Bulletin of Insectology ***56*** *(1): 7-23, 2003*
ISSN 1721-8861

Minutes of the meeting

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8th International Symposium of the ICP-BR Bee Protection Group
OPENING SESSION

The Chairman of the Bee Protection Bee Group, *John Stevenson*, welcomed everyone to the meeting. He reported that there were 105 delegates present from 13 countries. The meeting was being held under the auspices of the International Commission for Plant-Bee Relationships (ICP-BR) and he conveyed greetings from its Chairman, Prof. Ingrid Williams. He then introduced the Bee Protection Group's Vice-Chairmen, Pieter Oomen and Dietrich Brasse, together with the Secretary Gavin Lewis and the meeting's hosts in Bologna. Stevenson announced that for the first time there would be a prize for the best presentation from a young scientist (<40 years old), thanks to the generosity of James Devillers. He then introduced *Prof. Pietro Catizone* (Director, Dipartimento di Scienze e Tecnologie Agroambientali (DiSTA), Università di Bologna) who welcomed everyone to Bologna. He emphasised the importance of the subject of the meeting both for the Mediterranean area and the work of his department. *Prof. Giorgio Stupazzoni* then added his own welcome to Bologna as President of the Istituto Nazionale Apicultura (INA) and thanked everyone for coming. Stevenson thanked both for enabling the arrangements for the meeting to be made and for the excellent organisation. *Anna Gloria Sabatini* (Director, INA) hoped everyone would have an enjoyable and interesting meeting and outlined the programme, with the oral and poster sessions as detailed in the programme and a tour of the city on Thursday afternoon followed by the symposium dinner.

Prof. Giorgio Celli (DiSTA) then gave a short talk on the work relating to honey bees, pesticides and the environment that has been conducted at his laboratory since the 1970's. Initially, they had focussed on the high level of pesticide use, which had resulted in large impacts on honey bees, for example in cherries where bees were used for pollination. The first work was carried out on endosulfan, which had been labelled as harmless to bees so that it could be used during flowering. However, work in the lab. and field had shown it to be harmful as a result of its persistence and they had made a strong case for change. In the 1980's problems emerged with the health of farmers which might have been due to the high level of pesticide usage and they raised the possibility of bees being used as indicators of environmental and human health. Different insect species are sensitive to individual factors but bees are sensitive to a wide range of chemicals and so are the best choice. The signs given by bees for environmental health and the presence of pesticides include mortality which can be measured at the hive. Where they are not killed directly (e.g. fungicides and herbicides) there is a need for residue analysis of honey and other products. This approach has been tested on the large scale from 1983 to 1986 with contemporarily 300 stations spread from North to South Italy, monitoring pesticide residue levels and heavy metals contamination: this last investigation showed that it is not necessarily healthy to live in cities such as Florence. So bees are important for agricultural production, give important products and also are good indicators for our health – where they survive we can relax.

SESSION 1: THE EFFECTS OF IMIDACOLPRID ON HONEY BEES

Efficacy and nicotinic acetylcholine receptor binding of imidacloprid and its metabolites in *Apis mellifera* (Hymenoptera: Apidae): toxicological and biochemical considerations (Nauen, R., Ebbinghaus-Kintscher, U., *Schmuck, R.*). This paper looked at the effect of imidacloprid in terms of its toxicity to honey bees and its pharmacological profile. The acute oral and contact toxicity to adult honey bees (*A. mellifera* L. var. *carnica*) was reported for imidacloprid and a number of its metabolites that had been reported to occur in plants after seed dressing. The binding affinity of imidacloprid and its metabolites to the nicotinic acetylcholine receptor was investigated and found to correlate well with the observed toxicity. These in turn corresponded well with whole cell voltage clamp measurements (using neurons isolated from the antennal lobe of *A. mellifera*). On the basis of the results obtained it was concluded that only olefine and 5-OH imidacloprid have to be considered as relevant metabolites.

Plant metabolism of imidacloprid (*Sur, R.*). This paper reviewed a series of studies looking at the uptake, translocation and metabolism of imidacloprid in plants. The uptake of imidacloprid into plants from the soil after granular application or seed treatment was very low in most species: translocation occurs by acropetal transport via the xylem – phloem mobility was very low. After translocation, imidacloprid was found to degrade in nearly all plant parts via three different routes. The nature of the residue in bee relevant matrices (nectar and pollen) was imidacloprid only, with no metabolites being found above the limit of detection. In field trials (with corn, sunflower and rape) no residues were found in pollen and nectar.

Medrzycki (IT) asked which carbon atom had been labelled and did it end up in the metabolites. *Sur* (DE) replied that only one central carbon atom had been labelled but that it passed into both of the 2 main degradation pathways.

Note: names *in italics* refer to people who spoke at the meeting e.g. as presenters of platform papers, to ask questions or to contribute in the discussion sessions. All discussion sessions were chaired by Pieter Oomen.

Toxicity of imidacloprid feedings on honeybee (*Apis mellifera*) colonies (Faucon, J-P., Aurières, C., Drajnudel, P.,Ribière, M., Martel, A-C., Zeggane, S., *Chauzat, M-P.*, Aubert, M.). In order to test the hypothesis that imidacloprid is responsible for bee mortality leading to the weakness or loss of honeybee hives reported in France over recent years, colonies were fed with syrup containing imidacloprid at various concentrations over a full year. Groups of eight hives each were fed syrup alone or imidacloprid at 0.5 or 5 ppb; a fourth, negative control group was unfed. The colonies were fed on 13 occasions (July-August, 3x/week, 1 L/hive) and their summer development and winter development followed. Assessments included mortality, colony weight, capped brood area and incidence of diseases. Population development and capped brood area showed a similar development in all colonies with no statistical differences between the colonies even at the higher dose of 5 ppb. Other parameters (e.g. mortality colony weight, diseases) also did not show any significant differences between the treatments.

Mühlen (DE) asked what the total dose had been per hive over the course of the colony feedings and *Chauzat* (FR) replied that as the total volume given had been 13 litres this could be calculated. *Mühlen* also asked why American foulbrood had not been looked for but *Chauzat* said that they had checked for all known diseases including American foulbrood. *Schmuck* (DE) asked why there was a conclusion of known effects from Gaucho in sunflowers if no significant effects had been found. *Chauzat* said this was based on the known problems occurring with sunflowers and that imidacloprid was one of the possible factors.

Safety of imidacloprid seed dressings to honey bees. A comprehensive overview and compilation of the current state of knowledge (*Maus, C.*, Curé, G., Schmuck, R.). This paper presented a review of the "French Bee Malady", where a link has been proposed between imidacloprid seed dressings and incidents reported by French beekeepers. A summary of the key ecotoxicological studies showed a consistent picture, with a high level of toxicity in the laboratory (LD_{50} values in the range 40 to >81 ng a.s./bee). However, under more realistic, semi-field conditions, in which colonies were directly fed 'spiked' honey, no adverse effects (mortality, behaviour, colony status, brood etc) were seen at 20 ppb. Laboratory tests on perception and cognitive function (e.g. proboscis extension reflex) had shown a No Effect Concentration (NOEC) of 10 ppb while effects on dancing behaviour had given a No Observed Adverse Effect Concentration (NOAEC) of 20 ppb (i.e. no bees lost). Field feeding studies, looking at orientation effects had also given an NOAEC of 20 ppb. Overall, it was considered that under field conditions, the NOAEC was 20 ppb i.e. no mortality or antifeedant effects, no loss of bees or impaired colony function and only transitory, short-term behavioural effects. Bee relevant plant matrices (e.g. nectar and pollen) had been analysed and in most cases residue levels were below the limit of detection for parent (1.5 – 5 ppb) and metabolites (1.5 – 3 ppb). It was therefore concluded that imidacloprid seed dressings were of low risk to bees and that the "French Bee Malady" was probably caused by a range of other factors.

Mühlen (DE) asked if there any studies had been conducted to look for synergistic effects and *Maus* (DE) said that in France possible synergism with EBI fungicides had been investigated but none had been found.

Effects of imidacloprid administered in sub-lethal doses on honey bees' (*Apis mellifera* L.) behaviour. Laboratory tests (*Medrzycki, P.*, Montanari, R., Bortolotti, L., Sabatini, A.G., Maini, S., Porrini, C.). Given the high acute toxicity of imidacloprid to honey bees, this paper investigated the possible effects at sub-lethal doses. Laboratory tests were described in which groups of bees were fed either in single doses or *ad libitum* at dose levels of 100 and 500 ppb (in 50% sucrose solution). It was found that there was a statistically significant increase in the number of bees remaining stationary 1 to 2 hours after treatment, compared to untreated bees (not for the first half hour). In the second hour there were also statistically significant reductions in the level of walking and running in the treated bees as well. This negative effect on bee mobility was dose dependant and also found both in bees fed in single doses or *ad libitum*, although it was short-term i.e. no effect was seen 6 hours after dosing. It was then considered if this decline in mobility could have a negative effect on social behaviour e.g. by decreasing communicative capacity. It was suggested that despite the short-term nature of the effects seen, there could be significant effects in the field e.g. bees finding difficulty in returning to the hive and colony health being impaired though disrupted social behaviour. However, it was recognised that in the work reported, high concentrations had been tested.

Schmidt (DE) asked about the high concentrations that had been tested in relation to the levels found in nectar and pollen and also if the effects seen had been transitory with a return to normal behaviour. *Medrzycki* (IT) said that this was the case and that they had not considered the levels related to actual flower contamination.

Effects of sub-lethal imidacloprid doses on honey bees' (*Apis mellifera* L.) homing rate and foraging activity (*Bortolotti, L.*, Montanari, R., Marcelino, J., Medrzycki, P., Maini, S., Porrini, C.). Further work to investigate the possible connection between honey bee mortality and declining hive populations in France and Italy and imidacloprid was reported. Bees under field conditions were trained to use feeders. In a first experiment these were placed 200 m from the hive and the bees fed 50% sucrose solution containing 100 and 1000 ppb imidacloprid. The bees returned immediately to the hive and regurgitated their honey crop before entering the hive and so there were no effects. In a second experiment, the feeder was moved to 500 m from the hive and the bees were held for 1 hour after feeding at 100, 500 and 1000 ppb, before being marked and released. Due to an antifeedant effect, fewer bees were marked at

the two higher dose levels. There was a slight reduction in the number of bees returning to the hive at 100 ppb in the first 2 hours after their release while none returned at the higher test concentrations. A similar pattern was seen at 4-5 and 24 hours after release. The numbers of bees seen at the feeder was reduced at 100 ppb after 2 and 4-5 hours but this effect had gone after 24 hours, while none were seen at any time interval at the higher concentrations.

Stevenson (UK) referred to pyrethroids and the fact that in the case of deltamethrin (for example), no effects are seen at 10-12.5 g a.s./ha but at 25 g a.s./ha, effects start to appear. How close, he asked, are field exposure levels to 100 ppb *Oomen* (NL) referred to the bees lost at 50 and 1000 ppb when released 1 hour after feeding and whether this would happen if the bees were immediately released. *Bortolotti* (IT) said no but that the delayed release was seen as being more realistic. *Schmidt* (DE) suggested that an opposite conclusion could have been reached if there had been a spray application at 500 and 1000 ppb, which would have resulted in a protective antifeedant effect (while at 100 ppb there was no effect). *Bortolotti* said that there had been some feeding at the higher concentrations, especially 500 ppb, so that this would not necessarily have been the case. *Florelli* (FR) asked if any of the bees that disappeared had ever been found and *Bortolotti* replied that although they had only continued the assessments for 24 hours, longer observations of the hives had been made for other reasons and there had still been no sign of the bees. *Schmidt* (DE) pointed out that although Confidor can be sprayed at concentrations >100 ppb, this did not occur during flowering but *Bortolotti* thought that it would not be possible to exclude some use during bee activity resulting in exposure and also that other work showed antifeedant effects at 100 ppb. *Brasse* (DE) thought that a link might be possible at 100 ppb and asked if the levels in nectar and pollen were much lower but *Bortolotti* said that this had not been assessed.

Risk of environmental contamination by the active ingredient imidacloprid used for treating corn seed. Preliminary data (*Greatti, M.*, Barbattini, R., Rossi, S., Sabatini, A.G.). This paper considered the possible risk of environmental contamination from the sowing of imidacloprid treated corn seeds using pneumatic (air aspirated) seed drills. In order to detect the possible loss of imidacloprid during sowing filter papers were fixed to the centrifugal fan drain of a pneumatic drill and additionally samples were taken of flowers and grass in areas adjacent to the treated field. Imidacloprid residues were found on the filter papers: 44 mg/kg after 30 seconds, 60 mg/kg after 60 seconds and 128 mg/kg after 120 seconds. They were also found on the grass samples (e.g. 0.048 mg/kg) and the flowers (e.g. 0.026/0.039 mg/kg). Traces of 6-chloronicotinic acid were also found on all samples. It was suggested that these results indicated a potential risk to honey bees although it was recognised that data for nectar contamination would be necessary for a full risk assessment. It was suggested that better ways could be found to 'stick' the pesticide to the seeds. This work is ongoing.

Oomen (NL) asked if the quantity of material lost from the seeds per ha was known and *Greatti* (IT) said no but it could be calculated as the number of seeds per m^2 is known. *Schmuck* (DE) said that similar work had also been done by Bayer and found a loss of about 4%, equivalent to 4 g a.s./ha and since some of the work reported at the meeting indicated a no effect level of 100 ppb this should cause a serious problem but in practice, applications of 12 g a.s./ha (3x the level expected) showed no effects. *Medrzycki* (IT) suggested that it is not possible to relate the concentrations seen in this work to the concentrations used in the tests since they did not reflect actual exposure.

Imidacloprid, honey bees and potatoes in Atlantic Canada: is there a connection? (*Kemp, J.R.*, Rogers, R.E.L.). A possible connection between imidacloprid and honey bee health has also been investigated in Canada. Some beekeepers in Prince Edward Island and New Brunswick had complained of similar problems to those reported in France following the use of imidacloprid on clover and there were similar concerns with its regard to its use on potatoes. In the first phase of the investigation, a residue study was conducted in 2001 using a protocol jointly developed with the stakeholders. Samples were taken of soil, cover leaves and flowers, wildflowers and honey bee matrices (pollen and nectar collected from foraging honey bees and uncapped honey) and analysed for residues of imidacloprid and two if its metabolites. The sampled fields had been treated up to 2 years previously: potato fields (Year 1), underseeded grain fields (Year 2) and first and second flowering clover fields (Year 3). Residues were found in all soil samples and in a few cases, on clover leaves (first flowering only). However, no detectable residues (<2 ppb) were found on any of the bee matrices (nectar, pollen, honey) as well as on any of the flowers (clover and wildflowers). Thus, there was no evidence that imidacloprid posed a threat to honey bees when used as directed but the beekeepers were concerned that their problems were continuing (and possibly increasing). Accordingly, a second phase had been instigated for 2002/2003, involving an extensive monitoring approach to consider as many possible factors as possible. Thus, interviews are to be held with beekeepers (experiences, management practices, perception etc) and surveys made of vegetation, pesticide usage and apiary suitability. Additionally, samples will be taken for residue analysis (bees, wax, honey and flowers) and the occurrence of pests and diseases recorded. This study involves a high level of cooperation between industry, government, food producers, beekeepers (including the Canadian Honey Council) and farmers.

Discussion Session

Following these presentations addressing various aspects of the potential risk of imidacloprid to honey bees, a discussion session was held. *Oomen* (NL) started by briefly summarising the history of this issue, starting with the problems that had been experienced by French beekeepers over a number of years. As a result of this it

had been hypothesised that the use of imidacloprid had resulted in the bee losses observed and the general decline in colony health. *Stadler* (ARG) reported a study that had been conducted in Argentina in 2000 assessing the possible impact of sunflower seeds treated with imidacloprid on honey bees (presented in a poster). No significant differences were found between colonies on treated and untreated areas and in particular, no residues of imidacloprid and its main metabolites were found in hive components (wax, honey and pollen) 10 days after exposure to treated sunflowers. *Kemp* (CAN) found this encouraging as it supported their work in Canada. *Schmuck* (DE) then pointed out that there had been 6 years of work and that while sub-lethal effects had been shown in the laboratory, under field conditions (e.g. numerous field studies and the monitoring work of Kemp and Stadler) none of the effects reported by French beekeepers had been seen. He therefore concluded that imidacloprid had no effect on honey bees when used as a seed treatment.

Oomen (NL) returned to those studies (as presented at this meeting) where concerns had been raised. *Schmuck* (DE) pointed out that in the study of Faucon *et al.*, no effects had been seen and there was no other data available which demonstrated a concern. He did not consider the effects seen at 100 ppb surprising, given that they (Bayer) had set a field NOAEC at 20 ppb, and there was nothing to suggest a problem. However, *Chauzat* (FR) said that they needed a study such as that reported from Canada as an effect could be dependant on the health of the colony i.e. an interactive effect. *Schmuck* (DE) said that in 1998, the hypothesis had been that there was a link between imidacloprid and the reported problems with bee health. However, no data had been produced to support this link and so the hypothesis had been successively modified on a number of occasions rather than question its accuracy. He stressed that it was important not to look at one factor but that a holistic approach was needed, such as that taking place in Canada, to look at all possible factors. *Stadler* (ARG) agreed, and pointed out that in their independent field studies in Argentina, it had been concluded that "Gaucho" (a.s. - imidacloprid) was not harmful to bees

Brasse (DE) indicated that he did find some of the conclusions in the papers presented a little surprising. These appeared to be based on theoretical assumptions rather than actual field data e.g. poisoning incidents (field monitoring), while the residue studies had not found imidacloprid in bee relevant matrices. He wondered if the problems of bee health might be linked to other causes e.g. diseases, rather than pesticides. *Bortolotti* (IT) pointed out that the reported studies were not complete and suggested that the monitoring studies had found residue levels that were close to those found to have effects in field studies. *Brasse* (DE) responded that in the honey stomachs of dead honey bees collected during a tunnel test, no residues of imidacloprid had been found. He added that all relevant samples of poisoning incidents have been investigated for residues of imidacloprid, but all possibly available residues have been below the detection limit.

Medrzycki (IT) asked if it was possible that imidacloprid was taken up by a bee and rapidly metabolised i.e. within a few minutes. Thus, in those studies where effects had been seen, if the honey stomach contents had been analysed for imidacloprid residues, the measured values would be much lower than the initial exposure levels. This might also apply to contact toxicity e.g. on wild flowers following contamination resulting from the losses during seed sowing. *Brasse* (DE) pointed out that in cage tests, honey bees had been offered only contaminated nectar and the colonies had grown in a very healthy manner without any indication of effect. *Kemp* (CAN) added that although adsorption from the honey stomach was possible, in his study the bees had been frozen within 1 minute of field sampling and the residue levels were still <2 ppb.

Oomen (NL) suggested reviewing the regulatory decision with regards to imidacloprid and honey bees. The initial decision would have been taken by the authorities on the basis of the precautionary principle. Now there might be enough information to review that decision and to see if there was any link between imidacloprid and the reported problems of bee health. He went on to say that some studies might show behavioural effects e.g. disorientation, which could have an effect. However, *Schmuck* (DE) referred to the long distance (500 m) field feeding study reported earlier, in which effects at 100 ppb were relatively small and transitory. It was important to distinguish between seed treatments and foliar sprays. He agreed that there had been no link shown with imidacloprid but emphasised that there was clearly a problem of declining bee health in some areas over recent years. He said that there was a need for a detailed, systematic approach to be carried out which could identify possible causes (similar to the work of Kemp in Canada and Stadler in Argentina). He suggested that the Bee Protection Group could help in addressing this.

Stadler (ARG) pointed out that the quality of crop hybrids can effect bee development and that the effects could occur over a wide area if the bees had no choice of pollen. *Brasse* (DE) asked if anyone had found any link between imidacloprid seed dressings and bee poisoning incidents. *Fletcher* (UK) replied that in the UK, while imidacloprid was not used to a great extent there had been no incidents to date. *Medrzycki* (IT) asked if anyone had looked at the effects on honey bee brood. *Schmidt* (DE) replied that this had been looked at in a number of field studies (both seed dressings and foliar sprays) and no effects had been seen e.g. in the paper presented by Chauzat earlier. *Schmuck* (DE) added that in the long-term field trial where bees had been exposed to 'spiked' pollen or honey at 20 ppb, no effects on brood had been seen.

Giffard (FR) agreed with Oomen and said that one of the problems in France was that beekeepers appeared not to want to look at other possible causes but that it

was important to look at all factors. *Florelli* (FR) drew a comparison with the earlier experiences of the development of deltamethrin, where it had been blamed for disorientation and other effects. A substantial amount of work had shown that it was not a problem and that some pyrethroids could be used on flowering crops. With regards to the potential effects of imidacloprid, it was necessary to show a causal mechanism for this as well as to consider all possible factors. *Tasei* (FR) said that he was working closely with the French officials on this subject and that he found the Canadian experience very interesting – this might well be of value for the French beekeepers. *Oomen* (NL) concluded by saying that it was first necessary to identify the nature of the problem in France, Italy, Canada etc e.g. is it real and how is it manifested. Then, it was necessary to investigate all possible factors in order to make the assessment more objective.

The issue of imidacloprid and honey bees and the general problem of declining bee health was returned to in a discussion session on the last day of the symposium. In order to find a constructive way of addressing this problem, *Oomen* (NL) proposed setting up a working group to look at it (in accordance with the Bee Protection Group approach for such issues). It was clear that there were real concerns for beekeepers but it was not clear if imidacloprid played any part in the problems seen with colony performance. A similar concern about a general decline in bee health had been seen in France, Italy, Canada and other countries but the problem had not been well defined or understood. Accordingly, he suggested a working group could be set up to try and get to grips with the problem.

Stevenson (UK) said that a lot of progress had been made in the first session of the symposium, addressing this issue, but regulatory authorities and beekeepers clearly needed more guidance and thus the Bee Protection Group could make a valuable contribution. *Oomen* (NL) suggested that as the problem had been first identified in France, a French leader for the working group might be appropriate. He asked Dr Tasei if he would be prepared to do this. *Tasei* (FR) indicated that he could lead the working group at the start but would want to pass this role on to another person in due course.

Oomen (NL) asked if he thought that the first issue was to address the nature of the problem i.e. what are the possible causes for the decline in bee health. *Tasei* (FR) replied that in France they were looking for other factors that might be involved and that one of the problems of this was the need for expert support e.g. in the case of bee diseases. He wondered what the scope of the working group would be as the expertise would primarily be in the effects of pesticides. There was thus an issue concerning the type of people that needed to be in the group and how to manage it. He did not think that the people present at the symposium would be sufficient for the working group and thus it should be launched to a wider pool of participants. Oomen thought that this would be a good idea as a similar problem was being experienced in several countries and so this would allow knowledge to be shared in order to define the problem and looking for the causes.

Giffard (FR) suggested that as there was already a group dealing with this issue in France it would be better to invite this group to present their opinion to the Bee Protection Group rather than creating a new group with different ideas. He stressed the importance of involving French officials and beekeepers. *Tasei* (FR) said that he was a member of the French group and it was certainly willing to receive external advice so the deliberations of the Bee Protection Group would be very useful. *Brasse* (DE) suggested that the working group should not only consider imidacloprid but should also include other systemic insecticides. The objective of the group would thus be how to conduct registrations of compounds with high toxicity to bees and high systemic activity. *Stadler* (ARG) agreed that an alternative approach would be to create a working group to study the side-effects of systemic pesticides e.g. developing appropriate methodologies. He pointed out that it would not be possible to prove the reason for the decline of bee colonies unless the causal factor(s) could be identified (pesticides and others).

Medrzycki and *Sabatini* (IT) said that in Italy there was an apicultural environment that hadn't collaborated with outside agencies in the past. However, the Apicultural Institute in Bologna was now co-ordinating research all over Italy and the spectrum of researcher's interests was getting wider, including looking at the losses of bees in vineyards. He indicated that he would be very interested in collaborating with the working group. *Kemp* (CAN) also indicated that he and his colleague Dr Rogers would be happy to join such a group and share ideas. *Oomen* (NL) indicated that the meeting should return to this issue at the end of the symposium.

It was generally concluded by the meeting that the cause of the decline in bee health and colony performance, reported in a number of countries, was unlikely to have a single cause. Instead, it was probably due to a number of interacting factors, which could include disease, *Varroa* (including use of varroacides), colony management, pesticides, genetic factors, agricultural practice etc. These could act either independently of each other (i.e. in an additive fashion) or in combination to produced an enhanced (i.e. synergistic) effect.

SESSION 2: TEST METHODOLOGY AND STUDIES OF EFFECTS OF PESTICIDES ON HONEY BEES

Honey bee brood ring-test in 2002: method for the assessment of side effects of plant protection products on the honey bee (*Apis mellifera* L.) under semi-field conditions (tunnel test) (*Schur, A.*, Tornier, I., Brasse, D., Mühlen, W., von der Ohe, W., Wallner, K., Wehling, M.). A number of methods were described which are currently available to investigate the possible effects of plant protection products on honey bee brood.

However, in terms of a sequential testing scheme for honey bee brood there is a gap, which the work presented in this paper is designed to fill by the development and validation of a cage or 'semi-field' test. The work had been conducted by the "Arbeitsgemeinschaft Bienenschutz" (a German group comprising the BBA, bee institutes, companies and contract testing facilities), which had been formed in 1994 to discuss bee issues and develop new testing methods. In this case, a ring-test had been carried out in 2002 at 5 test facilities. The method involved the use of "Mini-Plus Beuten" hives (small conies of about 6000 bees and with 3 brood combs) placed in tunnel cages (at least 50 m^2) containing flowering *Phacelia*. There were two treatments, a water-treated control and the reference substance Insegar 25 WG (a.i.: fenoxycarb). The bees were exposed to the treated crop for 7 days during which time they were assessed for mortality (dead bee tray and on paths), flight intensity and colony assessments (e.g. strength of colony and comb area covered with different brood stages). In addition, special attention was paid to the brood, with the development of 100 eggs in marked cells being followed at intervals for up to 22 days (assessing stages up to emergence). A brood development index was produced showing development at successive expected stages, which for the control was slightly reduced at 11 and 16 days but in the reference treatment showed a marked reduction up to 16 days after marking (with recovery in a second generation at 22 days). Another measure of effect was the brood termination rate, which over the 5 trials conducted ranged between 8 and 43% for the control but was between 94 and 100% for the toxic reference. In conclusion, it was considered that the test method had a number of advantages (e.g. realistic exposure, sensitive, consistent with EPPO 170, quantitative results) although there were a few disadvantages (e.g. preparation of special colonies and fixed timetable).

Schmuck (DE) asked if the test had been replicated and whether it was considered to be sufficiently robust. *Schur* (DE) replied that it had not been but that it would be sufficiently robust with 2 replicates, which could be separated in time to cover a range of conditions.

A semi-field approach to testing effects of fresh or aged pesticide residues on bees in multiple rate test designs (*Bakker, F.*, Calis, J.). Another semi-field cage test design was described which is designed to produce reproducible results with multiple treatments (including residue ageing) suitable for statistical analysis. The design comprises 4 replicate 20 m^2 cages, each containing 108 flowering *Phacelia* plants and a single, standardised hive with a queen, about 2000 young bees and 1 comb each with 7-day old brood and pollen/honey. Daily assessments are made of mortality and foraging (number of bees on flowers over 20 seconds) before and after application, as well as colony performance (weight) and brood development. The method was tested with Dursban 75 WG, a micro-encapsulated formulation of chlorpyrifos applied at 1000 g a.s./ha and Reldan an EC formulation of chlorpyrifos applied at 2700 g a.s./ha, in comparison with a toxic standard (Penncap M) and a water-treated control. The treatments were applied with a hand-held sprayer in a volume of 300 l/ha. Comparisons were made with different age classes of bees as well as with different residue ageing periods (achieved by replacing untreated plants with treated plants on the evening before the initiation of exposure). No increase in mortality was found with Dursban, while some occurred with Reldan (greatest following the in-flight application) but the highest increase occurred with the toxic standard. Some reductions in foraging were found, but these were for shorter periods with Reldan and Dursban (especially with increasing residue age), while Dursban showed a slower response, which could have been due to the micro-encapsulation delaying exposure. A significant weight loss was only found in the Penncap M colonies. It was considered that the standardised test method allowed the use of powerful statistical analysis for the comparison of different exposure scenarios. In particular it gave excellent reproducibility, allowing the detection of a 30% reduction in foraging and a 16% increase in mortality.

Oomen (NL) asked about the comparability of this method with the classic cage method according to EPPO 170. *Bakker* (NL) said that he was not familiar with the EPPO 170 method, although cage and colony size differed so a comparison would be difficult. Oomen added that a comparison might be worthwhile although he recognised that there might be problems with confidentiality. *Schmuck* (DE) asked if the assessment of brood effects would need a larger cage area and Bakker replied that this aspect had only been looked at qualitatively but it could be included in a modified test design. *Miles* (UK) pointed out that the test had been designed to work with specific products, particularly with regards to the duration of residual toxicity, and had been very useful in this case.

The effects of IGRs on honeybee populations (*Thompson, H.*, Wilkins, S.). The longer-term effects of insect growth regulators (IGRs) were investigated for fenoxycarb (a juvenile hormone agonist) and diflubenzuron (a chitin synthesis inhibitor), applied at their maximum field rate onto flowering crops. Brood effects were assessed by full colony assessments (every 2 weeks during the season and monthly over winter) and following the development of individual cells marked up to 5 weeks after application on a weekly basis. The mean egg replacement/removal for the control was 19%, 47% for fenoxycarb and 44% for diflubenzuron (nearly 100% at week 0). Effects were also seen on older brood but no effects were seen in drone sperm counts in any of the treatments. Adult populations in the diflubenzuron colonies remained at about the control level in the longer term, while those in the fenoxycarb hives decreased to a greater extent over winter with effects being seen into the following spring, reflecting the lower brood levels. Queen viability was checked by allowing drafted larvae to pupate in the exposed colonies and then transferring them to new colonies and caging them on frames for 24 hours to assess their egg production.

Diflubenzuron showed a significant reduction compared to the control. Planned future work includes repeating the tests with tebufenozide and azadirachtin as well as conducting further assessments on egg production and following colony development for up to 1 year. This will produce comparable data which will allow advice to be given to PSD (UK) on the use of IGRs.
Oomen (NL) asked if the IGR dose levels used were the recommended field rates and *Thompson* (UK) confirmed that this was the case.

The effects of spinosad, a naturally occurring derived insect control agent to the honeybee (*Apis mellifera*) (*Miles, M.*). A summary was presented of the testing that had been conducted according to the EPPO 170 sequential pathway to assess the effects of spinosad (a fermentation product) on honeybees. Laboratory tests had shown that it has a high acute toxicity to bees by both the contact and oral route of exposure. Laboratory residual toxicity tests with alfalfa and kiwifruits had shown that it has a very short duration of residual toxicity, becoming non-toxic to bees within about 2-3 hours ageing. Two semi-field (cage) tests had been conducted: in the first, spinsosad had been applied early morning (before bee flight) at rates of 144 and 540 g a.s./ha. Mortality had only been slightly increased at the higher rate while foraging showed a rate-related reduction, with a small reduction over 1-2 days at 144 g a.s./ha but a stronger reduction over 7 days at 540 g a.s./ha. There were no effects on the brood at either rate. The toxic standard (dimethoate) had shown clear effects on both mortality and foraging. In the second cage test, application was carried out during bee flight at a rate of 216 g a.s./ha (1 and 4 applications) but the results had been affected by less favourable weather. There had been a small peak in mortality 1-2 days after application in both spinosad treatments, but this was much less than the increase seen in the toxic standard. Foraging had been very variable although the levels seen in the spinsoad treatments were in-between those seen in the control and dimethoate treatments. Again, the brood had not been affected. A series of field trials had been conducted in a variety of crops, with application occurring in all cases outside bee flight. In alfalfa at 70 and 175 g a.s./ha there had been no effects on mortality, foraging or brood, with similar results being found in almonds citrus and kiwifruit and also avocado (where only brood had been assessed. It was concluded that while spinsoad showed high toxicity to bees in the laboratory, this was of short duration and effects were minor under semi-field conditions while no adverse effects were seen in the field. Additionally, spinosad has been widely used and no adverse effects on honeybees have been reported.

Brasse (DE) asked how spinosad should be labelled and *Miles* (UK) replied that it could be applied to flowering crops but direct exposure (application during bee flight) should be avoided. *Oomen* (NL) asked what the explanation was for the lack of effects in the field given the high risk predicted from the laboratory data? *Miles* (UK) said that similar results had been found with bumblebees (following use in glasshouses) and with solitary bees and that this was possibly due to strong binding to foliage thereby rapidly reducing exposure.

Modeling the acute toxicity of pesticides to *Apis mellifera* (*Devillers, J.*, Pham-Delègue, M-H., Decourtye, A., Budzinski, H., Cluzeau, S., Maurin, G.). The use of Quantitative Structure-Activity Relationships (QSAR) methodology has been widely developed with over 15,000 methods available but there is only 1 for honeybees. The aim of this work was to produce a QSAR model to allow the simulation of acute toxicity to honeybees. A training set of 89 compounds (from Atkins *et al.*, 1981) was used to develop the model. In conclusion, it was considered that a QSAR model is a complimentary tool to better estimate acute toxicity of pesticides to *A. mellifera* (but cannot replace testing) and could help with the design of safer pesticides (i.e. compounds not yet synthesised). They can also be used to better understand toxicity mechanisms and to simulate the effects of xenobiotics, which affect honeybees (not just pesticides).

Devillers (FR) confirmed that a QSAR model, based on primary laboratory data, could contribute to the risk assessment in response to a suggestion from *Oomen* (NL), who then asked if the model could replace the primary data rather than being used as secondary data. Devillers said that QSAR models were used for aquatic toxicity assessments, to save time and to see if a molecule showed any potential toxicity in which case it would be necessary to conduct the appropriate tests.

Introduction of indices for the evaluation of tent tests and field tests with honeybees (*Schmidt, H-W.*, Brasse, D., Künast, C., Mühlen, W., von der Ohe, W., Tornier, I., Wallner, K.). This talk on the use of indices for the evaluation of semi-field (cage) and field tests was presented on behalf of the German working group "Bee Protection" (see talk by Schur *et al.*, above). The sequential testing scheme for assessing risk to bees (e.g. EPPO 170) was reviewed and some of the advantages and disadvantages considered. It was pointed out that in semi-field (cage) and field studies, the bees are introduced to the cage or field prior to the pesticide application in order to allow them to acclimatise to the new conditions. Assessments are carried out to demonstrate this but these can also show differences between individual colonies. One way to evaluate assessments of a treatment is to compare with the control (e.g. Abbott's formula for mortality) but this is only appropriate if there are no pre-treatment differences between the colonies. However, this is not always the case and so by comparing the same factors assessed before and after application, it is possible to identify any differences and hence and effects of the treatment. An example was given for two pyrethroids, in which a mortality quotient was calculated (average number of dead bees over 5 days after application / average number of dead bees before application). If the quotient is >1 then it demonstrates an increase in mortality after treatment and it was suggested that a value of ≥2 would indicate that there was a significant treatment-related effect or some other

factor responsible which should be identified. A similar quotient can be calculated for foraging, indicating the degree of any repellency (a value of ≤0.5 i.e. a reduction of at least 50%, was suggested as a threshold value). It can also be used for brood assessments and the use of a 'clearing quotient' to compare the quotient values for the treated and control colonies, was suggested. Overall, it was considered that the use of the quotient had a number of advantages: compares pre-and post conditions; transforms absolute values into relative ones; condenses complex data; addresses differences between colonies and clarifies any effects; allows for comparison of repeat runs; assists interpretation of results.

A method to feed bees known amounts of pesticides (*Ladurner, E.*, Bosch, J., Maini, S., Kemp, W.). A method was described to feed known amounts of pesticides to bees in order to help improve oral toxicity testing (currently carried out with group feeding, with tropholaxis sharing out the dose). Three methods were tested on 3 bee species (*Apis mellifera*, *Osmia lignaria* and *Megachile rotundata*): (1) black file canister with hole over glass slide; (2) glass vial with feeding tube inserted at one end; (3) polythene tube inserted into flower calyx. Four light regimes were tested: natural; artificial; artificial (plant growth); darkness. Bee feeding was found to be highest for all species using the flower method (varying from 90-95% under natural light to 45-70% in darkness) and was highest with *A. mellifera*. Also, no evaporation was observed with the flower method and so it gave a more reliable LD_{50} value and a better indication of repellency. It was considered that the flower method could help standardise oral testing and due to a greater efficiency, save time.

Bakker (NL) asked if artificial flowers could be used. *Ladurner* (IT) replied that they had tried this with *Osmia* but it hadn't worked. They had also tried just using petals but this had resulted in a low feeding efficiency and they had therefore concluded that the whole flower was needed.

Discussion Session

Oomen (NL) started the discussion session for Session 2 by briefly summarising the talks that had been presented, ranging from developments in semi-field test design and interpretation to a modelling approach for estimating the acute toxicity of pesticides. *Giffard* (FR) pointed out that just as laboratory tests have disadvantages, they are useful and in the same way while semi-field and field tests also have disadvantages this should not be a reason for not doing them. He was interested by the use of indices for semi-field and field tests, and suggested that large cages (tents) gave better results because of the better quality data obtained. The problem with small cages and colonies is that they don't provide good agricultural and scientific conditions. However, *Bakker* (NL) felt that small cages could provide useful data and pointed out that they are part of a sequential testing scheme. His work showed that standardised, reproducible data could be obtained with smaller cages and so they should be included. Indices could be used with the data from small cages but the variability must be low and he pointed out that if there were zero values (e.g. number of dead bees before application) then the index would become infinity.

Medrzycki (IT) asked how the decrease in mortality in the example given could be explained as it was about 3-fold in the control but 7-fold in the pyrethroid insecticide treatment. *Schmidt* (DE) replied that this was within the range of variation found in their trials and so was not unusual. *Medrzycki* responded that on this basis an increase of the same amount should therefore also be considered normal and yet the proposed threshold for significant effects had been given as 2. *Candolfi* (CH) agreed that considering an increase in mortality of only 2x e.g. from 5 to 10 bees, would not be right and that it was necessary to consider other factors as well e.g. climate and timing. He therefore did not think that the index approach was the right way. *Florelli* (FR) said that there was a problem with small numbers which could have a large effect on the indices and so it would be necessary to consider variability to avoid any experimental artefacts. *Schmidt* said that with regards to differing levels of mortality there was the same problem with Abbott's correction. With regards to the index approach, he thought that it should be seen as a useful tool for investigation and that it would be better to consider the advantages e.g. handling lots of data, rather than concentrating on the disadvantages.

Tornier (DE) reminded the meeting that the issue of cage size had been discussed over many years and that at the last meeting it had been agreed to recommend a size of at least 40 m^2 as this would give better results, with good numbers for foraging and mortality. *Bakker* said that he had no experience with large cages but over 3 years of using the small cages he had described (e.g. 20 m^2), they had found low control mortality, high foraging levels (30-60 bees/m^2) and a constant response to the toxic standard. *Giffard* agreed that small cages have a role to play but urged caution as he did not consider that they were representative of normal bee behaviour, which was more valid with larger cages. *Coulson* (UK) warned against putting too much emphasis on methodology. He asked about the two parameters Q (treated) and K (untreated) and if the thresholds were only based on Q. *Schmidt* replied that it was necessary to consider Q and K in order to calculate the 'clearing quotient' (Q / K), which can be used with the same trigger values. *Coulson* suggested that the triggers could be used only with Q but *Schmidt* thought that both should be used. *Brasse* (DE) said that the use of indices should not replace the judgement of a regulator but should allow someone who had not been at a trial to interpret the data. They would be useful to support a regulatory interpretation and assess the significance of any differences found.

Oomen concluded the discussion session by suggesting that the index approach to data evaluation should not be a prescribed method but was a useful approach and that

it could be considered for incorporation into the EPPO honey bee risk assessment scheme. He also referred to the other papers that had been presented in this session and said that the ring test approach for the honey bee brood test was a valuable approach and that as no points had been raised about this he assumed that everyone was in support. He also said that the long-term consideration of IGR effects on honey bees was important and asked that the Bee Protection Group be kept informed and again as there had been no points raised on this topic he assumed that everyone was in support. The meeting agreed.

SESSION 3: SYNERGISM

Assessment of the synergy and repellency of pyrethroid/fungicide mixtures (*Wilkins, S.*, Thompson, H.,). A presentation was given on work funded by the UK Pesticide Safety Directorate (DEFRA) to assess the synergism and repellency of pyrethroid-fungicide mixtures. The Pesticide Usage Survey was used to identify pesticide combinations and rates used on flowering crops, while the Wildlife Incident Investigation Scheme identified hazardous incidents involving bees. Pyrethroids show high toxicity to bees but repellency allows their use on flowering crops, while fungicides show low toxicity but incidents have been reported from mixtures of the two. This could be due to an increase in toxicity (synergism) or a decrease in repellency. To investigate this, two 'bee-safe' pyrethroids were used (λ-cyhalothrin and α-cypermethrin), together with 8 fungicides. Three of the fungicides showed an increase in toxicity with α-cypermethrin and 6 with λ-cyhalothrin. A repellency test was developed comprising a small cage into which filter paper dosed with the test solution was placed and a pre-weighed feeder containing sucrose solution was placed in the middle. Bees were introduced into the cage, their feeding behaviour observed and the feeder weighed after 4 hours. Three fungicides were found to significantly decrease the repellency of the pyrethoids (but not the same ones in each case). Combining the changes in toxicity and repellency for the mixtures allowed an assessment of the changes in risk for the various pyrethroid-fungicide mixtures. The greatest increases were found with α-cypermethrin and chlorothalanil and with λ-cyhalothrin and prochloraz. The next steps in this investigation is to see if fungicides affect the toxicity of pyrethroids used as varroacides (preliminary data indicates that this is the case for some combinations) and then to provide advice to PSD on practical approaches to addressing this issue.

Discussion Session

Mühlen (DE) asked about the 100% mortality seen in tent tests with varroacides but *Wilkins* (UK) replied that this data was not from a tent test but was preliminary laboratory data using varroacide-treated strips and topical application of fungicides. *Tornier* (DE) said that as varroacides were normally used in the winter when brood was not present, there needn't be any concern but Wilkins pointed out that if the treatment was considered acceptable then it could be applied all year. *Schur* (DE) asked about how the summarised data for the risk increase had been produced and *Wilkins* explained that it was the increase in toxicity x the reduction in repellency (equivalent to increase in exposure). *Florelli* (FR) asked about the reproducibility of the repellency results and Wilkins said that this had been checked by running the tests on different days with different bees and in all cases similar results had been obtained. He also explained in response to a question from *Mühlen* that the repellency had been assessed by comparing the amount of sucrose consumed in comparison with a control.

Stevenson (UK) asked how many bee poisoning incidents had been recorded e.g. in UK and Germany, in which synergism had been a factor. *Fletcher* (UK) said that in the UK there had been 4 or 5 cases (including 1 with bumble bees). *Brasse* (DE) said that in Germany there had been 47 cases recorded in 1999 but that as a result of this new labelling (limiting acceptable tank-mixes) had been introduced so that in the last 3 years there had only been 11. *Oomen* (NL) asked if the conditions under which bees might be exposed to pesticide mixtures are known. *Brasse* replied that a positive list of fungicides which can be used with insecticides is put on the label but for pyrethroids that can be used during bee flight then the mixtures can only be used after activity has finished and so long as there is no contamination of neighbouring crops. *Oomen* then asked if there are cases known where bees from colonies where pyrethroid varroacides had been used have been exposed to fungicides in the field. *Brasse* said that while this is possible, currently only one case is known

SESSION 4: HONEY BEE POISONING INCIDENTS AND MONITORING SCHEMES

Bee pesticide poisoning incidents in the UK (*Fletcher, M.*, Barnett, L.). In the UK bee poisoning incidents are investigates within the Wildlife Incident Investigation Scheme (WIIS). The purpose of this is to validate and improve the risk assessment process; for post registration surveillance and to enforce laws for chemical usage and the protection of environment. The scheme relies on beekeepers to recognise possible poisoning incidents; to report incidents and to submit bee samples. The field investigation addresses aspects relating to the bees e.g. condition of the colonies, scope of the incident and symptoms, as well as the agronomic factors e.g. crops present, pesticide use and weather conditions. The Central Science Laboratory provides bee laboratory diagnostics (diseases, etc) and chemical analysis (for all major groups of compounds). In recent years there has been a decline in incidents possibly reflecting better awareness by beekeepers and farmers but also beekeepers may be reluctant to report incidents. Most pesticides identified are insecticides (15 organo-phosphates, 2 organo-chlorines, 5 carbamates and 9 pyrethroids) but occasionally other compounds as well. Approved use incidents have included specific tank mixes (e.g. syner-

gism); application to heavy aphid infestations (i.e. honey dew); flight path though spraying; destruction of GM contaminated crops; contaminated drinking water; wood treatments. Misuse incidents have included spraying crops with flowering weeds present; feral bee control; over-spraying non-target plants; wax moth control; insufficient tank cleaning; contaminated smokers. Overall, 18% of uses have been from approved uses and 4% from abuse (with 46% being unspecified). The results of the incident investigations provides valuable information to regulators, companies, beekeepers, farmers and enforcement authorities and also gives confidence to the public that pesticides are being monitored. A final conclusion was that it would be very helpful to pool resources and data sets from other countries in the EU.

Poisoning incidents involving honeybees in Germany (1999-2002) and new problems for beekeeping (*Brasse, D.*). This talk was a continuation of the report given in Avignon (1999) in which the scheme of the ICPBR monitoring sub-group was presented. In Germany there has been a relatively low number of incidents in recent times with an average of 82 per year over the last 10 years. This represents a significant decrease compared to about 25 years ago when, in particular, there were problems with a small part of the vine growing area. Also, the crops involved are changing, with a decline in incidents in vine, an increase in broad beans and other crops (e.g. cereals, potatoes and asparagus) also showing changes. Only in fruit crops and oilseed rape have the incidents remained at a significant level. Deliberate poisoning has also increased. Overall, lindane is the most frequent active substance involved, although as it is not registered in Germany, this probably reflects imported wax material as the route of entry into the hive. Parathion has been replaced by dimethoate as the 2nd most common, while fenoxycarb incidents have declined, reflecting improved advice given to farmers by extension services. As mentioned in a previous talk, there have been 11 incidents in the last 3 years involving synergism of pyrethroids and ebi fungicides, which represents a significant decline following improved labelling. In general then, the situation in Germany is improving but there is now a new problem – residues in honey. A low value of 0.01 ppm has been set where no specific value has been fixed in order to protect consumers. Streptomycin, used in fruit crops, has been found to exceed this (12 out of 183 samples, >0.05 mg/kg). However, this level is much lower than for other foodstuffs and the ADI is 2.5x higher. Investigations of fungicide residue levels in honey have found some high levels but although most are generally at a low level, consumers are expressing concern.

Coulson (UK) asked in the increase in incidents seen in potatoes was due to exposure to aphid honeydew. *Brasse* (DE) confirmed that this was the case but said that in some cases, honey bees must have visited potato flowers for pollen collection, which is very unusual.

The death of honey bees (*Apis mellifera* L.) and environmental pollution by pesticides: the honey bees as biological indicators (Porrini, C., Sabatini, A.G., Girotti, S., Fini, F., Monaco, L., Celli, G., *Bortolotti, L.*, Ghini, S.). Six levels of environmental monitoring with honey bees were outlined, ranging from communication from beekeepers to the authorities to effects on pollination and then monitoring at increasing levels of complexity (and effort). In particular, from 1983 to 1986, a network of monitoring stations (>300) was operated in Italy, each comprising 2 hives fitted with dead bee traps. Pesticides were found in 581 bee samples, predominantly organo-phosphates (e.g. 15.6% dimethoate and 14.6% parathion). Since this time there has been no national monitoring but local programmes have been operated as here in the Bologna province. Improved methods have been incorporated into a pesticide monitoring protocol: new dead bee traps; a critical threshold of 250 dead bee/week/station requiring further analysis; chemical and pollen analyses; descriptions of foraging and crops present in affected area; data processing using an index of pesticide toxicity (taking into account toxicity and persistence). A graph was shown of the number of incidents found with different chemicals, identifying a number of key factors: unnecessary treatments e.g. dimethoate against aphids on wheat; the use of obsolete or hazardous chemicals e.g. methidathion; use of prohibited chemicals e.g. fenoxycarb; uses of chemicals where not approved e.g. lindane (only approved for stored products). This information can be used to guide growers and improve pest control strategies and an example was given where bee poisoning incidents had declined in the Forli province after training sessions had been given. It was concluded that honey bees can be killed when pesticides are mis-used or used incorrectly and that it was important to check Currently, in Italy there is no national network for monitoring poisoning incidents but the Italian EPA is promoting the creation of one and it is hoped that funds will be generated in the near future.

Titera (CZ) asked about the number of hives per station to which the threshold applied and *Bortolotti* (IT) confirmed that it is two but that it was not fixed and could vary and further, in reply to a question from *Wallner* (DE), that they were maintained by beekeepers who get paid for this. *Oomen* (NL) asked what is happening to the information generated by the scheme and *Bortolotti* said that it had not been published but was being used to help growers. *Mühlen* (DE) found the idea of monitoring very interesting but was concerned if 250 bees for the threshold was the right number as this was at the hive and reflects a high level of damage (as many bees will die in the field) and also there is the possibility of sub-lethal effects. *Bortolotti* agreed that there are now new types of pesticides with more subtle effects compared to 20 years ago when the scheme was established but that as always, there had to be a compromise between quality and cost.

Studies to improve the performance of dead honey bees collection traps for monitoring bee mortality (Porrini, C., *Medrzycki, P.*, Bentivogli, L., Celli, G.). Following on from the previous talk, the importance of

dead bee traps was emphasised both in relation to monitoring schemes as well as specific studies. Over the years a lot of traps have been developed and a brief review was given: some are reliable but complicated while others are simple but of low efficiency. One of the most commonly used is the 'underbasket' design, in which dead bees are collected at the hive. However, there are problems with this: mortality in the field is not accounted for and there may be loss of the collected bees e.g. due to predation. Work was described on the development of a new trap design, described as a 'barrier' trap. This comprises two sections, the 1st part being a barrier to trap the bees and the 2nd part for dead bee collection. The bees are forced to exit the hive through narrow holes, preventing undertaker bees from removing dead bees, which fall into the collecting section (a wire mesh basket to avoid predation), so that the bees are then free to exit through a further set of holes. The efficiency of the barrier trap was assessed by the daily introduction of 20 marked dead bees and making weekly counts of dead bees per trap. In two trials conducted under different conditions, the barrier trap was found to be more efficient (nearly 80%) then the underbasket design. Further trials are to be undertaken to refine and assess the traps performance. It was concluded that the barrier trap was an efficient design, capturing dying as well as dead bees, and was not affected by seasonal factors.

Mühlen (DE) said he was happy to talk about dead bee traps and was very interested in the developments presented but pointed out that the Münster design allows free bee flight whereas with the barrier trap it is more difficult. In a similar way, pollen traps can limit bee movement and thus affect colony development and he was concerned at their use in field trials. Also, there can be affects from neighbouring colonies as the bees rob them due to the loss of pollen. *Medrzycki* (IT) replied that the holes in the barrier trap are 6 mm in diameter (compared to 4.6 mm in pollen traps) and that the bees don't appear to have any difficulty getting through them. Also, in some areas there is a big problem with wasps, which try to remove dead bees and this had prevented the holes being any larger. *Mühlen* pointed out that dead bee traps only assess a proportion of bees dying at the hive but those dying in the field aren't addressed. One possibility is the Bee Scanner, which can count activity over many weeks but is very expensive. He suggested that research is needed to assess the significance of the loss of foragers in the field to the colonies. *Medrzycki* agreed but said this is an expensive option (also requiring a computer) and needs to be left in the field for several weeks. *Oomen* (NL) asked if it is possible to validate dead bee traps with the Bee Scanner and thought that it would be.

Discussion Session

Oomen (NL) reviewed the presentations on the bee monitoring schemes and asked if it was thought that in Italy it would be a good idea for the information to be passed onto the registration authorities. *Medrzycki* and *Porrini* (IT) agreed it would be. *Brasse* (DE) asked if there had been any incidents reported in relation to imidacloprid. *Tasei* (FR) said that he knew of no such information in France but that currently the monitoring scheme is limited to certain areas. This involves reports of incidents being made by apicultural technicians and then officials from the Plant Protection and Veterinary Services have to visit the site to take samples of plants and bees for pollen identification, disease and residue analysis. He said that he hoped to be able to report back on the development of this scheme at the next meeting. *Bortolotti* (IT) said that in Italy imidacloprid has not been included in their scheme as they have not had a sufficiently sensitive method of analysis. However, that they do now have a new method although this is not used all the time as it is too complex - bees are analysed when imidacloprid is suspected but no data is currently available. *Oomen* (NL) pointed out that these experiences stressed how important incident schemes are and that addressing the concerns expressed by French beekeepers with regards to pesticides might be helped by incident schemes to show that something is being done. *Fletcher* (UK) applauded the French efforts but pointed out that this still meant that only four EU countries had bee monitoring schemes. He urged more countries to join in and suggested that different countries could exchange information to help improve methodology. *Oomen* asked if other countries could visit the UK to see how the scheme works there and *Fletcher* said that they would be welcome. *Stadler* (ARG) added that they are starting a monitoring scheme in Argentina based on the UK and German models and that he thought it would be useful to exchange information with other countries.

SESSION 5: EFFECTS OF PESTICIDES ON BUMBLE BEES AND OTHER BEE SPECIES

Laboratory assessment of pesticide toxicity to bumblebees (Marletto, F., Patetta, A., *Manino, A.*). Work that had been carried out over recent years to develop laboratory methodology to assess pesticide toxicity to bumblebees was described. Five medium sized *Bombus terrestris* adults selected from two colonies (commercially reared) were introduced into disposable cages with plastic feeders, to avoid contamination. A number of insecticides and acaricides were tested – commercial products were tested at the highest rates on the label as dispersions in syrup (oral) or water (contact). In the oral tests, individual bees starved for 3 hours were placed in film canisters and a 10 µl drop of test solution placed near a hole in the base and the bee checked after 15 minutes (if not feeding, the bee was stimulated with sucrose solution). In the contact test, the bees were anaesthetised using dry ice (carbon dioxide), from which they showed good recovery, and dosed with aqueous test solutions containing a wetting agent (10 µl/bee). Indirect contact toxicity was tested by spraying 1.1 ml of a water dispersion onto the bottom of the cage using a hand-held sprayer and the bees introduced after air drying. The bees were removed from exposure after 3 hours. In all cases, mortality was assessed after 3, 6, 24, 48 and 72 hours and this was used to produce LD_{50} val-

ues (usually most mortality occurred within 24 hours) and these can then be used to calculate hazard ratios. Colony variability was assessed using 30 pairs of colonies (i.e. a total of 60) and the number of living and dead bees compared for each pair after dosing. In 5 out of the 30 pairs a significant difference was found and so it was considered necessary to take colony variability into account in these tests.

Van der Steen (NL) asked if bee size had been taken into account and *Manino* (IT) replied that only medium sized bees had been used and there were therefore no significant differences in size. *Van Vliet* (NL) asked about the comparative sensitivities of honey and bumble bees but *Manino* said that although they had some experience with honey bees they had only included bumble bees in the laboratory tests and so any differences weren't known.

An extended laboratory test to evaluate the effects of pesticides on bumblebees (*Incerti, F.*, Bortolotti, L., Porrini, C., Micciarelli, S., Sbrenna, G.). While bumble bees are an important pollinator of relevance to the environment and agriculture there are only a few studies assessing the effects of pesticides on them and hazard assessment often uses honey bee data. Given the morphological and biological differences between the two bee groups, work was carried out under the AMA project (funded by the Italian Ministry of Agriculture), to improve the understanding of pesticide effects on bumble bees. Following the sequential testing pathway, only limited semi-field and field work has been conducted and so the aim of the work described here was to develop a semi-field method. Large cages were used (0.5 m square and 1 m high), to allow room for the bees to fly, with two cages per test unit. Two cucumber plants (10-12 flowers/plant), sprayed with 25 ml of test solution, were placed in each cage when dry and then 30 bumble bees were introduced. Mortality, abnormal behaviour and temperature were recorded every 24 hours for three days. Five organo-phosphates, two pyrethroids an 2 azotorganics were tested, together with a water-sprayed control. The organo-phosphates showed the highest toxicity and the pyrethroids the lowest, while imidacloprid did not cause high mortality but reduced activity for several hours. A comparison of the results with those from acute contact toxicity tests showed a reduction in the level of effects. Residue ageing was also assessed by introducing the bees into the cages 24 hours after spraying and in some cases a marked reduction in toxicity was found e.g. dimethoate, while in others there was only limited change e.g. imidacloprid. It was concluded that bumble bees showed good adaptability to the cage test, which provided useful, reproducible results in addition to the usual laboratory toxicity tests. However, it was stressed that this is a first step and that further development and testing is required. A useful development for the cage tests would be to assess effects on colonies (e.g. brood development) but this would require the use of mini-colonies. However, the number of flowers present is not sufficient for larger numbers of bees so that supplementary feeding would be required and in addition there can be a problem with the production of suitable plants (timing of flowering).

Tasei (FR) asked what behavioural observations were made and whether these could identify repellency effects. *Incerti* (IT) replied that only foraging was assessed but that repellency could be observed with these. *Schmuck* (DE) thought that there could be differences in the spray deposition between laboratory and field and suggested that the application could be made outside. *Incerti* agreed that this might be the case but pointed out that they were to trying to assess field effects.

Teflubenzuron effects on the red mason bee (*Osmia rufa* L.): a preliminary test set up in microcosm (*Ferrazzi, P.*, Elia, E.). Solitary bees (*Osmia* spp.) play an important pollination role in orchards and glasshouses, although not much is know about this. Accordingly, the effects of an IGR insecticide, Nomolt (a.i.: teflubezuron) has been investigated using a terrestrial microcosm. This consists of two cells (1.5 m^3 each) for the control and test treatments, respectively. Biological communities comprising detritus consumers, primary producers (*Trifolium repens* and *Phacelia tanacetifolia*) and primary consumers (*Osmia rufa*) were established in the cells. Eleven *Osmia* males (just emerged) and 15 female cocoons (about to emerge) were introduced into each cell and then a solution of 1.5 ml Nomolt/l was sprayed on the vegetation. The results showed a significant difference between the control and treated cells: only five bees emerged in the Nomolt cell, compared to 13 in the control (although this difference diminished due to high female mortality in both cells). While the males appeared to be more active, differences were seen in their numbers in the later assessments. Behavioural differences were also seen, with no nest visiting seen in the Nomolt cell as well as other effects e.g. involuntary mandible trembling and repeated cleaning movements. One problem that had been identified with the test method was mortality caused by bees drowning in the water provided (even though cork floats had been added) and this aspect needed to be addressed. It was concluded that previous work with IGR compounds had not identified damage to adults and in particular, behavioural effects of pollutants on *Osmia* had not been considered and this work has shown this should be considered. It was pointed out that this is preliminary work and that work was necessary to better control the environmental parameters (e.g. humidity), which could influence *Osmia* mortality.

Method to evaluate the interference of pesticides on pollination activity of *Bombus terrestris* to protected cultures (*Nucifora, S.*, Vasquez, G.). *Bombus terrestris* has been used in Sicily with glasshouse crops since 1990 but since that time the need for farmers to use insecticides has increased. However, there are no procedures available for this use when bumble bees are present and results obtained from field trial have been varied and contrasting. The aim of this work, therefore, has been to identify safety intervals that farmers should observe after application before releasing bumble bees.

Testing was carried out in plastic greenhouses of about 500 m^2 containing cherry tomatoes and various aspects of the methodology were discussed. Each experiment was conducted with three different safety intervals and a control and the applications were timed to give the required periods so that the bumble bees were released at the same time in all treatments. Observations were made at intervals of flower visitation frequency, mortality, flying activity (continuous monitoring using cameras) and hive activity (using BeeScan). Imidacloprid was tested with safety intervals of 1, 2 and 3 weeks, while spinosad and indoxacarb were tested with intervals of 24 hours and 3 and 7 days. The results for imidacloprid showed a marked reduction in the percentage of flowers pollinated but that this effect was reduced with increasing safety interval. In the case of spinosad and indoxacarb there was no difference in the percentage of flowers pollinated compared to the control, no effect on flying activity (over the first five days) and no increase in mortality (dead larvae). It was concluded that the pollination of tomatoes should not be started until three weeks after the application of imidacloprid but that *B. terrestris* could be released 24 hours after the application of spinosad and indoxacarb (and shorter intervals might be possible).

Bortolotti (IT) asked if it is possible to remove the colonies when the brood is checked to get an accurate picture of any effects. *Nucifora* (IT) said that this could only be done at the end of the study. *Bakker* (NL) noted that in the results there had been two columns, labelled 'total flowers' and '% pollinated' – he asked what the total numbers are and how the percentage pollinated is assessed. *Nucifora* replied that the number of flowers could vary between the treatments depending on how much the crop had grown and so a count was made in each case and the number visited determined by assessing bite marks on the flowers. *Schmidt* (DE) asked about the product rates that had been applied to the foliage and also about the timing of application in relation to flowering. *Nucifora* said they were applied during flowering and that recommended rates were used (although in the case of spinosad, which is not registered in Italy, the recommended rate from other countries was used). *Scalabrino* (IT) wanted to know if the treated and control plots of tomatoes had been in the same greenhouse and *Nucifora* confirmed that this was the case and that they had been separated by a cleared area and a mesh screen. She then asked if this was sufficient to prevent contamination of the control colonies and *Nucifora* said that it was sufficient for separating the bees.

SESSION 6: CONTROL OF BEE PARASITES

The effects of antagonistic micro-organisms on the brood of honeybees (*Apis mellifera* L.) and bumblebees (*Bombus terrestris* L.) (*van der Steen, J.J.M.*, Dik, A.J.). The use of honey and bumble bees to transport spores of micro-organisms, which are antagonistic to plant pathogenic fungi, from the hive into flowers has been considered. However, it is inevitable that these micro-organisms would enter the brood nest and that they could have harmful effects. A brood test has therefore been developed for both bee groups. In the honey bee brood test, 5 µl of sucrose solution containing a known amount of the micro-organisms and a cultivation medium were introduced into individual marked brood cells containing eggs, young larvae or old larvae (25 cells of each stage). The cells were checked at intervals up to 23 days to check development and emergence and samples of larvae and pupae had been rinsed, homogenised and cultured on growth medium to check for the presence of micro-organisms. In the bumble bee brood test, it was not possible to treat individual brood cells and so 2 ml of the test solutions was sprinkled over the brood nest after removing the queen so that no new brood was produced. A second application was made after 7 days, resulting in exposure of different brood stages. Samples of brood were taken during the larval and pupal phases and also of adults at intervals reflecting the emergence of the different stages initially exposed (all adults were removed on these occasions so as to isolate the different phases of emergence). The larvae were rinsed then homogenised and cultured on a growth medium, while the adults were rinsed and the water inoculated on the medium, while their fat bodies dissected to check for the presence of micro-organisms. Micro-organisms could not be cultivated from either the treated honey bee or bumble bee brood and emergence occurred within the normal time period with no dead brood remaining. It was considered that honey bee colonies show a natural resistance to micro-organisms (e.g. due to pH of food, high sugar concentrations etc.). It was recognised that the bumble bee test is cruder than the honey bee test and that it would be better to infect and monitor development of individual cells. It was suggested that a new working group is needed to develop such a bumble bee brood test. It was concluded that honey and bumble bees could be used to safely spread the antagonistic micro-organisms.

Becker (DE) asked if the spread of micro-organisms in relation to the field had been investigated but *van der Steen* (NL) replied that this work had not considered this aspect. *Oomen* (NL) wanted to know more about the micro-organisms and what they were antagonistic against. *Van der Steen* said that he couldn't reveal this information because of commercial sensitivities. *Scalabrino* (IT) asked if the size of the emerging adults had been affected but *van der Steen* said that this hadn't been assessed.

***Varroa destructor* resistance to pyrethroid treatments in the UK** (*Thompson, H.M.*, Ball, R.F., Brown, M.A., Bew, M.H.). The *Varroa* mite was first detected in the UK in 1992 and by 2002 was widespread throughout the mainland. There are currently two registered treatments (both pyrethroids): Apistan (a.i.: tau-fluvalinate) and Bayvarol (a.i. flumethrin). These have now been used for 20 years in the EU and so there is the possibility of resistance, especially as beekeepers may put in more strips than recommended. Routine monitoring was started in 2000 for the detection of resistance as part of

a policy of contingency planning. A field kit has been developed, which contains Apistan bee strips at a lower concentration than used with bees. Test kits have been issued to beekeepers so that they can test their own colonies (with all data sent to the Central Science Laboratory) and a monitoring system has also been established for Bayvarol. The results from the field are confirmed with laboratory bioassays in which mites are exposed to paraffin wax impregnated with pyrethroid and then put into Petri dishes with bee larvae for food for 24 hours. A dose-response relationship for the varroacide is produced for individual apiaries. Resistance was first detected in August 2001, but due to the monitoring this was before widespread colony collapse had resulted and is currently confined to south-west England. A typical dose-response curve for a fluvalinate resistant strain shows a reduced response by a factor of 10 compared to a susceptible strain. The other available treatment, flumethrin, shows similar response levels. However, the dose-response curve for coumaphos, amitraz and cymiazol indicates that there is no cross-resistance, so there are three treatment options that could be used for the resistant mites. Current work is designed to establish if there is a mutation in the sodium channel gene of the pyrethroid resistant *Varroa* mites (as found in the US). This would allow the development of a rapid and sensitive DNA-based test enabling early detection as pat of a resistance management strategy to prolong the life of varroacides.

Tasei (FR) asked if any natural resistance of honey bees to the *Varroa* mite had been found as has been claimed to occur in France. *Thompson* (UK) said that while it is possible there is currently no evidence for this in the UK but in South Africa they leave the bees alone and do not treat them in order to try and select resistant bees. *Schmidt* (DE) suggested that pyrethroid residues might be deposited in wax at low concentrations and as it would not be effective against the mites could lead to resistance. He therefore asked if they have sampled wax from the colonies for residue analysis. *Thompson* replied that they would always recommend regular exchange of wax as part of good beekeeping practice in order to limit the development of diseases.

Discussion Session (including Working Groups)

Before the discussion session started, the prize for the best presentation from a young scientist was made: the first prize went to Edith Ladurner and equal second prize went to Laura Bortolotti and Piotr Medrzycki. James Devillers was thanked for his generosity.

Oomen (NL) introduced the discussion session by suggesting that the two sessions (no. 5 - effects of bees on bumble bees and other species and no. 6 - control of bee parasites) be kept separate. He briefly summarised the papers that had been presented in session no. 5 and then pointed out that the protection of bumble bees in the past had been on the basis that they showed the same sensitivity to pesticides as honey bees and so were equally protected. However, this hypothesis had not been rigorously tested and he asked if it would be possible to do so. *Manino* (IT) said that there are differences between bumble and honey bees in terms of the toxicity of pesticides and their behaviour in field and glasshouse crops. He thought that experiments should be carried out in these environments to take into account these differences. *Brasse* (DE) thought that while we have many years of experience with honey bees, it would be worthwhile to include bumble and solitary bees in national and EU regulatory testing in order to have a complete test system. However, he pointed out that it was important to link this to an assessment of risk e.g. with the '3-step' system used for honey bees before being introduced into regulatory procedures. *Oomen* also thought that it would be useful to collect information at all three levels in order to compare laboratory and field effects and then consider appropriate risk assessment and management. *O'Leary Quinn* (UK) agreed it would be important to have a testing system but thought that it is also important to know if there is a need for this information e.g. a comparison of toxicity and exposure between honey and bumble bees to see if testing is necessary.

Oomen said that EPPO has asked if the honey bee protection scheme can be extended to other pollinator species. He suggested that the Bee Protection Group could do this using a working group to extend honey bee risk assessment and management to the other groups. A bumble bee group had worked in the past and he suggested re-starting this, again under van der Steen, to compare the bee groups and come up with a proposal for them. *Van der Steen* (NL) thought that there is a big difference between honey and bumble bees and that there should thus be separate testing. He agreed to lead a working group to look into this. However, *Bakker* (NL) was not sure what the objective for such a group would be – there is a well developed role for honey bees and non-target arthropods and now it is proposed to extend the groups to include other pollinators. He asked why bumble bees could not be considered as a large mite or *Aphidius* and be included in the non-target arthropods. *Oomen* said that on this basis the honey bees could also be included in the non-target arthropod scheme but that while this is designed to protect arthropods in general, the honey bee and pollinator scheme is designed to protect the pollinator function.

Miles (UK) agreed that it is useful to look at other pollinators but pointed out that in other non-target groups, an indicator species is used together with a safety factor, to represent all relevant species. Before considering adding additional species to the regulatory scheme we should be clear where we are going and first consider if the honey bee is sufficiently robust for the group as a whole. *O'Leary Quinn* agreed that this is a useful point to discuss and said that in the UK, research is being carried out to see if *Daphnia* represents other aquatic invertebrates. It would be similarly useful to see if interspecies variation between pollinators species could be covered by honey bees and also if exposure is suffi-

ciently different to need consideration of other species. *Brasse* proposed that only those species which are actively introduced where pollination is needed should be considered and species from other systems should be excluded e.g. syrphids (which have a link to the non-target arthropods). *Oomen* agreed that honey bees are the main species for risk assessment but the aim of the regulatory concern is to protect pollinators against undesirable effects of pesticides and so we should consider other species on this basis. The following people offered to join van der Steen in this group: Thompson (UK), Brasse (DE), Bortolotti (IT), Ladurner (IT) and Stadler (ARG). It was agreed that the working group would report back to the Bee Protection Group at the next meeting.

Oomen then turned to the second session on bee parasites and said that he was surprised that there was only one paper on *Varroa*, looking at the resistance to pyrethroids in the UK, and asked if similar problems had been experienced in other countries. *Van der Steen* said that while Apistan could be used in the UK all year round, in the Netherlands he thought that it could only be used in winter. *Thompson* (UK) said that as far as she was aware varroacides could be used all year in the EU but beekeepers were advised to monitor and only use when mite numbers had passed a threshold. She pointed out that if colonies entered winter in a weakened state this could lead to serious problems and any treatment could be too late. *Stevenson* (UK) was also surprised that there had only been one paper on *Varroa* as he considered this was an important problem, which should not be ignored and he encouraged further investigation. *Oomen* pointed out that this was an EU-wide problem and information should be shared e.g. on alternative treatments. He suggested that a working group dealing with *Varroa* control, resistance etc should be set up and asked who would be prepared to join. *Wallner* (DE) said that resistance control had been introduced in one area of the Rhine valley and they were now trying to extend this. *Titera* (CZ) said that Amitraz had been used throughout the Czech Republic for 20 years, although since 1992 only in November and December and no resistance had been found up to now. *Chauzat* (FR) said that he was just starting to work with *Varroa* so couldn't join at the moment but he would keep in touch with Thompson. *Oomen* asked if he might be able to find another colleague and *Charriere* (CH) said that he has been working in *Varroa* control for 15 years and would like to join. In response to *Oomen* asking about Italy, *Manino* said that in Italy there was resistance to fluvalinate and coumaphos so it would be a good idea to collaborate. Thompson said she was prepared to lead the group and it was agreed that it would report back at the next meeting.

Oomen asked the meeting if it considered that the Bee Protection Group and its work was considered to be of continued importance (certainly this was indicated by the attendance here). This was agreed together with the need for a symposium to be held every 3 years as at present. He then asked that if there was a need for any further working groups, in addition to the two already set up, and specifically he mentioned the issue of imidacloprid, discussed earlier and the concerns of French beekeepers. Issues concerning bee health had been reported from France, Italy, Canada and other countries but the problem was not well defined or understood and a working group could be set up to address this topic. *Stevenson* said that there had been a lot of progress in the first session on this topic but that governments and beekeepers needed more guidance and a working group from this meeting could make a valuable contribution.

Oomen suggested that as the issue of bee health had been first identified in France, a French leader might be appropriate and he asked Tasei (FR) if he was prepared to do this and how it might approach the work. *Tasei* replied that he could start the work but would then wish to pass the leadership to another person. He agreed that it was necessary to look at all possible factors and pointed out that a lot of expert support would be needed e.g. to assess any role pathogens might have. As this group was specialised for pesticides he was not sure what its scope would be. *Medrzycki* (IT) said that there was a similar problem in other countries and this emphasised the need to share knowledge in order to define the problem and then look for the causes. *Giffard* (FR) pointed out that a group addressing this problem already existed in France and that it might be better to invite this group to give their opinion rather than investing in a new group. *Tasei* added that he was in the French group and that it was willing to accept external advice so the ICPBR group would be very useful. *Brasse* emphasised that the working group should not only consider imidacloprid but also other systemic pesticides. The aim of the group would thus be how to regulated compounds with high toxicity and high systemic activity. However, *Stadler* thought that it wouldn't be possible to prove the cause of the decline in colony health if we didn't know the origin of the cause (pesticide and/or others). This would need the creation of a working group to study the side effects of systemic pesticides e.g. to develop new methodologies etc. *Medrzycki* and *Sabatini* said that the Apicultural Institute in Bologna is now working all over the country with the support of the beekepers associations and that they would be very interested in working with the group. *Kemp* added that he and Dr Rogers would also be happy to join and share ideas. *Oomen* said that a conclusion on this issue would be reached after lunch.

Oomen proposed one further working group to address the issue of bee monitoring schemes. *Fletcher* said that he would be prepared to co-ordinate this group and asked who would be interested in joining. The following people offered to join: Tasei (FR), Chauzat (FR), Medrzycki (IT), Kemp (CAN) and Stadler (ARG). Again, it was agreed that the working group would report back to the Bee Protection Group at the next meeting.

SESSION 7: EPPO TEST GUIDELINE

Honey bee (*Apis mellifera* L.) testing in southern Europe: from the laboratory to the relevant crop in

the field (*Tornier, I.*, Kling, A., Schur, A.). Honey bee testing is carried out in northern Europe using *Apis mellifera carnica* and in southern Europe using *A. mellifera mellifera*. Laboratory tests had been carried out according to OECD 213/214 to see if there were any differences in the toxicity of dimethoate between the two types of honey bee. Oral and contact acute toxicity tests were conducted in Germany (*mellifera*) and Spain (*carnica*). In all cases, control mortality was less than 10% and no significant differences were found in the susceptibility of the two types of honey bee. It was concluded that honey bee tests could be carried for most of the year i.e. when seasonal constraints prevent testing in northern Europe it is possible to carry on testing in southern Europe. However, it was pointed out that this would require a change to the test guidelines, which currently recommend that the collection of bees should be avoided in early spring and late autumn but this is the best time in southern Europe. Cage tests have also been conducted in southern Europe using *A. mellifera mellifera* and a standard flowering crop (*Phacelia tanacetifolia*), which begins growing in February allowing an experimental phase between April and June. In addition, cage tests have been conducted using relevant crops e.g. citrus, apples and sunflowers. Similarly, field test have been conducted in southern Europe with *A. mellifera mellifera*, again using *Phacelia* or relevant crops e.g. apple, citrus, melon, plum and peach. The main difference between testing in the south, compared to the north, is that a lot of protection is needed as the bees are more aggressive. It was concluded that honey bee testing according to the sequential scheme is possible in southern Europe, although some aspects of EPPO 170 need to be amended.

Brasse (DE) asked if every crop can be considered as potentially relevant and *Tornier* (DE) replied that yes, although it must be attractive to honey bees to qualify.

Registration of systemic insecticides and EPPO guidelines (*Tasei, J-N.*, Pham-Delègue, M-H., Belzunces, L.). As a result of the concerns of French beekeepers about systemic seed treatments possibly endangering bees, attention was being given to ways of addressing this issue in the regulatory and test procedures. A review of the development of regulatory bee protection in Europe and France was presented. A number of general remarks about systemic insecticides were made, particularly in relation to their increasing use and the study of their effects on bees. A number of questions were raised by consideration of systemic activity: do foraging bees experience chronic exposure to low doses when visiting flowers; do they experience sub-lethal effects (e.g. behavioural, adult longevity, queen quality, larval survival, synergy); are there appropriate test methods available. In the EPPO decision-making scheme, indirect effects (e.g. systemic activity) should be assessed using special tests. Possible laboratory tests were reviewed, starting with the standard dose-response (LD_{50}) tests. Larval survival could be assessed using brood feeding tests, e.g. the Wittman test in which larvae are fed in individual cells. Adult longevity and other sub-lethal effects could be investigated using oral sub-chronic tests in which bees are kept in small cages. The learning process can be assessed in a conditioned proboscis extension test. If negative effects are seen in these laboratory tests, then a higher tier risk assessment is necessary, using cage or tunnel tests to assess brood survival, sub-chronic mortality and altered foraging behaviour. It was concluded that systemic seed treatments are used more widely than in the past, resulting in increased exposure of colonies to low concentrations of new active ingredients resulting in the possibility of sub-lethal effects. As a result, special laboratory and semi-field (cage) tests that are relevant to this mode of action and that have been validated are needed. This work is underway in France and it is hoped that there will be discussion and collaboration with other European scientists.

Becker (DE) wondered how a negative effect with a proboscis extension test would be described as if bees avoid an odour this could result in repellency i.e. not harmful. *Tasei* (FR) emphasised that the reduction in response measured in the proboscis extension test did not necessarily reflect a change in bee behaviour and validation would be needed in a cage test to relate this to any reduction in flower visitation. *Schmuck* (DE) asked if a field step was also included and *Tasei* replied that in the French Bee Protection Group it was considered that there were problems with interpreting field trials and so it was more reliable to only used tunnel tests. *Schmuck* responded that field trials assess whether there are any negative effects under practical conditions of use and that this was a reasonable final step for regulatory purposes. *Tasei* agreed that field trials were needed but that when they had considered their use in 1998 it was felt that the appropriate standard test conditions were not yet available. *Schmuck* pointed out the concerns about imidacloprid had come from the field but no effects had been seen under controlled field conditions and asked why this was not acceptable. *Tasei* said that this was why the French Ministry of Agriculture had set up an enquiry to consider as many factors as possible.

Brasse (DE) suggested that as the proboscis extension test has a behavioural endpoint there is too much scope for interpretation and asked what the basis is for maintaining consistency. *Tasei* replied that this depends on the production of a standardised and validated protocol. *Brasse* said that if the proboscis extends, it is not clear if this is a negative or positive response and that this laboratory test would need to be related to foraging behaviour. *Oomen* (NL) had similar reservations about the difficulty of interpreting this specific test and deciding if affects are acceptable or not. He pointed out that in the EPPO guideline, systemic activity can be assessed using cage tests or field trials with intensive exposure in order to assess the risk. *Schmidt* (DE) added that foraging is subject to a number of cues e.g. visual, and that proboscis extension could lead to induced foraging. He wanted to know how the proboscis extension test could be used to identify possible damage to colonies. *Tasei* replied that a threshold level of reduction could be related to a

significant reduction of foraging but that this would need to be validated in cage tests. *Brasse* agreed that the test could be used if a standardised protocol is produced that is validated against semi-field trials.

Tornier (DE) pointed out that only field trials provide normal conditions for bees and that in laboratory and semi-field tests their behaviour is affected. Also, brood effects need to be seen in field. He recalled that the IGR working group did not recommend the Wittman test, as it could not easily obtain consistent results. *Tasei* said that they recognised that the test was not easy but pointed out that some North American workers have produced good results with a modified design. *Candolfi* (CH) suggested that a series of ring-tests could be conducted to validate the test but this would take some time. He asked when the ideas that had been presented would be translated into regulatory requirements in France. *Tasei* replied that the Ministry of Agriculture had agreed to the larval test, as they wanted laboratory data on larval susceptibility, but this would depend on the ability of test facilities to provide this.

Discussion session

Oomen (NL) introduced the final discussion session by reminding the meeting that the EPPO test methods had been revised in 2001, incorporating the changes proposed at the Avignon meeting and it is expected that this version will last for several years. Also, the decision-making scheme would be published at the end of the year after final administrative checks. He noted that following the presentation from Tornier, some final changes would be needed to accommodate southern European testing. He then asked what the situation in France is with regards to the EPPO guidelines. *Tasei* (FR) noted that the EPPO guidelines refer to special tests e.g. for IGR compounds, behavioural effects etc. He would like to have methods approved and recommended for these special tests, although he recognised that some were more important than others and this would need prioritisation. *Oomen* replied that new methods should be developed and validated and can be included in the EPPO guideline references. *Brasse* (DE) thought that the term 'recommend' is too strong as he considered that the main emphasis in the scheme should be on the three-stage assessment and that special effects should be kept separate and included as needed. *Schmuck* (DE) asked what the criterion is for a method to be included in the EPPO guideline references. *Oomen* replied that it would need to be demonstrated to the Bee Protection Group meeting that a method is reliable and reproducible. There would also need to be a basis for evaluating the results. *Schmidt* (DE) said that the main emphasis should be on the sequential testing scheme (laboratory – semi-field – field) as special tests would not be able to show if colonies will be harmed.

Finally *Stevenson* (UK) thanked Oomen for chairing the discussion sessions and reminded the presenters of the timetable for the report. With regards to the next meeting, he said that a number of options are being considered, particularly in countries that had not hosted the meeting before (e.g. eastern Europe, Greece etc) and that everyone would be informed of the venue in due course. He thanked the people from Avenue Media who had worked hard maintaining the administrative functions of the meeting so effectively. He also thanked our Italian hosts for organising such an enjoyable meeting and he presented Sabatini, Bortolotti and Porrini with gifts in appreciation of their efforts. *Oomen* then reminded the meeting that this was the 8th symposium of the Bee Protection Group and that the Chairman, John Stevenson, had been involved in all of them and he presented him with a gift to thank him for all his work. *Stevenson* closed the meeting by saying that he hoped to see everyone in three years time.

MEETING RECOMMENDATIONS

A number of working groups were set up over the course of the meeting to address specific issues that it was felt needed further consideration:

(1) Following the earlier discussions, it was agreed that a working group should be set up to look at the problem of declining bee health currently being experienced by beekeepers in a number of countries. The objectives of the group would be: (1) define the nature of the problem; (2) investigate all possible causes and identify critical factors. *Kemp* (CAN) agreed to co-ordinate the group and the following people offered to help him with its work: Chauzat (FR), Stadler (ARG), Tasei (FR), von der Ohe (DE), Brasse (DE), Porrini (IT) and Sabatini (IT).

(2) While it was recognised that honey bees are the main species for risk assessment, as the aim of regulatory concern is to protect pollinators against undesirable effects of pesticides, it was considered appropriate to consider other species as well. The following people offered to join van der Steen as co-ordinator of a working group to address this issue: Thompson (UK), Brasse (DE), Bortolotti (IT), Ladurner (IT) and Stadler (ARG).

(3) A working group was set up to address the issue of bee monitoring schemes. This group will be co-ordinated by Fletcher (UK) and the following people expressed an interest in joining: Tasei (FR), Chauzat (FR), Medrzycki (IT), Kemp (CAN) and Stadler (ARG).

(4) The control of *Varroa* was seen as an EU-wide problem and that information should be shared e.g. on alternative treatments. It was agreed that a working group dealing with *Varroa* control, resistance etc should be set up and *Thompson* (UK) agreed to lead this. Wallner (DE), Chauzat (FR), Charriere (CH) and Manino (IT) agreed to join this group.

It was agreed that the working groups would report back at the next Bee Protection Group symposium in 3 years time.