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L'EVALUATION DU RISQUE POUR LES ABEILLES DANS UN CADRE REGLEMENTAIRE

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RÉSUMÉ
Que ce soit dans le cadre d'un dossier d'homologation d'un produit de protection des plantes ou dans le cadre d'une demande de mention abeille, des études spécifiques pour déterminer la toxicité de la substance active ou du produit sur les abeilles et des évaluations de risque sont requises ainsi que le devenir de la substance dans les produits apiaires (miel, pollen). Dans un premier temps, les différents tests nécessaires pour caractériser l'effet sur les abeilles seront décrits : des études de toxicité aiguë par voie orale ou par contact jusqu'aux études en champ en passant par les études chroniques sur les adultes ou les stades larvaires. Ensuite les évaluations de risque seront explicitées et la procédure de demande de mention abeille sera détaillée. Enfin la méthodologie du suivi des résidus dans les produits apiaires sera décrite.
Mots-clés : évaluation du risque, abeille, écotoxicologie, résidus dans les produits apiaires, études de laboratoire, essais en cage ou en tunnel, essais au champ, règlements européens, mention abeille

SUMMARY
Risk assessment for bees in a regulatory framework

Whether in the context of a registration dossier for a plant protection product or in the context of a request for a bee mention in France, specific studies and risk assessments are required to determine the ecotoxicological effects of the active substance and/or the product on bees, as well as data on residue levels in pollen and bee products (namely honey) for human consumption. In this presentation, first the different tests necessary to assess effects on bees will be described: laboratory studies on acute (contact or oral) and chronic (oral) toxicity to adult or larval honey bees and other bee species through to higher tier semi-field and field studies. Secondly, the ecotoxicological risk assessment process for EU dossiers and the procedure for requesting a 'bee mention' in France will be explained. Finally, the methodology for determining pesticide residues in honey and setting Maximum Residue Levels in honey and other apiculture products will also be described.
Keywords: risk assessment, bee, ecotoxicology, residues in apiary products, laboratory studies, cage and tunnel tests, field tests, European regulations, bee mention

For the approval of chemical active substances, the requirements to assess the effects on bees and to evaluate the risks are listed in Regulation (EU) No. 283/2013.
Where bees are likely to be exposed, acute oral and contact toxicity to adult bees shall be assessed, as well as chronic oral toxicity to adults and honey bee larvae.
At product level, for authorisation, the data requirements are listed in Regulation (EU) No. 284/2013 and testing on the product is required if it contains more than one active substance or the toxicity cannot be reliably predicted to be either the same or lower than the active substance. If necessary, higher tier studies may also be required to refine the risk assessment.

Tier 1 (laboratory studies)

Honey bee (Apis mellifera) Acute oral toxicity test:
This laboratory study is conducted according to the OECD Test Guideline 213 (1998).

Adult worker honeybees are either exposed to a range of doses of the test substance dispersed in sucrose solution or, if low acute toxicity is expected, to one single dose (limit test. Once the treated sucrose solution has been consumed, the bees are then fed the same diet, free of the test substance. Mortality is recorded daily during at least 48 hours and compared with control and toxic standard values. The end-points acute oral 48h-LD50 (median lethal dose causing 50% mortality) and NOEC (no observed effect concentration) are expressed as µg test substance/bee. Sub-lethal effects, if observed, are also reported.

Honey bee (Apis mellifera) Acute contact toxicity test:
This laboratory study is conducted according to the OECD Test Guideline 214 (1998).
Adult worker honeybees are exposed to a range of doses of the test substance dissolved in appropriate carrier or to one single dose (limit test). Anaesthetized bees are individually treated by topical application. A volume of 1 µL of solution containing the test substance at the suitable concentration should be applied with a microapplicator to the dorsal side of the thorax of each bee. Mortality in the test substance treatments is recorded (at minimum at 4-, 24- and 48-hour timepoints) and compared with control and toxic standard values. The end-points acute contact 48h-LD50 (median lethal dose causing 50% mortality) and NOEC (no observed effect concentration) are expressed as µg test substance/bee. Sub-lethal effects, if observed, are also reported.

Honey bee (Apis mellifera) chronic oral toxicity test (10-day feeding)
This laboratory study is conducted according to the OECD Test Guideline 245 (2017).
The aim of this study is to assess the effect of the test substance over an oral exposure period of 10 days. The test can be performed either as a dose-response or a limit test. Young adult worker bees are exposed to 50 % (w/v) aqueous sucrose solution containing the test substance by continuous and *ad libitum* feeding over a period of 10 days. In addition, appropriate control and reference substance groups are also tested. Mortality and behavioural abnormalities are observed and recorded daily during the 10-day test period. Analytical verification of the test substance is required at least for the lowest and highest concentration and the stock solution.
The test is designed for the determination of the following endpoints LC_{50} (median Lethal Concentration) and the LDD_{50} (median Lethal Dietary Dose) values after 10 days of exposure, and NOEC (No Observed Effect Concentration) and NOEDD (No Observed Effect Dietary Dose). In addition, in order to comply with Regulation (EU) No. 283/2013 the chronic oral LC_{10} and LC_{20} values should also be estimated, where possible.

Honey bee (Apis mellifera) larval toxicity test following repeated exposure
This laboratory study is conducted according to the OECD Series on Testing and Assessment guidance document No. 239 (ENV/JM/MONO(2016)34)
The aim of this 22-day study is to assess the effect of the test substance following repeated oral exposure of honey bee larvae on days 3, 4, 5 and 6 of the study. From day 3 (D3) until day 6 (D6) of the test, the test chemical is administered daily to the larvae at a constant concentration equivalent to increasing test chemical doses per larva per day with the diet resulting in a cumulative dose on day 6 (for each treatment level) in a range of at least five increasing test concentrations, or at one concentration in case of a limit test. Data reported includes larval mortalities from D3 to D8, pupal mortalities from D8 to D15 and emergence rate on D22.
Analytical verification of the test substance is required at least for the stock solution used to prepare the lowest and highest concentration. Where possible analytical verification in the larval diet itself is performed.

The NOEC/NOED values for adult emergence at D22 are reported. If data allows, the EC_{50}/ED_{50}, and/or any EC_{10}/ED_{10} and EC_{20}/ED_{20} values including the associated lower and upper confidence intervals are also determined for adult emergence on D22.

Other available tests on other bee species
Acute oral toxicity test (OECD Test Guideline 247 (2017)) on bumble bees and acute contact toxicity test (OECD Test Guideline 246 (2017)) on bumble bees.

Draft or proposed bee tests
Proposal of a new OECD guideline for the testing of chemicals (18 May 2020) -Honey bee (*Apis mellifera* L.) homing flight test, using single oral exposure to sublethal doses of test chemical

Acute contact toxicity test for solitary bees (*Osmia* spp.)

Higher tier bee studies

When acute or chronic effects on colony survival and development cannot be ruled out based on acute and chronic toxicity tier 1 laboratory tests, further testing shall be required (e.g. cage and tunnel tests and/or field tests)

Cage and tunnel tests
According to Regulation (EU) No. 284/2013, the test shall provide sufficient information to evaluate:
— possible risks from the plant protection product for bee survival and behaviour, and
— impact on bees resulting from feeding on contaminated honey dew or flowers.
Sub-lethal effects are addressed, if necessary, by carrying out specific tests (for example foraging behaviour).

Field tests

The test shall have an adequate statistical power and shall provide sufficient information to evaluate possible risks from the plant protection product on bee behaviour, colony survival and development. Sub-lethal effects are addressed, if necessary by carrying out specific tests (for example homing flight).
The design of higher tier studies to be used shall be discussed with the relevant competent authorities.

2) Risk assessment

Available EU Guidance documents for bee risk assessment

Guidance Document (GD) on Terrestrial Ecotoxicology (SANCO/10329/2002 rev 2 final). However, this GD only addresses the acute risk to honey bees and there is no formally agreed risk assessment scheme for the chronic risk to honey bee adults and larvae.

EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees) (EFSA Journal 2013;11(7):3295)

The EFSA 2013 GD has still not yet been formally noted and agreed at EU level and is currently undergoing revision.

In December 2015 EFSA published a technical report on the "Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology" (EFSA Supporting publication 2015:EN-924). In this report, in the absence of alternative approaches taken note by risk managers, EFSA proposed that the EFSA 2013 GD should be used to evaluate studies and perform a Tier 1 risk assessment for honey bees. For bumble bees and solitary bees, it was agreed that if any data are submitted, they should be evaluated. However, currently it cannot be recommended to routinely perform a risk assessment.

The EFSA 2015 report is also not a formally noted document, however EFSA and some Member States (e.g. Netherlands and Belgium) are now routinely requesting that honey bee acute and chronic risk assessments are performed and evaluated in accordance with Tier 1 of the EFSA 2013 GD. Other Member States (e.g.

UK, Germany) continue to use only the SANCO/10329/2002 GD and whilst chronic adult and larval toxicity studies are required to address the data requirements, chronic risk assessments are not currently required.

It should be noted that many substances of low toxicity to bees, including herbicides, will likely fail the EFSA Tier 1 risk assessment, particularly for the chronic risk to larvae and adult honey bees, because the trigger values required by EFSA are highly conservative. This is widely acknowledged by industry (e.g. ECPA) and it remains unclear how to address these failures because feasible higher tier options are not yet agreed.

First tier risk assessment

The risk assessment is made according to EFSA Journal (2013;11(7):3295). The risk assessment is based on the single application rate (no addition of the effect of several applications). The single application rate is multiplied by a short-cut value to give the exposure value. There are different short-cute values for acute and chronic exposure and for adults and larvae. The exposure value is then compared to the toxicity by calculating the Hazard Quotient which is the ratio between exposure value and measured toxicity.

When the Hazard Quotient (or ETR) is below the mandatory triggers, the Tier 1 risk is acceptable. If not refined assessment is required. For that tunnel tests, semi-field or field tests are settled in order to determine the effect of the active substance in more and more realistic conditions.

Refined risk assessment

If the risk is not acceptable, it is refined by taking into account the results of higher tier tests such as tunnel, semi-tunnel and field tests, which show the effects of the product on a population and not at an individual level.

Bee mention (French risk mitigation, Anses 2010)

Regulatory background

The Article 2 of the French Arrêté (2003) provides that "in order to protect bees and other pollinating insects, treatments with insecticides and acaricides are prohibited during the entire flowering period, and during the period of production of exudates, regardless of the products and the applicator device used, on all forest stands and all crops visited by these insects".

The bee mention makes it possible to derogate from this prohibition and to apply the product on one or more crop (s) during flowering or during the period of production of exudates, apart from in the presence of bees and other pollinating insects.

According to this Decree, 3 types of mentions can be attributed:

- "authorized use during flowering, apart from in the presence of bees";
- "authorized use during periods of exudate production, apart from in the presence of bees";
- "authorized use during flowering and during periods of exudate production, apart from in the presence of bees".

Risk assessment

To assess the risk in order to obtain a bee mention, the effects of the product on bees are investigated in laboratory tests (i.e. honey bee acute oral and acute contact toxicity and chronic toxicity to adult honey bees honey bee larvae. Moreover, where required tunnel, semi-tunnel or field tests are performed often on *phacelia* to observe the real effects of single or multiple applications during flowering and during production of exudates.

The risk assessment is conducted according to EFSA Journal (2013;11(7):3295).

If the acute and chronic risks are acceptable for all scenarios of exposure or if based on higher tier tests (cage, tunnel and field tests), it can be concluded that no adverse effects are expected on bee colonies after applications of the product during flowering and/or during the production of exudates even with multiple

applications, it is considered appropriate to grant an exemption for the product from the safety phrase Spe8, for the proposed uses as follows:

- "authorized use during flowering, apart from in the presence of bees";
- "authorized use during periods of exudate production, apart from in the presence of bees";
- "authorized use during flowering and during periods of exudate production, apart from in the presence of bees".

II Residue studies for MRL setting in honey

Honey can potentially contain residues of plant protection products (PPP) because honeybees may be exposed to such products either directly or indirectly by collection of nectar and pollen. Results of honey residue monitoring programmes show that sometimes residue levels are above the default Limit of Quantification (LOQ) value of 0.05 mg/kg. It was therefore necessary to adopt a harmonized methodology in Europe, enabling the setting of MRL values in honey in the interest of consumer protection.

1) EU guidelines

Technical guidelines for determining the magnitude of pesticide residues in honey and setting Maximum Residue Levels in honey (SANTE/11956/2016 rev. 9) were finalised in September 2018. These guidelines were implemented from the 1st of January 2020 to fulfil EU data requirements concerning the placing of plant protection products on the market (Regulation (EU) No. 283/2013, Annex 6.10).

A default MRL value can be set at the LOQ level when residues in honey after pesticide application are not expected:
- If it is demonstrated that there are no residues in the 'aerial parts' of the crop,
- If the crop is not attractive to bees,
- If the crop is harvested before flowering,
- In case of soil treatments / seed treatments (where the active / metabolites are not systemic).

Data from aerial parts sampled during the attractive period of the crop or its weeds can be used to determine residue levels in aerial part of the crop (four trials are considered sufficient). For direct to crop spray applications the aerial parts should be sampled typically within 1 day after drying of the residue. For other application types sufficient data must be available to ensure that likely worst case residues in aerial parts can be determined.

If the HR is < 0.05 mg/kg, then the MRL is set by default at LOQ or at 0.05 mg/kg.
If the HR is ≥ 0.05 mg/kg and < 0.5 mg/kg, then the MRL is set according to the HR (highest residue) from aerial parts of the crop.

If the HR is ≥ 0.5 mg/kg, or if residue levels in aerial part of the crop are not available, then specific studies (field or tunnel trials or transfer from syrup to honey) detailed below shall be conducted. The choice of the appropriate study type to be conducted is dependent on the active substance properties, intended use and available data.

2) Residue in honey studies: design and requirements

The objective of these studies is to determine the inadvertent residue in honey arising from pesticide use, in order to allow a dietary risk assessment and to establish scientifically-based MRLs.

Field residue study

Guidance on the study conduct is provided in Appendix IV of SANTE/11956/2016 rev. 9 (14 September 2018).

The study is conducted in open fields (at least 4 trials with 2 bee hives/site) during one season if it is implemented at various test sites.

The test substance is applied in a realistic worst-case scenario:

Highest total application rate

On a highly melliferous crop (i.e. bee attractive crop), such as oilseed rape or *phacelia*

The field size is at least 3 ha with no other flowering crops in the surrounding 2-3 km area; the test lasts up until the honey is mature (approx. 7-21 days following application or start of flowering). The honey samples are taken at 3 different spots on at least 2 different combs per hive and at least 500 g of honey per trial. The pollen samples are taken for plant variety determination and also to determine residue levels. To analyse honey (pollen and the treated crop, if desired) for the relevant residue, a suitable validated analytical method should be chosen. It is desirable to achieve a limit of quantification as low as possible. A value of 0.05 mg/kg per analyte is favoured.

The colony health is assessed prior to introduction to the fields and at the end of the test, after honey collection.

Tunnel residue study

Guidance on the study conduct is provided in Appendix V of SANTE/11956/2016 rev. 9 (14 September 2018).

The study is conducted in at least 4 trial sites. Trial sites must be a least 10 km apart. One control and one treated tunnel (at least 120 m^2 in size) shall be placed on each crop field site. The tunnels are placed on a field containing a melliferous crop, such as oilseed rape or *phacelia*. One beehive is placed in each tunnel. The study is conducted during one season. The test substance is applied in a worst-case scenario (highest total application rate) as for the field trials. The application is made the day after introduction of the hive into the tunnel. The test lasts up until the honey is mature (approx. 7-14 days following application) or at the end of flowering. At least 100 g of honey per tunnel is sampled.

Based on the residue in honey obtained in the field or tunnel studies, an MRL proposal could be made based on the OECD calculator.

Syrup feeding residue study

Guidance on the study conduct is provided in Appendix III of SANTE/11956/2016 rev. 9 (14 September 2018).

The aim of the study is to simulate the residue transfer from nectar to honey. The trials are conducted in tunnels which do *not* contain melliferous crops, so bees do not have access to another nectar source. Each trial consists on one control and four test item tunnels (at least 40 m^2 in size), with one beehive placed in each tunnel. Bees are fed on sugar solution (50% sucrose), which is dosed with the test substance relevant for the plant residue definition. The appropriate dose concentration for the test substance is ideally determined based on tunnel study data (residue levels in honey sacs from homing foragers on the day of application) or the likely residue levels in the aerial part of the treated crop. The colony of bees is fed for 4 days with a total of 8 L of sugar solution (2 L/day). Protein supplements/pollen (between 50 and 100 g/day) also need to be supplied. The study ends when the honey has reached commercial maturity (approx. 1-2 weeks).

From the syrup feeding study and if the residue amount in honey is higher than 0.05 mg/kg, an MRL can be defined by using data on transfer factor from syrup to honey: Highest Residue (HR) [in plants, according to enforcement residue definition] x median transfer factor (from syrup to honey).

Strengths and weaknesses of each study

The field study is the most realistic of the three residue study types as it is the most comparable to the real crop situation. Sufficient quantities of honey should be obtained, and it could potentially be combined with residue field trials. But the implementation of the study is restrictive due to the field size, and due to the

requirement of an isolated field (no other melliferous crops/production of honey dew around the trial site: 2-3 km radius)
The tunnel study is less stringent in requirements on trial location. It is more realistic than the syrup feeding study (see below, uses treated crops with incurred residues), but it is difficult to obtain sufficient quantities of honey for testing and there are potential colony health issues and impact on normal honey bee behaviour due to the confinement of the bees in tunnels.
Finally, the syrup feeding study avoids the need for the data to be generated on a particular crop. It can be used to derive worst-case residue transfer factors for honey. But there are potential colony health issues and impact on normal bee behaviour. It is only practical if the active and/or metabolites are soluble or can be suspended in the sucrose feeding solution for several days and are stable in the solution. Finally, only transfer factors from syrup to honey are obtained and should be applied to residue levels in aerial parts of the crop.
In each case, the residue study requires the development of a specific analytical method in honey with appropriate validation; and analyses should be performed within 30 days otherwise a storage stability study in honey is required to confirm stability of the residues.

Conclusions

For the ecotoxicology risk assessment of plant protection products, a complete data package is required to assess the effects of the active substance or product to bees. A standard tier 1 data package investigates acute effects *via* the oral and contact routes and chronic effects to adult and larval honey bees. Where necessary additional higher tier studies such as cage, tunnel or field tests which show the effects of the test substance on a population and not just an individual may be required.

In order to choose the most appropriate honey residue trials, to fulfil the new data requirements, it is important to assess the available active substance data package.

For both higher tier bee effects and residue trials data studies, it is advisable to discuss the intended approach with the RMS for the active/product. Where higher tier field trials data are required, it is important to remember that such studies can only be undertaken during the correct season of the year and thus such studies need to be planned prior to the spring/summer season. Reliable experienced Contract Research Organisations (CRO) can quickly become fully booked and it is imperative to think and plan ahead.

Bibliography

EFSA Journal (2013;11(7):3295). Guidance on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees)

EFSA Supporting publication 2015:EN-924 - Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology

ANSES, 2010 - Notes d'information aux pétitionnaires - L'objet de cette note d'information est de préciser le contenu type d'un dossier constitué par un pétitionnaire et déposé en vue de l'obtention d'une mention « abeille ».
French Arrêté (2003) - Arrêté du 28 novembre 2003 relatif aux conditions d'utilisation des insecticides et acaricides à usage agricole en vue de protéger les abeilles et autres insectes pollinisateurs
OECD Series on Testing and Assessment guidance document No. 239 (ENV/JM/MONO(2016)34) - Guidance Document on Honey Bee Larval Toxicity Test following Repeated Exposure

OECD Test Guideline 213 (1998). OECD Guidelines for the testing of chemicals - Honeybees, Acute Oral Toxicity Test

OECD Test Guideline 214 (1998). OECD Guidelines for the testing of chemicals - Honeybees, Acute Contact Toxicity Test

OECD Test Guideline 245 (2017). OECD Guidelines for the testing of chemicals - Honey Bee (Apis Mellifera L.), Chronic Oral Toxicity Test (10-Day Feeding)

OECD Test Guideline 247 (2017). OECD Guidelines for the testing of chemicals - Bumblebee, Acute Oral Toxicity Test

OECD Test Guideline 246 (2017). OECD Guidelines for the testing of chemicals - Bumblebee, Acute Contact Toxicity Test

SANCO/10329/2002 rev 2 final - Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC – 17 October 2002

SANTE/11956/2016 rev. 9 (14 September 2018). Technical guidelines for determining the magnitude of pesticide residues in honey and setting Maximum Residue Levels in honey.

Regulation (EU) No. 283/2013; COMMISSION REGULATION (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market.

Regulation (EU) No. 284/2013; COMMISSION REGULATION (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market