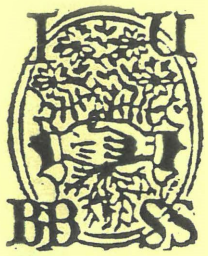

INTERNATIONAL COMMISSION FOR BEE BOTANY

SECOND SYMPOSIUM OF THE HARMONISATION OF METHODS FOR TESTING THE TOXICITY OF PESTICIDES TO BEES, HOHENHEIM, WEST GERMANY 21-23 SEPTEMBER, 1982

REPORT OF THE MEETING

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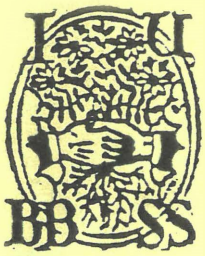

INTERNATIONAL COMMISSION FOR BEE BOTANY

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SECOND SYMPOSIUM ON THE HARMONISATION OF METHODS FOR TESTING THE TOXICITY OF PESTICIDES TO BEES, HOHENHEIM, WEST GERMANY

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REMERCIEMENTS.

Au nom de l'International Commission for Bee Botany, et plus particulièrement de son Groupe de Travail "Protection des abeilles", je tiens à adresser mes plus vifs remerciements à tous ceux et celles qui ont rendu possible la réalisation de ce compte-rendu.

Le Dr. J.H. STEVENSON, tout comme à Wageningen en 1980, a assuré le secrétariat de ce second "Symposium sur l'harmonisation des méthodes pour tester la toxicité des pesticides à l'égard des abeilles" avec la plus grande compétence. Sa tâche ne fut ni simple ni légère car la participation fut nombreuse et les rapports abondants. Il a bénéficié de l'aide patiente et experte de sa collègue Mrs. Dawn WELLS pour la dactylographie et la présentation ; je les remercie tous deux très sincèrement d'avoir pris en charge et mené à bien ce travail. Sans les facilités qui nous ont été accordées par la Shell International Petroleum Maatschappij en la personne du Dr. J.C. FELTON, nous aurions probablement dû renoncer à l'édition de ce Compte-rendu dans la forme très complète où il se présente aujourd'hui. Qu'il en soit remercié.

Nous exprimons enfin notre reconnaissance à la Société Agrishell qui a bien voulu nous faire profiter dans de généreuses conditions des facilités offertes par son atelier de reprographie. Nous remercions tout spécialement M. J.A. VIEL, Directeur du Service Recherche et développement et M. Ph. DEBRAY auprès de qui nous avons rencontré le plus sympathique accueil.

J. LOUVEAUX.

Président de l'I.C.B.B.

ACKNOWLEGMENTS

On behalf of the International Commission for Bee Botany and that of the "BEE PROTECTION WORKING GROUP", I with to express my most sincere thanks to all those who contributed to prepare these proceedings.

Thanks to Dr. J.H. STEVENSON who handled the recording of the minutes of this second "Symposium for the harmonization of methods for testing the toxicity of pesticides to bees". He has assumed this task with the same competence as that provided in Wagenigen, in 1980, in spite of the numerous presentations and interventions which took place. Thanks also to Mrs. Dawn WELLS whose expert and patient help provided both lay out and typing.

Thanks to Dr. J.C. FELTON, SHELL INTERNATIONAL PETROLEUM Maatschappij, for the help provided without which these proceedings would not have been procuded in such completeness.

Finally we wish to express our thanks to both Mr. J.A. VIEL and Ph. DEBRAY - Research and Development Department - AGRISHELL, for arranging the printing of this document.

J. LOUVEAUX

President I.C.B.B.

OPENING ADDRESS

Dr. J. Louveaux, President of the International Commission for Bee Botany

It was two years almost to the day since the Dutch authorities organised at Wageningen a symposium on Harmonisation of Methods for Testing the Toxicity of Pesticides to Bees under the aegis of the International Commission for Bee Botany. At the conclusion of the meeting, it was agreed to continue the work that had been undertaken and to hold a second meeting of the group in 1982. Our colleague Dr. Vorwohl having proposed Hohenheim, we accepted without hesitation because of our complete confidence in his competence and organising abilities; the same confidence that we had in Ir. Pettinga who, having been charged with the task of setting up the first symposium, had done so with such remarkable efficiency.

Therefore now that we had assembled in response to these proposals, Dr. Louveaux had the great honour and pleasure of opening this second symposium which, like the first, was taking place in scientific surroundings which are the raison d'etre of the I.C.B.B.

Although most of the participants had been at Wageningen, and were fully aware of the quality of the work done there, he thought it would not be inappropriate to summarise this first symposium.

First we had got to know one another and agreed procedures. Then we had carefully analysed all the factors which must be taken into account to produce valid test methods, thus providing an analytical document which would provide an excellent basis for discussion and improvement. He was certain participants would support him in thanking Dr. Stevenson for the remarkable work he had done in drawing up the final report. They had all appreciated its clarity and precision.

If one examined this report carefully, it emerged that, beneath the apparent diversity of the methods, it was easy to see a unity of ideas. Variations were often only adaptations rendered necessary by a particular context. In principle, all the methods showed a common concern for efficacy, simplicity and reproducibility; each attempting to produce suitable test conditions for the biological material and apparatus.

At the same time, one was sometimes astonished at the empirical nature of some procedures. When the question was asked why one method was preferred to another, the reply was not always scientifically based. This could arise because toxicity tests on bees generally resulted from extrapolation of those made on other insects, without taking into account the fundamental biological differences which exist between solitary phytophagous insects and social pollinators.

Very important research remained to be done to understand the detailed mechanisms of bee poisoning. It was not only the physiological action of the poison which must be studied, but also, and above all, the effect of this poison on the individual behaviour of the insect when visiting the flower and social behaviour in the midst of the colony.

Thus the debate was broadening. The idea of the bee as just a factor in the apicultural industry was superceded by its consideration as an indispensible link at the centre of complex and fragile biological ecosystems. Likewise the concept of toxicity of an active material was progressively being substituted for that of hazard from application in specific circumstances.

Thus these developments provided an important change in emphasis for one of the many themes for this second symposium.

Dr. Louveaux said he would not delay the discussions further. He thanked the participants for their attendance, and particularly thanked the staff at Hohenheim who were in charge of the organisation of the symposium.

INTRODUCTION

Ir. J.J. Pettinga, Symposium Chairman

Ir. Pettinga thanked the president of the I.C.B.B., Dr. J. Louveaux for his opening address. He pointed out that most of the participants had met the previous evening at Gasthof Jagershof, and the work of the symposium could therefore begin without delay.

The reasons for the meeting were fully explained in the report of our first symposium in Wageningen. Copies of this report may be obtained from Dr.G. Vorwohl at the Universitat Hohenheim. (See Appendix 2 for address).

The first symposium and the publication of its report had stimulated much activity. Interest in harmonisation of methods and in developing new procedures had increased greatly, even beyond the actual participants.

A new revised edition of the official publication of methods for use in the Federal Republic of Germany had been issued. A new manual of testing methods for France had been produced, and would be introduced at the meeting by Dr. Louveaux. (See Appendix 16).

The increased interest in the test methods and in the harmonisation of methods was also demonstrated by the number of enquiries which had been received from outside our circle and by the demand for copies of the Wageningen report.

The group meeting in Hohenheim had built up good contacts and cooperation between government researchers and colleagues from commercial companies. Therefore it was a pity that we lacked contact with the European Plant Protection Organization (EPPO). The Secretary-General, Mr. Mathis, was invited to Wageningen, but had been prevented from attending. We still needed this contact with EPPO, especially to reach our main goal: the harmonization of methods.

In concluding his introduction, Ir. Pettinga thanked Dr. J.H. Stevenson for agreeing once again to act as secretary, and to compile the report of this symposium.

CONCLUSIONS OF THE MEETING (Prepared by Dr. J.H. Stevenson)

1. Further standardisation of methods discussed at first symposium

1.1 Pollen nourishment and sensitivity to pesticides

O. Wahl: Influence of pollen nourishment and physiological condition on sensitivity to pesticides of the honeybee Apis mellifera carnica. SEE APPENDIX 3.

This valuable paper described tests with 19 pesticides (5 herbicides, 11 fungicides, 2 insecticides and 1 growth inhibitor). Well fed young bees were less sensitive than overwintering bees, bees over 50 days old, or bees with an inadequate pollen (i.e. protein) diet. During a lengthy discussion the following points were agreed:

1.1.1 It is better to avoid using overwintered bees which may be present in colonies in the spring. However it was pointed out that use of winter bees could make tests more sensitive.

1.1.2 Consistency between tests is remarkably good and most of the differences shown in Dr. Wahl's work were less than a factor of two. See 1.2.

1.1.3 Standardisation of material should therefore not be too sophisticated, but it is important to note origin and type of bees used.

1.1.4 The two insecticides used (phosalone and endosulfan) have low toxicity to bees and results with more toxic compounds would be valuable. Dr. Wahl agreed to test some more toxic insecticides.

1.2 Standardisation of laboratory LD50 values

J.M. Bull and W.W. Wilkinson: Dimethoate: Laboratory determination of the seasonal variation in acute oral and contact toxicity to honeybees. SEE APPENDIX 4.

R. Knight (presented by J.C. Felton): A laboratory record of the activity of parathion as a standard insecticide in tests against the honeybee, Apis mellifera. SEE APPENDIX 5.

Mr. Wilkinson reported that contact and oral laboratory tests on bees collected at Bracknell on four different occasions between early June and mid October 1981 gave similar results, which agreed with those obtained at Rothamsted. Mr. Felton reported that contact and oral laboratory tests of parathion at Sittingbourne over 11 years from 1968 gave similar results. The main points from the discussion were:

1.2.1 The primary purpose of a standard is to check consistency of results, it is therefore not necessary for all laboratories to use the same standard, but it is necessary for each laboratory to use a standard to check consistency at regular intervals.

1.2.2 Although results of laboratory tests with bees were considered to be remarkably consistent, it is desirable to check this. Dr. Stute would therefore provide 0.5g samples of technical ethyl-parathion to as many participating laboratories as possible, where this insecticide would be tested for contact and oral toxicity during the coming year using each laboratory's

standard procedure (i.e. before September 1983). The results would be collected and summarised by Dr. Stute.

1.2.3 Several members now agreed that the use of minimal quantities of carbon dioxide to anaesthetise bees did not significantly affect results, and there was therefore no objection to its use.

1.2.4 Special laboratory tests may sometimes be reouired to investigate any effects of wetters. For this purpose, a sprayer method such as the Potter Tower is preferred, rather than topical application.

1.3 **Group and individual feeding in laboratory oral toxicity tests**

J.M. Bull and W. Wilkinson: Food sharing amongst groups of 10 bees. SEE APPENDIX 6.

Tests with radio-labelled compounds in sucrose fed to groups of 10 bees showed that there was variation in the quantity received by each bee at first, but, after several hours, distribution was acceptable. Statistical calculations suggested that with unequal sharing, the LD50 value obtained would be reduced by 10% which is not significant. In discussion it was agreed:

1.3.1 Standard errors for oral LD50 values are usually slightly larger than for contact tests, but quite acceptable.

1.3.2 The group feeding method is satisfactory because dose sharing occurs fairly quickly.

1.3.3 Individual feeding is too time consuming for most purposes, and can cause higher control mortalities because of greater stress and longer handling times.

2. **Laboratory tests of toxicity of pesticides to honeybee larvae**

H. Rembold and CH. Czoppelt: The influence of different pesticides and growth regulators on the larval development in vitro. SEE APPENDIX 7.

D. Wittmann: Testing the toxicity of pesticides to honeybee larvae. SEE APPENDIX 8.

Prof. Rembold said the larvae were particularly sensitive because all food and residues are retained until pupation. Standard larval rearing was achieved by using groups of 60 placed in appropriately sized "thimbles' with a standard diet. Detailed studies of effects of feeding with 'unconventional' pest control compounds such as diflubenzuron, azadirachtin and precocene could be made, including mortality, injury and production of interclasses. Additional yeast in the diet increased growth rate and produced a larger proportion of queens.

At the University of Tubingen, Dr. Wittmann demonstrated an LC50 test in which test compounds were fed in a basic diet to brood cells which were returned to the colony and later inspected to count mortality. He also showed an in vitro test in disposable trays of 'thimbles'; this was a most informative technique but too complex for routine use.

The main points of discussion on these presentations were:

2.1 Observations during field tests should reveal gross effects of pesticide applications to brood. Laboratory tests on larvae would be most valuable to examine specific problems, e.g. growth regulatory compounds or when a pesticide application method was thought to be particularly hazardous to larvae.

2.2 Care was needed in interpreting results because positive results in laboratory tests could occur when there is little or no hazard in the field, e.g. with diflubenzuron.

2.3 Prof. Rembold had not yet tested conventional insecticides with his technique, but proposed to do so.

3. The hazard to honeybees of pyrethroid insecticides

The possibility that pyrethroid insecticides may be much less hazardous to honeybees in the field than laboratory toxicity data would suggest, had been mentioned in the report of the first symposium. Since then, much work, especially in the field, has concentrated on studying the "repellent" effects of pyrethroids and the consequent reduction of hazard. Seven papers were presented and discussed.

3.1 C. Bos and C. Masson: Laboratory tests of the toxicity of synthetic pyrethroids to honeybees. Methodological aspects. SEE APPENDIX 9.

Laboratory topical application tests were described and the LD50 values obtained are summarised in appendix 9.

In tests for repellency, worker honeybees were offered a choice between sugar solution only and sugar solution plus formulated or unformulated compound. Experiments were made either in a large cage with a nucleus colony and six dishes arranged on a circular table, or in a choice chamber with a perforated screen between two containers. For deltamethrin, the formulation materials were shown to make a large contribution to repellency.

3.2 J. van der Steen and J.J. Pettinga: Investigations about the size and the duration of toxicity to bees of the pesticide Ambush (permethrin), Decis (deltamethrin), Gusathion (azinphos-methyl) and Pirimor (pirimicarb). SEE APPENDIX 10.

Laboratory toxicity tests, and tests in cages and glasshouse to determine mortality and repellency were described. Due to repellent effects, low mortalities and some reversible paralysis were seen after treatment with permethrin and deltamethrin; bees would not be harmed if spraying during flight is avoided. Pirimicarb had no lethal effect. Azinphos-methyl was highly toxic and should not be sprayed on a crop less than four days before bee flight.

3.3 J. Bacilek: Comparison of some pyrethroids.

In laboratory tests bees were confined on 120mm petri dishes, on which formulations of deltamethrin, permethrin, cyfluthrin and flucythrinate were deposited at the equivalent of recommended field application rates. Deltamethrin gave the lowest mortality, and cyfluthrin the highest. In cage tests, repellency was demonstrated especially for deltamethrin, but less for permethrin and cypermethrin.

3.4 J. Ch. Bocquet, M..L'Hotellier, F. Fevre and R. Baumeister: A five year study of the effect of deltamethrin on bees under natural conditions. SEE APPENDIX 11.

White mustard plots of 1500 m^2 (30 strips of 50 x 1 m^2, separated by 0.7m weed free alleys to facilitate collection of dead bees) were planted at 15-20 day intervals to provide sequentially flowering plots for treatment with deltamethrin; after each experiment the plot was ploughed so as not to interfere with the next trial. At 35g/h deltamethrin caused some bee mortality, but there was very little at 21.2g/h and the hazard at 12.5g/h was less than phosalone which is recognised in France as "harmless to bees". Comparisons with other insecticides have also been made.

Application of deltamethrin at 7.5g/h to large areas of white mustard (6 ha) and winter rape (4 and 14ha) did not reveal any hazard to foraging bees.

3.5 L. Gerig: Field trials with Cymbush (cypermethrin) and Cybolt (flucythrinate) in Switzerland during May 1982. SEE APPENDIX 12.

For the trials, flucythrinate and cypermethrin were applied to flowering rape during bee flight,although this is not normally permitted in Switzerland. After treatment the crop was repellent to bees for two and six hours respectively. After this, there was no effect on foraging or bee development apart from a very small increase in dead bees collected from the flucythrinate treatment, and the compounds were classified as causing "minimum risk".

Previous work with permethrin gave similar results.

3.6 G. Vorwohl: Field trials with Ambush (permethrin) and Decis (deltamethrin) in Baden-Württemberg.

Permethrin and deltamethrin are classified as dangerous to bees in Germany. Ambush and Decis were applied to rape before the flowers opened. In the tests organized by the Landesanstalt fur Pflanzenschutz Stuttgart and the Landesanstalt fur Bienenkunde with Decis during the first two years (1980 and 1981) no clear difference in colony strength and the number of dead bees was observed. In 1982 under excellent weather conditions the colonies of the experimental group were apparently weaker at the end of the flowering period than the control group (ca. 30%). In field tests of other plant protection departments of Baden-Württemberg with permethrin aggressive behaviour was observed.

3.7 L.E. Smart and J.H. Stevenson: Laboratory estimation of toxicity of pyrethroid insecticides to honeybees: relevance to hazard in the field. SEE APPENDIX 13.

Although laboratory tests classify pyrethroids as very toxic to bees, field tests indicate that they may not be hazardous in practice. A number of factors could account for this, notably repellency and the low field application rates being recommended.

3.8 Discussion

3.8.1 The papers presented, and other evidence available, strongly support the view that for application to many flowering crops where bees are at risk, the hazard of pyrethroids will be small enough to be acceptable. Safety depended on repellency and application rate (3.4, 3.7) and field trials were

therefore essential to investigate each proposed application.

3.8.2 The relevance of comparative data from laboratory, cage and field should always be considered. For these compounds field data is essential for a final decision on hazard.

3.8.3 When colonies were brought to field trials immediately before treatment, bee deaths due to injury during transport and closure might be confused with pesticide poisoning. It might therefore be better to establish colonies a few days before treatment.

3.8.4 In field trials, treatments should be separated as far as possible from each other. In countries with reliable, constant climates it might be possible to use observations on the day before treatment as the "untreated control".

4. Methods of assessing pesticide hazards to honeybees used in France

J. Louveaux: Methodes d'essais destinees a connaitre les effets des insecticides sur l'abeille domestique (Apis mellifera L.). SEE APPENDIX 14.

4.1 French legislation dating from 1956 and subsequently modified and revised provides for effective protection of pollinating insects, especially honeybees, from insecticide usage. In effect, this legislation derives from the principle that all insecticides are toxic to bees and therefore forbids their use on nectar producing plants in flower. This is a general prohibition. However specific plant protection formulations can be exempted from this general prohibition when it has been shown with appropriate tests that they are not dangerous to bees under practical agricultural conditions. This is why it is essential to define tests which show precisely the toxicity of active materials to honeybees and the danger to pollinating insects of their use in agriculture. The most recent edition of "Methodes d'essais destinees a connaitre les effets des insecticides sur l'abeilles domestique (Apis mellifera L.)" describes these procedures (See Appendix 14). It is the result of collaboration of a number of specialists over a number of years. It was referred for approval to the Commission des essais biologiques de la Societe francaise de Phytiâtrie et de Phytopharmacie. [These methods were officially approved at a meeting of the appropriate committee on 17 February 1983].

4.2 This most important paper also reflected the conclusions reached at our first meeting in Wageningen, particularly the need for harmonised contact, oral, cage and field tests, and for specialised tests to investigate particular points. In the subsequent discussion, there was general agreement on the points raised.

5. Revision of recommendations for harmonisation of methods for testing the toxicity of pesticides to bees

5.1 At the end of the meeting the document agreed at Wageningen was discussed in detail and revised. This revision forms Appendix 1. Two points arising from this discussion merit mention here:

5.2 The use of a toxic standard in field trials to confirm that bees are really at risk inevitably does great damage to the colonies concerned, and the necessity for this was questioned. However it was agreed that toxic standards really are necessary to validate tests, particularly in countries and districts with uncertain climates, where honeybee foraging behaviour

cannot be guaranteed on a particular day.

5.3 The establishment of hazard or safety to honey bees was the most important aim of our work, and the best way of describing this was the subject of considerable discussion. Two categories were required, but "non-hazardous" was not an accurate expression, and could not easily be translated into other languages. The terms "Dangerous" and "Not Dangerous" were agreed. The second category would include both formulations that are completely non toxic to honeybees and those (such as some insecticide granules) which cause a very small mortality or other slight effects which are acceptable to the beekeeper. Some pyrethroids, applied under specified conditions might be included in this second category.

6. **Conclusions**

6.1 The Chairman, Ir. J.J. Pettinga

Ir. Pettinga warmly thanked Dr. Stevenson for his important part in the programme as a discussion leader about the subjects dealt with in point 5 and again for his work here and in the next months at home as secretary.

He announced the reception in the library of the Landesanstalt. The meeting was welcomed there by our "real Host", Professor Dr. W. Steche, Director of the Landesanstalt fur Bienenkunde in Hohenheim. Ir. Pettinga responded warmly to the welcome which gave him the opportunity to thank all the members of the Anstalt for their contribution to the symposium and its organisation, especially the "ladies team" for serving so generously coffees, teas and juices.

6.2 The President, Dr. J. Louveaux

Dr. Louveaux was very pleased with the progress made since the first symposium in Wageningen and conveyed the gratitude of I.C.B.B. to Dr. Vorwohl as well as to all those who had contributed to the very successful second symposium.

The continuing advances of the plant protection industry and improvement of techniques for control of crop damage convinced him that problems of protection of pollinating insects from pesticides would continue to arise in the future, probably in new forms. In these circumstances it seemed desirable to hold a third Symposium, in two years time (1984). Dr. Louveaux proposed France as the host country unless any other suggestion was put forward. His proposal was enthusiastically agreed.

6.3 Finally Mr. Felton, speaking for all participants, warmly thanked all officials and organisers, especially Dr. Vorwohl, for the excellent arrangements which had ensured such a successful meeting. These remarks were endorsed with acclamation.

APPENDIX 1

Revised recommendations for harmonisation of methods for testing the hazard of pesticides to bees

These were agreed at the Second Symposium on the Harmonisation of Methods for testing the Toxicity of Pesticides to Bees, Hohenheim, September 21-23, 1982.

Significant changes from the original recommendations agreed at Wageningen in 1980 are marked by a vertical line in the right-hand margin.

1 GENERAL CONSIDERATIONS

It is important to distinguish between "Harmonisation" and "Standardisation": and between "Toxicity" and "Hazard".

Harmonisation can be defined as comparison of different standardised test methods in order to reach agreement on the conditions whereby the results obtained by different methods can be generally accepted and compared. This was the principle objective of the meetings.

Standardisation implies the definition of a test method in such a way that following the test will lead to reproducibility of results. While the meetings made significant progress towards standardisation of methods of testing toxicity of pesticides to bees, no attempt could be made to produce definitive versions of tests.

Toxicity is the inherent property of a chemical to cause adverse biological effects at adequate dosages. The toxicity of pesticides to honeybees can be defined by the laboratory tests discussed at the meetings.

Hazard is the possibility of producing an adverse effect in specific circumstances. The hazard of pesticides usage for honeybees can be assessed by the cage and field tests discussed at the meetings.

2 LABORATORY TESTS

2.1 General conditions

2.1.1 Source of bees They should be adequately fed and from a healthy queen-right colony. The following categories were considered:

2.1.1.1 Foraging bees collected from the flight board at the hive entrance.

2.1.1.2 Bees of unknown age taken from frames without brood.

2.1.1.3 Bees reared in an incubator, feed with fresh or well preserved pollen from several sources and sucrose solution and therefore of known age (e.g. 7 to 8 days).

2.1.2 Age of bees

Young bees are reported to be more susceptible to pesticides than older ones.

2.1.3 Season for testing

2.1.3.1 Susceptibility to insecticides may vary at different seasons in which

case ideally the most susceptible stage should be tested, although variation was not thought to be great.

2.1.3.2 The ideal time would vary with climatic conditions in different countries.

AGREED that uniform, young bees are essential, preferably those listed under 2.1.1.2 and 2.1.1.3 above; collection in early spring and late autumn should be avoided, and the natural kill in "control" treatments during tests should be very low. They should be taken in a standardised way (e.g. from frame). The methods used, age and date of experiment should be clearly stated in the test report.

2.1.4 Differences in races of honeybees are probably not important, but the race should be recorded.

2.1.5 State of health of bees is very important. The greater danger from Nosema etc. in spring was mentioned.

2.1.6 AGREED that an appropriate reference compound should always be included regularly to check consistency of results, and each laboratory might choose its own compound (parathion and dimethoate were mentioned). A less toxic standard might also be useful for some purposes such as testing herbicides.

However, we should work towards a common standard, and to this end Dr. Stute would organise and report on a collaborative experiment in which as many laboratories as possible would include ethyl-parathion in their tests, so that a comparison could be achieved.

2.1.7 Anaesthetisation with carbon dioxide is acceptable if used carefully. Amount used and time of exposure should be kept to a minimum. It is important to ensure that application does not lower the temperature of the holding cage and the bees.

2.1.8 AGREED that holding cages should be well ventilated and easily cleaned. Plastic should be avoided, unless disposed of after use, because of possible contamination and wood should be used with caution. Cages should not affect control mortality.

2.1.9 AGREED to store bees at 25 $\pm$ 2^{o}C after treatment.

2.1.10 AGREED that observations of toxic effects and kill be made up to 24 hours after treatment, and longer if necessary.

2.2 Feeding test

2.2.1 Pure compounds or commercial formulations could be tested

2.2.2 AGREED to feed with sterilised 50% sucrose solution, although candy and water might be used after dosage.

2.2.3 AGREED to starve bees for up to two hours before tests if necessary.

2.2.4 Bees could be dosed individually or in groups of 10 to 50 depending on the size of cage. The majority preferred groups of 10, because 50 bees are difficult to observe, and individual feeding is time consuming. AGREED that bees must not be confined individually for more than one hour.

2.2.5 AGREED on need for replication. At least three groups of 10 or more bees to be used at each concentration, and a suitable number of concentrations to provide a regression line and LD50.

2.2.6 AGREED to dose at 10 or 20 cmm per bee.

2.2.7 AGREED to supply fresh 50% sucrose solution after dose has been taken and to change daily.

2.3 Contact tests

2.3.1 AGREED that tests with measured drop, measured spray or measured dust, where exact amount of compound that is applied to the bee can be measured are preferred.

2.3.2 AGREED that contact with sprayed paper or leaves is also useful, and may assist estimation of hazard as well as toxicity.

2.3.3 Some participants suggested doing 2.3.1 and proceeding to 2.3.2 if necessary.

2.3.4 Solution in acetone was acceptable.

2.4 Duration of toxicity

This test was optional depending on results of previous tests. Bees could be confined on sprayed flowers, foliage or an inert surface, e.g. paper.

2.5 Fumigation test

This may not always be necessary. AGREED to use German test.

2.6 Tests on larvae

AGREED that direct tests on larvae should be undertaken if there are special reasons for wanting this information. (See Appendix 8, and Wittmann, D., 1981. Bestimmung der LC50 von Dimilin 25 WP fur Bienenbrut mit einem neuen Apis - Larven - Test. Zeitschrift für angewandte Entomologie 92: 165-172.

2.7 Systemic properties

AGREED on potential importance of this, and to await results of research by Professor Drescher.

3. CAGE TESTS

3.1 AGREED minimum cage size to be 2 x 2 x 3 m.

3.2 AGREED to use small colony of at least three full frames or a "nucleus".

3.3 AGREED minimum 3 mm mesh size for cage to prevent escape of bees, but allow adequate ventillation.

3.4 Ideally no field bees should be introduced into the cage to reduce "trapping" on ceiling. Plastic coated netting on the roof can also be used to discourage "trapping".

3.5 Plants growing in soil are preferred, but potted plants are sometimes used.

3.6 Glasshouses are seldom used now. There may be a need for such tests for specific reasons.

3.7 AGREED to apply pesticide spray during day with bees flying, unless there are special reasons, such as a residual toxicity test, to do otherwise. Study of overall effect of the pesticide application is the main reason for cage tests; spraying of the cage walls should be avoided.

3.8 AGREED to use dead bee traps and to count bees dying in rest of cage.

3.9 AGREED to use a water control and toxic standard.

3.10 AGREED that Borago, Phacelia and Sinapis may be suitable test crops.

3.11 AGREED that feeding of colonies may be necessary.

3.12 AGREED to record foraging activity.

3.13 AGREED to record temperature and humidity.

3.14 Simultaneous treatment preferred, but may not always be possible. It is essential in countries with an unpredictable climate

4. FIELD TESTS

4.1 These most nearly test the practical hazard to bees of pesticide applications. They are expensive and will only be necessary if a proposed use of a pesticide may put foraging bees at risk.

4.2 Sequential treatments in which test chemical and control treatments are applied on subsequent days, save space, which is extremely important in some countries, but are impossible if the climate is unpredictable.

4.3 AGREED simultaneous treatments (i.e. within at most two hours) should be well separated to avoid bees foraging on the wrong plot. The minimum distances should be at least 1 km.

4.4 A total area of at least 1500 m^2 was desirable for each plot.

4.5 A toxic standard and an untreated control are desirable to confirm that bees are at risk.

4.6 AGREED to spray when bees are foraging actively, unless there are special reasons e.g. the evaluation of residual effects.

4.7 AGREED to use dead bee traps and observe dying bees around hives, and elsewhere if possible, e.g. flight corridor.

4.8 AGREED on value of also counting bees on frames and estimating effect on brood.

4.9 AGREED on value of using pollen traps on some hives.

4.10 AGREED to use at least 4 colonies per treatment. They should be healthy, well fed, queen-right, in normal condition and contain at least 10,000 to 15,000 bees according to season. Each colony should cover at least 10 to 12 frames, including at least 5 to 6 brood frames.

4.11 AGREED to estimate foraging bees in the crop.

5. ADAPTATIONS TO TEST SPECIFIC OR "UNCONVENTIONAL" PROPERTIES

5.1 Special formulations Where special formulations such as micro-encapsulated products, micro-fibres or granules are proposed, tests with these are necessary if risk to honeybees is possible.

AGREED to rely on carefully observed field tests, extending observation times up to 12 weeks to allow for delayed effects if risk to honeybees is suspected.

5.2 Herbicides Many herbicides showed low initial toxicity in laboratory feeding tests, but deaths after three or more days had been recorded and non-acute effects were suspected.

AGREED to attempt to record mortality in laboratory tests of such herbicides for one to two weeks after application.

AGREED to extend periods of observation in cage and field tests of herbicides.

5.3 Pyrethroid insecticides There is evidence that acute toxicity tests of these compounds in the laboratory are unrealistic because the high toxicity shown has not necessarily led to serious hazard to bees in the field. The "repellent" effects of pyrethroids and low application rates are probably major factors.

AGREED that data from field tests on the hazards of pyrethroids are essential.

5.4 Repellent compounds A repellent could be considered a useful means for additional protection of bees. When these become available, they should be included in formulations for field tests, and specialised laboratory tests will be required.

6 INTERPRETATION OF RESULTS

6.1 Hazard AGREED that for practical purposes, the hazard of formulations should be assessed on field data and that there could be only two categories: "Dangerous" and "Not dangerous". This second category would include both formulations that are completely non-toxic to honeybees and those (such as some insecticide granules) which cause a very small mortality or other slight effects which are acceptable to the beekeeper.

6.2 Toxicity Classification of toxicity data is useful to specialist research workers, but would not necessarily help pesticide users who must be guided by the assessment of hazard mentioned above. A series of categories for the results of acute laboratory test based on LD50 values (µg active

ingredient) was discussed:

	>100	µg/bee	virtually non-toxic
10	- 100	"	slightly toxic
1	- 10	"	moderately toxic
	<1,0	"	highly toxic

AGREED that a classification of toxicity to guide research workers is needed, but it must never become a guide for practical use, or be used as a basis for legal conclusions.

6.3 Comparability AGREED that one or two pesticides should eventually be chosen for testing by all available methods so that proper comparisons could be made. To this end a collaborative experiment is being organised by Dr. Stute, see 2.1.6.

APPENDIX 2

REPORT OF THE SECOND SYMPOSIUM ON THE HARMONISATION OF METHODS FOR TESTING THE TOXICITY OF PESTICIDES TO BEES, HOHENHEIM, SEPTEMBER 21-23, 1982.

List of participants

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APPENDIX 3

O. WAHL, Fischbachau:

Influence of pollen nourishment and physiological condition on the sensitivity to pesticides of the honey bee *Apis mellifera carnica*

The project will be reported fully in English in 1983 in "Oecologia" (Springer Verlag). It was motivated by heavy bee losses in late April/early May 1965 and 1966 in Hesse, FRG., when road verges were being treated with weed killer. The spray used was a mixture of the grass growth inhibitor MH 30 (maleic acid hydracid) and the hormone weed killer U 46 KV-Combi Fluid (MCPP+2.4-D) plus the wetting and adhesive agent Citowett, all warranted harmless for bees. As the dead bees showed no signs of disease it was suspected that they were old, weakened winter bees, victims of the otherwise harmless preparations. The investigations were intended to probe the extent of possible links between the physiological condition of the bees and their sensitivity to pesticides.

Methods

To establish the influence of pollen nourishment on poison sensivity, newly emerged bees were fed variously for 7-8 days in the incubator, some on sugar candy only, some receiving additional quantitatively or qualitatively different pollen feed or pollen substitute. Older bees of various categories were obtained by keeping small re-queened colonies in flight cages on different-quality proteins. Brood was removed from these colonies before emergence and yielded young bees bred with different protein diets. Many tests were made with hive bees of unknown age from colonies of normal size. Some of these had been prepared by locating them in places with different honey flow. To establish how the ages of summer bees influenced their pesticide sensivity, nucs were formed with bees emerging from Carnica combs, re-queened with Aurea queens or by adding several thousand newly emerged Carnica bees to artificial Aurea swarms. The poison sensivity of Carnica bees of known age was tested regularly. In October, Carnica colonies were re-queened with Aurea for tests on old

winter bees in spring.

Sensitivity to pesticides was measured as LD 50 and LD 10 per os, and tested with 5 hormone herbicides (active ingredients 2.4-D, MCPP, 2.4, 5-T), the fungicide Cupravit (copper-oxychloride), 10 organic fungicides (9 different active ingredients, mostly thiocarbamates), the insecticides Thiodan(Endosulfan) and Rubitox (Phosalon), ruled harmless to bees (FRG), as well as the grass growth inhibitor MH 30 and the wetting agent Citowett. All these act mainly or exclusively as stomach poisons.

I have to thank Dr. K. ULM of the Institut für medizinische Statistik und Epidemiologie der technischen Universität, München, for the final computation and statistical processing of the results by means of the Probit and Logit analyses.

Results

1) Tested with the same pesticide, young bees adequately nourished on high quality pollen are less sensitive than bees from the same colony and emergence fed inadequate amounts of pollen, poor quality pollens or pollen substitute; most sensitive are bees fed sugar only.

2) This result also applies for bees up to at least 50 days old, fed from emergence ad lib. with different quality pollens or with pollen in a comparison with pollen substitute.

3) Adequately pollen-fed bees are heavier than same-age bees fed on quantitatively or qualitatively inadequate pollen or pollen substitute. Exclusively sugar-fed bees are lightest. But different LD 50 values of a pesticide persist for differently protein-fed bees, even when these values are based on grams/bee weight.

4) The fungicide Maneb, a manganese-thiocarbamate, breaks the rule that poorly pollen-fed bees are more poison sensitive than optimally fed bees. Bees fed with inferior pollen or pollen substitute are not more sensitive to Maneb than well-fed comparable bees, in fact if

anything they are less sensitive. Tests with a manganese-free thiocarbamate and with $MnSO_4$ compared with $ZnSO_4$ showed that the manganese content of Maneb is responsible for this exceptional effect.

5) Young bees emerging in August are less sensitive to the same pesticides than same-age bees identically fed emerging in June or early July.

6) The quality of proteins available for the brood has no significant influence on the poison sensitivity of imagines (fed uniformly after emerging).

7) The honey flow has an influence however. Hive bees exploiting an ample early honey flow , and young bees emerged during this time, were less sensitive to the same pesticides than hive and young bees from colonies initially of the same strength having had only a moderate spring flow at their disposal , or none at all.

8) In summer bees, sensitivity to pesticides increases with age. Although these results are from a single year, they are based on tests with bees of known age from 8 colonies. In some colonies however the influence of a Nosema infection, worsening considerably with the bees' age, cannot be excluded.

9) Old winter bees, having cared for brood for ca. 3 weeks, and still Nosema-free according to customary methods of examination, were already as sensitive to the same pesticides at the beginning of March as older summer bees. In late March/early April the winter bees proved strongly Nosema-infected, and their poison sensitivity was significantly higher than that of the oldest, likewise Nosema-infected, summer bees.

Discussion

A detailed evaluation of results from bee-physiological and toxicological aspects will be given in the comprehensive publication. It is merely mentioned here that, according to earlier results of HAYDAK, de GROOT, MAURIZIO, WAHL and BACK the rise in poison sensitivity in

inadequately pollen-fed bees can be attributed to protein deficiency. In human toxicology a few investigations have been published on the influence of a protein-deficient diet on the poisonous effects and decomposition of drugs in mammals and man. It can be deduced from their results that protein deficiency in the bee probably suppresses the enzymatic decomposition of the pesticides administered. A more precise conception is impossible without a biochemical investigation.

Inferences for practical bee protection

The high poison sensitivity of old winter bees should be important in praxis. As long as large numbers of winter bees are present in the colonies in early spring, an increased danger of pest control measures must be expected.

In the course of the warm season, from spring and early summer to late summer, sensitivity to pesticides lessens. Scarcity of forage, particularly of pollen forage, raises poison sensitivity; it is lowered by brood-promoting forage, e.g. fruit and rape bloom.

Specifically, a pesticide's danger to bees depends on the height of the lethal dose and the concentration used. In all organic fungicides used in these tests - with the exception of Maneb and for MH 30, the LD 50 per bee lies over 1000 mcg, sometimes over 2000 and 3000 mcg. These preparations are in effect non-poisonous, i.e. harmless for bees, as practicable concentrations do not reach a lethal level.

The danger for bees of the other preparations tested is discussed with reference to a Table grading the LD 50 and LD 10 values ascertained with normal hive bees. On the basis of our tests with young bees, the LD 50 and LD 10 for each preparation were calculated for poorly protein-fed bees.

The resulting values indicate that all tested hormone herbicides, the wetting agent Citowett and the fungicide Maneb can be graded as harmless for the bee in practice. But the harmlessness of Cupravit,

Thiodan and Rubitox cannot it seems be relied upon. A decision is only possible on the basis of the approved concentration. Calcium arsenate will serve here as criterion. According to BORCHERT (1929) its LD 50 lies by 8 mcg per bee, the concentration used in fruit farming (when arsenic preparations were still approved) was 0.4%. Calculation shows that the LD 50 is reached with 2 µl. Based on this estimate, the LD 10 for Cupravit used in a 0.5% concentration is contained in 2 µl for normally fed bees, and in 1.2 µl for inadequately protein fed bees, so that bee losses are not out of the question. Yet such losses are unknown so far in practice (comm.by letter STUTE).

The Thiodan concentration sprayed with ground equipment is 0.75%, with aircraft 7.5%, but Thiodan is approved up to 12.5% as harmless for bees. At 0.75% the amount to be ingested for the LD 50 lies over the critical limit, as with Cupravit. But the LD 10, with 1.5 µl, is reached even by normally fed bees. With 7.5% and 12.5%, amounts of 0.6 and 0.4 µl suffice already for the LD 50 for optimally fed bees. The LD 50 can therefore be surpassed all too easily, and it is understandable that Thiodan is not approved as harmless for bees in all countries outside the FRG.

Rubitox is used in 0.15–0.2% concentrations in fruit and grape cultivation, but is classed as harmless up to 10% for aircraft spraying. At 0.2% it is admittedly harmless for bees in practice, but at 10% the critical dose drops far below even the LD 50 for optimally fed bees (0.16 µl). It follows that Rubitox can present a danger to bees, even in concentrations of 0.8–1% — overdoses which must be reckoned with in the praxis (LD 50 for normally fed bees contained in 2.0–1.6 µl). Thus objections to the certification of Rubitox as harmless for bees are not unfounded.

The pesticide mixture used in Hesse in 1965 and 1966, and mentioned initially, contains U 46 KV-Combi as the ingredient most affecting bees. Its effect on bees as a stomach poison is just as strong as the employed

concentration of this hormone herbicide alone. According to REHM (unpub.diss. 1968), the mixture was sprayed at that time in a 4.7% preparation containing 1.33% U46 KV-Combi. If the danger to bees of the used concentration of the herbicide is calculated by the above method, it is apparent that although the LD 50 for neither summer nor old winter bees was reached, with 1.9 µl the LD 10 for old winter bees was certainly reached. Insofar as the colonies still contained winter bees in late April/early May, these could have suffered approx. 10% mortality.

According to REHM (and confirmed by our tests) the mixture is clearly effective as a contact poison when bees are sprayed directly. This is mainly due to the addition of Citowett. Possibly any bees directly and thoroughly wetted by the spray were also killed, even if sommer bees were involved. Hard-hit beekeepers report that, in flying weather, the mixture was sprayed on weeds already in bloom and strongly visited by the bees.

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APPENDIX 4

DIMETHOATE: LABORATORY DETERMINATION OF THE SEASONAL VARIATION IN ACUTE ORAL AND CONTACT TOXICITY TO HONEYBEES.

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SUMMARY

At the Wageningen symposium in 1980 it was agreed that a toxic standard should be used in laboratory bee toxicity tests, the choice of compound to be left to individual laboratories. Compounds suggested, and already in use by some workers, included lindane (γHCH), parathion and dimethoate.

Dimethoate was chosen as a representative organophosphate insecticide, having the advantage of being readily soluble in water and organic solvents. Published work showed that it is highly toxic to bees, and has similar oral and contact LD50 levels. The mean LD50 of tests in three different years showed less than 10% variation and its order of toxicity relative to other pesticides was not changed. Five different strains of honeybees were affected to a similar extent by the compound.

During discussion at the Wageningen meeting it was suggested by some workers that, because the response of bees to pesticides can be affected by their physiological state, there might be interpretation problems caused by seasonal variation. It was therefore decided that the susceptibility of bees to it at different seasons should be investigated. The dates chosen fell within the period during which tests have been carried out in recent years at Jealotts Hill.

The oral and contact toxicity of technical dimethoate was measured in early June, late July-early August, early September and mid-October of 1981 using the standard laboratory methods. Twenty-four hour LD50 values were (given in the same order) Oral, 0.142, 0.155, 0.207, 0.174 µg a.i./bee; Contact 0.152, 0.158, 0.149, 0.140 µg a.i./bee. These values are similar to those obtained at Rothamsted Experimental Station (Oral 0.15, Contact 0.12 µg a.i/bee). Variation between tests at a given time was low; 95% confidence limits were around 15% above and below the mean for oral and 10% for contact tests.

Contact test results showed almost no seasonal variation. Oral test results were slightly more variable, probably as a result of dietary and physiological differences between bees.

Because of the consistency of results obtained over one season and supporting data of other workers, dimethoate was adopted at Jealotts Hill for use as a standard for laboratory acute toxicity tests on honeybees.

As four series of tests in one season are unlikely to encompass all variations of external conditions and bee physiological state, some future LD50s can be expected to fall outside the range recorded in 1981. If, however, the dimethoate standard results in a future test differ from those of 1981 by much more that a factor of 2, the state of the bees and conditions of the experiment should be investigated, and consideration given to repeat tests.

APPENDIX 5

A laboratory record of the actrivity of parathion as a standard insecticide in tests against the honey bee, Apis mellifera.

R.J. Knight
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Sittingbourne Research Centre
(Presented by J.C. Felton)

SUMMARY

Bee toxicity tests at the Sittingbourne Research Centre involve topical application and oral administration techniques using ethyl parathion as the standard in each test. The data available at SRC, from 1968 to the present time represents a valuable record of the consistency of performance of a standard insecticide against the honey bee and is presented for use in furthering the harmonisation of bee toxicity work with other organisations.

There is reasonable agreement between parathion mean LD_{50} values obtained in different years for each method, with little evidence of any consistent change in the susceptibility of the bees to parathion. The mean LD_{50} range indicates greater variabililty in the feeding tests (0.045-0.21 ug/bee) than in those for contact toxicity (0.072-0.17 ug/bee). The small differences between contact and oral LD_{50} values show that the method of administration is not critical for parathion and demonstrate the suitability of the compound as a standard insecticide in bee toxicity work of this kind.

TEXT:

INTRODUCTION

Laboratory toxicity data against bees has been a regulatory requirement for new pesticides in many countries for some time. The variety of methods used and the different interpretations put on the results has, however, sometimes led to confusion regarding the potential danger to bees of applications of pesticides. In recent years there has been a desire among interested parties to harmonise the various methods used in the different countries, in order to reach agreement on the conditions whereby the results obtained by different methods can be generally accepted and compared. This was the principle objective of a symposium held by the International Commission of Bee Botany, 23rd-25th September 1980. The concept of harmonisation through the inclusion of a standard insecticide such as parathion or dimethoate was accepted at the symposium.

Laboratory toxicity bioassays with honey bees have been conducted annually at the Sittingbourne Research Centre from 1968 to the present (except in years 1973 and 1978-80). Standardised tests involving topical application and oral administration techniques have been performed with candidate compounds using ethyl parathion as the standard in each test to enable the susceptibility of each batch of bees to be monitored. The data available at SRC for parathion therefore represents a valuable record of the consistency of performance of a standard insecticide against the honey bee and is presented here for use in furthering the harmonisation of bee toxicity work.

MATERIALS AND METHODS

(i) Test insects

Honey bee toxicity tests were normally undertaken between the months of May and September thus avoiding the use of insects early or late in the season when elderly and/or winter bees might be included. On the morning prior to testing worker bees were collected from the upper combs of the Sittingbourne Research Centre hives by an experienced bee-keeper who was also responsible for their maintenance. In the laboratory the bees were held at $23^{o}C \pm 2^{o}C$, ambient humidity in muslin cages placed in the airstream from an electric fan to ensure adequate ventilation. The food provided was 20% honey solution taken up in cotton wool pads. The mean weight of the bees when determined was calculated from the combined weight of 20 individuals.

(ii) Contact toxicity tests

Topical application tests were undertaken using acetone solutions of parathion to give a range of concentrations of the toxicant. Using an Agla micrometer syringe 1 µl of solution was applied to the ventral abdomen of individual workers lightly anaesthetised with carbon dioxide. After treatment the bees were held at $23^{o}C \pm 2^{o}C$ in groups of 10 in cylindrical metal gauze cages measuring 10 x 3.5 cm. The bees were fed with 20% honey solution in the cages which were placed in the airstream of an electric fan. Mortality was recorded after 24 hours.

(iii) Oral toxicity tests

Oral administration tests utilised dispersions of parathion in 20% honey solution, 0.2 ml being presented in a glass feeding vial to groups of 10 workers. A range of concentrations of the toxicant were tested and when the bees had taken all the parathion solution they were treated in the same way as those for the contact toxicity tests. It was assumed that the bees would share the 0.2 ml of solution equally and so receive comparable doses. In this respect Stevenson (1968) has shown that when bees are fed individually with dimethoate, the LD_{50} value obtained (0.19 µg/bee) is similar to that obtained with group feeding.

RESULTS

Each method as described provides a dosage/mortality curve from which an LD_{50} can be obtained by probit analysis. The LD_{50}s obtained by topical application and oral administration of parathion are given in Tables 1 and 2 respectively. The data have been produced by three different technicians in the period 1968-1982 although the methods used were the same in each case.

DISCUSSION

The amount of data generated for parathion in any one year was dependent on the number of candidate compounds requiring bee toxicity tests. The greatest number of assays occurred in 1976 when 10 contact and 10 oral tests were undertaken.

There is reasonable agreement between parathion mean LD_{50} values obtained in different years for each method. The mean LD_{50}s for contact and oral tests in 1982, 0.17 and 0.11 µg/bee respectively, are within a factor of two of the corresponding values obtained in 1968 when the tests first began, hence there is little evidence of any consistent change in susceptibility of the bees to parathion. The mean LD_{50} range from 1968-1982 indicates greater variability in the feeding tests (0.045-0.21 µg/bee) than in those for contact toxicity (0.072-0.17 µg/bee).

The small differences between contact and oral LD_{50} values and the consistency of performance of parathion demonstrate the suitability of the compound as a standard insecticide in bee toxicity work of this kind. The results show that the method of administration is not critical for parathion although this has not proved the case for some other compounds. For example, Stevenson (1968) found that disulfoton was appreciably more active by topical application than oral administration, hence it remains important to use both methods when evaluating the toxicity of a pesticide to bees under laboratory conditions.

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Table 1 Activity of parathion by topical application to the honey bee, Apis mellifera

Year of Test	Mean wt 20 bees (mg)	LD_{50} (µg/bee)										
		(i)	(ii)	(iii)	(iv)	(v)	(vi)	(vii)	(viii)	(ix)	(x)	Mean
1968	-	0.11	0.10	0.12								0.11
1969	-	0.16	0.20	0.14								0.17
1970	119	0.14	0.099	0.21	0.14							0.15
1971	-	0.090	0.061	0.065								0.072
1972	114	0.12	0.055	0.20	0.10	0.036	0.075	0.12				0.10
1974	138	0.14	0.11	0.095	0.14							0.12
1975	138	0.14	0.10	0.22	0.13	0.15	0.16	0.15	0.14	0.22		0.16
1976	135	0.16	0.19	0.23	0.18	0.098	0.10	0.13	0.14	0.10	0.056	0.14
1977	-	0.12	0.090	0.10	0.11							0.11
1981	131	0.095	0.11									0.10
1982	134	0.21	0.15	0.13	0.14	0.16	0.22					0.17

Table 2 Activity of parathion by oral administration to the honey bee, Apis mellifera

Year of Test	Mean wt 20 bees (mg)	LD_{50} (µg/bee)										
		(i)	(ii)	(iii)	(iv)	(v)	(vi)	(vii)	(viii)	(ix)	(x)	Mean
1968	-	0.087	0.081	0.071								0.080
1969	-	0.15										0.15
1970	119	0.25	0.14	0.24								0.21
1971	-	0.058										0.058
1972	114	0.056	0.28	0.072	0.11	0.14	0.045	0.074				0.11
1974	138	0.034	0.056									0.045
1975	138	0.15	0.13	0.17	0.092	0.15						0.14
1976	135	0.14	0.12	0.092	0.10	0.10	0.092	0.11	0.17	0.067	0.046	0.10
1977	-	0.081	0.084	0.096								0.087
1981	131	0.12	0.20									0.16
1982	134	0.11	0.14	0.062	0.13	0.11	0.12					0.11

APPENDIX 6

FOOD SHARING AMONGST GROUPS OF 10 BEES

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SUMMARY

The acute oral toxicity results obtained by giving caged groups of 10 bees a shared food source have been satisfactory over many years. Following the discussions at the Wageningen symposium in 1980, however, we tried to determine the pattern of food shring in such groups under test conditions.

Radiolabelled compounds were fed in sucrose solution, and groups were killed at different times after administration to determine the level of radioactivity in individual bees. Comparison was made between bees (1) not starved, (2) starved for 1 hour and (3) starved for 2 hours before the test. Assessments were made at intervals of 1,2,3,4,8 and 24 hours.

The main problems encountered were (1) metabolism of labelled compounds and (2) difficulty in killing bees without causing regurgitation (immersion in liquid nitrogen was adopted).

The results are very complex and are being studied by statisticians. Examples were shown which suggested (1) more initial variation among groups not starved and (2) more equalisation after 3 hours. There was variation in the actual amounts present in the bees on every occasion. This would be expected because of the known activities of bees in begging and offering food. The overall exposure of a bee over the whole test period cannot be known, and this criticsm applies also to bees fed individually then kept as a group.

A statistician has calculated that unequal sharing would result in a change in the dose-response curve, resulting in over-estimation of LD50 (i.e. lower toxicity), but that this is unlikely to be greater than 10%.

APPENDIX 7

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The influence of synthetic and botanical insect growth regulators on the development of honeybee larvae in vitro

The toxicity of pesticides to honeybee brood is usually tested through the alimentary chain: the compound is added to sugar solution in a certain concentration and is then introduced into the colony either directly or through the foraging bees. This procedure has several disadvantages besides an undefined dilution during the feeding process. The most uncontrolled interference comes from the nursing bees which prepare the larval food as a mixture of glandular secretion, honey, and pollen and which remove the larvae form their cells if they are weak or dead. These disadvantages, which mainly come out from the food chain and nursing behaviour, can be overcome by rearing the larvae under laboratory conditions in a completely controlled environment.

The method has been improved and standardized over the years (Rembold, Lackner, 1981). In principle, a mixture of diluted royal jelly and yeast extract is fed to the honeybee larvae during their whole growth period from L1 to L5. Each larva is kept in a plastic thimble and each test consists of a group of 60 larvae. The compound can either be topically applied to the larvae at any time and as a single dose, or added to the food. Further growth and development is then followed through mortality, weight gain, morphogenesis, and caste formation. As such, also delayed

effects like from insect growth regulators can be detected. These compounds are non-toxic, but interfere with specific morphogenetic events like the moulting processes. A typical result from such in vitro assays is collated in table 1.

Diflubenzuron (Dimilin WP25) interferes in a still unknown way with larval chitin synthesis. We have studied this insect growth regulator as an acetone solution in its effect on honeybee larvae (Czoppelt, Rembold, 1981). After a single topical application during the third larval instar, weight gain and rate of survival are influenced in a dose-dependent way. From 105 acetone-treated (or untreated) larvae, 95 % reached L5 stage, only 15 %, however, after topical application of 0.1 µg Dimilin. Even more drastic is the effect on pupation. Only 5.7 % of 70 test larvae became pupae in the highest dose group and then also developed to adults. The half lethal dose after topical application of Dimilin was found at 30 ng under standard test conditions. After continued feeding with the standard food, the half lethal Dimilin dose is at about 100 ng per larva.

Azadirachtin inhibits morphogenesis in most insects. The compound has therefore also been included in our honeybee tests (Rembold et al., 1980, Rembold, Czoppelt, 1981). It interferes with feeding only in its highest concentration of 0.5 µg per larva (Tab. 1). The effect on larval rate of survival is also low up to a dose of 0.25 µg azadirachtin, where the number of pupae and of adults is clearly reduced already. The substance obviously inhibits pupation at L5 even if applied at L3 already.

Precocene is another botanical insect growth regulator which interferes with juvenile hormone synthesis in some insect species. We have studied the effect of precocene II on bee larvae therefore in some detail (Rembold et al., 1979, Czoppelt, Rembold, 1978). If topically applied to L3 honeybees, a dose up to 30 µg precocene has no effect on growth and development (Tab. 1). Only near 50 µg per larva the number in survival of pupae and adults is reduced which may result from a toxic less than a growth regulating effect. It is clear from these results, that precocene II not induces a chemical allatectomy in honeybee larvae.

These few examples may demonstrate several advantages if growth and development of honeybee larvae are followed in vitro for testing the effect of pesticides. The development of each individual can be followed during and after contact with the pesticide without interference from the nurse bees. By a single topical application at different stages of development, sensitive phases can be evaluated. Last but not least, such an assay can be made independently from the season, if a winter flight room with honeybee colonies is available.

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Table 1. Evaluation of one synthetic (Dimilin) and two botanical (Azadirachtin, Precocene) insect growth regulators by rearing honey bee larvae in vitro.

µg Dimilin/L3[1]	Increase of larval body weight (%)[2]	Rate of survival (%) to L5[3]	Pupa	Adult
0[4]	777	94.3	86.6	82.9
Acetone	648	89.4	77.1	77.1
0.01[5]	671	92.9	62.9	60.0
0.03	368	72.9	44.3	38.6
0.05	491	25.7	14.3	12.9
0.1	331	14.3	5.7	5.7
µg Azadirachtin/L3[6]				
0	340	81.4	65.7	64.3
Methanol	366	90.0	70.0	64.3
0.05	324	94.2	70.0	68.5
0.1	396	94.2	64.2	60.0
0.25	394	75.7	42.8	37.1
0.5	293	42.8	14.2	14.2
µg Precocene II/L3[7]				
0	706	92.3	57.1	51.4
Acetone	741	90.4	60.0	55.2
5	638	86.6	64.7	58.0
10	688	85.7	57.1	56.1
25	629	82.8	52.3	45.7
50	597	59.8	19.6	16.8
75	563	54.2	20.0	20.0

1) 1 µl acetone/L3
2) increase within the first 48 h after application
3) fifth instar
4) 105 larvae for both untreated and treated control
5) 70 larvae were treated for each value
6) 0.5 µl methanol/L3, each value from 70 3rd instar larvae
7) 1 µl acetone/L3, each value from 105 3rd instar larvae

APPENDIX 8

THE APIS LARVAE TEST : STANDARD PROCEDURES FOR THE DETERMINATION OF LC_{50} AND LD_{50} AS WELL AS FOR THE ESTIMATION OF HAZARDS TO HONEYBEE BROOD RESULTING FROM PESTICIDE APPLICATION IN THE FIELD

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The Apis Larvae Test was developed as a method for the evaluation of pesticide toxicity to honeybee larvae. Up to this point there has been no standard procedure available for testing for pesticide effects on bee brood. Several attempts were presented on the occasion of the first harmonisation symposium held in Wageningen, September 1980, for establishing uniform test procedures (Wittmann and Engels, 1981).

The Apis Larvae Test for the determination of LC_{50} on brood frames in free flying bee colonies was described by Wittmann (1981). The Apis Larvae Test for the evaluation of LD_{50} using bee larvae reared in vitro was reported only recently (Wittmann and Engels, 1983,ms submitted). The complete procedure and equipment used for both test variations will be listed here. Used in conjuction with a field experiment, the Apis Larvae Test for LC_{50} determination can be expanded to estimate the hazard created by the application of pesticides for the bee brood.

Finally, conclusions based on experimentation with the described procedures and recommendations for future use of the Apis Larvae Test are discussed.

The LC_{50} Apis Larvae Test

The basic principle of the Apis Larvae Test is to solve the compound to be tested in larval food, which is directly applied to each individual larva in cells on brood frames kept in bee hives for the LC_{50} procedure.

Equipment for a test : 5 or more strong bee colonies, microbalance, glasfiber micro-beam light source, multipipette, pins, royal jelly, fructose, glucose, aqua bidest, pins, brood frame scheme paper, Gaussian integral probability paper, calculator.

Test Food

The semiartificial diet used as a test food consists of royal jelly and an aquaeus sugar solution to which the test compound (normally formulations) is added. As commercial samples of royal jelly contain batches of different origin and composition, any charge has to be mixed well before use. Recipe for the test food:

50,0 % royal jelly
6,25 % glucose
6,25 % fructose
37,5 % aqua bidest (to which the compound is added)
The test food is prepared and stirred well immedieately before the test starts.

Treatment of Larvae in Test Areas

For the test, strong bee colonies(with at least two supers)with large brood nests are selected. On a brood frame, a test area containing 50 larvae of approximately the 3rd instar are marked with pins. The position of the test areas on the frame and the number of larvae in it as well as their estimated age are noted on the frame scheme paper. 10 µl of the test food is applied to each larva using a multipipette(e.g. Eppendorf multipipette 4780).

Treatment of Controls

The larvae in control areas receive the diet without the compound to be tested. Further controls are left untreated to detect the spontaneous mortality which might occur in the colonies due to lethal factors or climatic changes during the test. Test and control areas should never be situated at the margins of a brood frame where nursing of the larvae is often suboptimal. The test frames are then put back into the colonies where they are kept until the brood cells are sealed.

Evaluation of Mortality and Determination of LC_{50}

A day after the cells are sealed, the frame is removed from the colony again. For the calculation of the mortality due to the pesticide effect, the surviving larvae in the test areas are counted. The sealed brood cells of the test and control areas have to be opened and inspected. This is necessary because it is known that dead prepupae and pupae are not always removed by the nurse bees(Rothenbuhler, 1964). The mean values are corrected against the average survival rate in the controls using Abbot's formula.

$$M = \frac{Sk - St}{Sk} \times 100$$

M: Mortality due to pesticide effect
Sk: Number of surviving larvae in control
St: Number of surviving larvae in test

For the determination of the LC_{50}, the brood mortality rate is plotted on probability paper(Fa. Schleicher u. Schüll 298 1/2). The regression line is calculated. By drawing the perpendicular line from its intersection with the 50 % level, the LC_{50} can be read from the x - Axis.

The LD_{50} Apis Larvae Test

The principle of this in vitro test method is the direct application of a small amount of test diet to larvae reared in vitro in an incubator.

Equipment for the test: Colonies with large brood nests, microbalance, glasfiber microbeam light source, multipipette for application of 5 µl, disposo trays, grafting spoon, 2 incubators, stencil drawing of circles of 3,8 - 4,2 mm Ø, royal jelly, glucose, fructose, aqua bidest, yeast extract, probability paper, calculator.

In Vitro rearing of larvae

Two day old larvae are transfered from their cells into UV-sterilized disposo trays(Linbro 76-356-05; Fa.Flow Laboratories)with a grafting spoon. They are reared on 0,2 ml of a semiartificial diet up to the early 4th instar in an incubator at 35°C and at 99% rel. humidity. Twice a day, the larvae are fed an additional 0,05 ml of the diet.

50,0 % royal jelly
6,25 % glucose
6,25 % fructose
1,25 % yeast extract (Difco 0127 - 02)
36,25% aqua bidest (in which the compound is solved to prepare the test diet)

Selection of Larvae for the Test

For the test, only those larvae which fit a circle of 4 mm diameter are selected. The average weight of the size selected larvae, which should be 20 mg, is checked by weighing a sample of 10 larvae. As weighing might affect them, these specimen are not taken for the test. Dead larvae and those with retarded development are discarded. The food is then carefully sucked off with a pasteur pipette connected to a vacuum pump.

Treatment of larvae during the test.

In front of the mouthparts of the larvae, a droplet of 5 µl of test diet is placed with a syringe (Hamilton microliter syringe 725 LT) attatched to a repeating dose device (Hamilton PB 600 - 1). The larvae are then placed back into the incubator. 6 hours after application, the food uptake is inspected at 1/2 hour intervals. The larvae which have finished their food receive 0.2 µl of non-contaminated diet again. They are kept on this diet for 24 hours.

Evaluation of mortality and determination of LD_{50}

24 hrs. after intoxication, the larvae are inspected under a stereomicroscope at 40x magnification. Dead larvae can be recognized by the color of their cuticula which is yellow or brownish. Due to the lack of muscular tension, the notches between the segments are not distinct. Some larvae, especially those which have died only a short time before inspection, do not show these symptoms clearly. In cases of uncertainty, those larvae which do not react to repeated touches with body contraction or movement of the stigmas should be considered dead.

The mean number of alive larvae is corrected against the control values with Abbots formula. For the determination of the LD_{50}, the data are treated in the same way as in the LC_{50} test.

Proposal for a field test

Predictions about possible pesticide hazard to honeybee larvae can be made if the LC_{50} test is combined with a field test.

1. Bee colonies are transfered to the test field. They are left undisturbed for 4 - 5 days so that they can get used to the new environment.
2. The spontaneous mortality in the colonies is determined. To this end, control areas containing 50 3rd instar larvae each are marked with pins. The number of of surviving larvae is determined a day after the cells have been sealed.
3. Test areas are plotted on the frames in the same way.
4. The pesticide is sprayed in the test field on the same day.
5. A day after the cells have been sealed, the number of surviving larvae is counted. After correction of the data with Abbot's formula, (see LC_{50} test) one obtains the pesticide induced mortality in percent.
6. Refering to the graph with which the LC_{50} was determined, one can now derive the average pesticide concentration which caused the brood mortality in the field test.
7. From this data one can estimate which range of pesticide dosage in field application can be expected to be hazardous for bee brood.

The Apis Larvae Test has been used in our lab for several years. The results obtained with this test, using the procedures described, have proven to be highly reproducible. In the future, the Apis Larvae Test should be applied to quantify toxic effects of pesticides to honeybee brood, and also could be used to screen for larvicide effects of new compounds.

All pesticides and other chemicals used in and around the bee hive, especially acaricides used for Varroa control, should be required to pass the Apis Larvae Test.

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Acknowledgement

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APPENDIX 9

TOXICITY AND REPELLENT EFFECT OF THE SYNTHETIC PYRETHROIDS ON BEES METHODOLOGICAL ASPECTS

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INTRODUCTION

In order to study the possible relationships between neurotoxic effects of a pesticide formulation and the real effect in fields on honeybees, we used three different and complementary experimental approaches

* First, by topical application, a Lethale Dose 50 (LD 50) strictly reliable (i.e. which can be reproduced in identical experimental conditions) was established and analyzed. Furthermore the different parameters which could modulate and modify this LD 50 (Lethale Dose) were analyzed.

* Secondly, an attempt to quantify the repellent effect of the considered pyrethroïds was performed. Simultaneously the part of the chemical mixture responsible for such a repellent effect was studied.

* In a third set of experiments the difficult problem of the structure-activity relationship was considered by mean of electrophysiological technics ; in other words we tried to determine the limits of the specific chemical interactions between the chemicals studied and the proteinaceous receptors of the chemical sensory detectors of the bees.

The final aim of these three complementary approaches is to predict the danger for the bee when applying pyrethroids in field. Such an evaluation was previously established by ATKINS *et al* (1977) for classical pesticides only using LD 50 tests and field experiments. But with the pyrethroids the evaluation of the danger is complicated by the very weak concentration of pure compound applied per acre and by the repellent effect of such a type of formulation.

I - LD 50 Evaluations

The LD 50 was established at 48 h on 12-18 days old bees ; these bees before and after the LD 50 test were reared in controlled conditions, in an incubator at 32 °C and 50 % of relative humidity. The pesticide application, the doses determination and the statistical interpretation were made from LOUVEAUX, 1982. The following synthetic pyrethroids were tested BIORESMETHRINE, DELTAMETHRINE, CYPERMETHRINE, DEPALLETHRINE, FENVALERATE, PERMETHRINE. Furthermore phosalone was tested as a reference.

Bioresmethrine	0,04 < LD 50 < 0,06
Deltamethrine	0,01 < LD 50 < 0,03
Cypermethrine	0,02 < LD 50 < 0,03
Depallethrine	0,06 < LD 50 < 0,15
Fenvalerate	0,22 < LD 50 < 0,45
Permethrine	0,07 < LD 50 < 0,10
Phosalone "O.P."	1,40 < LD 50 < 2,15

Table I : Statistical estimation of the LD 50 (µg/bee)

Temporal variation of the LD 50

The LD 50 was calculated from 1 to 8 days after the intoxication of the bees. The results obtained lead to subdivide the pyrethroïds studied into two groups :

* The first one, including bioresmethrine, cypermethrine and deltamethrine, in which the LD 50 was stable in duration. Those pesticides are lightning neurotoxic.

* The second one, including fenvalerate, depallethrine and permethrine ; for which the LD 50 slowly decreased between 4 and 8 days after the pesticide application (BOS and MASSON, 1982)

Seasonal variation of the LD 50.

. On the one hand, for all pyrethroids, the LD 50 studied in laboratory conditions at 20 °C temperature is twice less important, than it is at 32 °C temperature.

. On the other hand, a statistical significant difference of the LD 50 level has been demonstrated between the winter honeybees (from October to March) and the summer honeybees (from April to September) : for all the pyrethroïds studied, the winter honeybees always give a standard deviation twice superior to that of the summer honeybee.

Moreover, considering the sensibility to pyrethroïds, the summer honeybees were more homogeneous than the winter ones (e.g. the cypermethrine : winter bees : 0,005 < DL 50 < 0,08, summer bees : 0,01< LD 50 < 0,3)

II - Behavioural study of the repellent effect in flying cage.

The high toxicity of the pyrethroïds is modulated by the repellent effect observable in field. In laboratory we reproduced such an effect in strickly controlled conditions to analyze it.

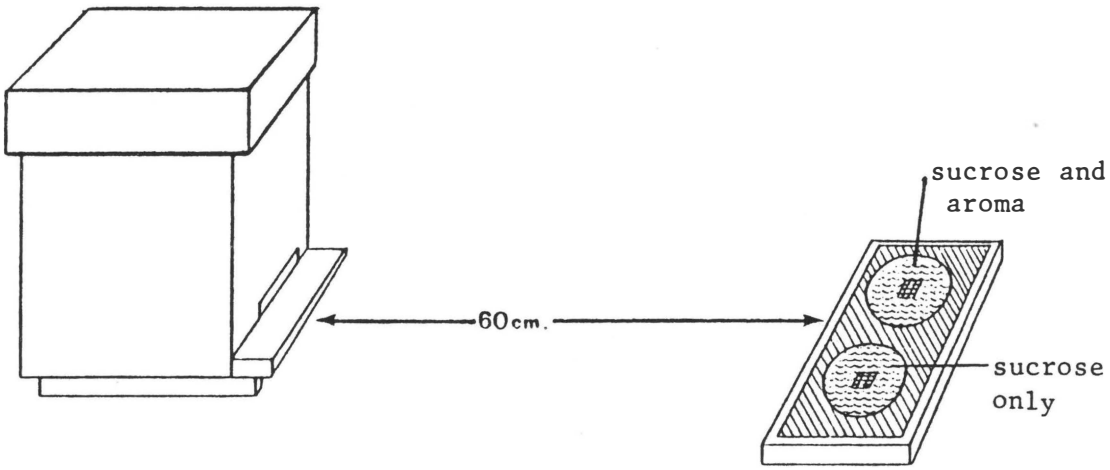

Figure 1 : Experimental device in flying room

The experimental device shown on Figure 1 was used to test commercial formulation of pesticides, pure compound and blank of formulation. During the test the bees had the choice between two dishes ; the one containing a sucrose solution and an empty aroma diffuser, the other containing a sucrose solution and an aromma diffuser filled with the tested odorant.

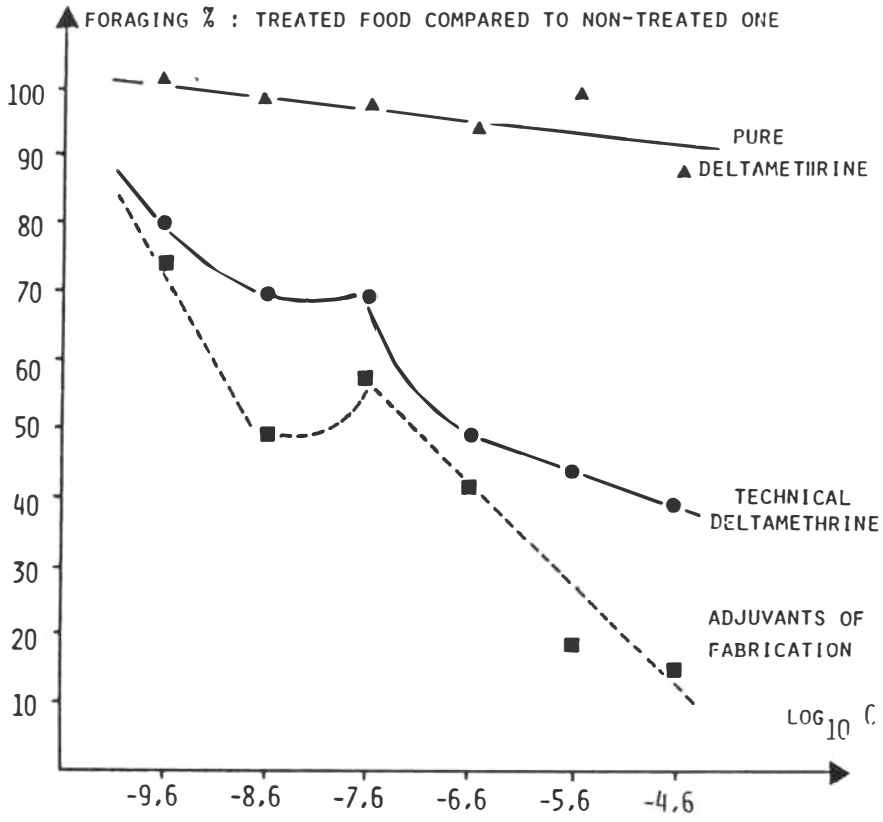

Figure 2 : A comparative study of the repellent power of the different formulation of Decis ®.

As shown in the Figure2 the adjuvant of fabrication and the commercial formulation gave the same response. The pure deltamethrine a.i. was not repellent for the bee, consequently the adjuvants seemed to be responsible for such a repellent effect (See BOS and MASSON, 1982).

III - Study of the repellent effect by coupled electrophysiological and behavioural techniques.

The aim of such a approach is to find a relationship between the molecular structure and the repellent (or attractant) effect. To reach this aim it was, at least, necessary to possess biological parameters (EAG's amplitude or number of bee repel by a chemical substance) and the chemical parameters of the tested repellent. Then it was possible to draw curves of correlation between biological and chemical parameters. We tested series of different compounds such as alcohols, acids, heterocycles... and other components which can be found in commercial formulation of pesticides. To illustrate this approach we will just present here some preliminary results

gathered with one chemical serie (alcohols) tested on different biological parameters.

Methods

Double_cage.

In small double cage (12 x 10 x 10 cm) we determined the repellent characteristics of the alcohols for bees.

Electrophysiological_tests (see MASSON and BROSSUT, 1981).

The technique used was that of the electroantennogram (EAG) (SCHNEIDER, 1957). The electrical activity recorded is a slow monophasic negative change from the antennal sensory fibres generated by populations of olfactory neurons which were simultaneously electrically reacting to the same set of molecules (here alcohols in gazeous form). It is considered to be an odor-induced summated receptor potential of many sense cells lying, more or less, in series.

The amplitude response of the receptor cells was correlated with the number of specific interractions between the molecules and the receptors.

Results

The alcohol components of the serie considered, differ only by the length of the carbon chain (C2 to C8) ; tested by EAG lead to the results shown on figure 2.

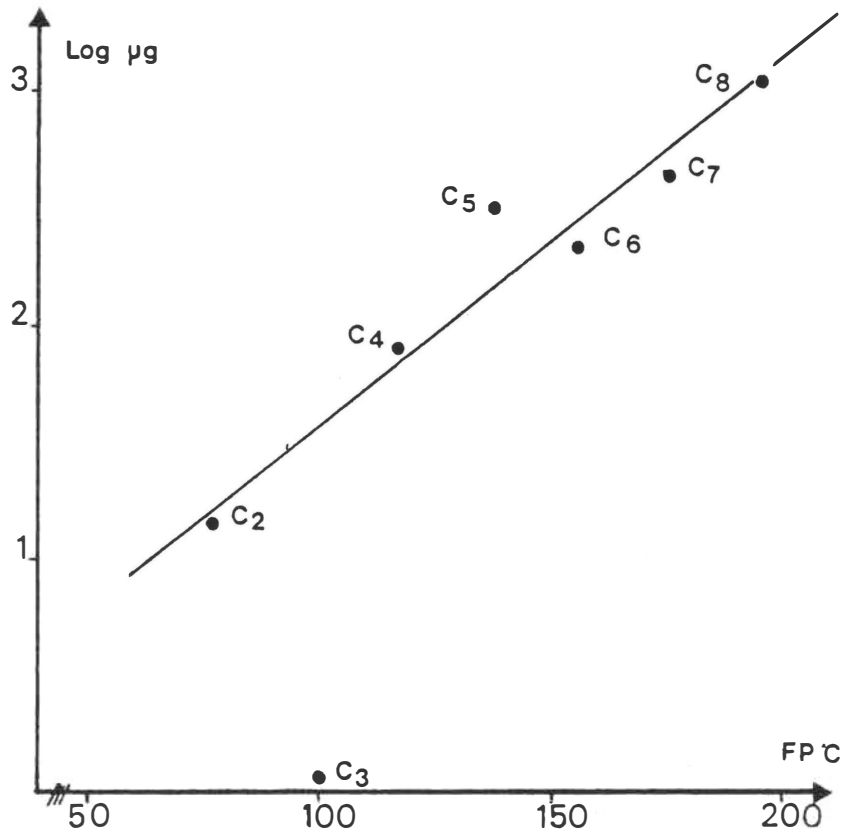

Figure 2 : Amplitude of the EAG of 8 alcohols fonction of fusion point

There was a positive correlation between the fusion point and the logarithm of the EAG's amplitude. The C_3 compound was out of the regression line.

The same type of correlation was found by behavioural experiments, all the compounds of this serie of alcohols were determined as repellents, only one exception the C_3 compound. Consequently the electrophysiological tests and the behavioural tests gave the same data for this serie of alcohols. Experimental tests are in progress to find a statistical positive correlation between EAG and behavioural tests.

Conclusion.

To conclude about these preliminary convergent results get by different and complementary experimental approaches, one can suggest that it will be certainly possible in a near future to predict by laboratory experimental tests the repellent power of a compound or of a technical formulation of pesticide.

Now using specific computer programms we are trying to join the "repellent effect" with the intrinsic toxicity (LD 50). Such relationships will be useful tools to estimate the potential hazard for bees when pesticides are applied in field conditions. In this way the field test might be only a verification of the previous estimation.

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APPENDIX 10

INVESTIGATIONS ABOUT THE SIZE AND THE DURATION OF TOXICITY TO BEES OF THE PESTICIDES AMBUSH, DECIS, GUSATHION AND PIRIMOR.

J. van der Steen and Ir. J.J. Pettinga

1. Introduction

For about 20 years, research has been done at the Experimental Station "Ambrosiushoeve" range of toxicity of pesticides to bees with the LD_{50} test.

In preceding years a start has been made with investigations about the duration of the toxicity by means of the so-called simulation tests, originally developed by our colleague Dr. Gerig in Bern-Liebefeld. This research started with Ambush and Gusathion (Bijenteelt 83(1981) nr 6, pg 131, in Dutch).

The simulation test gives an estimate of the duration of residual toxicity of pesticides to bees. It also gives an impression of secondary effects such as paralysis and knockdown; nevertheless it does not test repellent effects. These so-called side-effects may have great influence on the hazard of pesticides to bees.

In 1981 we have enlarged the investigations with tests in cages and repellency-tests; the cage-tests resemble more closely the method of applying pesticides in the field.

With four different tests: LD_{50}, simulation-test, cage-test and repellency-test, we have tried to form a complete picture of the influence of pesticides on bees, under the conditions in our country, especially for duration of toxicity.

2. Methods

2.1 LD50 test This test has been performed under the conditions laid down at the symposium in Wageningen 1980.

Also an LD50 test was performed with overwintering bees. The bees were fed individually with 10 mm^3 50% sucrose solution, with or without a pre-determined concentration of the pesticide.

2.2 Simulation test In these tests the bees are kept in contact with sprayed flowers in a plastic cage (disposable) as described in the results below. With this test we determine the duration of the toxicity and changes of behaviour of the bees.

Standards are flowers, sprayed with water and with a pesticide with a known toxicity, also in cages.

2.3 Tests in cages These are performed to test the influence of pesticides to bees under conditions used in practical application. Nucleus hives on 6 frames (without foraging bees) are placed in cages of 2x2x3 metres. After several days, when a sufficient number of field bees are available, spraying is done during bee foraging.

The dead bees are collected in a Gary-trap in front of the hive entrance. Pesticides being tested are applied at the recommended concentration in 1000 l.water/ha; i.e. 0,6 l. liquid is used in a cage of 6 m^2.

2.4 Repellency-tests

In a glasshouse, with bee-forage in flower, and a bee-colony within, squares of 1 m^2 are marked. The visiting bees are recorded several times a day during 5 minute periods. During bee-flight, one half of the glasshouse is sprayed with the pesticide under test at the advised concentration. Immediately after spraying visiting bees numbers are recorded (in 5 minute periods). These records are also continued during the following days.

3. Experiments and results

The pesticides tested were:

Decis (deltamethrin)	formulation 25 gr. active substance/1000 ml concentration to be appl. 0,02%
Ambush (permethrin)	formulation 250 gr. a.s./1000 ml. concentration to be appl. 0,025%
Pirimor (pirimicarb)	formulation 50% concentration to be appl. 0,05%
Gusathion (azinphos methyl)	formulation 225 gr.a.s./1000 ml. concentration to be appl. 0,15%

3.1 LD50 tests:

The LD50 of Decis was obtained with standard bees 6,5 ug/bee and with winter bees: 6.1 ug/bee. The LD50 of Ambush, Pirimor and Gusanthion obtained before 1981 were with overwintering bees.

3.2 Simulation tests: 3 tests were performed:

	I	II	III
date of spraying	14.1.1981	28.7.1981	7.9.1981
date of starting	15.7.1981	28.7.1981	7.9.1981
crop	Phacelia	Phacelia	Phacelia
pesticides	Decis 0.02% Gusathion 0,15%	Decis 0,02% Gusathion 0,15% Ambush 0.025%	Decis 0,02% Gusathion 0,15%

The results of these tests are classified according to time between spraying and time of introduction of bees to the flowers.

I. Period 0 - 60 minutes:

Ambush: Within two hours all the bees were paralysed, but none were dead. Some time later however 90% of the bees had died.

Decis: Within one hour about one half of the bees are paralysed. This paralysis diminished rapidly. After four hours about 30% of the bees had died.

Gusathion: Contrary to the pyrethroids the bees died rapidly. The period of paralysis was brief and within one hour almost all the bees had died.

II. Period 20 - 24 hours:

Ambush: A great number of the bees were rapidly paralysed. This condition also rapidly diminished. The majority of these paralysed bees recovered.

Decis: About 20% of the bees were paralysed. The number of dead bees was as great as in the standard.

Gusathion: Within two hours about 20% of the bees died; afterwards none.

III. Period 2 x 24 hours:

Decis: After one hour of contact with the sprayed flowers some of the bees were paralysed. Many of the paralysed bees recovered quickly. Mortality was the same as in the standard.

Gusathion: Mortality is the same as in the standard.

IV. Period 3 x 24 hours:

Decis: No particular effects.

Gusathion: As Decis.

3.3. Tests in cages

The total results of 4 experiments in cages are classified in periods after spraying. The results for the standard (blank), Gusathion and Ambush is the mean of four experiments. The results of Decis and Pirimor are the average of two experiments.

Ambush: This pesticide causes mortality immediately after application. This mortality decreases rapidly. After one day the death rate is the same as in the standard.

Decis: Spraying with decis gives no increase in mortality.

Pirimor: Also Pirimor gives no increase in mortality.

Gusathion: Just after spraying there is a high mortality. Even after two days the number of dead bees is greater than in the standard. After three days there is still some effect.

3.4. Repellency tests

These tests were performed once with Ambush and once with Decis. The repellent effect of Ambush occurs immediately after spraying and continues for some days. The repellent effect of Decis is clear after spraying; after some hours it diminishes.

4. Discussion

About methods: The LD50 is a typical laboratory-test; only the mortality

is recorded and only the oral toxicity is determined. The standard bees showed no ill effects. It is striking that there is a difference between the LD50 of standard bees and winter bees. Repeated tests are required to show if the difference is consistent or if there is another cause.

The contact-toxicity test was not done separately. With the simulation test, it is possible to detect (if present) any striking contact-toxicity.

The well-known repellency of Ambush (from literature and our own observations) is not quite clear in our simulation test. These tests are not the ideal ones to demonstrate repellency.

It is also necessary to provide sufficient sucrose solution 50% to the bees in simulation tests. This has no influence on the behaviour of the bees.

Repellency is perceptible in the cages, but difficult to measure. Also number of foragers differed between cages.

With the Gary-traps only those bees are trapped which have died inside the hives. The bees which died inside the cages or were knocked down are not recorded. We will try to solve this problem in the future by using another type of trap and/or to change the arrangement of the crop inside the cage.

In the repellency test we recorded in nearly all the observations more flying bees on the standard plots inside the glasshouse than on the treated ones. It may be the altitude of the sun had some influence. To exclude this, we shall have to change the situation of the plots.

About results

Decis: The LD50 is 6.5 - 6.1 ug/bee. This means that Decis belongs to the moderately toxic pesticides. The simulation tests show that this pesticide can still result in about 20% of the test bees being paralysed after 24 hours. Later on this effect diminishes rapidly.

Dr. L. Gerig has pointed out that this knock-down is reversible. We have made the same observations in our tests; it is however not complete. In the cage test Decis gives no increased mortality. Re pellency is recorded directly after application. After some hours this effect has disappeared.

Ambush: It has a LD50 of 0.9 ug/bee and therefore it belongs to the highly toxic pesticides. Simulation tests in 1980 have shown that bees may still become paralysed even after 4 days. This effect may be reversible.

In the cage tests there was a raised mortality some hours after application. This mortality diminished rapidly.

Repellency clearly occurs directly after application and continues for some days. How long could not be determined from our tests. According to Dr. Gerig it may be 2 or 3 days. This repellency is not complete.

Pirimor: The LD50 of this pesticide is 1 ug/bee. This is contrary to the other determinations, which are made with commercial formulations. Our figure is determined in 1970.

In two cage-tests there was no mortality at all. This is a confirmation of former investigations and experience in practice. Pirimor causes no mortality at the recommended concentration for application in practice. No research has been done on secondary effects.

Gusathion: The LD50 is smaller than 1 ug/bee. It is a highly toxic pesticide for bees. Simulation-tests in 1980 have shown, that this pesticide after 4 days has still a slight lethal effect on bees. In cage tests there is after 3 days still a raised mortality compared with the standard. After that it rapidly diminishes (cage test I and II). This may be due to the degradation of this pesticide under influence of the sunshine.

5. Conclusions and Recommendations

Decis: is not toxic to bees, in that they are not killed directly. Nevertheless its not harmless in view of the paralyses.

Ambush: The toxicity can be avoided for most bees by means of repellency but not for all the bees, in view of the increased mortalilty in the cage tests.

Conclusion: Ambush is not harmless to bees.

Pirimor: In the cage-tests this pesticide in the advised concentration causes no lethal effect on bees.

Gusathion: It is highly toxic to bees. Even 3 days after application it has still a killing effect. After this time this lethal effect diminishes rapidly.

For the two pyrethroids (Ambush and Decis) harm to bees can be avoided by not spraying during bee-flight, but preferably some days before bee-flight starts:

Decis: (decamethrin) two days before bee-flight

Ambush: (permethrin) 3-4 days before bee-flight

Harm by applying Gusathion can be avoided by spraying some days before bee-flight starts, at least 4.

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Literature: L. Gerig: Bienengiftigkeit der synthetische Pyrethrine

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APPENDIX 11

A FIVE-YEAR STUDY ON THE EFFECT OF DELTAMETHRIN ON BEES UNDER NATURAL CONDITIONS

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Summary

A five-year study with deltamethrin on bees under natural conditions has been carried out. A new methodology, usable on experimental stations to determine the effect of pesticides on bees is described. The results obtained with deltamethrin, parathion and phosalone are discussed.

In a second part, the effect of deltamethrin at 7.5 g a.i./ha on bees under practical conditions is then described.

Pyrethroid insecticides, and especially deltamethrin, are toxic to bees under laboratory conditions by topic applications (ATKINS, 1976). Nevertheless, the hazard of deltamethrin to bees seems limited under cage and semi-natural conditions (ATKINS 1976, GERIG 1978).

It was for that reason that, in collaboration with Mr. J. LOUVEAUX, Chairman of the "Station de Recherche sur les Abeilles et les Insectes sociaux - Institut National de la Recherche Agronomique", a methodology was developed which made it possible to carry out a study to determine the hazards of deltamethrin to bees under conditions as near as possible to those encountered in practice.

EXPERIMENTATION CARRIED OUT ON EXPERIMENTAL FIELD STATION

The experimental method presented during the 1980 Symposium in Wageningen has been tested for 5 years period (1978-1982) and is now accepted broadly as a guide-line for the working group "Commission des Essais biologiques" CEB in France.

The main points of this experimental method are detailed in this paper.

1) Environment

The trials were carried out by the Agricultural Experimentation Department of ROUSSEL-UCLAF near Paris, on its Experimental Field Station at Gouzangrez in the Vexin region. During the trial period, the environment was not or only slightly attractive to bees, except for the flower crop used in the trial *Sinapis alba* (white mustard), very attractive to bees (LOUVEAUX 1980).

2) Field experimental arrangement

In order to obtain suitable conditions for experimentation, the trial was carried out on crops which flower throughout the summer and with a number of bees representative of practical conditions.

2.1. Crop

1,500 m2 plots were sown with white mustard every 15-20 days from the end of March. Each of this plots consisted of 30 strips (50 m x 1 m) separated from one another by 0.70 m-wide alley. All these alleys were completely free from weeds and tamped down so as to obtain a flat surface on which the insects could be collected. Each plot was used only once and was then ploughed before sowing a new trial.

2.2. Bees

The bees concerned were "Italian race x Caucasian race" hybrids *(Apis mellifera lingustica x Apis mellifera caucasia)* from movable frame hives supplied by a professional bee-keeper. In general, during each trial and consequently for each application rate, 4 hives were placed close to the mustard plot. The bees from 3 of these hives remained in the trial area for about 20 days, which avoided the occurrence of any possible disturbance or weakening of the hive, the result of a choice of pollen and nectar being limited to mustard. The 4th hive remained during the whole trial period to be subjected to several treatments that could occur under practical conditions.

3) Treatments

The whole trial plot, i.e. 1,500 m2, was treated when the number of foraging bees reached its maximum, i.e., generally speaking, between 12 a.m. and 2 p.m. The treatments were carried out either with a van der Weij knapsack sprayer with an output of 500 l/ha under a constant pressure of 3 bars (1978, 1979, 1980), or with a sprayer used in practice (Tecnoma type) with a 10 m-wide spray boom, a constant pressure of 4 bars and a water output of 250 to 300 l/ha (1981, 1982).

4) Assessment of results

4.1. At the hives

4.1.1. On the ground, in front of the hives : 2 counts were made daily (at 8 a.m. and 5 p.m.) in order to determine the number of dead bees (a distinction was made between males, workers and nymphs). During this count, observations were made on the behaviour of bees (entering and leaving the hive, aggressiveness). These observations and counts were made before treatment and 1 hour and 3 hours later on, every day treatment was given. Moreover, each time the bee-keeper was on the station, he made observations on the behaviour and activity of the brood-comb.

4.1.2. At the hive : during the 1981 and 1982 experimental work a trap for collecting all the dead bees was placed in position, in order to check whether the cumulated mortality for the 4 hives exactly showed what had actually occured (the trap for dead bees enabled all the dead insects to be collected).

4.1.3. Pollen trap : a pollen trap was placed on some of the hives. This arrangement was not used for all the hives, as it had a tendency to weaken colonies by depleting them of a part of the pollen they had collected. This observation permitted to determine daily the amount of pollen collected, which in fact reflected the activity of a hive.

4.2. On the crops

All the counts and observations were made on 3 strips chosen at random from the 30 useful strips of each trial.

4.2.1. Count of foraging bees :

+ pre-treatment phase : a count was made at least 4 times a day (at 10 a.m., 12 a.m., 2 p.m. and 4 p.m.) on the selected 3 strips. Two technical assistants, on both sides of each strip, counted the bees foraging on the crop, walking to its end and returning to their starting point. Each strip had an area of 50 m2. Thus, the total treated surface on which the counts were made was 50 m x 2 x 3 = 300 m2.

+ treatment day : the daily counts were made at 10 a.m. and 12 a.m. Just before treatment a count was made following the same methodology. The treatment was made and further counts given at T + 15 minutes, T + 1 hour and T + 3 hours.

+ post-treatment phase : during the days following application, the same counts were made at 10 a.m., 12 a.m., 2 p.m. and 4 p.m. In addition to the counts made on flowers, 3 "sampling surveys" were made by strip at the base of the crop (the technical assistant drew the vegetation aside, in order to count any insect on the ground).

4.2.2. Behaviour of foraging bees :

+ pre-treatment phase : numerous observations permitted an estimate to be made of the behaviour of the working bee in relation with weather conditions.

+ day of treatment : immediately behind the spray boom, two technical assistants observed the behaviour of bees that had been reached by the insecticide mixture (temporary disappearing under the crop, disturbed foraging, abnormal flushing out, "wiping reflex", aggressiveness, ...)

+ post-treatment phase : observations similar to those made in pre-treatment phase, were made.

4.3. On the alleys

Simultaneously with the counts made on the crop (Cf. above, pre- and post-treatment phases, day of treatment), counts were also made on alleys in order to determine the number of dead bees or of bees which could no longer fly away. These counts were made on the alleys, on each side of the chosen 3 strips, i.e. 0.70 x 50 x 2 x 3 = 210 m2 (which corresponds, taking into account the intervals on a 300 m2 crop, to a strip + 2 half strips).

Between the hives and the trial, sampling surveys were made in order to check a possible mortality under the "flight corridors" of the bees. Theoretically, a normal trial lasts at least 5 days:

- the first two days, observations before treatment
- the third day, observations during treatment
- the 4th and 5th day, post-treatment observations.

In fact, the duration of a trial is much longer (weather changes, delay in the flowering of a crop, ...) and varied under the trial conditions from 10 to 27 days.

All these counts made it possible :

- to know how many bees were visiting the crops, according to the weather conditions checked at each count (pre-treatment phase).

- to detect a possible repellent effect of the insecticide (diminution of the number of bees/m2) and estimate its duration (under similar weather conditions).

5 - Results

From 1978 to 1982 24 trials were carried out with different rates of deltamethrin, water, parathion and phosalone. The following table summarizes the tested rates.

Year	Product and rate tested
1978	Water Deltamethrin at 3.75 - 5 - 6.25 - 7.5 g a.i./ha
1979	Water Deltamethrin at 5 - 6.25 - 7.5 - 8.75 - 10 g a.i./ha
1980	Parathion M at 500 g a.i./ha Phosalone at 1,200 g a.i./ha Deltamethrin at 7.5 - 10 - 12.5 - 12.5 - 17 g a.i./ha
1981	Deltamethrin at 21.2 and 35 g a.i./ha
1982	Code number

PRODUCT AND RATE TESTED

Instead of reporting each trial separately, it was considered better to discuss results obtained with water, parathion, phosalone and deltamethrin at 7.5 - 12.5 - 17.5 -21.2 and 35 g a.i./ha given in tables I to VIII which are at the end of the paper.

5.1. Results obtained with water

Treatment at 300 l/ha of water did not kill the bees, either on the crop or at the hives. Immediately after spraying, the number of foraging bees decreased ; the bees flew away normally or hid under the flowers (1.44 bee/m2 before, 0.45 bee/m2 behind the boom, 0.91 at T + 10 minutes, 0.81 at T + 20 minutes !) These data show that the treatment with water disturbed temporarily the behaviour of the bees as a result of physical shock.

5.2. Results obtained with parathion

The results of the treatments carried out on 3/6/80 at 2.30 p.m. (3.9 bees/m2, fine weather, 28°C) were the following :

- the behaviour of the bees on the crop was greatly affected after the passage of the boom. Most of the bees remained on the crop, moved very slowly and those which took off had a very heavy flight

- there was no massive rapid take off of the bees, but only a slight decrease in the number of bees on the crop after treatment.

- the bee mortality on the crop and in the alleys was high on the treatment day and the following day (30 bees on the treatment day in the alleys, 25 at T + 1, 24 at T + 2 and 21 at T + 3, i.e. a theoretical mortality/ha of 2.381 - 1.905 - 1.905 and 1.667 respectively).

- the bee mortality at the hives was very high in the evening, as soon as the insecticide had been applied and during the following 3 days.

To sum up, this trial confirmed the validity of the operating procedure, as a rate of 500 g a.i./ha parathion M applied on 1,500 m2 caused great mortality, both on the crop and at the hives.

5.3. Results obtained with phosalone

The results of an application of phosalone 1,200 g a.i./ha are the following :

- immediately behind the spray boom, the bees flew away but came back about 5 minutes later (1.1. bee/m2 at T 0 - 1.3 at T + 5 - 1.1. at T + 15 - 1.1. at T + 60 minutes and 1.1. at T + 2 hours).

- the behaviour of the bees on the crop did not change.

- no change in the behaviour of the bees at the hive and no appreciable mortality.

- a slight mortality was found in the alleys during the day following the treatment.

- the pollen weight collected did not change under similar weather conditions.

To sum up, the selectivity of phosalone applied at a rate of 1,200 g a.i./ha on foraging bees under the experimental conditions was confirmed.

5.4. Results obtained with deltamethrin

5.4.1. Observations on mortality

5.4.1.1. On the crop :

+ at a rate of 7.5 g a.i./ha, no bees were killed on the crop, either during the treatment day or the following days ; likewise, the mortality detected in the alleys remained very low.

+ at 12.5 g a.i./ha during the trial a very slight mortality was noted (336 bees/ha for 3 days) which is negligible, taking into account the great number of bees visiting the crop.

+ at a rate of 17.5 g a.i./ha, a slight mortality was revealed which, although not great, was greater than that observed at 12.5 g a.i./ha.

+ at 21.2 and 35 g a.i./ha, the mortality on the crop exists but is not so great.

Times	Deltamethrin					Phosa-lone 1,200	Para-thion 500
	7.5	12.5	17.5	21.2	35		
T + 1	48	96	576	67	166	240	2,143
T + 2	48	96	288	0	100	192	1,905
T + 3	0	144	768	134	333	384	1,867

Mortality observed with the selected products (in bees/ha)

5.4.1.2. At the hives :

The daily mortalities for each rate are reported in Tables II to VIII. The Table below summarizes the figures obtained for all the tested rates for a period of 3 days before and 4 days after application.

Times	Deltamethrin					Phosa-lone 1,200	Para-thion 500
	7.5	12.5	17.5	21.2	35		
T - 3	-	22 + 1	-	-	69 + 9	-	4 + 0
T - 2	0 + 0	18 + 0	-	-	-	-	7 + 0
T - 1	18 + 0	13 + 2	13 + 6	-	-	10 + 1	21 + 0
T 0	24 + 1	26 + 2	24 + 14	97 + 22	341 + 24	10 + 4	> 2,000
T + 1	14 + 0	9 + 9	24 + 71	93	97 + 15	29 + 4	850 + 0
T + 2	5 + 1	0 + 0	23 + 11	82 + 6	96 + 12	26 + 1	214 + 1
T + 3	4 + 1	-	9 + 0	125 + 8	220 + 11	49 + 9	133 + 3
T + 4	0	-	7 + 1	90 + 6	128 + 8	19 + 4	-

Daily mortality (workers + males) 3 days before and 4 days after treatment (for 4 hives)

From this Table, it is seen that :

- deltamethrin at 7.5 - 12.5 and 17.5 g a.i./ha and phosalone at 1,200 g a.i./ha do not give any abnormal mortality in front of the hives. On the other hand, mortality is very high with parathion. Although lower than the one of parathion, deltamethrin at 21.2 and 35 g a.i./ha causes a marked mortality.

5.4.2. Behaviour of the bees

+ At the hives : at all the selected rates, no change in the behaviour of the bees was observed ; moreover, the bees during the trials in the summer showed normal behaviour at the end of the following winter ; they recovered a good activity, a normal brood comb, ...

+ On the crop : the behaviour of the foraging bees was normal after application rates of 5 and 12.5 g a.i./ha. At 17.5, 21.2 and 35 g a.i./ha some bees crawled on the flowers and showed signs of "sluggsihness" which did not last more than one hour after application. However, it was noted that the bees during the sluggishness period were able to fly again when touched.

5.4.3. Repellent effect :

At the rates of 7.5 to 17.5 g a.i./ha, bees flew away immediately and rapidly behind the spray boom. This behaviour was not observed with lower rates or after a water treatment.

The visiting frequency of foraging bees on the crop decreased for 2 - 3 hours after insecticide application. ATKINS noted this effect, but for a longer duration and under different experimental conditions.

This effect, which can be qualified as repellent, involved no change in the pollination of the visited crop and seemed specific to Decis, as it was not observed either with parathion or with phosalone.

6) Conclusion

This extensive experimental work carried out under natural conditions led to the conclusion that the methodology applied is very suitable for testing the hazards relating to the use of pesticides (the toxicity of parathion was tested).

Because the rate of 35 g a.i./ha caused a not to be neglected mortality on treatment day as well as on the following days, especially at the hives, it can be considered that this rate does not show a sufficient "safety factor" to be applied directly on bees when foraging.

Under these conditions, it is seen that deltamethrin applied directly to foraging bees is not hazard to bees up to 21.2 g a.i./ha.

EXPERIMENTATION CARRIED OUT UNDER FIELD CONDITIONS

In order to confirm the suitability of this methodology used on the experimental field station, and to test the effect of deltamethrin under practical conditions, a large scale trial was carried out 3 times, respectively in 1980, 1981 and 1982.

1) Environment

At the time the trial was carried out, a check was made that the environment was not or only slightly attractive to bees (in 1980 and 1981, after a detailed survey with a car round the chosen field, in 1982 with a plane).

2) Field experimental arrangement

The following Table gives us general informations concerning the trials.

Year	1980	1981	1982
Location	Gouzangrez (95)	Avernes (95)	Avernes (95)
Crop	White mustard	Winter rape	Winter rape
Acreage of the treat. field	6 ha	4 ha	14 ha
Treatment day	26/8/80	7/5/81	11/5/82
Rate (g a.i./ha)	7.5	7.5	7.5
Amount of water/ha	300 l	300 l	220 l
Number of bees/m2 before treatment	≈ 1	1	0.8
Crop stage	End of flowering	End of flowering	Full flowering
Number of hives	4	4	8

For these 3 trials, there was no control or any repetition. The whole plot of an area of 4,6 or 14 ha was treated. 4 or 8 hives were placed in the middle of the plot, at the place where the crop was previously destroyed, so that observations concerning mortality on the soil could be made.

3) Observations

3.1. At the hives

+ the mortality was regularly checked every morning and evening in front of the hives.

+ a pollen trap placed on one of the four hives (or two of the eight hives) permitted the quantity of pollen collected, which in fact is a reflection of the bees activity, to be weighed.

+ the behaviour of bees (aggressiveness, ...) was observed during each visit to the hive.

+ the hives were also checked by the bee-keeper after they had been removed from the trial plot.

+ in 1982 the brood comb activity, the honey yield and the residue on the pollen were determined, in collaboration with Mr. J. LOUVEAUX's laboratory.

3.2. On crops

+ The treatment day, the foraging intensity of bees was determined by counting the insects present on a 30 m2 strip (30 x 1 m).

+ likewise, behind the spray boom, the behaviour of bees was observed : aggressiveness, wiping reflex, flushing out effect,

4) Results

4.1. Results obtained in 1980

The results obtained after the treatment were the following :

- no great change in the behavicur of the bees on the crop was reported (some bees showed signs of sluggishness) and the sampling surveys made at the base of the crop revealed the absence of mortality after the passage of the spray boom.

- the results of the mortality checked for the hives are shown in the following Table.

Day	Time	Mortality at the 4 hives			
		8 h	10 h	14 h	17 h
25/8	-	-	-	-	1
26/8	T O	-	32	29	-
27/8	T + 1	-	41	-	130
28/8	T + 2	116	-	-	92
29/9	T + 3	8	-	-	-
01/9	T + 6	9	-	-	13
02/9	T + 7	33	-	-	4
03/9	T + 8	2	-	-	6
04/9	T + 9	10	-	-	3
05/9	T + 10	-	1	2	-

When examing these data, it is seen that the mortality observed at the hives remains low. The already advanced flowering stage of the crop at the treatment time did not permit this trial to be carried out under as satisfactory conditions as it could have been wished (fall in the visiting intensity of the crop from 29/8/80 ; the study stopped on 5/9/80, i.e. 10 days after treatment), but under these conditions, it was nevertheless confirmed that the rate of 7.5 g a.i./ha remained non-toxic to bees.

4.2. Results obtained in 1981

The results are shown in Tables IX and X in the form of a chronological summary of observations on bee mortality in front of the hives, the visiting frequency on the crop, the weight of pollen collected and the weather conditions. When reading these Tables, it was confirmed that deltamethrin applied directly to foraging bees does not involve any abnormal modification of the colonies studied during the trial.

4.3. Results obtained in 1982

These are shown in Table XI, which gives the daily weight of pollen, number of bees visiting the crop and daily mortality for the 8 hives. The treatment was made during good weather on a full flowering crop : after the treatment, the number of bees foraging the crop decreased during one hour (repellent effect) ; subsequently, their behaviour was normal. All the observations concerning the comb did no show any effect of the treatment ; the yield of honey per hive varied from 16 to 28 kg. Once again, it was confirmed that deltamethrin applied at 7.5 g a.i./ha directly on bees foraging the crop is without hazard to bees.

CONCLUSION

Extensive trials carried out under Experimental Station or open field conditions for 5 years with several rates confirmed that the use of deltamethrin does not present a hazard to bees under practical conditions, while it revealed some specific properties (repellency).

Although slight mortality was observed at the 21.2 g a.i./ha rate, this remained much below that of parathion, classified as toxic. The 12.5 rate reveals a less toxic effect than phosalone which is officialy recognized as "harmless to bees".

These results are in full agreement with those obtained in Great-Britain at the rate of 10 g a.i./ha (Agrisearch Field Development 1980) and in France at the rate of 8 g a.i./ha (MASSON, 1981) on rape, and in practise (La Défense des Végétaux, 1982).

The conclusion from all these results is that there is a safety margin which allows the use of deltamethrin on rape or cereals in full flower at the rate of 6.25 g a.i./ha or less, although the rate of 17.5 g a.i./ha is still safe.

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TABLE I - Changes obtained in visiting intensity on the crop, following various applications of deltamethrin, parathion and phosalone

Product	Rate g a.i./ha	Application conditions				Number of bees after treatment				
		Day	Time	Number of bees/m2	T°	Behind boom	T + 10 mn to T + 15mn	T + 20 mn to T + 30 mn	T + 1 h	T + 2 h
Water	300 l	22/6/79	13 h 40	1.4	20°C	0.45	0.91	0.81	-	1.56
Deltamethrin	7.5	14/5/80	12 h	1	19	0.4	-	0.3	0.2	0.4
"	12.5	16/7/80	14 h	2.1	19	0.1	0.8	-	0.3	0.9
"	17.5	20/8/80	14 h	1.3	23	0.2	0.5	-	0.5	2.1
"	21.2	15/6/81	14 h	2.3	26	0.6	0.5	-	-	0.01
"	35	13/7/81	14 h	1.3	16	0.41	0.17	0.15	1	0.7
Parathion	500	03/6/80	14 h 30	3.9	23	3.5	0	-	1.1	-
Phosalone	1200	08/8/80	14 h 10	1.1	25	1.3	1.1	-	1.1	1.1

TABLE II - Results obtained with deltamethrin at 7.5 g a.i./ha

Day (1980)	10 h		12 h		14 h		16 h		Mortality at hives (3)	Mortality on alleys		
	Number of bees/m2	T°C (1)	Number of bees/m2	T°C	Number of bees/m2	T°C	Number of bees/m2	T°C		10 h	16 h	After treatment
12/05	-	18° → (1)	0.6	21° →	0.9	21° →	2.0	26° →	0	-	-	-
13/05	0.8	17°	1.1	22°	0.6	(24° W)	0.7	(23° W)	18 + 0	-	-	-
14/05 (2)	0.9	17°	1.0	19°	0.2	(21° W)	0.4	(18,5° W)	24 + 1	1 worker	1 worker	2 workers
15/05	0.1	13°	0.6	16°	0.7	18°	0.5	19,5° →	14 + 0	1 worker	0	-
16/05	0.2	13°	0.6	(17°)	0.9	(19°)	0.4	(19°)	5 + 1	1 worker	0	-
17/05	0.6	18°	-	-	-	-	0.6	19°	4 + 1	0	0	-
18/05	-	-	1.0	20°	-	-	0.5	(17°)	0	0	1 worker	-
19/05	0.9	18°	1.0	25°	1.1	23°	1.0	24°	11 + 1	0	0	-
20/05	0.7	17,5°	0.9	21° →	0.9	20°	0.7	(20°)	6 + 3	0	0	-
21/05	0	[14°]	0	[15°]	0.06	[17°]	0.08	[18°]	10 + 0	1 worker	0	-
22/05	0.1	(14°)	0.8	(17,5°)	0.8	20° →	1.0	20° →	10 + 1	0	0	-
23/05	0.04	(12°)	0.1	(14°)	0.8	17° →	0.8	17° →	15 + 1	3 workers	0	-

(1) 18° fine weather ; 21° → wind ; (24° W) stormy and cloudy ; [17°] rainy weather

(2) treatment day 14/5/80 at 14 hours

(3) workers + males : cumulated mortality for day.

TABLE III - Results obtained with deltamethrin at 12.5 g a.i./ha

Day (1980)	Hive	10 h		12 h		14 h		16 h		Mortality in hives (3)	Mortality in alleys			
	Pollen weight	Number of bees/m2	T°C	Number of bees/m2	T°C	Number of bees/m2	T°C	Number of bees/m2	T°C		10 h	16 h	Nb. bees after tr.	Mortality/ha(4)
10/07	18 g	0	(15°) (1)	0.1	(17°)	0	18° →	0	18° →	76 + 0	0	0		0
11/07	25 g	NCO*	-	0.5	19°	0.5	19°	0.2	19°	20 + 4	0	0		0
12/07	6 g	0.3	[19°]	rain						22 + 1	0	0		0
13-14/07	20 g	0	[19°]	0	[19°]	0	[19°]	0	[19°]	18 + 0	0	0		0
15/07	0	0	[18°]	0	[19°]	0	[19°]	0	[19°]	13 + 2	0	0		0
16/07 (2)	11 g	0	(16°)	0	(16°)	2.1	19°	0.9	19°	26 + 2	0	2	0	96
17/07	7.5 g	0.7	17°	1.7	18°	0.6	20° →	0.2	20° →	9 + 9	0	2 workers		96
18/07	6 g	0	(17°)	0	(18°)	0	18°	0	[18°]	0 + 0	1 worker	2 workers		144
19-20/07	0 g	NCO		NCO		NCO		NCO		333	following handling in hives by bee-keeper			
21/07	37 g	0.3	17° →	0.3	18° →	0.2	20° →	0.2	20° →	66 + 7	5 workers	2 workers		336
22/07	37 g	0.6	19°	0.5	20°	0.5	21°	0.5	22°	74 + 1	1 worker	1 worker		96
23/07	38 g	0.7	21°	0.4	22° W	0.5	25° W	0.7	27° W	30 + 4	1 worker	0		48

(1) (15°) cloudy sky ; [19°] rain ; 19° sun ; 22° W stormy ; 17° → wind.

* NCO = not carried out (rain)

(2) 16/07/80 : treatment at 14 hours

(3) workers + males : cumulated mortality for the day

(4) theoretical mortality reduced to per ha, according to counts on alleys.

TABLE IV - Results obtained with deltamethrin at 17.5 g a.i./ha

R_3 = hive n° 33 ; R_4 = hive n° 836

Date (1980)	Hive		10 h		12 h		14 h		16 h		Mortality in hives (3)	Mortality in alleys			Mortality/ha (4)
	Weight of pollen		Number of bees /m2	T°C (1)	Number of bees /m2	T°C	Number of bees /m2	T°C	Number of bees /m2	T°C		10 h + 12 h	14 h + 16 h	After treatment	
	R_3	R_4													
19/08	0	24	0.8	20°(1)	0.8	22°	1.42	(23°)	0.9	(24°)	13 + 6	0	0	-	0
20/08 (2)	1	18	1.04	18°	0.9	20°	1.3	23°	2.1	25°	24 + 14	0	3	0	144
21/08	6	13	0.4	(21°)	0.5	(22°)	0.6	(24°)	1.3	(23°)	24 + 71	8	4	-	576
22/08	1	3	0.6	16°	0.9	19°	1.1	19°	1.09	19°	23 + 11	2	4	-	288
23/08	1	9	0.1	15°	2.3	19°	3.2	20°	2.1	20°	9 + 0	12	4	-	768
25/08	1	14	0.4	17°	0.8	18°	1.1	22°	1.02	22°	7 + 1	7	0	-	336
26/08	3.4	6.9	0.3	17°	0.5	20°	-	21°	0	[16°]	15 + 6	-	4	-	192
27/08	3.9	15.8	-		-		-		-		9 + 1	-	-	-	-
28/08	-	-	2.2	(21°)	2	23°	1.6	27°	1.5	(27°)	26 + 13	-	-	-	-
29/08	-	-	0.5	18°	0.2	17°	-		-		7 + 2	5 (10 h)	-	-	240
01/09	18	15	0	16°	0.8	20°	0.4	20°	0.4	23°	35 + 8	0	-	0	0
02/09	8	7	-		0.6	23°	-		-		10 + 0	0	-	-	-
03/09	11	7	0.2	19°	0.4	22°	0.5	28°	0.6	28°	4 + 0	0	0	-	-
04/09	3	5	0.01	(18°)	0.05	(20°)	0.2	(21°)	0.2	(21°)	32 + 2	2	12	-	672

(1) 20° = fine weather ; (21°) = sky overcast ; [16°] rain ; 23° = variable sky + wind.

(2) Treatment carried out on 20/08/80 at 14 hours.

(3) Workers + males : cumulated mortality

(4) Theoretical mortality reduced to per ha, according to counts on alleys

TABLE V - Results obtained with deltamethrin at 21.2 g a.i./ha

Day (1981)	Hive N° 370 weight pollen collec.	10 h		12 h		14 h		16 h		Cumulated mortality 4 hives workers+males	Mortality in trap	Mortality in alleys			Theoretical mortal./ha (5)
		Number of bees/m2	T°C (1)	Number of bees/m2	T°C	Number of bees/m2	T°C	Number of bees/m2	T°C			10 h	16 h	After treatt.	
12-14/06	130	Hives placed in position on 12/06/81													
15/06 (2)	34.9	-	-	1.5	23°	2.3	26°	0.01	26°	97 + 22	-	-	-	2	67
16/06	40	0.6	13°	0.6	15°	0.8	16°	0.5	16°	93	12	0	0	0	0
17/06	17	0.12	12°	0.01	13°	0.01	12°	0.14	15°	82 + 6	10	4	0	0	134
18/06	33	0.06	11°	0.14	12°	0.6	14°	0.1	15°	125 + 8	31	0	0	0	0
19-20)21/06	160	0.5	11.5°	0.6	12°	0.8	12.5°	0.3	13°	90 + 6	20	0	8	0	268
22/06	92.5	0.1	11°	0.3	12°	0.5	20°	0.4	24°	189 + 9	42	0	3	0	100
23/06	56	1	11°	1.5	13°	2	15°	2	27°	76 + 9	12	0	0	0	0
24/06	24	1	10°	1.7	12°	-	12°	-	12°	80 + 52	21	0	0	0	0
25/06	24	-	11°	-	12°	-	13°	-	13°	-	100 (3)	0	0	0	0
26/06	-	-	10°	-	11°	-	11°	-	13°	130 + 16	25	0	0	0	0
29/06	50	-	9°	-	9°	-	9.5°	0.3	10°	167 + 22	82	0	0	0	0
30/06	13	0.6	10°	1.6	12°	1.8	11	0.7	14°	87 + 32	59	0	0	0	0
01/07	150	0.7	12°	1.5	15°	1.1	15	1.3	14°	126 + 27	24	0	0	0	0
02/07	78	-	14°	1.2	15° W	-	15	-	15°	548 + 9	140 (4)	0	0	0	0

(1) fine weather = 13° ; overcast sky + wind = 13° ; rain = 13° ; changing sky = 13°) ; stormy = 13° W
(2) day of treatment
(3) incident : retention of rain waters in trap for dead bees.
(4) treatment on wheat with endosulfan + maneb at a rate not specified by the bee-keeper.
(5) theoretical mortality per ha, according to counts on alleys.

TABLE VI - Results obtained with deltamethrin at 35 g a.i./ha

Day (1981)	Hive N° 570 weight pollen collect.	10 h		12 h		14 h		16 h		Cumulated mortality 4 hives workers + males (3)	Mortality in trap	Mortality in Alleys			Theoretical mortality/ha (4)
		Number of bees/m2	T°C	Number of bees/m2	T°C	Number of bees/m2	T°C	Number of bees/m2	T°C			10 h	16 h	Behind treat.	
09/07	120	0.7	21° (1)	1.1	22°	1.4	25°	0	19° W	59 + 9	19	0	0	0	0
10/07	30	0	(17°)	0.2	(19°)	0.6	19°	1.1	20°	69 + 9	44	0	0	0	0
11/07						no observation									
12/07						"	"								
13/07 (2)	32	0	(15°)	0.7	(15°)	1.3	16°	0.7	18°	341 + 24	97	0	0	0	0
14/07	10	1.1	16°	1.2	18°	2.1	20°	1.5	19°	97 + 15	53	0	5	0	166
15/07	28	0.5	(14°)	1.4	(17°)	2	17°	0.8	19°	96 + 12	39	2	1	0	100
16/07	15	0.5	11°	0.8	16°	1.6	17°	1.4	18°	220 + 11	77	7	3	0	333
17/07	22	0.7	(13°)	1.2	(15°)	1.1	(15°)	1.3	(15°)	128 + 8	90	0	0	0	0
18/07 (5)	10	-	(14°)	0.2	14°	-	[13°]	1.6	17°	1053 + 10	513	0	6	3	300
19/07	15	-	-	-	-	-	-	0.8	16°	449 + 46	104	-	7	-	233
20/07	40	-	-	-	-	0.9	(18°)	0.7	19°	77 + 28	37	-	3	-	100
21/07	40	-	-	-	[15°]	-	17°	0.8	(17°)	149 + 21	66	2	1		100
22/07	65	-	-	-	-	1.14	19°	0.5	18°	91 + 14	37	3			100
23/07	30	-	-	-	-	-	rains	-	-	204 + 8		5			166
24/07	0	-	-	-	-	-	rains	-	-	81 + 4	43				
27/07	97	0.5	18°	0.5	18°	0.6	19°	0.5	18°	227 + 91	127	2			66
28/07	82	0.8	20°	0.6	20°	1.1	22°	1	25°	51 + 32	19				

(1) fine weather = 13° ; overcast weather = (13) ; rain = [13] ; variable sky = 13) ; stormy = 13 W
(2) day of treatment
(3) workers + males (mortality cumulated for the day)
(4) theoretical mortality calculated for 1 ha (according to the countings in the alleys).

TABLE VII - Results obtained at 500 g a.i./ha (Parathion)

Day (1980)	10 h		12 h		14 h		16 h		Mortality at hives (3)	Mortality on alleys			Dead bees /ha (4)
	Number of bees/m2	T°C	Number of bees/m2	T°C	Number of bees/m2	T°C	Number of bees/m2	T°C		10 h	16 h	After treatment/ha	
27/05	0.02	(13°)	0.02	(16°)	0.5	(18°)	0.7	18°W	10 + 0	13	9	-	1746
28/05	0.1	(15°)	0.3	(17°)	1.0	(18°)W	1.1	(19°)	2 + 0	9	0	-	714
29/05	0.01	(15°)	0.03	(16°)	0.08	(17°)W	0	[15°]	4 + 0	8	0	-	635
30/05	0.1	(15°)	0.3	(16°)	0.5	(16°)	0.5	17°)	7 + 0	1	0	-	80
02/06	0.5	18°	0.7	19°	1.1	20°	0.8	(20,5°)W	21 + 0	0	0	-	0
03/06 (2)	0.8	19°W	2.5	21°W	3.9	23°W	1.1	26°W	> 2000	14	3	27	2381
04/06	0.04	23°	0.2	25°	0.2	(26°)W	0.4	28°W	850 + 0	9	16	-	1985
05/06	0.03	23°	0.7	25°W	1.3	26°W	1.1	29°W	214 + 1	2	22	-	1905
06/06	0	18°	0	(20°)W	0		1.2	(18°)W	133 + 3	18	3	-	1667

(1) (13°) = cloudy ; 18° = fine weather ; [15°] = rain ; 17°) = variable sky + wind ; 20°W = thundery weather.

(2) treatment on 3/06/80 at 14.30 hours.

(3) workers + males : cumulated mortality for the day.

(4) theoretical mortality expressed per ha, after the counts on the alleys.

TABLE VIII - Results obtained with phosalone at 1200 g a.i./ha

Date (1980)	Weight of pollen		10 h		12 h		14 h		16 h		Hive mortality (3)	Mortality in alleys			Mortality /ha (4)
	Hive 386	Hive 570	Number of bees/m2	T°C	Number of bees/m2	T°C	Number of bees/m2	T°C	Number of bees/m2	T°C		10 h	16 h	After treat.	
07/08	4 g	0 g	-	-	1.3	24° (1)	1.2	26°	0.1	25° W	10 + 1	0	0		0
08/08 (2)	15	12	0.7	22°	0.9	23°	1.1	25°	1.1	26°	10 + 4	1	2	0	144
09/08	4	14	0.6	(21°)	0.4	(23° W)	0.3	(25° W)	0.6	(25° W)	29 + 4	2	3		240
10/08	37	35	-	-	0.9	(19°)	-	-	0.8	(18°)	26 + 1	3	1		192
11/08	2	1	1.1	23°	0.8	23°)~	0.6	(24° W)	0.5	(24° W)	49 + 9	4	4		384
12/08	4	10	0	[18°]	0	[18°]	0.3	18°)~	0.3	18°)~	19 + 4	0	0		0
'3/08	1	6	0	[18°]	0	[21°]	0.2	[23°]	0.2	[24°]	27 + 1	0	0		0
14/08	3	19	0.4	23°	0.3	26°	0.5	27°	0.2	28°	6 + 3	2	1		144
18/08	1	12	0	19°	0.01	21°	0.1	21°	0	21°	23 + 9	6	1		336

(1) 24° = fine weather ; (23°) = cloudy weather ; [19°] = rain ; 23°)~ = variable sky + wind

(2) treatment on 8/08/80 at 14.10 hours.

(3) workers + males : cumulated mortality for the 4 hives.

(4) theoretical mortality per ha, according to counts on alleys.

TABLE IX - Summary of observations made during the open field trials 1981 With deltamethrin 7.5 g/ha

Date	Mortality in front of the hives		Number of bees visiting the crop/m2	Weight of pollen	Vegetative stage of the crop	Weather conditions	Observations
	Morning	Evening					
	06/04/81 : Treatment of the plot with deltamethrin at 0.3 l (control of pollen beetles, stage "tied buds")						
13/04/81					Beginning of the blossom		Hives placed in position
16/04/81		5	No activity at all		Blossom 50 %	cold	Activity around the hives
17/04		1	"		" "	"	
22/04		23	"		Full blossom 100 %	"	Very medium activity around the hives
27/04		22	"		" "	rain	
30/04		67	Slight activity		" "	rain 14°C	
04/05		16	No actitity at all	1-2-3-4-5 may 136 g	Blossom	rain	
06/05	5					cold + rain cloudy till 06/05	
07/05/81 Treatment at 14 h, 18°C, deltamethrin 300 cc/ha	3	71	14 h before T_1 = 1 bee/m2 T + 6 mn 0.5 (bees/m2) T + 12 mn 0.2 T + 30 mn 0.1 T + 1 h 0.2 T + 1 h 30 0.4 T + 2 h 0.4 T + 3 h 0.5	332 g	End of blossom (first pods already formed)	Sunny with some clouds 14 h = 18°C 17 h = 22°C	In front of the hives, no mortality : normal behaviour of bees. Some bees on ground KD, then fly back. On the crop, no repellent effect behind the boom. Repellent effect 12 mn after treatment. End 2-3 hours after treatment.

TABLE X - : Summary of observations made during the open field trials 1981 with deltamethrin at 7.5 g/ha

Date	Mortality in front of the hives		Number of bees visiting the crop/m2	Weight of pollen	Vegetative stage of the crop	Climatic conditions	Observations
	Morning	Evening					
09/05/81 T + 2		77 W 8 ♂	at 14 h = 0.7	270 g 9/05 + 10/05	End of blossom	stormy 16°	Normal activity around hives
11/05 T + 4	17 W		0	3.5 g	Petals fall	overcast rain	Low activity around hives
12/05 T + 5	10 W	31 W 2 ♂	at 15 h = 0.5	77 g	Petals fall	overcast/morn. sunny/evening	Normal activity around hives
13/05 T + 6	10 W	48 W	at 16 h = 0.8	87.5 g	Petals fall	overcast/morn. sunny/evening	Normal activity around hives
14/05 T + 7	4 W	59 W 1 ♂	at 16 h = 1	37.5 g	50 % of petals fallen	sunny	High activity around hives
15/05 T + 8	20 W	51 W 2 ♂	at 12 h = 0.7	36.9 g 15-16-17/05	50 % of petals fallen	sunny	Normal activity around hives
18/05 T + 11	9 W	29 W 1 ♂	at 16 h = 0.5	61 g	80 % of petals fallen	sunny	Normal activity around hives
19/05 T + 12	39 W 2 ♂	40 W 1 ♂	at 14 h = 0.7	28.5 g	80 % of petals fallen	sultry and stormy	High activity around hives
20/05 T + 13	30 W	19 W	at 14 h = 0.4	28.5 g	80 % of petals fallen	overcast, then sun at 16 h	Low activity
21/05 T + 14	7 W	35 W	at 10 h = 0.2	14.5 g	20 % flowers, 80 % pods	overcast + wind	Medium activity near hives
22/05 T + 15	6 W	19 W	at 16 h = 0.2	13.5 g	90 % pods	cloudy + wind	No activity at all to medium activity
25/05 T + 18	15 W	3 W	at 16 h = 0	0	90 % pods	changeable + wind	No activity at all near hives
26/05 T + 19	8 W	18 W	at 16 h = 0	0	Rape without any more flowers	rain	No activity at all

TABLE XI - Summary of observations made during the open field trial in 1982 with deltamethrin at 7.5 g/ha

Day (1982)	Weight of pollen		Number of bees visiting the crop								Mortality for 8 hives (workers only)		
	hive I	hive II	10 h	T°C (2)	12 h	T°C	14 h	T°C	16 h	T°C	8 h	18 h	daily mortality
04/5	18.9	13	0.01	(9°)	0.03	(10°)	0.11	12.5°)	0	[9°]	113	166	279
05/5	7	9.5	-	-	0	12°)	0	13°)	0	[12°]	208	257	465
06/5	16.4	17.8	0	8°	0	(12°)	0.08	15°)	0.07	(14°)	96	110	206
07/5	10	13.5	0.06	10°)	0.01	13°)	0.006	12°)	-	-	221	83	304
08-09/5	0	0	-	-	-	-	0	[10°]	-	-	-	202	202
10/5	70.7	37	0	10°)	0.05	13°)	0.09	(15°)	0.06	15°)	143	242	385
11/5 (1)	132.1	53.6	0.004	13°	0.09	18°)	0.13	20°	0.25	21°	419	202	621
12/5	188.7	71.3	0.09	12°	0.2	18°	0.68	23°	0.33	24°	256	45	301
13/5	107.4	28.6	0.04	12°	0.2	21°	0.29	25°	0.11	22°	115	44	159
14/5	122	30	0.08	15°	0.25	27°	0.35	31°	0.22	31°	108	57	165
15/5	115	40.8	0.17	17°	0.31	24°	0.27	33°	0.41	33°	199	91	290
16/5	168	79.5	-	-	-	-	-	-	-	-	-	-	-
17/5	85.7	22.8	0.07	14°	0.19	22°	0.45	25°→	-	24°	167	-	167

(1) Treatment day at 14 hours (0.8 bees/m2)

(2) (9°) = cloudy ; 12.5°) = variable sky ; [9°] = rainy weather

APPENDIX 12

Field trials with Cymbush (Cypermethrin) and Cybolt (Flucythrinate) in Switzerland during May 1982

(Extracts from the trial reports to be published in 1983 in Schweiz. Bienen-Zeitung, 105 NF)

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According to our field experiences in 1979 and 1980 with "Ambush" (Permethrin) applied in flowering rape during bee flight and flowering raspberry early in the morning out of the flight activity, we used the following criterions:

1. Measurement of the population density

2. Counting of the bees visiting the blossoms within 1 square meter areas (6 square meters in the treated and 2 square meters in the non treated plot of the experimental field)

3. Spraying during the flying activity on flowering rape (an area of about 10% inside the experimental field as a check plot has not been treated)

4. Counts of dead bees in a) the beehive entrance trap for dead bees, b) on a linen sheet on the floor in front of the colonies and c) on the ground in the field beneath the field counting areas

5. Fractionated collection of pollen loads the days before and after treatment as well as the day of treatment

The 6 observation hives in a trailer (Cymbush) and the 12 hives in the beehouse (Cybolt) were placed on the boarder of the rape field of 1 ha and 0.85 ha surface, respectively.

Main results

1. Population density

The 3 measurements (2 preceding and 1 following the treatment) did not show any measurable influence due to the treatments. The weight of the scale hive in the Cymbush field trial increased by 2 kg during the 3 days before and after the treatment.

2. Flight activity on the flowering rape

On 3 successive days we counted every half an hour in three 3-minute counts the blossom visiting bees within a marked one-square meter area. Slight deviations were probably due to the changing weather (full sunshine, winds and some very short rainfalls). This method gave a very objective range of the flight activity the days of treatment as well as before and after spraying.

3. Behaviour after treatment

After spraying the flowering rape during the main bee flight activity - a normally not allowed application in Switzerland - we observed the following reactions:

a) Cymbush (50 g ai/ha) sprayed at 1.30 p.m.: The field bees were frightened away by the spray beam, returned to the blossoms just behind the tractor where they remained for half a minute and then flew away.

Repellent effect: The flight activity slowly decreased during one hour after treatment to about 15-20% of the pretreatment activity. This depressed activity lasted the whole afternoon, i.e. during four hours. In the untreated plot we noticed a similar behaviour. In another nontreated field 400m away the bee flight activity was quite normal.

b) Cybolt (30g ai/ha) sprayed at 10.45 a.m.: Most of the rape blossom visiting bees returned to the flowers after having been disturbed by the spray machine. For this product, we did not observe any special reaction of the field bees.

Repellent effect: In the treated plot the flight activity decreased only during the following 2 hours to about 40%, in the untreated plot to about 55% of the morning level.

4. Mortality of bees

The number of dead bees a) in the traps and b) on the linen sheet in front of the hives lies more or less in a normal range of mortality.

In the Cymbush trial we observed an unusual high mortality (probably because of the frosty weather) 4 days before the treatment. Subsequently, it decreased steadily also during the observed 2 days after the treatment. About half an hour after spraying we observed in the 2 traps 6/2 trembling and staggering bees; 4 and 1 of them respectively recovered during the following hour. 75 minutes after spraying there were 16/6 other trembling and staggering bees, 10/5 of them recovered.

For Cybolt the mortality increased 5-fold 2 days after treatment and afterwards decreased to a normal level again.

On the linen sheet we made the following observations after treatment by sequences of time-lapse cinematography.

In the case of Cymbush the first bees with anormal behaviour appeared 75 min after treatment. During the following 3 hours of observation 18 bees died. With Cybolt we saw the first dead bee already during spraying. 2 other trembling and staggering bees recovered after 15 min. A second bee died 75 min afterwards and during the following 7 hours we counted 15 dead bees.

In both trials there were about 20 to 30 trembling and staggering bees, recovering partially within 15 to 75 minutes.

Beneath the 1-m^2 areas (c) we saw casually 3 dead or abnormal bees in the Cymbush field and none in the Cybolt field.

5. Pollen-gathering activity

From our intensive research on pollen collection behaviour we know that due to the individuality of the populations there are great differences

from hive to hive in the amount and type of pollen gathered.

In the Cymbush trial the gathering activity generally decreased in the rape field during the afternoon, probably because of the rainy weather in the mornings and later noons. The pollen-hive-bees gathered about 50% more fruit pollen than on the two other days. The depression for rape pollen after treatment was about 75%.

For Cybolt we noticed a post treatment depression of 42% at the beginning and 20% at the end of the afternoon. In this trial, too, the pollen-hive-bees gathered 50% more fruit pollen after treatment.

In all separated samples of rape pollen we did not find in the bioassay with Grillus domesticus any lethal degree of contamination. The lifespan of newly emerged bees fed with such pollen was not reduced remarkably either.

Conclusion

With our large field trial methods we could show again that the pyrethroids, the toxicity of which is known from laboratory trials, are less to non-hazardous in field application. But where should we draw the limit between " hazardous" and "non-hazardous" pyrethroids? (Comparatively, phosalone as a control product seems to involve "minimum risk").

As the pyrethroids belong to a newer group of insecticides with a) limited experiences on extended field application and b) no serious necessity for use as a non toxic product, we decided in autumn 1982 in Switzerland to continue to classify all pyrethroids as "hazardous for bees".

To be published in "BEE WORLD", late 1982. *

APPENDIX 13

LABORATORY ESTIMATION OF TOXICITY OF PYRETHROID INSECTICIDES TO HONEYBEES: RELEVANCE TO HAZARD IN THE FIELD

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Summary

Laboratory tests indicate that pyrethroid insecticides are very toxic to honeybees (Apis mellifera), but at the low field application rates recommended and considering other factors such as repellency, the actual hazard may be much less than expected.

Introduction

The toxicity of pesticides to honeybees (Apis mellifera) can be determined by suitable laboratory tests, but the hazard from the formulated pesticide is associated with specific circumstances in the field which must be considered in estimating the potential danger to honeybees and other non-target species[6]. This hazard is a function of the intrinsic toxicity of the pesticide, the field rate at which it is applied, the proportion of the dose which is available for transfer to the foraging bee, and the behaviour of the bee itself. Important factors include weather conditions in the two or three days before spraying as well as during application, the state of flowering of the crop and its attractiveness to bees which may in turn be influenced by other flowering crops, or wild flowers in the vicinity. For example, phorate if applied as a granular formulation[3,10], can be used to control Aphis fabae in flowering field beans with little risk to foraging honeybees; and bees placed in a flowering apple orchard may prefer to forage in a neighbouring oil seed rape crop.

Until the introduction of photostable pyrethroids, most insecticides were applied at similar rates, so that a tendency had developed for relative toxicities determined in the laboratory to be considered as indicating directly the hazard to honeybees in the field. On this basis, pyrethroids such as cypermethrin, deltamethrin and permethrin are similar to compounds known to present a serious hazard in the field. However, pyrethroids are used at much lower field rates than previous classes of insecticides, and this amongst other factors will clearly affect hazard.

We have therefore compared the toxicities of pyrethroid and other insecticides to honeybees and, where available, related these data to recommended or suggested field application rates.

Methods and Results

The acute contact toxicities of eight pyrethroid insecticides to worker honeybees were determined by the method recommended by the British Ministry of Agriculture, Fisheries and Food's Pesticide Safety Precaution Scheme. These values were compared (Table 1) with data previously obtained for pyrethroids, other insecticides known to be hazardous to honeybees in the field, and those considered to present a low risk.[7,10] Thre other insecticides quoted have been used in England on field bean and oil seed rape crops where foraging honeybees are known to be at risk.

Typical recommended or suggested application rates for these crops are included in Table 1, and the ratio in the last column represents the number of honeybee LD50 doses applied per hectare.

Discussion

The results in Table 1 show that because of low application rates, quantities of pyrethroid insecticides deposited on the crop, measured as numbers of toxic doses to honeybees, are intermediate between rates for insecticides known to be hazardous and those considered to present a low

* Bee World, 63 (4): 150-152 (1982)

risk. Thus pyrethroid insecticides may be less dangerous to honeybees in the field than expected from consideration of only laboratory toxicity data.

Other factors mentioned above, in particular repellency, may also protect honeybees. There is increasing evidence that some pyrethroids repel bees when applied to flowering crops before or during foraging. For instance Gerig[4,5] reported that foraging honeybees avoided flowering Phacelia and oil seed rape after early morning application of pyrethroid insecticides, and application to oil seed rape during foraging resulted in suspension of activity and diversion to non-sprayed areas of the crop. Atkins[1] described permethrin as a chemical repellent (though highly toxic to bees directly contacted) and advocated its use mixed with bee-toxic insecticides for early morning application to reduce significantly bee kills during subsequent foraging. Bos and Masson[2] reported repellency of formulated deltamethrin which they suggested was partly due to formulation. Such effects significantly reduce the hazard to honeybees caused by the use of pyrethroid insecticides, and are clearly important because they might allow these valuable compounds to be used in a wider range of circumstances with the minimal risk to pollinating insects.

Most significantly Shires and Debray[8] found that the hazard from cypermethrin applied to flowering oil seed rape was much less than might be expected by considering only intrinsic toxicity and attributed this to observed repellent action which markedly restricted foraging immediately after application without causing serious mortality; but their results may also reflect the low field application rate.

Hazards to foraging honeybees cannot be confidently predicted using simple data like those in Table 1. Whenever insecticide applications are proposed under conditions where bees are at risk, field trials[7,8,10], taking account of all the above factors, are essential for proper assessment.

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TABLE 1. The acute contact toxicity of some insecticides to honeybees, compared with suggested field application rates.

	LD50 µg per bee	n	Suggested field rate g.a.i.per ha	Ratios x 10^{-6}
Azinphos-methyl[a]	0.063	2	460	7300
Triazophos[a]	0.055	3	400	7300
Malathion[a]	0.27	2	1300	4800
Dimethoate[a]	0.12	3	350	2900
HCH[a]	0.20	6	280	1400
Demeton-S-methyl[a]	0.26	3	240	920
"Cyhalothrin"[d,e]	0.027	2	12.5	460
Cypermethrin[d]	0.056	2	25	450
Permethrin[d]	0.11	2	50	450
"Flucythrinate"[d,f]	0.27	1	75	280
Fenvalerate[d]	0.23	3	50	220
Deltamethrin[d]	0.051	3	10	200
Endosulfan[b]	7.1	4	470	66
Phosalone[b]	8.9	1	460	52
Pirimicarb[b]	>50		140	<3
"NRDC 181"[d,g]	0.23	1	c	
"NRDC 185"[d,h]	0.38	3	c	

a Hazardous to bees
b Low risk to bees, but may not be effective against some pests.
c Not commercially developed, therefore no field rate available
d Pyrethroid insecticides
e (S)-α-cyano-3-phenoxybenzyl (1R,cis)-3-(Z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate, generously provided by ICI (UK) Ltd.
f [RS]-α-cyano-3-phenoxybenzyl (S)-2(4-difluoromethoxyphenyl)-3-methylbutyrate, generously provided by American Cyanamid
g [RS]-α-cyano-3-phenoxybenzyl [RS]-2(4-chlorophenyl)cyclopropylacetate
h [RS]-α-cyano-3-benzoylbenzyl [1R,cis]-3-(2,2-dibromovinyl)-2, 2-dimethylcyclopropanecarboxylate
n Number of regression lines used to obtain LD50. In previous work, using the same technique, the mean standard deviation per test was 23%[9]. To obtain a measure of per cent standard error for an LD50, divide 23 by $\sqrt{n}$. Thus for azinphos-methyl the value would be $23/\sqrt{2}$ = 16%.

APPENDIX 14

Methodes d'essais destinees a connaitre les effets des insecticides sur l'abeille domestique (Apis mellifera L.)

J. Louveaux

These test methods have been established by members of the Commission des Essais Biologiques de la Société Française de Phytiâtrie et de Phytopharmacie.

The Commission consists of representatives of specialist departments of the French Ministry of Agriculture: Institut National de la Recherche Agronomique, Service de la Protection des Végétaux, de l'Association Française de Normalisation (AFNOR); of the pesticides industry and of professional agricultural organisations.

In their present state (June 1983) they should be considered as recommended protocols for research and for decisions on the control of pesticide usage.

The two editors are J. Louveaux and A. Perrot but it is a collective work for which they have called upon a number of collaborators.

The contents are:-

This report of 20 pages, plus appendices, is produced by the Société française de Phytiâtrie et de Phytopharmacie. It can be obtained, price 20 French Francs, from the following address:-

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