Hazards of pesticides to bees - 10th International Symposium of the ICP-Bee Protection Group

References

- Babendreier D., Kalberer N. K., Romeis J., Fluri P., Mulligan E., Bigler F. (2005). Influence of Bt-transgenic pollen, Bt-toxin and protease inhibitor (SBTI) ingestion on development of the hypopharyngeal glands in honeybees. *Apidology* 36: 584-594.
- 2 Deseyn J., Billen J. (2005) Age-dependent morphology and ultrastructure of the hypopharyngeal gland of Apis mellifera workers (Hymenoptera, Apidae). Apidology 36: 49-57.
- 3 Gupta P.R., Chandel R. S. (1994) Effects of diflubenzuron and penfluron on workers of Apis cerana and Apis mellifera L. Apidology 26: 3-10.
- 4 Huang Z. Y., Otis G. W. (1989) Factors determining hypopharyngeal gland activity of worker honey bees (*Apis mellifera* L.). Insectes sociaux 36(4): 264-276.
- 5 Hrassnigg N., Crailsheim K. (1998) Adaptation of hypopharyngeal gland development to the brood status of honeybee (*Apis mellifera* L.) colonies. *Journal of insect Physiology* 44: 929-939.
- 6 Malone L. A., Todd H. J., Burgess E. P. J., Chriteller J. T. (2004) Development of hypopharyngeal glands in adult honey bees fed with Bt toxin, a biotin-binding protein and protease inhibitor. *Apidology*, 35: 655-664.
- 7 Pernal S. F., Currie R. W. (2000) Pollen quality of fresh and 1-year-old single pollen diets for worker honey bees (*Apis mellifera* L.). *Apidology* 31: 387-409.
- 8 Sagili R. R., Pankiw T., Zhu-salsman K. (2005) Effects of soybean inhibitor on hypopharyngeal gland protein content, total midgut protease activity and survival of the honey bee (*Apis mellifera* L.). *Journal of insect Physiology* 51: 953-957.
- 9 Sagili R. R., Pankiw T. (2007) effects of protein-constrained brood food on honey bee (*Apis mellifera* L.) pollen foraging and colony growth. *Behavioral Ecology and Sociobiology* 61:1471-1478.

V. Honey bee poisoning incidents and monitoring schemes

Review of honeybee pesticide poisoning incidents in Europe – evaluation of the hazard quotient approach for risk assessment

Helen M Thompson^{1*}, David Thorbahn²

¹CSL, Sand Hutton, York, UK YO41 1LZ

² Biologische Bundesanstalt für Land- und Forstwirtschaft, Untersuchungsstelle für Bienenvergiftungen, Messeweg 11-12, 38104 Braunschweig, Germany

*email: h.thompson@csl.gov.uk; Tel: 44 1904 462515; fax 44 1904 462515

Abstract

<u>Background</u>: Honeybee risk assessment is required in Europe for all pesticides where bees may be exposed. This is well established for sprayed products where the hazard quotient (HQ), calculated by dividing the application rate of the sprayed product active ingredient by the LD50, is less than 50 the product is considered safe to bees (unless it is an IGR). In the UK, Germany and the Netherlands post-registration monitoring schemes on the poisoning of honeybee by pesticides collate data on honeybee incidents.

<u>Results</u>: The incident schemes have been invaluable in identifying agronomic practices resulting in honeybee mortality and changes have been made to labelling to address such issues. The decrease in the numbers of incidents reported supports the assertion that such schemes have positively contributed to the regulatory process and also provide confidence in the risk assessment approaches.

<u>Conclusion</u>: This review of incidents in Europe over the last 25 years suggests that the HQ approach to risk assessment for honeybees offers an appropriate level of protection.

Keywords: honeybee, pesticide, hazard quotient, risk assessment

Introduction

In Europe EU Directive 91/414 requires honeybee risk assessment to be undertaken for all pesticides where bees may be exposed. For sprayed products the basis of the risk assessment is the generation of a hazard quotient (HQ) calculated by dividing the application rate of the sprayed product active ingredient by the contact or oral LD50 (whichever is the lower). The Directive then requires under 2.5.2.3: "no authorization shall be granted if the hazard quotients for oral or contact exposure of honeybees are greater than 50, unless it is clearly established through an appropriate risk assessment that under field conditions there are no unacceptable effects on honeybee larvae, honeybee behaviour, or colony survival and development after use of the plant protection product according to the proposed conditions of use".

The result of the calculated hazard quotient is therefore a key decision making point in the risk assessment process in determining if further work is required or the product can be safely used in the presence of foraging honeybees without further evaluation. In the UK, Germany and the Netherlands post-registration monitoring schemes have been established to permit reporting of suspected cases of poisoning of honeybees (incidents) and to inform the regulatory process. This paper reviews the incident data available over the last 25 years from these countries to determine the robustness of the currently used HQ value of 50.

Materials and methods

At the outset of the analysis it was determined that a pesticide incident (i.e. a report from a single use) would be the basis of the comparison with the HQ. An incident often included impacts on more than one colony (in some cases several hundred) but was considered a more robust measure of the impact of a pesticide than the total number of colonies affected which is directly linked to the number present at the time and related to the availability of suitable forage.

Data on the numbers of honeybee poisoning incidents and the pesticide residues detected were collated from countries where there were established monitoring schemes (the UK, the Netherlands and Germany). The details of the national schemes have been reviewed^{1.4}. There were no equivalent data available from other European countries. The data available for the UK spanned 1981-2006, for the Netherlands 1989-1998 and 2005-2007 and Germany 1985-2004. These data from the national co-ordination schemes clearly spanned the time period when Directive 91/414 was introduced and therefore provided data for a broad range of pesticides. The incidents were ascribed to pesticides only when residues of the pesticides were detected in bees or, in Germany only, other related materials such as plants on which the bees had been foraging.

There were also differences in national schemes in their approach to pesticide analysis. In Germany, herbicides and fungicides were routinely screened and used as a 'fingerprint' to establish a link between bee and plant material. In the UK, fungicides and herbicides were only screened for when field evidence suggested that they may be implicated. Thus, in Germany multiple pesticides were more likely to be detected in an incident and, whilst a reflection of the relative presence of the pesticides in incident samples, overestimated the true number of incidents directly resulting from its use.

LD50 data for honeybees and application rates were collated and hazard quotients for each pesticide calculated by dividing the application rate (g ai/ha) by the contact and/or oral LD50 (µg ai/bee). In most cases the crop was either not known or was not reported and therefore the application rate used in the HQ was the highest rate for which data were available (liaison.csl.gov.uk). This application rate was unlikely to be significantly different from that actually used at the time although it must be recognised that if rates were higher it may be considered to underestimate the worst-case. The HQ for each pesticide was then compared to the reported number of incidents in which the pesticide was detected.

Results And Discussion

As with any reactionary scheme the incident data were dependent on the willingness of beekeepers to report incidents. It has been well recognised that the willingness of beekeepers to report incidents is affected by their perception of the scheme's/ regulators ability to solve any problems, the need to retain apiary sites and

the effect of any follow-up action on their relationship with landowner/farmer. In both the UK and Germany the reason, where known, for the incident was reported, i.e. normal use, misuse (often caused by misunderstanding of the label, e.g. use on flowering crop) or abuse (deliberate poisoning). However, in many cases, around 70% in 1999-2003 in the UK and up to 41% in Germany, the reason for the incident remained unknown due to problems in identifying both the crop that the bees were foraging on and the particular pesticide application. In the UK deliberate abuse of pesticides accounted for less than 5% of the reported incidents whereas in Germany from 2001-2006 the abuse cases represented up to 32% of the total. There were no reports of pesticide abuse in the Netherlands.

Data were collated (Table 1) for 74 pesticides with a wide range of modes of action. The number of samples in which residues were detected ranged from 1 to 1488 (sulphur). In the UK and Netherlands the number of incidents per year (Figure 1) appeared to be decreasing. For Germany, the graph represents the total number of incidents which were reported to the investigation office by the beekeepers. More than 50% of these incidents were caused by diseases as Varroa, Nosema, virus infections or by deliberate poisoning. The total number of incidents decreased from more than 400 per year at the beginning of the 1980s to less than 100 per year since the middle of the 1990s and remained on this low level except for the year 2003 with 178 incidents (including 645 bee and plant samples).

Pesticide class	Number of chemicals	Number incidents/samples	% incidents/samples
Insecticide	43	4250	
OP	21	2075	24.0
Carbamate	7	289	3.3
OC	7	1213	14.0
Pyrethroid	8	673	7.8
Herbicide	3	14	0.2
Fungicide	23	3418	39.5
Veterinary medicines varroacides ¹	4	964	11.1

Table 1 Classes of pesticides detected in incidents

¹coumaphos (40%), fluvalinate (18%), benzylbenzoate (13%) and bromopropylate (29%)



Figure 1 Reported pesticide incidents for the UK and the Netherlands and reported incidents ascribed to pesticides in Germany during the period 1981- 2006

Julius-Kühn-Archiv 423, 2009

Insecticides were detected in 49% of the samples with fungicides detected in 40%. Of the insecticides organophosphorus compounds contributed to 49% and organochlorines 29%. The major organophosphorus pesticide residues were dimethoate (23%); parathion (17%); triazophos (14%); methyl parathion (14%) and phosalone (13%). The major contributor to the organochlorine related residues was gamma HCH (53%) and in the pyrethroid class the major contributors were cypermethrin (42%); l-cyhalothrin (25%) and alphacypermethrin (16%). Of the carbamate residues detected 53% were fenoxycarb (an IGR which is relatively non-toxic to adult bees but results in brood mortality); 19% were bendiocarb and 14% were carbaryl. Carbaryl was banned in Germany in 1982, as it had caused the serious losses of honeybee populations in German vine growing areas and was involved in more than 50% of the incidents at that time

The most frequently detected pesticides in incidents reported in each country were sulphur, lindane/gamma HCH, vinclozolin and coumaphos (Germany); dimethoate, parathion and methyl parathion (the Netherlands) and triazophos, dimethoate. lindane/gamma HCH and bendiocarb in the UK. Of these sulphur was used as fertiliser and fungicide in agriculture and against the waxmoth in apiculture. Coumaphos was used as a varroacide by beekeepers. Lindane residues may derive from wax from non-EU countries where it is still used in agriculture. In Germany it was banned in 1977 (West) and 1990 (East), respectively. Vinclozolin is a fungicide of low toxicity to honeybees (>200 µg /bee) that may be present in incidents with other pesticides.

Bendiocarb contributed more than 50% of the reports related to carbamate insecticide use and all were in the UK. Use in the UK is restricted to the treatment of feral bee and wasp nests and poor sealing of the treated colonies is known to lead to robbing and mortality at colonies in the vicinity.

The number of incidents reported were compared with the calculated hazard quotient (HQ) values and shown in Figure 2. The low correlation coefficient (r^2 =0.03) showed there was no linear relationship between the HQ and the number of reported incidents but it is the threshold value of 50 that is of importance in this context. There were two pesticides which were detected in over 100 incidents but had HQ values below 50. These were captan with an oral LD50 of 91 µg ai/bee and application rate of 2900 g ai/ha (HQ 32) and fluvalinate with an LD50 of 4.8 µg ai/bee and application rate of 48 g ai/ha (HQ=10). All the other pesticides with HQs lower than 50 (9) showed less than 100 reported incidents over the 25-year period in all three countries.



Figure 2 Comparison of the total number of reported incidents involving pesticides and the hazard quotient (HQ) for each pesticide ($R^2 = 0.03$)

The high number of samples reported with fluvalinate residues (169 samples in Germany) was related to its use as a varroacide rather than as an insecticide. Fluvalinate residues have been regularly reported in the UK but residues were very low and not attributed to pesticide poisoning¹. If the incidents were caused by the agricultural formulation such as Mavrik which is applied at 48 g ai/ha and with an LD50 of 4.8 μ g ai/bee then exposure of the bee to over 17 μ l of the applied product would have caused mortality. This was

compared with a pesticide known to cause incidents - dimethoate, application rate 336 g ai/ha, volume 200 l/ha and LD50 0.12 μ g/bee - 0.007 μ l would have resulted in mortality.

Captan was a widely used phthalimide fungicide and therefore it is likely that as with the wide range of fungicides reported in samples (e.g. sulphur, vinclozolin) it purely demonstrated exposure. Based on an application rate of 2900 g/ha and an application volume of 200 l/ha this was equivalent to 14.5 g/ l or 14.5 μ g/µl. With an LD50 of 91 µg ai/bee a bee would need to be exposed to 6.2 µl of the applied product to result in mortality. In some cases, however, fungicide exposure may have increased the toxicity of other pesticides present, e.g. the pyrethroids. The azole fungicides are of particular concern in this regard and were reported in a total of 311 samples (9% of the fungicides³ and the 3 UK alphacypermethrin incidents and a single deltamethrin incident between 1994-2003 also contained fungicides¹.

Use of dimethoate on field beans and triazophos and gamma-HCH on oilseed rape were reported as the major causes of incidents and had high HQ values. The use of dimethoate on oilseed rape was withdrawn in the UK in 2000. All products using gamma-HCH were withdrawn in the UK in 2001 with all uses ceasing in 2003. In the Netherlands the use of dimethoate and parathion on potato crops to control aphids resulted in mortality due to bees foraging on aphid honeydew or flowering weeds within the crop. A similar issue related to bees foraging on aphid honeydew and flowers (including weeds) in potato crops occurred in Germany due to the misuse of bee hazardous products containing dimethoate, parathion, chlorpyrifos, cypermethrin or methamidophos. In these cases incidents occurred when bees were exposed to spray coating covering the honeydew which had become wet due to the humidity in the air the morning after the application. As a consequence the aphid control thresholds in potatoes were reduced and a publicity campaign was undertaken with farmers. In 2006 during similar weather conditions only 17 incidents in potatoes were reported instead of 119 incidents ascribed to potatoes in 2003.

The presence of several pesticides within a sample doesn't necessarily implicate all the pesticides in the incident but does demonstrate that bees may be exposed to a range of compounds whilst foraging on treated crops. In Germany up to 5 different pesticides were detected in samples of honeybees and up to 3 in plant samples⁴. In the UK pesticide mixtures were found in 6% of incidents where multiple residue detection was undertaken between 1981 and 1991 and 4% between 1994 and 2003.

The high hazard quotient of the pyrethoid insecticides would suggest that incidents would occur in the field due to their widescale use and a significant number of incidents have been reported. However, field studies have widely demonstrated that repellency to the pyrethroid applications occurs on treated crops and therefore far lower mortality than may be expected occurs in the field⁵). For some pyrethroid insecticides applied at low rates it has been demonstrated in large-scale field studies that when applied alone during bee flight no increased mortality or other impact on honey bee colonies occurred. The number of incidents may be due to application of tank mixes of pyrethroids with EBI fungicides during times when bees are actively foraging on the crop (the label recommends application early morning or late evening). Of the 11 incidents between 1994 and 2003 half contained residues of pyrethroid insecticides and fungicides. The EBI fungicides increase the toxicity of pyrethroid insecticides by blocking their cytochrome P450 dependent metabolism⁶. The increased toxicity of the combination may result in exposure to a toxic dose before repellency can become effective thus increasing the risk to the bees⁷. In Germany the number of incidents reported has resulted in labelling that either prohibits the use of mixing of pyrethroids and EBI fungicides generally during flowering or allows application during flowering but only in the evening after bee flight³.

Conclusions

This review of incidents in Europe over the last 25 years suggests that the HQ approach to risk assessment for honeybees offers an appropriate level of protection. The incident schemes have been invaluable in identifying agronomic practices resulting in honeybee mortality and changes have been made to labelling to address such issues, e.g. not using tank mixes of pyrethroids and EBI fungicides during bee flight, ensuring absence of flowering weeds in non-flowering crops, spraying early morning or late evening. Without these incident schemes honeybee incidents are likely to go undetected or the cause unproven. The decrease in the numbers of incidents reported supports the ascertain that such scheme positively contribute to the regulatory process and can provide confidence in the risk assessment approaches.

Acknowledgements

Beekeepers for sending in samples from suspected poisoning incidents; the UK Pesticides Safety Directorate for funding of the study (project number PS2335); Pieter Oomen, Plant Protection Service, PO Box 9102, 6700 HC Wageningen, The Netherlands for the provision of the data from the Netherlands and comments on a draft manuscript.

References

- Barnett, EA, Charlton, AJ, and Fletcher, MR, Incidents of bee poisoning with pesticides in the United Kingdom, 1994-2003. Pest Management Science 63: 1051-1057 (2007)
- 2 Oomen, PA, Honeybee poisoning incidents over the last ten years, as reported by beekeepers in the Netherlands. In: Hazards of Pesticides to Bees ed. Belzunces, LP, Pelissier, C, and Lewis, GB, Avignon France, INRA Les Colloques 98, pp129-135 (1999)
- 3 Brasse, D., Overview about the poisoning incidents in honeybee populations and their clarification in Germany 1996-1998. In: Hazards of Pesticides to Bees ed. Belzunces, LP, Pelissier, C, and Lewis, GB, Avignon France, INRA Les Colloques 98, pp141-147 (1999)
- 4 Seefeld, F, Chemical detection of damage to honeybees caused by pesticides Nachrichtenbl. Deut. Pflanzenschutzd. **58**: S59-66 (2006)
- 5 Brobyn, PJ, Possible synergistic effects on honeybees of pyrethroids and fungicides: the UK regulatory consideration In: Hazards of Pesticides to Bees ed. Belzunces, LP, Pelissier, C, and Lewis, GB, Avignon France, INRA Les Colloques 98, pp97-111 (1999)
- 6 Pilling, ED, and Jepson, PC, Synergism between EBI fungicides and a pyrethroid insecticide in the honeybee (Apis mellifera) Pestic. Sci. **39**: 293-297 (1993)
- 7 Thompson, HM, and Wilkins, S, Assessment of the synergy and repellency of pyrethroid fungicide mixtures. Bulletin of Insectology **56**: 1-4 (2003)

Periodical honey bee colony losses in Germany: preliminary results from a four years monitoring project

Martina Janke^{1*}, Peter Rosenkranz², Working group of German bee institutes³

¹LAVES Institut für Bienenkunde Celle, Herzogin-Eleonore-Allee 5, D-29221 Celle, Germany

² Universität Hohenheim, Landesanstalt für Bienenkunde, August-von-Hartmann Straße 13, 70599 Stuttgart ³ http://www.ag-bienenforschung.de

*phone: 0049(0)51419050356, fax: 0049(0)51419050344, e-mail : martina.janke@laves.niedersachsen.de

Abstract

Within the framework of the German Bee Monitoring Project winter losses of bee colonies were evaluated from the database of 120 beekeepers and 1200 bee colonies by assessing the following parameters: data on the apiary (site, nuclei, movement of colonies, *Varroa* treatment), strength of the colonies in autumn and spring, honey yields, residues in bee bread (stored pollen), bee disease analysis.

During the last four years the winter losses of the monitoring beekeepers were between 8 and 16% and showed regional differences. The loss rates were clearly lower than those of non-monitoring beekeepers.

In 215 bee bread samples analysed with a sensitive multi-method, more than 55 active ingredients were found. Most active ingredients were found in traces but often in combinations. Primarily fungicides, varroacides and herbicides were found. Clothianidin was not found in any sample. Imidacloprid was found in one sample at the limit of detection.