IOBC
OILB

WPRS / SROP

Hazards of pesticides to bees

Impacts des pesticides sur l'abeille

editors :

C. Pélissier & L. P. Belzunces

IOBC wprs Bulletin
Bulletin OILB srop

Vol. 23 (3) 2000

International Organization for Biological and Intergrated Control of Noxious Animals and Plants
Organisation Internationale de Lutte Biologique et Intégrée contre les Animaux et les Plantes Nuisibles
West Palaearctic Regional Section / Section Régionale Ouest Paléarctique

Notes :

The complete texts of oral/posters presentations and the minutes of discussions will be edited in a forthcoming book published by INRA-Editions, Paris, in the series Les Colloques de l'INRA.
The following abstracts are printed as received from authors.

Les textes complets des présentations orales et affichées ainsi que les minutes des discussions seront édités dans un ouvrage publié par INRA-Editions, Paris, dans la série Les Colloques de l'INRA.
Les résumés suivants sont reproduits tels que soumis par les auteurs.

The IOBC/WPRS Bulletin is published by the International Organization for Biological and Integrated Control of Noxious Animals and Plants, West Palearctic Regional Section (IOBC/WPRS)

Le Bulletin OILB/SROP est publié par l'Organisation Internationale de Lutte Biologique et Intégrée contre les Animaux et les Plantes Nuisibles, Section Régionale Ouest Paléarctique (OILB/SROP)

Address General Secretariat :
INRA – Centre de Recherches de Dijon
Laboratoire de Recherches sur la Flore Pathogène dans le Sol
17, rue Sully – BV 1540
F-21034 DIJON CEDEX
France

ISBN 92-9067-118-1

INRA **ACTA**

Abstracts of the 7th International Symposium of the ICPBR Bee Protection Group co-organized by INRA and ACTA

Résumés du 7ème Symposium International du Groupe Protection de l'Abeille de l'ICPBR co-organisé par l'INRA et l'ACTA

INTERNATIONAL COMMISSION FOR PLANT-BEE RELATIONSHIPS

Hazards of pesticides to bees

Impacts des pesticides sur l'abeille

at

Université d'Avignon, France
07 - 09 Septembre 1999

Edited by Colette Pélissier & Luc P. Belzunces

IOBC wprs Bulletin **Vol. 23 (3) 2000** **Bulletin OILB srop**

INRA

The Institut National de la recherche Agronomique was set up in 1946 and became a national public scientific and technological establishment in 1984, under the joint authority of the Ministries of Higher Education, Research and Technology and of Agriculture and Fisheries. The research carried out by the INRA contributes to ensure better nutrition for man and preserve his health, to sensibly develop and manage land and environment, to promote scientific and technological innovation, particularly in the life sciences, while remaining vigilant and responsible, to understand and control the complexity of our biological, economic and social systems.

L'Institut National de la recherche Agronomique a été fondé en 1946. C'est un établissement public à caractère scientifique et technologique depuis 1984, placé sous la tutelle conjointe du Ministère de l'Education Nationale, de la Recherche et de la Technologie, et du Ministère de l'Agriculture et de la Pêche. Ses orientations de recherche sont de mieux nourrir les hommes et préserver leur santé, aménager et gérer avec sagesse leurs espace de vie, innover sur le front des sciences et des technologies, notamment celle du vivant, en restant vigilants et responsables, comprendre et piloter la complexité de nos sytèmes biologiques, économiques et sociaux.

INRA
147 Rue de l'Université
75338 Paris Cedex 07
France

ACTA

The 17 agricultural and technical centres and institutes (Instituts et Centres Techniques Agricoles -ICTA) and their 1.000 scientists and technicians are at the heart of the French applied research system.They keep up to date with and anticipate changes by providing an interface betwen the needs of farmers and of the food processing industries and carrying out upstream research. Created and directed by farmers, the ICTAs work together under the umbrella of Association for Technical Co-ordination in Agriculture (Association de Coordination Technique Agricole -ACTA), which represents them with professional authorities and at the national and European levels. The ACTA organizes and directs collaboration between institutes and the implementation of programmes in areas of common interest.

Les 17 Instituts et Centres Techniques Agricoles (ICTA), avec leur 1 000 ingénieurs et techniciens, sont au cœur du dispositif français de Recherche Appliquée. Ils accompagnent et anticipent ces évolutions en jouant un rôle d'interface entre les besoins des agriculteurs, des industriels de l'agrofourniture et de l'agroalimentaire, en partenariat avec la recherche d'amont. Créés et gérés par les agriculteurs, les ICTA se concertent dans le cadre de l'ACTA - Association de Coordination Technique Agricole - qui les représente auprès des instances professionnelles et des pouvoirs publics nationaux et européens. L'ACTA organise et anime la concertation inter-instituts et la mise en œuvre d'actions sur des thèmes d'intérêts communs.

ACTA
149 Rue de Bercy
75595 Paris Cedex 12
France

Co-organized by

Supported by

Symposium Organizing Committee

Comité d'organisation du Symposium

Chairman : John STEVENSON, ICPBR Bee Protection Group (Harpenden, England)

- Luc P. BELZUNCES, INRA Laboratoire de Toxicologie Environnementale (Avignon, France), Coordinator
- Colette PELISSIER, INRA Zoologie et Apidologie (Avignon, France), Co-coordinator
- Gavin LEWIS, JSC International Ltd (Harrogate, England)
- Gilbert MAURIN, ACTA (Paris, France)
- Jean-Noël TASEI, INRA Station de Zoologie (Lusignan, France)
- Sèverine PELCOQ, ACTA (Paris, France)
- Sophie CLUZEAU, ACTA (Paris, France)
- Michèle AGUILA, INRA Unité de Zoologie et Apidologie (Avignon, France)

For further information contact

Dr.Luc.P. BELZUNCES
Institut National de la recherche Agronomique,
Unité de Zoologie et Apidologie
Laboratoire de Toxicologie Environnementale
Site Agroparc, Domaine Saint-Paul
84914 Avignon Cedex 9 - FRANCE
Email: belzunce@avignon.inra.fr

Colette PELISSIER
Institut National de la recherche Agronomique,
Unité de Zoologie et Apidologie
Documentation
Site Agroparc, Domaine Saint-Paul
84914 Avignon Cedex 9 - FRANCE
Email: cpeliss@avignon.inra.fr

Foreword

I am delighted to learn of the success of the seventh Symposium of the ICP-BR Bee Protection Group and I congratulate everyone concerned with the organisation of a particularly important meeting and with the production of this excellent report.

We are most grateful to Institut National de la Recherche Agronomique (INRA) for financial help and for undertaking the organisation of the meeting. We also thank Association de Coordination Technique Agricole (ACTA) for their contribution to the organisation, the University of Avignon for the use of their facilities, and the following companies and organisations for generous support:

AgrEvo France
American Cyanamid Co
Bayer SA
Elf AtoChem Agri SA
JCS International Ltd
Novartis Agro SA
Parthena
Rhône Poulenc Agro France
SOPRA
UIPP

The Bee Protection Group provides a forum where representatives of industry, National Regulatory Authorities and Government and University Research Departments come together to discuss the assessment of the hazards to bees of crop protection operations and to ensure that the farmer and the beekeeper can remain in harmony.

The Group has been working on the methodology for identifying and assessing these hazards since its first meeting in 1980, and it was a major acheivement that the final form of the EPPO **"Guideline for the efficacy evaluation of plant protection products SIDE EFFECTS ON HONEYBEES"** was agreed at the Symposium.

Professor Ingrid H. Williams PhD
Chairman ICP-BR

October 1999

IACR - Rothamsted
Herpenden
Hertfordshire, AL5 2JQ
England

Contents

Appendixes

Oral presentations

(order of programme)

Hazards of pesticides to bees, ICPBR 7th Bee Protection Symposium, Avignon, France, 07-09 September 1999. *IOBC wprs Bulletin*: 23 (3), 2000

A semi field test to evaluate the side effects of pesticides on brood in honeybee colonies (*Apis mellifera* L.)

Boris Leymann[1,2], Werner Mühlen[1] and Alois Edelmann[2]

1 : Landwirtschaftkammer Westfalen-Lippe, Fachbereich Bienenkunde
Postfach 5980, 48147 Münster, Germany
Fax : (+49) 0251 2376 551
e-mail : boris.leymann@biologie.uni-bielefeld.de
2 : Universität Bielefeld, Fak. Biologie, Abt Morphologie und Systematik der Tiere
33501 Bielefeld, Germany

For the registration of pesticides tests on honeybee colonies (*Apis mellifera* L., Hymenoptera, Apidae) under field conditions are required by the European Union to evaluate the side effects of these chemicals (EPPO 1992).
As yet there is no method to evaluate quantitatively the development of brood in honeybee colonies. The EPPO-Guideline 170 describes no standards on quantifying brood loss in cage or field tests. No suitable method is available to evaluate brood damages as might result from the application of IGR.
A test method is introduced to evaluate brood loss in honeybee colonies. In this semi field test large flight cages (4x12x2 meters) are used. As crops *Phacelia* and *Sinapis* were used, which provide a beehive with enough pollen and nectar. Three cages were used for one test, (control, test product and reference product known to present a high hazard). Each tent contained a small bee colony in an observation hive. In each hive about one hundred eggs and young larvae were marked on an overhead folie taped to the window of the hive. These windows made it easy to study the development of individual marked brood without disturbing the colony. It was possible to divide in cells with normal development and cells with disturbed development or dead larvae.
Our study shows a high risk for brood within the reference Alsystin WP 25 (800 g/ha) of 94.9% dead larvae, the control had a brood loss of 14.5 % the test substance NeemAzal T/S (6l/ha) showed similar values with 17.2% brood loss. The difference in percentage of well developed cells and disturbed cells in each test unit gave a clue for evaluating the side effects of pesticides on the brood of honeybees.

Hazards of pesticides to bees, ICPBR 7th Bee Protection Symposium, Avignon, France, 07-09 September 1999. *IOBC wprs Bulletin*: 23 (3), 2000

Joint effects of pyrethrinoid insecticides and azole fungicides on honey bee thermoregulation

Luc P. Belzunces[1], Rémy Vandame[1], Xingfa Gu[2]

1 : INRA Institut National de la Recherche Agronomique
Laboratoire de Toxicologie Environnementale
Unité de Zoologie et Apidologie
Site Agroparc, 84914 Avignon cedex 9, France
Fax : (+33) (0)4 32 72 26 02
e-mail : belzunce@avignon.inra.fr
2 : INRA, Unité de Bioclimatologie
Site Agroparc, 84914 Avignon cedex 9, France
Fax : (+33) (0)4 32 72 26 02

Pyrethroid insecticides present two particular features. They produce synergies when associated with azole fungicides and they have a negative temperature coefficient by eliciting a toxicity inversely proportional to the temperature. At neural level, this negative temperature coefficient would be due to the fact that pyrethroids block action potential more efficiently at low temperatures than at high temperatures (Wang, 1972). However, in matter of toxicity, the negative temperature coefficient might be partly due to an inhibition of thermoregulation by blocking the flight muscles involved in honey bee thermogenesis. To study an eventual effect of pyrethroids on honey bee thermoregulation, a non traumatizing method, involving infrared thermography, was used. The bees were treated at 22°C with sublethal doses of pyrethroids and then kept at 22°C with a 500 $g.l^{-1}$ sucrose solution *ad libitum* and monitored by infrared thermography for 4 hours. The pyrethroids used were bifenthrin, esfenvalerate, deltamethrin, cypermethrin, fluvalinate, alphamethrin and lambda-cyhalothrin. The effects of pyrethroids on thermoregulation were compared by treating the bees with a same sublethal dose of 10 pmol per bees corresponding to 5 ng of deltamethrin per bee. At 10 pmol per bee, deltamethrin, cypermethrin and lambda-cyhalothrin elicited a hypothermia of about –10°C while alphamethrin elicited a hypothermia of about –7°C. At the same dose, fluvalinate, bifenthrin and esfenvalerate did not induce a significant hypothermia. In a second time, deltamethrin was associated either to prochloraz, an imidazole fungicide, or to difenoconazole, a triazole fungicide, two molecules known to induce synergies with pyrethroids. Deltamethrin at 0.5 and 1.5 ng per bee did not induce a significant hypothermia whereas the doses of 2.5 and 4.5 ng per bee elicited a serious hypothermia whose effect was very marked 2 hours after the treatment. Similarly, prochloraz and difenoconazole did not induce a significant effect on thermogenesis at doses up to 850 ng per bee but elicited a serious hypothermia at 1250 ng per bee. When associated with prochloraz and difenoconazole at 850 ng per bee, deltamethrin elicited a serious hypothermia at doses that did not have a significant effect on thermoregulation when it was used alone.

Reference :

Wang C.M., Narahashi T., and Scuka M. (1972). Mechanism of negative temperature coefficient of nerve blocking action of allethrin. *J. Pharmacol. Exp. Therap.* 182, 442-453.

Sublethal effects of Imidacloprid on learning and memory in honeybees

David Guez[1], Séverine Suchail[1], Ryszard Maleszka[2], Monique Gauthier[3] and Luc P. Belzunces[1]

1 : INRA Institut National de la Recherche Agronomique
Unité de Zoologie et Apidologie
Site Agroparc, Domaine Saint-Paul
84914 Avignon cedex 9, France
Fax : (+33) (0)4 32 72 26 02
e-mail : guez@avignon.inra.fr
2 : Visual Sciences
Research School of Biological Sciences
The Australian National University
Canberra ACT 0200, Australia
fax: 61 2 6249 3784
e-mail: maleszka@rsbs.anu.edu.au
3 : Laboratoire de Neurobiologie de l'Insecte
Université Paul Sabatier (Toulouse 3)
118 Route de Narbonne 31062 Toulouse cedex, France
Fax : 05 61 55 84 44
e-mail : gauthier@cict.fr

We examined the effects of sub-lethal doses of a new neonicotinoid insecticide, Imidacloprid in field and in laboratory conditions.
In field conditions we tested the honeybees foraging behaviour (1 mg/l and 0.1 mg/l of Imidacloprid in sugar solution). Imidacloprid alters foraging behaviour in field conditions when the delivered sugar solution contains1 mg/l of Imidacloprid (1 ppm), whereas Imidacloprid does not act on foraging behaviour if the delivered solution contains 0.1 mg/l (100 ppb) during the time of the experiment.
In laboratory assays, Imidacloprid was tested on habituation (0.1 ng, 1 ng and 10 ng per animal) of the proboscis extension reflex (PER) in honeybees (*Apis mellifera*) reared under laboratory conditions. Imidacloprid alters the number of trials needed to habituate the honeybee response to multiple sucrose stimulations. Treatment with Imidacloprid leads to an increase in the number of trials necessary to abolish the response in 7-day old bees, and to a reduction in the number of trials for habituation in 8-day old bees. The temporal effects of Imidacloprid in both 7-day and 8-day old bees suggest that, 4 hours after treatment, the observed effects are due to one or several Imidacloprid metabolite(s), rather than to Imidacloprid itself. Our results suggest the existence of two distinct subtypes of nicotinic receptors in the honeybee that have different affinity to Imidacloprid and that are differentially expressed in 7-day and 8-day old individuals.

Results of a comprehensive field research programme with the systemic insecticide imidacloprid (Gaucho)

Gaëlle Curé[1], Hans-Werner Schmidt[2] and Richard Schmuck[2]

1 : Bayer S.A. Division Agro
49 - 51 quai de Dion Bouton
92815 Puteaux Cedex, France
Fax : (+33) (0) 1 49 06 51 47
e-mail : gaelle.cure.gc@bayer-ag.de
2 : Bayer Ag, Plant Protection
51368 Leverkusen, Germany
Fax : (+49) 2173 383215
e-mail : HANS-WERNER.SCHMIDT.HS@bayer-ag.de

Gaucho® is a registered trademark for plant protection products which contain the chloronicotinyl insecticide imidacloprid and which are used for seed dressing, e.g. on sunflower seed. Sunflower seed are treated with 0.7 mg a.i./grain which is equivalent to 50 g a.i./ha at a planting density of 70,000 plants /ha. In 3 tunnel and 8 field tests it was examined whether this seeddressing could have an influence on honeybees at the time of flowering. Residue analysis of flowers and honeybees were performed to determine the exposure of these pollinating insects during flowering. The residue levels were then compared with effect concentrations as determined in controlled feeding experiments.
In none of the 3 tunnel and 8 field tests did the seeddressing with imidacloprid affect the vitality, the foraging activity or the behaviour of the honeybees. From these results it can be reliably concluded that a seeddressing of sunflower seed with imidacloprid has no biological relevance for honeybees.
Pollen and nectar of sunflower plants were analysed for the presence of imidacloprid and its relevant metabolites. No residue levels were found at a limit of quantitation of 10 ppb.
In the controlled feeding experiments under field conditions, no adverse effects were observed on bees fed with spiked sugar solution up to 20 ppb imidacloprid. At higher concentrations (50-100 ppb) honeybees only reduced transitorily the foraging activity which indicates that honeybees realise the presence of imidacloprid at these levels. When these results are compared with the residue levels found in the relevant parts of the sunflower plants it is evident that the findings of the tunnel and field tests are fully confirmed and that no adverse effects on honeybees must be expected from the use of Gaucho in sunflowers.

Acute and chronic toxicity of imidacloprid and its metabolites in *Apis mellifera*

Séverine Suchail[1], David Guez[1] and Luc P. Belzunces[1]

1 : INRA Institut National de la Recherche Agronomique
Laboratoire de Toxicologie Environnementale
Unité de Zoologie et Apidologie
Site Agroparc, Domaine Saint-Paul
84914 Avignon cedex 9, France
Fax : (+33) (0)4 32 72 26 02
e-mail : suchail@avignon.inra.fr

Imidacloprid (1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine) belongs to a new chemical family of chloronicotinyl compounds whose mode of action on the insect nervous system differs from that of traditional neurotoxic products. Imidacloprid, a strong systemic compound, is effective against several sucking and mining pests.
The acute and chronic toxicities of imidacloprid and its main metabolites (5-OH-parent, 4,5 -dihydroxy-parent, guanidine, 6-chloronicotinic acid, olefin and urea) after oral application to *Apis mellifera* were investigated. Intoxication by those active compounds induces rapid (ca 30 min) behavioural abnormalities such as movement coordination problems, trembling and tumbling like most of the neurotoxic symptoms. For acute toxicity studies, bees were treated with doses of toxic compounds ranging from 1 to 1000 ng.bee^{-1}. The acute oral test revealed important characteristics. LD50 values of imidacloprid were about 67 ng.bee^{-1} at 48 h and at 72 h and at 96 h were about 50 ng.bee^{-1} for *A. m. mellifera*. The two main imidacloprid metabolites, 5-OH-parent and olefin were highly toxic to bees. At 48 h and at 72 h, LD50 values of imidacloprid and olefin were similar but at 96 h olefin had a higher toxicity. 5-OH-parent showed less toxic than imidacloprid. Urea metabolite appeared also as a toxic compound with about 50% of imidacloprid toxicity. For chronic toxicity, bees were fed during 10 days with sucrose solutions containing 0.1, 1 and 10 µg.l^{-1} of imidacloprid. The chronic oral tests showed that imidacloprid and its all studied metabolites were toxic and induce mortality 72 h after the begining of intoxication.

Honey bee poisoning incidents in the Netherlands over the last ten years.

Pieter A. Oomen

Plant Protection Service
PO Box 9102, 6700 HC, Wageningen, The Netherlands
Fax : (+31) 317 421701
e-mail : P.A.Oomen@pd.agro.nl

The Netherlands does not have a formal honey bee poisoning incidents monitoring scheme. But since ten years a voluntary monitoring is functioning. Bee keepers inform the national Unions of beekeepers of any poisoning incident of which they suspect pesticides as being the cause. The Unions bring this information together in a standardised database. Independently, the Agricultural Inspection Service (AID) investigates all incidents brought to their knowledge and about which there are good indications that the Pesticide Act has been violated. The information from the AID is added to the database. The database is analysed every year in order to verify the effectivity of the measures to protect honey bees from pesticide hazards.
Over the last ten years (1989-1998), the number of incidents appear to vary between 21 (in 1994) and 175 (in 1996). Incidents occurred mainly in the Eastern and Southern provinces of the Netherlands where agriculture is most intensive. Arable crops, in particular potato, are the crops most involved in incidents. Insecticides, in particular organophosphates are most often given as the cause of poisoning.
The voluntary monitoring, although evidently not at all as reliable as investigative monitoring, gives a reasonable overview of the character and size of the actual honey bee incidents caused by pesticides as experienced by bee keepers. It has served already for the Board for the Registration of Pesticides to review the risk mitigation regulations for use of organophosphate insecticides in potato crops.

Hazards of pesticides to bees, ICPBR 7th Bee Protection Symposium, Avignon, France, 07-09 September 1999. *IOBC wprs Bulletin*: 23 (3), 2000

First draft of "Field inquiry into suspected poisoning incidents involving honeybees"

Dietrich Brasse

BBA Institüt für Pflanzenschutz in
Ackerbau und Grünland
Messeweg 11-12
38104 Braunschweig, Germany
Fax : (+49) 5 31 2 99 30 08

On the last meeting of the group Sept. 1996 at Braunschweig (Germany) there was presented a "Monitoring scheme for investigating suspected pesticide poisoning of honeybees". The scheme is listing the different steps of the performance of assessment, investigation and report of poisoning incidents. The different steps are linked with each other in a logical sequence. A very important part of the scheme is the filling of a detailed questionnaire. As it is supposed, that normally no official person is present, when a poisoning incident happens or when it is noticed first, the informations of the questionnaire will be the basis for all decisions, actions and investigations to be done later. Therefore the informations given normally by the beekeeper must be as detailed and precise as possible.

The presented "Field inquiry into suspected poisoning incidents involving honeybees" is basing in corresponding questionnaires from Great Britain (identical title), the Netherlands (title: Form for reporting spraying incidents – to use when honeybees are presumably killed as consequence of exposure to plant protection products) and Germany (title: Application form for investigation of poisoning incidents of honeybee populations).

The questionnaire is containing a lot of questions, which may enable the investigators, to form an impression about the circumstances of the causes of the poisoning incident, without having seen the local conditions. Moreover the detailed questions respectively their precise answers should make the detection of the causes of the incidents for the investigators easier and faster. Otherwise when no or vague informations are given the investigations are like a search for a needle in a haycock. A missing or vague answering of the questions also makes the investigations much more expensive.

Finally the answers of the questionnaire could be part of legal conflicts between farmer and beekeepers, when the beekeeper is demanding a compensation for the loss of his populations. A complete and precise answering may help all involved persons (policemen, judges, lawyers) who are mostly inexperienced in beekeeping and plant protection, to give them a better understanding of the situation.

Overview about the poisoning incidents in honeybee populations and their clarification in Germany from 1996 to 1998

Dietrich Brasse

BBA Institüt für Pflanzenschutz in
Ackerbau und Grünland
Messeweg 11-12
38104 Braunschweig, Germany
Fax : (+49) 5 31 2 99 30 08

A comparison of the poisoning incidents in honeybee populations in Germany from 1996 to 1998 shows that the damages mainly had been distributed to three cultures: fruits and potatoes in 1996, rape in 1997 and 1998. The clarification of the incidents showed that there had been a main reason for the poisoning in each culture and year. The incidents in fruit cultures of 1996 were caused by the misuse of Fenoxycarb at the beginning flowering period and the incidents in potatoes were caused by the application of organophosphorous compounds to honeydew. The incidents in rape in 1997 and 1998 were caused by a combination of Pyrethroids classified as not hazardous for bees with fungicides of the group of Ergosterol-Biosynthesis-Inhibitors (EBI). The development of the incidents show that poisoning incidents may always arise in cultures, where they are not expected. An actual and complete information of the farmers by the plant protection service and a correct promotion for the products by the approval holders may prevent a great part of the incidents.

Hazards of pesticides to bees, ICPBR 7th Bee Protection Symposium, Avignon, France, 07-09 September 1999. *IOBC wprs Bulletin*: 23 (3), 2000

The use of toxic standards in the honey bee acute toxicity test

Gavin B. Lewis[1], H J Gough[2], C Künast[3], Helen M. Thompson[4] and John H. Stevenson[5]

1 : JSC International Ltd., Osborne House, 20 Victoria Avenue
Harrogate, North Yorkshire, HG1 5QY, United Kingdom
Fax : (+44) (0) 1423 520297
e-mail : GavinLewis@compuserve.com
2 : Zeneca Agrochemicals, Jealott's Hill Research Station
Bracknell, Berkshire RG42 6ET, United Kingdom
3 : BASF Aktiengesellschaft, Landwirtschaftliche Versuchsstation
PO Box 120, 67114 Limburgerhof, Germany
Fax : (49+) (0)621 6027214
e-mail : christoph.Kuenst@basf-ag.de
4 : MAFF Central Science Laboratory
Sand Hutton, York YO4 1LZ, United Kingdom
Fax : (+44) 1 904 462 240
e-mail : H.Thompson@csl.gov.uk
5 : 16 Old Rectory Close, Harpenden, Herts., AL5 2UD, UK
Fax : (+44) 1 582 712 316

The value of toxic standards in validating laboratory acute toxicity tests, in terms of the sensitivity of the test organisms and the precision of the test procedures, is widely recognised. The new OECD guidelines for honey bee acute contact and oral toxicity tests[1] incorporate the use of a suitable toxic standard. In particular, dimethoate is recommended for this purpose and expected ranges of LD_{50} values for the contact and oral tests are included to provide guidance for assessing the validity of individual tests. However, these ranges are based on an exercise conducted at only one laboratory[2]. At the last meeting of the International Commission for Plant-Bee Relationships 'Bee Protection Group'[3], it was pointed out that this published set of LD_{50} values for dimethoate do not necessarily represent the variability shown by the various strains of bees used in different laboratories and countries. Accordingly it was agreed that it is necessary to conduct a validation exercise for the use of dimethoate as a toxic standard in the honey bee acute toxicity tests using data from as wide a range of sources as possible. This paper considers the results of this exercise and the issues that have been identified.

References :

1 OECD Guidelines No. 213. Honey bees, acute oral toxicity test.
OECD Guidelines No. 214. Honey bees, acute contact toxicity test.

2 H J Gough, E C McIndoe and G B Lewis, (1994). The use of dimethoate as a reference compound in laboratory acute toxicity tests on honey bees (*Apis mellifera L.)* 1981-1992. *Journal of Apicultural Research* 33 (2):119-125.

3 Proceedings of the 6th International Commission of Plant-Bee Relationships Symposium on Hazards of Pesticides to Bees, BBA Braunschweig, 17-19 September 1996.

Field evaluations of non-pesticide chemicals as honey bee repellents

Dan F. Mayer[1], J.D. Lunden[1] and G. Kovacs[2]

1 : Department of Entomology
Washington State University
IAREC, 24106 N. Bunn road, Prosser
WA 99350, USA
Fax : (+1)(509) 786 9370
e-mail : mayerd@wsu.edu
2 : Ecotoxicological Laboratory
Facankert, Hungary
Fax : (36+) 74 440148
e-mail : ecotox@terrasoft.hu

Bee poisoning from pesticides is a serious problem worldwide. Major concern exists for the safety of honey bees (*Apis mellifera* L.) as valuable pollinators of many horticultural crops. One way of reducing the pesticide hazard to bees is to apply a chemical repellent that will discourage bees from foraging on crops for an interval after a bee hazard pesticide has been applied.

During 1990-1998, we conducted field tests on blooming apples (*Malus domestica* Borkh.), dandelions (*Taraxacum officinale* G. Weber, in Wiggers), buckwheat (*officinale*) and white Dutch clover (*officinale*) plants to evaluate their repellent effect to foraging honey bees.

Evaluations were made by slowly walking through the plots and counting the number of honey bees (30 s/6.7 m/0.91 m swath) except for apples where they were counted by slowly moving around and counting the number of honey bees (30 s/1 tree) at 1 and 4 h. after application.

We evaluated about 240 non-pesticide chemicals. Eleven chemicals significantly reduced the number of honey bee foragers at 1 h. after application but not at 4 h. In some tests, but not all, 10 chemicals significantly reduced the number of honey bee foragers at 1 h. after application but not at 4 h. One chemical significantly reduced the number of honey bee foragers at 1h. and 4 h. after application. In some tests, but not all, 2 chemicals significantly reduced the number of honey bee foragers at 4 h. after application but not at 1 h.

Are allelochemicals risk-free to use on flowering bee-pollinated crops? A field bioassay with the sexual pheromone used in mating disruption of the codling moth.

Bernard E. Vaissière[1], Myriam Matti[1], Nicolas Morison[1], Benoît Sauphanor[2]

1 : INRA Institut National de la Recherche Agronomique
Laboratoire de Pollinisation Entomophile
Unité de Zoologie et Apidologie
Site Agroparc, Domaine Saint-Paul
84914 Avignon cedex 9, France
Fax : (+33) (0)4 32 72 26 02
e-mail : vaissier@avignon.inra.fr
2 : INRA Institut National de la Recherche Agronomique
Laboratoire de Lutte Intégrée
Site Agroparc, Domaine Saint-Paul
84914 Avignon cedex 9, France
Fax : (+33) (0)4 32 72 26 02
e-mail : sauphano@avignon.inra.fr

The objective of our study was to assess the potential risks for entomophilous pollination which could result from using mating disruption to manage populations of the codling moth, *Cydia pomonella* (L), in orchards during flowering. We used the synthetic sexual pheromone of that species released by Isomate C dispensers and tested its effects on the foraging activity and pollinating effectiveness of honey bees, *Apis mellifera* L., under plastic tunnels planted with a monoecious cultivar of cantaloupe, a strictly entomophilous crop. We used a sequential experimental design with 4 replications to avoid the confounding effects of the weather conditions. The release of the codling moth sexual pheromone did not affect honey bee foraging nor pollination. In more general terms, the bioassay we developped using a semi-natural environment and sequential design could be useful for the registration of allelochemicals which are considered for mating disruption against insect pests during the flowering of entomophilous crops.

Assessing the exposure and toxicity of pesticides to bumble bees

Helen M. Thompson

National bee Unit, CSL, Sand Hutton, York YO41 1LZ, UK
Fax : (+44) 1904 462240
e-mail : H.Thompson@csl.gov.uk

Recently, there has been concern about the potential impact of pesticides on both long-tongued and short-tongued species of bumble bee (*Bombus*). There has been a severe decline in the abundance of bumblebees in the last thirty years, particularly in southern Britain, and it is possible that this is due in part to the use of certain pesticides. Bumble bees are important pollinators of many crops and wild flowers and, therefore, there are both conservation and economic reasons for taking action to assess the impact of pesticides on bumblebees.

This paper highlights the differences in the potential risk posed by pesticides to bumblebees from that of honeybees. This is based on their exposure through use of crops and flowering weeds and on the limited available data on toxicity of pesticides to a small number of bumble bee species. Pesticide risk assessments for honeybees are based on hazard ratios which rely on application rates and toxicity data and are unlikely to be appropriate for bumblebees. This paper will show that bumblebees are active at different times to honeybees, are likely to visit many of the same and some additional crop species and are active on many weed species found around crops. Therefore bumble bees are, likely to have different exposure profiles to honeybees. This paper also reviews deaths of bumble bees reported through the UK bee poisoning incident scheme and shows that, unlike honeybees, deaths of bumble bees due to pesticides are unlikely to be reported, since the bees are not kept domestically and will die in small numbers.

Hazards of pesticides to bees, ICPBR 7th Bee Protection Symposium, Avignon, France, 07-09 September 1999. *IOBC wprs Bulletin*: 23 (3), 2000

Does Gaucho (Imidacloprid) seed coating of Sunflower affect the bumblebee *Bombus terrestris* ?

Jean-Noël Tasei[1], G. Ripault[1], E. Rivault[1]

1: INRA Institut National de la Recherche Agronomique
Laboratoire de Zoologie
86600 Lusignan, France
Fax : (+33) (0)5 49 55 60 88
e-mail : tasei@lusignan.inra.fr

Seed coating treatments of Sunflower by the systemic insecticide Gaucho was suspected of affecting honeybees and bumblebees. Hypothesis raised was that Gaucho could migrate into nectar and pollen, then modify flower attractiveness, homing behaviour and colony development.

We report greenhouse and field experiments with *Bombus terrestris* and aiming at comparing a /: the behaviour of workers foraging on treated and control plants blooming in a greenhouse and cultivated in pots disposed in an alternating pattern, b/ : the homing rate of 10 colonies placed for 9 days in a treated field and 10 colonies in a control field, by using marked workers, c/ : the development of these 20 colonies under laboratory conditions after withdrawal from fields.

In greenhouse, workers visited blooming heads of treated and control plants at the same rate and the mean duration of their visits was similar.

In field colonies :

- Analysis of pollen carried by worker hairs and pellets showed that 98% of nectar foragers visited exclusively Sunflower in either field, whereas only 26% and 29% of pollen gatherers collected Sunflower pollen in the control and the treated field respectively.
- Forager activity at nest entrances was similar in both fields.
- After 9 days, 23% and 33% of the marked foragers did not return to hives in the control and treated field respectively. This difference was not significant.
- During the 26 day period under laboratory conditions the population increase rate of the 20 colonies was 3.3 and 3.0 workers/day in hives of the control and treated field respectively. This difference was not significant.
- New queens were produced in 8 colonies in either field. The mean number of new queens per hive was 17 and 24 in the control and treated field respectively. Their mating rate was the same .

It was concluded that applying Gaucho at the registered dose, as a seed coating of Sunflower cultivated in greenhouse or in field, did not affect the foraging behaviour of *B. terrestris*, its homing capability and its colony development.

Hazards of pesticides to bees, ICPBR 7th Bee Protection Symposium, Avignon, France, 07-09 September 1999. *IOBC wprs Bulletin*: 23 (3), 2000

The effect of the size of the bumblebee (*Bombus terrestris* L) on the susceptibility for the pesticide Dimethoate 40%.

Joseph J.M. van der Steen

Research Centre for Insect Pollination and Beekeeping "Ambrosiushoeve"
Ambrosiusweg 1
5081 NV Hilvarenbeek
the Netherlands
Fax : (+31) 13 5439155
e-mail : ambrosiushoeve@wxs.nl

The draft methods on the acute toxicity of pesticides for bumblebees in the laboratory were presented at the ICPBR symposium in Braunschweig 1996.
In order to define the methods more precisely, the effect of size, weight and age of worker bumblebees on the susceptibility to pesticides was determined. These tests were done with Dimethoate 40%. It appeared that the group of bumblebees of 0.15 to 0.23 gram is homogeneous in their reaction to Dimethoate 40%. There is a tendency that old bumblebees are more susceptible than young ones. Based on these data, we made in a more detailed description of the methods. To test the reproducibility of the methods, a ring test with Decis and the positive control Dimethoate is done. The methods to determine the acute oral LD_{50}, acute contact LD_{50} and, as far as available, the results of the ring test will be presented.

Side effects of an Insect Growth Regulator on bumble-bees and honey-bees

Ingo Tornier

GAB Biotechnologie GMBH
Eutinger -Str. 24
75223 Niefern-Oeschelbronn, Germany
Fax : (+49) 7233 4697
e-mail : ingo.tornier@GAB-BIOTECH.de

Methods to test acute toxicity of plant protection products on honey-bees have been extensively described in BBA-, EPPO-, and now also in OECD-guidelines. They are conducted as a matter of routine in accordance with GLP for the registration of pesticides. Concerning effects on larval development of bees, in particular the honey-bee (*Apis mellifera*) and the bumble-bee (*Bombus terrestris* L.), only a few established methods are known.
In the following a test method for bumble-bees in a greenhouse and honey-bees in tunnel tents will be presented:

Bumble-bee Test : In small greenhouse compartments four queen-right colonies with approximately 30 worker bumble-bees and a similar amount of brood will be used per variant. Before the application and at the end of the test period pictures of the brood will be taken. In order to assess the mortality of adult bumble-bees and the development of new worker bumble-bees during the test all adult bumble-bees are counted and colour-marked before placing the hives into the compartments. A trap for collecting dead bees and larvae will be fixed at the entrance of the hives.
During the test period, the following parameters are also recorded:

- Consumption of sugar solution
- The weight of the colonies before and after application
- The wing size of emerged bumble-bees

Honey-bee Test:
Tents covered with light plastic gauze will be placed over flowering *Phacelia tanacetifolia* areas, with a size of about 60 m^2. For the test, small healthy colonies («Mini-Plus-Beuten») with 12 combs and at least 6 brood frames will be used. In order to guarantee a detailed brood assessment foliage will be put on the comb for marking different brood stages.

Subgroup Persistence Testing: Report of the coordinator to the ICPBR Bee Protection Group

Pieter A. Oomen

Plant Protection Service
PO Box 9102, 6700 HC, Wageningen, The Netherlands
Fax : (+31) 317 421701
e-mail : P.A.Oomen@pd.agro.nl

At the ICPBR Braunschweig meeting in 1996 it was decided that the working group on persistence testing (started in 1993) would investigate the need and perspective for a standard procedure for laboratory persistence testing. Such a procedure is currently required by the EPPO/Council of Europe risk assessment scheme for honey bees (1993) and consequently also by the Uniform Principles of the EU (Harmonisation Directive 91/414/EEC).
Since 1996 dr. Harold Gough of Zeneca reported his extensive findings to the working group. Gough intends to report about this directly to the ICPBR Bee Protection Group. Based on his findings, the group concluded that a practicable and reliable test method is still remote. At the same time, for several reasons, it is expected that an adequate persistence testing method would contribute little to the current risk assessment scheme. Therefore, it is the advice of the subgroup that the residual testing step should be skipped from the honey bee risk assessment scheme, and that no further effort be spent to developing and applying a persistence testing methodology.

New technical aspects in bee toxicity tests, discussion on residue testing

Géza Kovacs

Ecotoxicological laboratory
Facankert 7136, Hungary
Fax : (+36) 74 44 01 48
e-mail : ecotox@terrasoft.hu

1. **Cages and feeders in acute tests :** presentation of advantages of the disposable cages and feeders used at the Ecotoxicological Laboratory. The cages are made from Petri dishes, circular insert formed from strip of plastic or metal screen and feeders from Eppendorf tubes.
2. **Presentation of a new brood testing in bee brood test :** the principle of the method is the application of the modified Jenter Queen rearing cage. The observations performed at the intervals of 7 days starting at the eggs laying of the caged queen and following all brood stages.
3. **Residue testing** : raising some aspects of the residue testing. Discussion of the test conditions such as our type of cages, exposure time, timing of the spraying (daytime and season).

A bi-tunnel method developed to investigate the side-effects of systemic seed dressings or systemic soil treatments on honeybees, *Apis mellifera*

Marco Candolfi[1] and E. Servajean[2]

1 : Novartis Crop Protection AG, Ecotoxicology
WRO-1058.3.64, 4002 Basel, Switzerland
Fax : (41+) 61 697 59 22
e-mail : marco.candolfi@cp.novartis.com
2 : Envirotests S.A. 14 rue Paul Bert, 64000 Pau, France
Fax : (33+) (0)5 59 84 80 55
e-mail : Envirotests@wanadoo.fr

A semi-field method was developed to assess the side-effects of systemic seed dressings or systemic soil treatments with plant protection products on honeybees (*Apis mellifera*), which allows a direct pre- and post-treatment comparison of the effects. The proposed methodology takes also into account the requirements outlined in the EPPO 170, BBA VI,23-1 and C.E.B. 129 guidelines. The study design is based on the release of bees in a bi-tunnel system: two adjacent tunnels, each 20 m x 8 m wide and a height of 3.5 m at the highest point, covered with a white fine mesh plastic netting. Three replicated bi-tunnels per treatment and the control are set up. In all the control plots, both tunnels of each replicate are untreated and therefore planted only with control plants, while in the test item replicates one tunnel is untreated (i.e. planted with control plants) and the second tunnel is treated (e.g. dressed seeds, treated soil). In order to prolong the exposure time of the bees (flowering plants available over a longer period), the crop planting is staggered. Bee colonies headed by sister queens with a size of approximately 10000-15000 bees will be set up at one end of the bi-tunnel between the 2 tunnels. Each hive is provided with a pollen trap, a dead bee trap and Apiscan. A removable netting between the two tunnels allows to control in which tunnel(s) the bees will be able to forage. The complete study consists of 3 successive periods of exposure (each exposure 5-7 days) of the bees: 1) bees will be allowed to invade the untreated half of the bi-tunnel and the behavior of each individual colony will be assessed, 2) the netting separating the 2 tunnels will be removed and the bees will be allowed to freely invade the treated and untreated areas (dynamics of invasion and the behavior of colonies will be assessed in order to study potential repellent effects) and 3) the bees will be confined in the second tunnel (treated tunnel) and behavior of the colonies observed.

Seminar

Discussion meeting on honey bee testing methodology

dr. Vlasta Zlof (EPPO) and dr. P.A.Oomen (EPPO/CoE Working Group Honey Bees)

1. **Zlof**: Introduction about the role of EPPO, the EPPO Guideline 170 on testing honey bees and the EPPO procedures for improvement of the guideline.
2. **Oomen**: Comments received from different countries by EPPO on the Guideline 170 (to be included in annex which is to be sent to participants before the meeting – to be prepared yet).
3. **Oomen**: Discussion on each separate comment. Mandate to Oomen as coordinator of the EPPO/CoE group to adapt the guideline accordingly, including the recommendations of previous ICPBR meetings, and to present the adapted guideline on behalf of ICPBR to EPPO for final processing.

The revision of EPPO standard for the evaluation of side-effects of plant protection products on honeybees

Vlasta Zlof

EPPO European and Mediterranean Plant Protection Organization
1 rue Le Nôtre
75016 Paris, France
Fax: (+33) (0)1 42 24 89 43
e-mail: zlof@eppo.fr - Web: www.eppo.org

EPPO has published over the last 20 years over 200 "Guidelines for the Efficacy Evaluation of Plant Protection Products". They provide common standards to assess product efficacy in the framework of registration, and are now considered as a reference in Directive 93/71/EEC of the European Union amending Directive 91/414 . The preamble of this Directive mentions that they are "the best available basis for setting the minimum requirements to be applied in all EU Member States with regard to the guidelines used for efficacy testing". Revision of certain guidelines had been requested by the European Commission, after consultation of the Member States. One of the guidelines concerned is on side-effects on honeybees (PP 1/170(2)). This standard describes the conduct of trials for the evaluation of side-effects of plant protection products on honeybees. It is important that plant protection products should be authorized for use only in ways which minimize the risk of harm to honeybees. For this purpose it may be necessary to provide evidence during the registration process to enable the safety of the product in question to be evaluated. This guideline presents several different types of tests (laboratory tests, cage tests, field tests and tunnel tests) which can be used to provide such evidence. However, some other tests which are sometimes used, such as tests on inhalation and long-term contact, are not described. EPPO Secretariat had received comments on this guideline from several EU countries. The EPPO Panel on Efficacy Evaluation of Fungicides and Insecticides considered that the ICPBR Symposium would be the right place to present comments and to revise and improve this guideline.

Hazards of pesticides to bees, ICPBR 7th Bee Protection Symposium, Avignon, France, 07-09 September 1999. *IOBC wprs Bulletin*: 23 (3), 2000

Possible synergistic effects on honeybees of pyrethroids and fungicides: the UK regulatory consideration

Patricia Brobyn

Pesticide Safety Directorate
King's Pool, 3 Peasholme Green, York, Y01 7PX, United Kingdom
Fax : (+44) (0)1 904 455 711
e-mail : p.j.brobyn@psd.maff.gov.uk

There is much evidence from the published literature to show that certain fungicides act synergistically with pyrethroid insecticides and increase their toxic action on bees. Effects may be very marked in laboratory studies but less clear cut in higher tier studies. Such research together with field evidence from sources such as the UK Wildlife Incidents Investigations Scheme has been considered in order to reach a decision on an appropriate regulatory approach to this situation.

Hazards of pesticides to bees, ICPBR 7th Bee Protection Symposium, Avignon, France, 07-09 September 1999. *IOBC wprs Bulletin*: 23 (3), 2000

A study of undertaking behaviour of honey bees (*Apis mellifera* L.) by use of different bee traps

Ingrid Illiès[1,2], Werner Mühlen[1], Gerti Dücker[2] and Norbert Sachser[2]

1 : Landwirtschaftkammer Westfalen-Lippe, Fachbereich Bienenkunde
48147 Münster, Germany
Fax : (+49) 0251 2376 551
e-mail : illiesi@uni-muenster.de
2 : Westfälische Wilhelms-Universität Münster, Abteilung für Verhaltensforschung
48149 Münster, Germany

Honey bees carry dead bees out of the hive. This behaviour is defined as "undertaking behaviour" by VISSCHER (1988). Bee traps are devices at the hive entrance. They are constructed to collect all dead bees that were carried out by the undertakers. The number of dead bees found there is an important criterion for the estimation of hazards of pesticide to bees (EPPO, 1992). In this study the efficiency of three types of bee traps commonly in use (Gary-Trap (Gary, 1960), a modified Gary-trap, and the IPSAB-Trap) and their influence on undertaking behaviour was tested comparatively for twelve bee hives. Hives with only the flight board represented the control. Fifty dead marked bees were placed on the floorboard to the hive. Ten times in 24 hours the dead bees in the trap and on a linen sheet (1.2 m^2) in front of the hive were counted.
The use of different bee traps leads to different and uncomparable results. In the container of the Gary-Trap a lot of strayed and worn-out bees died. Bees clear the modified Gary-trap from dead bees, especially during good flight conditions.They consider the trap as a part of the hive. Dead bees disappear from the IPSAB-Trap because of predators and wind. The two Gary-Traps have a negative effect on undertaking behaviour. The bees showed a large number of different behaviours and they needed more time to transport the dead ones than in the IPSAB-Trap, which showed the most similar results to the control. Based on these results an "Optimal Bee trap" should provide a confortable hive entrance and a container for dead bees offering protection from predators and an escape route for strayed bees.

Hazards of pesticides to bees, ICPBR 7th Bee Protection Symposium, Avignon, France, 07-09 September 1999. *IOBC wprs Bulletin*: 23 (3), 2000

Predicting the hazard of insecticide applications to bees

Jeffrey Lunden[1] and Dan F. Mayer[1]

1 : Washington State University
IAREC, 24106 N. Bunn road, Prosser
WA 99350, USA
Fax : (+1)(509) 786 9370
e-mail : mayerd@wsu.edu

The objective of this study was to predict the degree of toxicity hazard to honey bees in the field when an insecticide is applied. We used two types of data and compared the two to each other. The LD_{50} µg a.i./bee was determined for honey bees (*Apis mellifera* L.), alfalfa leafcutter bees (*Megachile rotundata* (Fabr.)) and alkali bees (*Nomia melanderi* Cockerell) for 25 insecticides. In addition, the LC_{50} ppm for the same insecticides was determined for the three species.
The LD_{50} µg/bee data was converted to a LD_{50} of µg a.i./g body weight and the LC_{50} ppm was converted to kilograms per hectare (kg/ha) in order to make for comparison.
In general, no correlations were found between the two types of data. In most cases, the LD_{50} kg/ha required to kill 50% of the bees was significantly lower as compared to the LD_{50} of µg a.i./body weight.

Managing nuclei in insect-proof tunnel as an observation tool for foraging bees

Marc-Edouard Colin[1], Yves Le Conte[1] and Jean-Paul Vermandere[1]

1 : INRA Institut National de la Recherche Agronomique
Unité de Zoologie et Apidologie
Site Agroparc, 84914 Avignon Cedex 9, France
Fax : (33+) (0)4 32 72 26 02
e-mail : colin@avignon.inra.fr

A 8 x 20 m insect-proof tunnel is used to separate the experimental bees from those of the nearby colonies and to make certain of their food source. Nuclei enclosing one-fourth Dadant frame are made wih Plexigas sides allowing a complete observation of the insects. After the nuclei are filled with pollen, brood and 1000 adult bees, foraging bees are trained to visit a feedind station placed at the center of the tunnel, which means a 10 m distance from the entrance of the nuclei. The day-time of the disposal of the sugar solution is to be strictly regarded. When the foragers are well trained to forage a sucrose solution, a contamined one is offered, the following days. Criteria of observation can be (i) the foragers recruitment, (ii) the number of feeding bees and their behavior at the feeder, (iii) the return flights, (iv) the trophallaxis inside the nucleus. Such a protocol has been put into practice to demonstrate the alteration of the homing flight in bees exposed to sublethal doses of deltamethrin (Vandame et al., 1995). It has been improved to reveal some features of the toxicity of the imidacloprid at sublethal doses.

Possibilities and limitations of monitoring the flight activity of honeybees by means of BeeSCAN beecounters.

Marc Struye

Lowland Electronics bvba
Langekoeistalstraat 2, 8432 Leffinge, Belgium
Fax : (+ 32) (0)59 27 97 93
e-mail: lowland@unicall.be

As part of an environmental study where honeybees are involved in, it can be important to know the amount of flight activity. In such circumstances electronic beecounters can be used.
Here we describe the use of BeeSCAN counters for flight activity measurements of honeybees in the field.
This presentation explains the most important characteristics a beecounter has to possess, in order to obtain the wanted precision for accurate measurements of flight activity. It is often very important to know the amount of losses that a bee colony has every day. Therefore the counter has to be bi-directional and obtain a high precision. Nevertheless the counter shouldn't disturb the normal behaviour of the bee colony.
The reliability of a counter can easily be verified by means of the 'robbers-test'. This test is also described during this presentation.
Not only the counter is important; the correct use is even more crucial to obtain good results. Some aspects of manipulation and installation of these counters are described.
Bad interpretation of the obtained data can lead to incorrect conclusions. Therefore quick and accurate analysis is necessary in this matter.

Hazards of pesticides to bees, ICPBR 7th Bee Protection Symposium, Avignon, France, 07-09 September 1999. *IOBC wprs Bulletin*: 23 (3), 2000

Posters

(alphabetical order)

Hazards of pesticides to bees, ICPBR 7th Bee Protection Symposium, Avignon, France, 07-09 September 1999. *IOBC wprs Bulletin*: 23 (3), 2000

Effect of pesticides on the bumblebee, *Bombus terrestris* L. in the laboratory

Laura Bortolotti[1], E.Grazioso[1], C. Porrini[2] and G. Sbrenna[1]

1 : Department of Biology, University of Ferrara
Via Borsari 46
Ferrara, Italy
Fax : (39+) 0532 249761
e-mail : laurabortoli@hotmail.com
2 : Institute of Entomology "Guido Grandi", University of Bologna
Bologna, Italy

Until some years ago, few data were available on toxicity of pesticides on bumblebees (*Bombus terrestris* L.) and the normal procedure was to transfer to this species the data obtained on honeybees. Because of the increasing relevance and commercial use of bumblebees as pollinating insects, it became necessary to more precisely assess the pesticide risks on these insects. For this purpose, specific guidelines were established in the VI International Symposium on Hazard of Pesticides to Bees, held in September 1996 in Braunschweig, Germany. We applied these guidelines, introducing some changes.
Our research on pesticide effects on bumblebees was part of the Italian national project A.M.A., financially supported by the Italian Ministry of Agricultural Resources and Forestry. The aim was to determine the toxicity of several pesticides towards the bumblebee. The pesticides chosen for the test are among those commonly used in greenhouses in Italy. Since the research was specifically aimed to the open field situation, the following variations were introduced in the guidelines: a) the pesticides were diluted in water (instead of acetone) to mimic the open field situation; b) all doses tested were equal or fractions of the field dose; c) the mortality was observed for a longer period (up to 10 days). All pesticides were initially tested at the field dose by both the contact and the oral test, and those resulting the most toxic ones were tested also at lower (fractional) doses. The results revealed that some pesticides are highly toxic for bumblebees even at very low doses. The different approaches for a suitable toxicity test for bumblebees are discussed and compared.

Impact of novel herbicide resistant oilseed rape on honey bee colonies in semi-field conditions

Nicolas Châline[1], Axel Decourtye, D. Marsault, Minh Hà Pham-Delègue, M. Lechner, Xavier Van Waetermeulen[2] and D. Viollet.

1 : INRA Institut National de la Recherche Agronomique
Laboratoire de Neurobiologie Comparée des Invertébrés
La Guyonnerie, BP 23
91440 Bures-sur-Yvette, France
Fax : (+33) (0)1 69 07 50 54
e-mail: chaline@jouy.inra.fr
2 : AgrEvo France
Les Algorithmes, Imm. Thalès – St Aubin
91197 Gif Sur Yvette Cedex, France
Fax : (33+) (0)1 69 85 63 05
e-mail : xavier.vanwaetermeulen@agrevo.com

Over the last ten years, research on transgenic crops have proliferated but little is known about their interactions with non-target organisms. Therefore, it is essential to assess their impact on beneficial insects such as bees, not only under laboratory conditions but also in more realistic semi-field or field conditions.
This experiment was carried out under semi-field conditions to study the impact of a transgenic oilseed rape cultivar tolerant to the herbicide Glufosinate on honey bee colonies. The purpose of this study was:
1) to compare both the activity and development of bee colonies foraging either on transgenic oilseed rape (OSR) treated with Glufosinate or control OSR treated with Colzor and Fervinal.
2) to test their foraging preferences between the two cultivars.
The amounts of herbicide and gene product residus was tested in a variety of samples (bees, honey, and pollen). In addition, in order to investigate possible indirect pleiotropic effects on other plant cues in honey bee-plant relationships, we compared nectar quality between the two OSR cultivars.
The experiment consisted of 2 types of tunnels: monocrop tunnels with either control or transgenic OSR, and choice tunnels containing 2 plots of transgenic OSR and 2 plots of control OSR. Two bee colonies were introduced in each monocrop tunnel: one colony was monitored with a bee counter to assess general activity and colony development and the other was used to provide samples for residue analysis. In the choice tunnels, bee foraging preferences were studied by repeated counting of foragers on each OSR cultivar.
The results showed no major differences between GM herbicide resistant and control OSR. The influence of other important parameters, such as plant growth stage, is discussed together with the choice of a procedure to standardize this bioassay for risk assessment of GM plants on honey bees.

Hazards of pesticides to bees, ICPBR 7th Bee Protection Symposium, Avignon, France, 07-09 September 1999. *IOBC wprs Bulletin*: 23 (3), 2000

Impairment of olfactory learning performances of *Apis mellifera* L. by long term ingestion of imidacloprid

Axel Decourtye[1], M. Le Metayer, H. Pottiau, M. Tisseur[2], Jean-François Odoux and Minh Hà Pham-Delègue

1 : INRA Institut National de la Recherche Agronomique
Laboratoire de Neurobiologie Comparée des Invertébrés, INRA
La Guyonnerie, BP 23
91440 Bures-sur-Yvette, France
Fax : (+33) (0)1 69 07 50 54
e-mail : decourty@jouy.inra.fr
2 : ACTA
La Saulsaie
01120 Montluel, France
Fax : (33+) (0)4 78 06 62 76

Although the traditional means of assessing toxicity of pesticides in honey bees has involved the determination of mortality in acute tests, it is also important to examine the effect of ecologically relevant sublethal exposure on various aspects of the honey bee behaviors. Two standardized bioassays to evaluate sublethal effects of pesticides on the behavior of *Apis mellifera* L. were used to test the insecticide molecule : imidacloprid (chloronicotinyl). Methods used to study learning processes and foraging behavior were adapted to test the sublethal effects of imidacloprid at both the individual and colony levels under confined conditions. At the individual level, we studied the effects of long term ingestion of imidacloprid (11 days administration) on olfactory learning performances using the olfactory conditioning of proboscis extension on restrained bees. At the colony level, a sugar solution containing 50 ppb of imidacloprid was fed to a colony in an outdoor flight room (14 days administration) to determine the effects on foragers recruitment activity, the flight activity as measured with an activity counter set at the hive entrance and olfactory discrimination performances on an artificial feeder. The olfactory conditioning procedure applied to restrained individuals showed that honey bees surviving the diet contaminated with 4-40 ppb of imidacloprid had reduced olfactory learning performances. Only the concentration of 4 ppb showed a percentage of mortality not significant by different from the control diet after long term ingestion. In the flight room, administration of imidacloprid induced a decrease in the foragers recruitment activity as well as in the flight activity and the olfactory discrimination performances. Thus, the decrease in the learning performances induced by imidacloprid at the individual level was confirmed at the colony level. However, it would be necessary to conduct further work on the dose-reponse relations or the sublethal effects of differents pesticides, before concluding about the hazard of imidacloprid on honey bees.

Hazards of pesticides to bees, ICPBR 7th Bee Protection Symposium, Avignon, France, 07-09 September 1999. *IOBC wprs Bulletin*: 23 (3), 2000

Bee selectivity of MAVRIK® (tau-fluvalinate) in tank mix with ERIA® (Difenoconazole, Ergosterol Biosynthesis Inhibitor - EBI). Short, medium and long term effects under semi-field conditions.

Bruno Lefebvre[1] and D. Bassand

1 : Novartis Crop Protection AG
Environmental Safety Assessments
R1058-P58
4002 Basel, Switzerland
Fax : (+41) 61 697 71 51
e-mail: bruno.lefebvre@cp.novartis.com

Ergosterol biosynthesis-inhibiting (EBI) fungicides have been found to synergize the toxicity of pyrethroids to the honeybees. In order to study this phenomenon between tau-fluvalinate (MAVRIK®) and ERIA® [formulation containing difenoconazole (EBI)], 2 semi-field studies were conducted: a tent trial (5x5m) according to the German BBA method (*Phacelia*, all crop surface treated) and a tunnel trial (20x8m) according to the French CEB method (white mustard, 2 treated + 2 untreated plots of 2x8m each placed in staggered rows per tunnel).
In both studies, 1 application was made either with tau-fluvalinate alone (0.2L/ha MAVRIK®, 48g tau-fluvalinate/ha) or with tau-fluvalinate (0.2L/ha MAVRIK®, 48g tau-fluvalinate/ha) + ERIA® at 2L/ha (126g difenoconazole/ha + 250g carbendazime/ha). Effects were compared to a control group (deionized water) and either a toxic reference (800g dimethoate/ha, tent study) or a harmless reference (600g phosalone/ha, tunnel study).
In the tent study, mortality levels with the tank mix or tau-fluvalinate alone did not differ from those observed in the water control; foraging activities were also not different to that in the control.
In the tunnel study, mortality was negligibly higher (1%) with the tank mix compared to tau-fluvalinate alone. Foraging activity was markedly decreased on the treated plots just after treatment but was completely restored 3 hours later with both treatments. Medium (before winter) and long term (after winter) effects were also investigated in the tunnel study. No effects were observed either on mortality and behaviour of adult bees, or on the development of the hive (brood, youngs, food reserves). In all cases, the toxicity with the tank mixture did not differ from the compound used alone, being as safe for bees as tau-fluvalinate itself. Bees, at no time, demonstrated any signs of disorientation, enhanced aggressiveness or excitability.
No synergistic effects of tau-fluvalinate (MAVRIK®) and ERIA® could be observed in these semi-field trials carried out in either France or Germany.

Load test of *Acidum tannicum* for honey bees

Enikö Matray[1], Zsuzsanna Szel[1], Katalin Turi[1]

1 : Institute for Small Animal Research
Department of Bee-breeding and Bee-biology
Isaszegi ut, POB 417, Gödöllö, Hungary
Fax : (+36) 28 430 184
e-mail : szel@sunserv.katki.hu

In our experiment bees were fed with sugar syrup which contained different concentration of *Acidum tannicum*. *Acidum tannicum* is a preparation against *Ascosphaera apis*. Bees were kept in small cages in thermostat at 24 °C, and they were fed every day and number of bee carcass was counted every day also. This experiment takes for 30 days.
There were 6 groups. Group A got sugar syrup which contained 0.05% *Acidium tannicum*; group B: 0.10%; C: 0.15%; D: 0.20%; E: 0.25% respectively, and there was one control group. The survival of bees was in group A 72%; in group B: 80%; in group D: 26%; and in group E: 18%. The experiment was finished in group C on the 28th day. In the control cage the survival of bees was 90%. The results show there was not important bee loss at 0.05 and 0.10% concentration. Bees killed in group C only after the 25th day, but in group E 82% of bees were killed on the 16th day. There was significant difference only in group E according to the statistical calculation.

The use of electronic bee counters as a tool to study the influence of environmental factors on bee behaviour

Marc Struye

Lowland Electronics bvba
Langekoeistalstraat 2, 8432 Leffinge, Belgium
Fax : (+ 32) (0)59 27 97 93
e-mail: lowland@unicall.be

This presentation describes some situations where electronic beecounters can be used to study the influence of different kind of environment influences on the amount of bee activity. An important parameter in evaluating the activity of a honeybee colony is given by the number of bees leaving and entering the hive as a function of time.For example the influence of pesticides treatments,weather conditions, can be studied. The electronic beecounters can be of different shape to adapt perfectly to the beehives used for experimental purpose. By means of some graph-presentations we demonstrate the usefulness of these instruments. Also a description of the 'robbers-test', which can easily be used for verifying the reliability of the counters, is given. Some photographs show the use in the field under different circumstances.

Hazards of pesticides to bees, ICPBR 7th Bee Protection Symposium. Avignon, France, 07-09 September 1999. *IOBC wprs Bulletin*: 23 (3), 2000

Degradation of Imidacloprid in *Apis mellifera*

Séverine Suchail[1], David Guez[1], Luc P. Belzunces[1]

1 : INRA Institut National de la Recherche Agronomique
Laboratoire de Toxicologie Environnementale
Unité de Zoologie et Apidologie
Site Agroparc, Domaine Saint-Paul
84914 Avignon cedex 9, France
Fax : (+33) (0)4 32 72 26 02
e-mail : suchail@avignon.inra.fr

A high-performance liquid chromatography tandem mass spectrometry-mass spectrometry (LC/MS/MS) assay was performed to analyse in *Apis mellifera,* imidacloprid biodegradation and formation of its two main metabolites, 5-OH-imidacloprid and olefin. Bees were treated orally with 2 ng or 5 ng of imidacloprid per bee. The observation times were 20 min (immediately after ingestion), 4 h, 6 h, 24 h, 30 h and 48 h. Residues are extracted from 2 g of bees with a mixture of methanol/water (3/1; v/v). After filtration and concentration, aqueous solution is partitioned against dichloromethane using a ChemElut® column. A second clean up is performed on a silica gel column followed by elution with acetonitrile/water (1000/1; v/v). The residues dissolved in a mixture of acetonitrile/water (2/8; v/v) were injected and quantified by reversed phase HPLC with electrospray MS/MS-detection. The retention times were 4.5 min, 5.5 min and 9 min for olefin metabolite, 5-OH-imidacloprid and imidacloprid, respectively. Imidacloprid and its metabolites were fortified at 10 $mg.kg^{-1}$, recoveries (mean ± SE) were about 99 ± 4 % for imidacloprid, 85 ± 6 % for 5-OH-imidacloprid and 79± 12 % for olefin. Imidacloprid was metabolised relatively quickly and thoroughly. Twenty minutes after total imidacloprid ingestion, about 60 % of the given real dose was either eliminated or transformed. After 6 h for 2 $ng.bee^{-1}$ dose, and 24 h for 5 $ng.bee^{-1}$ dose, no imidacloprid can be detected. Half-life of imidacloprid in *A. mellifera* was approximately 2,5 and 3,5 for 2 $ng.bee^{-1}$ and 5 $ng.bee^{-1}$, respectively. The appearance of 5-OH-imidacloprid and olefin was very fast. The peak of 5-OH-imidacloprid and olefin appears 4 hours after oral ingestion. From 24 hours, less than 2 $\mu g.kg^{-1}$ occur in bees for the two metabolites at the two tested doses. Twenty minutes following imidacloprid ingestion, these two major metabolites represent about 10 % for the two tested doses. Thus, it appears that it is very difficult to ascertain an intoxication diagnosis 24 h after intoxication with imidacloprid.

Effects of wetting agent selection on the contact toxicity of a dimethoate formulation to honeybees

Helen M. Thompson

National Bee Unit, CSL, Sand Hutton, York YO41 1LZ, UK
Fax : (+44) 1904 462240
e-mail : H.Thompson@csl.gov.uk

A range of wetting agents are suggested by OECD Guideline 214 for assessing the contact toxicity of pesticides to honeybees. This study aimed to determine the effect of wetting agent selection on the contact toxicity of an EC formulation of dimethoate. A number of wetting agents were used; Triton X100, Tween 20, Igepal, Span 20, Brij 35 and polyoxyethylene W1 all at 1g/l and the results compared with dimethoate formulation dissolved in acetone. All dilutions were prepared within two hours of use, except Triton X100 and acetone where an additional set of dilutions were prepared 16 hours in advance. All bees were dosed in groups of ten with 3 replicates per dose at dose levels of 0.25, 0.125 and 0.063 µg ai dimethoate/ bee. All tests were run concurrently with a wetting agent control (30 bees) to provide data on the toxicity of the wetting agent alone. Mortality was assessed at 4, 24 and 48 hours after dosing and probit analysis performed to determine the 48 hr LD_{50}, 95% confidence limits and slope of the dose-response. The results of this study will be presented and the effects on choice of wetter agent discussed.

Hazards of pesticides to bees, ICPBR 7th Bee Protection Symposium, Avignon, France, 07-09 September 1999. *IOBC wprs Bulletin*: 23 (3), 2000

Assessing the effects of glasshouse application of a novel insect growth regulator on bumble bee colonies

Helen M. Thompson[1] and Katie A. Barrett[1]

1 : National Bee Unit, CSL, Sand Hutton, York YO41 1LZ, UK
Fax : (+44) 1904 462240
e-mail : H.Thompson@csl.gov.uk

Bumble bees are widely used as pollinators for glasshouse crops, particularly tomatoes. It is important that pesticide applications made to glasshouse crops have no significant adverse effects on the bumble bees on which they are dependent for pollination. This study aimed to determine the effects of a novel insect growth regulator applied to flowering tomatoes on introduced commercial bumble bee colonies. The study comprised five 5m by 3m glasshouse cubicles each containing 26 tomato plants and a single bumble bee queen right colony containing 100-200 workers. The tomato plants in the cubicles were treated at 10 day intervals. Pollen patties in each glasshouse were sprayed once at the same rate as the plants to provide a supplementary treated pollen supply for the colonies. Two cubicles were treated with water as a control, two cubicles were sprayed with the novel IGR (0.05% a.i.) and one cubicle was a positive control treated with 0.03% a.i. diflubenzuron. Numbers of dead adults, dead larvae, flying bees, foraging bees and open flowers present and general colony appearance of the colonies were monitored in each glasshouse at pre-determined intervals from day -3 to day 23 (first spray applied day 0). Colonies in both the novel IGR and diflubenzuron treated cubicles showed high levels of brood mortality with larvae ejected from the colonies. Highest numbers of dead larvae were observed within the first 15 days after spraying. The treated colonies showed no significant adult mortality or adverse effects on numbers of flying or foraging bees. An assessment of the number of fruit present on day 23 compared to the total number of flowers open in previous assessments, a measure of pollination efficiency, showed no statistically significant differences between the treated and control glasshouses.

Hazards of pesticides to bees, ICPBR 7th Bee Protection Symposium, Avignon, France, 07-09 September 1999. *IOBC wprs Bulletin*: 23 (3), 2000

Toxicity and repellency to honeybees of pyrethroid insecticide/fungicide mixtures

Helen M. Thompson[1] and Helen Folkard-Ward[1]

1 : National Bee Unit, CSL, Sand Hutton, York YO41 1LZ, UK
Fax : (+44) 1904 462240
e-mail : H.Thompson@csl.gov.uk

A short series of studies were performed investigating the contact toxicity of two pyrethroid formulations Fastac (alphacypermethrin) and Hallmark (lambda-cyhalothrin) in combination with a range of six benzimidazole, azole, triazole and dicarboximide fungicides. The ratio of pyrethroid to fungicide was chosen to replicate the usual field application rates of the compounds and therefore the exposure which may occur in the field, e.g. due to tank mixing by farmers.
At realistic application rates a combination of Fastac and Tilt (propiconazole) resulted in no increase in contact toxicity over that which could be explained by additivity which suggests that alphacypermethrin metabolism is not affected by the presence of the fungicide. No other combinations of Fastac and fungicide formulations resulted in increased toxicity. Hallmark and Tilt showed a slight increase in contact toxicity. No other combinations of Hallmark and fungicide formulations resulted in increased toxicity. Further investigations showed that the increase in toxicity between Hallmark and Tilt was ratio dependent. Some of the WIIS honeybee reports have involved alphacypermethrin which could not be explained in the laboratory toxicity studies even using the same formulations, e.g. Fastac and Compass, at realistic application rates. Fastac was shown to be repellent in a laboratory choice test using sucrose feeders placed on pyrethroid and control treated filter papers. Addition of the fungicide Compass (iprodione and thiophanate-methyl) reduced the apparent repellency of Fastac to the level of the control, i.e. the bees ate the same amount from the feeders on the treated filter paper as those on the untreated paper. These are very preliminary studies but suggest that there may be some effect of the fungicides on the repellency of the pyrethroid and requires further investigation, e.g. in semi-field studies. This reduced repellency may increase the exposure of bees in the field to pyrethroids, and thus mortality following crop treatment, by reducing their avoidance of treated crops.

Hazards of pesticides to bees, ICPBR 7th Bee Protection Symposium, Avignon, France, 07-09 September 1999. *IOBC wprs Bulletin*: 23 (3), 2000

Tests regarding the danger of the seed disinfectant, GAUCHO, for bees

Klaus Wallner

Universität Hohenheim
Landesanstalt für Bienenkunde (730)
70593 Stuttgart, Germany
Fax : (+49) 711 459 2233
e-mail : BIENEWA@UNI-HOHENHEIM.DE

The hazard of the systemic seed disinfectant, Gaucho WS (a.i. Imidacloprid), on bees was examined with the use of *Phacelia tanacetifolia* in tent and field tests. The substance is suspected to be responsible for the decrease in the crops of sunflower honey. The test criteria included: foraging activity, orientation, mortality in front of the hive entrances, honey sac weight of returning forager bees, the amount of Imidacloprid in the honey sac load, in the honey itself, and in the bee bread, the toxic effect on larvae, and the foraging activity on flower clusters in the field.
In the tent, the bees (2 colonies with 5 combs) on the disinfected area (120 sqm, 0,005 g a.i./m^2) showed no symptoms of toxic effects or disorientation. Flight activity (10 observations: 5 min./5 days), honey crop, and the daily mortality (7 days) remained unchanged. The analysis of the honey sac (40 bees pooled) content showed that *Phacelia* plants excrete traces of this substance with the nectar ($3<x<10$ ppb). Imidacloprid was also detectable in the bee bread ($3<x<10$ ppb). The attractiveness of disinfected *Phacelia* did not change under field conditions. A negative influence on honeybees could not be determined.

Hazards of pesticides to bees, ICPBR 7th Bee Protection Symposium, Avignon, France, 07-09 September 1999. *IOBC wprs Bulletin*: 23 (3), 2000

Appendixes

Hazards of pesticides to bees, ICPBR 7th Bee Protection Symposium, Avignon, France,
07-09 September 1999. *IOBC wprs Bulletin*: 23 (3), 2000

Appendix I

EPPO / OEPP

A - PP 1/170(2) English, Under revision 99/7541

This is the revised draft of EPPO Guideline for the evaluation of side-effects of plant protection products on honeybees (PP 1/170) as approved by the ICP-BR Bee Protection Group at its meeting in Avignon (September 1999). It will be finalized and published in due course, subject to the EPPO approval procedure.

EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES

PP 1/170(2) English

Under revision 99/7541

Guideline for the efficacy evaluation of plant protection products

SIDE-EFFECTS ON HONEYBEES

Specific scope

This standard describes the conduct of trials for the evaluation of side-effects of plant protection products on honeybees.

Specific approval and amendment

First approved in September 1991.
Aligned with revised standard text in 1998.
Revised in ...

It is important that plant protection products should be authorized for use only in ways which minimize the risk of harm to honeybees. For this purpose it may be necessary to provide evidence during the registration process to enable the safety of the product in question to be evaluated. This guideline presents several different types of tests (laboratory tests, cage test, field trial and tunnel test) which can be used to provide such evidence. However, some other tests which are sometimes used, such as tests on inhalation and long-term contact, are not described. The description of these methods is based upon the "Recommendations for harmonization of methods for testing hazards of pesticides to honeybees", decided by the International Commission for Plant Bee Relationships at the Symposia on the harmonization of methods for testing the toxicity of pesticides to bees, held in Wageningen, NL (1980), Hohenheim, DE (1982), Harpenden, GB (1985), Rez, CZ (1990), Wageningen, NL (1993), Braunschweig, DE (1996) and Avignon, FR (1999).
The laboratory tests examine oral toxicity and contact toxicity of the plant protection product. The semi-field cage test and the full field trial study the effects of application of the product during bee flight. The tunnel test can be used to study certain hazards to honeybees which are virtually impossible to study by field trials, such as the effects on bees foraging the honey dew from aphids.

While recognizing that no single test method can provide sufficient information to classify the side-effects of plant protection products on honeybees, it is important also to stress it is not envisaged that all these tests must be followed. Because field testing is time-consuming and costly, the laboratory tests or semi-field test may serve to classify many products as definitely harmless or harmful without having recourse to field trials. The decisions on which tests to perform and on whether to proceed from one test to another will depend on the characteristics of the plant protection product, its use pattern and on the tests already performed. These decisions can be derived from a logically constructed sequential decision-making scheme (Oomen, 1986). A joint EPPO/Council of Europe Panel on Environmental Risk Assessment of Plant Protection Products has now developed such schemes, including one for honeybees (OEPP/EPPO, 1993).

I. Laboratory tests

1. Experimental conditions

1.1 *Principle of the trial*

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

1.2 *Trial conditions*

Keep bees in holding cages that are well ventilated and easily cleaned. Do not use plastic cages unless disposed of after use, because of possible contamination. Avoid re-use of wooden cages unless very well cleaned and sterilized. Cages should not cause control mortality. Store bees after treatment at a temperature of 25 ± 2°C. Relative humidity during the test should be recorded.

Bees should be kept in darkness during the whole trial period, except during assessments.

1.3 *Preparation of the bees*

Use preferably uniform, young adult worker bees. Bees should be adequately fed and from a healthy and queen-right colony. Where applicable, the last varroacide treatment should be identified and the timing recorded. The treatment should have ended at least 4 weeks before the start of the test. Collect bees in a standardized way. Avoid collection in early spring or late autumn. Bees collected from frames without brood or from the flight board at the hive entrance are suitable. Bees may also be reared in an incubator, fed with fresh or well preserved pollen and sucrose solution. The method of collection used, the age and (if known) the race of bees, and date of the experiment should be reported.

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

1.4 *Design of the trial*

Treatments: either formulated products or active substances are tested. Include a control treated with the dosing vehicle and an appropriate toxic standard to check consistency of results (e.g. parathion, dimethoate).

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

Replicates: at each concentration, use at least 3 groups of 10 bees. For limit tests, number of groups should be increased to 5.

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

2. Application of treatments

2.1 *Oral toxicity test*

2.1.1 *Test product(s)*

Use the formulated product or active substance in 200-500 gram/litre final concentration of sucrose solution. Dissolve or disperse formulations without additional solvents if possible.

2.1.2 *Mode of application*

Starve bees for up to 2 h before tests. Dose at 10 or 20 µl of test solution per bee through feeders. By group feeding, bees will share the test solution between themselves and so receive similar doses. There should be a maximum period of dosing (e.g. 4-6 hours) to avoid mortality due to starvation.
If at the end of this period there is still test dose remaining, the amount should be measured (this allows the precise dose taken by the bees to be determined, which is more accurate for the LD50 calculation and provides information on distastefulness/repellency).
Supply fresh sucrose solution after dose has been taken and change daily if test period exceeds 48 h.

2.2 *Contact toxicity test*

2.2.1 *Test product(s)*

Dissolve the active substance in acetone where possible. Use other solvents only if the active substance is insoluble in acetone. Formulated material should be delivered in an aqueous dispersion using an appropriate wetting agent where necessary.

2.2.2 *Mode of application*

Treat anaesthetized bees individually by topical application. Dose a measured amount of product to the thorax of each bee. Supply fresh sucrose solution after application and check daily (replenish if necessary).

3. Mode of assessment

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

4. Results

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

II. Cage tests (including tunnel tests)

The cage test can also be modified for specific tests with honeybees e.g. repellency or the evaluation of the hazard of the application of plant protection products to honeybees foraging the honeydew secreted by aphids. In these cases, the cage test can be modified to a field tunnel test.

1. Experimental conditions

1.1 *Principle of the trial*

Bees from small colonies are forced to forage on a flowering crop in field cages. The test products and a toxic standard known to present a high hazard to bees are sprayed in separate cages during bee flight, while other cages are left as untreated controls. The toxic standard is used to confirm that bees are at risk. In case the trial conditions do not allow the use of a toxic standard, it should be demonstrated otherwise that bees had been at risk. The effects of the treatment on bees are assessed just before and several times after application.

1.2 *Trial conditions*

It is recommended to use cages (tunnels) with a minimal size of 40 m². The cage should have a maximal mesh size of 3 mm. Plastic coating on the roof may be used to prevent trapping of the bees.
Suitable test crops are *Borago, Brassica, Phacelia, Sinapis,* and other flowering crops attractive to bees on which use of the test product is proposed.
On cereals, where aphid honeydew is being simulated, sucrose solution is sprayed onto a suitable crop e.g. wheat, in such manner as to maintain sufficient attraction.

1.3 *Preparation of the bees*

Use one small healthy queen-right colony per cage, of at least three full frames, or a nucleus.
Feeding of the colonies during the trial may be necessary and water should be offered.

1.4 *Design of the trial*
Treatments: test product(s), toxic standard known to present a high hazard to bees (e.g. parathion, dimethoate) and a control without plant protection product. The control may or may not receive a water spray.
Test units: cages with one colony.
Replicates: sufficient to enable appropriate statistical analysis.

2. Application of treatments

2.1 *Test product(s)*
Use formulated products only.

2.2 *Mode of application*
Apply products during the daytime when bees are foraging most actively. Avoid spraying the cage walls.
The number of foraging bees per m^2 , and how the assessments are carried out, should be recorded.

2.3 *Doses*
The product should normally be applied at the highest dose specified for the intended use in flowering crops; if desired, an additional higher rate may also be tested.

3. Mode of assessment

Pre-treatment assessments should be sufficient to demonstrate a stable background mortality and to show that the bees have acclimatised to the test conditions and are actively foraging on the crop.
Record effects just before and at several intervals, preferably 0, 1, 2, 4 and 7 days after treatment. Record foraging activity and the behaviour of bees on the crop and around the hive. Count the bees in dead-bee traps and those dying in the rest of the cage. Record temperature and humidity. Other assessment e. g. effects on brood, should be made as appropriate to the type of test product.

4. Results

Repeat tests where control mortality is considerable in comparison with the toxic standard and also where mortality in the toxic standard treatment is low.
Mortality data must always be provided and any other data which is relevant to the properties of the product being tested.
Original (raw) data should be available. Statistical analysis should normally be used, by appropriate methods which should be indicated. If statistical analysis is not used, this should be justified.

III. Field tests

1. Experimental conditions

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

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

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

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

1.5 *Design and lay-out of the trial*
Treatments: product(s) to be tested and an untreated or water-sprayed control; reference products known to present a low or high hazard to bees may also be included. A toxic standard does not have to be included, but if not, honeybee exposure should be otherwise demonstrated, e.g. by evidence based on assessments of foraging bees before and after application (collecting pollen and marking bees in the field may also provide useful information in this respect).
Plot size: at least 1500 m². Full-strength colonies require larger areas. Plots should be well separated to avoid bees foraging on the wrong plot. The plots should not be close to other flowering crops which are attractive to bees. The distance between plots should be recorded.
Replicates: although very desirable, replication is often not feasible because of requirements of separation. Use at least 3 colonies per treatment.

2. Application of treatments

2.1 *Test product(s)*
Use formulated products only.

2.2 *Reference product(s)*
Choose products registered for a use similar to the intended use of the test product, if required.

2.3 *Mode of application*
Apply the products during the daytime when bees are demonstrated to be actively foraging on the test crop. Apply treatments simultaneously, i.e. within at most 2 h. Follow the recommendations for a application specified for the intended use.
The number of foraging bees per m² , and how the assessment is done, should be recorded.

2.4 *Doses*
The products should normally be applied at the highest dose recommended for the practical field use. Volume of application and nozzle type should be as recommended and should be recorded.

3. Mode of assessment and recording

3.1 *Meteorological data*
Temperature and humidity should be recorded throughout the trial period. Rainfall and sunshine or cloud cover should also be reported.

3.2 *Type, time and frequency of assessment*
3.2.1 *Type*

Estimate or record the following parameters: number of foraging bees in the crop, behaviour of bees on crop and around hives, mortality of bees (using dead-bee traps).
It is desirable to estimate also: pollen collection (using pollen traps), pollen in collected honey. In special situations it may be necessary to estimate the number of bees on frames, brood status in frames, and to study residues in dead bees, pollen, wax and honey. Brood status should always be assessed at test initiation and test termination.

3.2.2 *Time and frequency*

Pre-application assessment: at least twice; the second assessment should be immediately before application or, at most, one day before.
Post-application assessment: at several intervals, preferably 0, 1, 2, 4, 7 14 and 28 (only for brood) days after application.
All assessments should be performed approximately at the same time of a day. Assessment may be continued for longer intervals for up to 3 months after application.

4. Results

The trial is invalid and should be repeated if the exposure at the time of application cannot be convincingly demonstrated e.g. through the use of a toxic standard or from the foraging assessments carried out immediately pre-treatment. Also, repeat trial if mortality in the control treatment is considerable (generally above 15%). Original (raw) data should be available. Mortality data must always be provided and any other data which is relevant to the properties of the product being tested. Statistical analysis should normally be used, by appropriate methods which should be indicated. If statistical analysis is not used, this should be justified.

References

Gough, H.J., McIndoe, E.C., Lewis, G.B. (1994) The use of dimethoate as a reference compound in laboratory acute toxicity tests on honey bees (*Apis mellifera* L.) 1991-1992. Journal of Apicultural Research 33 (2) 119-125.

ICBB (1980) Conclusions of the meeting. In *Symposium on the harmonization of methods for testing the toxicity of pesticides to bees*. Wageningen (NL).

ICBB (1982) Revised recommendations for harmonization of methods for testing the hazard of pesticides to bees. In *Second Symposium on the Harmonization of methods for testing the toxicity of pesticides to bees* Appendix I, pp. 11-16. Hohenheim (DE).

ICBB (1985) Recommendation for harmonization of methods for testing hazards of pesticides to honeybees. In *Third symposium on the harmonization of methods for testing the toxicity of pesticides to bees* Appendix I. Harpenden (GB).

ICPBR (1990) Proceedings of the Fourth International Symposium on the Harmonization of Methods for Testing the Toxicity of Pesticides to Bees. May 15-18,1990, Rez, Czechoslovakia.

ICPBR (1993) Discussion and Recommendations of the Fifth Meeting, p.10-14 in: Harrison, E.G. (1993) Proceedings of the Fifth International Symposium on the Hazards of Pesticides to Bees. October 26-28, 1993, Wageningen, The Netherlands.

ICPBR (1996) Recommendations of the meeting, p; 31-35 in: Lewis, G.B. (Ed, 1996) Proceedings of the 6th International Symposium on Hazards of Pesticide to Bees. September 17-19, 1996, Braunschweig, Germany

OEPP/EPPO (1993) Decision-making scheme for the environmental risk assessment of plant protection products. Chapter 10: honeybees. *Bulletin OEPP/EPPO Bulletin* **23**, 151-165.

OOMEN, P.A. (1986) A sequential scheme for evaluating the hazard of pesticides to bees, *Apis mellifera*. *Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* **51**, 1205-1213.

SHIRES, S.W., LE BLANC, I., DEBRAY, P., FORBES, S. & LOUVEAUX, J. (1984) Field experiments on the effects of a new pyrethroid insecticide WL-85871 on bees foraging artificial aphid honeydew on winter wheat. *Pesticide Science* **15**, 543-552.

B - PP 1/170(2) English

EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES

PP 1/170(2) English

Guideline for the efficacy evaluation of plant protection products

SIDE-EFFECTS ON HONEYBEES

Specific scope

This standard describes the conduct of trials for the evaluation of side-effects of plant protection products on honeybees.

Specific approval and amendment

First approved in September 1991.
Aligned with revised standard text in 1998.

It is important that plant protection products should be authorized for use only in ways which minimize the risk of harm to honeybees. For this purpose it may be necessary to provide evidence during the registration process to enable the safety of the product in question to be evaluated. This guideline presents several different types of tests (laboratory tests, cage test, field trial and tunnel test) which can be used to provide such evidence. However, some other tests which are sometimes used, such as tests on inhalation and long-term contact, are not described. The description of these methods is based upon the "Recommendations for harmonization of methods for testing hazards of pesticides to honeybees", decided by the International Commission for Plant Bee Relationships at the Symposia on the harmonization of methods for testing the toxicity of pesticides to bees, held in Wageningen, NL (1980), Hohenheim, DE (1982) and Harpenden, GB (1985). The laboratory tests examine oral toxicity and contact toxicity of the plant protection product. The semi-field cage test and the full field trial study the effects of application of the product during bee flight. The tunnel test can be used to study certain hazards to honeybees which are virtually impossible to study by field trials, such as the effects on bees foraging the honey dew from aphids.
While recognizing that no single test method can provide sufficient information to classify the side-effects of plant protection products on honeybees, it is important also to stress it is not envisaged that all these tests must be followed. Because field testing is time-consuming and costly, the laboratory tests or semi-field test may serve to classify many products as definitely harmless or harmful without having recourse to field trials. The decisions on which tests to perform and on whether to proceed from one test to another will depend on the characteristics of the plant protection product, its use pattern and on the tests already performed. These decisions can be derived from a logically constructed sequential decision-making scheme (Oomen, 1986). A joint EPPO/Council of Europe Panel on Environmental Risk Assessment of Plant Protection Products has now developed such schemes, including one for honeybees (OEPP/EPPO, 1993).

I. Laboratory tests

1. Experimental conditions

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

1.2 *Trial conditions*

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

1.3 *Preparation of the bees*

Use preferably uniform, young adult worker bees. Bees should be adequately fed and from a healthy and queen-right colony. Collect bees in a standardized way. Avoid collection in early spring or late autumn. Bees collected from frames without brood or from the flight board at the hive entrance are suitable. Bees may also be reared in an incubator, fed with fresh or well preserved pollen and sucrose solution. The method of collection used, the age and (if known) the race of bees, and date of the experiment should be reported.
Bees may be anaesthetized with carbon dioxide for testing of contact toxicity. Keep the amount used and times of exposure to a minimum, but ensure anaesthesia is complete. Ensure that application does not lower the temperature of the holding cage and the bees.

1.4 *Design of the trial*

Treatments: either formulated products or active substances are tested. Include a control treated with the solvent and an appropriate reference product to check consistency of results (e.g. parathion, dimethoate).
Test units: dose bees individually or in groups of at least 10. Bees should not be confined individually for more than 1 h.
Replicates: at each concentration, use at least 3 groups of 10 (or more) bees.
Concentrations: use a suitable range and number of concentrations in order to provide a regression line and LD50.

2. Application of treatments

2.1 *Oral toxicity test*

2.1.1 *Test product(s)*

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

2.1.2 *Mode of application*

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

2.2 *Contact toxicity test*

2.2.1 *Test product(s)*

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

2.2.2 *Mode of application*

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

3. Mode of assessment

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

4. Results

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

II. Cage tests

1. Experimental conditions

1.1 *Principle of the trial*
Bees from small colonies are forced to forage on a flowering crop in field cages. The test products and a reference product known to present a high hazard to bees are sprayed in separate cages during bee flight, while other cages are left as untreated controls. The reference product is used to confirm that bees are at risk. In case the trial conditions do not allow the use of a hazardous reference product, it should be demonstrated otherwise that bees have been at risk. The effects of the treatment on bees are assessed just before and several times after application.

1.2 *Trial conditions*
Use cages with a minimal size of $2 \times 2 \times 3$ m. The cage should have a maximal mesh size of 3 mm. Plastic coating on the roof may be used to prevent trapping of the bees.
Suitable test crops are *Borago, Phacelia, Sinapis,* and other flowering crops attractive to bees on which use of the test product is proposed.

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

1.4 *Design of the trial*
Treatments: test product(s), reference product known to present a high hazard to bees (e.g. parathion, dimethoate) and a control without plant protection product. The control may or may not receive a water spray.
Test units: cages with one colony.
Replicates: sufficient to enable appropriate statistical analysis.

2. Application of treatments

2.1 *Test product(s)*
Use formulated products only.

2.2 *Mode of application*
Apply products during the day when bees are flying. Avoid spraying the cage walls.

2.3 *Doses*
The product should normally be applied at the highest dose specified for the intended use in flowering crops; if desired, an additional higher rate may also be tested.

3. Mode of assessment

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

4. Results

Repeat tests where control mortality is considerable (generally above 15%) and also where mortality in the reference treatment is low. Original (raw) data should be available. Statistical analysis should normally be used, by appropriate methods which should be indicated. If statistical analysis is not used, this should be justified.

III. Field tests

1. Experimental conditions

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

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

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

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

1.5 *Design and lay-out of the trial*
Treatments: product(s) to be tested, reference product known to present a high hazard to bees (e.g. parathion, dimethoate), reference product known to present a low hazard to bees or an untreated control.
Plot size: at least 1500 m^2. Full-strength colonies require larger areas. Plots should be well separated by at least 500-1000 m^2 to avoid bees foraging on the wrong plot. The plots should not be close to other flowering crops which are attractive to bees.
Replicates: although very desirable, replication is often not feasible because of requirements of separation. Use at least 3 colonies per treatment.

2. Application of treatments

2.1 *Test product(s)*
Use formulated products only.

2.2 *Reference product(s)*
Choose products registered for a use similar to the intended use of the test product.

2.3 *Mode of application*
Apply the products during the day when bees are actively foraging. Apply treatments simultaneously, i.e. within at most 2 h. Follow the recommendations for application specified for the intended use.

2.4 *Doses*
The products should normally be applied at the highest dose recommended for the intended use for the crop/pest situation under test; if desired an additional higher rate may also be tested. Volume of application and droplet size should be as recommended and should be recorded.

3. Mode of assessment and recording

3.1 *Meteorological data*
Temperature and humidity should be recorded throughout the trial period.

3.2 *Type, time and frequency of assessment*

3.2.1 *Type*

Estimate or record the following parameters: number of foraging bees in the crop, behaviour of bees on crop and around hives, mortality of bees (using dead-bee traps).
It is desirable to estimate also: pollen collection (using pollen traps), pollen in collected honey, number of bees on frames, brood status in frames, and residues in dead bees, pollen, wax and honey.

3.2.2 *Time and frequency*

Pre-application assessment: one day or just before application.
Post-application assessment: at several intervals, preferably 0, 1, 2, 4, 7 and 14 days after application.
Assessment may be continued at larger intervals for up to 3 months after application.

4. Results

Repeat trial if mortality in the non-hazardous reference treatment is considerable (generally above 15%) and also if mortality in the hazardous reference treatment is low. Original (raw) data should be available. Statistical analysis should normally be used, by appropriate methods which should be indicated. If statistical analysis is not used, this should be justified.

IV. Tunnel tests

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

1. Design of the trial

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

2. Experimental conditions and application of treatments

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

3. Mode of assessment and recording

As in field tests.

References

ICBB (1980) Conclusions of the meeting. In *Symposium on the harmonization of methods for testing the toxicity of pesticides to bees*. Wageningen (NL).
ICBB (1982) Revised recommendations for harmonization of methods for testing the hazard of pesticides to bees. In *Second Symposium on the Harmonization of methods for testing the toxicity of pesticides to bees* Appendix I, pp. 11-16. Hohenheim (DE).
ICBB (1985) Recommendation for harmonization of methods for testing hazards of pesticides to honeybees. In *Third symposium on the harmonization of methods for testing the toxicity of pesticides to bees* Appendix I. Harpenden (GB).
OEPP/EPPO (1993) Decision-making scheme for the environmental risk assessment of plant protection products. Chapter 10: honeybees. *Bulletin OEPP/EPPO Bulletin* **23**, 151-165.
OOMEN, P.A. (1986) A sequential scheme for evaluating the hazard of pesticides to bees, *Apis mellifera*. *Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* **51**, 1205-1213.
SHIRES, S.W., LE BLANC, I., DEBRAY, P., FORBES, S. & LOUVEAUX, J. (1984) Field experiments on the effects of a new pyrethroid insecticide WL-85871 on bees foraging artificial aphid honeydew on winter wheat. *Pesticide Science* **15**, 543-552.

C - PP 3/10(1) English

EPPO Standards

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

CHAPTER 10
HONEY BEES

PP 3/10(1) English

oepp
eppo

Organisation Européenne et Méditerranéenne pour la Protection des Plantes
1, rue Le Nôtre, 75016 Paris, France

APPROVAL
EPPO Standards are approved by EPPO Council. The date of approval appears in each individual standard.

REVIEW
EPPO Standards are subject to periodic review and amendment. The next review date for this set of EPPO Standards is decided by the EPPO Working Party on Plant Protection Products.

AMENDMENT RECORD
Amendments will be issued as necessary, numbered and dated. The dates of amendment appear in each individual standard (as appropriate).

DISTRIBUTION
The EPPO/Council of Europe decision-making scheme for the environmental risk assessment of plant protection products is distributed by the EPPO Secretariat to all EPPO member governments. Copies are available to any interested person under particular conditions upon request to the EPPO Secretariat.

SCOPE
The EPPO/Council of Europe decision-making scheme for the environmental risk assessment of plant protection products is intended to be used by National Plant Protection Organizations or equivalent authorities, in their capacity as bodies responsible for the registration of plant protection products, including an evaluation of the environmental risks arising from their use.

REFERENCES
OEPP/EPPO (1993) Decision-making scheme for the environmental risk assessment of plant protection products. Chapters 1-6, 8 & 10. *Bulletin OEPP/EPPO Bulletin* **23**, 1-165.

OEPP/EPPO (1993) Decision-making scheme for the environmental risk assessment of plant protection products. Chapters 7, 9 & 11. *Bulletin OEPP/EPPO Bulletin* **24**, 1-87.

OUTLINE OF REQUIREMENTS
The decision-making scheme for the environmental risk assessment of plant protection products was developed by a joint Panel of EPPO and the Council of Europe and provides guidelines on how to assess the potential impact of a particular plant protection product on various different elements of the environment. The assessment scheme is for use by agrochemical companies and by regulatory authorities, and aims to:

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

The scheme is a set of flexible procedures that can be adapted for use in various ways according to the priorities in different states, yet retain the consistency of a common framework. It is not based on a series of fixed, automatic 'triggers' for testing requirements, but is able to take full account of the particular features of each plant protection product, and to make use of expert judgement when necessary.

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

PP 3/10(1) English

Decision-making scheme for the environmental risk assessment of plant protection products

CHAPTER 10
HONEYBEES

Specific scope

This standard provides an assessment of risk presented by plant protection products to honeybees.

Specific approval and amendment

First approved in September 1992.
Edited as an EPPO Standard in 1998.

Introduction

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

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

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

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

Decision-making scheme

Details of the product and its pattern of use

1. Take from Chapter 2 the basic information on the product and its pattern of use.
   In addition, enter the following information:
   -time of treatment in relation to crop flowering
   -any special directions for use.

   Go to 2

Possibility of exposure

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

Preliminary screening based on toxicity

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

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

4. Assess how long residues remain active on foliage (see Note 3).
   if persistence is short (e.g. LT50 < r h) — **Go to 5**
   if persistence is longer (e.g. LT50 > r h) — **Go to 15**

Identification of stages at risk

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

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

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

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

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

Cage and field trials

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

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

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

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

Categories of risk

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

12. Categorize as negligible risk to bees

    Go to 19

13. Categorize as low risk to bees

    Go to 16

14. Categorize as medium risk to bees

    Go to 16

15. Categorize as high risk to bees

    Go to 18

Analysis of uncertainty

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

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

17. Consider whether the lower risk category reached by preliminary assessment, or the higher category in the repeat assessment, is more appropriate as a basis for classification and approval of the product's use.

    Go to 19

18. Review the data which led to the high-risk category and check whether the conclusions are correct.
    if yes, confirm assessment **Go to 19**
    if no, obtain more information as needed **Go to 3**

Risk management

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

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

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

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

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

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

Explanatory notes

Note 1. Possibility of exposure

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

Note 2. Toxicity tests

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

The ratio between application rate and toxicity (sometimes referred to as a hazard ratio) gives an approximation of how closely the likely exposure of bees is to a toxicologically significant level. In calculating the ratio (dose per ha/LD50), dose per ha is the highest application rate in g ha^{-1}, and LD50 is measured in µg a.i. per bee. The upper (q) and lower (p) thresholds are determined on the basis of bee toxicity, dosage rate and an independent classification of risk verified by extensive practical experience of plant protection products. Suggested values are q=2500 for the upper threshold and p=50 for the lower threshold.

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

Note 3. Residual toxicity

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

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

Note 4. Bee brood feeding tests

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

Note 5. Indirect effects

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

Note 6. Cage trials

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

Note 7. Field trials

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

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

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

Note 8. Significance of field results

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

Note 9. Additional testing

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

References

Council of Europe (1992) Chapter V. Guidance on environmental phenomena and wildlife data. In *Pesticides* (7th ed.). Council of Europe, Strasbourg (FR).

FAO (1989) *Revised Guidelines on Environmental Criteria for the Registration of Pesticides*. FAO, Rome (IT).

Felton, J.C., Oomen, P.A. & Stevenson, J.H. (1986) Toxicity and hazard to honey bees: harmonization of test methods. *Bee World* **67**, 114-124.

Gerig, L. & Oomen, P.A. (1993) Methods for testing the duration of activity of pesticide residues on bees. *Bulletin OEPP/EPPO Bulletin* (in press).

OEPP/EPPO (1992) Guideline on test methods for evaluating the side-effects of plant protection products. No. 170. Honeybees. *Bulletin OEPP/EPPO Bulletin* **22**, 203-216.

Oomen, P.A., De Ruijter, A. & van der Steen, J. (1992) Method for honeybee brood feeding tests with insect growth-regulating insecticides. *Bulletin OEPP/EPPO Bulletin* **22**, 613-616.

Wittman, D. & Engels, W. (1981) [Development of test procedures for insecticide-induced brood damage in honey bees.] *Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie* **3**, 187-190 (in German).

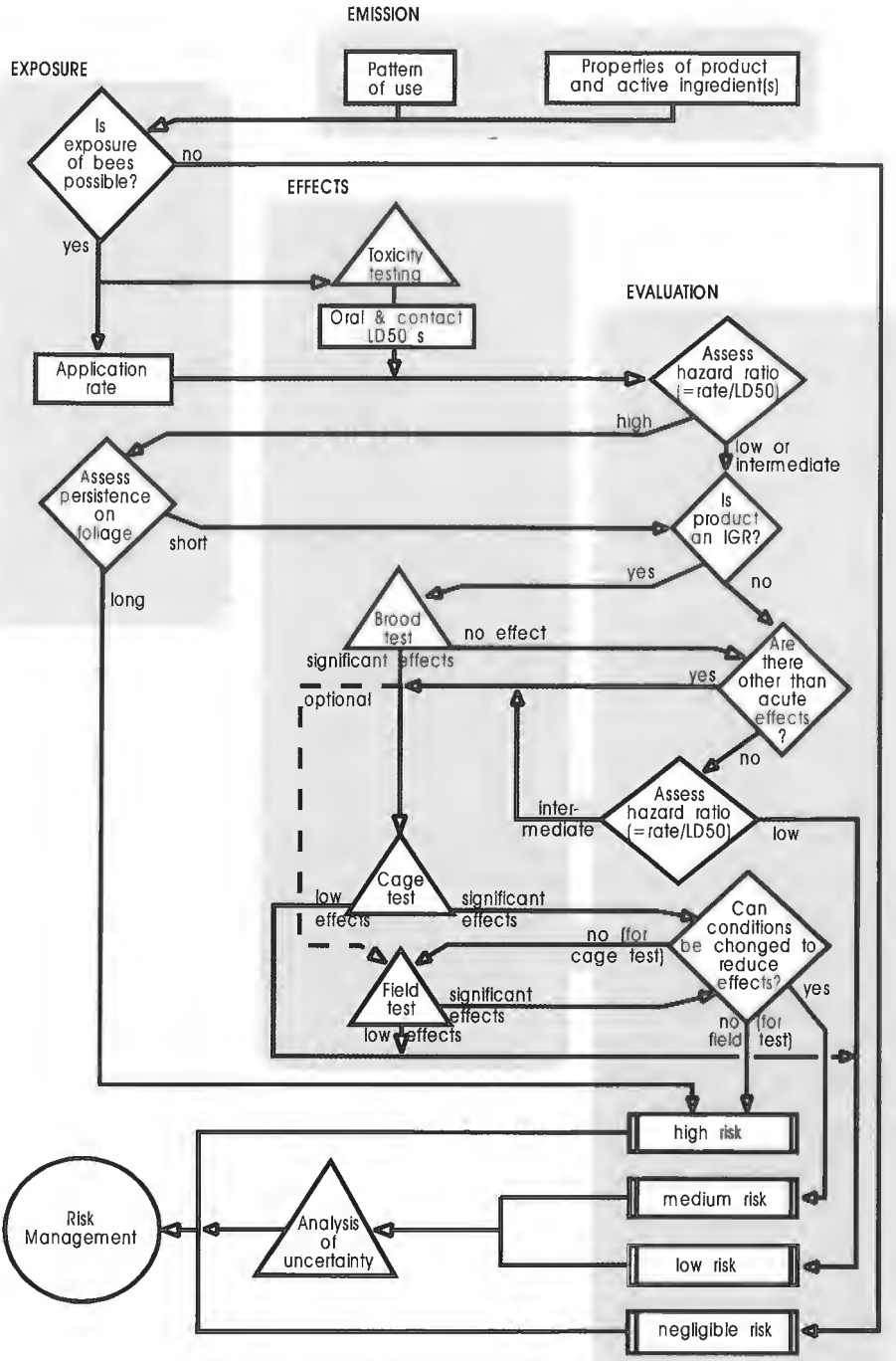

Fig. 10. Simplified diagram of the sub-scheme for evaluation of the risk of a plant protection product to honeybees.

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

Reprinted from : *Bulletin OEPP / EPPO Bulletin* **22**, 613-616 (1992)

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

by P.A. OOMEN, A. DE RUIJTER* and J. VAN DER STEEN*

Plant Protection Service, Wageningen (The Netherlands)
* Research Centre for Insect Pollination and Beekeeping, Hilvarenbeek (The Netherlands)

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

Introduction

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

1. Experimental conditions

1.1 Principle of the trial

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

1.2 Trial conditions

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

1.3 Design of the trial

Treatments: test product, reference product (IGR of which the dose/effect relation on bee brood is known, e.g. fenoxycarb, diflubenzuron), non-pesticide control (pure sugar solution).
Test units: sound medium-size bee colonies.
Replicates: at least three colonies per product and per concentration. All colonies of a trial should be placed in one location.

1.4 Preparation of the bees

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

2. Application of treatments

2.1 Products to be tested

Use formulated products only.

2.2 Mode of application

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

2.3 Dosage

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

2.4 Time and frequency of application

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

3. Mode of assessment

3.1 Brood development

One day (not longer than 24 h) before the start of feeding, mark in each colony at least 100 cells with eggs, 100 cells with young larvae and 100 cells with old larvae by means of a transparent (overhead) sheet. Clear combs are preferred for marking cells. Avoid if possible the outer part of the brood nest, as bees sometimes have difficulty nursing the outer cells during cold weather. Brood development in all marked cells is checked weekly until 3 weeks after application. Inspection is then ended, since normal brood development takes 3 weeks.
A practical way to describe brood development is as follows. The pretreatment positions of egg, young and old larvae cells are indicated on a first overhead sheet by numbers in a colour code for each stage. The date, colony number and a mark how the sheet was placed over the comb are also indicated. The first sheet is then copied several times to serve as a mother copy for later inspections. In these, the different juvenile stages are again indicated by their own colour code.

3.2 Mortality of adult bees and brood

Dead-bee traps are placed in front of the beehives; suitable traps are gauze boxes of 100x75x50 cm, open at the upper side. The traps are inspected daily to count dead adult bees, and to collect dead larvae and pupae, which are examined in the laboratory for specific effects of IGRs (white eye rims, malformations).

3.3 Other effects

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

3.4 Data on meteorology and environment

From the day of first observation, record meteorological and environmental data. This includes temperature (average, maximum, minimum in °C), rainfall in mm, relative humidity (maximum, minimum). Environmental

data include the main sources of nectar near the colonies, time of the year, and a description of the near surroundings.

4. Results

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

Acknowledgements

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

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

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

[cyrillic abstract]

References

OEPP/EPPO (1992) Guideline on test methods for evaluating the side-effects of plant protection products on honeybees. *Bulletin OEPP/EPPO Bulletin* 22, 203-216.

GREIG-SMITH, P. (1991) Environmental risk assessment of plant protection products: an approach to the development of guidelines. *BulletinOEPP/EPPO Bulletin* 21, 219-225.

OOMEN, P.A. & GERIG, L. (1993) Method for testing the duration of activity on honeybees of residues on leaves. *Bulletin OEPP/EPPO Bulletin* 23 (in preparation).

Note from Dr. Oomen, Feb. 2000 :

The last reference (Oomen & Gerig, 1993, in preparation) was not published actually.

Acknowledgements :

The Organizing Committee thank EPPO for the authorization to reprint these documents, and Dr. Vlasta ZLOF for her efficient collaboration.

Appendix II

OECD / OCDE

OECD's Guidelines for the Testing of Chemicals

Section 2 - Effects on Biotic Systems

Adopted Test Guidelines

Number 213

Honeybees, Acute Oral Toxicity Test
(Original Guideline, adopted 21st September 1998

Number 214

Honeybees, Acute Contact Toxicity Test
(Original Guideline, adopted 21st September 1998)

These Guidelines are available at OECD, Paris :

OECD
Environmental Health and Safety Division
2, rue André-Pascal
75775 Paris cedex 16
France

http://www.oecd.org/ehs/

Appendix III

EEC / CEE

No L 65/20 EN Official Journal of the European Communities 15. 3. 96

COMMISSION DIRECTIVE 96/12/EC

of 8 March 1996

amending Council Directive 91/414/EEC concerning the placing of plant protection products on the market

(Text with EEA relevance)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market (1), as last amended by Commission Directive 95/36/EC (2), and in particular Article 18 (2) thereof,

Whereas Annexes II and III to Directive 91/414/EEC set out the requirements for the dossier to be submitted by applicants respectively for the inclusion of an active substance in Annex I of that Directive and for the authorization of a plant protection product;

Whereas it is necessary to indicate, in Annexes II and III to Directive 91/414/EEC, to the applicants, as precisely as possible, any details on the required information, such as the circumstances, conditions and technical protocols under which certain data have to be generated; whereas these provisions should be introduced as soon as available in order to permit applicants to use them in the preparation of their files;

Whereas it is now possible to introduce more precision with regard to the data requirements concerning ecotoxicological studies on the active substance provided for in Part A, point 8, of Annex II to Directive 91/414/EEC;

Whereas it is also now possible to introduce more precision with regard to the data requirements concerning ecotoxicological studies on the plant protection product provided for in Part A, point 10, of Annex III to Directive 91/414/EEC;

Whereas the measures provided for in this Directive are in accordance with the opinion of the Standing Committee on Plant Health,

HAS ADOPTED THIS DIRECTIVE:

Article 1

Directive 91/414/EEC is amended as follows:

1. In Part A of Annex II, point 8 'Ecotoxicological studies on the active substance' is replaced by Annex I hereto;
2. in Part A of Annex III, points 10 'Ecotoxicological studies' and 11 'Summary and evaluation of points 9 and 10' are replaced by Annex II hereto.

Article 2

Member States shall bring into force the laws, regulations and administrative provisions necessary to comply with this Directive by 31 March 1997. They shall immediately inform the Commission thereof.

When Member States adopt these measures, these shall contain a reference to this Directive or shall be accompanied by such reference at the time of their official publication. The procedure for such reference shall be adopted by the Member States.

Article 3

This Directive shall enter into force on 1 April 1996.

Article 4

This Directive is addressed to the Member States.

Done at Brussels, 8 March 1996.

For the Commission

Ritt BJERREGAARD

Member of the Commission

(1) OJ No L 230, 19. 8. 1991, p. 1.
(2) OJ No L 172, 22. 7. 1995, p. 8.

ANNEX I

'8. ECOTOXICOLOGICAL STUDIES

Introduction

(i) The information provided, taken together with that for one or more preparations containing the active substance, must be sufficient to permit an assessment of the impact on non-target species (flora and fauna), likely to be at risk from exposure to the active substance, its metabolites, degradation and reaction products, where they are of environmental significance. Impact can result from single, prolonged or repeated exposure and can be reversible or irreversible.

(ii) In particular, the information provided for the active substance, together with other relevant information, and that provided for one or more preparations containing it, should be sufficient to:

— decide whether, or not, the active substance can be included in Annex I,

— specify appropriate conditions or restrictions to be associated with any inclusion in Annex I,

— permit an evaluation of short- and long-term risks for non-target species — populations, communities, and processes — as appropriate,

— classify the active substance as to hazard,

— specify the precautions necessary for the protection of non-target species, and

— specify the hazard symbols, the indications of danger, and relevant risk and safety phrases for the protection of the environment, to be mentioned on packaging (containers).

(iii) There is a need to report all potentially adverse effects found during routine ecotoxicological investigations and to undertake and report, where required by the competent authorities, such additional studies which may be necessary to investigate the probable mechanisms involved and assess the significance of these effects. All available biological data and information which is relevant to the assessment of the ecotoxicological profile of the active substance must be reported.

(iv) The information on fate and behaviour in the environment, generated and submitted in accordance with points 7.1 to 7.4, and on residue levels in plants generated and submitted in accordance with point 6 is central to the assessment of impact on non-target species, in that together with information on the nature of the preparation and its manner of use, it defines the nature and extent of potential exposure. The toxicokinetic and toxicological studies and information submitted in accordance with points 5.1 to 5.8 provide essential information as to toxicity to vertebrate species and the mechanisms involved.

(v) Where relevant, tests should be designed and data analysed using appropriate statistical methods. Full details of the statistical analysis should be reported (e. g. all point estimates should be given with confidence intervals, exact p-values should be given rather than stating significant/non significant).

Test substance

(vi) A detailed description (specification) of the material used, as provided for under point 1.11 must be provided. Where testing is done using active substance the material used should be of that specification that will be used in the manufacture of preparations to be authorized except where radiolabelled material is used.

(vii) Where studies are conducted using active substance produced in the laboratory or in a pilot plant production system, the studies must be repeated using active substance as manufactured, unless it can be justified that the test material used is essentially the same, for the purposes of ecotoxicological testing and assessment. In cases of uncertainty, appropriate bridging studies must be submitted to serve as a basis for a decision as to the possible need for repetition of the studies.

(viii) In the case of studies in which dosing extends over a period, dosing should preferably be done using a single batch of active substance if stability permits.

Whenever a study implies the use of different doses, the relationship between dose and adverse effect must be reported.

(ix) For all feeding studies, average achieved dose must be reported, including where possible the dose in mg/kg body weight. Where dosing via the diet is utilized the test compound must be distributed uniformly in the diet.

(x) It may be necessary to conduct separate studies for metabolites, degradation or reaction products, where these products can constitute a relevant risk to non-target organisms and where their effects cannot be evaluated by the available results relating to the active substance. Before such studies are performed the information from points 5, 6 and 7 has to be taken into account.

Test organisms

(xi) In order to facilitate the assessment of the significance of test results obtained, including the estimation of intrinsic toxicity and the factors affecting toxicity, the same strain (or recorded origin) of each relevant species should, where possible, be used in the various toxicity tests specified.

8.1. Effects on birds

8.1.1. Acute oral toxicity

Aim of the test

The test should provide, where possible, LD_{50} values, the lethal threshold dose, time courses of response and recovery and the NOEL, and must include relevant gross pathological findings.

Circumstances in which required

The possible effects of the active substance on birds must be investigated except where the active substance is intended solely to be included in preparations for exclusive use in enclosed spaces (e.g. in glasshouses or in food storage practice).

Test conditions

The acute oral toxicity of active substance to a quail species (Japanese quail *(Coturnix coturnix japonica)* or Bobwhite quail *(Colinus virginianus)* or to mallard duck *(Anas platyrhynchos)* must be determined. The highest dose used in tests need not exceed 2 000 mg/kg body weight.

Test guideline

Setac — Procedures for assessing the environmental fate and ecotoxicity of pesticides (1).

8.1.2. Short-term dietary toxicity

Aim of the test

The test should provide the short term dietary toxicity (LC_{50} values, lowest lethal concentration (LLC), where possible no observed effect concentrations (NOEC), time courses of response and recovery) and include relevant gross pathological findings.

Circumstances in which required

The dietary (five-day) toxicity of the active substance to birds must always be investigated on one species except where a study in accordance with the provisions of point 8.1.3 is reported. Where its acute oral NOEL is $\leq$ 500 mg/kg body weight or where the short-term NOEC $<$ 500 mg/kg food the test must be performed on a second species.

Test conditions

The first species to be studied must be either a quail species or mallard duck. If a second species must be tested it should not be related to the first species tested.

Test guideline

The test must be carried out in accordance with OECD Method 205.

8.1.3. Subchronic toxicity and reproduction

Aim of the test

The test should provide the subchronic toxicity and reproductive toxicity of the active substance to birds.

(1) Society of Environmental Toxicology and Chemistry (Setac), 1995. *Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides*, ISBN 90-5607-002-9.

Circumstances in which required

The subchronic and reproductive toxicity of the active substance to birds must be investigated, unless it can be justified that continued or repeated exposure of adults, or exposure of nest sites during the breeding season is unlikely to occur.

Test guideline

The test must be carried out in accordance with OECD Method 206.

8.2. **Effects on aquatic organisms**

The data of the tests referred to in points 8.2.1, 8.2.4 and 8.2.6 have to be submitted for every active substance even when it is not expected that plant protection products containing it could reach surface water following the proposed conditions of use. These data are required under the provisions of Annex VI to Directive 67/548/EEC for the classification of the active substance.

Data reported must be supported with analytical data on concentrations of the test substance in the test media.

8.2.1. Acute toxicity to fish

Aim of the test

The test should provide the acute toxicity (LC_{50}), and details of observed effects.

Circumstances in which required

The test must always be carried out.

Test conditions

The acute toxicity of the active substance must be determined for rainbow trout *(Oncorhynchus mykiss)* and for a warm water fish species. Where tests with metabolites, degradation or reaction products have to be performed the species used must be the more sensitive of the two species tested with the active substance.

Test guideline

The test must be carried out in accordance with the Annex to Commission Directive 92/69/EEC [1] adapting to technical progress for the 17th time Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification and labelling of dangerous, substances, Method C1.

8.2.2. Chronic toxicity to fish

Circumstances in which required

A chronic toxicity study must be carried out unless it can be justified that continued or repeated exposure of fish is unlikely to occur or unless a suitable microsom or mesocosm study is available.

Expert judgment is required to decide which test has to be performed. In particular for active substance for which there are indications of particular concerns (related to the toxicity of the active substance for fish or the potential exposure) the applicant shall seek the agreement of the competent authorities on the type of test to be performed.

A fish early life stage toxicity test might be appropriate where bioconcentration factors (BCF) are between 100 and 1 000 or where EC_{50} of the active substance < 0,1 mg/l.

A fish life cycle test might be appropriate in cases where

— the bioconcentration factor is greater tan 1 000 and the elimination of the active substance during a depuration phase of 14 days is lower than 95 %,

or

— the substance is stable in water or sediment (DT_{90} > 100 days).

It is not necessary to perform a chronic toxicity test on juvenile fish when a fish early life stage toxicity test or a fish life cycle test has been performed; it is likewise not necessary to perform a fish early life stage toxicity test when a fish life cycle test has been performed.

8.2.2.1. Chronic toxicity test on juvenile fish

Aim of the test

The test should provide effects on growth, the threshold level for lethal effects and for observed effects, the NOEC and details of observed effects.

[1] OJ No L 383, 29. 12. 1992, p. 113.

Test conditions

The test must be conducted on juvenile rainbow trout, following exposure of 28 days to the active substance. Data on the effects on growth and behaviour must be generated.

8.2.2.2. Fish early life stage toxicity test

Aim of the test

The test should provide effects on development, growth and behaviour, the NOEC and details of observed effects on fish early life stages.

Test guideline

The test must be carried out in accordance with OECD Method 210.

8.2.2.3. Fish life cycle test

Aim of the test

The test will provide effects on reproduction of the parental and the viability of the filial generation.

Test conditions

Before performing these studies the applicant shall seek the agreement of the competent authorities on the type and conditions of the study to be performed.

8.2.3. Bioconcentration in fish

Aim of the test

The test should provide the steady-state bioconcentration factors, uptake rate constants and depuration rate constants, calculated for each test compound, as well as relevant confidence limits.

Circumstances in which required

The bioconcentration potential of active substances, of metabolites and of degradation and reaction products, likely to partition into fatty tissues (such as log $p_{ow} \geq 3$ — see point 2.8 or other relevant indications of bioconcentration), must be investigated and be reported, unless it can be justified that exposure leading to bioconcentration is not likely to occur.

Test guideline

The test must be carried out in accordance with OECD Method 305E.

8.2.4. Acute toxicity to aquatic invertebrates

Aim of the test

The test should provide the 24 and 48-hour acute toxicity of the active substance, expressed as the median effective concentration (EC_{50}) for immobilization, and where possible the highest concentration causing no immobilization.

Circumstances in which required

The acute toxicity must always be determined for *Daphnia* (preferably *Daphnia magna*). Where plant protection products containing the active substance are intended to be used directly on surface water additional data have to be reported on at least one representative species from each of the following groups: aquatic insects, aqautic crustaceans (on a species not related to *Daphnia)* and aquatic gastropod molluscs.

Test guideline

The test must be carried out in accordance with Directive 92/69/EEC, Method C2.

8.2.5. Chronic toxicity to aquatic invertebrates

Aim of the test

The test should provide where possible EC_{50} values for effects such as immobilization and reproduction and the highest concentration at which no effect such as on martality or reproduction occurs (NOEC) and details of observed effects.

Circumstances in which required

A test on *Daphnia* and on at least one representative aquatic insect species and an aquatic gastropod mollusc species must be carried out unless it can be justified that continued or repeated exposure is not likely to occur.

Test conditions

The test with *Daphnia* must be continued for 21 days.

Test guideline

The test must be carried out in accordance with OECD Method 202, Part II.

8.2.6. Effects on algal growth

Aim of the test

The test should procide EC_{50} values for growth and growth rate, NOEC values, and details of observed effects.

Circumstances in which required

Possible effects on algal growth of active substances must always be reported.

For herbicides a test on a second species from a different taxonomic group has to be performed.

Test guideline

The test must be carried out in accordance with Directive 92/69/EEC, Method C3.

8.2.7. Effects on sediment dwelling organisms

Aim of test

The test will measure effects on survival and development (including effects on emergence of adults for *Chironomus*), the relevant EC_{50} values and the NOEC values.

Circumstances in which required

Where environmental fate and behaviour data required in point 7 report that an active substance is likely to partition to and persist in aquatic sediments, expert judgement should be used to decide whether an acute or a chronic sediment toxicity test in required. Such expert judgement should take into account whether effects on sediment dwelling invertebrates are likely by comparing the aquatic invertebrate toxicity EC_{50} data from points 8.2.4 and 8.2.5 with the predicted levels of the active substances in sediment from data in Annex III, point 9.

Test conditions

Before performing these studies the applicant shall seek the agreement of the competent authorities on the type and conditions of the study to be performed.

8.2.8. Aquatic plants

A test on aquatic plants has to be performed for herbicides.

Before performing these studies the applicant shall seek the agreement of the competent authorities on the type and conditions of the study to be performed.

8.3. **Effect on arthropods**

8.3.1. Bees

8.3.1.1. Acute toxicity

Aim of the test

The test should provide the acute oral and contact LD_{50} value of the active substance.

Circumstances in which required

Potential impact on bees must be investigated, except where preparations containing the active substance are for exclusive use in situations where bees are not likely to be exposed such as:

— food storage in enclosed spaces,

— non-systemic seed dressings,

— non-systemic preparations for application to soil,

— non-systemic dipping treatments for transplanted crops and bulbs,

— wound sealing and healing treatments,

— rodenticidal baits,

— use in glasshouses without pollinators.

Test guideline

The test must be carried out in accordance with EPPO Guideline 170.

8.3.1.2. Bee brood feeding test

Aim of the test

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

Circumstances in which required

The test must be carried out when the active substance may act as an insect growth regulator unless it can be justified that it is not likely that bee brood would be exposed to it.

Test guideline

The test must be carried out in accordance with ICPBR Method (e.g. P. A. Oomen, A. de Riujter and J. van der Steen. Method for honeybee brood feeding tests with insect growth-regulating insecticides. *EPPO Bulletin*, Volume 22, pp 613 to 616, 1992.)

8.3.2. Other arethropods

Aim of the test

The test should provide sufficient information to evaluate the toxicity (mortality and sublethal effects) of the active substance to selected arthropod species.

Circumstances in which required

Effects on non-target terrestrial arthropods (e.g. predators or parasitoids of harmful organisms) must be investigated. The information obtained for these species can also be used to indicate the potential for toxicity to other non-target species inhabiting the same environment. This information is required for all active substances except where preparations containing the active substance are for exclusive use in situations where non-target arthropods are not exposed such as:

— food storage in enclosed spaces,

— wound sealing and healing treatments,

— rodenticidal baits.

Test conditions

The test must be performed initially in the laboratory on an artificial substrate (i.e. glass plate or quartz sand, as appropriate) unless adverse effects can be clearly predicted from other studies. In these cases, more realistic substrates may be used.

Two sensitive standard species, a parasitoid and predatory mite (e.g. *Aphidius rhopalosiphi* and *Typhlodromus pyri)* should be tested. In addition to these, two additional species must also be tested, which should be relevant to the intended use of the substance. Where possible and if appropriate, they should represent the other two major functional groups, ground dwelling predators and foliage dwelling predators. Where effects are observed with species relevant to the proposed use of the product, further testing may be carried out at the extended laboratory/semi-field level. Selection of the relevant test species should follow the proposals outlined in Setac — Guidance document on regulatory testing procedures for pesticides with non-target arthropods (1). Testing must be conducted at rates equivalent to the highest rate of field application to be recommended.

Test guideline

Where relevant, testing should be done according to appropriate guidelines which satisfy at least the requirements for testing as included in Setac — Guidance document on regulatory testing procedures for pesticides with non-target arthropods.

8.4. Effects on earthworms

8.4.1. Acute toxicity

Aim of the test

The test should provide the LC_{50} value of the active substance to earthworms, where possible the highest concentration causing no mortality and the lowest concentration causing 100 % mortality, and must include observed morphological and behavioural effects.

(1) From the Workshop European Standard Characteristics of beneficials Regulatory Testing (Escort), 28 to 30 March 1994, ISBN 0-95-22535-2-6.

Circumstances in which required

Effects on earthworms must be investigated, where preparations containing the active substance are applied to soil, or can contaminate soil.

Test guideline

The test must be carried out in accordance with Commission Directive 88/302/EEC (1) adapting to technical progress for the ninth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances, Part C, Toxicity for earthworms: Artificial soil test.

8.4.2. Sublethal effects

Aim of the test

The test should provide the NOEC and the effects on growth, reproduction and behaviour.

Circumstances in which required

Where on the basis of the proposed manner of use of preparations containing the active substance or on the basis of its fate and behaviour in soil (DT_{90} > 100 days), continued or repeated exposure of earthworms to the active substance, or to significant quantities of metabolites, degradation or reaction products, can be anticipated expert judgement is required to decide whether a sublethal test can be useful.

Test conditions

The test must be carried out on *Eisenia foetida*.

8.5. **Effects on soil non-target micro-organisms**

Aim of the test

The test should provide sufficient data to evaluate the impact of the active substance on soil microbial activity, in terms of nitrogen transformation and carbon mineralization.

Circumstances in which required

The test must be carried out where preparations containing the active substance are applied to soil or can contaminate soil under practical conditions of use. In the case of active substances intended for use in preparations for soil sterilization, the studies must be designed to measure rates of recovery following treatment.

Test conditions

Soils used must be freshly sampled agricultural soils. The sites from which soil is taken must not have been treated during the previous two years with any substance that could substantially alter the diversity and levels of microbial populations present, other than in a transitory manner.

Test guideline

Setac — Procedures for assessing the environmental fate and ecotoxicity of pesticides.

8.6. **Effects on other non-target organisms (flora and fauna) believed to be at risk**

A summary of available data from preliminary tests used to assess the biological activity and dose range finding, whether positive or negative, which may provide information with respect to possible impact on other non-target species, both flora and fauna, must be provided, together with a critical assessment as to its relevance to potential impact on non-target species.

8.7. **Effects on biological methods for sewage treatment**

Effects on biological methods for sewage treatment must be reported where the use of plant protection products containing the active substance can give rise to adverse effects on sewage treatment plants.'

---

(1) OJ No L 133, 30. 5. 1988, p. 1.

ANNEX II

'10. **ECOTOXICOLOGICAL STUDIES**

Introduction

(i) The information provided, taken together with that for the active substance(s), must be sufficient to permit an assessment of the impact on non-target species (flora and fauna), of the plant protection product, when used as proposed. Impact can result from single, prolonged or repeated exposure, and can be reversible, or irreversible.

(ii) In particular, the information provided for the plant protection product, together with other relevant information, and that provided for the active substance, should be sufficient to:

— specify the hazard symbols, the indications of danger, and relevant risk and safety phrases for the protection of the environment, to be mentioned on packaging (containers),

— permit an evaluation of the short- and long-term risks for non-target species — populations, communities, and processes as appropriate,

— permit an evaluation of whether special precautions are necessary for the protection of non-target species.

(iii) There is a need to report all potentially adverse effects found during routine ecotoxicological investigations and to undertake and report such additional studies which may be necessary to investigate the mechanisms involved and assess the significance of these effects.

(iv) In general, much of the data relating to impact on non-target species, required for authorization of plant protection products, will have been submitted and evaluated for the inclusion of the active substance(s) in Annex I. The information on fate and behaviour in the environment, generated and submitted in accordance with points 9.1 to 9.3, and on residue levels in plants generated and submitted in accordance with point 8 is central to the assessment of impact on non-target species, in that it provides information on the nature and extent of potential or actual exposure. The final PEC estimations are to be adapted according to the different groups of organisms taking in particular into consideration the biology of the most sensitive species.

The toxicological studies and information submitted in accordance with point 7.1 provide essential information as to toxicity to vertebrate species.

(v) Where relevant, tests should be designed and data analysed using appropriate statistical methods. Full details of the statistical analysis should be reported (e.g. all point estimates should be given with confidence intervals, exact p-values should be given rather than stating significant/non significant).

(vi) Whenever a study implies the use of different doses, the relationship between dose and adverse effect must be reported.

(vii) Where exposure data are necessary to decide whether a study has to be performed, the data obtained in accordance with the provisions of Annex III, point 9 should be used.

For the estimation of exposure of organisms all relevant information on the plant protection product and on the active substance must be taken into account. A useful approach for these estimations is provided in the EPPO/Council of Europe schemes for environmental risk assessment (1). Where relevant the parameters provided for in this section should be used. Where it appears from available data that the plant protection product is more toxic as the active substance, the toxicity data of the plant protection product have to be used for the calculation of relevant toxicity/exposure ratios.

(viii) In the context of the influence that impurities can have on ecotoxicological behaviour, it is essential that for each study submitted, a detailed description (specification) of the material used as provided for under point 1.4, be provided.

(ix) In order to facilitate the assessment of the significance of test results obtained the same strain of each relevant species should where possible be used in the various toxicity tests specified.

(1) OEPP/EPPO (1993). Decision-making schemes for the environmental risk assessment of plant protection products. *Bulletin OEPP/EPPO Bulletin 23*, 1-154 and *Bulletin 24*, 1-87.

10.1. **Effects on birds**

Possible effects on birds must be investigated except where the possibility that birds will be exposed, directly or indirectly, can be ruled out such as for use in enclosed spaces or wound healing treatments.

The acute toxicity/exposure ratio (TER_a), the short term dietary toxicity/exposure ratio (TER_{st}) and the long term dietary toxicity/exposure ratio (TER_{lt}) must be reported, where:

TER_a = LD_{50} (mg a.s./kg body weight) / ETE (mg a.s./kg body weight)
TER_{st} = LC_{50} (mg a.s./kg food) / ETE (mg a.s./kg food)
TER_{lt} = NOEC (mg a.s./kg food) / ETE (mg a.s./kg food)

where ETE = estimated theoretical exposure.

In the case of pellets, granules or treated seeds the amount of a.s. in each pellet, granule or seed must be reported as well as the proportion of the LD_{50} for the a.s. in 100 particles and per gram of particles. The size and shape of pellets or granules must be reported.

In the case of baits the concentration of a.s. in the bait (mg/kg) must be reported.

10.1.1. Acute oral toxicity

Aim of the test

The test should provide, where possible, LD_{50} values, the lethal threshold dose, time courses of response and recovery, the NOEL, and must include relevant gross pathological findings.

Circumstances in which required

The acute oral toxicity of preparations must be reported, where TER_a or TER_{st} for the active substance(s) in birds are between 10 and 100 or where results from mammal testing give evidence of a significantly higher toxicity of the prepration compared to the active substance unless it can be justified that it is not likely that birds are exposed to the plant protection product itself.

Test conditions

The study must be conducted on the most sensitive species identified in the studies provided for in Annex II, point 8.1.1 or 8.1.2.

10.1.2. Supervised cage or field trials

Aim of the test

The test will provide sufficient data to evaluate the nature and the extent of the risk in practical conditions of use.

Circumstances in which required

Where the TER_a and TER_{st} are > 100 and when there is no evidence of risk from any further study on the active substance (e.g. reproduction study) no further testing is required. In the other cases, expert judgement is necessary to decide whether there is a need to carry out further studies. This expert judgement will take into account, where relevant, foraging behaviour, repellency, alternative food, actual residue content in the food, persistence of the compound in the vegetation, degradation of the formulated product or treated produce, the amount of predation of the food, acceptance of bait, granules or treated seed and the possibility for bioconcentration.

Where TER_a and $TER_{st} \leq 10$ or $TER_{lt} \leq 5$, cage or field trials must be conducted and reported unless a final assessment is possible on the basis of studies according to point 10.1.3.

Test conditions

Before performing these studies the applicant should seek the agreement of the competent authorities on the type and conditions of the study to be performed.

10.1.3. Acceptance of bait, granules or treated seeds by birds

Aim of the test

The test will provide sufficient data to evaluate the possibility of consumption of the protection product or plant products treated with it.

Circumstances in which required

In the case of seed dressings, pellets, baits and preparations which are granules and where $TER_a \leq 10$, acceptability (palatability) tests must be conducted.

10.1.4. Effects of secondary poisoning

Expert judgment is required to decide whether the effects of secondary poisoning should be investigated.

10.2. **Effects on aquatic organisms**

Possible effects on aquatic species must be investigated except where the possibility that aquatic species will be exposed can be ruled out.

TER_a and TER_{lt} must be reported, where:

TER_a = acute LC_{50} (mg a.s./l)/realistic worst case PEC_{sw} (initial or short-term, in mg a.s./l)

TER_{lt} = chronic NOEC (mg a.s./l)/long term PEC_{sw} (mg a.s./l)

10.2.1. Acute toxicity to fish, aquatic invertebrates or effects on algal growth

Circumstances in which required

In principle tests should be carried out on one species from each of the three groups of aquatic organisms as referred to in Annex II, point 8.2 (fish, aquatic invertebrates and algae) in case the plant protection product itself can contaminate water. However where the available information permits to conclude that one of these groups is clearly more sensitive, tests on only the most sensitive species of the relevant group have to be performed.

The test must be performed where:

— the acute toxicity of the plant protection product can not be predicted on the basis of the data for the active substance which is especially the case if the formulation contains two or more active substances or formulants such as solvents, emulgators, surfactants, dispersants, fertilizers which are able to increase the toxicity in comparison with the active substance, or

— the intended use includes direct application on water

unless suitable studies referred to under point 10.2.4 are available.

Test conditions and test guidelines

The relevant provisions as under the corresponding paragraphs of Annex II, points 8.2.1, 8.2.4 and 8.2.6 apply.

10.2.2. Microcosm or mesocom study

Aim of the test

The tests must provide sufficient data to evaluate the essential impact on aquatic organisms under field conditions.

Circumstances in which required

Where $TER_a \leq 100$ or where $TER_{lt} \leq 10$, expert judgment must be used to decide whether a microcosm or mesocom study is appropriate. This judgment will take into account the results of any additional data over and above those required by the provisions of Annex II, point 8.2 and of point 10.2.1.

Test conditions

Before performing these studies the applicant shall seek the agreement of the competent authorities on the specific aims of the study to be performed and consequently on the type and conditions of the study to be performed.

The study should include at least the highest likely exposure rate, whether from direct applicaiton, drift, drainage or run-off. The duration of the study must be sufficient to permit evaluation of all effects.

Test guideline

Appropriate guidelines are included in:

Setac — Guidance document on testing procedures for pesticides in freshwater mesocosms/Workshop Huntingdon, 3 and 4 July 1991

or

Freshwater field tests for hazard assessment of chemicals — European Workshop on Freshwater Field Tests (EWOFFT).

10.2.3. Residue data in fish

Aim of the test

The test will provide sufficient data to evaluate the potential for occurrence of residues in fish.

Circumstances in which required

In general data are available from bioconcentration studies in fish.

Where bioconcentration has been observed in the study performed in accordance with Annex II, point 8.2.3 expert judgement is required to decide whether a long-term microcosm or mesocosm study has to be carried out in order to establish the maximum residues likely to be encountered.

Test guideline

Setac — Guidance document on testing procedures for pesticides in freshwater mesocosms/Workshop Huntingdon, 3 and 4 July 1991.

10.2.4. Additional studies

The studies referred to in Annex II, points 8.2.2 and 8.2.5 may be required for particular plant protection products where it is not possible to extrapolate from data obtained in the corresponding studies on the active substance.

10.3. **Effects on terrestrial vertebrates other than birds**

Possible effects on wild vertebrate species must be investigated except where it can be justified that it is not likely that terrestrial vertebrates other than birds are exposed, directly or indirectly. TER_a, TER_{st} and TER_{lt} must be reported, where:

TER_a = LD_{50} (mg a.s./kg body weight) / ETE (mg a.s./kg body weight)

TER_{st} = subchronic NOEL (mg a.s./kg food) / ETE (mg a.s./kg food)

TER_{lt} = chronic NOEL (mg a.s./kg food) / ETE (mg a.s./kg food)

where ETE = estimated theoretical exposure.

In principle the evaluation sequence for the assessment of risks to such species is similar to that for birds. In practice it is not often necessary to perform further testing as the studies conducted in accordance with the requirements of Annex II, point 5 and Annex III, point 7 would provide the required information.

Aim of the test

The test will provide sufficient information to evaluate the nature and the extent of risks for terrestrial vertebrates other than birds in practical conditions of use.

Circumstances in which required

Where TER_a and $TER_{st} > 100$ and where there is no evidence of risk from any further study no further testing is required. In the other cases, expert judgment is necessary to decide whether there is a need to carry out further studies. This expert judgment will take into account, where relevant, foraging behaviour, repellency, alternative food, actual residue content in the food, persistence of the compound in the vegetation, degradation of the formulated product or treated produce, the amount of predation of the food, acceptance of bait, granules or treated seed and the possibility for bioconcentration.

Where TER_a and $TER_{st} \leq 10$ or $TER_{lt} \leq 5$ cage or field trials or other appropriate studies must be reported.

Test conditions

Before performing these studies the applicant shall seek the agreement of the competent authorities on the type and conditions of the study to be performed and whether the effects of secondary poisoning should be investigated.

10.4. **Effects on bees**

The possible effects on bees must be investigated except where the product is for exclusive use in situations where bees are not likely to be exposed such as:

— food storage in enclosed spaces,
— non-systemic seed dressings,
— non-systemic preparations for application to soil,
— non-systemic dipping treatments for transplanted crops and bulbs,
— wound sealing and healing treatments,
— rodenticidal baits,
— use in glasshouses without pollinators.

The hazard quotients for oral and contact exposure (Q_{HO} and Q_{HC}), must be reported:

Q_{HO} = dose/oral LD_{50} (µg a.s. per bee)

Q_{HC} = dose/contact LD_{50} (µg a.s. per bee)

where

dose = the maximum application rate, for which authorization is sought, in g of active substance per hectare.

10.4.1. Acute oral and contact toxicity

Aim of the test

The test should provide the LD_{50} values (by oral and contact exposure).

Circumstances in which required

Testing is required if:

— the product contains more than one active substance;

— the toxicity of a new formulation cannot be reliably predicted to be either the same or lower than a formulation tested according to the provisions of Annex II, point 8.3.1.1 or of this point.

Test guideline

The test must be carried out according to EPPO Guideline 170.

10.4.2. Residue test

Aim of the test

The test should provide sufficient information to evaluate possible risks to foraging bees from residual traces of plant protection products remaining on crops.

Circumstances in which required

Where $Q_{HC} \geq 50$, expert judgment is required to decide whether the effect of residues must be determined unless there is evidence that there are no significant residual traces remaining on crops which could affect foraging bees or unless sufficient information is available from cage, tunnel or field tests.

Test conditions

The median lethal time (LT_{50}) (in hours) following 24-hour exposure to residues on leaves aged during eight hours must be determined, and reported. Where LT_{50} is more than eight hours, no further testing is required.

10.4.3. Cage tests

Aim of the test

The test should provide sufficient information to evaluate possible risks from the plant protection product for bee survival and behaviour.

Circumstances in which required

Where Q_{HO} and Q_{HC} are < 50, further testing is not required except if significant effects are observed in the bee brood feeding test or if there are indications for indirect effects such as delayed action or modification of bee behaviour; in those cases cage and/or field tests shall be carried out.

Where Q_{HO} and Q_{HC} are > 50, cage and/or field testing is required.

Where field testing is conducted and reported in accordance with point 10.4.4, it is not necessary to conduct cage tests. However, cage tests where conducted, must be reported.

Test conditions

The test should be carried out using healthy bees. If bees have been treated, e.g. with a varroacide, it is necessary to wait for four weeks before using the colony.

Test guideline

The tests must be conducted in accordance with EPPO Guideline 170.

10.4.4. Field tests

Aim of the test

The test should provide sufficient information to evaluate possible risks from the plant protection product on bee behaviour, colony survival and development.

Circumstances in which required

Field tests must be conducted where on the basis of expert judgement, taking into account the proposed manner of use and the fate and behaviour of the active substance, significant effects are observed in cage testing.

Test conditions

The test should be carried out using healthy honeybee colonies of similar natural strength. If bees have been treated, e.g. with a varroacide, it is necessary to wait for four weeks before using the colony. The tests shall be conducted under conditions reasonably representative of the proposed use.

Special effects (larval toxicity, long residual effect, disorienting effects on bees) identified by the field tests may require further investigation using specific methods.

Test guideline

The tests must be conducted in accordance with EPPO Guideline 170.

10.4.5. Tunnel tests

Aim of the test

The test should provide sufficient information to evaluate the impact on bees resulting from feeding on contaminated honey dew or flowers.

Circumstances in which required

Where it is not possible to investigate certain effects in cage or field trials, a tunnel test should be carried out, e.g. in the case of plant protection products intended for control of aphids and other sucking insects.

Test conditions

The test should be carried out using healthy bees. If bees have been treated, e.g. with a varroacide, it is necessary to wait for four weeks before using the colony.

Test guideline

The test must be carried out in accordance with EPPO Guideline 170.

10.5. **Effects on arthropods other than bees**

The effects of plant protection products on non-target terrestrial arthropods (e.g. predators or parasitoids of harmful organisms) must be investigated. The information obtained for these species can also be used to indicate the potential for toxicity to non-target species inhabiting the same environment.

10.5.1. Laboratory, extended laboratory and semi-field tests

Aim of the test

The test should provide sufficient information to evaluate the toxicity of the plant protection product for selected arthropod species that are relevant to the intended use of the product.

Circumstances in which required

Testing is not required where severe toxicity (> 99 % effect on the organisms compared to control) can be predicted from relevant available data or where the plant protection product is for exclusive use in situations where non-target arthropods are not exposed such as:

— food storage in enclosed spaces,

— wound sealing and healing treatments,

— rodenticidal baits.

Testing is required when significant effects on the organisms in comparison with the control are reported in the laboratory tests at the maximum recommended dose, conducted in accordance with the requirements of Annex II, point 8.3.2. Effects on a particular test species are considered to be significant when they exceed the threshold values as defined in the EPPO schemes for the environmental risk assessment unless species-specific threshold values are defined in the respective test guidelines.

Testing is also required if:

— the product contains more than one active substance,

— the toxicity of a new formulation cannot be reliably predicted to be either the same or lower than a formulation tested according to the provisions of Annex II, point 8.3.2 or of this point,

— on the basis of the proposed manner of use or on the basis of the fate and behaviour continued or repeated exposure can be anticipated,

— there is a significant change in the proposed use, e.g. from arable crops to orchards, and species relevant to the new use have not previously been tested,

— there is an increase in the recommended application rate, above that previously tested under Annex II.

Test conditions

Where significant effects were observed in the studies performed in accordance with the requirements of Annex II, point 8.3.2, or in the case of change of use such as arable crops to orchards, the toxicity of two additional relevant species must be investigated and reported. These must be different to the relevant species already tested under Annex II, point 8.3.2.

For a new mixture or formulation, the toxicity should initially be assessed using the two most sensitive species as identified in studies already performed for which the threshold values were exceeded but effects still remain below 99 %. This will enable a comparison to be made; if it significantly more toxic two species relevant to its proposed use must be tested.

Testing must be conducted at a rate equivalent to the maximum rate of application for which authorization is sought. A sequential testing approach should be adopted, i.e. laboratory, and if necessary extended laboratory and/or semi-field.

Where there will be more than one application per season, the product should be applied at twice the recommended application rate unless this information is already available from studies performed in accordance with Annex II, point 8.3.2.

Where on the basis of the proposed manner of use or on the basis of the fate and behaviour continued or repeated exposure can be anticipated (such as the product is to be applied more than three times per season with a re-application of 14 days or less), expert judgment is required to examine whether further testing is required, beyond initial laboratory testing, which will reflect the proposed use pattern. These tests may be performed in the laboratory or under semi-field conditions. When the test is done in the laboratory a realistic substrate such as plant material or a natural soil should be used. However it may be more appropriate to carry out field tests.

Test guideline

Where relevant testing should be done according to appropriate guidelines which satisfy as least the requirements for testing as included in Setac - Guidance document on regulatory testing procedures for pesticides with non-target arthropods.

10.5.2. Field tests

Aim of the test

The tests should provide sufficient information to evaluate the risk of the plant protection product for arthropods under field conditions.

Circumstances in which required

Where significant effects are seen following laboratory and semi-field exposure, or where on the basis of the proposed manner of use or on the basis of the fate and behaviour continued or repeated exposure can be anticipated expert judgment is required to examine whether more extensive testing is necessary to permit an accurate risk assessment.

Test conditions

The tests must be conducted under representative agricultural conditions and in accordance with the proposed recommendations for use, resulting in a realistic worst case study.

A toxic standard should be included in all tests.

Test guideline

Where relevant testing should be done according to appropriate guidelines which satisfy at least the requirements for testing as included in Setac — Guidance document on regulatory testing procedures for pesticides with non-target arthropods.

10.6. **Effects on earthworms and other soil non-target macro-organisms, believed to be at risk**

10.6.1. Effects on earthworms

The possible impact on earthworms must be reported except where it can be justified that it is not likely that earthworms are exposed, directly or indirectly.

TER_a and TER_{lt} must be reported where:

TER_a = LC_{50} (mg a.s./kg)/realistic worst case PEC_s (initial or short-term, in mg a.s./kg)

TER_{lt} = NOEC (mg a.s./kg)/long term PEC_s (mg a.s./kg).

10.6.1.1. Acute toxicity tests

Aim of the test

The test should provide the LC_{50}, where possible the highest concentration causing no mortality and the lowest concentration causing 100 % mortality and must include observed morphological and behavioural effects.

Circumstances in which required

These studies are only required where

— the product contains more than one active substance,

— the toxicity of a new formulation cannot be reliably predicted from the formulation tested according to the provisions of Annex II, point 8.4 or of this point.

Test guideline

The tests must be conducted in accordance to OECD Method 207.

10.6.1.2. Tests for sublethal effects

Aim of the test

The test should provide the NOEC and the effects on growth, reproduction and behaviour.

Circumstances in which required

These studies are only required where

— the product contains more than one active substance,

— the toxicity of a new formulation cannot be reliably predicted from the formulation tested according to the provisions of Annex II, point 8.4 or of this point,

— there is an increase in the recommended application rate, above that previously tested.

Test conditions

The same provisions as under the corresponding paragraphs of Annex II, point 8.4.2 apply.

10.6.1.3. Field studies

Aim of the test

The test should provide sufficient data to evaluate the effects on earthworms in field conditions.

Circumstances in which required

Where $TER_{lt} < 5$ a field study to determine effects under practical field conditions must be conducted and reported.

Expert judgment is required to decide whether residue contents of earthworms should be investigated.

Test conditions

Fields selected shall have a reasonable earthworm population.

The test must be carried out at the maximum proposed application rate. A toxic reference product must be included in the test.

10.6.2. Effects on other soil non-target macro-organisms

Aim of the test

The test should provide sufficient data to evaluate the impact of the plant protection product on macro-organisms that contribute to the breakdown of dead plant and animal organic matter.

Circumstances in which required

Testing is not required where in accordance with Annex III, point 9.1, it is evident that DT_{90} values are less than 100 days, or the nature and manner of use of the plant protection product are such that exposure does not occur or when data from studies on the active substance performed in accordance with the provisions of Annex II, points 8.3.2, 8.4 and 8.5 indicate that there is no risk for soil macrofauna, earthworms or soil microflora.

Impact on organic matter breakdown must be investigated and reported, where the DT_{90f} values determined in field dissipation studies (point 9.1) are > 365 days.

10.7. **Effects on soil non-target micro-organisms**

10.7.1. Laboratory testing

Aim of the test

The test should provide sufficient data to evaluate the impact of the plant protection product on soil microbial activity in terms of nitrogen transformation and carbon mineralization.

Circumstances in which required

Where the DT_{90f} values determined in field dissipation studies (point 9.1) are > 100 days, impact on soil non-target micro-organisms must be investigated through laboratory testing. Testing is, however, not required if in the studies performed in accordance with the provisions of Annex II, point 8.5 deviations from control values in terms of metabolic activity of the microbial biomass after 100 days is < 25 %, and such data are relevant to the uses, nature, and properties of the particular preparation to be authorized.

Test guideline

Setac — Procedures for assessing the environmental fate and ecotoxicity of pesticides.

10.7.2. Additional testing

Aim of the test

The test should provide sufficient data to evaluate the impact of the plant protection product under field conditions on microbial activitiy.

Circumstances in which required

Where at the end of 100 days, measured activity deviates by more than 25 % from the control, in the laboratory testing further testing in the laboratory, under glass and/or in the field may be necessary.

10.8. **Available data from biological primary screening in summary form**

A summary of available data from preliminary tests used to assess the biological activity and dose range finding whether positive or negative, which provides information with respect to possible impact on non/target species, both flora and fauna, must be provided, together with a critical assessment as to its relevance to potential impact on non-target species.

11. **SUMMARY AND EVALUATION OF POINTS 9 AND 10**

A summary and evaluation of all data presented in points 9 and 10 should be carried out according to the guidance given by the competent authorities of the Member States concerning the format of such summaries and evaluations. It should include a detailed and critical assessment of those data in the context of relevant evaluative and decision making criteria and guidelines, with particular reference to the risks for the environment and non-target species that may or do arise, and the extent, quality and reliability of the data base. In particular the following issues should be addressed:

— predicting distribution and fate in the environment, and the time courses involved,

— identifying non-target species and populations at risk, and predicting the extent of potential exposure,

— evaluation as to the short- and long-term risks for non-target species — populations, communities, and processes — as appropriate,

— evaluation as to the risk of fish kills, and fatalities in large vertebrates, or terrestrial predators, regardless of effects at population or community level, and

— identification of precautions necessary to avoid or minimize contamination of the environment, and for the protection of non-target species.'

Index of authors

Index of main keywords

List of Registered Persons

ALWAN, Moayed
Iraqi Bee Keepers Association
Al-Kerrada 909/57/13 - Iraq
Tel : (964+) 171 77 577; Fax : (964+) 171 90 456

AUDEMARD, Henri
Club Protection Raisonné
9, allée Innocent VI
84000 Avignon - France
Tel : (33+) (0)4 90 87 60 26; Fax : (33+) (0)4 90 87 60 26
henri.audemard@wanadoo.fr

BARCIET, Francis
Rhône Poulenc Agro France
55, rue René Cassin
69337 LYON Cedex 09 - France
Tel : (33+) (0)4 72 85 41 71; Fax : (33+) (0)4 72 85 41 58
francis.barciet@ladargoire.rhone-poulenc.com

BELZUNCES, Luc P.
Laboratoire de Toxicologie Environnementale
Inra Unité de Zoologie
Site Agroparc
84914 Avignon Cedex 9 – France
Tel : (33+) (0)4.32.72.26.04; Fax : (33+) (0)4.32.72.26.02
belzunce@avignon.inra.fr

BERGE, Jean-Baptiste
INRA
Unité Santé Végétale et Environnement
123, bd F. Meilland – BP 2078
06606 Antibes Cedex - France
Tel : (33+) (0)4 93 67 89 32; Fax : (33+) (0)4 93 67 89 55
berge@antibes.inra.fr

BERNARD, Thierry
ISK Biosciences
5, chemin du Jubin – BP 32
69570 Dardilly - France
ISK-tbe@isk-biosciences.fr

BONMATIN, Jean-Marc
CNRS
Centre de Biophysique Moléculaire
Avenue Charles Sadron
45100 Orléans Cedex 2 - France
Tel : (33+) (0)2 38 25 55 87; Fax : (33+) (0)2 38 63 15 17
bonmatin@cnrs-orléans.fr

BORTOLOTTI, Laura
University of Ferrara
Via Borsari 46
44100 Ferrara - Italy
Tel : (39+) (0)5327291748; Fax : (39+) (0)532249761
laurabortolotti@hotmail.com

BOUTEILLE, Agnès
Cyanamid Agro
14, chemin Professeur Depéret
69160 Tassin La Demi Lune - France
Tel : (33+) (0)4 72 32 45 45; Fax : (33+) (0)4 78 34 28 86

BRASSE, Dietrich
Biologische Bundesanstalt
Messeweg 11/12
D-38104 Braunschweig - Germany
Tel : (49+) 0531/299-4525; Fax : (49+) 0531/299-3008

BROBYN, Patricia
Pesticides Safety Directorate
King's Pool, 3
Peasholne Green
York YO1 7PX - U.K.
Tel : (44+) (0)1904 455918; Fax : (44+) (0)1904 455711
p.j.brobyn@psd.maff.gov.uk

BRUNEAU, Étienne
CARI asbl
Place Croix du Sud n°4
1348 Lauvain La Neuve - Belgium
Tel : (32+) (0)10/47 34 16; Fax : (32+) (0)10/47 34 94
bruneau@ecd.uk.ac.be

CANDOLFI, Marco
Novartis Crop Protection AG
WRO-1058.3.64
CH-4002 Basel - Switzerland
Tel : (41+) 61 697 60 50; Fax : (41+) 61 697 59 22
marco.candolfi@cp.novartis.com

CHALINE, Nicolas
Inra Lab. Neurobiologie Comparée des Invertébrés
La Guyonnerie – BP 23
91440 Bures Sur Yvette - France
Tel : (33+) (0)1 69 29 87 53; Fax : (33+) (0)1 69 04 50 54
chaline@jouy.inra.fr

CHARRIÈRE, Jean-Daniel
FAM, Section apiculture Liebefeld
3003 Bern - Switzerland
Tel : (41+) 31 323 82 02; Fax : (41+) 31 323 26 02
Jean-Daniel.Charrière@fam.admin.ch

CLUZEAU, Sophie
ACTA
149, rue de Bercy
75595 Paris Cedex 12 - France
Tel : (33+) (0)1 40 04 50 42; Fax : (33+) (0)1 40 04 50 11
cluzeau@acta.asso.fr

COLIN, Marc-Édouard
Inra Zoologie
Site Agroparc
84914 Avignon Cedex 9 - France
Tel : (33+) (0)4 32 72 26 03; Fax : (33+) (0)4 32 72 26 02
colin@avignon.inra.fr

COPPEN, Donald
Cyanamid Agro SA
Chaussée de Tirlemont, 105
B-5030 Gembloux - Belgium
Tel : (32+) 32 10 494 832; Fax : (32+) 32 10 494 833
coppenD@AHP.be

COURANT, Christiane
Edition d'Apidologie
Inra Unité de Zoologie
Site Agroparc
84914 Avignon Cedex 9 - France
Tel : (33+) (0)4.32.72.26.19; Fax : (33+) (0)4.32.72.26.02
courant@avignon.inra.fr

CURÉ, Gaëlle
Bayer SA
49-51, quai de Dion Bouton Cedex
92815 Puteaux - France
Tel : (33+) (0)1 49 06 57 21; Fax : (33+) (0)49 06 51 47
gaelle.cure.gc@bayer-ag.de

DECOURTYE, Axel
Inra Lab. Neurobiologie Comparée des Invertébrés
La Guyonnerie – BP 23
91440 Bures Sur Yvette - France
Tel : (33+) (0)1 69 29 87 66; Fax : (33+) (0)1 69 07 50 54
decourty@jouy.inra.fr

DRESCHER, W.
Lindenweg, 7
53125 Bonn - Germany
Tel : (49+) (0)228 251717; Fax : (49+) (0)228 9101903

DUNIER-THOMANN, Muriel
INRA - SSM
10 Route de Saint Cyr
78026 Versailles Cedex - France
Tel : (33+) (0)1.30.83.31.66; Fax : (33+) (0)1.30.83.31.19
mdunier@versailles.inra.fr

FLÉCHÉ, Cécile
AFSSA – Sophia Antipolis
105, route des Chappes – BP 111
06902 Sophia Antipolis - France
Tel : (33+) (0)4 92 94 37 00; Fax : (33+) (0)4 92 94 37 01
c.fleche@sophia.afssa.fr

GIFFARD, Hervé
Testapi
Sarré
49350 Gennes – France
Tel : (33+) (0)2.41.52.68.94; Fax : (33+) (0)2.41.52.62.08
testapi@compuserve.com

GODET, Fabrice
Zeneca-Sopra
18, rue Grange Dame Rose, BP 141
78148 Vélizy Cedex – France
Tel : (33+) (0)1 34 63 50 00; Fax : (33+) (0)1 34 63 51 86
Fabrice.Godet@ageurope.zeneca.com

GOMOND, Stéphane
ACTA
La Saulsaie
01120 Montluel - France
Tel : (33+) (0)4 78 06 60 82; Fax : (33+) (0)4 78 06 62 76

GOUZOU, Laurent
Rhône Poulenc Agro France
55, avenue René Cassin
69337 Lyon Cedex 09 - France
Tel : (33+) (0)4 72 85 43 01; Fax : (33+) (0)4 72 85 43 87
laurent.gouzou@rhone-poulenc.com

GUEZ, David
Laboratoire de Toxicologie Environnementale
Inra Unité de Zoologie
Site Agroparc
84914 Avignon Cedex 9 – France
Tel : (33+) (0)4.32 72 26 29; Fax : (33+) (0)4.32 72 26 02
guez@avignon.inra.fr

HARRISON, Libby
Zeneca Agrochemicals
Jealott's Hill Research Station
Bracknell, Berkshire, RG40 6ET - UK
Tel : (44+) 1344 414179; Fax : (44+) 1344 413688
libby.harrison@aguk.zeneca.com

HELLER, Jean-Jacques
Elf Atochem Agri SA
1, rue des Frères Lumière
78370 Plaisir - France
Tel : (33+) (0)1 30 81 73 55; Fax : (33+) (0)1 30 81 72 51
Jheller@agri.ato.com

HERNO, Valérie
Bayer SA
49-51, quai de Dion Bouton
92815 Puteaux - France
Tel : (33+) (0)1 49 06 54 88; Fax : (33+) (0)1 49 06 51 47
valérie.herno.vh@bayer-ag.de

ILLIES, Ingrid
Landwirstschaftskammer Westfalen-Lippe
Fachbereich Bienenkunde
Postfach 5980
DE-48135 Münster - Germany
Tel : (49+) (0)251 2376 663; Fax : (49+) (0)251 2376 551
illiesi@uni-muenster.de

KAPETANAKIS, Evangelos
School of Agriculture Technological Education Institute, PO Box 140
Heraklion Crete GR 71110
Tel : (30+) 81 31 92 82; Fax : (30+) 81 31 92 82
ekapet@lyttos.admin.teiher.gr

KOVACS, Géza
Écotoxikological Laboratory
Facankert – Hungary
H-7136, Facankert - Hungary
Tel : (36+) 74 440145; Fax : (36+) 74 440148
ecotox@terrasoft.hu

KÜNAST, Christoph
BASF Aktiengesellschaft
BASF Agricultural Center
P.O. Box 120
64114 Limburgerhof - Germany
Tel : (49+) (0)621 6027576; Fax : (49+) (0)621 6027214
christoph.kuenst@basf-ag.de

LEBLANC, Jean
FMC France SA
614, rue Benoît Mulsant
69400 Villefranche/Saone - France
Tel : (33+) (0)4 74 68 35 46; Fax : (33+) (0)4 74 60 04 47
jean_leblanc@fmc.com

LECONTE, Florence
Calliope SA
Route d'Artix – BP 80
64150 Noguères - France
Tel : (33+) (0)5 59 60 92 25; Fax : (33+) (0)5 59 60 92 19
cal20@calliope-sa.com

LEFEBVRE, Bruno
Novartis Crop Protection AG
Environmental Safety Assessment
R1058-P58
4002 Basel - Switzerland
Tel : (41+) 61 697 71 51; Fax : (41+) 61 697 40 44
bruno.lefebvre@cp.novartis.com

LEWIS, Gavin
JSC International LTD
Osborne House, 20 Victoria Avenue
Harrogate, North Yorkshire, HGI 5QY - UK
Tel : (44+) 1423 520245; Fax : (44+) 1423 520297
Gavin.Lewis@compuserve.com

LEYMANN, Boris
Landwirstschaftskammer
Westfalen-Lippe
Fachbereich Bienenkunde
Postfach 5980
DE-48147 Münster - Germany
Tel : (49+) (0)251 2376 663; Fax : (49+) (0)251 2376 551
Boris.leymann@biologie.univ-bielefeld.de

LUNDEN, Jeffrey
Washington State University
24106 North Bunn Road
Prosser Washington 99350 - USA
Tel : 509 786 2226; Fax : 509 786 9370
mayerd@wsu.edu

MARTEL, Anne-Claire
AFSSA Sophia Antipolis
105, route des Chappes – BP 111
06902 Sophia Antipolis Cede - France
Tel : (33+) (0)4 92 94 37 00; Fax : (33+) (0)4 92 94 37 01
ac.martel@sophia.cneva.fr

MATHIEU, Laetitia
AFSSA Sophia Antipolis
Lab. Pathologie des Abeilles
105, route des Chappes – BP 111
06902 Sophia Antipolis Cedex - France
Tel : (33+) (0)4 92 94 37 13; Fax : (33+) (0)4 92 94 37 01
l.mathieu@sophia.afssa.fr

MAYER, Dan
Washington State Univversity
24106 North Bunn Road
Prosser WA 99350 - USA
Tel : (1+) 509 786 2226; Fax : (1+) 509 786 9370
mayerd@wsv.edv

MÜHLEN, Werner
Landwirstschaftskammer
Westfalen-Lippe
Fachbereich Bienenkunde
Postfach 5980
DE-48147 Münster - Germany
Tel : (49+) (0)251 2376 663; Fax : (49+) (0)251 2376 551
0251-23760-0006@t-online.de

NAVARRO, Dominique
Cyanamid Agro
Base de recherche
Rue de la Fosse Corbin
27120 Le Plessis Hebert - France
Tel : (33+) (0)2 32 22 34 25; Fax : (33+) (0)2 32 36 93 00

OOMEN, Pieter A.
Plant Protection Service
P.O.Box 9102
6700 HC Wageningen - The Netherlands
Tel : (31+) 317 496868; Fax : (31+) 317 421701
P.A.Oomen@pd.agro.nl

PASCUAL, Juan
Rhône Poulenc Agro
355, rue Dostoievski – BP 153
06903 Sophia Antipolis Cedex - France
Tel : (33+) (0)4 92 94 34 52; Fax : (33+) (0)4 93 65 39 24
juan.PASCUAL@SOPHIA.RHONE-POULENC.com

PELISSIER, Colette
INRA, Unité de Zoologie
Site Agroparc
84914 Avignon Cedex 9 - France
Tel : (33+) (0)4.32.72.26.06; Fax : (33+) (0)4.32.72.26.02
cpeliss@avignon.inra.fr

PRANDIN, Luciana
Istituto Zooprofilattico Sperimentale delle Venezie
Via Romea, 14/a
35020 Legnaro (PD) - Italy
Tel : (39+) (0)49 8084344; Fax : (39+) (0)49 8830572
fmutinelli.izsv@interbusiness.it

PFLUGER, Wolfgang
Bayer AG Agrochemical Division
Landwirtschaftszentrum Monheim
Alfred-Nobel-Strass.
51368 Leverkusen Bayerwerk - Germany
Tel : (49+) 2173 383888 (3880); Fax : (49+) 2173 38491
wolfgang.pflueger.wp@bayer-ag.de

RIOU, Géraldine
Philagro France
2, rue Claude Chappe
69370 Saint Didier Au Mont D'Or - France
Tel : (33+) (0)4 78 64 32 30; Fax : (33+) (0)4 78 64 14 19

ROEDERER, Jeanne
Makhteshim Agan France
Imm. « Le Seine Saint-Germain »
12, boulevard des Iles
92441 Issy Les Moulineaux - France
Tel : (33+) (0)1 41 90 16 96; Fax : (33+) (0)1 46 42 71 17

SCHMIDT, Hans-Werner
BAYER Ag, Plant Protection
51368 Leverkusen - Germany
Tel : (49+) 2173 383060; Fax : (49+) 2173 383215
hans-werner.schmidt.H@bayer-ag.de

SCHMITZER, Stephan
Ibacon
Industrie Strasse, 1
64380 Rossdorf - Germany
Tel : (49+) 6154 695005; Fax : (49+) 6154 800055
stephan.schmitzer@ibacon.com

SCHMUCK, Richard
Bayer AG, Plant Protection, PF-E/OE
51638 Leverkusen - Germany
Tel : (49+) 2173 383132; Fax : (49+) 2173 384981
richard.schmuck.rs@bayer-ag.de

SCHOEN, Leen
Agri Phyto Méditerranée
Chambre d'Agriculture
19 Avenue de Grande Bretagne
66025 Perpignan cedex - France
Tel : (33+) (0)5.68.35.74.00; Fax : (33+) (0)5.68.34.65.44

SCHUR, Andrea
GAB Biotechnologie Gmbh
Eutinger strasse, 24
D-75223 Nierfern-Oeschelbronn - Germany
Tel : (49+) 7233 9627 17; Fax : (49+) 7233 4697
andrea.schur@GAB-Biotech.de

SERVAJEAN, Elisabeth
Société Envirotests
14, rue Paul Bert
64000 Pau - France
Tel : (33+) (0)5 59 84 79 91; Fax : (33+) (0)5 59 84 80 55
Envirotests@wanadoo.fr

STEEN (van der), Joseph J.M.
Research Centre for Insect Pollination and Beekeeping « Ambrosiushoeve »
Ambrosiusweg 1
5081 NV Hilvarenbeek - The Netherlands
Tel : (31+) 135425888; Fax : (31+) 135439155
ambrosiushoeve@wxs.nl

STEVENSON, John H.
CHAIRMAN
ICPBR Bee Protection Group
16 old Rectory Close
Harpenden, Herts AL5 2UD - UK
Tel : (44+) 1582 712316; Fax : (44+) 1582 712316

STRUYE, Marc
Lowland Electronics bvba
Langekoeistalstraat, 2
B-8432 Leffinge - Belgium
Tel : (32+) 59 27 93 92; Fax : (32+) 59 27 97 93
lowland@unicall.be

SUCHAIL, Séverine
Laboratoire de Toxicologie Environnementale
Inra Unité de Zoologie
Site Agroparc
84914 Avignon Cedex 9 – France
Tel : (33+) (0)4 32 72 26 29; Fax : (33+) (0)4 32 72 26 02
suchail@avignon.inra.fr

SUTEAU, Patrick
Rhône Poulenc AGRO
355, rue Dostoievski – BP 153
06903 Sophia Antipolis Cedex - France
Tel : (33+) (0)4 92 94 34 56; Fax : (33+) (0)4 93 65 39 24
patrick.suteau@rhone-poulenc.com

SZEL, Zsuzsanna
ISAR
Department of Bee-Breeding and Bee-Biology
Gödöllo, Isaszegi ùt, POB : 417 - Hungary
Tel : (36+) 28 420 366/124; Fax : (36+) 28 430 184
szel@sunserv.katki.hu

TASEI, Jean-Noël
INRA Zoologie
86600 Lusignan - France
Tel : (33+) (0)5.49.55.60.90; Fax : (33+) (0)5.49.55.60.88
tasei@lusignan.inra.fr

TISSEUR, Michel
ACTA
La Saulsaie
01120 Montluel - France
Tel : (33+) (0)4 78 06 60 82; Fax : (33+) (0)4 78 06 62 76

THOMPSON, Helen
National Bee Unit
Central Science Laboratory
Sand Hutton
York YO4 1LZ - UK
Tel : (44+) 1904 462515; Fax : (44+) 1904 462240
H.Thompson@csl.gov.uk

TORNIER, Ingo
GAB Biotechnologie GMBH
Eutinger – Strasse, 24
D-75223 Niefern-Oeschelbronn - Germany
Tel : (49+) 7233 962712; Fax : (49+) 7233 4697
Ingo.tornier@GAB-BIOTECH.de

TURI, Katalin
ISAR
Department of Bee-Breeding and Bee-Biology
Gödöllo, Isaszegi ùt, POB : 417 - Hungary
Tel : (36+) 28 420 366/124; Fax : (36+) 28 430 184
turi@sunserv.katki.hu

VAISSIERE, Bernard
Laboratoire de Pollinisation Entomophile
Inra Unité de Zoologie
Site Agroparc
84914 Avignon Cedex 9 - France
Tel : (33+) (0)4 32 72 26 37; Fax : (33+) (0)4 32 72 26 02
Vaissier@avignon.inra.fr

VAN WAETERMEULEN, Xavier
AgrEvo France
Les Algorithmes
Imm. Thalès – St Aubin
91197 Gif Sur Yvette Cedex - France
Tel : (33+) (0)1 69 85 63 40; Fax : (33+) (0)1 69 85 63 05
xavier.vanwaetermeulen@agrevo.com

VERGNET, Christine
INRA
SSM – Centre INRA de Versailles
Route de Saint-Cyr
78026 Versailles Cedex - France
Tel : (33+) (0)1 30 83 31 15; Fax : (33+) (0)1 30 83 31 49
Christine.Vergnet@versailles.inra.fr

WALLNER, Klaus
Universität Hohenheim
Landesanstalt für Bienenkunde (730)
D-70593 Stuttgart - Germany
Tel : (49+) 711 459 2662; Fax : (49+) 711 459 2233
BIENEWA@UNI-HOHENHEIM.DE

WALTERSDORFER, Anna
AgrEvo GmbH
Environmental Sciences, H872
Brüningstrasse, 50
65926 Frankfurt - Germany
Tel : (49+) 69 305 4415; Fax : (49+) 69 305 7342
anna.waltersdorfer@agrevo.com

WYNESS, Louis
Covance
Otley Road, Harrogate
HG3 OLP - UK
Tel : (44+) 1423 848438; Fax : (44+) 1423 569595
Louis.Wyness@COVANCE.com

ZLOF, Vlasta
EPPO
1, rue Le Nôtre
75016 Paris - France
Tel : (33+) (0)1 45 20 77 94; Fax : (33+) (0)1 42 24 89 43
zlof@eppo.fr

ICPBR

The International Commission for Plant-Bee Relationships (ICPBR) was founded in 1950 by the swiss scientist Anna MAURIZIO, whose outstanding work was mainly devoted to bees and their relationships with plants. Since 1980 this Commission - which is affiliated to the International Union of Biological Sciences (IUBS) - has regularly organized in Europe working sessions on the harmonization of methods for testing the toxicity of pesticides to bees.
ICPBR develops the scientific process preceding decisions from European administrative Authorities, EPPO (European and Mediterranean Organization for Plant Protection) and OECD (Organization for Economic Cooperation and Development). ICPBR symposia are thus always expected with great interest since they represent the first step in the evolution of legislation concerning bee protection related to the use of plant protection products.

La Commission Internationale pour les Relations Plante-Abeille a été fondée en 1950 par Anna MAURIZIO, chercheur suisse dont l'œuvre figure aux premiers rangs des travaux sur les relations entre les abeilles et les plantes. Cette Commission, affiliée à l'IUBS (International Union of Biological Sciences), organise régulièrement en Europe depuis 1980 des sessions de travail sur l'harmonisation des méthodes d'évaluation de la toxicité des pesticides vis-à-vis des abeilles.
L'ICPBR développe la démarche scientifique se plaçant en amont des instances administratives européennes, de l'OEPP (Organisation Européenne et Méditerranéenne pour la Protection des Plantes), et de l'OCDE (Organisation de Coopération et de Développement Economiques). Ainsi, ces symposiums sont toujours attendus avec grand intérêt car ils représentent la première étape dans l'évolution de la législation sur la protection de l'abeille relative à l'utilisation des produits phytopharmaceutiques.

For further information contact
Dr John STEVENSON
16 Old Rectory Close,
Harpenden,
Herts, AL5 2UD, England
Fax: +44 (0)1582 712316

The IOBC/WPRS Bulletin is published by the International Organization for Biological and Integrated Control of Noxious Animals and Plants, West Palearctic Regional Section (IOBC/WPRS)

Le Bulletin OILB/SROP est publié par l'Organisation Internationale de Lutte Biologique et intégrée contre les Animaux et les Plantes Nuisibles, section Régionale Ouest Paléarctique (OILB/SROP)

Address General Secretariat :
INRA - Centre de Recherches de Dijon
Laboratoire de Recherches sur la Flore Pathogène dans le Sol
17, rue Sully - BV 1540
F-21034 DIJON CEDEX
France

ISBN 92-9067-118-1