6.6 Monitoring effects of pesticides on pollinators - a review of methods and outcomes by the ICPPR working group

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Abstract

Background: Monitoring studies, in the context of Regulation (EC) 1107/2009, are recommended as a complement to the risk assessment. They are used to verify the conditions of exposure and of occurrence of risks in the field, as well as the efficacy of risk mitigation measures. No guidance is currently available for performing monitoring studies at the EU level, for honey bees or other pollinating insects. An inventory was thus undertaken in order to examine current methodologies and propose recommendations for the implementation and use of such studies in risk assessment and decision making.

Results: The inventory gathered 58 references, 41% on honey bees and 59% on wild bees. Monitoring studies in honey bees measure mortality, together with the occurrence of diseases, health status, and in some studies pollination. For wild bees, studies usually examine bee presence in relation to habitat, habitat changes or the influence of farmland.

Conclusion: This analysis indicates the need to shape monitoring studies on the basis of all the factors that influence the composition of bee communities within a landscape, including land use, floral diversity and agricultural practices. A first set of the critical traits for further monitoring studies is proposed for the two groups of pollinators.

Key words: pesticides, monitoring, honey bee, bumblebees, solitary bees, Regulation (EC) 1107/2009.

Introduction

Monitoring, in the context of the environmental assessment of Plant Protection Products (PPP) or pesticides, aims at getting feedback regarding the fate and/or effects of active substances and/or their relevant degradation products in/on the environment, when PPP are used under realistic conditions for crop protection. These studies complement the risk assessment performed in application of Regulation 1107/2009/EC and previously Directive 91/414/EEC[1,2], with the aim to characterise the conditions of exposure of organisms in the environment, the conditions of occurrence of risks and eventually to verify the efficacy of risk mitigation measures.

Monitoring effects of pesticides on honey bees has been getting more importance over the last five years and has been recommended along with approval decisions for some active substances[3]. There is however, no harmonized guidance on monitoring methodology for honey bees or other pollinating species, nor is there any guidance on the use of generated data in support of risk assessment or decision making.

This paper summarizes the work undertaken by the International Commission on Plant-Pollinator Relationship to review existing monitoring of the effects of pesticides on managed and wild bees and propose guidance on good monitoring practices.

Experimental methods

Focus of the inventory

Our inventory gathered studies being available as published data or as studies undertaken by regulatory authorities and industry and made available to the working group.

The literature review was performed using the following key words: *honey bee, bumble bees, bees, pollinator, pesticide, insecticide, monitoring, effects, residues, cultivated area, crops, agronomic area, agricultural landscape*.

Residue monitoring looking at the presence of pesticides, veterinary products and other compounds in bees and/or hive products were also considered, the study design being often similar to the design followed in effect monitoring studies.

Regarding wild bees, pesticide-focused monitoring studies were more limited than for honey bees and therefore the inventory was extended to research on the relationship between pollinator communities and their habitat, so that methodologies currently in use for monitoring purposes could be accounted for.

The studies retained in this inventory examined honey bees and other pollinating insects at the field scale or at the farm/landscape scale. Regional or national scale surveys were not included as they usually do not record pollinator fauna concomitantly to practices in the field or the farm practices, which makes it difficult to relate to a particular product or a practice, including the use of pesticides.

For each study, the materials and methods section was reviewed and the following variables were reported (Table 1):

Table 1: Parameters looked at in the review of existing monitoring studies on honey bees and other pollinating insects

Species	Information reported
Honey bees	Purpose of the study
	Country
	Year(s) when the study was performed
	Duration of the study
	Crops/area monitored
	Number of fields monitored per study/area sampled
	Surface of the fields/area monitored
	Variable recorded (including landscape variables)
	Sampling method for each variable recorded
	Expression of the results
Other pollinating insects	As for honey bees +
	Taxonomic level at which pollinating insects were recorded

All the parameters listed above were systematically evaluated and reported in our database. When no detail was provided in the paper or report on a parameter, it was described as “not addressed” in our analysis.

Results

Honey bees

The inventory gathered a total of 24 studies, performed between 2006 and 2014. In most cases the monitoring was implemented in one country, and two studies have implemented monitoring in several countries[4]. Ten countries in total were represented in this inventory (table 2).

Twenty of these studies monitored the effects and / or exposure of honey bees to pesticides. Nine of these studies focused on insecticides of the neonicotinoid group some of them were part of national monitoring requested by the European regulation[3,5-13]. Five studies looked at the potential effects of pesticides on bee health and conducted analysis of residues in bee products[5,9-11, 13, 14-17]. Residues were then monitored in bee matrices as well as in pollen, nectar and flowers[5,6, 14-16, 18-20].

The four remaining studies did not look at effects of pesticides on honey bees but rather aimed at describing patterns of their presence in cultivated landscapes[21-24].

Most of the studies covered a period of 1 (6 studies) or 3 years (6 studies), with observations running over a season or two per year. Five studies extended the observation time window to the overwintering period.

As regards the landscapes where these monitoring were undertaken, arable crops ranked first (9 studies), while orchards (3 studies) or forest areas (1 study) were less investigated. In arable crops 7 studies were implemented in maize cultivation, which was driven by the concerns related to neonicotinoid insecticides[3]. The remaining arable crops monitored, sometimes in the same projects, were oilseed rape (3 studies) and sunflower (1 study). The number of sites and area covered by the studies was not always documented in much detail.

In the studies that included “honey bee health” as an observed parameter, the term was not homogeneously defined. The variables recorded were mortality of adult bees, colony development, brood surface and brood quality. In half of these studies only, a dedicated disease analysis in colonies was undertaken together with other records.

An overview of the honey bee monitoring studies is provided in table 2.

Table 2 Parameters recorded in honey bee monitoring studies. Source: 24 monitoring studies performed between 2006 and 2014.

<table>
<tr><th>Parameter</th><th>Outcome for each parameter (and corresponding number of studies)</th></tr>
<tr><td>Purpose of the study</td><td>Effect of pesticides on honey bee health (7)
Exposure to pesticides and effects on honey bee health (5)
Residues analysis in bee/hive products (4)
Studies focused on one pesticide group (9)
Study of interaction/synergies pathogens (multi factorial studies) (4)</td></tr>
<tr><td>Country</td><td>United States (5), France (5), Germany (3), Italy (2), Austria (2), Belgium (1), Canada (1), Kenya (1), Spain (1), Switzerland (1)</td></tr>
<tr><td>Year(s) when the study was performed</td><td><table><tr><td>Year</td><td>2006</td><td>2007</td><td>2009</td><td>2010</td><td>2011</td><td>2012</td><td>2013</td><td>2014</td><td>ongoing</td></tr><tr><td>Nb of studies</td><td>1</td><td>3</td><td>2</td><td>3</td><td>4</td><td>1</td><td>2</td><td>4</td><td>4</td></tr></table></td></tr>
<tr><td>Duration of the study</td><td><table><tr><td>Study duration</td><td>One season</td><td>1 year</td><td>2 years</td><td>3 years</td><td>4 years</td><td>Not documented</td></tr><tr><td>Nb of studies</td><td>3</td><td>6</td><td>3</td><td>6</td><td>1</td><td>5</td></tr></table></td></tr>
<tr><td>Crops/area monitored</td><td>Agricultural landscape (6)
Arable fields (9), of which maize (7), oilseed rape (3) and sunflower (1)
Orchards (2)
Forest/agroforestry (1)</td></tr>
<tr><td>Number of fields monitored per study/area sampled</td><td><table><tr><td>Nb of sites</td><td>1 to 10</td><td>10 to 20</td><td>> 20</td><td>Not documented</td></tr><tr><td>Nb of studies</td><td>5</td><td>5</td><td>5</td><td>9</td></tr></table></td></tr>
<tr><td>Surface of the fields/area</td><td><table><tr><td>Surface monitored</td><td>m^2</td><td>ha</td><td>km^2</td><td>Not documented</td></tr><tr><td>Nb of studies</td><td>2</td><td>4</td><td>2</td><td>16</td></tr></table></td></tr>
<tr><td>Variable recorded (including landscape variables)</td><td>Colony health (8)
Colony development (8)
Residue analysis in bee matrices and pollen/nectar/flowers (8)
Overwintering (5)
Landscape variables (18)</td></tr>
</table>

Other pollinating insects

The inventory gathered a total of 34 studies, performed between 1998 and 2014. As for honey bees, the monitoring was implemented in one country, but four studies implemented monitoring in several countries. Seventeen countries in total were represented in this inventory.

All these studies were performed by research organizations and published.

Contrary to honey bees, monitoring of pollinating insects appeared to be mainly driven by interests in pollinator-habitat relationship (table 3)[21-22, 25-32]. In particular these studies evaluated pollinators' responses to habitat[33-43] or to flower[21, 29]. Factors behind spatio-temporal diversity[44-47] or distribution among pollinating species are also a major topic. Two thirds of the studies looked at one of the following aspects: inter-species competition[48], relationship of pollinator community to crop yields, or responses to habitat loss. One study was dedicated to sampling issues. Only three studies were dedicated to pesticide effects in cultivated landscapes[49-51].

As regards study location, the great majority of monitoring took place in agricultural landscapes (32 out of 34 studies). This involved cereals/arable crops (7 studies), vegetable or fruit crops (5 studies), orchards (3 studies) and vineyards (3 studies). The remaining studies were performed in other permanent crops, pastures or forests. One study was conducted in uncultivated fields and 2 in urban environments[52-53.]

Study duration ranged between 1 month and 20 years, most of the studies comprised between 1 season and 3-6 years (table 3). One season usually covers 3 to 6/7 months, depending on the crop. Protocols were also usually designed to allow for observations over the period of activity of pollinators within the crop/landscape studied.

The number of sites involved was highly variable amongst the monitoring and ranges from 1 to more than 20 sites per study although described in more details than in monitoring involving honey bees. A site was usually treated as a replicate. The size of a site ranged from 9 m^2 to several km^2, with a majority of studies monitoring sites in the range of hectares (22 studies).

The species and taxonomic groups being monitored are detailed in table 3. Community approaches were usually preferred although it could include a focus on *Bombus* spp [25, 26, 30, 31, 34, 44, 45] *Osmia* spp[42, 54] or *Megachile* spp[42, 48]. The variables recorded belong to common ecological indices, such as species abundance, species diversity, species richness as well as records of flower visits or foraging activity.

The environment or landscape was most often described, through a characterisation of the vegetation type (i.e land occupation with details on the use), species abundance, species diversity or species richness. In some cases it also included records of crop yields or crop pollination as in [21, 25, 43, 55]. The monitoring always reported parameters describing pollinator communities and/or populations and landscape descriptors, and analysis plotted against landscape descriptors in an attempt to explain patterns of pollinators' presence as a function of the presence of non-cropped area and food/habitat resource.

Table 3 Parameters recorded in pollinating insects monitoring. Source: 34 monitoring studies performed between 1998 and 2014.

<table>
<tr><th>Parameters</th><th>Outcome for each parameter (and corresponding number of studies)</th></tr>
<tr><td>Purpose of the study</td><td>Response to habitat management (14)
Spatio-temporal diversity or distribution (9)
Species richness and or abundance in relation to flower abundance (4)
Effects of pesticides on communities/populations (3)
Response to habitat loss (1)
Competition between native and incoming species (1)
Pollinator-crop yields relationship (1)
Sampling method (1)</td></tr>
<tr><td>Species/taxonomic group</td><td>Bombus spp (12), Butterflies (4), Hoverflies (4), Osmia spp (3), Megachile spp (2), Solitary bees (2), Trap nesting bees (2), Apoidea (1), Chelostoma spp (1), Herriades spp (1), Hylaeus spp (1)</td></tr>
<tr><td>Country</td><td>United Kingdom (9), USA (7), Netherlands (5), Switzerland (4), Germany (3), Hungary (3), Spain (3), Denmark (2), Italy (2), Belgium (1), Brazil (1), Canada (1), Ecuador (1), France (1), Indonesia (1), New Zealand (1), Sweden (1)</td></tr>
<tr><td>Year(s) when the study was performed</td><td><table>
<tr><td>Year</td><td>1998</td><td>2000</td><td>2001</td><td>2003</td><td>2004</td><td>2005</td><td>2006</td></tr>
<tr><td>Nb of studies</td><td>1</td><td>2</td><td>1</td><td>2</td><td>1</td><td>2</td><td>3</td></tr>
<tr><td>Year</td><td>2008</td><td>2009</td><td>2010</td><td>2011</td><td>2012</td><td>2013</td><td>2014</td></tr>
<tr><td>Nb of studies</td><td>4</td><td>3</td><td>3</td><td>5</td><td>3</td><td>1</td><td>3</td></tr>
</table></td></tr>
<tr><td>Duration of the study</td><td><table>
<tr><td>Study duration</td><td>1 month</td><td>1 season</td><td>8 months</td><td>1 year</td><td>2 years</td><td>3 years</td><td>3 - 6 years</td><td>20 years</td></tr>
<tr><td>Nb studies</td><td>1</td><td>9</td><td>1</td><td>9</td><td>4</td><td>7</td><td>2</td><td>1</td></tr>
</table></td></tr>
<tr><td>Crops/area monitored</td><td>Agricultural landscape (32), of which cereals/arable crops (7), vegetables/fruits (5), orchards (3), vineyards (3), other permanent crops (2), pasture/meadows/grassland (2), forest (2), uncultivated fields (1)
Urban to rural gradient (2)</td></tr>
<tr><td>Number of fields monitored per study/area sampled</td><td><table>
<tr><td>Nb of sites</td><td>1 to 10</td><td>10 to 20</td><td>> 20</td><td>Not documented</td></tr>
<tr><td>Nb of studies</td><td>30</td><td>10</td><td>22</td><td>-</td></tr>
</table></td></tr>
<tr><td>Surface of the fields/area</td><td><table>
<tr><td>Surface monitored</td><td>m²</td><td>ha</td><td>km²</td><td>Not documented</td></tr>
<tr><td>Nb of studies</td><td>13</td><td>22</td><td>11</td><td>-</td></tr>
</table></td></tr>
<tr><td>Variable recorded (including landscape variables)</td><td>Pollinators:
Species abundance (25), species diversity (11), Species richness (15), record of flower visits or foraging (11)
Flora:
Species abundance (7), species diversity (5), species richness (8), vegetation type (18), crop yields (3)</td></tr>
</table>

Discussion

Honey bees

Most of the studies reviewed in this paper have focused on an assessment of pilot colonies placed in fields in agricultural landscapes. Little emphasis was however given to the description of the landscape itself, i.e. describing the composition of the surrounding habitat. Habitat quality and food resource have however been identified as primary factors in honey bee health, according to wide scale surveys in Europe and the US[56, 57]. The diversity of the factors identified as influential on honey bee survival and colony sustainability over time drive their inclusion in the list of parameters to be monitored in order to be able to isolate pesticide effects from other confounding factors. Monitoring studies for other pollinating insects usually include a description of the surrounding environment, using Geographical Information System (GIS) -based characterisation of land use in most cases, as well as ecological indices for field margin flora (see table 3). Such data would be of great value in honey bee monitoring studies to better interpret the results. These data would also be useful when deciding upon the size of the sites to be monitored and the size of the apiary(ies) to be placed on the sites.

Sites should be selected of comparable size and land use, and contain similar proportions of non-cropped area so that the main difference between them would consist in the application of the product the effects of which are monitored. Again GIS data may be used for the selection of the sites as well as preliminary field visits in order to collect landscape information.

The size of the sites will also determine the number of colonies to be placed in the apiaries. Ideally each apiary should count a minimum of 10 colonies in order to allow reliable statistics, but it should not exceed 25 colonies in order to avoid side effects such as robbing or drift. Where effects of a pesticide applied on a crop are monitored - i.e. focuses on an exposure via foraging on that crop, the fields within the sites should be defined so that they may host enough colonies. For example, in oilseed rape an average of 5 colonies/ha seems to emerge from published data[58]. Thus to be able to monitor apiaries of 10 colonies the sites should be selected to contain oilseed rape fields of 2 hectare size.

The level of floral diversity within the sites will depend on the purpose of the study. In studies focused on the effects of a pesticide used on crops, the sites should contain a sufficient proportion and size of these treated crops so that they represent a significant food resource to honey bees. Where monitoring aims at reflecting the conditions of exposure that honey bee encounter where the product is used, i.e. in the conditions of use and farming encountered in a specific area then other food resources are to be taken into account.

As regards colony health observation, the status of good health should be defined *a priori* in the study.

A colony in “good health” should for example be free from clinical symptoms of diseases and its development should take place within the natural range during the season, and succeed to overwinter. This implies to track the pathogens and symptoms, including when no clinical signs are observed. The same approach should be adopted for pesticides. Indeed pathogens and pesticides are often looked for in symptomatic bees only, while for most of them the thresholds, expressed as individual residue/pathogen level for clinical signs is poorly documented. This way it may be possible to determine the levels of pathogens and pesticides that may be recorded in honey bees without symptomatic effects and in healthy colonies. This is particularly critical as these factors are most often observed together, which makes impossible the interpretation of the data.

When the investigated crop is of interest for honey production, then honey production may also be considered. Pollination success, as evaluated for example though crop yields, should be included in the studies where crops directly depend on honey bee pollination activity.

The study duration should cover the flowering period of the crop and may be extended to the next spring to cover the overwintering period. The flowering period of weeds in field margins and on the farm area may also be taken into account where an exposure cannot be excluded.

Weather data should be recorded as they may influence flight activity even in crops being highly attractive to honey bees.

Other pollinating insects

Few studies in our review have focused on the effects of pesticides. Studies monitoring pollinating insects in agricultural landscapes most often compared the composition of communities between farming practices, using for this ecological indices representing species abundance, diversity and richness, and their relationship to the landscape features differentiating the farming practices. The landscape was described with various levels of accuracy as regards abundance and diversity of the flora and again GIS-based landscape description has been increasingly used.

A similar approach as for honey bees may be adopted in order to identify sites containing a significant proportion of cropped land on which the product of interest is in use. Then the same conditions as regards the proportion of non-cropped land and size of the sites as for honey bees may apply in order to isolate the treatment-factor i.e. the size of the sites should reflect the common practice i.e. typical land use as regards cropped vs non-cropped surfaces, the sites should be of comparable size and proportion of cropped/non-cropped area in order to emphasis differences on the use of the product of concern.

As for honey bees, the number of sites should be defined in order to represent the diversity of landscapes around a crop and/or the effect of special landscape features as in the implementation of Agri-Environmental Schemes (AES) or risk mitigation measures, where relevant. The sites may be treated as replicates, within which several sampling spots may be included, to represent intra-site variability.

Representative groups such as bumble bees were also often considered as a focus, either as indicative species, because of their natural abundance in the sites monitored, or due to their expected presence as a result of the implementation of specific landscape features (such as flowering field margins of special interest to bumble bees, for example). As for honey bees, habitat quality and food resource are identified as the primary factors shaping pollinators composition, provided by the cropped area but also by the non-cropped area in the farmland and both the crop(s) and landscape features will shape the fauna of interest. The monitoring period should cover the flowering period of the crop and may include flowering weeds in the surrounding area where an exposure through them cannot be excluded.

The number of variables to be monitored may be significantly influenced by the number of sites monitored, as relying on human resources. The variables monitored should in general allow to describe abundance, diversity, richness and relation to vegetation type in the surroundings. Yield measurements may be performed where crop pollination depends on local species.

As before, weather data should be recorded.

A summary of the recommendations is proposed in table 4.

Table 4 Recommendations as regards monitoring studies for honey bees and other pollinating insects

Parameters	Honey bees	Other pollinating insects
Area surface and number of sites	At least 2-3 ha per field/orchard with crops representative for the area. Non-cropped area and neighbouring fields should be described if attractive for honey bees. At least two fields/orchards per treatment.	Cultivated area with fields representative of the area. Non-cropped area representative of the landscape and practices (i.e. implementation of risk mitigation measures / AES if relevant).
No. of colonies to be monitored / sampling and description of pollinating insects	At least 10 colonies per apiary, one apiary per site, not more than 25 colonies per apiary	One to several sampling per site to describe pollinator communities occurring in the area. Sampling should allow to reflect the abundance, richness and diversity within the sites. Taxonomic levels recorded should be driven by community patterns and landscape characteristics. For social species being brought to sites then number of colonies should be managed as for honey bees.
Parameters to be recorded	• Colony health (free of clinical symptoms, bee samples taken at beginning of the study for disease analysis if necessary) • Colony development (Liebefeld method) • Overwintering success • Honey production if crop with apicultural interest • Landscape variables (heterogeneity, other bee attractive crops/weeds) • Crop yields where relevant • Weather recordings	• Species richness, abundance and diversity, at the relevant taxonomic level • Where relevant the number of nests occupied • Landscape variables (heterogeneity, other bee attractive crops/weeds) • Crop yields where relevant • Weather recordings
Study duration	Flowering period of the crop and of the surrounding vegetation if an exposure cannot be excluded Monitoring over the overwintering period	Flowering period of the crop and of the surrounding vegetation if an exposure cannot be excluded

Conclusions

A significant experience has been gained in monitoring studies on honey bees and other pollinating insects over the past 15 years, with an increasing interest over time in research organisations, but also regulatory authorities and phytopharmaceutical companies.

The analysis of this study inventory revealed distinct approaches depending on the species monitored and on the purpose of the study. Honey bees are indeed managed organisms being placed in the agricultural landscape, and they are therefore monitored as such, effects being recorded taking this initial presence as a baseline. Other pollinating species are monitored as components of an ecosystem naturally occurring and there is less *a priori* on their relative abundance or diversity when a study is initiated. The occurrence of a species is dependent on environmental descriptors which are usually recorded in monitoring. This relationship to the landscape is however eminently important for honey bees as well and the main recommendation of this analysis may well be to record environmental descriptors in honey bee monitoring studies.

This inventory is being pursued in order to refine our recommendations on methodological aspects of monitoring as a function of study objectives. Additional recommendations as regards the use of monitoring outcome are also in preparation.

Acknowledgements

The authors would like to thank Libby Barnett (FERA), Anja Bartels (AGES), Connie Hart (PMRA), Jean-Michel Laporte (Syngenta), Brigitte Maurizi (DGAl), Christian Maus (Bayer CropScience), Mark Miles (Bayer CropScience), Jacoba Wassenberg (Ctgb), Christof Schneider (BASF) for their support in the working group. The authors particularly thank Mark Miles for his review of this document.

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