Evaluation Manual for the Authorisation of Plant protection products and Biocides

NL part

Plant protection products

Chapter 7 Ecotoxocology: terrestrial; bees

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Chapter 7 Ecotoxicology; terrestrial; bees

Category: Plant protection products

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GENERAL INTRODUCTION

This chapter describes the data requirements for estimation of the effects on bees of a plant protection product and its active substance in the NL framework (§2 - §2.5).

2. NL FRAMEWOWRK

The NL framework (§2 - §2.5) describes the authorisation procedure for Plant protection products based on existing substances, included in Annex I, and new active substances.
A new substance is a substance not authorised in any of the Member States of the EU on the 25th of July 1993.
The pesticide that contains such substances may be authorised if the criteria laid down in the Wgb (Plant protection products and Biocides Act) 2006 [1] are met. The product is tested against the Plant protection products and Biocides Regulations (RGB) [2]. The evaluation dossiers must meet Annex II and III to Directive 91/414/EEC (see Application Form and corresponding instructions).

A Member State may on the basis of agricultural, phytosanitary and ecological, including climatological, conditions deviate from the EU evaluation methodology.

The NL framework describes the data requirements (§2.2), evaluation methodologies (§2.3), criteria and trigger values (§2.4) for which specific rules apply in the national approval framework or when the national framework has been elaborated in more detail than the EU framework.

The NL procedure described in §2 - §2.5 of this chapter can also be used for evaluation of a substance for inclusion in Annex I in case no European procedure has been described

2.1 Introduction

The assessment as regards the risk to bees follows the EU framework; for the NL assessment reference is therefore made to the EU assessment.

The decision tree with corresponding explanatory notes is presented in Appendix II-1. This decision tree summarises the evaluation as regards bees.

2.2 Data requirements

The data requirements for chemical Plant protection products are in agreement with the provisions in EU framework (see §1.2 of the EU part). The question numbering of the NL Application Form has also been included in §1.2 of the EU part.

Experiments carried out after the 25th of July 1993 must have been carried out under GLP.

There may be no doubt about the identity of the tested product or the purity of the tested substance for each study.

The studies must be carried out in compliance with the applicable guidelines. A review of the guidelines and whether or not these are required for particular fields of use is given in Appendix A to Chapter 7.

The applicant should always submit data about the risk to bees in case a chance of exposure of bees or bumblebees exists. This is the case when the product is applied:

- on plants (including weeds) - and during the flowering period of those plants - on which honeybees are flying for pollination or honey collection (specified in Annex II-2);
- in crops in which flowering weeds occur to a more than incidental extent;
- on plants that are to a considerable extent affected by aphids as a result of which they become attractive for bees by the presence of honeydew. These are, e.g., potatoes, cereals, and nursery stock, public parks and gardens (*Acer, Betula, Euonymus, Hedera, Hydrangea, Ilex, Rosa, Taxus* and *Tilia*);
- on plants with extra-floral nectar glands (nectar outside the flower). These are, e.g., broad beans and field beans, all *Prunus* species, *Cymbidium* orchids in greenhouses.

In many other cases exposure of bees is not likely, e.g., when the product is used during winter, when bees are not flying; use in closed spaces; use in greenhouses with insect screens where bees of bumblebees do not serve as pollinators; use on non-flowering crops (apart from the exceptions mentioned above), use on flowering crops that are not visited by bees of bumblebees such as grasses; seed coatings and granules, unless such products have a systemic effect; pre-emergence herbicides; products for dipping.

2.3 Risk assessment

The evaluation methodologies for chemical Plant protection products comply with the description under EU framework (see §1.3 of the EU part).

The national evaluation is in line with the European risk assessment for bees as elaborated in the Guidance Document on Terrestrial Ecotoxicology [**Fout! Bladwijzer niet gedefinieerd.**].

On national level, a method has been developed for determination of the combination toxicity (see below). Combination toxicity is not relevant in the EU evaluation process because active substances are evaluated and not products.

Combination toxicity

Combination products are formulated Plant protection products that contain more than one active substance. Combinations of Plant protection products of which, in accordance with the recommendation in the directions for use, the user prepares a combination in a tank (tank mix) are also considered as combination products. When evaluating the side effects of combination products on non-target organisms the question arises whether the risk must be estimated on the basis of a toxicity test with the combination product or whether a reasonable risk estimate can be made on the basis of the toxicity data of the separate active substances. There is no European guidance as regards combination toxicology.

It is possible to base the risk assessment of a combination product on toxicity tests with the formulation. The *acute* toxicity test may lead to variable results because quantity and quality of the co-formulants may not be constant and the formulation may alter the availability of the active substances. For the acute risk assessment, the combination toxicity on the basis of the tests with the product are compared with the combination toxicity based on the toxicity research with the separate active substances. The lowest combination toxicity value is then used in the risk assessment.

Combination toxicity is determined on the basis of concentration addition.
In theory, three different effects are to be expected when two or more substances are used in a mixture:
- the substances may weaken each others' toxic effects (antagonism)
- the effects of the substances may be additive
- the substances may potentiate each others' toxic effects (synergism).

Although the effects of mixtures of active substances in Plant protection products have only been studied to a very limited extent and not for all relevant species and toxicological endpoints it is expected that active substances in a combination product or tank mix together contribute to the toxicity of that product or that tank mix.
The extent to which the active substances are contributing is poorly known. The available data indicate that also in case of partial addition the extent of combination toxicity does not deviate strongly from concentration addition. In view of these considerations the evaluation of the toxicity data of combination products or tank mixes is based on concentration addition. In case of concentration addition each substance contributes to the total toxicity of a mixture in proportion to its concentration. The calculation method is given in Appendix C to Chapter 7.

2.4 Approval

Risk assessment for bees has been laid down in regulations. The Wgb (Plant protection products and Biocides Act) 2006 [1] stipulates in Art. 28 (1) (b4 and b5): "no unacceptable effect on the environment where in particular the consequences for non-target species are taken into account".

The evaluation of products on the basis of existing active substances already included in Annex I, or new substances, has been laid down in the Plant protection products and Biocides Regulations (RGB) [2] in which it is elaborated that these products are evaluated according to the Uniform Principles (UP).

2.4.1 Criteria and trigger values

For the criteria and trigger values for bees for the national authorisation reference is made to the EU part (§1.4.1).

2.4.2 Decision making

For decision making as regards the risk to bees for the national authorisation reference is made to the EU part (§1.4.2).

2.5 Developments

- Bumblebees:

The risk evaluation for bumblebees is fully based on extrapolation from the evaluation of bees, based on the hypothesis that they are equally or less sensitive than bees.
This hypothesis seems plausible because they are closely related, ánd because bumblebees have a more favourable surface/mass ratio. The hypothesis, however, needs validation.
Work on this is done in international ICPBR (International Commission for Plant-Bee Relationships) framework, in which the Bee Unit of the Dutch Organisation for Applied Plant Research (PPO) is participating. This yields indications that bees and bumblebees may react very differently to substances.

Artemis argues that new chemical products should for the following reasons also be tested separately on bumblebees:
- extrapolation from toxicity data for bees is dangerous; among the reasons are the difference in feeding habits (tropholaxis) of bees, the differences in size between the animal species (in view of the oral and contact LD_{50} which are expressed in microgrammes per bee) and in view of the physiological differences as a result of which certain products are toxic to bees and not toxic to bumblebees.
- the test protocols (EPPO, CEB, BBA) that have been developed for bees cannot be used for bumblebees. Separate test guidelines for bumblebees are under development.

It should be considered to what extent bees and bumblebees need a separate risk evaluation; consultations about this will be held with the competent experts.

- Insect Growth Regulators
  The risk evaluation of IGR needs further optimisation, in particular the evaluation via cage and field experiments. Work on this is done in international ICPBR framework, in which the Bee Unit of the Dutch Organisation for Applied Plant Research (PPO) is participating

- It is desirable to draw up a list of flowering crops that are not visited by bees or bumblebees.

3. APPENDICES

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Appendix II-1 Explanatory notes decision tree risk to bees

The decision tree for the risk to bees covers the harmfulness of Plant protection products, applied in accordance with the WG/GA (Statutory Use Instructions/Directions for Use), for bees.

Explanatory notes decision tree

1. The applicant must always provide data on the risk to bees whenever a possibility exists that bees are exposed. For a description of these cases, see §2.2.

2. 'Insect Growth Regulators' (IGRs) are substances that disturb the growth and/or ecdysis of insects. Examples of such compounds are the acyl-urea compounds (diflubenzuron, teflubenzuron), insect-hormone mimetics (e.g. phenoxycarb) and cyromazine.

3. By means of bee-brood testing, a first screening takes place whether substances have an IGR effect on bees. For this test, an internationally approved test protocol for the bee-brood test can be used [3]. Insufficient reference exposure data are available to relate larval toxicity data to exposure in the field and damage to the brood.

4. A cage test or field test is therefore always required if the NOAEL is exceeded at the field dose. This is the case if at the end of the test period growth disturbances are found in the brood. Possible effects on adult bees or bumblebees will then also show up in the cage test or field test. The brood test is not required where cage or field test data are available.

5. Through this step in the decision tree, effects other than acute effects (such as poisoning through nectar or pollen; delayed activity such as for micro-encapsulation; effects on the behaviour of bees) can be assessed by means of a suitable test and evaluation procedure (expert judgement) (via a cage test adapted for the expected effects). Evaluation of other risks, such as larval toxicity, long-term residual effects or disorientating effects on bees, is only possible with data from test conditions that sufficiently resemble the field situation, i.e., field, tunnel or cage experiments
   The risks can, e.g., be expected on the basis of the substance properties.

6. The contact and oral toxicity values (LD_{50}) of a product are usually of the same order of magnitude. Large differences between them may indicate unreliable data. Since direct contact is the major route of exposure to acute toxic substances, the contact LD_{50} is usually the most relevant parameter for the risk assessment of insecticides.
   The oral LD_{50} is more relevant for the risk assessment of substances of low toxicity (for bees), such as herbicides.
   The toxicity tests must be carried out in accordance with EPPO guideline 170 [4] or the OECD guidelines 213 and 214.

   The ratio "g per ha/LD_{50}" is used to estimate the risk. "G per ha" is the highest single recommended practical dose (in g a.s./ha) for the crop concerned. "LD_{50}" is the lowest value of the contact and oral LD_{50} (in microgram a.s./bee).

7. If a plant protection product contains several active substances, the combination toxicity must be determined. For these Plant protection products, it is desirable to test the toxicity of the product or the formulation. If this has not been done and only toxicity data of the individual active substances are available, the combination toxicity may be calculated on the basis of concentration addition (see Appendix C of Chapter 7 Ecotoxicology).

   The trigger value for the dose/toxicity ratio of 50 (when using the above-mentioned units for dose per ha and LD_{50}) has been laid down in the Uniform Principles of Directive 91/414/EEC. This means: if the dose/toxicity ratio is > 50, a cage trial is required.

8. Good field trials are more representative for risk assessments than cage trials. Results from good field trials are therefore given precedence over results from cage trials.

9. The cage test must be performed according to methods described in the above-mentioned EPPO guideline 170 [4]. Apart from that, the design of the trial may be need modification in order to investigate specific effects observed during previous studies.

   Exposure to Plant protection products is more intensive in cages and tunnels than in the field. The plant protection product will, therefore, be classified as having a LOW Risk to bees (or RISK PRESENT after *limited* exposure) if the effects on survival and development of the colony show no (statistically) significant difference from those in the control situation without the plant protection product. The use of a reference product is necessary to demonstrate that the bees were at risk under the environmental conditions. If the effects are significantly higher than those in the control situation, a field test is required.
   See also the explanatory notes on the evaluation of field trials under point 10.

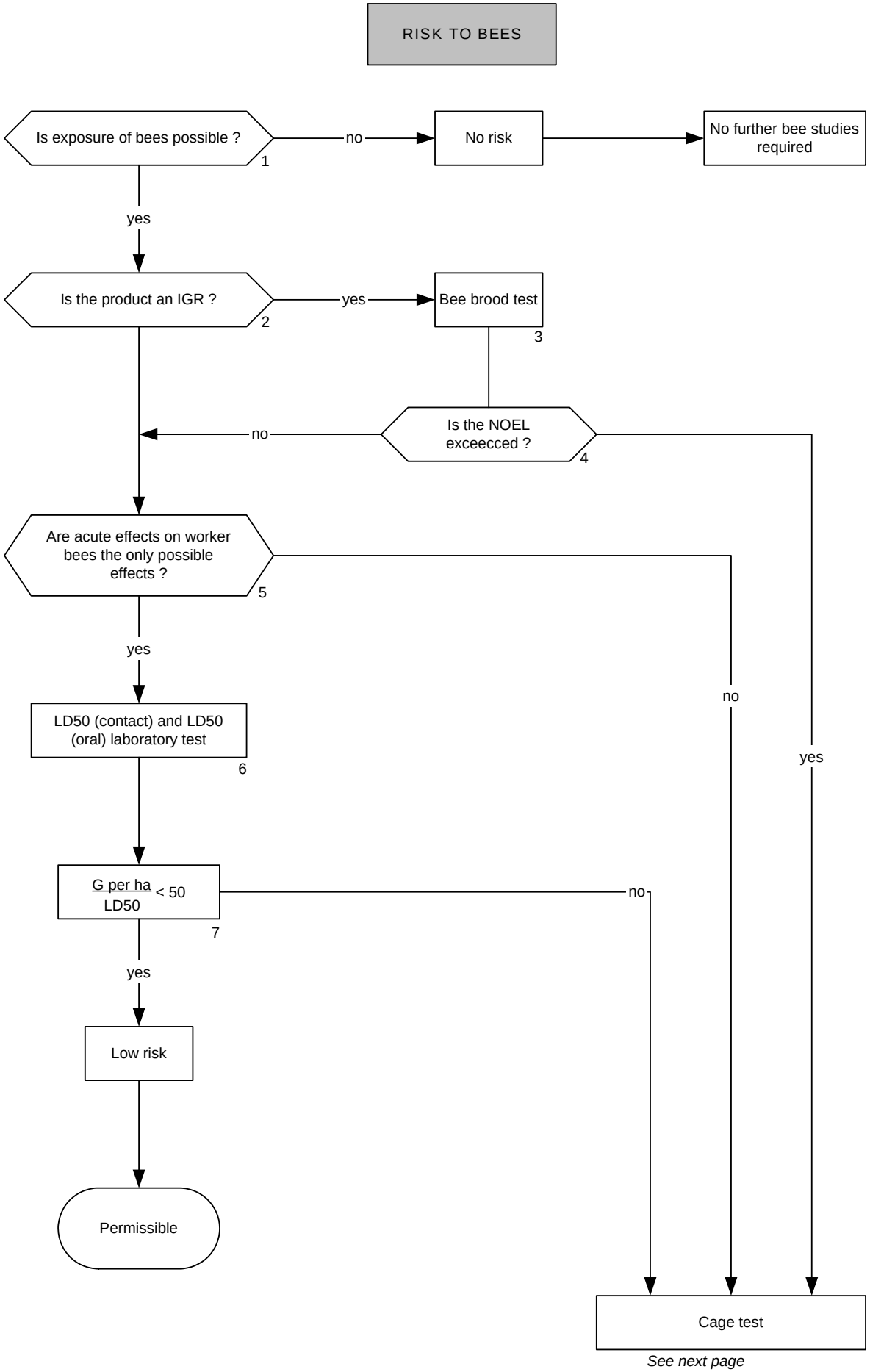
10. The above-mentioned guideline 170 [4] also describes the method for performing a field trial. In this trial, a harmful reference product is also essential to demonstrate that the bees were effectively exposed under the environmental conditions (particularly weather conditions) of the trial. In addition, a harmless reference is used to evaluate the effect of the test substance on the survival and development of the bee colony.
    The conditions under which the cage and field test are conducted should be reasonably representative of the prescribed use. In practice, exposure of bees may be controlled ('risk management') by restrictions on the use, such as a limiting the use to application at dusk only, after bee flights have ceased. The product should then be tested under these circumstances.
    For certain purposes, which are difficult to realise in the open field, such as studies into the effects via honeydew, tunnel trials can replace field trials. Further guidelines for tunnel trials are given in EPPO guideline 170 [4].

[3] A reference product is a product authorised for use on the same crop against the same disease or pest as the test product (other active substances).
[4] A harmful/harmless reference product is a product of which it is known from practice or earlier tests that it is harmful/harmless for the same test organism under the same conditions as during the test.

A HIGH RISK to bees exists if the effects observed in a well-conducted field trial are significantly higher than those of the harmless reference product: If no effects are observed under specific prescribed application restrictions, but they are during full exposure, the substance/product is considered to be possibly dangerous, but not dangerous under the conditions of use to be prescribed (RISK PRESENT).
Effects smaller than or equal to those for the harmless reference product during full exposure, lead to the classification LOW RISK.

11. Effects resulting from experimental treatments may be difficult to identify, and it may be difficult to distinguish them from e.g. natural mortality. The criterion used is therefore that the mortality observed should show no statistically significant difference from that of the untreated population. A prerequisite is that the trial design was statistically correct. Usually a statistical analysis of the results resolves this problem. Experience shows however, that studies with bees (in particular cage and field trials) are not always suitable for such analyses, in view of the special demands on the isolation between the trial fields and the scale of the experiments. However, cage and field trials provide supplementary data on exposure by:
    - the usual procedures in studies with bees, including the use of a reference product harmful to bees (particularly to demonstrate that indeed exposure occurred);
    - the collection of pollen, followed by residue analysis;
    - direct observation of foraging behaviour of foraging bees.

    For these reasons, the desired interpretation of the results is still possible. In a number of cases, however, expert judgement will be required to decide whether “effects” in cage or field trials are significant or not important.



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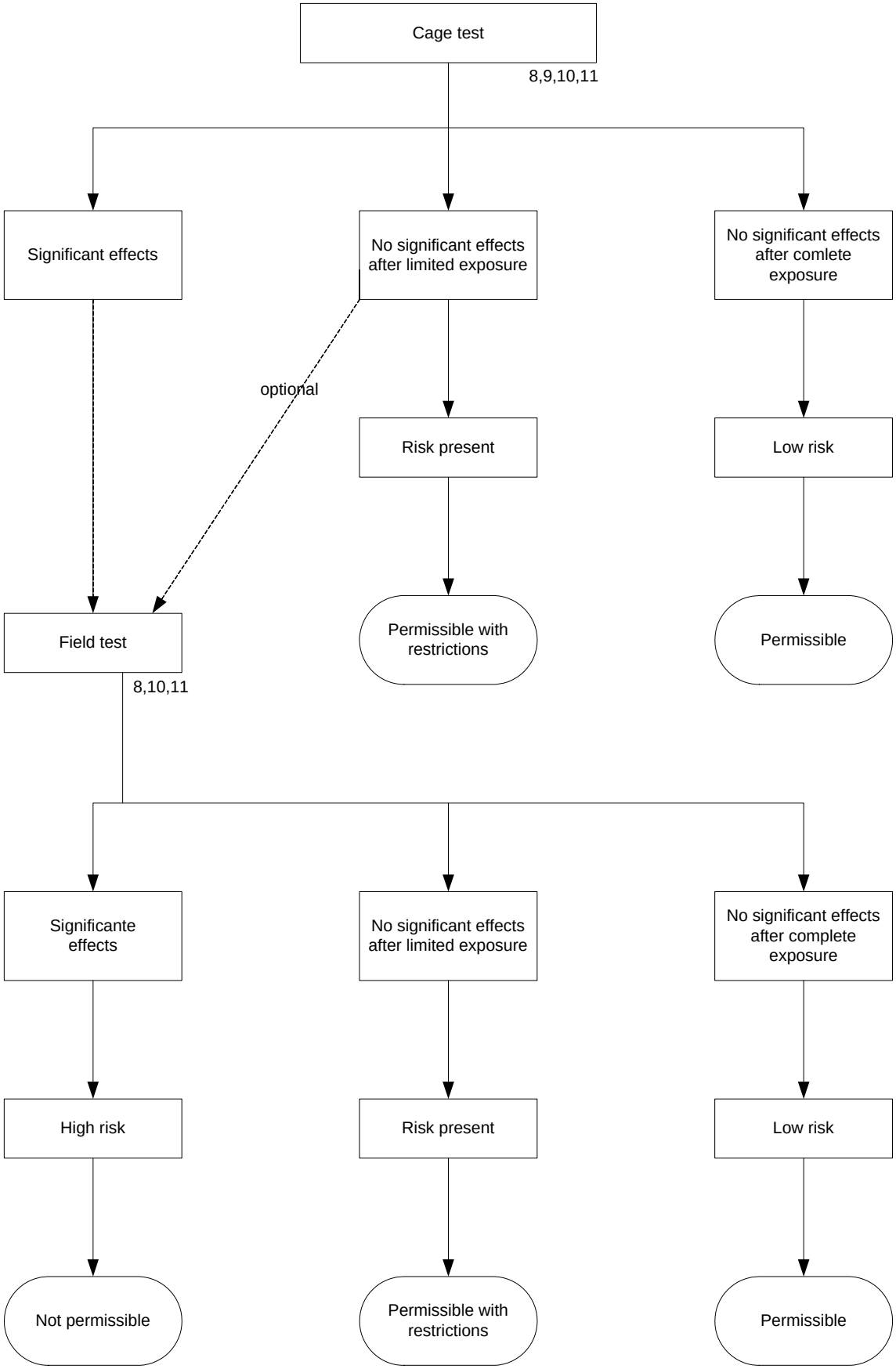
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Significant effects
No significant effects after limited exposure
No significant effects after comlete exposure
optional
Risk present
Low risk
Field test
8,10,11
Permissible with restrictions
Permissible
Significante effects
No significant effects after limited exposure
No significant effects after complete exposure
High risk
Risk present
Low risk
Not permissible
Permissible with restrictions
Permissible

Appendix II-2 Crops used by honeybees for pollination or nectar collection

(Annex A8.3.1 / P10.4 of Application Form)

Field crops

Soft fruit:	berry, strawberry, blackberry, raspberry
Top fruit:	apple, cherry, pear, plum
Vegetables:	asparagus, gherkin, beans, courgette, broad beans.
Arable crops:	Chinese radish, phacelia, caraway, white clover, lupine, lucerne, oilseed rape and mustard seed etc., evening primrose, field bean, flax, sunflower
Flower bulbs:	crocus, hyacinth, (botanic) tulip and miscellaneous bulbs and corms.

Summer flowers.

Seed production of various vegetables, herbs and flowers, including: endive, brassicae, leek, onion, chicory, carrot.

Nursery stock, public parks and gardens.

Under protection

Vegetables and fruit: blackberry, raspberry, strawberry, berries, peach, plum, gherkin, courgette, aubergine, sweet pepper and melon.

Seed production of various vegetables, herbs and flowers, including: endive, asparagus, gherkin, brassicae, chicory and carrot.

Appendix

Tree species that are important nectar plants and that are sometimes treated with Plant protection products against insects: lime, willow.

Weeds that are important nectar plants and that are sometimes treated with Plant protection products: dandelion, creeping thistle, and weeds growing in agricultural crops (e.g. redshank, sheep sorrel).

4 REFERENCES

[1] Regeling voor de toelating, het op de markt brengen en het gebruik van gewasbeschermingsmiddelen en biociden (Wet gewasbeschermingsmiddelen en biociden) (Plant protection products and Biocides Act, Wgb 2006);
NL acts, decisions, orders, etc. can be obtained via http://wetten.overheid.nl/;

[2] Regeling van de Minister van Landbouw, Natuur en Voedselkwaliteit van 26 september 2007, nr. TRCJZ/2007/3100, houdende nadere regels omtrent gewasbeschermingsmiddelen en biociden (Plant protection products and Biocides Regulations (RGB), published in the Government Gazette (Staatscourant) 188 of 28 September 2007 came into effect on 17 Oktober 2007;
including
Regeling van 20 oktober 2009 tot wijziging van de Regeling gewasbeschermingsmiddelen en biociden in verband met de aanwijzing van beoordelingsmethoden), published in the Government Gazette (Staatscourant) 16032 of 26 Oktober 2009 came into effect on 1 January 2010;
NL acts, decisions, orders, etc. can be obtained via http://wetten.overheid.nl/

3 Oomen, P.A., De Ruijter, A. & van der Steen, J. (1992) Method for honeybee brood feeding tests with insect growth-regulating insecticides. Bulletin OEPP/EPPO Bulletin 22, 613-616.

4 EPPO (1992). Guideline on test methods for evaluating the side-effects of plant protection products. No. 170. Honeybees. Bulletin OEPP/EPPO Bulletin 22, 203-216.