirimicarb (see Table 3)

The newer pyrethroid compounds were first detected in honeybee incidents in 1985, and since then residues of these compounds have been found in a total of seven incidents. These are deltamethrin (one incident, 1985); cypermethrin (one incident, 1988); permethrin (one incident, 1990); fenvalerate (one incident, 1990); alpha-cypermethrin (two incidents, 1991); and delta-cypermethrin (one incident, 1991).

Residues of other compounds found during the analysis of bee samples include the herbicides, paraquat (two incidents, 1985 and 1988) and diquat (one incident, 1986); the acaricide, bromopropylate (one incident, 1987); the fungicide, prochloraz (one incident, 1990); and napthol (found as residues with triazophos in one incident, 1983).

In some incidents, residues of more than one compound were detected. For instance, omethoate residues were detected in incidents involving dimethoate.

DISCUSSION

Pesticide poisoning of honeybees can be attributed to one of four categories of use (Fletcher *et al.*, 1992).

Deliberate abuse arises when there is an intentional, malicious poisoning of honeybees using chemicals not approved for this purpose.

Misuse occurs when a chemical is used in a manner not approved, but there is no purposeful intent to kill honeybees. An example would be spraying a crop in flower when the recommendations for use of the compound states that it should only be used after petal fall.

The **approved use** of a compound may result in honeybee deaths. It is this category of use that is fed back to the approvals system so future problems can be anticipated.

Any pesticide poisoning incidents that cannot be attributed to one of the above categories, usually through lack of evidence as to its cause, is classified as **unspecified use**.

The incident scheme was set up in its present form mainly as a result of the aerial spraying of winter sown oilseed rape with triazophos to control the brassica pod midge (*Dasineura brassicae*) and cabbage seed weevil (*Ceutorhynchus assimilis*). Only ground spraying is now allowed. Triazophos incidents tend to occur when the oilseed rape is sprayed when in flower. Instructions on the label now clearly state that it should only be applied after 90% petal fall. Problems are found when flowering is patchy, often caused by woodpigeon (*Columba palumbus*) or rabbit (*Oryctolagus cuniculus*) damage earlier in the season or flooding leading to uneven crop growth. This results in the damaged patch flowering at a later date, and although less than 10% of the field may present a large area on which bees may forage. A small number of incidents involving triazophos have arisen from the spraying of vegetable crops in summer.

As can be seen from Table 2, triazophos residues have been found in honeybees over the ten year period. However, in recent years the number of incidents has declined. This may be due to several factors such as a better educated farming community to the problems of spraying and bees; the issuing of spray warnings to beekeepers: the use of newer less toxic compounds to bees; and new varieties of oilseed rape.

Over the ten year period several incidents have occurred as the result of spraying with the insecticide dimethoate. These have involved applications to beans, peas and, to a lesser extent, cereal crops. These incidents occur when the beans or peas are sprayed when in flower.

Residues of other organophosphorus compounds have been implicated in bee incidents (see Table 2). These have arisen as a result of crop or fruit spraying operations, either with spray drift affecting nearby flowering plants, or by crops or fruit being sprayed when in flower, or being sprayed when weeds are in flower around and amongst the crop or fruit. Additionally, malicious poisoning of swarms or bee colonies using organophosphorus insecticides has occurred where they are seen as a perceived nuisance.

With the organochlorines, only residues of gamma-HCH have been found in the ten year period (see Table 3). These have resulted from applications to crops, timber treatments to roofs visited by bees, and malicious poisoning.

Three carbamate compounds have been found to be involved with honeybee poisoning (see Table 3). Carbaryl poisoning occurred in association with its use as a blossom thinner to orchard crops at times when bees were foraging there. Bendiocarb is used as public health product as a spray against masonry bees, wasps, feral bee colonies and ants. Incidents are thought to occur as a result of honeybees robbing these colonies after they have been treated. Malicious use again may also be involved. There was a single incident involving pirimicarb, a compound considered to be safe to honeybees. In this incident it is thought that a very large dose of spray may have been applied to roses in a garden where the bees were foraging.

Pyrethroids are considered to be relatively non-toxic to bees (Smart and Stevenson, 1982). However, residues have been detected in honeybees submitted to the Scheme. In many incidents, the significance of these residues is unknown and it is difficult to ascribe the deaths to these compounds. In some incidents, residues were found with those of organophosphorus compounds, the latter being associated with the deaths of the bees. In view of reports of synergism between pyrethroids and other pesticides, these incidents involving more than one residue will receive particular attention in future.

Of the other compounds detected in honeybees, the herbicides paraquat and diquat may have been taken in by bees as a source of moisture after they had been applied. The residue of the fungicide prochloraz was found to be much lower than the LD50 and is considered to reflect exposure. The naphthol residue was found to be present with residues of triazophos, which was the likely cause of death. Several bees died after the acaricide, bromopropylate, had been correctly applied to hives.

CONCLUSIONS

The WIIS has highlighted problems with pesticides poisoning of honeybees and steps have been taken in light of these findings to change the approval of chemicals. It remains a valuable way of monitoring agricultural compounds after registration and the results should be fed back into the registration system as part of the risk assessment process. Additionally, where there have been cases of infringement of the legislation, prosecutions have be taken.

The Scheme provides a useful means of monitoring the presence of residues of pyrethroids in honeybees and can assess any significant problems, as they arise, with these compounds and others that are cleared for use.

The Scheme is reactive and relies on beekeepers to submit samples of dead bees for analysis.

The value of monitoring activity such as this could be enhanced by pooling information, where available, from different countries of Europe, and perhaps further afield. However, this will not be straightforward, due to variations in beekeeping practices, likelihood of mortality being reported, and the degree of sophistication in the investigation procedures. As a minimum, a catalogue of large-scale incidents throughout Europe, which are notified and investigated to some extent (whether systematically or as an *ad hoc* response) would be useful. This would provide an indication of possible problems that require deeper investigation. Such a collection of case histories only needs someone to accept the task of collating and summarising it, and would be a valuable addition to existing pre- and post-registration procedures.

To go further, and derive robust indices of the relative numbers of incidents in different countries, caused by different pesticides, would need much more. This would depend on the existence of a standardised formal response scheme, and therefore is probably only applicable at the moment to a handful of countries. In addition, it would be vital to document

a) usage of pesticides, by quantity and/or frequency of use;

b) statistics on beekeeping;

c) number of cases reported;

d) numbers attributed to poisoning, with a detailed explanation of what tests and analyses were carried out, and their sensitivity.

The exercise of defining these aspects will also help to provide a protocol by which to judge whether it might be feasible to establish new schemes in other countries, or perhaps even organise international collaboration in investigations, such that all analyses might be done by one laboratory, for example.

Pooling of information in this way would require not only an organisational framework, but also establishment of quality assurance procedures to ensure the compatibility and consistency of the data. ICPR would be well placed to take the initiative in this area.

The 'Authorisation Directive' (91/414/EEC) from the EEC is concerned with the placing of plant protection products on the market. It requires that member states shall put in place a system to carry out post registration control and monitoring of plant protection products that may have effects on populations or the environment. Included is residue analysis to evaluate "the residues of the active substance, metabolites, degradation or reaction products, resulting from authorised uses of the plant protection product, and which are of toxicological or environmental significance." The Scheme used in the UK provides a useful and well-tried method of investigating problems with pesticides and honeybees that may arise post-registration.

Apart from the regulatory role the Scheme provides a valuable means of reassuring the public, including beekeepers, that something is being carried out to monitor the effects of pesticides after approvals have been granted.

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Appendix 19

F. Wimmer: Monitoring of bee poisoning incidents in France, 1982-1986.

RECENSEMENT DES CAS DE MORTALITÉ D'ABEILLES de 1982 à 1986

F. WIMMER

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RESUME

De 1982 à 1986, 477 cas de mortalité d'abeilles ont été portes à la connaissance du groupe de travail « Traitement phytosanitaire et protection des abeilles ».

300 échantillons d'abeilles mortes sur les 477 analysés ont révélé la présence d'un ou plusieurs produits. Les parathions, le lindane, la deltaméthrine et le diméthoate ont été le plus souvent mis en évidence.

Pourtant, la relation de cause à effet n'est pas toujours clairement définie, les doses retrouvées étant limites par rapport à la DL50 ordinairement admise.

Ce constat pose le problème du diagnostic d'intoxication, *a priori*, difficile s'il reste basé sur le seul critere DL50.

Mots-clés : Traitements phytosanitaires, Intoxication, Analyse, DL50.

SUMMARY

Between 1982 and 1986, the working group of « the phytosanitary treatment and the conservation of bees » were informed of the mortality of bees in 477 apiaries.

Of the 477 cases examined, 300 samples of the dead bees analysed revealead the presence of one or several chemical products.
(notably : « parathions », « lindane », « deltamethrine », and « dimethoate ») in most of the cases.

However, the relation between the cause and effect has not always been clearly defined, since the doses found were just at the limit of the DL50 (lethal dose 50) which is normally admitted.

This fact emphaseses the problem of diagnosing poisoning which is difficult to establish if it is only based on the criterion of DL50.

Keywords : Phytosanitary treatment, Poisoning, Analyse, LD50.

PRÉAMBULE

Une recrudescence des accidents de ruchers était signalée en 1980 par les apiculteurs mettant en cause les traitements phytosanitaires pratiqués par les agriculteurs.

Le manque d'informations précises relatives à ces mortalités d'abeilles empêchait toute analyse objective des faits.

Ainsi, était créé en 1981 un groupe de travail coordonné par l'A.C.T.A. intitulé « Traitement phytosanitaire et Protection des abeilles ». L'un de ses objectifs était donc de recenser le plus finement possible les cas de mortalité afin d'en définir les causes.

Pour ce faire, le Service de la protection des végétaux, membre du groupe, était chargé de centraliser et d'analyser tous les renseignements transmis par les différents partenaires impliqués.

Dans la pratique, chaque cas de mortalité signalé par l'apiculteur devait faire l'objet d'une enquête de terrain au niveau du rucher et des cultures avoisinantes se concrétisant par la rédaction d'une fiche de renseignements. Parallèlement, un échantillon d'abeille morte et, si possible, de plantes prélevées sur les cultures suspectées étaient dirigés vers un laboratoire à des fins d'analyse.

Deux laboratoires étaient sollicités, à savoir, pour la zone Nord, le Laboratoire Central d'Hygiène Alimentaire (Paris), et pour la zone Sud, le Laboratoire national de pathologie des petits ruminants et des abeilles (Nice).

Nous présentons ici le bilan de cinq années de suivi des cas de mortalité d'abeilles portés à la connaissance du groupe.

Communication présentée à la F.N.G.P.C. à Paris le 18.12.86.

MORTALITÉ D'ABEILLES : BILAN de 1982 à 1986

Avant d'entrer dans le vif du sujet, on gardera présent à l'esprit que ce bilan n'a pas la prétention d'être exhaustif, la principale raison étant que le groupe « Traitement phytosanitaire et Protection des abeilles » n'a pas été informé de tous les cas de mortalites observés par les apiculteurs. On accordera donc à ce bilan seulement une valeur d'indicateur de tendance.

Nombre de cas de mortalité recensés

Les dégâts subis par les apiculteurs vont de l'affaiblissement des ruchers avec perte de butineuses jusqu'à la destruction totale des colonies.

Le tableau 1 présente, année par année, le nombre de cas analysés par les laboratoires précités. Les fiches de renseignements accompagnant les échantillons ont permis de les classer en fonction des cultures suspectées.

— Seulement 36 % des 477 cas recensés concernent des accidents où une culture était suspectée. Les traitements sur colza sont le plus souvent mis en cause ; ils représentent 27 % des analyses pratiquées.

— Lorsqu'il y a mortalité d'abeilles, avec présomption d'intoxication par un traitement phytosanitaire, il est très difficile pour l'enquêteur de mettre en évidence le ou les parcelles *a priori* responsables des dégâts : les 307 cas, soit 64 % où la culture est inconnue, confirment ce constat. L'inconvénient majeur est de ne pouvoir guider le laboratoire dans son travail analytique, l'obligeant à une recherche systématique, longue et coûteuse.

Quels produits ont été décelés à l'analyse ?

Sur 477 analyses pratiquées, 177 ont un résultat négatif. Il reste donc 300 analyses où au moins un produit a été mis en évidence.

La figure 1 présente, pour chaque pesticide, le nombre de fois où il a été mis en évidence, étant entendu qu'un échantillon peut en contenir un ou plusieurs (407 détections pour 300 échantillons positifs).

Par ordre d'importance décroissante, on classe les produits de la façon suivante :

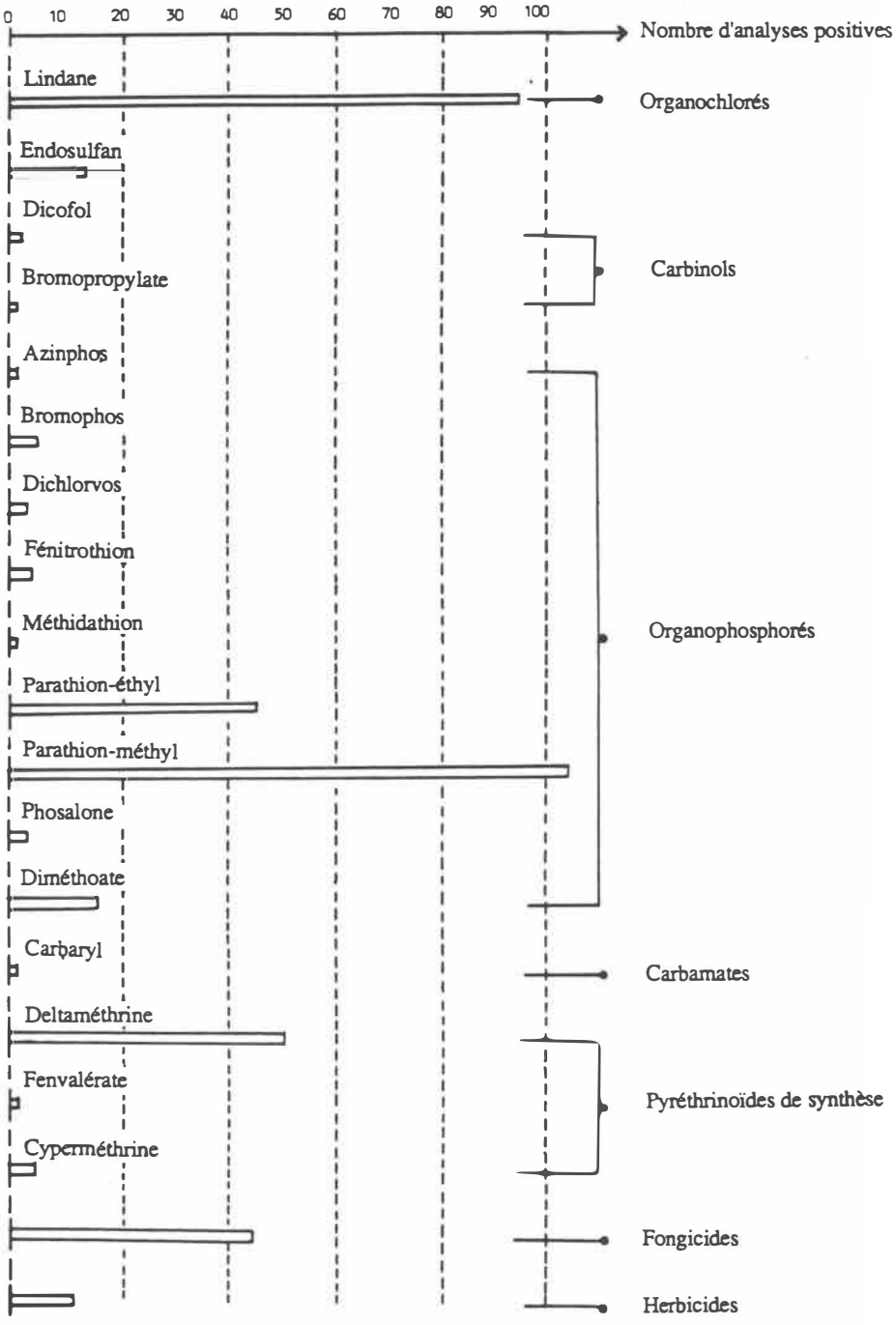
— parathion méthyl : 105 fois, soit 35 % des échantillons
— lindane : 95 fois, soit 31 % des échantillons
— deltaméthrine : 51 fois, soit 17 % des échantillons
— parathion éthyl : 46 fois, soit 15 % des échantillons
— diméthoate : 16 fois, soit 5 % des échantillons
— autres produits : 10 fois, soit 3 % des échantillons

Tableau 1.

Culture suspectée	Nombre de cas ayant été analysés au laboratoire					
	1982	1983	1984	1985	1986*	Total
Arboriculture fruitière	12	12	2	8		34
Céréales	1	4	3	3		11
Colza	22	15	43	29		109
Vigne			5	11		16
Culture non connue	43	59	83	68	54	307
Total	78	90	136	119	54	477

* Le détail des accidents recensés en 1986 n'est pas encore connu. Les 54 cas analysés ont donc été placés dans le groupe culture non connue.

Figure 1 - Nombre d'analyses positives par produit.



INTERPRÉTATION DES ANALYSES

Prouver que le produit mis en evidence par l'analyse est responsable de la mortalité constatee des abeilles revient à comparer son dosage à la DL50 ordinairement admise.

En ce sens, un dosage egal ou superieur a la DL50 confirme sans conteste l'intoxication. Par contre, pour les dosages inférieurs, ce qui etait le cas pour bon nombre des 300 analyses positives, le laboratoire n'est pas en mesure d'affirmer si le produit decele est responsable des mortalites observees.

En fait, on est pratiquement certain de ne pas retrouver a l'analyse les quantites de produits effectivement absorbees par l'abeille. D'ailleurs, un essai interlaboratoire, mene en 1985, a prouve que des echantillons d'abeilles, prealablement intoxiques au parathion methyl, parathion ethyl et fenvalerate, donne un resultat d'analyse avec un taux de recuperation compris entre 3,6 et 39 %.

Pratiquement plusieurs hypotheses sont emises, notamment :

— echantillon preleve trop longtemps apres la date presumee de l'intoxication,

— acheminement de l'echantillon vers le laboratoire dans de mauvaises conditions,

— metabolisation et/ou fixation, plus ou moins irreversible, du produit chez l'abeille.

CONCLUSION

De 1982 à 1986, 477 cas de mortalite d'abeilles ont ete signales au groupe de travail « Traitement phytosanitaire et protection des abeilles ».

Les analyses pratiquees ont revele la presence de pesticides dans 300 situations. Le parathion, le lindane, la deltamethrine et le dimethoate sont les produits le plus souvent detectes.

La relation de cause à effet, basee sur la seule comparaison du dosage mis en evidence par l'analyse avec la DL50, ne semble pas être suffisante.

Des travaux axés sur la recherche d'autres parametres ou indicateurs semblent necessaires pour poser valablement un diagnostic d'intoxication.

ÉCHOS ET NOUVELLES

Le Ministère de l'Agriculture communique :

Le progrès de la biotechnologie va permettre de mettre en marché des produits nouveaux attendus dans tous les domaines de l'économie. Ces produits seront soumis, comme les autres, aux procédures d'homologation ou d'autorisation de mise en marché qui sont en vigueur actuellement.

Cependant, pour parfaitement maîtriser leur fabrication et leur emploi, le ministre de l'agriculture vient de constituer une commission du génie biomoléculaire auprès de lui.

Son avis portera sur les conditions d'emploi et les précautions à prendre pour l'utilisation des produits issus des biotechnologies, conduisant à une dissémination dans le milieu naturel d'organismes modifiés génétiquement, notamment par les techniques du génie génétique, et capables de se multiplier.

Pour contrôler totalement l'expérimentation de ces produits et permettre aux entreprises et aux laboratoires d'orienter leurs recherches vers la définition de produits présentant toute garantie d'inocuité, l'avis de la commission du génie biomoléculaire pourra être sollicité à tout stade des recherches et du développement par tous ceux qui le souhaitent.

La commission du génie biomoléculaire sera formée de chercheurs renommés dans les matières de la biotechnologie, de la santé et de la protection de l'environnement, ainsi que d'industriels et de personnalités compétentes dans les domaines du travail, de la consommation et du droit. La présidence de la commission a été confiée au professeur Pierre Royer, président du centre international de l'enfance et président de la fondation française pour la nutrition.

VIENT DE PARAITRE

NÉMATODES DES PLANTES CULTIVÉES

Les Nématodes provoquent des dommages importants à de nombreuses cultures. Pratiquement invisibles à l'œil nu, ils ne peuvent être décelés de manière certaine que par une analyse de terre ou de racines, en laboratoire. Cette détermination est indispensable pour repérer les parcelles contaminées et établir un plan de lutte en temps utile.

L'ACTA vient de publier un dossier pour l'identification des principaux groupes de Nématodes par l'examen des dégâts sur cultures. Il est composé de 5 fiches illustrées réunies sous jaquette :
- Nématode à kyste de la betterave.
- Nématode des tiges et des bulbes,
- Nématodes du maïs,
- Nématodes des céréales et des graminés.
- Nématodes des galles de racines.

Chaque fiche présente au recto des dessins en couleurs très précis et détaillés des organes atteints et au verso un descriptif des symptômes, des dégâts provoqués et de la biologie des diverses espèces.

Les moyens de lutte sont développés en pages intérieures de la jaquette.

Un tel dossier met à la disposition des agriculteurs et techniciens une somme d'informations les plus récentes réunies par l'ACTA auprès des meilleurs spécialistes de ces parasites (I.N.R.A., S.P.V., Instituts techniques, U.I.P.P.).

Pour se procurer dès maintenant ce dossier « NÉMATODES DES PLANTES CULTIVÉES », adresser la commande à : ACTA - Publications, 149, rue de Bercy, 75595 PARIS Cedex 12. (Joindre le règlement de 45 F T.T.C.).

DEMAIN : QUELS DÉBOUCHÉS POUR NOS BOIS ?

La production française de bois, en volume, pour les quatre prochaines décennies est assurée, particulièrement pour les résineux.

En 1984 on récoltait trois millions de mètres cubes de bois résineux : **dès 2009, il sera possible d'en récolter annuellement 15 millions.**

Mais ces bois trouveront-ils preneurs ? qui les achètera ? de quels types de produits le marché sera-t-il demandeur ? L'appareil industriel pourra-t-il s'adapter ? saurons-nous vendre et surtout exporter notre production ?

S'il peut paraître périlleux de faire de la prospective économique sur trente ou quarante ans, il est néanmoins nécessaire de tenter de répondre à ces questions car c'est sur ces points que se jouera demain l'avenir de la forêt française.

L'Institut pour le Développement Forestier, dans le numéro 36 de Forêt-Entreprise consacre à ces questions un important dossier réalisé à partir d'études économiques françaises et européennes.

Pour recevoir ce numéro « Les producteurs face au défi du marché des bois » au prix de 45 F T.T.C. port inclus, s'adresser à : I.D.F. Diffusion, 23, avenue Bosquet, 75007 PARIS. Tél. (1) 45 55 23 49, en joignant le règlement à la commande.

Appendix 20

C. Gretenkord and W. Drescher: Development of a cage test method for the evaluation of pesticide hazards to the bumble bee *Bombus terrestris* L.

Development of a cage test method for the evaluation of pesticide hazards to the bumble bee *Bombus terrestris* L.

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1. Introduction

For more than 40 years the undesirable effects of pesticides on pollinators have been investigated almost exclusively with honey bees. But what about pesticide hazards to other bee species? Does the german "decree for the protection of honey bees (1992)" protect them sufficiently?
The successful method of rearing *Bombus terrestris* in captivity now offers the opportunity to test another native bee species with regard to its sensivity to pesticides. In 1992 we started to develop a cage test method, following the scheme used for honey bees. So far, we investigated 10 different insecticides of 4 chemical groups and carried out 36 single tests.

2. Material and Methods

The test cages had a size of 3 x 4 x 2 m. As test crop we used *Phacelia tanacetifolia* Benth. Under favourable weather conditions the colonies (in insolation boxes) were placed in the cages. When the temperature was high, especially in July and August, we placed the nest boxes in the ground, outside the cages, to protect the colonies from overheating.
We only used healthy and queen-right colonies, consisting of at least 100 worker bees. They were fed with sugar syrup, offered in the anteroom of the nesting chamber *at libitum*. In addition, we regularly placed a mixture of pollen and sugar syrup on the comb, so that there was always a small supply of pollen in the nest. Otherwise it is possible that larvae are carried out. The bumble bees needed 7 to 11 days to get used to the cage conditions, that is to reach a good foraging activity.
Pesticides were applied around noon. We always used the double of the highest dose recommended for use in flowering crops (Tab.1), while the control was treated with water. The observations were made according to the guidelines of the "Biologische Bundesanstalt" (STUTE 1991) for the test with honey bees. For assessing the effects of the treatments on the brood, we took pictures of the colonies shortly before placing them into the cages, before the application and daily during the observation period. In the case of Insegar™ the colonies were observed for 2 weeks after the cage test (1 week) in a climatic room. Then we froze the whole colonies and investigated the larvae and pupae.
Pesticides, which had an effect on the bumble bees, were tested in 3 replicates, if there was no effect, we made 2 replicates.

3. Results

Pirimor™, Unden™, Thiodan™ and Rubitox™ had no effect on *B. terrestris*, neither on mortality nor on foraging activity. Insegar™ caused no damage to the brood. E 605™ caused a

high mortality on the first day after application, a moderate mortality on the second day. Metasystox™ induced a high mortality on the first day and had a long-term effect (at least 1 week). With Decis™ we observed a high mortality on the first day and a repellent-effect. Karate™ caused a high mortality until the second day after application and also had a repellent-effect. Roxion™ induced a moderate mortality. The mortality in the control was always very low. Raw data and statistical analysis will be published in another paper.

It became evident, that the monitoring of pesticide-effects on the brood of bumble bee colonies is very difficult in cage tests. The reasons for this are:

1. The number of larvae is difficult to determine. 2. The development of individual larvae can not be traced, since the structure of the comb changes permanently. 3. Slight losses of brood are quickly compensated and are therefore difficult to recognize. 4. In comparison to the amount of pollen which has to be offered additionally, only a small amount of contaminated Phacelia-pollen was collected by the worker bees. As sugar syrup is fed *ad libitum*, nectar is collected from the crop only to a limited degree.

In some tests, however, there was an unusually high number of larvae carried out of the nest box after the treatment (compared to the control). But it is not clear whether it was the result of poisoning, since there are some other factors, which may cause a reduction of brood: For instance the competition between queen and worker bees or among worker bees, which are normal events in the development of bumble bee colonies. It is also possible that the supply of pollen was too small.

4. Discussion

Our investigations show, that cage tests with bumble bees are possible. Compared to the test method used for honey bees, some modifications are required. The effect of pesticide treatment on mortality, foraging activity and the behaviour of worker bees and males (if present) can be determined. But pesticide-effects on the brood are difficult to assess.

The results of our cage tests do not allow a final assessment of the sensivity of *B. terrestris* to pesticides in comparison with honey bees. Further investigations are needed, especially laboratory experiments, which were already started. Concerning insect growth regulators, like Insegar™, a larval test has to be developed.

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GRETENKORD, C. and DRESCHER, W., 1993: Effects of four pesticides (Decis™, Metasystox,™ Pirimor™, Rubitox™) on the bumble bee *Bombus terrestris* L.: Determination of the oral LD_{50} and first experiences with semi-field tests. 40. Jahrestagung der Arbeitsgemeinschaft der Institute für Bienenforschung e. V., 30.03.-01.04.1993 in Bad Sassendorf Ostinghausen (in press)

Tab. 1: Insecticides tested in Cage Experiments and Effect on *B. terrestris*

Insecticide-group	**Trade Name (in Germany)**	**Active Ingredient**	**Honey Bee Hazard Classification**	**Tested Dosage (+ 600 l Water)**	**Effect on *B. terrestris***
Carbamates	PIRIMOR-Granulat	Pirimicarb (50 %)	B 4	900 g/ha	no effect
	UNDEN flüssig	Propoxur (200 g/l)	B 1	2400 ml/ha	no effect
Chlorinated Hydrocarbons	THIODAN 35 flüssig	Endosulfan (352 g/l)	B 4	1200 ml/ha	no effect
Organophosphorus Compounds	E 605 forte	Parathion (500 g/l)	B 1	420 ml/ha	high mortality
	METASYSTOX R	Oxydemeton-methyl (250 g/l)	B 1	1200 ml/ha	high mortality; long-term effect
	ROXION	Dimethoate (400 g/l)	B 1	1200 ml/ha	moderate mortality
	RUBITOX Spritzpulver	Phosalone (30 %)	B 4	2400 g/ha	no effect
Pyrethroids	DECIS flüssig	Deltamethrin (25 g/l)	B 2	1000 ml/ha	high mortality repellent-effect
	KARATE	Lambda-Cyhalothrin (50 g/l)	B 2	400 ml/ha	high mortality; repellent-effect
Juvenoids	INSEGAR	Fenoxycarb (25 %)	B 1	1200 g/ha (+ 1500 l Water)	no effect

Appendix 21

J.N. Tasei: A sequential study on the effects of deltamethrin (Decis CE) on bumble bees.

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A sequential study on the effects of deltamethrin (Decis CE) on bumble bees

SUMMARY

Several methods were used to estimate the effects of low doses of deltamethrin on bumble bees. First the relation between dose and mortality was established through topical tests which indicated that 0.06 µg/worker caused little mortality. Spraying the insecticide on flowers grown under a tunnel proved that 12.5 g a.i./ha resulted in few dead foragers. Residues detected on sprayed workers ranged from 0.02 to 0.11 µg/bee. Residues in anthers, pollen loads and nectar reached a peak concentration the day of treatment, 0.6, 0.3 and 0.016 mg/kg respectively whereas in the bumble bee nests honey contained only 0.005 mg/kg. Topical application of 0.01 and 0.02 µg/worker did not affect the longevity of bumble bees but increased their syrup consumption by 40 % to 100 %. Artificial contamination of syrup by 0.2 mg/kg of deltamethrin did not affect the longevity of workers but reduced food uptake by 59 %. A syrup contamination with 0.01 g a.i./kg had no influence on the size of the first egg-batch of queens or the larval development. It is suggested that enzymatic degradation occurs in the crop of queens thus partly detoxifying the larval food. Further experiments with topical applications of sublethal doses on queens should be undertaken in standard conditions with regard to colony development.

INTRODUCTION

Scientific literature gives informations on how low doses of insecticides can affect honey bees. Parameters concerned are : waggle dances (Schricker and Stephen, 1970), learning power (Taylor *et al.,* 1987) feeding behaviour (Mamood and Waller, 1990), longevity (Mackenzie and Winston, 1989) fecundity (Lensing, 1987) larval growth and pupation (Czoppelt and Rembold,1988). In bumble bees no similar researches have been reported yet, despite the concern of several scientists about the protection of these species (Stevenson and Racey, 1967 ; Plowright *et al.,* 1978 ; Mayer and Johansen, 1985 ; Drescher and Geusen-Pfister, 1991 ; Trottin-Caudal and Trapateau, 1992). For this reason this paper presents a sequential study leading to an estimation of the effects of sublethal doses of deltamethrin on bumble bees (*Bombus terrestris*). The objectives were to evaluate :

1) the dose under which no mortality occured through experiments in laboratory and applications on flowers,

2) the residues which were deposited on plants and insects after a sublethal spray,

3) how sublethal deposits on insects or in food could affect longevity, food consumption and the initiation phase of the colony.

MATERIAL and METHODS

a) **Topical tests.** We used workers picked up randomly from colonies of *B. terrestris* reared in glasshouse. After a 30 sec narcosis with CO_2 a 1 µl drop of an acetonic solution of Decis CE was applied on their thorax (doses ranged from 0.01 to 2.5 µg/worker). Control insects received pure aceton. Then insects were introduced by groups of 8 to 10 in cardboard boxes of 1 dm^3 and fed with a solution at 35 % sugar. They were kept at 20°C in the dark. Mortality was checked every day.

b) **Tests in glasshouse.** We grew mustard (*Sinapis arvensis*) in 3 glasshouse compartments (3 x 2 m). In each compartment we introduced 2 colonies of *B. terrestris* which could forage on 2 m^2 of flowers. Every day dead workers were numbered. At the peak foraging hour the control plot was sprayed with water and the two others with Decis CE at the registered dose (6.25 g a.i./ha) and the double dose (12.58 g a.i./ha) respectively. Each treatment was repeated at a 7 day interval.

c) **Estimation of residues**. Two plots of 32 m^2 of rape (*Brassica napus oleifera*) grown under 2 screened tunnels were sprayed with Decis CE at 12.5 g a.i./ha. In one tunnel 3 colonies of bumble bees allowed the collection of foragers, pollen loads and contaminated honey. In the other we sampled flowers, anthers and nectar. Samples were collected the day before treatment (D - 1), the day of treatment (D) and at D + 1, D + 2 and D + 6. The weight of samples was close to 1 g. Deltamethrin analysis was performed according to the recommendations of L'Hotellier (1982) modified by Sabik (1991).

d) **Feeding tests on workers.** Workers collected and kept in the same conditions as described above (a/) fed on syrup contaminated with deltamethrin (0.2 mg/kg). The treatment comprised 4 x 8 workers. Every day live bees were counted and food uptake estimated after a correction of the evaporation.

e) **Feeding tests on queens.** Overwintered queens were caught in the nature before they initiate their nest in spring. They were reared individually in flight cages of 19 dm^3. Each cage was connected to a wooden nesting box (25 x 25 cm) half filled with cotton. Queens were fed with male flowers of *Salix* and a syrup at 35 % sugar. Six control queens were fed a syrup deprived of insecticide, others a syrup contaminated with deltamethrin at 0.01 mg/kg. This treatment was applied to 3 groups of 6, 5 and 4 queens, during 5, 10, and 25 days respectively. For each queen we noted the date of the first transfer of pollen loads (nest foundation), the date of emergence of the first worker and the number of workers in the first brood.

RESULTS

The experiments on acute toxicity of deltamethrin demonstrated that : **1.** The mean value of the L.D.50 at 20°C after 48 h was 0.9 µg/worker (0.65 < L.D.50 < 1.28) which is much more than that estimated in the honey bee (0.01 µg/worker). **2.** A topical application of 0.06 µg/worker resulted in less than 8 % mortality.

The application of the registered dose of Decis (6.25 g a.i./ha) caused no significant losses whereas the double dose resulted in a low mortality (Table 1) just above the significance level. (A chi-square test was applied to the total number of workers - 31 - which died within the 4 days following both sprays, $\chi^2_1 = 4.2$, $P = 0.04$).

Table 1. Mortality of *B. terrestris* workers according to treatments. Figures between brackets are the number of workers sprayed on the flowers.

	Number of dead workers per day		
	before spray	*during 3 days following*	
		spray n° 1	*spray n° 2*
control	0.4	0.3 (20)	1.0 (29)
deltamethrin 6.25 g/ha	1.3	1.0 (41)	1.3 (37)
deltamethrin 12.5 g/ha	0.9	4.7 (38)	5.0 (34)

With regard to the foraging activity and the mortality in the control (Table 1) we consider that 12.5 g a.i./ha is close to the sublethal threshold in field conditions. After a similar spray on rape, maximum residues of deltamethrin (observed the day of treatment, except for workers) ranged from 0.005 mg/kg in honey to 0.9 mg/kg in foragers (Table 2).

Table 2. Maximum weights of residues detected in 6 substratums after spraying flowers (Brassica napus) with deltamethrin at 12.5 g a.i./ha.

Substratum	*Honey*	*Nectar*	*Pollen loads*	*Whole flowers*	*Anthers*	*Workers*
Weight of deltamethrin (mg/kg)	0.005	0.016	0.3	0.4	0.6	0.9

Surprisingly, deltamethrin was less concentrated in honey than in nectar and more concentrated in foragers 6 days after the treatment which is not in concordance with other authors' observations (Bos, 1981 ; Haouar *et al.*, 1990). The residues in workers ranged from 0.15 to 0.90 mg/kg i.e. 0.02 to 0.11 µg/worker. In topical tests for studying sublethal effects of deltamethrin on workers we chose 0.01 and 0.02 µg/worker which are 10 % and 20 % of the maximum rate of residues detected on foragers and 17 % and 34 % of the dose causing less than 8 % mortality in laboratory. The longevity of workers treated with these doses was not affected but their syrup consumption increased significantly in both cases (Table 3).

Table 3. Food uptake (g/day/worker) by *B. terrestris* after topical applications of deltamethrin.

	WEEK	*1*	*2*	*3*	*4*
deltamethrin	0.01 g a.i.	0.40	0.33	0.17	0.12*
application	0.02 g a.i.	0.40	0.37	0.19*	0.12*
per worker	control	0.40	0.26	0.11	0.06

* values significantly different from the control (Dunnett "t", $P < 0.05$)

Such phenomenon was reported in blow flie by Haynes (1988). In the feeding test of workers we chose 0.2 mg/kg that is 66 % of the maximum rate of residues detected in pollen loads, but about 10 times the maximum concentration in nectar. Table 4 shows that the uptake was significantly reduced presumably owing to the repellent effect of the insecticide as already reported in honey bee with other ingredients by Lensing (1987) and Fiedler and Drescher (1984). No effect on longevity was observed.

Table 4. Consumption by *B. terrestris* (g/day/worker) of a syrup contaminated with deltamethrin at 0.2 mg/kg.

WEEK	*1*	*2*	*3*
contaminated syrup	0.24*	0.16*	0.08
control	0.53	0.39	0.17

* values significantly different from the control (Dunnett "t", $P < 0.05$)

In feeding tests of queens, the artificial contamination of the syrup was close to that of nectar. The duration of the treatments doubtless induced a greater intake of deltamethrin by queens than the foraging of a treated field. This presumable high contamination of queens did not significantly affect either the size of the first brood or the larval development (Table 5).

Table 5. Effect of a contamination by deltamethrin of a syrup fed to *B. terrestris* queens on their first brood. No difference appears between treatments (ANOVA test)

Duration of treatment (0.01 mg deltamethrin per kg)	*Number of workers emerged from the first brood*	*Time interval between egg-laying and emergence (days)*
control	4.7 ± 0.8	25.8 ± 1.1
5 days	4.5 ± 0.6	26.1 ± 1.8
10 days	4.6 ± 0.2	24.4 ± 0.9
25 days	5.5 ± 0.6	23.5 ± 0.9

Higher concentrations of deltamethrin (0.2 mg/kg) had no more effect. With regard to the very low weight of residues in honey pots we suggest that a break down of deltamethrin occurred in the crop of *B. terrestris*. This hypothesis was supported by an additional experiment showing that the break down speed of deltamethrin was similar in bumble bee honey and in a sugar solution, thus indicating that detoxication may be due to enzymes reacting in the bumble bee crop.

Our results bear evidence that deltamethrin is harmless to bumble bees through collecting nectar, thanks to detoxication process. Further investigations on deltamethrin pick up by *B. terrestris* on treated flowers would be required. Moreover it would be worth experimenting on sublethal doses applied topically to queens.

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Appendix 22

Copies of the Honeybee test guidelines;

- **Guideline on test methods for evaluating the side-effects of plant protection products on honeybees.**
- **Decision-making scheme for the environmental risk assessment of plant protection products.**
- **Method for honeybee brood feeding tests with insect growth-regulating insecticides.**

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Method for honeybee brood feeding tests with insect growth-regulating insecticides

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A method is proposed for testing the side-effects of plant protection products on honeybee brood, particularly aimed at products with insect growth-regulating properties. It is intended to complement the EPPO guideline on test methods for evaluating the side-effects of plant protection products on honeybees and to be used in the framework of the EPPO CoE decision-making scheme on environmental risk assessment.

Introduction

This test method is concerned with assessing the side-effects on honeybee brood of plant protection products with insect growth-regulating properties. It is modelled on the EPPO guideline on test methods for evaluating side-effects on honeybees (OEPP EPPO, 1992). The method is intended to be used within the framework of the Honeybee chapter in the EPPO CoE Decision-making Scheme for Environmental Risk Assessment (Greig-Smith, 1991), together with several other laboratory, cage and field tests (OEPP EPPO, 1992; Oomen & Gerig, 1993). It provides a qualitative screening of plant protection products in such a way that products causing no harmful effects to bee brood in the test are classified as posing a low risk to bee brood, while products causing harmful effects to bee brood need further testing in the field in order to assess the actual risk. The method is also under review by the International Commission for Plant-Bee Relations (ICPBR), Working Group on Honeybee Toxicology.

1. Experimental conditions

1.1 Principle of the trial

Colonies of honeybees are fed the insect growth-regulating insecticide (IGR) to be tested at the quantity of 1 litre per colony at the concentration recommended for field use. The IGR is presented as formulated product in sugar solution. A reference IGR and a pure sugar solution is fed simultaneously to other colonies. Brood development is followed by weekly inspection of individual brood cells; mortality of adult bees and brood is studied by use of a dead-bee trap in front of the colony.

1.2 Trial conditions

Bees from test colonies should be free-flying, with access to natural nectar sources. Natural nectar flow should not be heavy, otherwise the bees may store the insecticide-contaminated sugar with test compound rather than feed the fresh nectar to the larvae.

1.3 Design of the trial

Treatments: test product, reference product (IGR of which the dose effect relation on bee brood is known, e.g. fenoxycarb, diflubenzuron), non-pesticide control (pure sugar solution)

Test units: sound medium-size bee colonies.

Replicates: at least three colonies per product and per concentration. All colonies of a trial should be placed in one location.

1.4 Preparation of the bees

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

2. Application of treatments

2.1 Products to be tested

Use formulated products only.

2.2 Mode of application

Start feeding of all colonies simultaneously, preferably during the evening in order to prevent robbery. Feed 1 litre of sugar solution (50% sucrose) per colony until consumed. Do not add new solution after bees have finished the original quantity. Normally the solution will be finished within 24 h.

2.3 Dosage

Test products are fed at a concentration recommended for high-volume use. If resources permit, lower or higher concentrations expected to cause effects can usefully also be tested. Reference products known to cause effects at lower than field concentrations may be tested at these lower concentrations.

2.4 Time and frequency of application

Only single applications. Trials can be done during the whole season of normal nectar collection and brood development of honeybees. All development stages of brood (eggs, young larvae, old larvae, pupae) should be present.

3. Mode of assessment

3.1 Brood development

One day (not longer than 24 h) before the start of feeding, mark in each colony at least 100 cells with eggs, 100 cells with young larvae and 100 cells with old larvae by means of a transparent (overhead) sheet. Clear combs are preferred for marking cells. Avoid if possible the outer part of the brood nest, as bees sometimes have difficulty nursing the outer cells during cold weather. Brood development in all marked cells is checked weekly until 3 weeks after application. Inspection is then ended, since normal brood development takes 3 weeks.

A practical way to describe brood development is as follows. The pretreatment positions of egg, young and old larva cells are indicated on a first overhead sheet by numbers in a colour code for each stage. The date, colony number and a mark how the sheet was placed over the comb are also indicated. This first sheet is then copied several times to serve as a mother copy for later inspections. In these, the different juvenile stages are again indicated by their own colour code.

3.2 Mortality of adult bees and brood

Dead-bee traps are placed in front of the beehives; suitable traps are gauze boxes of 100 × 75 × 50 cm, open at the upper side. The traps are inspected daily to count dead adult bees, and to collect dead larvae and pupae, which are examined in the laboratory for specific effects of IGRs (white eye rims, malformations).

3.3 Other effects

Other parameters, such as flight intensity, bee family behaviour, queen behaviour etc., can usefully be kept under observation.

3.4 Data on meteorology and environment

From the day of first observation, record meteorological and environmental data. This includes temperature (average, maximum, minimum in °C), rainfall in mm, relative humidity (maximum, minimum). Environmental data include the main sources of nectar near the colonies, time of the year, and a description of the near surroundings.

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.

Acknowledgements

The authors are thankful to the members of the Honeybee subgroup of the EPPO CoE Joint Panel on Environmental Risk Assessment, and in particular to the experts of the Crop Protection and Soil Conservation Service Institute at Facankert (HU) for providing a description of the Hungarian testing method for bee brood.

Méthode d'évaluation des effets des insecticides régulateurs de croissance contaminant l'alimentation des larves d'abeilles

Une méthode est proposée pour l'évaluation des effets des produits phytosanitaires sur le développement des larves d'abeilles. Elle concerne particulièrement les insecticides dont le mode d'action est la régulation de la croissance des insectes. Elle doit compléter la directive OEPP sur l'évaluation des effets non intentionnels des produits phytosanitaires sur les abeilles et trouver son utilisation dans le cadre du système de décision OEPP/CoE sur l'évaluation des risques pour l'environnement.

Метод оценки влияния инсектицидов, регуляторов развития насекомых на питание потомства пчелиной матки

Предлагается метод оценки побочного воздействия агентов защиты растений на потомство пчелиной матки, причем особое внимание уделяется агентам, обладающим свойствами регуляторов развития насекомых. Метод позволит дополнить директиву ЕОЗР в отношении контрольных методов оценки побочных эффектов агентов защиты растений на организм пчелы. Предлагается использование метода в рамках утвержденной ЕОЗР/ЕС схемы принятия решений в отношении оценки риска для окружающей среды.

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EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPÉENNE ET MÉDITERRANÉENNE
POUR LA PROTECTION DES PLANTES

GUIDELINE ON TEST METHODS FOR EVALUATING THE SIDE-EFFECTS OF PLANT PROTECTION PRODUCTS ON HONEYBEES

It is important that plant protection products should be authorized for use only in ways which minimize the risk of harm to honeybees. For this purpose it may be necessary to provide evidence during the registration process to enable the safety of the product in question to be evaluated. This guideline presents several different types of tests (laboratory tests, cage test, field trial and tunnel test) which can be used to provide such evidence. However, some other tests which are sometimes used, such as tests on inhalation and long-term contact, are not described.

The description of these methods is based upon the 'Recommendations for harmonization of methods for testing hazards of pesticides to honeybees', decided by the International Commission for Plant Bee Relationships at the Symposia on the harmonization of methods for testing the toxicity of pesticides to bees, held in Wageningen, NL (1980), Hohenheim, DE (1982) and Harpenden, GB (1985). The laboratory tests examine oral toxicity and contact toxicity of the plant protection product. The semi-field cage test and the full field trial study the effects of application of the product during bee flight. The tunnel test can be used to study certain hazards to honeybees which are virtually impossible to study by field trials, such as the effects on bees foraging the honey dew from aphids.

While recognizing that no single test method can provide sufficient information to classify the side-effects of pesticides on honeybees, it is important also to stress it is not envisaged that all these tests must be followed. Because field testing is time-consuming and costly, the laboratory tests or semi-field test may serve to classify many products as definitely harmless or harmful without having recourse to field trials. The decisions on which tests to perform and on whether to proceed from one test to another will depend on the characteristics of the plant protection product, its use pattern and on the tests already performed. These decisions can be derived from a logically constructed sequential decision-making scheme (Oomen, 1986). A joint EPPO/ Council of Europe Panel on Environmental Risk Assessment of Plant Protection Products is currently developing such schemes, including one for honeybees.

I. Laboratory tests

1. Experimental conditions

1.1 *Principle of the trial*

Oral and contact toxicity of test compounds to adult worker honeybees 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 re-use 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, the age and (if known) the race of bees, and date of the experiment should be reported.

Bees may be anaesthetized with carbon dioxide for testing of contact toxicity. 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 should not be confined individually for more than 1 h.

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

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

2. Application of treatments

2.1 *Oral toxicity test*

2.1.1 *Test product(s)*

Use the formulated product or active ingredient in 20–50% sucrose solution. Dissolve formulations without additional solvents if possible.

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 *Test product(s)*

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 each 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 median 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 forced to forage on a flowering crop in field cages. The test products 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 used to confirm that bees are at risk. In case the trial conditions do not allow the use of a hazardous reference product, it should be demonstrated otherwise that bees have been at risk. The effects of the treatment on bees are assessed just before and several times after treatment.

1.2 *Trial conditions*

Use cages with a minimal size of 2 × 2 × 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: test product(s), reference product known to present a high hazard to bees (e.g. parathion, dimethoate) and a control without plant protection product. The control may or may not receive a water spray.

Test units: cages with one colony.

Replicates: sufficient to enable appropriate statistical analysis.

2. Application of treatments

2.1 *Test product(s)*

Use formulated products only.

2.2 *Mode of application*

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

2.3 *Doses*

The product should normally be applied at the highest dose recommended on the (proposed) label for use in flowering crops; if desired, an additional higher rate may also be tested.

3. Mode of assessment

Record effects just 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 assessment e.g. effects on brood, should be made as appropriate to the type of test product.

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 tests

1. Experimental conditions

1.1 *Principle of the trial*

Bee colonies are placed in or on the edge of large test fields of flowering crops. The fields are chosen so that bees can only forage in the field in which their hive is placed. Test fields should be well separated. The test product, and reference products known to present high and low hazards to bees, are applied in separate test fields during bee flight. If test conditions do not allow the use of a hazardous reference, it should be demonstrated otherwise that bees have been at risk. The effects of the treatments on bees are assessed shortly before and several times after treatment.

1.2 *Selection of crop*

Carry out the tests on the crop on which use of the test product is proposed. If not possible, rape, phacelia or another crop attractive to bees should be used as test plants. In any case, the crop should be in full flower.

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 in position 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 10–12 frames, including at least 5–6 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 or an untreated control.

Plot size: at least 1500 m². Full-strength colonies require larger areas. Plots should be well separated by at least 500–1000 m² 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 *Test product(s)*

Use formulated products only.

2.2 *Reference product(s)*

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 *Doses*

The products should normally be applied at the highest dose 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 just before treatment.

Post-treatment assessment: 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 trial if mortality in the non-hazardous reference treatment is considerable (generally 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 used 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 honeybees 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 solution 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. Experimental conditions and application of treatments

Trial conditions and methods described by Shires *et al.* (1984) are suitable.

3. Mode of assessment and recording

As in field tests.

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EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
COUNCIL OF EUROPE
ORGANISATION EUROPÉENNE ET MÉDITERRANÉENNE
POUR LA PROTECTION DES PLANTES
CONSEIL DE L'EUROPE

DECISION-MAKING SCHEME FOR THE ENVIRONMENTAL RISK ASSESSMENT OF PLANT PROTECTION PRODUCTS

Chapter 10
Honeybees

Introduction

This sub-scheme is concerned with the potential risks to pollinating insects from the use of plant protection products. It specifically addresses the assessment of risks to the honeybee (*Apis mellifera*) and their brood and colonies arising from exposure of worker bees to insecticides and other plant protection products while they are foraging away from their colonies.

There is also an increasing need to protect other important pollinators (e.g. bumble bees). In principle, this could be approached by adapting the sub-scheme so that it applies specifically to other species. However, there is insufficient information available about other pollinators to permit an assessment in comparable detail to that for honeybees. Also, populations of other pollinators are considerably more difficult to handle and study than honeybee colonies. Therefore it is preferable to make predictions for other species by extrapolation from the large body of data on honeybees. Preliminary validation of this approach is desirable, by examining correlations between species for susceptibility and exposure to existing products.

In its content and technical approach, the sub-scheme is compatible with the EPPO guideline on test methods for evaluating the side-effects of plant protection products on honeybees (OEPP/EPPO, 1992), which provides details of the main test protocols referred to in the sub-scheme. These are based on recommendations of the International Commission for Plant-Bee Relations (ICPBR), formerly the International Commission for Bee Botany (ICBB) (Felton *et al.*, 1986), and are fully in line with previous international guidelines (e.g. FAO, 1989; Council of Europe, 1992).

The sub-scheme adopts the assumption that the most reliable risk assessment is based on data collected under conditions which most resemble normal practice (i.e. by field tests or by monitoring the product in use). However, these tests are expensive, difficult to carry out and sometimes difficult to interpret. Laboratory and cage tests are convenient alternative shortcuts to classification. Nevertheless, field test results should be regarded as decisive when conclusions from laboratory or cage tests conflict with those from field tests. Experience has shown that such conflicts rarely occur.

Decision-making scheme

Details of the product and its pattern of use

Take from Chapter 2 the basic information on the product and its pattern of use.
In addition, enter the following information:

time of treatment in relation to crop flowering
any special directions for use.

Go to 2

Possibility of exposure

2. Is exposure of bees possible (see Note 1)?
   if yes — **Go to 3**
   if no (winter use, glasshouse, etc.) — **Go to 12**

Preliminary screening based on toxicity

Most products that are applied as sprays can be evaluated initially by considering the likely exposure of bees and the toxicity of the product. In cases where exposure relative to toxicity is high, the persistence of the chemical on foliage may determine the actual risk and should be taken into account.

3. Assess the toxicity of the product to worker honeybees by conducting LD50 (contact) and LD50 (oral) laboratory tests. Calculate the ratio between the application rate and toxicity (g ha $^{-1}$/LD50 in μg per bee) (see Note 2).
   if ratio < q — **Go to 5**
   if ratio > q — **Go to 4**

4. Assess how long residues remain active on foliage (see Note 3)
   if persistence is short (e.g. LT50 < r h)
   if persistence is longer (e.g. LT50 > r h)

Identification of stages at risk

Questions 5–8 identify cases in which honeybee larvae may be at risk, for which special tests may be appropriate, and allow indirect effects to be considered (e.g. intoxication through feeding on nectar or pollen, delayed action, and alteration of behaviour).

5. Is the product an insect growth regulator (IGR)?
   if yes — **Go to 6**
   if no — **Go to 7**

6. Conduct a bee brood feeding test (see Note 4). Are effects on bee brood significant?
   if yes — **Go to 10**
   if no — **Go to 7**

7. Are there any likely effects other than acute effects on worker bees? (see Note 5)
   if yes — **Go to 9**
   if no — **Go to 8**

8. Reexamine the ratio between application rate and toxicity (see Note 2).
   if ratio < p — **Go to 13**
   if ratio > p — **Go to 9**

Cage and field trials

The results of field tests are more directly relevant to practical conditions than those of cage tests. Therefore cage tests are not generally necessary if a field trial has been carried out. This stage of testing also provides an opportunity to develop means of minimizing effects, by extending the cage or field tests to examine patterns of use which would cause less exposure. Such extra testing is an optional supplement to the risk assessment procedure, which may aid risk management.

9. Has a field trial been carried out?
   if yes — **Go to 11**
   if no — **Go to 10**

10. Conduct a cage trial (see Note 6). Are effects on the colony significant?
    if yes — **Go to 11**
    if no — **Go to 13**
    if yes but eliminated under modified use (see Note 9) — **Go to 14**

11. Conduct a field trial in conditions representative of use. When effects through foraging on honeydew are studied, tunnel tests may replace field tests (see Note 7). Are effects on colony survival and development significant (see Note 8)?
    if no after full exposure — **Go to 13**
    if yes but eliminated under modified conditions of use (see Note 9) — **Go to 14**
    if yes — **Go to 15**

Categories of risk

The preceding stages of assessment allow uses of plant protection products to be allocated to four categories of potential risk to honeybees.

12. Categorize as negligible risk to bees

Go to 19

13. Categorize as low risk to bees

Go to 16

14. Categorize as medium risk to bees

Go to 16

15. Categorize as high risk to bees

Go to 18

Analysis of uncertainty

After completing the risk assessment based on data reflecting normal use of the product, it is necessary to consider whether errors in measurements, or variations in conditions of use, might alter the conclusions. This is appropriate for products initially categorized as medium or low risk to honeybees, to detect cases in which risks might be higher in practice.

16. Repeat the assessment, using values of toxicity, application rate, and persistence that represent realistic extremes of variation. Also consider whether the results or test conditions of cage and field trials are such that a significantly higher risk might have occurred under other plausible conditions. Is the risk category changed by the repeat assessment?
    if yes — **Go to 17**
    if no, confirm initial assessment — **Go to 19**

17. Consider whether the lower risk category reached by preliminary assessment, or the higher

category in the repeat assessment, is more appropriate as a basis for classification and approval of the product's use.

Go to 19

18. Review the data which led to the high-risk category and check whether the conclusions are correct.

    if yes, confirm assessment — **Go to 19**

    if no, obtain more information as needed — **Go to 3**

Risk management

19. The following points give guidance on the steps which might be appropriate in order to minimize effects on honeybees, for products in each of the categories of risk (see Note 9).

    If risk is low or negligible: set no restrictions on use.

    If there is a medium risk (i.e. no hazard in specified conditions): allow conditional use and specify conditions of use. For example, allow use only after the end of the daily bee flight, require monitoring of effects in use.

    If there is a high risk: specify conditions restricting use to situations in which bees will not be exposed. For example, allow use only before and after flowering of crop while weeds treated simultaneously should not be flowering, allow use only in specified crops (e.g. potatoes) while weeds treated simultaneously should not be flowering, allow use only in glasshouse crops where bees do not enter.

    If the use is on crops that bees regularly pollinate, restrictions may be stricter than on crops that are not attractive to bees (i.e. where exposure is accidental, rather than a predictable consequence of bees' foraging activity).

    It may be noted finally that lack of data at any stage in the sub-scheme can enable the product to be placed in the high-risk category. Experience has shown this feature to be useful in practice, when it may be preferable to adopt restrictions appropriate to the high-risk category rather than conduct field tests.

Explanatory notes

Note 1. Possibility of exposure

In some cases, exposure of bees is not possible, and there is no need for a detailed assessment of risks. Examples are: use during winter when bees are not flying; indoor use and use in glasshouses where bees are not used for pollination; seed dressings and granules except when there is systemic activity; products for dipping bulbs, etc. However, any crops in which there are flowering weeds, or which might be overflown by bees visiting other crops, may present a risk of exposure, even if the crops themselves are not attractive to bees. In such cases, it is prudent to regard exposure as possible and to continue with the assessment.

Note 2 Toxicity tests

Suitable methods for toxicity tests are described by OEPP/EPPO (1992). Contact and oral toxicities (LD50) tend to be of the same order of magnitude. Large deviations may indicate unreliability of the data. As the main route of hazardous exposure to acutely toxic compounds is through contact action, the contact LD50 is most important for insecticides, while the oral LD50 is more relevant for the assessment of compounds not acutely toxic, such as herbicides.

The ratio between application rate and toxicity (sometimes referred to as a hazard ratio) gives an approximation of how closely the likely exposure of bees is to a toxicologically significant

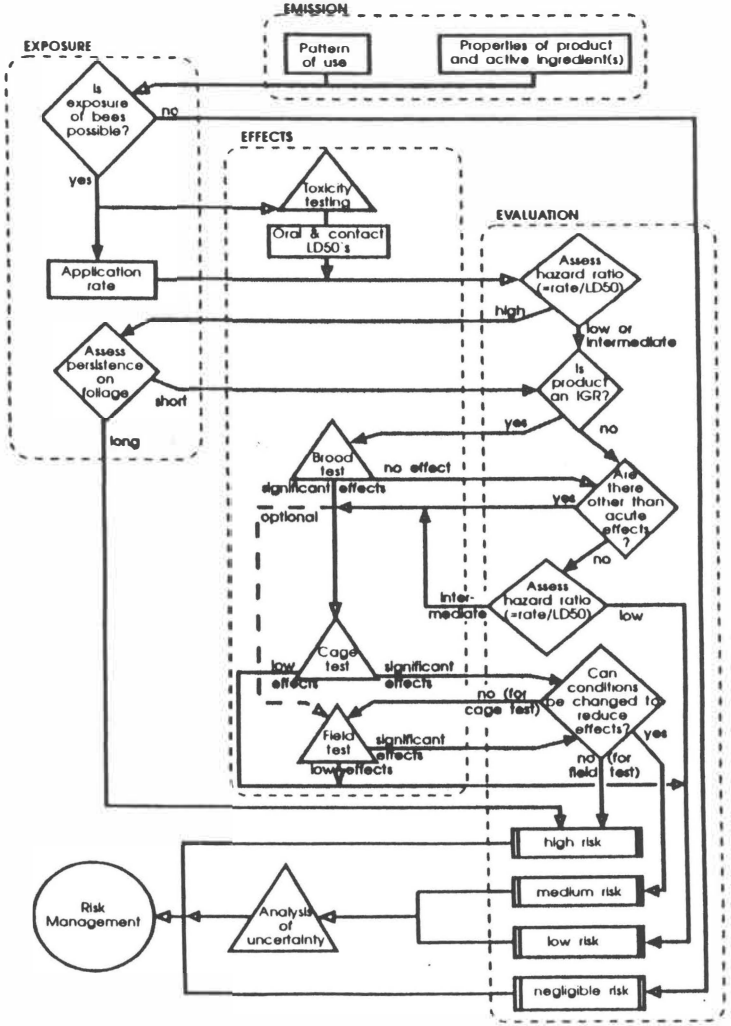

Fig. 10. Simplified diagram of the sub-scheme for evaluation of the risk of a plant protection product to honeybees.

level. In calculating the ratio (dose per ha/LD50), dose per ha is the highest application rate in g ha^{-1}, and LD50 is measured in μg a.i. per bee. The upper (q) and lower (p) thresholds are determined on the basis of bee toxicity, dosage rate and an independent classification of risk verified by extensive practical experience of plant protection products. Suggested values are $q = 2500$ for the upper threshold and $p = 50$ for the lower threshold.

This screening may be carried out either by expressing both toxicity and application rate as the active ingredient or as the formulated product. Pesticides containing mixtures of active ingredients should be evaluated by entering toxicity and rate of the formulated product only.

Note 3. Residual toxicity

If there is data which demonstrates that the residual toxicity of the a.i. declines rapidly enough to avoid significant exposure of bees, effects may be reduced. A suitable method for the determination of residual toxicity of chemicals on foliage is described by Gerig & Oomen (1993).

Toxic pesticides with short residual activity (LT50 < r h) may become harmless overnight. These cases should be verified by cage or field trials. A tentatively suggested criterion for the duration of residual activity is 50% mortality after 24 h exposure to residues on leaves aged during 8 h (i.e. r = 8 h).

Note 4. Bee brood feeding tests

Preliminary screening of IGRs is made by a bee brood feeding test. At present, there is still too little data on exposure to relate larval toxicity (method described by Wittman & Engels, 1981) with field application rate and brood damage. Therefore, if any effects are detected in a feeding test, cage and/or field testing is necessary. A suitable method is described by Oomen *et al.* (1992). In these tests possible effects on adult worker bees will be detected as well. Of course, a feeding test is not required when cage or field test data on broad effects are available.

Note 5. Indirect effects

This stage will identify indirect effects of all kinds (e.g. intoxication through nectar or pollen, delayed action of a toxic a.i., modification of bee behaviour) and allows the sub-scheme to take account of these effects, through special tests. However, unless test conditions resemble practical conditions sufficiently (e.g. in field and cage testing), interpretation of these special tests may be difficult, because of a lack of existing similar information.

Note 6. Cage trials

Suitable methods are outlined by OEPP/EPPO (1992) for cage or tunnel trials which may serve to identify a number of non-dangerous pesticides. The design of trials should be influenced by the characteristics of the chemical and its effects on bees, revealed by the earlier tests. Exposure in a cage or tunnel is more intensive than in the field. The product tested is therefore regarded as presenting a low risk if the effects on colony survival and development are similar to those in a non-pesticide control, provided that environmental conditions are suitable for the detection of hazards to bees (see also Notes 7 and 8).

Note 7. Field trials

Field trials serve to classify all remaining pesticides. Suitable methods are discussed in OEPP/EPPO (1992). The design of trials should be influenced by the characteristics of the chemical and its effects on bees, revealed by the earlier tests. Both cage and field trials should include a reference product known to present a high risk to bees, to demonstrate that the test bees were at risk under the environmental conditions (especially weather) of the trial. A reference product known to present a low risk (or a non-pesticide control) is also necessary, in order to enable evaluation of the effects of the test product on colony survival and development and arrive at an appropriate category of risk.

Special effects (larval toxicity, long residual effect, disorienting effects on bees, etc.) identified by the field test may require further investigation using specific methods. If field trials are virtually impossible (e.g. for evaluating the hazard to bees foraging on honeydew secreted by cereal aphids), tunnel trials may replace field trials.

Cage and field trials should be conducted under conditions reasonably representative of the uses to be prescribed. This allows also for testing under conditions of twilight, i.e. under conditions for use only after the end of daily bee flight (see also Notes 8 and 9).

Note 8. Significance of field results

Effects as a result of the experimental treatment in cage or field trials, and in bee brood tests may be difficult to assess and to distinguish from other sources of mortality. Statistical analysis of the results should normally solve this problem. However, experience has shown that studies with bees (particularly cage and field trials) do not lend themselves to this approach as a consequence of the necessary isolation and scale of the experiments. In the case of cage and field trials, it is considered that current procedures, including the use of a 'toxic standard' reference compound, pollen collection (including residue analysis) and direct observations of foraging behaviour should provide sufficient information concerning exposure to the test compound to enable reliable interpretation of results. Decisions on whether effects in cage and field trials should be considered as 'significant' requires expert judgement.

Note 9. Additional testing

As an aid to risk management, additional testing may be incorporated into cage or field trials, in order to examine whether effects on bees under normal recommended patterns of use can be reduced by changing the conditions of use (e.g. lower application rates, use during twilight only).

pesticides et sont décrites par OEPP/EPPO (1992). La conception des essais doit être influencée par les caractéristiques du produit et par ses effets sur les abeilles, révélés par les essais précédents. L'exposition est plus intensive en cage ou dans un tunnel qu'au champ. Ainsi, on considère que le produit à tester présente un risque faible si la survie et le développement de la colonie sont les mêmes que ceux d'un témoin non traité, pourvu que les conditions d'environnement (notamment climatiques) permettent la détection des risques pour les abeilles (voir également notes 7 et 8).

Note 7. Essais au champ

Les essais au champ servent à classer tous les pesticides restants. Les méthodes recommandées sont celles de OEPP/EPPO (1992). La conception des essais doit être influencée par les caractéristiques du produit et par ses effets sur les abeilles, révélés par les essais antérieurs. Les essais effectués en cage comme au champ doivent inclure un produit de référence dont on sait qu'il présente un risque élevé pour les abeilles, afin de montrer que les abeilles soumises à l'essai étaient exposées au risque dans les conditions d'environnement, notamment climatiques, de l'essai. Il est nécessaire en plus d'utiliser un autre produit de référence dont on sait qu'il présente un risque faible (ou un témoin non pesticide), afin de permettre l'évaluation des effets du produit testé sur la survie et le développement de la colonie et de parvenir à une classification adéquate du risque.

Des effets spéciaux (toxicité pour les larves, effet résiduel à long terme, effet désorientant sur les abeilles, etc.) identifiés à l'aide des essais au champ peuvent nécessiter des investigations supplémentaires faisant appel à des méthodes spécifiques. Si les essais au champ sont pratiquement impossibles (par ex. pour évaluer les risques pour les abeilles butinant la miellée des pucerons sur céréales), ils peuvent être remplacés par des essais en tunnel.

Les essais effectués en cage et au champ doivent être réalisés dans des conditions raisonnablement représentatives des usages prescrits. Ceci concerne également les essais effectués le soir après la période de vol des abeilles (voir également notes 8 et 9).

Note 8. Signification des résultats obtenus au champ

Il peut être difficile d'évaluer les résultats d'un traitement expérimental en cage ou des essais effectués au champ, ainsi que ceux des essais portant sur une génération d'abeilles, et de les distinguer des autres causes de mortalité. L'analyse statistique des résultats devrait normalement résoudre ce problème; toutefois, l'expérience montre que les études portant sur les abeilles (en particulier les essais effectués en cage et au champ) ne se prêtent pas à cette analyse du fait de l'isolement des parcelles et de leur dimension. Dans le cas des essais effectués en cage et au champ, on considère que les procédures actuelles, incluant l'utilisation d'une substance de référence en tant que 'témoin toxique', la récolte du pollen (y compris l'analyse des résidus) et l'observation directe du comportement de butinage, doivent fournir suffisamment d'informations sur l'exposition à la substance testée pour permettre une interprétation fiable des résultats. S'il est indispensable d'attribuer une signification statistique aux résultats des essais effectués en cage et au champ, il faudra faire appel au jugement d' experts.

Note 9. Analyses supplémentaires

Pour une meilleure gestion du risque, les essais effectués en cage et au champ peuvent incorporer des éléments supplémentaires visant à examiner si les effets sur les abeilles peuvent être réduits en modifiant les conditions d'usage (diminution de la dose, traitement uniquement au crépuscule)

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Appendix 23

W.W.M. Brouwer. International Commission for Plant-Bee Relationships - Recommendations from the fifth meeting. Sent to EEC, with reference to EC-directive 91/414.

INTERNATIONAL COMMISSION FOR PLANT-BEE RELATIONSHIPS

Recommendations from the fifth meeting
(26-28 October 1993, **Wageningen**, The Netherlands)

Recommendations with respect to the Uniform Principles

As honeybees, bumble bees and solitary bees might all serve the same goal of pollination in agricultural crops, we recommend to apply the relevant sections of both Part B on Evaluation and Part C on Decision making to bees in general and not only to honeybees.
This is also relevant because we think, bees should not fall under the natural enemies for which there are also sections in the Uniform Principles.

Recommendations with respect to Annex II (data requirements on the active ingredient)

1. **Aim of the test** (section 8.3.1.1)
   "The highest dose causing no mortality" can be deleted as nothing is done with it.

2. **Toxic standards versus reference compounds** (section 8.3.1.1)
   First of all we notice there is some misunderstanding about the use of these terms. Toxic standards serve to validate a test but do not function as references. Toxic standards are used in laboratory tests on acute toxicity (both on the active ingredient and the Plant Protection Product), and in cage and field tests with the Plant Protection Product.
   So in section 8.3.1.1, under 'test conditions', 'reference compound' has to be substituted by 'toxic standard'.

3. **Test guideline bee brood feeding test** (section 8.3.1.2)
   The ICPBR method was published as a scientific paper in EPPO Bulletin. It can be referred to as:
   P.A. Oomen, A. de Ruijter and J. van der Steen. Method for honeybee brood feeding tests with insect growth-regulating insecticides. *EPPO Bulletin*, Volume 22, 613-616, 1992.
   This method is under review now by ICPBR. There is no objection to refer to it already now.

Recommendations with respect to Annex III (data requirements on the Plant Protection Product)

1. **Aim of the test** (section 10.3.2.1)
   "The highest dose causing no mortality" can be deleted as nothing is done with it.

2. **Mortality in control** (section 10.3.2.1)
   We feel that under 'test conditions' the maximum allowed mortality in controls has to be 15% equally with the active ingredient in the acute toxicity test in Annex II.

3. **Toxic standards versus reference compounds** (section 10.3.2.1)
   First of all we notice there is some misunderstanding about the use of these terms. Toxic standards serve to validate a test but do not function as references. Toxic standards are used in laboratory tests on acute toxicity (both on the active ingredient and the Plant Protection Product), and in cage and field tests with the Plant Protection Product.
   So in section 10.3.2.1, under 'test conditions', 'reference compound' has to be substituted by 'toxic standard'.

4. **Toxic standards and reference compounds in cage and field tests**
   We think that besides a control and a reference treatment there should also be included a treatment with a toxic standard within this type of tests, unless there is another way to confirm that bees have been exposed to the pesticide. Alternative ways to show exposure are pollen assessment and bee counting. Therefore we suggest to add the following sentence, under "test conditions" of both section 10.3.2.3 (cage test) and 10.3.3 (field test):
   "A treatment with a toxic standard (e.g. dimethoate) has to be included, unless it can be shown in another way that honeybees have been exposed to the plant protection product".

5. **Necessity of cage tests**
   We think it makes little sense to perform a cage test, when there were significant effects detected in a bee brood feeding test, because a cage test will not provide the data needed in this situation.
   Therefore we suggest to delete the first sentence under 'circumstances in which required' of section 10.3.2.3.
   Dr. Oomen agreed to adapt the EPPO risk assessment scheme on this point.

6. **Reference compound in cage and field tests**
   Under "test conditions" of both section 10.3.2.3 (cage test) and section 10.3.3 (field test) dimethoate is given as the compound to be used as a reference product.
   We suggest to delete the word 'dimethoate', because it serves only as a toxic standard (see remark 4), but not as a reference product.

7. **Section 10.3.3, Circumstances in which required**
   'Hives' has to be changed into 'colonies'

8. **Use of bees in field tests**
   The ICPBR recommends to use healthy bees. It becomes however more and more difficult to get colonies that have not been affected by Varroa mites. Therefore, in most cases, bees have been treated with varroacides. Both due to infection by the Varroa mite and by the use of varroacides, bee colonies may become more sensitive to pesticides. For this we have the following recommendation. This will as soon as possible be included in the EPPO guideline 170. So for inclusion under 'Test conditions' we suggest:
   'It is necessary to wait for 4 weeks after the last treatment with varroacides, before using the colony in a field test'.

Appendix 24

W. Mühlen, R. Hintzen and R. Forster: Arguments for the necessity of multiple testing to evaluate the toxicity of pesticides to honeybees. A paper prepared after the symposium.

Arguments for the Necessity of Multiple Testing to Evaluate the Toxicity of Pesticides to Honeybees

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One topic this year's ICPBR symposium (ICPBR SYMPOSIUM: Hazards of Pesticides to Bees, Wageningen, Netherlands, 1993) concerns the question whether one single test of the LD_{50} oral and contact according to the EPPO-Guideline 170 (EPPO 1992) is sufficient to judge the toxicity of pesticides to bees, could not be discussed conclusively. If we support the single test a strong homogeneity among honeybees with regards to their sensetivity to pesticides is a basic assumption. Taking into account recent knowledge with regard to behaviour, physiology and geographical spread (breeds) there are distinct differences between the various European origins of bees (cf. RUTTNER 1993).

Thus GONTARSKY (1953) examined honeybees of various geographical desants with regard to their activity of two digestive enzymes and found out that portuguese desants take a physiologically determined special position. MAURIZIO (1961) found in further examinations differences in the activity of enzymes of *A.m. caucasica* in comparison to three other european breeds (*A.m. carnica, ligustica and mellifera).* Thus the activity of inverting enzymes in pharyngeal glands and midgut is dependend on different factors, such as age, physiological condition and breed of the tested bees. MAURIZIO explained that the effectivity of the pharyngeal glands and of midgut enzyme is strongly reduced during wintertime reaching the level of the summer bees not until springtime (april).

On the basis of these findings the question is raised to what extent physiological factors and specific differences in breed influence the susceptibility to pesticides. On a symposium on methods of testing insecticides on bees in 1958 ATKINS pointed to the fact that variations in the results of laboratory tests are caused among other factors by variation in honeybee resistence from colony to colony. Errors arising from bee resistence are primarily caused by age of bees, length of holding period before the bees are treated, and whether the bees are fed before or after exposure. Especially NAZER (1974), LADAS (1972) and WAHL AND ULM (1983) examined further the influence of physiological factors upon the insecticide resistence of the honeybee.

NAZER (1974) investigated the susceptibility of bees concerning different

physiological conditions. He showed that bees with less brain enzyme (older bees) were more susceptible to organophosphorus AChE inhibitors than younger bees, which had higher concentrations of brain enzyme.

LADAS (1972) found that young bees proved less resistent to DDT than older ones. Caucasian bees (*A.m. caucasica*) showed lesser susceptibility to DDT and Dipterex than bees of the Carniolan (*A.m. carnica*) and Italian (*A.m. ligustica*) race. Between Carniolian bees of different geographical origin significant differences in resistence could also be observed. Variation in susceptibility of younger and older bees were also found by GRAVES AND MACKENSEN (1965).

WAHL AND ULM (1972) investigated the influence of pollen feeding and physiological condition on pesticide sensitivity of carnica bees. They found that amount and quality of pollen inguested in the first days of life affected the pesticide sensitivity of bees of different ages. Honeybees that fed adequate high quality pollen are less sensitive than counterparts that fed inadequade of inferior pollen or pollen substitute. Pesticide sensitivity decreases generally from early to late summer. Poison sensitivity of summer bees increases with age; most sensitive are old winter bees which had to practice broodcare in early spring.

WAHL AND ULM (1972) found such differences if the LD_{50} was calculated for the same body weight. GERIG (1975) drew the same conclusion. He found significant differences in the susceptibility to pesticides in relation to the weight of the bees. He took a sample of 100 to 600 bees from a natural hive. Summer bees and winter bees of lower weight (96-103 mg) proved to be more sensitive to Orthene and Phosdrin than heavier bees (105-139 mg).

The communication of MAURIZIO (1957) describes the determination of the LD_{50} for bees of five fluorine compounds. At the same time the effects of the age and diet of the bees and of the quantity of the liquid containing the poison on the poisening value have been examined. Age and diet of the bees affect the poisoning value. Winter bees, summer flying bees and caged summer bees fed with pollen, are approximately equally susceptible, summer stock bees are less susceptible, caged bees kept on a diet of pure sugar are most susceptible to the substances tested. MAURIZIO concluded that in order to obtain comparable results in determining the poison values, the conditions of the experiments (age and nutritional conditions of the bees, concentration of liquids etc.) should be kept constant.

Comparable results found JOHANSEN (1979). According to his experiments the age of honeybees affects their tolerance to insecticides. Newly emerged bees are most susceptible to DDT, Dieldrin and Carbaryl and older bees to Malathion and Methylparathion. Apparently susceptibility is greater for smaller bees than for large ones. In working with colonies ATKINS AND ANDERSON (1962) reported that the colonies gradually changed in their susceptibility to a given dose of DDT from 65% to 15% kill over an eight-year period.

Conclusion

The literature has shown that for the evaluation of the toxicity of a pesticide the variability of the susceptibility of honeybees to pesticides with regard to age, physiological condition (nutrition, summer and winter bees) and origin/desant must be

taken into account.

To abstain from multiple tests does not consider the fact that there may occur faults in the evaluation of pesticides. There is no investigation that allowes the conclusion that results from single tests of the LD_{50} oral and topical are valid for all regions and origins of bees in the EC. Therefore, for single testing the proof of validity is necessary. STEVENSON (1978) reported that the standard deviation within a test is low, however, the variation between single tests is significant that this must be taken into account. According to STEVENSON the standard variation is at most 27% for topical application and 43% for oral uptake; thus STEVENSON suggests to do a series of tests.

The comparability of the relevant conditions with view to agriculture, plant protection and environment (incl. honeybees) is the basis for the acceptance of an official pesticide authorization in different member countries of the EC according to Guideline 91/414/EEC (Amtsblatt der Europäischen Gemeinschaft 1991). The comparability is not evident on the basis of the literature presented.

For the classification of the tolerance of pesticides to bees one must distinguish between toxicity and hazard. Data of toxicity (e.g. LD_{50}) are no sufficient basis for the evaluation of the risk. Even BERAN AND GLOFKE pointed out in 1959 that the LD_{50} for statistical reasons is necessary for comparative toxicity tests, however, it must not be regarded as a trigger. In the evaluation of risk which is suggested by the EPPO (EPPO 1993) LD_{50} is connected directly to the exposure because a hazard quotient (HQ) of 50 represents a an LD_{50} that is only two times higher than the exposure it is not possible to prove safely that a plant protection product could be classified as non toxic to bees. An additional series of tests is necessary if the HQ is located in the range of the trigger value. Experiences with the chemical Endosulfan confirm this interpretation.

On the background of this knowledge the BBA requires test results that are representative for different seasons and regions as well as for different origins (cf. STUTE 1991). On the basis of our experience there were in the past evident deviations between the results of different test facilities concerning the assessments of side effects of pesticides to bees. These deviations could have led to a different classification of the pesticide. A reduction of the testmethod to one single laboratory test in order to define the LD_{50} oral and contact for risk assessment is therefore not justifiable.

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Appendix 25

Copies of the working documents of EC-directive 91/414, Annexes II (30/07/93) and III (06/08/93), sections relevant to honeybee testing.

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(does not necessarily represent the views of the Commission services)

ANNEX II

8. **ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE**

(1) The information provided, taken together with that for one or more preparations containing the active substance, must be sufficient to permit an assessment to be made as to the impact for non-target species (flora and fauna), following use of preparations as proposed. Impact can result from single or prolonged exposure, and can be reversible, or irreversible. Direct effects must be considered, and where relevant and feasible be investigated.

(ii) The information provided, together with other relevant information, must be sufficient to:

- permit a decision to be made as to whether, or not, the active substance can be included in Annex I;
- specify appropriate conditions or restrictions to be associated with any inclusion in Annex I;
- permit an evaluation to be made as to short and long term risks for non-target species - populations, communities, and processes - as appropriate, within the limits imposed by current scientific and technical knowledge;
- classify the active substance as to hazard;
- specify the precautions necessary for the protection of non-target species; and
- specify the hazard symbols, the indications of danger, and the risk and safety phrases for the protection of the environment, to be included on packaging (containers).

To be decided when more experience is available (in particular in Germany).

8.2.9 Aquatic plants

To be decided when more experience is available (in particular UK, EPPO)

8.3 Effects on other non-target organisms

8.3.1 Honeybees

8.3.1.1 Acute toxicity

Aim of the test

The test will provide the acute oral and contact toxicity (LD_{50}) of the active substance and the highest dose causing no mortality.

Circumstances in which required

Potential impact on honey bees (*Apis mellifera*) must be investigated, except where preparations containing the active substance are for exclusive use in situations where honey bees are not exposed such as:

- food storage in enclosed spaces;
- use in winter in open air;
- non-systemic seed dressings;
- non-systemic preparations for application to soil;
- non-systematic dipping treatments for transplanted crops;
- wound sealing and healing treatments;
- rodenticidal baits
- animal repellents.

Test conditions

Where due to precipitation in the test medium, or difficulties in application the oral or contact toxicity of the active substance cannot be determined, a relevant formulated product may be used. Mortality in controls, should not exceed 10%, and must not exceed 15% after 48 hours. A study with a reference compound -eg dimethoate - recently conducted must be included in the tests to facilitate consistency of interpretation.

Test guideline

8.3.1.2 Bee brood feeding test :

Aim of the test

The test will provide sufficient information to evaluate possible risks from the plant protection product on honeybee larvae.

Circumstances in which required

The test must be carried out when the active substance is an insect growth regulator.

Test guideline

ICPBR Method

8.3.2 Other beneficial arthropods (eg predators)

Aim of the test

The test will provide sufficent information to evaluate the acute toxicity of the active substance to selected arthropod species.

Circumstances in which required

Effects on arthropod species other than bees, which are naturally occurring, and which are predators, or parasites of harmful organisms, must be investigated, except where preparations containing the active substance are for exclusive use in situations where arthropods are not exposed such as:

- food storage in enclosed spaces;
- dipping treatments for transplanted crops;
- wound sealing and healing treatments;
- rodenticidal baits;
- animal repellents.

Where, due to precipitation in the test medium, or difficulties in application, the acute toxicity of the active substance cannot be determined, a representative formulated product may be used.

Test conditions

The test must be performed in the laboratory on an artificial substrate (i.e. glass plate or quartz sand, as appropriate)to 4 indicator species representing different groups (ground dwelling predators, mite predators, specific aphid and plant dwelling predators and general parasites). Testing must be conducted at rates which include a maximum rate equivalent to

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ANNEX III

10 ECOTOXICOLOGICAL STUDIES

Introduction

(i) The information provided, taken together with that for the active substance(s), must be sufficient to permit an assessment to be made as to the impact for non-target species (flora and fauna), of the plant protection product, when used as proposed. Impact can result from single, prolonged or repeated exposure, and can be reversible, or irreversible. Direct effects must be considered, and where relevant be investigated.

(ii) The information provided, must be sufficient to :

- permit an evaluation to be made as to the short and long term risks for non-target species - populations, communities, and processes as appropriate;
- permit an evaluation to be made as to the need for special precautions necessary for the protection of non-target species; and. specify the risk and safety phrases for the protection of non-target species.

(iii) There is a need to investigate and report all potentially adverse effects found during routine ecotoxicological investigations and to undertake and report such additional studies as are necessary to clarify the mechanisms involved and assess the significance of these effects. Particular attention must be given to plant protection products to be authorized for use on an extensive scale, and to those containig active substances which are classified as being very toxic, toxic, persistent or which biorecumulate.

Circumstances in which required

Where signifiant effects are detected in the bee brood feeding test, cage and/or field test shall be carried out.

Where the hazard quotients for oral and contact exposure (Q_{HO} or Q_{HC}) are < 50, further testing is not required. In the other cases cage testing is required. Where field testing is conducted and reported in accordance with paragraph 10.3.3, it is not necessary to conduct cage tests. However, cage tests where conducted, must be reported.

Test conditions

Cage tests must include control and reference (dimethoate) treatments. Details of mortality and behavioural changes in bees, and developmental changes in the colonies, must be reported.

Test guideline

The tests must be conducted in accordance with EPPO Guideline 170.

10.3.3 Toxicity to foraging bees under field conditions

Aim of the test

The test will provide sufficient information to evaluate possible risks from the plant protection product on honeybee behaviour, colony survival and development.

Circumstances in which required

Field trials, using strong and well established hives, must be conducted, where on the basis of expert judgement significant effects are seen in cage testing.

Test conditions

The tests must include control and reference (dimethoate) treatments. The tests shall be conducted under conditions reasonably representative of the proposed use.

Special effects (larval toxicity, long residual effect, disorienting effects on bees) identified by the field tests may require further investigation using specific methods.

Where it is not possible to investigate certain effects in field trials, a tunnel test should be carried out, e.g. in the case of plant protection products intended for control of aphids and other sucking insects in order to investigate the impact on bees resulting from feeding on contaminated honey

dew.

Test guideline

The test must be carried out in accordance with EPPO Guideline 170.

10.3.4 Effects on beneficifal arthropods other than bees

The effects of plant protection products on species which are naturally occuring, and which are predators, or parasites of harmful organisms, must be investigated. Effects on species which are useful for biological control purposes or are relevant to recognized integrated control measures, are particularly important.

10.3.4.1. Laboratory tests or semi-field tests

Aim of the test

The test will provide sufficient information to evaluate the toxity of the plant protection product for selected arthropod species.

Circumstances in which required

Testing is not required where significant toxicity can be predicted from data on the active substance as provided for in Annex II point 8.3.2. or where the plant protection product is for exclusive use in situations where arthropods are not exposed such as :

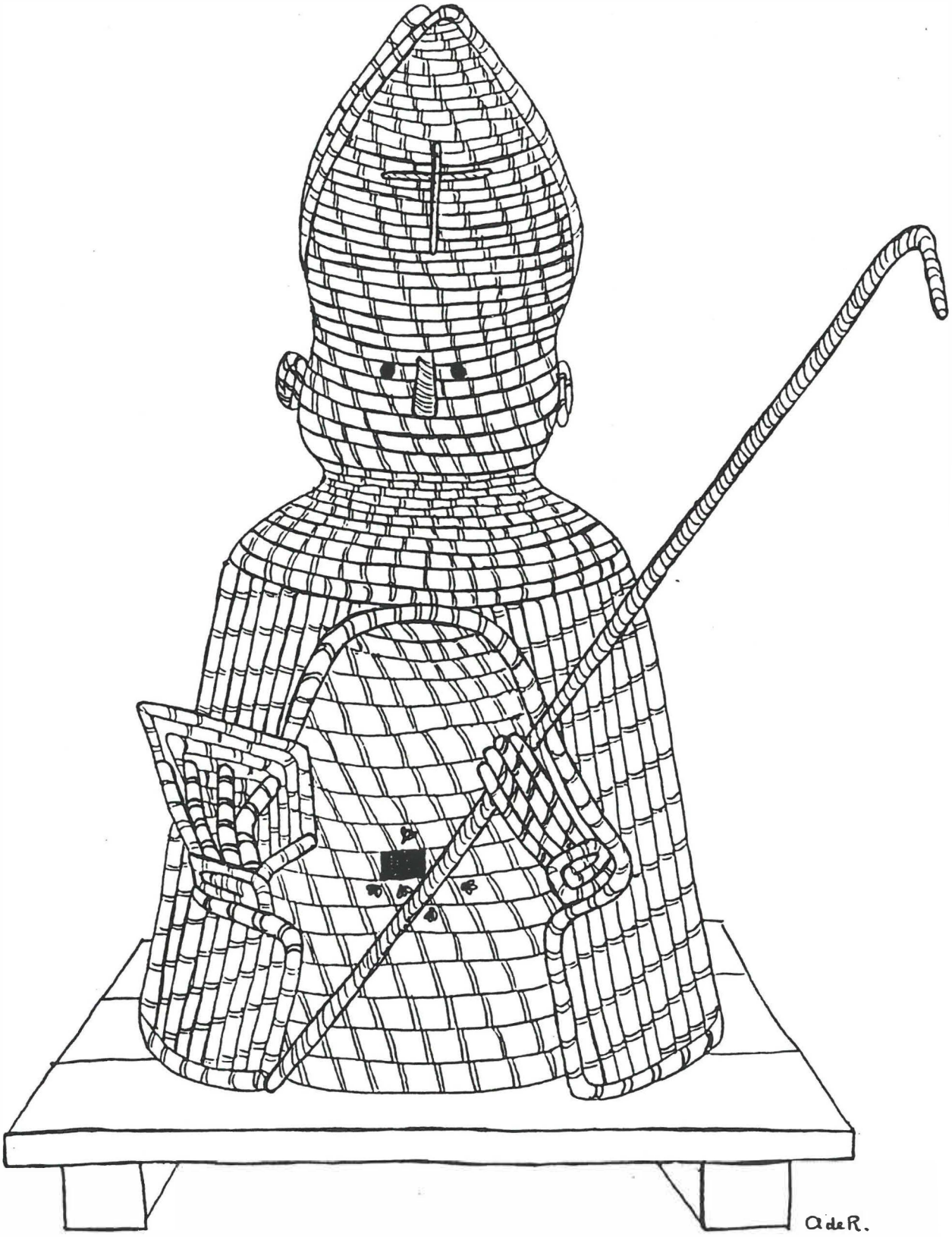
food storage in enclosed spaces ;
dipping treatments for transplanted crops.
wound sealing and healing treatments;
rodenticidal baits;
animal repellents

Testing is required if the product contains more than 1 active substance, or where the toxicity of the product may be enhanced compared to the active substance due to the action of co-formulants, or where the contact to the product is prolonged due to multiple application.

Testing is also required when between 30 and 99 % effect on the organisms in comparison with the control is reported in the laboratory tests at the maximum recommended dose, conducted in accordance with the requirements of Annex II point 8.3.2.

Test conditions

The acute toxicity to 2 to 6 species representing different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