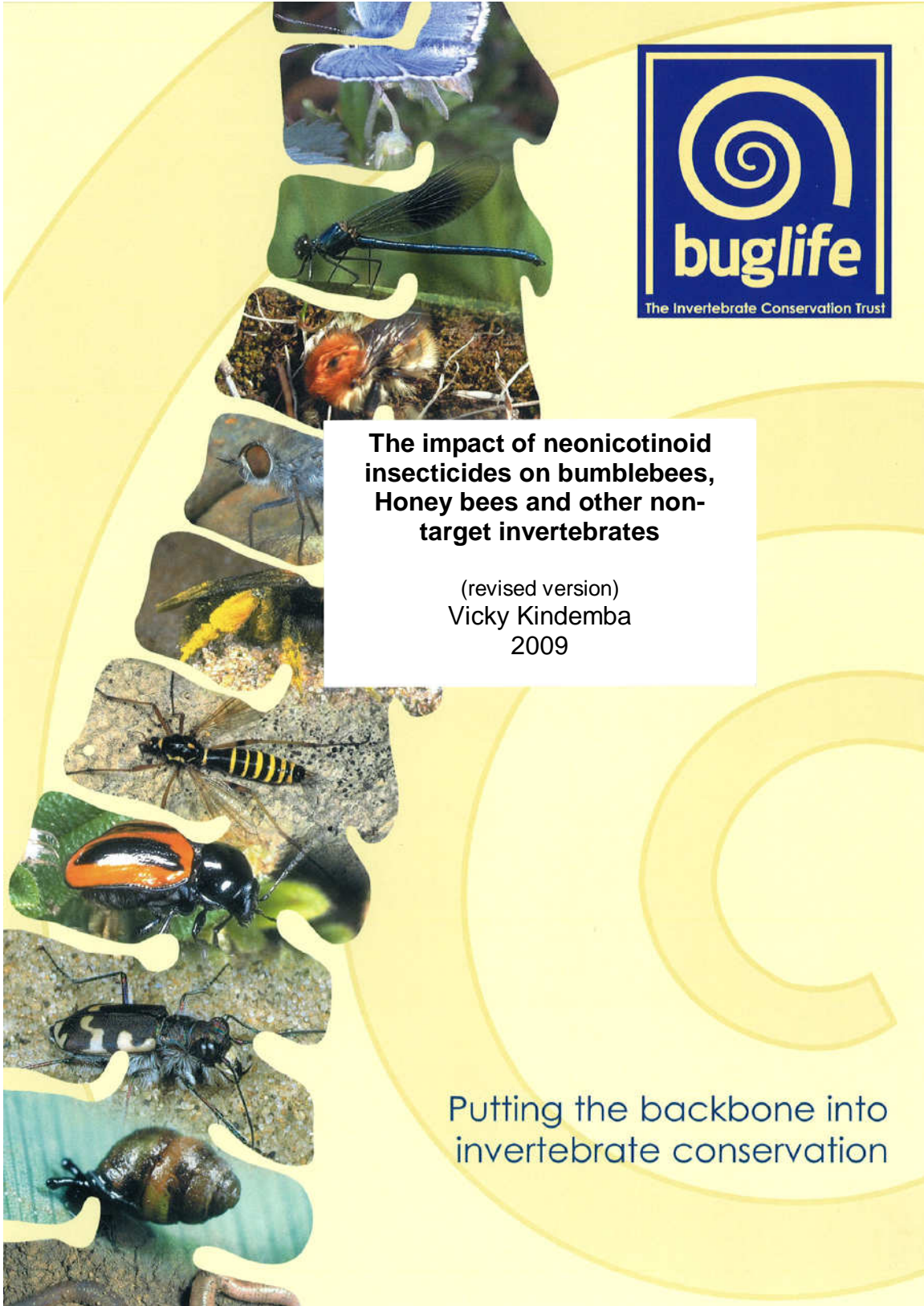




**The impact of neonicotinoid  
insecticides on bumblebees,  
Honey bees and other non-  
target invertebrates**

(revised version)  
Vicky Kindemba  
2009

Putting the backbone into  
invertebrate conservation





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October 2009

ISBN 978-1-904878-964



**Edinburgh Entomological Club**



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# The impact of neonicotinoid insecticides on bumblebees, Honey bees and other non-target invertebrates

## 1.0 Executive summary

The current declines being witnessed in both wild bees and Honey bees have been attributed to a number of possible factors, including: varroa mite infections, habitat loss, mobile phone masts and pesticides. Central to the global debate are neonicotinoid pesticides, banned to different degrees in a number of European countries, these pesticides have been regularly linked to bee declines.

This report covers neonicotinoid pesticides and fipronil, a chemically distinct pesticide that is grouped with neonicotinoids because it is also a systemic insecticide that acts on the nervous system of insects. The report reviews existing approvals research and independent research on the effects of these chemicals on Honey bees, bumblebees and other non-target invertebrates, and investigates the current approvals mechanism and its standards.

Findings reveal a disparity between independent research and the research that was undertaken by Bayer, the producer of the neonicotinoid pesticide imidacloprid, for the imidacloprid 'Draft Assessment Report' (DAR), the 2005 report that was the foundation of the EU regulatory approvals process for this pesticide. Independent research found significant negative impacts on bees that were not included in the DAR because the research was invalidated by the DAR. The basis for this invalidation is questionable, but it resulted in key evidence not being considered as part of the approvals process.

Additional research post-dating the DAR has provided more evidence that neonicotinoids may damage populations of bees and other non-target organisms.

These peer reviewed independent research papers show significant negative impacts of imidacloprid on bees and other non-target invertebrate occur at levels predicted to be present in the UK countryside. These predicted levels are based on imidacloprid application rates approved for use in the UK. Similar levels have been found present in hives of other countries when EU approved imidacloprid products were used for example Gregorc & Bozic 2004 found five samples of bees out of 12 hives tested in Slovenia were found to contain imidacloprid above 5 µg/kg<sup>42</sup> and Chauzat *et al.* 2006 found levels of 5.7 µg/kg in pollen from French hives<sup>55</sup>.

Papers on impacts at predicted environmental concentrations include:-

- Yang *et al.* 2008 - foraging Honey bees reduced their visits to a syrup feeder when it was contaminated with 3 µg/kg of imidacloprid<sup>48</sup>.
- Alexander, Heard & Culp 2008 - mayflies of the genera *Baetis* and *Epeorus* showed a reduction in reproductive success when exposed to concentrations of imidacloprid as low as 0.1 µg/l and in addition there were reductions in head length in *Baetis* and thorax length in *Epeorus*<sup>22</sup>.
- Alexander *et al.* 2007 - found that imidacloprid levels reduced survival, feeding and egestion in the mayfly *Epeorus longimanus* and aquatic worm *Lumbriculus variegatus* at concentrations between 0.5 and 10 µg/l<sup>23</sup>.
- Rose, Dively, & Pettis 2007 - an imidacloprid level of 10 µg/kg within pollen cakes in Honey bee colonies caused a 20% reduction in the number of brood cells<sup>54</sup>.

- Colin *et al.* 2004 - sub-lethal doses altered the behaviour of foraging Honey bees and 6 µg/kg of imidacloprid reduced the proportion of active bees<sup>51</sup>.
- Suchail, Guez & Belzunces 2001 – this study tested chronic toxicity on Honey bees using a 0.1 µg/l solution given every day for eight days. The LD50 was reached at a mean rate of 12 µl/d per bee; after 8 days this was 0.01 ng/bee (0.1 µg/kg), showing chronic toxicity to cause bee deaths at much lower concentrations of imidacloprid than acute toxicity<sup>35</sup>.

Test methods utilised for the approval process of imidacloprid were found to be insufficient for assessing sub-lethal effects and chronic exposure risks to Honey bees from imidacloprid. We found that overall the existing approval mechanisms for crop protection products controlled by Plant Protection Products Directive 91/414 are generally inadequate for assessing the impacts on non-target invertebrates, with no standards for sub-lethal effects and inappropriate assessment methods for systemic pesticides; this means that the product approval decisions which are made in the UK by the UK's Chemicals Regulation Directorate are reliant on inadequate research. There were also a number of exposure routes that had not been properly investigated, such as exposure from dust formed during the sowing of dressed seeds<sup>11,12</sup>.

There is a lack of independent research into the potential impacts on non-target organisms from other neonicotinoid pesticides and therefore the respective DAR research cannot be as thoroughly reappraised as has been possible for imidacloprid. It is highly likely that risks posed by imidacloprid will also be posed by related pesticides that are used in comparable circumstances.

The precautionary principle states that if there are reasonable scientific grounds for believing that a new product may not be safe, it should not be used until there is convincing evidence that the risks are small and outweighed by the benefits. This is enshrined in Directive 91/414 which states that "Member States shall ensure that a plant protection product is not authorized unless.....it has no unacceptable influence on the environment." "Authorizations may be reviewed at any time if there are indications that any of the requirements....are no longer satisfied."

Given the essential nature of pollination services provided by the Honey bee and wild bee populations and the current precarious state of these animals any additional risk to their populations from pesticide use constitutes an unacceptable influence on the environment. In addition we have identified generic key weaknesses in the European approval process in relation to imidacloprid making the approval research not comprehensive enough in regard to risks to bees. Buglife and the organisations that have signed onto this report call for the following action to be taken:

- A review of the inclusion of imidacloprid, other neonicotinoids and fipronil on the positive list of authorised substances in Annex I of Directive 91/414.
- A review of existing neonicotinoid and fipronil products authorised for outdoor use in the UK.
- Until the reviews are completed a precautionary suspension of all existing approvals for products containing neonicotinoids and fipronil where these products have been authorised for outdoor use in the UK.
- The development of international methodologies for assessing the effects of systemic pesticides and sub-lethal impacts on invertebrates.



# The impact of neonicotinoid insecticides on bumblebees, Honey bees and other non-target invertebrates

## 2.0 Aims and objectives

The aim of this report is to establish whether neonicotinoid and related pesticides are having a negative impact on Honey bees and wild bees in the UK, and are thus contributing to the bee declines currently being witnessed. This report examines this issue by reviewing all available research and examining the effectiveness of the EU regulatory approval process in assessing the impact of these chemicals on bees. Imidacloprid is the main focus for this report as it is the most thoroughly assessed and independently researched neonicotinoid pesticide and there is a large volume of scientific literature on imidacloprid and its impact on bees.

## 3.0 Introduction

Neonicotinoids are a set of nicotine-based insecticides that include the chemicals imidacloprid, clothianidin, acetamiprid, thiacloprid, thiamethoxam, dinotefuran and nitenpyram. Neonicotinoids are a type of insecticide, differing from conventional spray products in that they can be used as either seed dressings or as soil treatments and as a result they are dispersed into plant tissues resulting in a slower (chronic) exposure to non-target organisms. Neonicotinoids are one of the most widely used groups of insecticides globally, they are neurotoxins that act on invertebrates' information processing by affecting a specific neural pathway that is more common in invertebrates than other animal groups, making them popular insecticides<sup>1</sup>.

This set of insecticides has become an increasing concern to beekeepers and bee researchers, with many suspecting that neonicotinoids may be connected to current bee declines<sup>10,2</sup>, and this has led to either full or partial ban of some of these chemicals in a number of European countries, including France, Germany, Italy and Slovenia; and a large body of research investigating the issue.

In the UK five neonicotinoids are registered for use, these are: imidacloprid, clothianidin, acetamiprid, thiacloprid and thiamethoxam. These chemicals are mainly used in plant protection products, and are applied to a variety of crops in both commercial and non-commercial use. The volume of commercial neonicotinoids used in Great Britain has increased over the last six years; see Tables 1 and 2 for the total area of land treated.

Fipronil is included in this analysis as well. It is not chemically a neonicotinoid pesticide but it acts on the nervous system of insects and is used as a systemic insecticide, and therefore presents similar potential risks to bees and other non-target invertebrates.

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<sup>1</sup> Jones A.K., Raymond-Delpech V., Thany S.H., Gauthier M., & Sattelle D.B. (2006) The nicotinic acetylene gene family of the honey bee, *Apis mellifera*. *Genome Research* **16**, 1422–1430

<sup>2</sup> Vermandere P. (2002) Affaiblissement des colonies d'abeilles sur la miellee de tournesol, in AFSSA (Ed.) Analyse des phenomenes d'affaiblissement des colonies d'abeilles, Paris, pp. 12–18

**Table 1: Usage of neonicotinoids and fipronil on outdoor and indoor crops for commercial use, total active substance treated area (ha) (this figure is calculated by multiplying the basic areas by the number of times the area is treated)<sup>3</sup>, although registered for use in the UK there is no information on the use of acetamiprid as survey data was collected prior to acetamiprid products being approved for use in the UK**

	2000	2001	2002	2003	2004	2005	2006
Imidacloprid	346,813	347,058	540,207	540,546	777,890	783,054	770,053
Clothianidin							43,224
Thiacloprid		51	5,934	6,154	8,980	9,338	14,636
Thiamethoxam						1,213	1,213
Fipronil				87	87	562	562

**Table 2: Usage of neonicotinoids on outdoor and indoor crops, total weight applied (kg)<sup>3</sup>**

	2000	2001	2002	2003	2004	2005	2006
Imidacloprid	25,404	26,562	30,216	30,486	41,031	43,916	82,254
Clothianidin							5,980
Thiacloprid		5.6	682	790	1,165	1,213	1,677
Thiamethoxam						5.4	5.4
Fipronil				52	52	124	124

### 3.1 European regulation of plant protection products

Plant protection products used in the UK are governed by EU Directive 91/414<sup>6</sup> and the Plant Protection Product Regulations 2005. This legislation, which requires that all products available in EU Member States are to undergo a two-stage approvals process.

At the first stage, active substances contained in plant protection products are assessed at the European level in a 'Draft Assessment Report' (DAR) which assesses acute and chronic toxicity as well as sub-lethal effects, in order to ascertain the environmental risk posed by the active substances. The Draft Assessment Report is undertaken by the company which develops the active substance, without independent assessment during field and laboratory studies. The draft assessment report is then agreed by Member States through a review process. If the active substance meets certain criteria set out in the Directive, which includes 'to have no unacceptable influence on the environment', particularly with regard to its impact on non-target species, it will be included on a positive list of approved active substances which forms Annex I to Directive 91/414.

At the second stage, plant protection products containing the active substances must be approved at the national level. The Chemicals Regulation Directorate is the government body in the UK which is responsible for testing and ensuring the environmental safety of pesticides, and authorising plant protection products in the UK. Before approving the plant protection product, Member States must be satisfied that the active substances used in the product are contained in Annex I to Directive 91/414 and that certain

<sup>3</sup> Chemicals Regulation Directorate: Pesticide Usage Statistics <http://pusstats.csl.gov.uk/myindex.cfm>



requirements are met, for example that the use of the product will not have an unacceptable influence on the environment, having particular regard to its effect on non-target species. The assessment of whether the product will meet these requirements must be made pursuant to the Uniform Principles contained in Annex VI to Directive 91/414. The following Uniform Principles are of particular relevance to the approval of neonicotinoids:

- Member States shall ...ensure that the data submitted is acceptable in terms of quantity, quality, consistency and reliability.
- Member States shall consider other relevant technical or scientific information they can reasonably possess with regard to the performance of the Plant Protection Product or to its adverse effects.
- Member States shall consider possible elements of uncertainty in the information obtained during the evaluation.
- Member States shall evaluate the possibility of exposure of aquatic organisms to the Plant Protection Product.
- Member States shall evaluate short-term and long-term risk to Honey bees (Western or European honey bee (*Apis mellifera*)).

The approval of the plant protection product may be reviewed if there are indications that any of the relevant requirements are no longer satisfied. The approval must be revoked if the subsequent review concludes that the relevant requirements are no longer satisfied or the information supplied with the original application for approval was false or misleading.

The 'Draft Assessment Report' for imidacloprid has now been finalised and a directive has been adopted which will add imidacloprid to Annex I with effect from 1 August 2009. The following sections of this report outline a number of serious deficiencies in the Draft Assessment Report relating to imidacloprid, including:

### 3.1.1 *Environmental risk assessments*

Within the DAR an environmental risk assessment is carried out, using Honey bees as the test species, to assess oral and contact toxicity by measuring the LD50 (the lethal dose required to kill 50% of a test population). This amount is then compared to application level and then the hazard quotient (HQ) is calculated, i.e. the application rate in grams per hectare divided by the LD50 in terms of micrograms of test item per bee. This quotient has a trigger value of 50. If the HQ is less than 50, the risk is considered to be low and if the HQ is greater than 50 there is a risk present and further data or restrictions are required. As well as Honey bees, other non-target invertebrates are used as indicator species to assess the impact on invertebrates. For aquatic invertebrates the acute toxicities of the active substance and metabolites are assessed for the waterflea *Daphnia magna*. Earthworms and soil organisms (e.g. Collembola) have a similar assessment methodology but persistence is also considered in order to take into account potential accumulation. The risk to other non-target arthropods is assessed using the toxicity of two sensitive species – a predatory mite, *Typhlodromus pyri*, and an aphid parasitoid, *Aphidius rhopalosiphi* – to obtain a hazard quotient. These species are sensitive to chemicals in their environment and so they are used as indicators. If there is no impact on these species it is assumed that there is a very low risk to other non-target arthropods. During the approvals process ecotoxicity testing is split into two tiers. Tier 1 studies use single cohorts of test species, which are examined under standardised conditions, the standards of which are defined by EPPO (European and Mediterranean Plant Protection Organisation) standards and guidelines. Higher Tier

studies are employed if a substance breaches a trigger value. Higher Tier studies use more complex and flexible tests and a range of experimental techniques are employed to further assess substances, but these tests are not standardised. Currently there are only EPPO set standards for assessments in Tier 1 studies for the application of chemicals to the aerial parts of plants which have a rapid residual action of a few hours/days.

### 3.1.2 *Sub-lethal effects and systemic pesticides*

The action of systemic pesticides is covered by hazard quotient assessment, but this parameter is not suitable for systemic chemicals. Systemic pesticides behave very differently and result in the contamination of nectar and pollen causing chronic exposure to pollinators. The long-term exposure associated with the use of systemic pesticides poses a greater ecological risk than direct treatments. A lack of suitable standardised testing for chronic toxicity means there are no threshold values and this significant environmental risk is not considered appropriately during the approval process. The European assessment process is also weak at assessing the sub-lethal effects of pesticides. Sub-lethal effects are tested through the higher-level tests, which occur if the Hazard Quotient is triggered; but the tests are run on an adhoc basis and there are no internationally agreed, standardised assessment methods, and no validity criteria or toxic standards for them<sup>4,5</sup>. Sub-lethal effects do not cause immediate animal deaths but do alter behaviour and/or reduce reproductive capacity, which would lead to population decline.

### 3.1.3 *New Thematic Strategy*

A new Thematic Strategy for pesticides is currently under development, which includes a new regulation to replace the current pesticide authorisation process under Directive 91/414<sup>6</sup>. This new replacement regulation will see changes in environmental testing from a risk-based assessment to a hazard-based assessment; however, there will not be any changes to the current assessment standards for systemic pesticides and sub-lethal effects that are reviewed in this report.

## 4.0 Methodology

This report attempts to give a broad cross-section of relevant literature, looking at the European approval assessment for imidacloprid and independent research that has taken place. Papers were identified by web searching all current literature and sourcing references from relevant papers. Access to papers was limited and relied on sourcing from contacts or through contacting authors; not all relevant papers were sourced but the majority were and should provide an adequate cross-section of literature. This report focuses on independent research showing an effect particularly for Honey bees, as most studies showing no impact are detailed in the DAR. The main body of research occurred between 2002 and 2005 on imidacloprid and bees. The research examined in this report has not been assessed in relation to methodology; however, the tables in Appendix 1

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<sup>4</sup> Thompson H. & Maus C. (2007) The relevance of sub-lethal effects in honey bee testing for pesticide risk assessment. *Pest Management Science* **63**, 1058–1061

<sup>5</sup> DEFRA Research and Development, Theme: Environmental Effects of Pesticides: Non-target arthropods <http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=ProjectList&Completed=0&AUID=1213>

<sup>6</sup> Directive 91/414/EEC: [http://europa.eu/eur-lex/en/consleg/pdf/1991/en\\_1991L0414\\_do\\_001.pdf](http://europa.eu/eur-lex/en/consleg/pdf/1991/en_1991L0414_do_001.pdf)

outline the studies reviewed and their quality, and most of the studies cited are from peer-reviewed publications.

## 5.0 Imidacloprid

Imidacloprid is manufactured by Bayer Cropscience and is present in a range of crop protection products which are used for either soil, seed or foliar application to control pest invertebrates such as aphids, thrips, whiteflies, turf insects, soil insects and some beetles. For products containing imidacloprid on sale in the UK see Appendix 3<sup>7</sup>. Systemic imidacloprid is one of the world's most used insecticides, often used as a seed dressing on maize, sunflower and rape crops<sup>8</sup>. The chemical is very persistent in soils and has a half-life of approximately 1,000 days, depending on the soil type and environmental conditions<sup>9</sup>. In water imidacloprid has a half life of more than a year, which is also dependent on environmental conditions. Research has shown that imidacloprid has a high mobility in plants, and when used as a seed dressing becomes mobile and will migrate from the stem to the leaf tips and eventually into male flowers<sup>8</sup>; as a result imidacloprid residues have been detected in the pollen<sup>10</sup> and nectar<sup>15</sup> of a number of flowering crop plants, leading to prolonged exposure of non-target invertebrates to imidacloprid.

### 5.1 Products in the UK

Currently a total of 28 registered plant protection products contain imidacloprid and are available for use on a number of different crops in the UK (see Appendix 3).

### 5.2 Use on crops

In the UK in 2006 imidacloprid was widely used as a seed treatment for five different outdoor crops: wheat, oilseed rape, linseed, triticale and sugar beet. Table 3 shows the difference in treated hectares of insecticides containing imidacloprid on crops in the UK and Table 4 shows the difference in tonnage of active substance applied<sup>14</sup>. For tonnage applied, sugar beet has the highest tonnage, but sugar beet is cropped before it flowers, which removes the availability of imidacloprid through nectar and pollen to bees. However, this is not the only form of contamination; research studies have found that using pneumatic seed drills for the sowing of corn seeds causes the release of contaminated dust, filter papers on the fan drills showed residues of 120 to 240 µg per gram of filter paper used at 240 seconds, and as a result imidacloprid are found in grass and pollen in areas adjacent to sowing and that residual imidacloprid is found on flowers and grass at least 4 days after sowing<sup>11,12</sup>;

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<sup>7</sup> Exttoxnet: <http://exttoxnet.orst.edu/pips/imidaclo.htm>

<sup>8</sup> Bonmatin J.M., Marchand P.A., Charvet R., Moineau I., Bengsch E.R. & Colin M.E. (2005) Quantification of imidacloprid uptake on maize crops. *Journal of Agricultural and Food Chemistry* **53**, 5336–5341

<sup>9</sup> Baskaran S., Kookana R.S. & Naidu R. (1999) Degradation of bifenthrin, chlorpyrifos and imidacloprid in soil and bedding materials at termiticidal application rates. *Pesticide Science* **55**, 1222–1228

<sup>10</sup> Bonmatin J.M., Moineau I., Colin M.E., Fleche C. & Bengsch E.R. (2005) Behaviour of Imidacloprid in Fields: Toxicity for Honey Bees. *Environmental Chemistry Part V*, 483–494

<sup>11</sup> Greatti M., Sabatini A.G., Barbattini R., Rossi S. & Stravisi A. (2003) Risk of environmental contamination by the active ingredient Imidacloprid used for corn seed dressing. Preliminary results. *Bulletin of Insectology* **56** (1), 69–72

<sup>12</sup> Greatti M., Barbattini R., Stravisi A., Sabatini A.G. & Rossi S. (2006) Presence of the a.i. imidacloprid on vegetation near corn fields sown with Gaucho® dressed seeds. *Bulletin of Insectology* **59** (2), 99–103

contrastingly, a Bayer study found no release of imidacloprid during sowing<sup>13</sup>. Contamination of the wider environment can also occur through runoff and consequent impact on aquatic habitats. Evidence of this was presented in the DAR<sup>19</sup>. After sugar beet the next highest tonnage is applied to wheat, which is wind- or self-pollinated; then oilseed rape which is an insect-pollinated crop. Linseed is mainly self-pollinated with a small amount of insect pollination; oats and triticale are self-pollinating. Therefore the crops of most concern when considering impacts on pollinators are oilseed rape and linseed, as nectar and pollen from their flowers make imidacloprid available to pollinators. Using the figures from the 'Pesticide usage survey report' for 2006<sup>14</sup>, approximately 23 g of beta-cyfluthrin/imidacloprid per hectare was used on oilseed rape in 2006.

**Table 3: Usage of pesticides on arable crops grown in Great Britain 2006 (treated hectares)<sup>14</sup>**

Seed Treatments	Wheat	Oilseed Rape	Linseed	Oats	Triticale	Sugar Beet	Total
Bitertanol/ fuberidazole/ imidacloprid	216,537			5,380	158		222,075
Imidacloprid						91,827	91,827
Beta-cyfluthrin/imidacloprid		337,378	15,354				352,732

**Table 4: Usage of pesticides on arable crops grown in Great Britain 2006 (tonnes of active substance applied)<sup>14</sup>**

Seed Treatments	Wheat	Oilseed Rape	Linseed	Oats	Triticale	Sugar Beet	Total
Bitertanol/fuberidazole/ imidacloprid	36.23			0.73	0.02		36.98
Imidacloprid						55.18	55.18
Beta-cyfluthrin/imidacloprid		7.95	3.07				11.02

Set-aside land (land previously taken out of production) was impacted through pesticide use on bio-fuel crops categorised as set-aside. Detailed below is the number of hectares of set-aside using beta-cyfluthrin/imidacloprid as a seed treatment in 2006: industrial oilseed rape 48,074 ha, industrial linseed 3,610 ha, and game cover 143 ha. This gives a total of 51,827 hectares of set-aside treated with 1.87 tonnes of beta-cyfluthrin/imidacloprid<sup>14</sup>, approximately 36 g per hectare.

### 5.3 Crop residues

Research has been carried out on the residues of imidacloprid present in crops treated with systemic imidacloprid products. For maize samples, residue levels measured from less than 0.1 µg/kg to 33.6 µg/kg with average levels of 4.1 µg/kg in stems and leaves, 6.6 µg/kg in male flowers (panicles) and 2.1 µg/kg in pollen<sup>8</sup> and a French literature assessment that reviewed all scientific literature on imidacloprid and validated it (see

<sup>13</sup> Schnier H.F., Wenig G., Laubert F., Simon V. & Schmuck R. (2003) Honey bee safety of imidacloprid corn seed treatment. *Bulletin of Insectology* **56** (1), 73–75

<sup>14</sup> Garthwaite D.G., Thomas M.R., Heywood & Battersby (2006) Pesticide Usage Survey Report 213: Arable Crops in Great Britain 2006 (including aerial applications 2003–5). Pesticide Usage Survey Team, Central Science Laboratories, Sand Hutton, York

Appendix 4), found an average of 0.75–3.5 µg/kg for maize pollen. The French literature assessment by the French Scientific and Technical Committee that for sunflower seeds dressed with imidacloprid average residue levels were recorded at 3.3 µg/kg in flowers and 2.2 µg/kg in pollen<sup>15</sup>. Other research on sunflowers and maize has found higher levels in the flowers of imidacloprid-treated plants with average values of approximately 10 µg/kg<sup>16</sup>. Independent studies that detected residues of imidacloprid also assessed the risks associated with recorded levels, and concluded that the high residual levels could be related to colony losses in bees. The residue levels present in maize and sunflower crops are also similar to those present in oilseed rape<sup>8,16</sup>. In contrast studies used in the EU Draft Assessment Report (DAR) detected no residues of imidacloprid in sunflower nectar and pollen grown from seed-dressed crops above 1.5 µg/kg (the detection level)<sup>8,17</sup>. Bee exposure via honeydew is considered to be very low as acute oral toxicity for aphids is much lower than the toxicity levels for bees<sup>19</sup> as outlined in this report and so aphids would not survive to produce honeydew that is toxic to bees.

#### 5.4 Domestic use and amenity products

A large number of domestic and amenity products have imidacloprid as their active ingredient and are registered for use in the UK<sup>18</sup>. Some products are for indoor use only, but others are for outdoor use on ornamental plants and nursery stock and also for use on lawns and amenity turf.

#### 5.5 What are the effects of imidacloprid on non-target species?

##### 5.5.1 Summary of European Assessment research

The EU DAR<sup>19</sup> on imidacloprid determined that imidacloprid used on tomatoes and apples would have unacceptable effects on the aquatic environment, and recommended the use of buffer zones when spraying crops. The European Food Standards Authority (EFSA) peer review 29/05/08<sup>20</sup> concluded that spraying of imidacloprid poses a high risk and even with mitigation measures bees still will not be protected. The DAR conducted microcosm studies for seed dressings on tomatoes, apples and sugar beet crops and in a number of cases for tomatoes and apples the toxic exposure level was triggered indicating unacceptable damage to aquatic invertebrates; but imidacloprid is not licensed for outdoor use on these crops. For soil-dwelling and foliage-dwelling predators and parasitoids the assessment concluded that in principle the in-field situation is acceptable, taking into account the results of laboratory, semi-field studies, aged-residue studies, a field study and the half-life of the substance and corresponding re-colonisation potential. For off-field situations there was an acceptable risk to non-target arthropods considering the results of laboratory and field studies and drift mitigation. The larvae of the carabid beetle *Poecilus cupreus* was also tested and found to be very sensitive to imidacloprid. Despite, the rapporteur Member State deeming that the concentrations tested were too high for it to conclude no risk to carabids for use on sugar beet, there was no indication

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<sup>15</sup> Scientific and Technical Committee (2004) A multifactorial study on the disturbance of bees: Imidacloprid used in coating seed (Gaucho) and the disturbance to bees. Final Report

<sup>16</sup> Bonmatin J.M., Marchand P.A., Charvet R., Moineau I., Bengsch E.R., Colin M.E. (2003) Method for Analysis of Imidacloprid in soils, plants and pollens. *Analytical Chemistry* **75** (9), 2027–2033

<sup>17</sup> Schmuck R., Schoening R., Stork A. & Schramel O. (2001) Risk posed to honey bees (*Apis mellifera* L., Hymenoptera) by an Imidacloprid seed dressing of sunflowers. *Pest Management Science* **57**, 225–238

<sup>18</sup> Chemicals Regulation Directorate – Product Register: <https://secure.pesticides.gov.uk/PestReg/ProdSearch.asp>

<sup>19</sup> Draft Assessment Report: Initial risk assessment provided by the rapporteur Member State Germany for the existing active substance imidacloprid

of further research required<sup>19</sup>. For tests on earthworms, soil micro-organisms and soil degradation, results demonstrated that use of imidacloprid at environmentally applicable levels had no unacceptable impact. However, research in the DAR on the long-term impacts to soil-dwelling arthropods and earthworms was deemed inadequate by the peer review report and the need for further research was highlighted, particularly for long-term impacts on earthworms, by the EFSA peer review<sup>20</sup>.

#### 5.5.2 Non-target invertebrate independent research - relevant environmental levels

Aquatic invertebrates are particularly vulnerable to imidacloprid. The growth and size of mysid shrimps (Mysidacea) are known to be affected by imidacloprid concentrations of less than 1 µg/l<sup>21</sup>. Mayflies of the genera *Baetis* and *Epeorus* showed a reduction in reproductive success when exposed to concentrations as low as 0.1 µg/l, expressed through a reduction in reproductive success, through a reduction of head length in *Baetis* and thorax length in *Epeorus*<sup>22</sup>. The EU DAR predicts aquatic environmental concentrations of imidacloprid from seed dressing runoff as high as 1.6 µg/l<sup>19</sup>; at this level there would be significant damaging impacts on mayflies. Another study found that environmentally relevant imidacloprid levels reduced survival, feeding and egestion in the mayfly *Epeorus longimanus* and aquatic worm *Lumbriculus variegatus* at concentrations between 0.5 and 10 µg/l<sup>23</sup>. Predicted Environmental Concentration (PEC) for the DAR was 1.656 µg/l imidacloprid for the highest global maximum for seed dressings, therefore effect concentrations stated previously are below this level. Buffer zone mitigation was considered for sprays which had PECs much higher than seed dressings; seed dressings had PECs at levels below effect concentrations for test species in the DAR and so were considered low risk<sup>19</sup>. An effect has also been observed for carabid beetles: the EU DAR tested *Poecilus cupreus* and found it to be highly sensitive to imidacloprid. Imidacloprid has also been found to increase the fecundity and longevity of the Two-spotted spider mite (*Tetranychus urticae*)<sup>24</sup>.

#### 5.5.3 Non-target invertebrate independent research - effects above relevant environmental levels

Imidacloprid is toxic to earthworms; for example, the LC50 of *Eisenia fetida* is between 2 and 4 mg/kg in soil<sup>25</sup>, levels higher than those that are present due to authorised use. Sub-lethal effects on the development of mason bee *Osmia lignaria* were seen at 30 µg<sup>26</sup>. Effects on butterfly species have also been shown, with tests on adults of the Monarch (*Danaus plexippus*) and the Painted lady (*Vanessa cardui*) feeding on Bloodflower (*Asclepius curassavica*). Larval survival rate for both species was significantly reduced where imidacloprid residues in flower nectar reached 29 µg/kg and

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<sup>20</sup> European Food Standards Authority – Scientific Report (2008) 148, 1–120, Conclusion on the peer review of Imidacloprid

<sup>21</sup> Imidacloprid – Insecticide Factsheet (2001) *Journal of Pesticide Reform* **21**, No. 1

<sup>22</sup> Alexander A.C., Heard K.S. & Culp J.M. (2008) Emergent body size of mayfly survivors. *Freshwater Biology* **53**, 171–180

<sup>23</sup> Alexander A.C., Culp J.M., Liber K. & Cessna A.J. (2007) Effects of insecticide exposure on feeding inhibition in mayflies and oligochaetes. *Environmental Toxicology and Chemistry* **26** (8), 1726–1732

<sup>24</sup> James D.G. & Price T.S. (2002) Fecundity in Two-spotted spider mite (Acari:Tetranychidae) is increased by direct and systematic exposure to Imidacloprid. *Journal Economic Entomology* **95** (4), 729–732

<sup>25</sup> Luo Y. (1999) Toxicological study of two novel pesticides on earthworm, *Eisenia foetida*. *Chemosphere* **39**, 2347–2356.

<sup>26</sup> Abbott V.A., Nadeau H.A., Higo H.A. and Winston M.L. (2008) Lethal and sublethal effects of imidacloprid *Osmia lignaria* and clothianidin on *Megachile rotundata* (Hymenoptera: Megachilidae). *Journal of Economic Entomology* **101** (3), 784–796.



54 µg/kg<sup>27</sup>. This level is higher than those levels that would be found in nectar at its current authorised use; however, Lepidoptera are an under-researched group in relation to the effects of imidacloprid and in some countries outside Europe they are the target pest organisms; for example, in Hawaii, imidacloprid is used against butterflies to control turf caterpillars<sup>28</sup>. Imidacloprid is also used against a number of pest moth species<sup>29,30</sup> and so will be fairly toxic to Lepidoptera. Imidacloprid has been found to reduce numbers of beneficial crop invertebrates such as Coleopteran larvae, hister beetles and lacewings<sup>31,32</sup>. On exposure to turf plots treated with imidacloprid, the non-target carabid *Harpalus pennsylvanicus*, displays a range of neurotoxic problems, such as: paralysis, impaired walking and excessive grooming. These behaviours render individuals highly vulnerable to predation<sup>33</sup>. Termites *Reticulitermes flavipes* were more susceptible to entomopathogens when in soil contamination with imidacloprid (5,10 and 20 mg)<sup>34</sup>. All these levels are above environmental levels present when approved products are used correctly.

#### 5.5.4 Bee risk assessment – European Assessment

The EU Draft Assessment Report<sup>19</sup> on imidacloprid determines a Hazard Quotient (HQ) using the highest possible spray application, which is 150 g a.s./ha (active substance) and this gave an HQ for oral consumption of 40,540 and an HQ for contact with the active substance of 1,852. With a trigger level of 50 this gives a massive HQ for oral consumption of imidacloprid, 810% greater than the trigger. As imidacloprid breached the HQ for oral consumption this led to Higher Tier studies, because further assessments, including of sub-lethal effects, and also of risk mitigation for spraying is required for authorisation. Chronic toxicity had an LD50 of 24 µg and the DAR recorded no imidacloprid residue levels in plants at or above this level. Although there are no approved assessment methods for sub-lethal effects, because of the high hazard quotient for imidacloprid, the DAR included a number of semi-field and field tests to assess the side effects of seed dressing use. The results from the DAR field studies consistently concluded that there was no impact of imidacloprid and its main metabolites on Honey bee populations when imidacloprid is used as a seed treatment.

The DAR did not detect residues of imidacloprid in nectar and pollen. However, there was a limit to detecting levels of imidacloprid in nectar, pollen and soil with levels below

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<sup>27</sup> Rogers M. & Krischik V.A. (2003) Non-target effects of imidacloprid in nectar on the monarch butterfly, *Danaus plexippus*, and the painted lady butterfly, *Vanessa cardui*. Annual Meeting of the Entomological Society of America

<sup>28</sup> Deputy J. & Hara A. (2000) Destructive Turf Caterpillars in Hawaii. Insect Pests, Cooperative Extension Service CTAHR

<sup>29</sup> Matthes M. & Epperlein, K. (2004) Abundance dynamics and control strategies of the horse chestnut leaf mining moth. *Mitteilungen der Deutschen Gesellschaft für allgemeine und angewandte Entomologie* **14** (1–6), 361–364

<sup>30</sup> Sreekanth M., Ramesh B.T., Mirazam S. & Narasimha R.B. (2000) Evaluation of certain new insecticides against Lepidoptera pests of cabbage. *International Pest Control* **42** (4), 134–137

<sup>31</sup> Kunkel B.A., Held D.W. & Potter A.D. (1999) Impact of Halofenozide, Imidacloprid, and Bendiocarb on beneficial invertebrates and predatory activity in turfgrass. *Journal of Economic Entomology* **92** (4), 922–930

<sup>32</sup> Rogers M.A., Krischik V.A. and Martin L.A. (2007) Effect of soil application of imidacloprid on survival of adult green lacewing, *Chrysoperla carnea* (Neuroptera: Chrysopidae), used for biological control in greenhouse. *Biological Control* **42** (2), 172–177

<sup>33</sup> Kunkel B.A., Held D.W., & Potter D.A. (2001) Lethal and sub-lethal effects of Bendiocarb, Halofenozide, and Imidacloprid on *Harpalus pennsylvanicus* (Coleoptera: Carabidae) following different modes of exposure in turfgrass. *Journal of Economic Entomology* **94** (1), 60–67

<sup>34</sup> Ramakrishnan R., Suiter D.R., Nakatsu C.H., Humber R.A., & Bennett G.W. (1999) Imidacloprid-enhanced *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) susceptibility to the entomopathogen *Metarhizium anisopliae*. *Journal of Economic Entomology* **92** (5), 1125–1132

5 µg undetectable for both imidacloprid and 5-OH (one of its metabolites), and levels below 10 µg for olefin (another metabolite). In the DAR this was not an issue as no lethal or sub-lethal effects were observed below 5 µg. In the context of other independent research this detection level is insufficient as some research has found impacts at concentrations below 5 µg, specifically for chronic toxicity testing<sup>35</sup>.

#### 5.5.5 Ecotoxicology studies on bees

The toxicology results examined within this section are divided in relation to whether or not they show effects at environmentally relevant levels; the environmentally relevant levels are defined by the pollen and nectar levels given by peer review sources for maize and sunflowers, and similar levels to these are thought to present in oilseed rape plants<sup>8,15</sup>. Independent research results are also displayed in a table in Appendix 1 showing the quality and the source of the data quoted.

#### 5.5.6 Bumblebee toxicity research – above relevant environmental levels

Acute toxicity testing in Italy produced results which found the LD50 at 24 hours to be 0.04 µg/bumblebee (4,000 µg/kg) and at 72 hours 0.02 µg/bumblebee (2,000 µg/kg) for imidacloprid<sup>36</sup>.

#### 5.5.7 Bumblebee sub-lethal effects research - relevant environmental levels

A laboratory feeding test used pollen and nectar contaminated with imidacloprid at two concentrations, 10 and 6 µg/kg<sup>37</sup>. These were fed to *Bombus terrestris* individuals at both concentrations over an 12-week period. These concentrations were chosen as pollen collected by Honey bees foraging from treated sunflowers has not been found to reach concentrations of imidacloprid higher than 10 µg/kg. The study concluded that survival rate and reproductive capacity of *Bombus terrestris* was not likely to be affected by the prolonged ingestion of nectar from sunflowers with seeds dressed with imidacloprid. Although this was the conclusion, some significant negative impacts were observed during the investigation; for example both doses of imidacloprid affected worker survival rate by 10% in the first month and brood production was reduced in one treatment, when compared with the control, but not significantly enough to affect reproduction in the long term<sup>37</sup>.

A number of studies show no impact on bumblebees; for example, a study undertaken on *Bombus terrestris* and imidacloprid-treated sunflower seeds found that after 9 days of treatment there was no significant difference between the control and the treated fields. The number of workers returning did not differ and also no significant effect was seen for population increase rate and mating rate for the colonies<sup>38</sup>. However, it should be noted that the study was only conducted for 9 days, too short a time for assessing life-cycle effects. A further study, which used realistic levels of imidacloprid through chemical

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<sup>35</sup> Suchail S., Guez D. & Belzunces L.P. (2001) Discrepancy between acute chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera*, *Environmental Toxicology and Chemistry* **20**(11), 2482-2486

<sup>36</sup> Marletto F., Patetta A. & Manino A. (2003) Laboratory assessment of pesticide toxicity to bumblebees. *Bulletin of Insectology* **56** (1), 155-158

<sup>37</sup> Tasei J.N., Lerin J. & Ripault G. (2000) Sub-lethal effects of imidacloprid on bumblebees, *Bombus terrestris* (Hymenoptera: Apidae), during a laboratory feeding test. *Pest Management Science* **56** (9), 784-788

<sup>38</sup> Tasei J.N., Ripault G. & Rivault E. (2001) Hazards of Imidacloprid seed coating to *Bombus terrestris* (Hymenoptera: Apidae) when applied to sunflower seeds. *Journal of Economic Entomology* **94** (3), 623-627

seed and soil treatments, found that no lethal or sub-lethal colony or individual foraging effects were observed<sup>39</sup>.

*Bombus terrestris* bumblebees pollinating tomatoes sprayed with imidacloprid at product label doses, were harmed<sup>40</sup>. Field research on turf application and bumblebees in the US found that 28–30 days after the application of imidacloprid sprays on turf containing white clover there was no effect on colony vitality for *Bombus impatiens* (number of brood, workers and honey pots, and weights of queens, workers and whole colonies with hives), suggesting that spray treatments pose little hazard to bumblebees. In contrast, exposure to dry non-irrigated residues of a pesticide containing imidacloprid had a severe impact on colony vitality as individuals did not avoid insecticide-treated areas<sup>41</sup>. A similar type of contamination would occur from sowing of dressed seeds, which can result in the release of contaminated dust on to adjacent vegetation during drilling<sup>11,12</sup>.

#### 5.5.8 Honey bee toxicity research – above relevant environmental levels

A study examining the acute toxicity (LD50) values of imidacloprid to Honey bees found the LD50 to be approximately 60 ng/bee (600 µg/kg) at 48 hours and 40 ng/bee (400 µg/kg) at 72 hours and 96 hours<sup>44</sup>. However, a Bayer study found an LD50 at slightly higher levels, between 0.14 and 1.57 mg/kg (140 and 1,570 µg/kg)<sup>17</sup>. A further study found that much lower levels were acutely toxic with an oral LD50 of 3.7–20.6 ng/bee (37–206 µg/kg)<sup>42</sup>.

#### 5.5.9 Honey bee toxicity research - relevant environmental levels

Chronic toxicity was tested in a study using 0.1, 1 and 10 µg/kg solutions of imidacloprid and its metabolites for 10 days. LD50 was reached at 8 days, at a rate of 12 µl/day giving accumulated doses of 0.1, 1 and 10 µg/kg and so imidacloprid was toxic at doses 60 to 6,000 times lower than those required to produce the same effect in acute toxicity tests<sup>35</sup> a very significant result. The imidacloprid DAR chronic toxicity had a much higher toxicity with a laboratory LD50 of 24 µg/kg and a 'no observed lethal effect concentration' (NOLEC) of 20 µg/kg<sup>19</sup>. A recent field study found no evidence of honey-bee mortality in hives adjacent to imidacloprid treated maize fields<sup>43</sup>.

#### 5.5.10 Honey bee sub-lethal effects research - effects above relevant environmental levels

A number of higher imidacloprid levels have been found to have sub-lethal effects on Honey bees; however, some of these levels are higher than those found in pollen and nectar to date. A laboratory study found a level of 100 µg/kg imidacloprid made treated bees less active and their communicative capacity was impaired for a few hours after

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<sup>39</sup>Morandin L.A. & Winston M.L. (2003) Effects of novel pesticides on Bumblebee (Hymenoptera: Apidae) Colony health and foraging ability. *Environmental Entomology* **32** (3), 555–563

<sup>40</sup>Sterk G & Benuzzi M. (2004) New plant protection chemicals: tests of toxicity to bumble bees in the greenhouse. *Culture Protette – CAB Abstract*

<sup>41</sup>Jerome A.G., Held D.W. & Potter D.A. (2002) Hazards of insecticides to the bumble bees *Bombus impatiens* (Hymenoptera: Apidae) foraging on flowering white clover in turf. *Journal of Economic Entomology* **95** (4), 722–728

<sup>42</sup>Gregorc A. & Bozic J. (2004) Is honey bee colonies mortality related to insecticide use in agriculture? *Sodobno Kmetijstvo* **37** (7), 29–32

<sup>43</sup>Nguyen B.K., Saegerman C., Pirard C., Mignon J., Widart J., Thirionet B., Verheggen F.J., Berkvens D., De Pauw E. & Haugbrugge E. (2009) Does imidacloprid seed-treated maize have an impact on honey bee mortality. *Journal Economic Entomology* **102** (2), 616–623

treatment, potentially leading to a decline in social behaviour<sup>44</sup>. More detailed research examining imidacloprid effects on neuronal metabolism found it exerted a facilitatory or inhibitory effect depending on the dose<sup>45</sup>. In another study, feeding sugar solutions of 24 µg/kg of imidacloprid resulted in a reduction in the foraging activity on the food source as well as a reduction in activity at the hive entrance<sup>46</sup>. The same research found imidacloprid to negatively affect learnt olfactory discrimination tasks as well as learning performances for proboscis extension response<sup>46</sup>. At 48 µg/kg imidacloprid was seen to affect syrup consumption and foraging activity<sup>47</sup>; similarly another study found that 50 µg/l of imidacloprid caused an increase in the foraging interval and at 1,200 µg/l caused abnormalities in the revisiting of feeding sites<sup>48</sup>. Another study found a similar result, with 20–100 µg/kg reducing foraging activity as well as causing other behavioural changes, such as affecting the trembling dancing that discourages other bees from foraging. At higher concentrations it reduced the effectiveness of the waggle dance as the information communicated became less precise, but no population effects were seen<sup>49</sup>. Also a Bayer study assessing concentrations as high as 20 µg/kg used during the chronic toxicity tests had no adverse impact on colony development<sup>17</sup>.

#### 5.5.11 Honey bee sub-lethal effects research - effects at relevant environmental levels

A larger number of research papers have found that imidacloprid is toxic to Honey bees at sub-lethal doses of between 0.1 and 20 µg/kg. At this point it affects their vital functions<sup>10,35,51</sup>. Recorded effects of environmentally relevant imidacloprid levels on Honey bees include: apathy, laboured breathing, a lack of co-ordination and convulsion<sup>50</sup>. Sub-lethal doses have been seen to alter the behaviour of foraging insects and 6 µg/kg of imidacloprid induces a decrease in the proportion of active bees<sup>51</sup>. Foraging bees reduced their visits to a syrup feeder when it was contaminated with 3µg/kg, this may be due to reduced effectiveness of the waggle dance<sup>48</sup>. A study administering 30-minute oral treatment of 12 ng (0.012 µg) of imidacloprid found that olfactory learning performances were impaired, such as proboscis extension reflex

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- <sup>44</sup>Medrzycki P., Montanari R., Bortolotti L., Sabatini A.G., Maini S. & Porrini (2003) Effects of imidacloprid administered in sub-lethal doses on honey bee behaviour. Laboratory tests. *Bulletin of Insectology*. **56** (1), 59–62
- <sup>45</sup>Armengoud C., Lambin M., Gauthier M (2002) Effects of imidacloprid on the neural processes of memory in honey bees. Honey Bees. CABI Abstracts <http://www.cababstractsplus.org/google/abstract.asp?AcNo=20053057361>
- <sup>46</sup>Decourtye A., Devillers J., Cluzeau S., Mercedes C. & Pham-Delegue M.H. (2004) Effects of Imidacloprid and Delamethrin on associative learning in honeybees under semi-field and laboratory conditions. *Ecotoxicology and Environmental Safety* **57** (3), 410–419
- <sup>47</sup>Ramirez-Romero R., Chaufaux J. & Pham-Delegue M.H. (2005) Effects of Cry1Ab protoxin, deltamethrin and imidacloprid on the foraging activity and the learning performances of the honeybee *Apis mellifera*, a comparative approach. *Apidologie* **36**, 601–611
- <sup>48</sup>Yang E.C., Chuang Y.C., Chen Y.L. & Chang L.H. (2008) Abnormal foraging behaviour induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae). *Journal of Economic Entomology* **101** (6), 1743–1748
- <sup>49</sup>Kirchner W.H. (1999) Mad-bee disease? Sub-lethal effects of Imidacloprid (Gaucho) on the behaviour of honey bees. Association with of institute for bee research, 50 year anniversary 1949–1999 reports of the 46th seminar in Marburg, 23–25 March 1998. *Apidologie* **30**, 422–423
- <sup>50</sup>Moise A., Marghitas L.A., Dezmirean D. & Man M.(2003) Research concerning the effect of Imidacloprid on honeybees (*Apis mellifera carpatica*). *Buletinul Universitatii de Stiinte Agricole si Medicina Veterinara Cluj-Napoca, Seria Zootehnie si Biotehnologii* **59**
- <sup>51</sup>Colin M.E., Bonmatin J.M., Moineau I., Gaimon C., Brum S., & Vermandere J.P. (2004) A method to quantify and analyze the foraging activity of Honey Bees: Relevance to sub-lethal effects induced by systematic insecticides. *Archives of Environmental Contamination and Toxicology* **47** (3), 387–395

procedure and the medium-term olfactory memory<sup>52</sup>. When comparing the amounts of imidacloprid consumed over the development period for different categories of bees (e.g.: nurses, workers, etc.), development periods varied from 5 to 90 days and consumption ranged from 3.8 to 0.5 ng (0.038 to 0.0005 µg), and so there is a wide consumption variation between different categories. The study found that some categories of Honey bees are potentially exposed to lethal and sub-lethal doses of imidacloprid<sup>53</sup>. Another study found imidacloprid levels of 10 µg/kg within pollen cakes caused a 20% reduction in the number of brood cells, and so consumption was negatively affecting nurse bees or contaminating brood food<sup>54</sup>. A study of hives that were close to sunflowers treated with Gaucho® (contains imidacloprid) were studied and five out of the 12 hives tested contained imidacloprid concentrations above 5 µg/kg, considering degradation rates this level could be as high as 10 µg/kg when consumed<sup>42</sup>. The hives contaminated with imidacloprid were also infected with two parasites a mite, *Varroa destructor*, and a microsporidian, *Nosema apis*. The study could not rule out a connection between the insecticide and increased parasites and disease. It was thought that hives with imidacloprid above 5 µg/kg could be severely impacted and suffer colony death<sup>42</sup>. Another study showed that residues of imidacloprid were present in 69% of 125 bee colonies sampled. Of these, 11 samples had imidacloprid levels quantified and had values ranging from 1.1 to 5.7 µg/kg<sup>55</sup>. A Bayer study, using four independent research facilities tested Honey bees exposed to levels found in the environment, at 0.1, 1.0 and 10 µg/l 50% sucrose solution spiked with imidacloprid and its plant metabolites for 10 days and found no increase in treatment-related mortality or behavioural abnormalities<sup>56</sup>.

## 6.0 Clothianidin

Clothianidin is another neonicotinoid but the scientific research available for clothianidin is not as extensive as for imidacloprid. Clothianidin has an aerobic soil metabolism of 148 to 1,155 days. A summary of information regarding this neonicotinoid and its use in the UK is detailed below.

### 6.1 UK available products

Currently five products containing clothianidin are registered for use in the UK; these are: Deter, Poncho, Poncho Beta, Raxil Deter and Redigo Deter for crops<sup>88</sup>. These products are used on the following crops; barley (winter, seed), durum wheat (seed), oats (winter, seed), rye (seed), triticale (seed), wheat (winter, seed), forage maize, grain maize, sweetcorn, fodder beet (seed), sugar beet (seed). For sugar beet beta-cyfluthrin/clothianidin accounts for 5% for the area grown; however, as with imidacloprid

<sup>52</sup> Decourtye A., Armengaud C., Renou M., Devillers J., Cluzeau S., Gauthier M., & Pham-Delegue M.H. (2004) Imidacloprid impairs memory and brain metabolism in the honeybee (*Apis mellifera* L.) *Pesticides Biochemistry and Physiology* **78**, 83–92

<sup>53</sup> Rortais A., Arnold G., Halm M.P. & Touffet-Briens F. (2005) Modes of Honey bees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. *Apidologie* **36**, 71–83

<sup>54</sup> Rose R., Dively G.P. & Pettis J. (2007) Effects of Bt corn pollen on honey bees: emphasis on protocol development. *Apidologie* **38**, 368–377

<sup>55</sup> Chauzat M.P., Faucon J.P., Martel A.C., Lachaize J., Cougoule N. & Aubert M. (2006) A survey of pesticide residues in pollen loads collected by honey bees in France. *Apiculture and Social Insects* **99** (2), 253–262

<sup>56</sup> Schmuck R. (2004) Effects of a chronic dietary exposure of the Honeybee *Apis mellifera* (Hymenoptera: Apidae) to Imidacloprid. *Archives of Environmental Contamination and Toxicology* **47** (4), 471–478

it is only flowering crops that result in pollen and nectar residues and clothianidin is currently only applied to wind-pollinated and non-flowering insect-pollinated crops. However, contamination of the wider environment may occur during sowing and could affect other non-target species such as pollinators, this impact has been seen with other neonicotinoid systemic pesticides<sup>11,12</sup> and so could be a contamination route for other pesticides used as seed dressings.

## 6.2 EU Draft Assessment Report

The acute oral and contact toxicity tests had very high hazard quotients of 4,947–20,580 for acute oral toxicity and 424–1,762 for acute contact toxicity, again showing an unacceptable level of risk for spraying. For dressed seeds, which are most applicable to the UK crop products, all field and semi-field experiments showed no test-related impacts; although mortalities did occur in a large proportion of the tests, they were deemed to be not test-related<sup>57</sup>. An independent review by a Honey bee expert<sup>58</sup> of the test process for clothianidin noted that the test methods used colonies of 500 bees to assess the egg-laying capacity of queens. It noted that with queens continually laying up to 1,000 eggs per day, colonies would likely be much larger than this and so the colony size utilised is inadequate to assess whether egg-laying capacity is impaired<sup>58</sup>.

## 6.3 Research

There is very little independent research; however, one study has examined bumblebees, studying colonies exposed to clothianidin at 6 µg/l, representing the highest level found in field pollen, and at a higher level of 36 µg/l. Clothianidin did not affect newly emerged worker weights, the amount of brood or the number of workers, males and queens at either dose, and the study concluded that residue from seed-treated canola (oilseed rape) would not effect the health of bumblebee colonies<sup>59</sup>.

For Honey bees, a laboratory-based feeding experiment by Bayer found an LD50 dose of 3.9 µg<sup>60</sup>; and at a dose of 0.016 µg active substance (a.s.)/bee, or higher, bees rejected spiked sucrose solution. As a consequence the study recommended that clothianidin should be classified as highly toxic to Honey bees. A US government document states the LD50 for clothianidin as >0.0439 µg/bee and that it has the potential to cause toxic chronic exposure to Honey bees through the translocation of residues in nectar and pollen. The document also states that the effects of this toxic chronic exposure may include lethal and/or sub-lethal effects to larvae and also reproductive effects on the queen; therefore a full life-cycle study is required<sup>61</sup>. In a short 3-week field experiment, flowering canola seeds (oilseed rape) were treated with clothianidin, after which colonies were tested in the laboratory. During this time no effect was seen on Honey bee mortality, worker longevity or brood development, or on weight or honey yields of colonies. In addition the residues of clothianidin found on the crop were 8- to

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<sup>57</sup> Draft Assessment Report: Initial risk assessment provided by the rapporteur Member State Belgium for the existing active substance Clothianidin

<sup>58</sup> Kievits J. (2007) Bee gone. *Pesticide News* **76**, 3–5

<sup>59</sup> Franklin M.T., Winston M.L. & Morandin L.A. (2004) Effects of Clothianidin on *Bombus impatiens* (Hymenoptera: Apidae) colony health and foraging ability. *Journal of Economic Entomology* **97** (2), 369–373

<sup>60</sup> Schmuck R. & Keppler J. (2003) Clothianidin – Ecotoxicological profile and risk assessment. *Pflanzenschutz-Nachrichten Bayer* **56** (1), 26–58

<sup>61</sup> EPA (Environmental Protection Agency) Pesticide Fact Sheet for Clothianidin, May 30 2003, Office of Prevention. Pesticides and Toxic Substances



22-fold below the reported 'no observable adverse effects concentration', no residues were found in the bee wax and no adverse effects were witnessed on overwintering<sup>62</sup>.

## 7.0 Fipronil

### 7.1 UK available products

Another systemic pesticide that acts on the nervous system of insects is fipronil; there are four registered products in the UK containing fipronil. These are: Regent 1GR, Regent 1GR, Vil-Nil and Vil-Nil (Certis Europe), all of which are used for ornamental plant production (container-grown).

### 7.2 EU Draft Assessment Report

The EU DAR showed a high level of risk for acute toxicity, but none of the field and tunnel testing indicated a risk to Honey bee survival, foraging activity or colony status from either soil or seed treatments. Like imidacloprid there were issues with detection levels being high at 1 µg/kg<sup>63</sup>. An independent review<sup>64</sup> of the test process for fipronil noted that the Toxicity Exposure Ratio (TER) is based on the amount of nectar a bee is exposed to being 20 µl (25 mg); however, foraging and hive bees can consume between 100 and 900 mg in five days. This gave a much higher TER with bees consuming half the LD50 in just four days. Also noted were tunnel tests where the tunnels were only 10 metres long and not long enough to assess impacts on direction learning as only eyesight and smell is used at these short distances.

### 7.3 Research

There is very limited independent research for fipronil. French research on 125 Honey bee colonies found fipronil residues in 10 samples at an average concentration of 1.2 g/kg<sup>55</sup>. Another study found that a daily dose of fipronil at a rate of 0.1 ng/bee caused the death of all tested bees within a week and at 0.01 ng/bee they spent more time immobile in field apparatus and ingested significantly more water; and fipronil-treated bees' sensory abilities were affected<sup>65</sup>. One study on Honey bees observed a significant reduction in sucrose sensitivity for the dose of 1 ng/bee (0.001 µg/bee) 1 hour after application. A lower fipronil dose of 0.5 ng/bee (0.0005 µg/bee) impaired olfactory learning of the Honey bees. It was concluded that the olfactory memory process and sucrose perception of Honey bees is vulnerable to sub-lethal doses of fipronil<sup>66</sup>.

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<sup>62</sup>Cutler C.G. & Scott-Dupree C.D. (2007) Exposure to Clothianidin seed-treated Canola has no long-term impact on honey bees. *Journal of Economic Entomology* **100** (3), 765–772

<sup>63</sup>Draft Assessment Report: Initial risk assessment provided by the rapporteur Member State Germany for the existing active substance Fipronil

<sup>64</sup>Kievits J. (2007) Bee gone. *Pesticide News* **76**, 3–5

<sup>65</sup>Aliouane Y., El Hassani A.K, Gary V., Armengaud C., Lambin M. & Gauthier M. (2009) Subchronic exposure of honeybees to sublethal doses of pesticides: Effects on behaviour. *Environmental Toxicology and Chemistry* **28** (1), 113–122

<sup>66</sup>Hassani A.K.E., Dacher M., Gauthier M. & Armengaud C. (2005) Effects of sub-lethal doses of fipronil on the behaviour of the honeybee (*Apis mellifera*). *Pharmacology Biochemistry and Behaviour* **82** (1), 30–39

## 8.0 Acetamiprid

### 8.1 UK available products

Acetamiprid is present in eight products sold in the UK, including: Bugclear Ultra, Bugclear Ultra for Pots, Bugclear Ultra for Ready to Use, Bugclear Ultra Gun!, Bugclear Ultra Vine Weevil Killer and Gazella. These products are used on house plants, ornamental garden plants, apple, aubergine (indoor), cherry, ornamental plant production (outdoor), ornamental plant production (indoor), pear, pepper (indoor), plum, tomato (indoor).

### 8.2 Research

Independent research has found that acetamiprid has negative effects on Honey bee behaviour at sub-lethal doses in sucrose, by impairing long-term learning at 0.1 µg/bee and increasing locomotive activity at 0.1 µg/bee. In the same study an increasing antennal stimulation to sucrose at 1 µg/bee<sup>68</sup> was observed and in another study an increased responsiveness to water was witnessed at 0.1 µg/bee<sup>65</sup>.

## 9.0 Thiacloprid

### 9.1 UK available products

Thiacloprid is present in nine UK products: Agrovista Reggae, Baby Bio House Plant Insecticide, Biscaya, Calypso, Exemptor, Provado Ultimate Bug Killer Ready to Use, Provado Vine Weevil Killer 2, Standon Zero Tolerance and Ultimate Bug Killer Ready to Use. It is used on crops including: apple, herb (outdoor), herb (indoor), house plant, ornamental garden plants, combining pea, mustard, oilseed rape, potato (seed), potato (ware), vining pea, wheat, aubergine (indoor), cane fruit and bush fruit, cherry, courgette (indoor), cucumber (indoor), leaf brassica, lettuce (outdoor use only), pepper (indoor), tomato (indoor) and ornamental plant production.

### 9.2 Research

Bayer research found that thiacloprid had no adverse effect on earthworms at its maximum proposed use rates and was of limited risk to non-target arthropods<sup>67</sup>. It also found that the LD50 for oral consumption by Honey bees was between 4.1 and 17.9 µg a.s./bee, the research concluded that at proposed application rates thiacloprid had a low toxicity to Honey bees and so a reasonable level of risk to bees<sup>67</sup>.

## 10.0 Thiamethoxam

### 10.1 UK available products

Seven products are registered for use in the UK: Actara, Bug Attack Granules, Bug Attack Liquid Concentrate, Bug Attack Quick Sticks, Bug Attack Ready to Use, Centric and Cruiser SB. These products are used on a number of different crops including: potato, potato (seed crop), house plant (container-grown), ornamental garden plants (indoor, container-grown), house plant, house plant (container-grown), apple, pear, fodder beet (seed) and sugar beet (seed).

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<sup>67</sup> Schmuck R. (2001) Ecotoxicological profile of the insecticide thiacloprid. *Pflanzenschutz-Nachrichten Bayer* **54** (2), 161–184

## 10.2 Research

Thiamethoxam at field doses was found to be harmful to bumblebees (*Bombus terrestris*) in a study on tomato plants<sup>40</sup>. Other studies found this chemical to have no significant impact: for Honey bees sub-lethal doses of thiamethoxam at 0.1, 0.5 and 1 µg/bee were found to have no effect on behaviour<sup>68</sup>; also no effect on bumblebees used in tomato pollination when applied at 200 g active ingredient (a.i.) /ha<sup>69</sup> and no effect on bumblebee broods at 150 and 161 g a.i./ha<sup>70</sup>.

## 11.0 Other issues

Concern has been expressed in regard to the effects of combining neonicotinoids with fungicides; one study found that in combination with fungicides the toxicity of neonicotinoids to Honey bees increased for both acetamiprid and triflumizole this was as much as 1,141-fold<sup>71</sup>. Neonicotinoids such as imidacloprid are combined with fungicides in seed dressing but no research has been done on the effect of mixing these substances so this area needs further research. In tests on other pesticides significant differences have been found between the susceptibilities of bee colonies tested. This is due to other factors having permanent or semi-permanent effects on their susceptibility. It is unclear whether these factors were genetic or environmental<sup>72</sup>, but this colony variation could help in explaining some of the differences in results outlined in this report.

## 12.0 Global perspective of neonicotinoids and fipronil use and bans

In France imidacloprid-based insecticides have been banned from use on sunflower, maize and oilseed rape crops, because of their potential effects on bees. The French government has also banned six fipronil insecticides, because of their suspected impact on bees. The Advisory Commission on Pesticides for France (Commission de Toxiques) was charged to evaluate the impact of pesticides, and, after reviewing studies on Gaucho® (an imidacloprid-based BAYER product), it published the following three comments:

1. The examined data does not allow for a conclusion of the indisputable effect of imidacloprid or its metabolites on bees' production of honey.
2. Conversely, it is not possible to totally exclude the effect of imidacloprid and its metabolites, taking into account the toxic effects of minute doses, doses that are in keeping with those concentrations potentially present in the plants during the period of harvest.

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<sup>68</sup> El Hassani A.K., Dacher M., Gary V., Lambin M., Gauthier M. & Armenqaud C. (2008) Effects of sub-lethal doses of acetamiprid and thiamethoxam on the behaviour of honeybee (*Apis mellifera*). *Archives of Environmental Contamination and Toxicology* **54** (4), 653–661

<sup>69</sup> Alarcon A.L., Canovas M., Senn R. & Correia (2005) The safety of thiamethoxam to pollinating bumblebees (*Bombus terrestris* L.) when applied to tomato plants through drip irrigation. *Communications in Agricultural and Applied Biological Sciences*. **70** (4), 569–579

<sup>70</sup> Sechser B. & Freuler J. (2003) The impact of thiamethoxam on bumblebee broods (*Bombus terrestris* L.) following drip application in covered tomato crops. *Anzeiger fur Schallingskunde*. **76** (3), 74–77

<sup>71</sup> Iwasa T., Motoyama N., Ambrose J.T. & Roe M. (2004) Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*. *Crop Protection* **23** (5), 371–378

<sup>72</sup> Tahori A.S., Sobel Z. & M. Soller (1969) Variability in insecticide tolerance of eighteen honey-bee colonies. *Entomologia Experimentalis et Applicata* **12** (1), 85–98

3. That complementary research should be undertaken to clarify the following points:
  - a. The metabolism of the product in parts of the plant accessible to bees
  - b. The limit of the toxicity of the product and its metabolites for bees and the quantities present
  - c. The persistence of imidacloprid in the soil and its presence in crops that have not been treated<sup>73</sup>.

Germany has banned all neonicotinoid-based insecticides because of massive bee deaths<sup>74</sup>. This was caused by a pollution incident where the application rate of clothianidin was increased to deal with a high level of infestation of western corn rootworm. As a result the chemical had not bound properly to the maize seeds and, during sowing, dust was created containing clothianidin, which drifted onto and contaminated flowering plants, exposing foraging Honey bees to clothianidin. Neonicotinoids have remained banned in Germany because of a lack of clarity about to what extent and how bees come into contact with these chemicals, and because of new findings suggesting plant products consumed by bees may increase risk. In Italy the government has taken a precautionary measure and suspended the use of products containing imidacloprid, clothianidin, fiprinol, and thiamethoxam to be used on oilseed rape, sunflower and sweetcorn because of their potential impact on bees, and is to start a research programme to further investigate the recent bee death witnessed in the country<sup>75</sup>. Also, in Slovenia, sales of both clothianidin and imidacloprid have been banned. There have also been similar concerns over imidacloprid in Canada. Prince Edward Island beekeepers have reported serious losses of bees since 1995; thought to be linked to residues from imidacloprid. The potatoes on the island have been treated with soil applications of Admire (imidacloprid) to prevent Colorado potato beetle. It is believed that imidacloprid had accumulated in the soil through rotational clover and canola crops which contained sub-lethal residues of imidacloprid in the pollen and nectar. However, a comprehensive study looking at imidacloprid levels in plants and hives in this area did not find detectable levels of the chemical above 2 µg/kg (level of detection)<sup>76</sup>.

## 13.0 Discussion

### 13.1 Imidacloprid

Research results on the toxicology of imidacloprid shows a range of concentrations of imidacloprid having a range of impacts on non-target invertebrates<sup>22,23,24,34,35,37,40,41,46,48,49,51,53,54,61,etc.</sup>. The most significant result is that very low levels of imidacloprid have significant sub-lethal impacts on bees<sup>37,40,46,48,49,50,51,53,54</sup>, by altering behaviour and causing bee deaths through chronic toxicity, see Appendix 1. These low levels are environmentally relevant and relate to residues levels found in the pollen and nectar of

<sup>73</sup> [http://www.apiservices.com/articles/us/gaucho/manifestation\\_paris\\_us.htm](http://www.apiservices.com/articles/us/gaucho/manifestation_paris_us.htm)

<sup>74</sup> The Federal Office of Consumer Protection and Food Safety (BVL) website — Authorisations for neonicotinoids are still suspended due to the hazards to bees:

[http://www.bvl.bund.de/cln\\_027/nn\\_496790/sid\\_FFE204596E8096E5D0F6C6B9E657F9EA/EN/08\\_PresseInfothe\\_k\\_engl/01\\_Presse\\_und\\_Hintergrundinformationen/PI\\_Maissaatgut\\_Mesurool\\_engl.html\\_\\_nnn=true](http://www.bvl.bund.de/cln_027/nn_496790/sid_FFE204596E8096E5D0F6C6B9E657F9EA/EN/08_PresseInfothe_k_engl/01_Presse_und_Hintergrundinformationen/PI_Maissaatgut_Mesurool_engl.html__nnn=true)

<sup>75</sup> Italian Government press release on neonicotinoid ban <http://www.ministerosalute.it/>

<sup>76</sup> Rogers R.E.L & Kemp R.K. (2003) Imidacloprid, potatoes, and honey bees in Atlantic Canada: Is there a connection? *Bulletin of Insectology* **56** (1), 83–88

plants grown using imidacloprid treated seeds. These results are from independent research which achieved results with a higher sensitivity than those collected through the EU DAR. The higher sensitivity of independent research and the evidence they show of imidacloprid risk to bees has been noted by many studies<sup>77,78</sup>. There are a significant number of research projects showing harmful impacts to both Honey bees<sup>35,39,46,48,49,51,53,54</sup> and bumblebees<sup>37,40</sup> at environmentally relevant levels mainly in studies of chronic toxicity and sub-lethal impacts of imidacloprid. These are two main areas with no standard test method which are significant for invertebrates particularly social insects. This difference between independent research and the DAR calls into question the effectiveness of the current European regulations in assessing the impacts of systemic pesticides and sub-lethal effects. The evidence presented here suggests that the DAR has failed to accurately assess the active substance imidacloprid and its impacts when used as a seed dressing. Imidacloprid is now present and widely used in the UK countryside as a seed dressing, most commonly on oilseed rape crops; see Appendix 3 for a table of approved products.

The mix of results in relation to imidacloprid concentrations may be related to hive susceptibility with more vulnerable or stressed hives having a greater sensitivity to insecticide impacts; another possibility is species sensitivity. Hive susceptibility could be related to genetic or environmental variables such as poor weather conditions, drought, food shortage, disease or parasites<sup>72</sup>, (Darvill *pers. commun.*).

There has been insufficient consideration of other potential contamination routes beyond the crop plant itself. Research has shown that areas adjacent to sowing can be contaminated through dust during drilling<sup>11,12</sup>. This method of contamination was not fully considered during the DAR and a Bayer research report found conflicting results regarding this route of contamination<sup>13</sup>. Other areas of potential contamination that have not been investigated either by the neonicotinoid DARs or by independent research are transferral of contaminants through soil to non-crop plants and weeds within the treated field; and also the drinking of contaminated plant guttation water by bees and other non-target invertebrates has not been properly investigated.

Aquatic environments can be contaminated through runoff; aquatic invertebrates such as mayfly are very sensitive to imidacloprid contamination, with low levels present in the environment resulting in reduced reproductive success<sup>21,22,23</sup>.

In the amenity sector, nurseries and private garden products contain imidacloprid and are available as sprays, seed dressings and soil application products<sup>18</sup>, making imidacloprid available again through pollen and nectar, as well as through direct contact. Direct contact impacts through sprays can be mitigated by spraying early in the morning before pollinators are active, but this is difficult to regulate, particularly for amenity, nurseries and private garden products. Also research has shown non-irrigated sprays to be particularly toxic to bumblebees<sup>41</sup>. Research within the DAR generally focuses on

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<sup>77</sup> Maxim L. & Sluijs (2007) Uncertainty: Cause or effect of stakeholders' debates? Analysis of a case study: The risk for honeybees of the insecticide Gaucho®. *Science of the Total Environment* **376**, 1–17

<sup>78</sup> SCT (Scientific and Technical Committee for the Multifactor Study of the Honeybee Apiaries Decline). Imidaclopride utilisé en enrobage de semences (Gaucho®) et troubles des abeilles, rapport final. Paris: Ministère de l'Agriculture, de la Pêche et des Affaires Rurales; 2003. Available at: <http://www.agriculture.gouv.fr/spip/IMG/pdf/rapportfin.pdf>.

use in the agricultural systems; however, the variety of plant types treated in the amenity and amateur sector is much greater, which could lead to a new suite of exposure levels that are not as well considered as crop plants.

### **13.2 Other neonicotinoids and fipronil**

Clothianidin research is limited but what does exist is part of the Clothianidin DAR; the Honey bee research conducted in the DAR has been called into question by a review from a Honey bee expert as it was inadequate to fully assess the effect of Clothianidin on Honey bees<sup>58</sup>. Clothianidin is not used on any insect-pollinated flowering cereal or root crop plants, but there may be contamination of the wider countryside from dry residues during sowing. Exposure and the nature of that exposure from other crop types, e.g. fruits, ornamental plants etc., has yet to be thoroughly investigated; this is true for imidacloprid and all other neonicotinoids. There is also limited independent research for neonicotinoids other than imidacloprid and fipronil, so there is a lack of understanding of the levels present in the environment and the impact they might be having is unknown. The independent research that has taken place suggests these chemicals consistently alter bee behaviour<sup>46,49,50,51</sup>, with the exception of thiamethoxam where studies showed no effect.

### **13.3 Assessing the assessment process**

One very notable thing is that the data collected by the DAR<sup>19</sup> conflicts with independent research and another is that there are a number of important areas of research not covered by the DAR. For example, the experiments carried out by the imidacloprid DAR were too short to pick up long-term effects on Honey bee colonies. One of the biggest effects that imidacloprid could have would be to reduce the queen's ability to lay down fat reserves and so reduce survival. This could not be assessed in a 4–6-week experiment (Darvill B., *pers. commun.*). Also in the winter, and at times, for instance, of drought stress, when hives are more susceptible to chemical effects, nectar sources are low and the honey becomes more concentrated<sup>42</sup>, causing impacts not seen in good conditions. Winter bees and hive susceptibility are two areas of research not adequately covered by the DAR.

A report completed by the French Scientific and Technical Committee, 'For a multifactor study on the disturbance of bees: imidacloprid used in coating of seed (Gaucho) and the disturbance to bees', reviewed and validated current literature relating to the impact of imidacloprid on bees<sup>78</sup>. The results provided by these validated studies were used to evaluate the risks to bees; this was assessed using Predicted Exposure Concentration (PEC) together with Predicted No Effect Concentration (PNEC), and a range of scenarios were formed and assessed relating to different bee types and potential sources of contamination. The report concluded that, based on the current state of knowledge and the results from their scenarios, the PEC/PNEC relationships were demonstrating impacts including mortality in pollinators, behaviour disruption and winter mortalities. The report concluded that the coating of sunflower seeds with Gaucho® (active substance – imidacloprid) puts at risk bees of different ages, except for collectors making pollen balls. Corn seeds coated in Gaucho® are likely to lead to the mortality of nursing bees which would consequently lead to accrued mortality and weaken bee populations<sup>78</sup>. A translation of the summary of the report is in Appendix 4. They stated that the chronic exposure created by these products indicates that the European regulations need to provide a Hazard Quotient and threshold for systemic pesticides so that they can be thoroughly assessed. This issue is part of a study being undertaken as



part of a DEFRA research project that will be completed in 2009 on 'Systemic pesticide risk assessment for Honey bees'<sup>79</sup>.

A full assessment of the imidacloprid DAR was done by Natuur en Milieu, PAN-Europe, Inter Environment Wallonie, Nature et Progress and Movement pour le droit et le respect des générations futures (MNRGF)<sup>80</sup> and the results of this assessment were sent as a letter, see Appendix 5, to the European Commissioner for Health to request an internal review of Commission Directive 116/2008, including a review of the DAR for active substance imidacloprid. Their letter outlines concerns with the DAR, which are also set out in Appendix 2, including the following: necessary tests for each bee category (e.g.: larval tests) were not carried out, no bee brood feeding test was done so impacts on larvae were not assessed, and also the DAR states that there is no difference between larvae and adults but this is not backed up with scientific evidence. The need for comprehensive winter bee tests on pollen consumption is important due to differences in the nature of consumption between winter and summer bees. Also a chronic toxicity study showing low levels of imidacloprid and its metabolites impacting on bees was invalidated in the DAR based on weak reasoning, and another study looking at sub-lethal impacts estimated the No Observed Effect Level (NOEL) using an underestimation of the nectar consumption per bee and so the non-effect concentration was set too high. The DAR discredits results showing sub-lethal effects on insufficient grounds and the validation of studies is done without any validation criteria; the DAR also uses flawed consumption tests and colony sizes which are too small to test egg-laying, and sowing dust effects are not sufficiently measured. There is no consideration in the DAR of synergies between the active substance and bee pathogens and there are massive discrepancies between the results of different tests. The letter also notes that the DAR validation discredits non-favourable reports during the substance authorisation but the favourable reports are not thoroughly validated and this highlights the lack of scientific independence of the assessment and that the applicant is doing the assessment without any kind of independent assessment. The letter concludes that the DAR fails to respect Article 4 of Directive 91/414<sup>6</sup> and fails to demonstrate that there is no unacceptable impact on bees or on other foraging insect species and that seed coatings go against integrated pest management in the Framework Directive on the sustainable use of pesticides<sup>80</sup>. More information on these discrepancies can be seen in a copy of the letter which is in Appendix 5.

A critique of the DARs of non-imidacloprid neonicotinoids by a Honey bee expert<sup>58</sup> found that the colony size utilised was inadequate for assessing queen laying capacity for clothianidin, that the amount of nectar consumed was not sufficient for assessing Toxicity Exposure Ratio and that the tunnel size was inadequate for assessing learning behaviour in the fipronil DAR. Generally the critique found that tests also failed to prove that bees were consuming the test pollen and not their stores but the DARs of the other neonicotinoids have not been as fully assessed as imidacloprids.

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<sup>79</sup> DEFRA Science and Research Projects: Are pesticides risk assessments for honeybees protective of other pollinators? PS2337  
<http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=0&ProjectID=15390>

<sup>80</sup> Letter to the European Commissioner for Health from Natuur en Milieu, PAN-Europe, Inter Environment Wallonie, Nature et Progress and MNRGF. Re: Request for an internal review of commission regulation 116/2008  
[http://www.natuurenmilieu.nl/pdf/090120.01\\_digi\\_papier\\_brf\\_a\\_vassiliou\\_ec\\_health\\_brussel\\_request\\_internal\\_revew\\_116\\_2008.pdf](http://www.natuurenmilieu.nl/pdf/090120.01_digi_papier_brf_a_vassiliou_ec_health_brussel_request_internal_revew_116_2008.pdf)

With no standardised methods for sub-lethal effects and an assessment method that is inappropriate for systemic pesticides, the risk assessments produced as part of the substance approval process cannot be relied upon to detect the sub-lethal effects of pesticides or give an accurate assessment of their impacts, particularly for social insects. For sub-lethal effects research was carried out in 2001/02 on 'The relevance of sub-lethal effects in Honey bee testing for pesticide risk assessments' that reviewed the existing literature on sub-lethal effects and concluded that there are a wide range of impacts from different pesticide types on Honey bees. The report acknowledges that for these effects to be incorporated into risk assessments there needs to be greater understanding of their long-term consequences, especially for social insects such as bees, and that assessments would need to put greater emphasis on significant sub-lethal effects and their long-term consequences for colony survival and development. In 2005 the International Commission for Plant–Bee Relationships – Bee Protection Group held its ninth meeting. The following topics were discussed at the meeting, both sub-lethal and long-term effects, also the effects of systemic pesticides, establishing a test method for sub-lethal effects and validating a testing methodology. It was decided that an EU sequential risk assessment was needed to allow assessment of systemic pesticides and a working group was set up to do this<sup>81</sup>.

A number of studies have been carried out to develop an appropriate test methodology for systemic pesticides as the current Hazard Quotient method is not appropriate for these types of chemicals. A 2003 international study<sup>82</sup> recommended altering current testing guidelines and the decision-making scheme which is defined by the 'European and Mediterranean Plant Protection Organisation' (EPPO), in order to assess the direct effects of low-dose systemic insecticides on bees. Another study proposed a technique using Predicted Exposure Concentration (PEC) together with Predicted No Effect Concentration (PNEC) to assess risk, when studying Honey bees and the risk posed by imidacloprid, and the study found the risk calculated for Honey bees and imidacloprid to be alarming<sup>83</sup>. There are also another two studies that look at using a stepwise risk assessment scheme with trigger values and PNEC<sup>84,85</sup> as a test method. Risk assessments also routinely focus on only Honey bees because of Directive 91/414<sup>6</sup> Directive. It is unclear whether current testing adequately accounts for impacts on bumblebees<sup>86</sup> and other pollinators. A DEFRA research project entitled 'Are pesticide risk assessments for Honey bees protective of other pollinators'<sup>79</sup> is currently assessing this issue; a report was due in late 2008.

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<sup>81</sup> Lewis G., Thompson H & Smagghe G. (2007) In focus: Pesticides and honeybees the work of the ICP-BR Bee Protection Group. *Pest Management Science* **63** (11), 1047–1050

<sup>82</sup> Tasei J.N., Pham-Delegue M.H. & Belzunces L. (2003) Registration of systemic insecticides and European and Mediterranean Plant Protection Organisation (EPPO) guidelines. *Bulletin of Insectology* **56** (1), 189–191

<sup>83</sup> Halm M.P., Rortais A., Arnold G., Tasei J.N. & Rault S. (2006) New risk assessment approach for systemic insecticides: The case of honey bees and Imidacloprid (Gaucho). *Environmental Science and Technology* **40**, 2448–2454

<sup>84</sup> Bruneau E., Kievits J., Lortsch J.-A. & Szöke I.S. (2009) Systemic plant protection products needs a new assessment scheme: a beekeepers point of view. Proposal to ICP-BR

<sup>85</sup> Alix A. & Vergnet C. (2007) Risk assessment to honeybees: a scheme developed in France for non-sprayed systemic compounds. *Pest Management Science*. **63**, 1069-1080

<sup>86</sup> Goulson D., Lye G.C. & Darvill B. (2008) The decline and conservation of bumble bees. *Annual Review of Entomology* **53**, 191–208

## 14.0 Conclusions

We conclude that the DAR for imidacloprid is inadequate for assessing the impact of the test substance on bees; and current test methods utilised in EU DARs are inadequate for assessing systemic pesticides and sub-lethal impacts on non-target invertebrate species. This has been demonstrated by critiques on the DAR written by experts and also by a number of validated independent research studies<sup>78,80</sup>. The precautionary principle states that if there are reasonable scientific grounds for believing that a new product may not be safe, it should not be used until there is convincing evidence that the risks are small and outweighed by the benefits<sup>87</sup>. Independent research shows imidacloprid to have a significant impact on bees at environmentally relevant levels; to be highly toxic to bees when chronic toxicity through pollen and nectar is appropriately assessed; to have significant effects on other non-target invertebrates and a high risk of exposure through other routes. Imidacloprid is currently authorised for use as a systemic pesticide in the UK on the flowering crop oilseed rape as well as a number of amateur and amenity uses which include insect-pollinated flowering plants. Other neonicotinoids and fipronil are also used in the UK but are under-researched in comparison to imidacloprid; however, all neonicotinoids have a similar chemical structure, and alongside fipronil are mainly used as systemic pesticides; show a high toxicity to bees; and affect the nervous systems of invertebrates in the same way. Therefore, imidacloprid, and, potentially, other neonicotinoids and fipronil, may be a significant factor contributing to current bee declines and could also contribute to declines in other non-target invertebrate species. Therefore the current use of imidacloprid is counter to the objectives of Directive 91/414 as it is potentially having an unacceptable effect on the environment.

The inclusion of neonicotinoids such as imidacloprid and fipronil in Annex I to Directive 91/414 represents a breach of Article 5 of Directive 91/414, in view of the considerable evidence that the use of plant protection products containing these substances has an unacceptable influence on the environment, particularly in relation to non-target species. Buglife and the organisations that have signed onto this report therefore request, in accordance with Article 5(5) of Directive 91/414, that the inclusion of all neonicotinoids and fipronil in Annex I be reviewed, and that no further neonicotinoids or similarly acting chemicals be added to Annex I.

We also call for an immediate review, in accordance with Regulation 13(3) of the Plant Protection Product Regulations 2005 (the "Regulations"), of all existing authorisations of neonicotinoid and fipronil products for outdoor use in the UK, and a precautionary suspension of all existing approvals relating to neonicotinoid and fipronil products authorised for outdoor use in the UK until the reviews have taken place. The new European crop protection product Directive 116/2008, which is due to enter into force on 1 August 2009, gives Member States until 31 January 2010 to reevaluate existing authorisations of products containing imidacloprid. However, in light of the harm that the continued use of such products could cause to the environment, and of the requirements of Regulation 13, this review should be brought forward.

The evidence outlined in this report indicates clearly that these products do not satisfy the requirements set out in Regulation 6 of the Regulations, specifically that they must

have no unacceptable influence on the environment, having particular regard to their impact on non-target species. The studies outlined in this report clearly constitute “other relevant technical or scientific information” that must be taken into consideration in accordance with the Uniform Principles set out in Directive 91/414. A failure to conduct this review would be a breach of both the Regulations and Directive 91/414 and would be susceptible to judicial review.

Directive 116/2008 will include imidacloprid in Annex I with effect from 1 August 2009. However, the Directive states that, when assessing whether to authorise plant protection products containing imidacloprid, Member States must pay particular attention to the impact on aquatic organisms, non-target arthropods and the protection of Honey bees. Given the concerns that have been outlined in relation to these issues in this report, we call for a moratorium on all further authorisations of plant protection products containing imidacloprid because of the inadequacies of the approval regulations and independent research showing that imidacloprid, and potentially the other neonicotinoids and fipronil, may have significant environmental impacts leading to declines in populations of bees and to other non-target invertebrate declines.

We also call for internationally agreed test methodologies for systemic pesticides and sub-lethal impacts on non-target invertebrates to be developed and implemented by 2015.

## **15.0 Recommendations**

This report identifies key weaknesses in the European approval process in relation to imidacloprid and highlights risks posed by the continued use of neonicotinoids and fipronil in pesticides. This report therefore calls for the following action to be taken:

- A review of the inclusion of imidacloprid, other neonicotinoids and fipronil on the positive list of authorised substances in Annex I of Directive 91/414.
- A review of all existing authorisations of neonicotinoid and fipronil products authorised for outdoor use in the UK.
- Until the reviews are completed a precautionary suspension of all existing approvals for products containing neonicotinoids and fipronil where these products have been authorised for outdoor use in the UK.
- Development of international methodologies for assessing the effects of systemic pesticides and sub-lethal impacts on invertebrates.

## **16.0 Acknowledgements**

This report could not have been written without the support of Sarah Tuffin. Expert advice was given by Dr Alastair Burn, Dr Simon Potts, Dr Ben Darvill, Janine Kievits and Alan Andrews. Assistance was provided by colleagues at Buglife: Matt Shardlow, Alan Stubbs, and proofreading at various stages by Mike Shardlow, Gill Sanders and Jamie Roberts.

## 17.0 Appendices

### 17.1 Appendix 1

Summary tables of imidacloprid research separated into research groups for: environmental tests, other non-target invertebrates (non-bee), bumblebee and Honey bee

Table for studies on imidacloprid general environmental studies

Author	Study Title	Test Method	Key Findings	Quality Assessment
Baskaran, Kookana & Naidu 1999 <sup>9</sup>	Degradation of bifenthrin, chlorpyrifos and imidacloprid in soil and bedding materials at termiticidal application rates	Degradation rates were studied in laboratory conditions	Imidacloprid is persistent in soils with a half-life of approximately 1,000 days, but length is dependent on the soil type and environmental conditions	Peer-reviewed publication <i>Pesticide Science</i>
Bonmatin <i>et al.</i> 2005 <sup>8</sup>	Quantification of imidacloprid uptake on maize crops	Field samples from France during 2000–2003 were used to measure levels of imidacloprid in plant materials and products	Imidacloprid has a high mobility in plants, and when used as a seed dressing becomes mobile and will migrate from the stem to the leaf tips and eventually into flowers. Residue levels measured from less than 0.1 µg/kg to 33.6 µg/kg with average levels of 4.1 µg/kg in stems and leaves, 6.6 µg/kg in male flowers (panicles) and 2.1 µg/kg in pollen	Peer-reviewed publication <i>Agricultural and Food Chemistry</i>
Bonmatin <i>et al.</i> 2005 <sup>10</sup>	Behaviour of imidacloprid in fields toxicity for Honey bees	Developed of a method to determine low amounts of imidacloprid, in the µg kg <sup>-1</sup> range, in soils, plants and pollens using high pressure liquid chromatography – tandem mass spectrometry	Imidacloprid residues have been detected in the pollen	Peer-reviewed publication <i>Environmental Chemistry</i>
Greatti <i>et al.</i> 2003 <sup>11</sup>	Risk of environmental contamination by the active ingredient imidacloprid used for corn seed dressing - Preliminary results	Detection of air contaminants using paper filters at the output fan of pneumatic seed drills as well as samples from adjacent vegetation	However, this is not the only form of contamination; research studies have found that during sowing operations for corn seeds residues from 120 to 240 µg of imidacloprid are found in grass and pollen of areas adjacent to sowing	Peer-reviewed publication <i>Bulletin of Insectology</i>

Author	Study Title	Test Method	Key Findings	Quality Assessment
Greatti <i>et al.</i> 2006 <sup>12</sup>	Presence of the a.i. imidacloprid on vegetation near corn fields sown with Gaucho®-dressed seeds	Detection of air contaminants from sowing on imidacloprid-dressed seeds using paper filters at the output fan of pneumatic seed drills as well as samples from adjacent vegetation	However, this is not the only form of contamination; research studies have found that during sowing operations for corn seeds residues from 120 to 240 µg of imidacloprid are found in grass and pollen of areas adjacent to sowing residual imidacloprid on flowers and grass at least 4 days after sowing	Peer-reviewed publication  <i>Bulletin of Insectology</i>
Schnier <i>et al.</i> 2003 <sup>13</sup>	Honey bee safety of imidacloprid corn seed treatment	Two field trails in 2001 and 2002 in Germany to assess the abrasion rate from Gaucho®-treated seeds directing the outflow through a commercial car filter	Found no release of imidacloprid during sowing	Peer-reviewed publication  <i>Bulletin of Insectology</i>
Scientific and Technical Committee (France) (2004) <sup>15</sup>	A multifactorial study on the disturbance of bees: Imidacloprid used in coating seed (Gaucho) and the disturbance to bees	Literature review with a validation of studies undertaken	The assessment found an average of 0.75–3.5 µg/kg for maize pollen. The French literature assessment found that for sunflower seeds dressed with imidacloprid average residue levels were recorded at 3.3 µg/kg in flowers and 2.2 µg/kg in pollen. levels present in maize and sunflower crops are also similar to those present in oilseed rape	Peer-reviewed report
Bonmatin <i>et al.</i> 2003 <sup>16</sup>	Method for analysis of imidacloprid in soils, plants and pollens	Developed a method to determine low amounts of imidacloprid, in the µg kg <sup>-1</sup> range, in soils, plants and pollens using high pressure liquid chromatography – tandem mass spectrometry	Other research on sunflowers and maize has found higher levels in the flowers of imidacloprid-treated plants with average values of approximately 10 µg/kg	Peer-reviewed publication  <i>Analytical Chemistry</i>



Table of studies on imidacloprid and other (non-bee) non-target invertebrates at environmentally relevant levels

Author	Study Title	Species	Test Method	Key Finding	Quality Assessment
n/a <sup>61</sup>	Imidacloprid – Insecticide Factsheet	Mysid shrimps	U.S. EPA. Office of Prevention and Toxic Substances. 1992. NTN 33893 ecological effects data, response to Miles Inc.'s request to upgrade four aquatic studies. Memo from D. Urban, Ecological Effects Branch, to D. Edwards, Registration Div. Washington, D.C., Aug. 25	The growth and size of mysid shrimps (Mysidacea) are known to be affected by imidacloprid concentrations of less than 1 µg/l	Peer-reviewed publication  <i>Journal of Pesticide Reform</i>
Alexander, Heard & Culp (2008) <sup>22</sup>	Emergent body size of mayfly survivors	Mayflies of the genera <i>Baetis</i> and <i>Epeorus</i>	Field-deployed mesocosms were used to examine the effects of 12 hours of pulse and 20 days of continuous exposure of imidacloprid	Mayflies of the genera <i>Baetis</i> and <i>Epeorus</i> showed a reduction in reproductive success when exposed to concentrations as low as 0.1 µg/l, expressed through a reduction in reproductive success, through a reduction of head length in <i>Baetis</i> and thorax length in <i>Epeorus</i>	Peer-reviewed publication  <i>Freshwater Biology</i>
Alexander <i>et al.</i> (2007) <sup>23</sup>	Effects of insecticide exposure on feeding inhibition in mayflies and oligochaetes.	Mayfly <i>Epeorus longimanus</i> and Aquatic worm <i>Lumbriculus variegatus</i>	The oligochaete and mayfly were exposed to 24 hour pulse exposure and feeding and egestion were measured	Another study found that environmentally relevant imidacloprid levels reduced survival, feeding and egestion in the mayfly <i>Epeorus longimanus</i> and aquatic worm <i>Lumbriculus variegatus</i> at concentrations between 0.5 and 10 µg/l	Peer-reviewed publication  <i>Environmental Toxicology and Chemistry</i>
James & Price (2002) <sup>24</sup>	Fecundity in Two-spotted spider mite (Acari: Tetranychidae) is increased by direct and systematic exposure to imidacloprid	Two-spotted spider mite ( <i>Tetranychus urticae</i> )	Mites were exposed to sprays of varying concentrations containing imidacloprid and fed discs of plant material cut from imidacloprid treated bean plants	Imidacloprid has also been found to increase the fecundity and longevity of the Two-spotted spider mite ( <i>Tetranychus urticae</i> )	Peer-reviewed publication  <i>Journal Economic Entomology</i>

Table for displaying studies on imidacloprid on bumblebees, both toxic and sub-lethal effects at environmentally relevant levels

Author	Study Title	Test Method	Key Finding	Quality Assessment
Tasei, Lerin & Ripault (2000) <sup>37</sup>	Sub-lethal effects of imidacloprid on bumblebees, <i>Bombus terrestris</i> (Hymenoptera: Apidae), during a laboratory feeding test	A laboratory feeding test used pollen and nectar contaminated with imidacloprid at two concentrations, 10 and 6 µg/kg. These were fed to <i>Bombus terrestris</i> individuals both concentrations over an 12-week period.	The study concluded that survival rate and reproductive capacity of <i>Bombus terrestris</i> was not affected by the prolonged ingestion of nectar from sunflowers with seeds dressed with imidacloprid. Although this was the conclusion, some significant negative impacts were observed during the investigation; for example, both doses of imidacloprid affected worker survival rate by 10% in the first month and brood production was reduced in one treatment when compared with the control.	Peer-reviewed publication  <i>Pest Management Science</i>
Sterk & Benuzzi. (2004) <sup>40</sup>	New plant protection chemicals: tests of toxicity to bumble bees in the greenhouse	Three trails were undertaken involving contact with commercial formulations and ad libitum feeding of field doses in pollen and sugar solution	A study undertaken on bumblebees ( <i>B. terrestris</i> ) pollinating tomatoes treated with imidacloprid also found it to be harmful to bumblebees	Peer-reviewed publication  <i>Colture Protette – CAB Abstract</i>
Jerome, Held & Potter (2002) <sup>41</sup>	Hazards of insecticides to the bumble bees <i>Bombus impatiens</i> (Hymenoptera: Apidae) foraging on flowering white clover in turf.	Tested residual effects of turf treatments both irrigated and non-irrigated using caged field trails	Research on turf application and bumblebees in the US found that 28–30 days after the application of imidacloprid sprays on turf containing white clover, there was no effect on colony vitality for <i>Bombus impatiens</i> (number of brood, workers, and honey pots and weights of queens, workers and whole colonies with hives), suggesting that spray treatments pose little hazard to bumblebees. In contrast, exposure to dry non-irrigated residues of a pesticide containing imidacloprid had a severe impact on colony vitality	Peer-reviewed publication  <i>Journal of Economic Entomology</i>

Table for displaying studies on imidacloprid on Honey bees both toxic and sub-lethal effects at environmentally relevant levels

Author	Study Title	Test Method	Key Finding	Quality Assessment
Suchail, Guez & Belzunces (2001) <sup>35</sup>	Discrepancy between acute chronic toxicity induced by imidacloprid and its metabolites in <i>Apis mellifera</i>	Chronic toxicity was tested by worker bees being fed sucrose solutions containing 0.1, 1 and 10 µg/l of imidacloprid and its metabolites for 10 days	Chronic toxicity was tested in a study using a 0.1 µg/l solution given every day for eight days. It reached the LD50 at a mean rate of 12 µl/d per bee; after 8 days this is 0.01 ng/bee (0.1 µg/kg), a much lower concentration than the acute toxicity result	Peer-reviewed publication  <i>Environmental Toxicology and Chemistry</i>
Moise <i>et al.</i> (2003) <sup>50</sup>	Research concerning the effect of Imidacloprid on Honey bees ( <i>Apis mellifera carpatica</i> )	Not available	Recorded effects of environmentally relevant imidacloprid levels on Honey bees include: apathy, laboured breathing, a lack of co-ordination and convulsion	Technical report  <i>Buletinul Universitatii de Stiinte Agricole si Medicina Veterinara Cluj-Napoca, Seria Zootehnie si Biotehnologii</i>
Colin <i>et al.</i> (2004) <sup>51</sup>	A method to quantify and analyse the foraging activity of Honey bees: Relevance to sub-lethal effects induced by systematic insecticides	Video recording was used to quantify the foraging activity of colonies of Honey bees confined within insect-proof tunnels	Sub-lethal doses have been seen to alter the behaviour of foraging insects and 6 µg/kg of imidacloprid induces a decrease in the proportion of active bees	Peer-reviewed publication  <i>Archives of Environmental Contamination and Toxicology</i>
Kirchner (1999) <sup>49</sup>	Mad-bee disease? Sub-lethal effects of Imidacloprid (Gaucho) on the behaviour of Honey bees. Association with of institute for bee research, 50 year anniversary 1949–1999 reports of the 46th seminar in Marburg, 23–25 March 1998	n/a	Another study found a similar result with 20–100 µg/kg reducing foraging activity as well as causing trembling dancing that discourages other bees from foraging. At higher concentrations it reduced the effectiveness of the waggle dance as the information communicated became less precise but during the research no population effects were seen	Peer-reviewed publication  <i>Apidologie</i>
Yang <i>et al.</i> 2008 <sup>48</sup>	Abnormal foraging behaviour induced by sublethal dosage of imidacloprid in the Honey bee (Hymenoptera: Apidae)	The time interval was between two visits at the same feeding site was measured and some were treated orally with sugar water containing imidacloprid	Foraging bees reduced their visits to a syrup feeder when it was contaminated with 3 µg/kg, this may be due to reduced effectiveness of the waggle dance	Peer-reviewed publication  <i>Journal of Economic Entomology</i>

Author	Study Title	Test Method	Key Finding	Quality Assessment
Decourtye <i>et al.</i> 2004 <sup>52</sup>	Imidacloprid impairs memory and brain metabolism in the Honey bee ( <i>Apis mellifera</i> L.)	Administered 30-minute oral treatments of 12 ng (0.012 µg) imidacloprid	The study found that olfactory learning performances were impaired, such as proboscis extension reflex procedure and the medium-term olfactory memory	Peer-reviewed publication  <i>Colture Protette</i>
Rortais <i>et al.</i> (2005) <sup>53</sup>	Modes of Honey bees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees	Based on literature data modes of Honey bees exposure to systemic insecticides are proposed by estimating their pollen and nectar consumption. Estimates are given for larvae and for adults consuming the highest amounts of pollen (i.e.: nurse bees), and nectar (the wax-producing bees, the brood attending bees, the winter bees, and the foraging bees)	When comparing the amounts of imidacloprid consumed over a period of 5 to 90 days ranging from 3.8 to 0.5 ng for different categories of bees with a wide consumption variation between different categories, the study found that Honey bees are potentially exposed to lethal and sub-lethal doses	Peer-reviewed publication  <i>Apidologie</i>
Rose, Dively, & Pettis (2007) <sup>54</sup>	Effects of Bt corn pollen on Honey bees: emphasis on protocol development	In field studies, colonies foraging in sweet corn plots and fed Bt pollen cakes for 28 days	An imidacloprid level of 10 µg/kg within pollen cakes caused a 20% reduction in the number of brood cells, and so consumption was negatively affecting nurse bees or contaminating brood food	Peer-reviewed publication  <i>Apidologie</i>
Gregorc & Bozic (2004) <sup>42</sup>	Is Honey bee colonies mortality related to insecticide use in agriculture?	Hives that were close to sunflowers treated with Gaucho® (contains imidacloprid) were studied and tested for the presence of imidacloprid	Five out of the 12 hives contained imidacloprid concentrations above 5 µg/kg; which could have been as high as 10 µg/kg at the time of consumption. The hives were infected with two parasites a mite <i>Varroa destructor</i> and a microsporidian <i>Nosema apis</i> . The study could not rule out a connection between the insecticide and increased parasites and disease. It was thought that hives with imidacloprid above 5 µg/kg could be severely impacted and suffer colony death	Peer-reviewed publication  <i>Sodobno Kmetijstvo</i>

Author	Study Title	Test Method	Key Finding	Quality Assessment
Chauzat <i>et al.</i> 2006 <sup>55</sup>	A survey of pesticide residues in pollen loads collected by Honey bees in France	A field survey on five random colonies for 3 years, with visits four times a year and multi-residue analysis was conducted	Another study showed that residues of imidacloprid was present in 69% out of 125 bee colonies sampled, of these 11 samples had imidacloprid levels quantified and had values ranging from 1.1 to 5.7 µg/kg	Peer-reviewed publication  <i>Apiculture and Social Insects</i>

## 17.2 Appendix 2

Table showing uncertainties regarding the methods used to assess Honey bee testing in the imidacloprid DAR

Uncertainty	Reason	Source
All bee classes have the potential to be exposed but the tests on different classes of bee have not been carried out	Research has shown a difference in sensitivity between different classes of bee <sup>53</sup>	A letter to the European Commissioner for Health from Stichting Natuur en Milieu, PAN-Europe, Inter Environment Wallonie, Nature et Progres and MDRGF France <sup>80</sup>
No bee brood feeding tests have been done with the assumption made that the larvae have the same sensitivity as adults	Research has shown that some substances are more toxic to larvae	A letter to the European Commissioner for Health from Stichting Natuur en Milieu, PAN-Europe, Inter Environment Wallonie, Nature et Progres and MDRGF France <sup>80</sup>
No testing for the effects of consuming contaminated pollen during the winter	Wintering bees are different to summer bees in relation to pollen consumption	A letter to the European Commissioner for Health from Stichting Natuur en Milieu, PAN-Europe, Inter Environment Wallonie, Nature et Progres and MDRGF France <sup>80</sup>
Vulnerable times which cause stress to colonies, such as winter, drought, extreme weather conditions, are not assessed	These vulnerable times are when colonies will be most sensitive to chemicals	Darvill B. <i>pers. commun.</i>
Chronic toxicity testing is provided for only two metabolites and the research cited for choice of metabolites is not credible as it shows other metabolites are more toxic than those chosen	The metabolites that have not been tested have low acute toxicity levels and have been detected in pollen and nectar	A letter to the European Commissioner for Health from Stichting Natuur en Milieu, PAN-Europe, Inter Environment Wallonie, Nature et Progres and MDRGF France <sup>80</sup>

<b>Uncertainty</b>	<b>Reason</b>	<b>Source</b>
Invalidation of a study that showed chronic toxicity is much higher than acute toxicity, and bee mortality by metabolites at low concentrations. The study was invalidated due to discrepancies with other scientific literature	Test levels are much lower than other studies, as other studies did not test such low levels. The study was also peer-reviewed and validated by the French Comité scientifique et technique. A great variability in toxicity levels and mortality was acknowledged in other studies and so lower levels cannot be a reason for discrediting a study	A letter to the European Commissioner for Health from Stichting Natuur en Milieu, PAN-Europe, Inter Environment Wallonie, Nature et Progres and MDRGF France <sup>80</sup>
The NOEL (No Observed Effect Level) when looking at sub-lethal effects is based on a nectar consumption level that is an underestimate	A calculation of the characteristics of a regular colony demonstrates this	A letter to the European Commissioner for Health from Stichting Natuur en Milieu, PAN-Europe, Inter Environment Wallonie, Nature et Progres and MDRGF France <sup>80</sup>
A study that shows sub-lethal effects at very low toxicity levels was discredited due to techniques modifying behaviour	This invalidation is not credible as the study did employ a control to ensure that testing techniques did not effect behaviour	A letter to the European Commissioner for Health from Stichting Natuur en Milieu, PAN-Europe, Inter Environment Wallonie, Nature et Progres and MDRGF France <sup>80</sup>
The field and tunnel tests all used feed combs; there is no proof that the bees were consuming the contaminated food rather than the comb food, as pollen is often left at least 5 days, maybe even months, before consumption	No proof of consumption of contaminated food rather than comb food	A letter to the European Commissioner for Health from Stichting Natuur en Milieu, PAN-Europe, Inter Environment Wallonie, Nature et Progres and MDRGF France <sup>80</sup>
Small colony sizes of 500 bees to assess egg-laying. Normal queens lay 1,000 to 1,500 eggs per day and so there can be colonies of 50,000 bees	Egg-laying cannot be properly assessed in such small colonies	A letter to the European Commissioner for Health from Stichting Natuur en Milieu, PAN-Europe, Inter Environment Wallonie, Nature et Progres and MDRGF France <sup>80</sup>
The length of some of the studies at 4–6 weeks are not long enough to assess some long-term impacts such as queen survival	The ability of queens to lay down fat reserves for their own and the colonies' long-term survival	Darvill B. <i>pers.commun.</i>
No validity criteria when deciding on whether a study was valid or not	A mis-match in decisions when compared with the French Comité scientifique et technique which did define validation criteria	A letter to the European Commissioner for Health from Stichting Natuur en Milieu, PAN-Europe, Inter Environment Wallonie, Nature et Progres and MDRGF France <sup>80</sup>

<b>Uncertainty</b>	<b>Reason</b>	<b>Source</b>
The level of variability in results between tests did not result in a sufficient security margin for risks to bees	There is a discrepancy in the No Observed Effect Concentration (NOEC) between the DAR and the French Comité scientifique et technique due to the high level of result variability not being considered	A letter to the European Commissioner for Health from Stichting Natuur en Milieu, PAN-Europe, Inter Environment Wallonie, Nature et Progres and MDRGF France <sup>80</sup>
No consideration of synergies between the active substance and bee pathogens	This area of research should have been considered	A letter to the European Commissioner for Health from Stichting Natuur en Milieu, PAN-Europe, Inter Environment Wallonie, Nature et Progres and MDRGF France <sup>80</sup>

### 17.3 Appendix 3

Crop protection products registered for use in the UK that contains imidacloprid and the crops they are registered for<sup>88</sup>

<b>Product</b>	<b>Crops registered for</b>
Admire	Hops
Bayer UK 720	Ornamental plant production (outdoor, container-grown), ornamental plant production (indoor, container-grown)
Baytan Secur	Barley (winter), oats (winter), wheat (winter)
Bug Free Extra Chinook	Aubergine (indoor), house plant, ornamental garden plants, pepper (indoor), tomato (indoor)
Chinook (seed dressing)	Oilseed rape (seed)
Chinook Blue	Oilseed rape (seed)
Chinook Colourless	Oilseed rape (seed)
Couraze	Ornamental plant production (indoor, container-grown)
Gaicho	Sugar beet
Gaicho FS	Fodder beet (seed), sugar beet (seed)
Imidachem	Ornamental plant production (container-grown)
Imidasect	Container-grown hardy ornamental nursery stock, ornamental (container- and pot-grown), ornamental plant production (outdoor), ornamental plant production (indoor)
Imidasect 5GR	Ornamental plant production (container-grown), Ornamental plant production (indoor)
Imidasect 60 FS	Fodder beet (seed), sugar beet (seed)
Imidasect 70 WS	Sugar beet
Intercept 5 GR	Ornamental plant production (indoor, container-grown)
Intercept 70 WG	Ornamental plant production (container-grown hardy nursery stock), ornamental plant production (container-grown) (outdoor), ornamental plant production (container-grown) (indoor)

<sup>88</sup> Pesticide Register of UK Approved Products – Chemicals Regulation Directorate  
<https://secure.pesticides.gov.uk/PestReg/>



<b>Product</b>	<b>Crops registered for</b>
Merit Turf	Managed amenity turf
Mido 70% WDG	Ornamental plant production (outdoor, container-grown), ornamental plant production (indoor, container-grown)
Neptune	Hops
Nuprid 600FS	Fodder beet (seed), sugar beet (seed)
Provado Lawn Grub Killer	Lawn
Provado Ultimate Bug Killer	Ornamental plant production (indoor, outdoor, container-grown)
Provado Ultimate Bug Killer Concentrate (home market)	Aubergine (indoor), house plant, ornamental garden plants, pepper (indoor), tomato (indoor)
Provado Ultimate Bug Killer Concentrate (crop market)	Aubergine (indoor), house plant, ornamental garden plants, pepper (indoor), tomato (indoor)
Provado Bug Killer Ready to Use (home market)	Aubergine (indoor), house plant, ornamental garden plants, pepper (indoor), tomato (indoor)
Provado Vine Weevil Killer	House plant (container-grown), ornamental garden plants (container-grown)
Raxil Secur	Barley (winter)
Sibutol Secur	Oats (winter), wheat (winter)
Tripod Plus	Barley (winter, seed), oats (winter, seed), wheat (winter, seed)

#### 17.4 Appendix 4

Translation of a French report executive summary which evaluated literature and reassessed the risk to Honey bees<sup>92</sup>

### **Scientific and Technical Committee of a Multifactorial Study on the Disturbance/Disorder of Bees**

#### **Imidacloprid used in coating seed (Gaucho) and the disturbance to bees Final Report**

#### **SUMMARY**

In light of the volume of work and in order to avoid digression which can result from an analysis of multiple parameters, the work group focused on the possible role of Gaucho and imidacloprid in the disturbances which have been observed. This report is an assessment of the current state of knowledge of the risks posed to bees related to the use of imidacloprid as a treatment for sunflower and corn seeds. The report presents the conclusions of the Metrology Sub-Group, which were validated by the members of the Scientific and Technical Committee (CST). The report follows the classic outline of an environmental risk evaluation in that it distinguishes the analysis of exposure from the analysis of effects. In light of the problems encountered while validating data from different studies, a recommendations chapter was included in the hope of improving future studies. It is evident that this approach, which focused on one phenomenon, will expand in order to take other factors into account, such as other phyto-sanitary products,

and the combination of effects of those products with pathologies, with particular apicultural practices, in poor agricultural usage, etc.

## **FIRST PART: IMIDACLOPRID AND ITS METABOLITES**

### **1. List of reports and publications inventoried on the disturbances to bees**

In this paragraph we present the documents concerned with exposure and toxicity data which formed the basis of the literature review:

- 245 studies or associated documents provided by the Directorate General of Food
- 93 documents from the scientific and technical literature

### **2. Review of physico-chemical properties**

The principal physico-chemical, toxicological and environmental characteristics of imidacloprid are taken up in this chapter.

This section is dedicated to the analysis of exposure data, doses in pollen, nectar, soils and in plants, as well as the validation of this data. The data is extracted from 15 studies produced by both public and private laboratories in France and Europe.

#### **3.1 Amounts in pollen**

Validation of the data allows us to conclude that the levels of imidacloprid residues in the sunflower pollen (the seeds of which had been treated with Gaucho) were on average 3.3 parts per billion (ppb), while levels in the [pollen found in pollen traps in the hive] were on average 2.2 ppb. In terms of the concentrations in corn pollen, the data validated demonstrated an average content of imidacloprid of 0.75–3.5 ppb for the flower pollen and the traps respectively. Taking into account that the activity of the colony may be modified by the position/laying down of the pollen traps, only the amounts of imidacloprid obtained from flower pollen are considered to be representative of the quantities of imidacloprid entering the colony. 3.3 ppb is thus retained for use in the exposure scenarios and the evaluation of the risks linked to the use of imidacloprid-coated sunflower seeds. 3.5 ppb is used to assess the risks linked to the use of imidacloprid in corn seed coating.

#### **3.2 Amounts in the nectar**

Only one study which was analysed was validated in this category, which indicates that the content of residues in the sunflower nectar (of which the seeds were treated with Gaucho) was 1.9 ppb. The other studies did not meet the validation criteria (vague methods, high quantification limit, or number of weak samples).

#### **3.3 Amounts in soils**

Imidacloprid was detected in soils which had supported the cultivation of Gaucho-treated sunflowers during the year of sampling, at values averaging at 10.25 ppb. The year after the treatment, the quantities of imidacloprid decreased and were on average 4.4 ppb. However, it is not possible to conclude past one year, due to a poor sampling strategy.

#### **3.4 Amounts in plants**

The analysis of studies only partially validated one study due to poor sampling strategy. As a rough guide, an average content of 4.6 ppb of imidacloprid in a sunflower treated with Gaucho the year of sampling was calculated by pooling together the different samples of leaves, stems and flower heads. It was not possible to conclude when the soils had been planted with Gaucho-treated plants in the previous year. With regard to

the Gaucho-treated corn, the content of imidacloprid in the different vegetable parts were 3.7, 3 and 7.5 ppb in the leaves and stems together, the male parts and the panicles respectively.

### 3.5 Quantities of imidacloprid entering the hive

In this section, we evaluated the theoretical quantity of imidacloprid brought back to the hive in contaminated pollen and nectar. For the sunflower pollen brought annually to the colony, these quantities varied between 0.84 µg and 50 µg. For the sunflower nectar, the only study which was validated allowed us to estimate a quantity between 133 µg and 266 µg. Imidacloprid can also be brought back to the colony via corn pollen in variable quantities, ranging from 0.04 to 66 µg.

### 3.6 Quantities of imidacloprid present in other 'hive products'

At the moment, we do not have any data relating to residual amounts of imidacloprid in royal jelly, the [bouillie larvaire], the beebread, the wax, etc.

## 4. Toxicity data linked to the use of imidacloprid

The third part is dedicated to the analysis of the effects of imidacloprid on bees, through the examination of results issues from trials of acute, chronic and sub-lethal toxicity. The available results are subsequently validated or invalidated by the CST.

### 4.1 Mortality following a single administered dose of active substance (acute toxicity)

The results presented for acute toxicity by oral administration for imidacloprid are derived from standard experimental protocols and give results of LD50 ranging from 4 ng to 71 ng of imidacloprid per bee. All the available studies were validated. In terms of the acute toxicity by topical administration, we obtained values of LD50 from 6.7 ng to 242 ng of imidacloprid per bee. Metabolites of imidacloprid, olefin and hydroxyimidacloprid, are also toxic when administered orally. Olefin gave a LD50 ranging from 28 to >35.7 ng of active substance per bee while hydroxyimidacloprid produced a LD50 of 153–258 ng of active substance per bee. The other metabolites (chloronicotinic-6 acid, dihydroxyimidacloprid, [urea by-product] and guanidine) did not produce a particular toxicity (LD50 >1,000 ng of active substance per bee).

### 4.2 Mortality following repeated administration of active substance (chronic toxicity)

The studies pertaining to the chronic toxicity of imidacloprid and its metabolites demonstrate diverging results in part due to the heterogeneous nature of the protocols, resulting in studies that were difficult to compare and validate. Only two studies of repeated oral administration of imidacloprid were validated. One led to a LD50 of 12 pg per bee over 10 days (Suchail 2001) while the second study led to a NOEC of 1,700 pg per bee over 10 days for all the metabolites or to a NOEC between 2,740 and 8,000 pg per bee over 10 days for the [urea by-product] and the chloronicotinic-6 acid.

### 4.3 Sub-lethal effects

Numerous studies are interested in sub-lethal effects. These studies are very diverse and heterogeneous. They have studied sub-lethal effects in laboratories, caged, or in the field. In the laboratory, the validated data for acute oral toxicity demonstrated a NOEC of 940 pg of imidacloprid per bee for motor co-ordination and the knockdown effect. Following intoxication by repeated oral administration of imidacloprid, the NOEC was 200 pg of imidacloprid per bee over 10 days for the reflex of extending the proboscis. For the data on acute toxicity via topical administration, we obtained a LOEC

of 100 pg of imidacloprid per bee; no toxicity data by repeated topical administration was validated. The administration of metabolites of imidacloprid by one oral administration led to higher NOECs, from 1,200 to 7,000 pg of active substance per bee. The studies of flight cages and underground tunnels gave, after repeated oral administration of active substance, a LOEC of 75 pg of imidacloprid and hydroxyimidacloprid per bee each and 20 pg of olefin per bee for effects on the frequency and length of feeding. When imidacloprid or olefin were left in a feeder in the field, the results for intoxication by repeated ingestion of active substance demonstrated a NOEC of 250 ng of imidacloprid per bee for all observed behaviours and a NOEC of 250 ng of olefin per bee for the waggle dance.

## **SECOND PART: RISK EVALUATION**

### **5. Bee exposure scenarios to evaluate the risk of intoxication**

We proposed five scenarios corresponding to different possible modes of intoxication (oral or topical intoxication) during different stages in the bee life-cycle (larvae, nurses, foragers) for pollen (scenarios 1, 2, 3), nectar or the honey (scenarios 3, 4, 5), following either immediate consumption or deferred consumption.

### **6. Risk evaluation**

The risk evaluation consisted of comparing a predicted exposure concentration, referred to as 'Predicted Environmental Concentration' (PEC) to a concentration expected to have no effect for organisms in the environment, referred to as 'Predicted No Effect Concentration' (PNEC). A risk is thus present when the estimated value of PEC is superior to that of PNEC. The risk evaluation for bees linked to the use of imidacloprid seed coating was conducted in line with the approach of 'new and existing chemical substances' developed in the framework of the regulation of new and existing chemical substances (Directive 67/548). The phyto-sanitary approach developed in the framework of the regulation of phyto-sanitary products (Directive 91/414<sup>6</sup>) cannot be applied in the case of seed coating as it is based on doses per hectare which does not have a realistic meaning in our case.

#### **6.1 Evaluation of exposure (PEC)**

—For scenario 1 (feeding of larvae), in considering that the sugar making up the larval feed comes entirely from the harvested nectar, the quantity of imidacloprid ingested by a larva at the beginning of five days was estimated at between 1.1 and 87 pg, this quantity depending on the percentage of contamination of the sunflower nectar ingested. In addition, the quantity of ingested pollen was considered negligible with regard to the total quantity of food ingested by the larva.

—For scenario 2 (consumption of pollen by the nurse bees), in assuming total stability of imidacloprid following its stockpiling in the hive, the quantity of imidacloprid absorbed by the bees depends concurrently on the percentage of contaminated pollen which they have consumed and the concentration of imidacloprid in the pollen. It would be between 40 and 180 pg per bee (worst case, unlikely) when the nurse consumes the sunflower pollen and between 42 and 168 pg following the consumption of corn pollen. We note that the nurse bees could also become intoxicated by consuming contaminated honey (scenario 5).

—For scenario 3 (ingestion of pollen by the pollinators [butineuses], in arbitrarily estimating that 1% of the pollen is ingested during the making of pollen balls. Following the making of pollen balls, the quantity of imidacloprid ingested varies between 3.3 and

15 pg per bee for the sunflower pollen and between 3.5 and 16 pg for the corn pollen. Due to the fidelity of bees to flowers, the percentage of contaminated pollen ingested will be 0 or 100% depending on the field treatment (non-Gaicho or Gaicho). These foragers could equally intoxicate themselves in consuming honey to stockpile the energy necessary for flight (scenario 4).

—For scenario 4 (consumption of nectar by the foragers), the quantity of imidacloprid depends on the percentage of contaminated sunflower nectar that the forager ingests to provide the energy necessary for its flight, and the concentration of imidacloprid in the sunflower nectar. In using 12 hours as the average time for collecting, the collector ingests 131-655 pg of imidacloprid per bee.

—For scenario 5 (consumption of reserve honey by bees in the hive to assure thermoregulation), in assuming a total stability of imidacloprid following the transformation of nectar into honey, the quantity of imidacloprid absorbed by the bees depends on the percentage of contaminated honey they have ingested and the concentration of imidacloprid in the honey. Based on a consumption of 0.2–0.8 g of honey per bee to maintain a temperature of 15°C at the centre of the hive and 5°C at the periphery, the quantity of imidacloprid ingested by the bee varies between 190 and 3,800 pg depending on the percentage of contaminated sunflower nectar which would be used up in the production of honey.

## 6.2 Evaluation of effects (PNEC)

The PNEC is evaluated by associating the data (be it the acute, chronic or sub-lethal intoxication data) by assigning it an uncertainty factor determined on a case by case basis. This factor takes into account the following uncertainties:

- intra- and inter-laboratory variation
- extrapolation of short-term toxicity data to the long term
- extrapolation from the laboratory to the field.

The adaptation of the approach of ‘new and existing chemical substances’ to specific cases represented by the exposure of bees based on the different intoxication data led to the estimates presented in the table below: (*Table not translated*)

Based on our current state of knowledge and on the scenarios we developed to evaluate exposure, and based on the uncertainty chosen to evaluate the dangers, the relationships between the PEC/PNEC determined is worrying. They are in agreement with the field observations of numerous beekeepers in large farms of corn and sunflower relating to the mortality of pollinators (scenario 4), their disappearance, behavioural disturbances and certain winter mortalities (scenario 5). Consequently, the coating of sunflower seeds in Gaicho poses significant risks for bees of different ages, with the exception of the ingestion of pollen by the foragers during the making of a pollen ball (scenario 3). In terms of Gaicho-coated corn seeds, the relationship PEC/PNEC turns out to be, as for the sunflower, worrying in the case of pollen consumption by the nurse bees, which would lead to an accrued mortality of these and be one of the explanatory elements for the weakening of bee populations observed despite the ban on Gaicho on sunflowers. Finally, given that other factors can contribute to the weakening of bee colonies, research should be conducted on the frequency, mechanism and causes of these symptoms.

## **THIRD PART: RECOMMENDATIONS FOR ACQUISITION OF DATA LACKING FOR RISK EVALUATION**

### **8. Recommendations**

This chapter covers:

—the different problems encountered during the validation of data (poor sampling strategy, limits on detection and overly elevated quantifications, non-standardised protocols for toxicology studies).

—missing data: amounts of residues in the different hive products (the principal ones being honey, the larval feed, the honeycomb), data on the stability of imidacloprid in the pollen, nectar, and honey over the course of stockpiling in the hive, data on toxicity on the larvae and nursing. Finally, certain suggestions are made in order to remedy the problems which were encountered. These suggestions could also be applied in the case of other studies of phyto-sanitary molecules.

### **9. Work required to complete the multifactorial study**

The report must be progressively enriched by future work of members of the Metrology Sub-Group. It requires:

—the completion of an evaluation of the same type of risks as those conducted for imidacloprid for fipronil

—the analysis of the other factors implicated in the loss of bees (illnesses, apicultural and agricultural practices, genetic varieties of the cultivated and treated plants, influence of terpenes) in close collaboration with the Network Sub-Group

—an inventory of the disturbances to bees in other countries.

## **17.5 Appendix 5**

A letter to the European Commissioner for Health from Stichting Natuur en Milieu, PAN-Europe, Inter Environment Wallonie, Nature et Progres and MDRGF France expressing concerns regarding the methods used to assess risks to bees for the Draft Assessment Report (DAR)<sup>80</sup>.

Androulla Vassiliou  
European Commissioner for Health  
DG Health and Consumer Protection  
B-1049 Brussels  
Belgium

Utrecht, 21 January 2009

Our reference: HM/jc/090120.01-4.1.0.40.N08  
Contact: Mr. H. Muilerman

Subject: Re: Request for an internal review of Commission Regulation 116/2008

Dear Commissioner,

I am writing on behalf of undersigned organisations to submit a formal request for an internal review (based on Regulation 1367/2006, article 10) of Commission Directive

116/2008 of 15/12/2008 which was published in the Official Journal of 16/12/2008. It is the opinion of our organisations that Commission Directive 116/2008, including the active substance Imidacloprid in Annex I of Directive 91/414<sup>6</sup>, is not justified on several grounds and should be reviewed as a matter of urgency. Please find below the basis of our request.

Procedural criteria:

Our organisations are entitled to make this request because we fulfil the criteria laid down in article 11 of Regulation 1367/2006. Stichting Natuur en Milieu, PAN-Europe, Inter Environment Wallonie, Nature et Progres and MDRGF France are all independent non-profitmaking legal persons in accordance with the national law. Promoting environmental protection is one of our central objectives which we are actively pursuing.

Grounds for internal review:

Article 5 of Directive 91/414<sup>6</sup> on the inclusion of active substances in Annex I requires that active substances are expected to fulfill the condition of having no unacceptable influence on the environment as provided for in Article 4.1.b. IV and V (the article which is the basis for member states authorising the use of formulations based on the active substance). In our opinion, however, the evidence presented by the Commission does not support the conclusion that the effects of the use of imidacloprid on bees are acceptable.

EFSA concludes in its peer review of 29/5/2008 that spraying of imidacloprid (and its two main metabolites) poses a high risk to bees. The report recommends risk mitigation measures (no application during flowering, flowering weeds are removed) but concludes that bees will still be not be protected by the suggested risk mitigation measures. For tomato production, for example, the situation is even more acute, as the EFSA peer review states, because tomatoes are flowering all the time. In relation to all spraying applications it is clear that the Commission has not demonstrated that acceptable use is possible.

Regarding the use of imidacloprid as a seed coating we think the tests performed by the notifier are inadequate in relation to bees and more generally to other pollinators. Below we present further arguments and comment relating to the DAR by the Rapporteur Germany.

Annex VI of Directive 91/414 (Uniform principles) on testing determined in 2.5.2.3.: Where there is a possibility of Honey bees being exposed, no authorisation shall be granted if the hazard quotients for oral or contact exposure of Honey bees are greater than 50, unless it is clearly established through an appropriate risk assessment that under field conditions there are no unacceptable effects on Honey bee larvae, Honey bee behaviour, or colony survival and development after use of the plant protection product according to the proposed conditions of use.

Imidacloprid is simultaneously persistent, systemic, and highly toxic for bees (LD50 at 48 hours is 4.8 ppb (parts per billion). Used in seed coatings, imidacloprid is absorbed by the root system and transported by sap to all parts of the plant, including nectar and pollen. As the active substance is present in soil, even succeeding crops absorb



residues and become toxic (cf. Annex B.9. p. 919 and following, and several scientific papers<sup>89</sup>).

(1) Because contaminated nectar and pollen may be brought to the hive by the foragers, all the bee classes (drones, queens, nurses, larvae etc.) are potentially exposed to the active substance. The sensitivity of bees is now well understood and differs according to their class<sup>90</sup>. The necessary tests specific to each bee class have not been carried out. The closest estimates available document chronic toxicity (p. 965) in 'young bees' and 'old bees'. They show a difference of sensitivity between these two categories of bee.

(2) Another important point is that no bee brood feeding test has been carried out (point B.9.4.2., p. 926). Following the report: 'it has to be expected that the active substance and the formulated compounds are as toxic to larvae as to adult bees'. There is absolutely no scientific argument to support this assertion. The toxicity of a pesticide to one category of bees cannot be deduced from its toxicity to another; some substances are more toxic to larvae than to adult bees and vice versa<sup>91</sup>. Moreover, we recall the statement of the French State Council suspending the Maize authorisation of Gaucho, based on the absence of this test despite the very high HQ (Hazard Quotient) value of imidacloprid (40 540; cf. Reasoned statement of the overall conclusion, p. 57). Authorising imidacloprid without carrying out the larvae test is a clear violation of Directive 91/414 and unacceptable from a scientific viewpoint, as the innocuousness for larvae is not proved. If the European Authority estimates that the HQ is not a valid concept for seed coatings, a new assessment scheme has to be described before the substance concerned is assessed, and included in annex VI of the 91/414/EEC Directive. Until then, the clauses in Annex VI, point B and C 2.5.2.3 must fully be enforced. The HQ (or TER: Toxicity Exposure Ratio) concepts have been designed to avoid making higher tier tests with low risk substances. In no case can the arbitrary choice of discarding the HQ (this HQ validity not being investigated so far) for seed coating substances be used to lower the risk. Consequently all the prescribed tests should be realised. Moreover, imidacloprid is also formulated for spraying (e.g. Confidor). From a legal point of view, the lack of a bee brood feeding test is thus sufficient to definitely invalidate the assessment that the HQ is not relevant in this case.

(3) The report does not include any test about the effects of contaminated pollen consumption during wintering. The amount of pollen consumed by winter bees is unknown at this time. Every beekeeper knows that wintering may succeed only if the bee colony has collected important quantities of pollen during summer. Most of this pollen will disappear during winter and early spring: it has been consumed by the bees, and particularly by the nurses for feeding the early brood. The winter bees are not numerous (5,000–10,000) and, as they live much longer than summer bees, they will feed the brood for a long period. This means that pollen consumption per winter bee may be very important compared to summer bees. Thus the pollen toxicity for winter bees has to be tested specifically. Before carrying out this test, it is necessary to quantify

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<sup>89</sup> E.g. Bonmatin J.-M., Marchand P., Charvet R., Moineau I., Bengsch E.R. & Colin M.-E.. Quantification of imidacloprid uptake in maize crops. *Journal of Agricultural and Food Chemistry* **53**, 5336–5341

<sup>90</sup> Alix A. & Vergnet Chr. (2007) Risk assessment to honey bees: a scheme developed in France for non sprayed systemic compounds. *Pest Management Science* **63**, 1069–1080

<sup>91</sup> For instance, refer to: Alix A. & Vergnet Chr. (2007) Risk assessment to honey bees: a scheme developed in France for non-sprayed systemic compounds. *Pest Management Science* **63**, 1069–1080, point 4.2

the pollen amounts consumed by winter bees with great care in order to define their exposure.

(4) The dossier includes a point about chronic mortality, examining the LD50c of imidacloprid and several metabolites. This point does not allow the conclusion that imidacloprid is innocuous, for the following reasons:

(a) Data are provided for two metabolites only (urea and 6-chloronicotinic acid). No values are provided for olefin and 5-hydroxy-imidacloprid when these metabolites are hazardous for bees (they have a low acute LD50) and are detected in pollen and nectar. The explanation of this choice (point B.9.4.7.4.1 p. 962) is not credible: it is based on a scientific article<sup>35</sup> that on the contrary shows the significant lethal toxicity of olefin and 5-hydroxy-imidacloprid.

(b) The study<sup>35</sup> shows firstly that the chronic toxicity is significantly higher than the acute toxicity, and secondly that for most of the metabolites, the mortality is the same for all the tested concentrations including very low concentrations (0.1 ppb, more or less 0.1 ng/bee). This study is invalidated in the DAR (p. 961 et seq.), based on its discrepancy with other studies of the scientific literature. We cannot agree with this argument because:

(i) the study<sup>35</sup> tests substance amounts and concentrations that are significantly lower than the other studies and finds an equal substance toxicity for all the low concentration for most of the metabolites; if these other studies did not test such low concentration, it is logical that they only detect higher LD50s.

(ii) the study<sup>35</sup> is published and peer-reviewed; moreover, it was validated by the French Comité scientifique et technique, based on the validity criteria elaborated by this Comité for analysing the existing literature<sup>92</sup>. The DAR considers it non-valid by comparison with LD50c measured in other studies, without verifying these other studies' validity, which is not admissible from a scientific point of view.

(iii) An imidacloprid characteristic is the great variance shown by the mortality measurements, acute as well as chronic. For instance<sup>35</sup> itself concludes an acute toxicity of  $57 \pm 28$  ng/bee, that is to say to an acute LD50 significantly higher than the figure finally considered by the DAR (4.8 ng/bee). As a consequence a study cannot be invalidated on the single reason that the measured lethal toxicity figure departs from those found in other studies, without further verification.

(5) The substance is neurotoxic and can have sub-lethal effects, making the bee unable to perform all its behavioural schemes, which are necessary for the colony survival. The report estimates that the NOEL (No Observed Effect Level) is 1.2 ng per bee, leading to a non-effect concentration of 46 ppb (point B.9.4.7.3.1, p. 959). This figure is incorrect because it is based on a consumption of 20 Hl per bee, corresponding to the syrup amount given to each bee following the LD50 test design. In reality a bee consumes much more nectar than that. Rortais et al (2005)<sup>93</sup> estimate the forager consumption to be between 224 and 898.8 mg in 7 days. We can make a quick estimation: a colony harvests 60 kg of honey, that is to say 150 kg nectar, during one month (an average during the sunflower blossom). Two generations of foragers must be considered, or

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<sup>92</sup> CST (non daté): Imidaclopride utilisé en enrobage de semences (Gaucho®) et troubles des abeilles – pp. 50 and 61

<sup>93</sup> Rortais A, Arnold G, Halm MP, Touffet-Briens F, 2005 : Modes of honeybees exposure to systemic insecticides : estimated amounts of contaminated pollen and nectar consumed by different categories of bees, *Apidologie* 36 (2005), 71 – 83

about 20,000 foragers (it is commonly considered that a hive has about 10,000 foragers at any one time). In its life, each bee will harvest 7.5 g of nectar of which about 10% is used for the forager itself. So each bee will ingest about 750 mg in 2 weeks, or 107 mg in 48 hours. The other part of the nectar is brought to the hive, but is in contact with the oesophagus and the stomach of the bee and so can have a contact toxicity. So the calculated NOEC in real conditions should be at least 10 times lower than the value estimated in the report. The real calculated NOEC should be below 5 ppb.

(6) The DAR quotes the study<sup>94</sup> that shows significant sub-lethal effects on bees for concentrations between 0.1 and 10 ppb. This study is also peer-reviewed. However, the DAR (p. 960) turns down this study conclusion on the grounds that the bees were reared in an incubator and subjected to an ice narcosis, and that these practices are suspected to modify bees' learning abilities. This argument ignores the fact that the authors have observed the sub-lethal effects by comparison between a treated sample and a control sample that was subjected to the same rearing and narcosis; thus the argument is inadmissible and the study may not be dismissed.

(7) None of the field and tunnel tests submitted in the report proves that the treated pollen has really been consumed during the test. Pollen consumption is always postponed by the bees as pollen needs a lactic fermentation of at least 10 days to be digested, and may remain several months in the comb cells. During all tests, the hives that are put in the tunnel or fields contain feed combs. The submitted tests do not take this fact into account; they do not prove that the bees consume the contaminated food rather than the comb food; since it is more likely they use first the comb feed. Thus the tests conclude on the absence of effects while the real exposition is not proved. This is scientifically unacceptable.

(8) Several tests leave us in doubt. For example, it is impossible to assess the queen's egg-laying in a small colony of 500 bees, as described in several tests, particularly in cage tests (point B.9.4.4). The queen's egg-laying depends on the number of nurses able to take care of the larvae. A normal queen lays 1,000 to 1,500 eggs per day, assuming a colony of more than 50,000 bees, that is to say 100 times more than the small colonies used in the tests. So egg-laying cannot be fully assessed in such small colonies. Moreover, the small colonies are unable to develop all the behaviours needed for the survival of a normal colony (e.g. developing a drone population, vitality, sufficient cell production ...).

(9) All these concerns are closely linked to the irrelevance of the current assessment scheme for systemic insecticides since they are susceptible to remain in contact with bees for a long period of time because they potentially contaminate the foraged matrices: pollen and honey stocks. The scheme irrelevance has been emphasised by scientific papers<sup>95</sup> and is currently widely accepted. This definitely invalidates the global assessment process. Scientists admit today a PEC/PNEC (Predicted Environmental Concentration / Predicted No Effect Concentration) approach to be more relevant to

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<sup>94</sup>Guez D, Suchail S, Gauthier M, Maleszka R, Belzunces LP (2001) Contrasting effects of imidacloprid on habituation in 7- and 8-day-old honeybees (*Apis mellifera*). *Neurobiology Learning Memory* **76**, 183–191

<sup>95</sup>Alix A. & Vergnet Chr. (2007) Risk assessment to honey bees: a scheme developed in France for non- sprayed systemic compounds. *Pest Management Science* **63**, 1069–1080

Halm M.P., Rortais A., Arnold G., Tasei J.N. & Rault S. (2006) New risk assessment approach for systemic insecticides: The case of honey bees and imidacloprid (Gaucho). *Environmental Science & Technology* **40**, 2448–2454

assess the risk for bees of systemic, neurotoxic and persistent active substances or plant protection products (PPP). Halm et al (2006) and the French Comité Scientifique et Technique (CST) have tried this approach<sup>92</sup>; both of these papers agree on the fact that the imidacloprid PEC/PNEC ratio is alarming. We quote here the CST conclusions:

From what we know now, according to the scenarios developed to measure exposure and the uncertain factors chosen to measure the dangers, the PEC/PNEC reports obtained are worrying. They agree with observations from the field reported by numerous bee-keepers in areas of large-scale agriculture (maize, sunflower), concerning the death of forager bees (scenario 4), their disappearance, their behavioural abnormalities and specific winter mortalities (scenario 5). As a consequence, the seed dressing of sunflowers with Gaucho carries a significant risk for bees of different ages, with the exception of foragers because these ingest the pollen when there are making pollen balls. Regarding the seed dressing of maize with Gaucho, the PEC/PNEC report proves worrying, as for sunflowers, in the amount consumed by the nurses, which could lead to a growing mortality among them and could be one of the elements explaining the reduction of bee populations observed despite the ban on Gaucho on sunflowers.

(10) The sowing dust effects are not assessed in the report. It is, however, a very important way of contamination, already scientifically studied in Italy<sup>96</sup>. Recently high bee mortalities occurred in Italy (Padanian plain, during spring 2007 and 2008) and Germany (Land of Baden-Württemberg during spring 2008). The DAR's conclusions about the risk for bees (Reasoned statement of the overall conclusion, p. 58) are entirely based on the postulate that the bee exposure does not exceed a 5 ppb concentration. This postulate appears illusory. Investigating hive damage in the Lombardy plain (spring 2008), the Servizio Veterinario della Lombardia have found up to 144 ppb of imidacloprid in bees. A single pollen sample was analysed, giving the amazing imidacloprid concentration of 311 ppb. During the Baden-Württemberg incident, the regional veterinarian services asked the beekeepers to destroy feed combs because hive pollen was contaminated. The study of Chauzat et al. 2006<sup>97</sup>, showed that imidacloprid was detected in 40 pollen trap samples (81 samples were taken during this study, coming from 5 different regions). Imidacloprid persistence in the environment appears thus much more important than reported in the DAR (Draft Assessment Report).

(11) The DAR does not include studies about the potential synergies between the active substance and bees' pathogens. Imidacloprid shows such synergic properties with some pathogenic agents<sup>98</sup>. In the case of bees this hypothesis has never been investigated in detail but is likely to occur with *Nosema* spp., and could explain the increase of bee pathologies noted by beekeepers and scientists during recent years. A PPP called Premise 200SC, whose active substance is imidacloprid, is described as disorientating termites and making them ill by stopping their grooming behaviour. The PPP advertising paper explains that stopping the grooming behaviour allows soil fungi to attack termites.

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<sup>96</sup> Greatti M., Sabatini A.G., Barbattini R., Rossi S. & Stravisi A. (2003) Risk of environmental contamination by the active ingredient imidacloprid used for corn seed dressing. Preliminary results. *Bulletin of Insectology* **56** (1), 69–72  
Greatti M., Barbattini R., Stravisi A., Sabatini A.G. & Rossi S. (2006) Presence of the a.i. imidacloprid on vegetation near corn fields sown with Gaucho® dressed seeds. *Bulletin of Insectology* **59** (2), 99–103

<sup>97</sup> Chauzat M.P., Faucon J.P., Martel A.C., Lachaize J., Cougoule N. & Aubert M. (2006) A survey of pesticides residues in pollen loads collected by honey bees in France. *Journal Economic Entomology* **99** (2), 253–262

<sup>98</sup> See, for example: Cuthbertson A.G., Walters K.F. & Deppe C. (2005) Compatibility of the entomopathogenic fungus *Lecanicillium muscarium* and insecticides for eradication of sweet potato whitefly, *Bemisia tabaci*. *Mycopathologia* **160**(1), 35–41

What about bees? Some hive micro-organisms are fungi, for instance the genus *Nosema* (usual pathogen of the bees) or *Beauveria* (in normal conditions a non-pathogen for bees). In the same paper we can read (last page) that “independent trials in Japan have Found Premise SC to be effective for at least five years”. This fact is not in agreement with the provided Imidacloprid DT50.

(12) We have noticed amazing discrepancies between the results of the different tests: for example between the LD50 for Honey bees and for wild bees (*Bombus terrestris*). Both studies (De Ruijter 1999, p. 913) conclude that imidacloprid has effects for all tested doses, without correlation between the dose and the effect, including mortality. Similar discrepancies appear during cage, tunnel or field tests. Some tests highlight a lack of foraging activity (Bakker 2003, p. 943) where others find an increase of bee activity (Stadler 2000, p. 947). Such inconsistent results should be considered with caution: sufficient margins of security are necessary to consider the risk for bees. The conclusion on the acceptable risk for bees does not take such margins into account, since the figures accepted by the assessment report are a NOEC of 10 ppb, a nectar and pollen concentration of 5 ppb (the average of real concentration is 2 to 3.5 ppb, cf. report of the French CST).

(13) Importance of scientific studies' validity criteria

(a) The DAR considers the tests valid or not valid without having defined any validity criterion. The conclusions about the studies' validity do not tally with those of the French Comité scientifique et technique, which has defined validity criteria. Invalidation of several studies, such as Guez et al. (2001)<sup>94</sup> and Suchail et al. (2001)<sup>35</sup>, or of results published by Pham-Delègue and Cluzeau (1999)<sup>99</sup> is questionable when, for instance, the residues studies of Schmuck et al. (point B.9.4-5, p. 920) are considered valid, in spite of insufficient limits of detection (5 or 10 ppb). Schmuck et al. studies never detect any residue when other studies show a frequent presence of imidacloprid in the foraged matrices (for instance, following a study on the pollen contaminations in France, imidacloprid is detected in half of the trap pollen samples (40/81)<sup>100</sup>). The methods used in this laboratory should be analysed in order to verify that they are able actually to detect the substances in the foraged matrices, particularly in the pollen since the potential contamination is inside the grains.

(b) A statistical validation should be provided for the studies and their conclusions. Such validations are all the more necessary since the results variance is very great, for the acute and chronic LD50 and for the sub-lethal effects as well (see, e.g., Kirchner 1998<sup>101</sup> and 2000<sup>102</sup>, pp. 950 – 951).

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<sup>99</sup> Pham-Delegue M.H. & Cluzeau S. (1999) Effets des produits phytosanitaires sur l'abeille; incidence du traitement des semences de tournesol par Gaucho sur les disparitions de butineuses. Rapport final de synthèse au Ministère de l'Agriculture et de la Pêche

<sup>100</sup> Chauzat M.P. & Faucon J.P. (2007) Pesticide residues in beeswax samples collected from honey bee colonies (*Apis mellifera* L.) in France. *Pest Management Science* **63**, 1100–1106

<sup>101</sup> Kirchner W.H. (1998) The effects of sublethal doses of imidacloprid on the foraging behaviour and orientation ability of honeybees. *Unpublished Study Report*, University of Konstanz

<sup>102</sup> Kirchner W.H. (2000) The effects of sublethal doses of imidacloprid, hydroxy-imidacloprid and olefine-imidacloprid on the behaviour of honeybees. *Unpublished Study Report*, University of Bochum

(14) We observe that the DAR dismisses all the studies that seem to be unfavourable to the molecule authorisation, when the favourable studies obviously are not so carefully analysed from their validity point of view. Again this fact raises the problem of the scientific assessment independence since the assessment is integrally submitted by the applicant, without confirmation of any test by independent laboratories, even in the case of doubt. In the current context, where seed-coating insecticides remain the number one suspect in worldwide bee mortality, such a situation fosters suspicion, which is prejudicial to beekeepers, to the industry concerned, and above all to the public authorities, who fail to ensure the general interest protection and the necessary arbitration between the interests of the concerned sectors.

The conclusion is very clear: the report evaluation does not respect Article 4 of Directive 91/414/CEE. It definitely fails to demonstrate that there is no unacceptable impact on bees or on other foraging species.

Moreover, the European Authorities have recently moved to offer greater support to the wider adoption of 'Integrated Pest Management' in the Framework Directive on the sustainable use of pesticides. The major advantage of integrated pest management is to avoid permanent pesticide residues in soils and plants that may lead to the development of resistant pest populations, as well as unwanted effects on human health. Using pesticides only when needed, against a well-defined pathogen, with limited effect on non-target species and during a limited period of time is the basis of integrated management. Seed coatings are just following the opposite approach: they are used in all cases, even when there is no pathogen target to destroy. They use very persistent active substances in order to protect the plant from seeding to harvest. They remain in the soil and in the plant for very long periods. They are not specific and destroy non-target species.

Given the catalogue of serious flaws in the Commission's decision-making process, PAN Europe wishes to request for a review of Regulation 149/208 and asks you to withdraw or suspend this Regulation in preventing harm to consumers.

Yours sincerely,

Stichting Natuur en Milieu - Inter Environment Wallonie, Belgium  
PAN-Europe Network Europe, England,  
Pesticide Action MDRGF, Mouvement pour le droit et le respect des générations futures,  
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