TECHNICAL GUIDELINES[1]

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Technical guidelines for determining the magnitude of pesticide residues in honey and setting Maximum Residue Levels in honey

Implemented by 1 January 2020

[1] This document has been conceived as a guidance document of the Commission Services. It does not represent the official position of the Commission. It does not intend to produce legally binding effects. Only the European Court of Justice has jurisdiction to give preliminary rulings concerning the validity and interpretation of acts of the institutions of the EU pursuant to Article 267 of the Treaty.

1 INTRODUCTION

Honey can potentially contain residues of plant protection products (PPPs) since honeybees may be exposed to such products either directly or indirectly by collection of nectar and pollen. Residues of plant protection products are sometimes found in honey during residue monitoring and levels can vary from one substance to another. It is therefore appropriate to establish safe Maximum Residue Limits (MRLs) for consumers. Since a methodology on the data needed and the approach for using them to set appropriate MRLs is not available, the MRLs for honey have historically been set at a default level of 0.05 mg/kg. European data requirements (Regulation (EU) No 283/2013, Annex 6.10) require studies on residues in pollen and bee products for human consumption, but do not specify the type and conditions of the studies to be performed. According to the Regulation, type and conditions shall be discussed with national competent authorities. These Technical Guidelines will fill this gap and give further technical information on studies and data required, enabling EFSA and the Commission to refine MRLs for honey in the interest of consumer protection. These technical guidelines have been endorsed by the Standing Committee meeting of 18-19 September 2018.

MRLs for honey are also reflected in Article 16(c) and Annex I (part A and B) of Regulation (EC) No 396/2005 where the possibility to set temporary MRLs is given.

Honey has been considered as a food of animal origin (cf Annex I of Regulation 396/2005: code 1040000, under PRODUCTS OF ANIMAL ORIGIN -TERRESTRIAL ANIMALS). As a general rule, pesticides may be ingested or absorbed by livestock in three ways:

1. following direct application of the product to the animal,
2. as a result of treatment of their accommodation,
3. through residues in feeding stuffs.

Residues of pesticides arising from uses as veterinary medicinal products or after accommodation (beehive) treatment (cases 1 and 2) must be taken into consideration when setting MRLs for plant protection products.

In the first two cases MRLs have been set in the past by Council Regulation (EEC) No 2377/90 (now replaced by Regulation (EU) No 37/2010).

In case 3, pesticide residues may arise in honey from current pesticide uses. MRLs established in this case should in principle be set on the basis of appropriate supervised residue trials data.

The situation for honey is not comparable to other situations where supervised trials are carried out as residues may be taken up by the honeybees during collection of nectar and/or pollen when plant protection products are used while the treated crops or adjacent non-target plants are flowering or during collection of nectar and/or pollen from flowering rotational crops after the use of persistent systemic products.

As estimated from the data available in the EFSA Model for risk assessment of pesticides MRLs (PRIMo: Pesticide Residue Intake Model), the average consumption of honey *per capita* and per day in Europe is less than 5 g/capita/day and thus represents a very small part of the total diet. This would consequently not imply a significant contribution to the Theoretical Maximum Daily Intake (TMDI), usually calculated in order to assess the chronic risk of dietary exposure.

Considering the acute exposure, according to:

- the EFSA Model PRIMo (rev.3):
  - critical Large Portion of 1.38 and 3.58 g/kg bw respectively defined for adults (CZ males 15-17y) and children (NL toddler),
  - case 1 equation (International Estimate Short Term Intake (IESTI) = LP x HR / ARfD),
- an Acute Reference Dose (ARfD) of 1.5 x 10^{-4} mg/kg bw/day, which corresponds to the lowest ARfD established to date (for carbofuran), a maximum level of honey contamination can be set at 0.042 mg/kg.

This calculation shows that any MRL, even the default value of 0.05 mg/kg further proposed, should be checked for acute risk of dietary exposure, using PRIMo. The Limit Of Quantification (LOQ) needs to be set at a lower level in case a risk for consumers is identified at the default level of 0.05 mg/kg.

Honey, wax, pollen loads, drone larvae, propolis and royal jelly can be harvested from beehives.

Honey, honeycomb, pollen, royal jelly, wax and propolis have been considered most pertinent for consumers.

The composition of these products is described in literature. For the purpose of this exercise the content of water, lipids and sugars is important, The following table lists values from literature[2, 3, 4, 5].

Bee products composition

	Water content	Lipid content	Carbohydrate content
Honey[(a)]	16 - 22%	--	70 - 80%
Nectar[(a)]	40 – 50%		5 – 80%
Wax[(e) (f)]	--	64%-67% fatty acid esters, 12-15% fatty acids, ≤ 1% free alcohols	--
Pollen (air dried)[(g)]	7 - 11%	5%	32 – 37% (hand-collected 19%)
Propolis[(h) (i)]		25 - 50% (waxes and fatty acids)	Less than 5% (and 5% pollen)
Royal jelly[(k) (l)]	57 - 70%	3,5 - 19% of dry weight	18 – 52% of dry weight

Bee wax, honeycomb, pollen and raw propolis as well as royal jelly contain lipids in different amounts (all above 5%). Due to the lipid content of these matrices, it is expected that fat-soluble active substances will be found at higher levels than in honey. In addition, available monitoring data indicate that for the same substance pollen contains higher residues compared to honey.

Bee wax is used in cosmetics and pharmaceuticals for production of e.g. lip balm, lip gloss, hand creams, salves, and moisturizers, eye shadow, blush, and eye liner. In food industry, bee wax is used for cheese coatings and as food additive E901. In addition beekeepers recycle bee wax. They remove old brown combs at the end of breeding period for hygienic reasons. The wax is melted and cleaned from contaminants. The clean yellow wax is used to produce new walls for the bees to produce the combs. As a result of this recycling, fat-soluble substances may accumulate. Nevertheless, contamination with plant protection products of honey by transfer-back from contaminated wax is considered negligible (this also applies to lipophilic active substances (LogPow > 3)).

Worker bees produce royal jelly to feed the larvae. Due to its composition it is more likely to find water-soluble active substances in this matrix but it cannot be ruled out that fat-soluble substances will also be found in royal jelly.

The composition of propolis varies considerably from region to region along with vegetation, from season to season, and from hive to hive. Propolis being sold to consumers is not defined. The quality varies between products being more likely a raw propolis and products obtained after ethanolic extraction containing nearly no lipids.

On the other hand, nectar contains about 70% - 80% of water when bees collect it. During repeated transfer of nectar from one honeycomb cell to the next, an air stream is produced by the bees by wing flapping, which reduces the water content by 30-80% in the original nectar to below 20% in honey. In this case, we expect to find the water-soluble active substances more likely in honey.

Comb honey (honey on the honeycomb) may be consumed. Consumption of comb honey is considered to be covered by the MRL for honey. In the first instance, royal jelly could be used as a related product to honey due to its high water content (Böhme *et al*., 2017).

Consumption of pollen (including pollen present in honey), royal jelly, propolis, bee wax and honeycomb is negligible. Therefore there is no need to generate experimental residue data for these commodities .

[2] (a), (e). (k) J. Nitschman and J. O. Hüsing (ed.), 2002: Lexikon der Bienenkunde. Tosa Verlag, Wien, 2002.
[3] (f), (g), (h), (l) R. Krell, 1996: Value-added products from beekeeping. FAO AGRICULTURAL SERVICES BULLETIN No. 124. Food and Agriculture Organization of the United Nations Rome 1996.
http://www.fao.org/docrep/w0076e/w0076e00.htm#con, retrieved 31st October 2017
[4] (d) S. W. Nicolson, M. Nepi and E. Pacini (Ed.), 2007: Nectaries and Nectar. Published by Springer 2007. Page 9
[5] (i) N. Kunz, 2013: Propolisernte in Deutschland: Effiziente Gewinnung einer gleichbleibend guten Qualität. Diplomarbeit zur Erlangung des akademischen Grades Diplom-Biologin (Dipl.-Biol.). University Stuttgart-Hohenheim, April 2013.

This document gives guidance on the following issues:

- under which circumstances to consider residues/ MRLs in honey
- how to establish likely residues/an appropriate MRL and how to collect those data (including experimental and trial guidance)

2 RESIDUE DEFINITION FOR HONEY AND BEE PRODUCTS

Similar to other food products, a residue definition for risk assessment needs to be derived for honey which covers the toxicological relevant compounds present in honey resulting from the use of pesticides in crops foraged by bees.

Honey is produced by bees from sugary secretions of plants (mainly nectar) through regurgitation, enzymatic conversion and water evaporation and followed by storage in the bee hives for a certain time period.
In the absence of specific metabolism studies with honey bees, the residue definitions for risk assessment needs to be derived taking into account other sources of information such as studies investigating the nature of residues in primary crops (i.e. crops that were treated with the pertinent pesticide), the degradation during pasteurisation and studies investigating the nature of residues in rotational crops (i.e. residues taken up by plants from the soil).

The following metabolites may be relevant when proposing the risk assessment residue definition for honey:

- components (parent compound and metabolites) included in the risk assessment residue definition for primary crops
- degradation products formed during pasteurisation conditions
- metabolites included in the risk assessment residue definition for rotational crops in case of metabolites and/or an active substance remaining in the soil, after application of that active substance, which have the potential to be taken up by a following crop.

Potential residue definition components should then be considered based on magnitude and toxicological information, in line with the current EU guidance, to produce a simplified proposal.

To derive a residue definition for enforcement (residue definition for MRL setting/tolerance expression), the basic principles described in the relevant OECD guidance document should be taken into account. Thus, a few considerations should be made on which components of the residue definition for risk assessment are qualified to be included in the residue definition for enforcement:

- Would the enforcement residue definition derived for plant products be suitable?
- Is it possible to cover all components proposed to be included in the residue definition for enforcement for honey with a multi-residue method?
- Are analytical standards available for all components of the proposed enforcement residue definition?

When appropriate, the monitoring residue definition for honey should be preferably the same as the monitoring residue definition for plant products (primary crops).

3 DECISION-MAKING SCHEME

The proposed approach is divided into several successive steps as presented in Appendix I. The MRL will be set depending on the results obtained at each different step. Each step of this decision scheme is discussed below.

3.1 Are residues expected in honey after pesticide application?

Residues in honey can occur:

- When a substance is applied during the flowering stage (BBCH 60-69) of a crop which is foraged by bees (see Appendix II)
- When a substance with systemic properties[6] is applied prior to the flowering stage (before BBCH 60), including treatment of seeds, of a crop which is foraged by bees (see Appendix II) .
- from uses on non-target plants (in-field weeds and adjacent plants) when a substance is applied during the flowering period from April to September.
- from succeeding crops after application of a persistent and systemic active substance[7]
- via honeydew collected from plant-sucking insects in forestry (such as *Picea* spp., *Abies* spp, *Pinus* spp. and *Quercus* spp.)

[6] If metabolism studies in crops (studies conducted according to OECD guideline 501) clearly establish that neither the parent nor toxicologically-relevant metabolites are present in a non-treated part of the plant when the active substance is applied according to critical GAPs, then it can be considered that the active substance is not systemic. Indications can also be found in the rotational crop studies.

[7] DT_{90} (soil) > 100 days (trigger value for performing rotational crop residue studies)

A list of the main agricultural crops in Europe, from which it is possible to produce honey, via the presence of nectar and/or honeydew in/on the treated crop or in the surroundings can be found in Appendix II[8].

If residues in honey are **not** expected (the substance is applied on a crop from which it is not possible to produce honey, the substance has no systemic properties and is not applied during the flowering period or the substance has systemic properties but is applied after the flowering period and is not persistent), it is recommended to set a default MRL at the limit of quantification (LOQ) determined for the active substance in honey. In the absence of a specific LOQ in honey for the active substance under consideration, the default value of 0.05* mg/kg can be used.

If residues in honey are expected considering the proposed uses and the properties of the active substance, then further data on crop or field/tunnel trials are required. To this end, it is possible to consider a "worst case" situation, that is, to obtain these data by applying the most critical scenario on a crop representing a worst case in terms of residues in honey (for example, rapeseed (*Brassica napus*), phacelia, or any other crop with high melliferous capacity) even if this is not a proposed use. The highest total application rate defines the most critical scenario. For non-systemic substances only, the application rate to be tested can be limited to the use rates applied during flowering.

To achieve reliable result, residues in honey should be determined as soon as possible after sampling and at the latest 30 days after sampling. If this cannot be achieved, storage stability data (as described in OECD Test Guideline 506 "Stability of Pesticide Residues in Stored Commodities") are required concerning the stability of the residue in stored honey samples.

3.2 What is the "residue" level in aerial parts of the crop[9]?

Data from aerial parts sampled during the attractive period of the crop or its weeds can be used if available (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. It is recommended to sample for leaves 12 units/500 g and for flowers and nectar, a minimum of 20 units from 12 different locations in the field. Samples should be stored according to OECD Guideline 506. Analytical methods used should be fully validated according to SANCO/3029/99 or SANCO/825/00.

When calculating the HR in aerial parts of the plant, data pertinent to flowering parts of the plant (especially nectar data) should be preferentially used. Nectar can be sampled using micropipette or other tools (e.g., Corbet, 2002; McKenna, 1988; D. S. Morrant, 2008) or bees can be used for nectar collection (EFSA Journal 2013;11(7):3295). For spray applications sampling can be done within 1 day after drying of the residue. The sample size should not be fewer than 20 individual plants or bees. In case nectar contains a low level of sugar, the residue level has to be recalculated with a concentration factor by which the nectar is concentrated.

If the highest residue level measured in aerial parts of the crop at the time when the crop or the non-target plant is foraged by bees is below a threshold value of 0.05 mg/kg, then the residue level expected in honey is assumed to be below 0.05 mg/kg. A default MRL of 0.05 mg/kg can then be fixed, based on a transfer factor of 1 from aerial parts to honey. This level can be considered as conservative compared to data available in the literature (Kubik *et al.*, 1999; Bogdanov, S. (2006); Schur & Wallner, (1998, 2000)).

If the highest residue level in aerial parts of plants is equal to or above the threshold value of 0.05 mg/kg but below 0.5 mg/kg, an MRL proposal could be made based on the HR and on the hypothesis of a transfer factor of 1 from aerial parts to honey depending on the outcome of the risk assessment and if the MRL is safe for consumers.
However, when plant and honey residue definitions differ:

[8] Appendix II lists the so-called `melliferous` crops. These crops, besides being attractive to bees, provide enough pollen, nectar, propolis and/or honeydew to enable honeybees to yield honey from that crop.
[9] Aerial parts of the crop comprise leaves, flowers and/or nectar but not grains

- if additional metabolites included in honey definition come from processing studies, pasteurization transfer factor should be taken into consideration for residue calculation
- if metabolites come from rotational crop studies, data complying with residue definition in honey are required.

If no data in aerial parts are available or if the highest residue determined in relevant aerial parts is equal or higher than **0.5** mg/kg, more specific data are required in order to set an MRL at a level as low as possible.
It should be noted if ecotoxicological semi-field or field studies are performed on bees, data on pollen and nectar from these studies might be useful depending on comparison of the applied GAPs.

Residues in honey can be determined by:

- use of data from studies on transfer from syrup (see 3.2.1),
- use of data from field or tunnel residue trials (see 3.2.2)

3.2.1 Experimental studies via syrup feeding

Syrup trials aim to determine a worst case transfer of pesticides into honey by providing bees with sugar syrup dosed with parent and metabolites to which bees are expected to be exposed. At least 4 test tunnels and 1 control tunnel (using one bee colony for each tunnel) are considered necessary.

The syrup should be spiked according to the plant residue definition for risk assessment.

Further guidance on conducting syrup trials can be found in Appendix III.

The median transfer factor derived in these studies can then be used to calculate appropriate MRLs

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

If the MRL in honey based on these trials is considered safe for consumers, no further data is considered necessary.

3.2.2 Experimental field or tunnel data

Field and tunnel trials aim to determine the likely residues in honey based on the tested GAP, via direct foraging of bees on a treated crop. At least four trials are considered necessary.

Further guidance on conducting field and tunnel trials can be found in Appendix IV and V.

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

3.3 Is the active substance included in a veterinary medicinal product?

As a last step it should always be verified if an active substance is also used as a veterinary medicine for beehive treatment (mainly to control bee diseases or parasites).

If an MRL under Commission Regulation (EU) No 37/2010 is available, it is necessary to:

- compare both residue definitions for monitoring and risk assessment;
- Verify that the MRL set under veterinary legislation for honey also accommodates possible PPP uses

If the MRL set under the veterinary legislation is higher than the MRL to accommodate PPP uses, a consumer risk assessment, with the PRIMo model, with the MRL as defined under Commission Regulation (EU) No 37/2010 needs to be performed. If the veterinary MRL is safe to consumers, this MRL can be taken over into Regulation (EC) No 396/2005.

If the MRL set under the veterinary legislation is lower than the MRL that accommodates PPP uses, the procedure set out in chapters 3.1 and 3.2 should be used to define the appropriate level.

4 MONITORING DATA

Monitoring data might be a useful tool to provide additional information if such data are available. Article 16 of Regulation (EC) n° 396/2005 allows the setting of temporary MRLs in honey on the basis of monitoring data.

After authorization of a plant protection product, monitoring data can be used to achieve more realistic values thus complying with the ALARA principle (level as low as reasonably achievable). MRLs for honey based on monitoring data will always be temporary according to Article 16 of Regulation (EC) No 396/2005[10]. They can be reviewed at any moment to ensure the ALARA principle still applies but will be reviewed at the latest every 10 years.

The available monitoring data should:

- reflect the agreed residue definition;
- reflect different production areas.

An MRL from monitoring data can be derived according to the methodologies proposed by FAO in its "Plant production and protection paper 197" (FAO, 2009):

- FAO spice approach: The MRL is derived from the calculation of the upper 95th confidence limit for the 95th percentile, considering the samples with detectable residues only. A minimum of 58-59 values is recommended.
  This approach is described in Regulation (EU) No 283/2013, which request MRL proposals covered by the 95th percentile of the data population at the 95% confidence level[11].
- FAO extraneous MRL (EMRL) approach: This approach refers to "the chemicals which have been widely used as pesticides, are persistent in the environment for relatively long periods after use has been discontinued and are expected to occur in foods or feeds at levels of sufficient concern to warrant monitoring" (FAO, 2009). Since there is no internationally agreed level of acceptable violation rate, specific percentiles are not recommended by JMPR, but it is reported that "violation rates of 0.5 to 1% or greater are generally unacceptable". Therefore and based on the entire dataset including values below the LOQs, MRL are derived corresponding to violation rates of 0.5 and 1% (99.5th and 99th percentile respectively).

[10] Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. OJ L 70, 16.3.2005, 3.4.2013, p. 1.

[11] 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. OJ L 93, p. 1.

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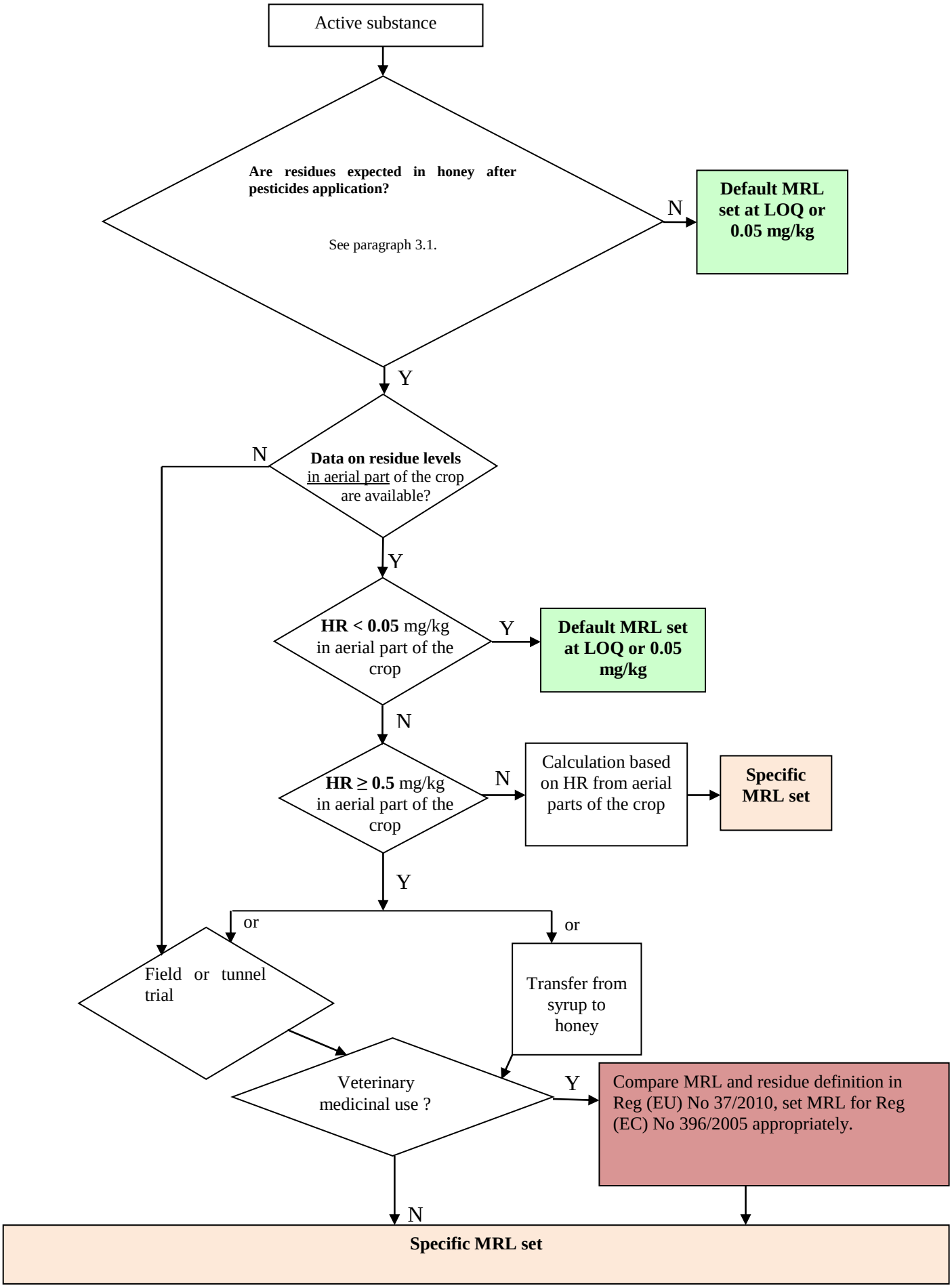
APPENDIX I

DECISION MAKING SCHEME FOR MRL SETTING IN HONEY



APPENDIX II

LIST OF MELLIFEROUS CROPS[1]

Code no. (1)	Groups to which the MRLs apply	Examples of individual products within the groups to which the MRLs apply	Scientific name (2)	Melliferous capacity[2]
100000	1. FRUIT FRESH OR FROZEN; NUTS	FRUIT (FRESH OR FROZEN)		
110000	(i) Citrus fruit	Citrus fruit		
110010		Grapefruit	*Citrus paradisi*	**Yes**
110020		Oranges	*Citrus sinensis*	**Yes**
110030		Lemons	*Citrus limon*	**Yes**
110040		Limes	*Citrus aurantifolia*	**Yes**
110050		Mandarins	*Citrus reticulata*	**Yes**
110990		Other citrus fruit		**Yes**
120000	(ii) Tree nuts (shelled or unshelled)	Tree nuts (shelled or unshelled)		
120010		Almonds	*Prunus dulcis*	**Yes**
120020		Brazil nuts	*Bertholletia excelsa*	**No data available**
120030		Cashew nuts	*Anacardium occidentale*	**Yes**
120040		Chestnuts	*Castanea sativa*	**Yes**
120050		Coconuts	*Cocos nucifera*	**Yes**
120060		Hazelnuts	*Corylus avellana*	**Yes**
120070		Macadamia	*Macadamia ternifolia*	**Yes**
120080		Pecans	*Carya illinoensis*	**No data available**
120090		Pine nuts	*Pinus pinea*	**Yes**
120100		Pistachios	*Pistachia vera*	**No**
120110		Walnuts	*Juglans regia*	**Yes**

[1] These crops, besides being attractive to bees, provide enough pollen, nectar, propolis and/or honeydew to enable honeybees to yield honey from that crop.
[2] Crops for which no data is available to indicate its melliferous capacity should be regarded as melliferous unless data is provided to indicate it does not have melliferous capacity. Not applicable to crops harvested before flowering.

Code no. (1)	Groups to which the MRLs apply	Examples of individual products within the groups to which the MRLs apply	Scientific name (2)	Melliferous capacity[2]
120990		Other tree nuts		**No data available**
130000	(iii) Pome fruit	Pome fruit		
130010		Apples	*Malus domesticus*	**Yes**
130020		Pears	*Pyrus communis*	**Yes**
130030		Quinces	*Cydonia oblonga*	**Yes**
130040		Medlar	*Mespilus germanica*	**Yes**
130050		Loquat	*Eriobotrya japonica*	**Yes**
130990		Other pome fruit		**Yes**
140000	(iv) Stone fruit	Stone fruit		
140010		Apricots	*Prunus armeniaca*	**Yes**
140020		Cherries	*Prunus cerasus, Prunus avium*	**Yes**
140030		Peaches	*Prunus persica*	**Yes**
140040		Plums	*Prunus domestica*	**Yes**
140990		Other stone fruit		**Yes**
150000	(v) Berries & small fruit	Berries & small fruit		
151000	(a) Table and wine grapes	Table and wine grapes		**Yes**
151010		Table grapes	*Vitis euvitis*	**Yes**
151020		Wine grapes	*Vitis euvitis*	**Yes**
152000	(b) Strawberries	Strawberries	*Fragaria* x *ananassa*	**Yes**
153000	(c) Cane fruit	Cane fruit		
153010		Blackberries	*Rubus fruticosus*	**Yes**
153020		Dewberries	*Rubus ceasius*	**Yes**
153030		Raspberries	*Rubus idaeus*	**Yes**
153990		Other cane fruit		**Yes**

Code no. (1)	Groups to which the MRLs apply	Examples of individual products within the groups to which the MRLs apply	Scientific name (2)	Melliferous capacity[2]
154000	(d) Other small fruit & berries	Other small fruit & berries		
154010		Blueberries	*Vaccinium corymbosum*	**Yes**
154020		Cranberries	*Vaccinium macrocarpon*	**Yes**
154030		Currants (red, black and white)	*Ribes nigrum, Ribes rubrum*	**Yes**
154040		Gooseberries	*Ribes uva-crispa*	**Yes**
154050		Rose hips	*Rosa canina*	**Yes**
154060		Mulberries	*Morus* spp*;*	**Yes**
154070		Azarole (mediteranean medlar)	*Crataegus azarolus*	**Yes**
154080		Elderberries	*Sambucus nigra*	**Yes**
154990		Other other small fruit & berries		**Yes**
160000	(vi) Miscellaneous fruit	Miscellaneous fruit		
161000	(a) Edible peel	Miscellaneous fruit (edible peel)		
161010		Dates	*Phoenix dactylifera*	**No**
161020		Figs	*Ficus carica*	**No**
161030		Table olives	*Olea europaea*	**No**
161040		Kumquats	*Fortunella species*	**No data available**
161050		Carambola	*Averrhoa carambola*	**Yes**
161060		Persimmon	*Diospyros kaki*	**Yes**
161070		Jambolan (java plum),	*Syzygium cumini*	**No data available**
161990		Other miscellaneous fruit (edible peel)		**No data available**
162000	(b) Inedible peel, small	Miscellaneous fruit (inedible peel, small)		
162010		Kiwi	*Actinidia deliciosa* syn. *A. chinensis*	**No**

Code no. (1)	Groups to which the MRLs apply	Examples of individual products within the groups to which the MRLs apply	Scientific name (2)	Melliferous capacity[2]
162020		Lychee (Litchi)	*Litchi chinensis*	**Yes**
162030		Passion fruit	*Passiflora edulis*	**No data available**
162040		Prickly pear (cactus fruit)	*Opuntia ficus-indica*	**Yes**
162050		Star apple	*Chrysophyllum cainito*	**No data available**
162060		American persimmon (Virginia kaki)	*Diospyros virginiana*	**Yes**
162990		Other miscellaneous fruit (inedible peel, small)		**No data available**
163000	(c) Inedible peel, large	Miscellaneous fruit (inedible peel, large)		
163010		Avocados	*Persea americana*	**Yes**
163020		Bananas	*Musa x paradisica*	**Yes**
163030		Mangoes	*Mangifera indica*	**Yes**
163040		Papaya	*Carica papaya*	**Yes**
163050		Pomegranate	*Punica granatum*	**Yes**
163060		Cherimoya	*Annona cherimola*	**No data available**
163070		Guava	*Psidium guajava*	**Yes**
163080		Pineapples	*Ananas comosus*	**No**
163090		Bread fruit	*Artocarpus altilis*	**No data available**
163100		Durian	*Durio zibethinus*	**Yes**
163110		Soursop (guanabana)	*Annona muricata*	**No data available**
163990		Other miscallaneous fruit (inedible peel, large)		**No data available**
200000	2. VEGETABLES FRESH OR FROZEN	VEGETABLES FRESH OR FROZEN		

Code no. (1)	Groups to which the MRLs apply	Examples of individual products within the groups to which the MRLs apply	Scientific name (2)	Melliferous capacity[2]
210000	(i) Root and tuber vegetables	Root and tuber vegetables incl. potaotes)		
211000	(a) Potatoes	Potatoes	*Tuber form Solanum Spp*	**No**
212000	(b) Tropical root and tuber vegetables	Tropical root and tuber vegetables		
212010		Cassava	*Manihot esculenta*	**No data available**
212020		Sweet potatoes	*Ipomoea batatas*	**Yes**
212030		Yams	*Dioscorea sp.*	**No data available**
212040		Arrowroot	*Maranta arundinacea*	**No data available**
212990		Other tropical root and tuber vegetables		**No data available**
213000	(c) Other root and tuber vegetables except sugar beet	Other root and tuber vegetables except sugar beet		
213010		Beetroot	*Beta vulgaris* subsp. *vulgaris*	**No**
213020		Carrots	*Daucus carota*	**No (Yes for seed production)**
213030		Celeriac	*Apium graveolens* var. *rapaceum*	**No**
213040		Horseradish	*Armoracia rusticana*	**No**
213050		Jerusalem artichokes	*Helianthus tuberosus*	**Yes**
213060		Parsnips	*Pastinaca sativa*	**No (Yes for seed production)**
213070		Parsley root	*Petroselinum crispum*	**Yes**
213080		Radishes	*Raphanus sativus* var. *saitvus*	**No (Yes for seed production)**
213090		Salsify	*Tragopogon porrifolius*	**Yes**

Code no. (1)	Groups to which the MRLs apply	Examples of individual products within the groups to which the MRLs apply	Scientific name (2)	Melliferous capacity[2]
213100		Swedes	*Brassica napus* var. *napobrassica*	**No (Yes for seed production)**
213110		Turnips	*Brassica rapa*	**No (Yes for seed production)**
213990		Other other root and tuber vegetables		**No data available**
220000	(ii) Bulb vegetables	Bulb vegetables		
220010		Garlic	*Allium sativum*	**No (Yes for seed production)**
220020		Onions	*Allium cepa*	**No (Yes for seed production)**
220030		Shallots	*Allium ascalonicum* (*Allium cepa* var. *aggregatum*)	**No (Yes for seed production)**
220040		Spring onions	*Allium cepa*	**No (Yes for seed production)**
220990		Other bulb vegetables		**No data available**
230000	(iii) Fruiting vegetables	Fruiting vegetables		
231000	(a) Solanacea	Solanacea		
231010		Tomatoes	*Lycopersicum esculentum*	**No**
231020		Peppers	*Capsicum annuum*, var *grossum* and var. *longum*	**Yes**
231030		Aubergines (egg plants)	*Solanum melongena*	**Yes**
231040		Okra, lady's fingers	*Hibiscus esculentus*	**Yes**
231990		Other solanacea		**No**
232000	(b) Cucurbits - edible peel	Cucurbits - edible peel		
232010		Cucumbers	*Cucumis sativus*	**Yes**
232020		Gherkins	*Cucumis sativus*	**Yes**

Code no. (1)	Groups to which the MRLs apply	Examples of individual products within the groups to which the MRLs apply	Scientific name (2)	Melliferous capacity[2]
232030		Courgettes	*Cucurbita pepo* var. *melopepo*	**Yes**
232990		Other cucurbits - edible peel		**Yes**
233000	(c) Cucurbits-inedible peel	Cucurbits - inedible peel		
233010		Melons	*Cucumis melo*	**Yes**
233020		Pumpkins	*Cucurbita maxima*	**Yes**
233030		Watermelons	*Citrullus lanatus*	**Yes**
233990		Other cucurbits - inedible peel		**Yes**
234000	(d) Sweet corn	Sweet corn	*Zea mays* var. *sacharata*	**No**
239000	(e) Other fruiting vegetables	Other fruiting vegetables		
240000	(iv) Brassica vegetables	Brassica vegetables		
241000	(a) Flowering brassica	Flowering brassica		
241010		Broccoli	*Brassica oleracea*	**No (Yes for seed production)**
241020		Cauliflower	*Brassica oleracea* var. *botrytis*	**No (Yes for seed production)**
241990		Other flowering brassica		**No (Yes for seed production)**
242000	(b) Head brassica	Head brassica		
242010		Brussels sprouts	*Brassica oleracea* var. *gemmifera*	**No (Yes for seed production)**
242020		Head cabbage	*Brassica oleracea* convar *capitata*	**No (Yes for seed production)**
242990		Other head brassica		**No (Yes for seed production)**
243000	(c) Leafy brassica	Leafy brassica		
243010		Chinese cabbage	*Brassica pekinensis*	**No (Yes for seed production)**

Code no. (1)	Groups to which the MRLs apply	Examples of individual products within the groups to which the MRLs apply	Scientific name (2)	Melliferous capacity[2]
243020		Kale	*Brassica oleracea* convar. *Acephalea*	**No (Yes for seed production)**
243990		Other leafy brassica		**No (Yes for seed production)**
244000	(d) Kohlrabi	Kohlrabi	*Brassica oleracea* convar. a*cephala*, var. *gongylodes*	**No (Yes for seed production)**
250000	(v) Leaf vegetables & fresh herbs	Leaf vegetables & fresh herbs		
251000	(a) Lettuce and other salad plants including Brassicacea	Lettuce and other salad plants including Brassicacea		
251010		Lamb's lettuce	*Valerianella locusta*	**No**
251020		Lettuce	*Lactuca sativa*	**No**
251030		Scarole (broad-leaf endive)	*Cichorium endiva*	**No**
251040		Cress	*Lepidium sativum*	**No**
251050		Land cress	*Barbarea verna*	**No**
251060		Rocket, Rucola	*Eruca sativa (Diplotaxis* spec.)	**No**
251070		Red mustard	*Brassica juncea* var. *rugosa*	**No**
251080		Leaves and sprouts of Brassica spp	*Brassica spp*	**No**
251990		Other lettuce and other salad plants		**No**
252000	(b) Spinach & similar (leaves)	Spinach & similar (leaves)		
252010		Spinach	*Spinacia oleracea*	**No (Yes for seed production)**
252020		Purslane	*Portulaca oleracea*	**No**

Code no. (1)	Groups to which the MRLs apply	Examples of individual products within the groups to which the MRLs apply	Scientific name (2)	Melliferous capacity[2]
252030		Beet leaves (chard)	*Beta vulgaris*	**No**
252990		Other spinach and similar		**No data available**
253000	(c) Vine leaves (grape leaves)	Vine leaves (grape leaves)	*Vitis euvitis*	**Yes**
254000	(d) Water cress	Water cress	*Nasturtium officinale*	**No**
255000	(e) Witloof	Witloof	*Cichorium intybus*. var. *Foliosum*	**No**
256000	(f) Herbs	Herbs		
256010		Chervil	*Anthriscus cerefolium*	**No data available**
256020		Chives	*Allium schoenoprasum*	**Yes**
256030		Celery leaves	*Apium graveolens* var. *seccalinum*	**Yes**
256040		Parsley	*Petroselinum crispum*	**Yes**
256050		Sage	*Salvia officinalis*	**Yes**
256060		Rosemary	*Rosmarinus officinalis*	**Yes**
256070		Thyme	*Thymus* spp.	**Yes**
256080		Basil	*Ocimum basilicum*	**Yes**
256090		Bay leaves (laurel)	*Laurus nobilis*	**Yes**
256100		Tarragon	*Artemisia dracunculus*	**Yes**
256990		Other herbs		**No data available**
260000	(vi) Legume vegetables (fresh)	Legume vegetables (fresh)		
260010		Beans (with pods)	*Phaseolus vulgaris,*	**Yes**
260020		Beans (without pods)	*Phaseolus vulgaris*	**Yes**
260030		Peas (with pods)	*Pisum sativum*	**Yes**
260040		Peas (without pods)	*Pisum sativum*	**Yes**

Code no. (1)	Groups to which the MRLs apply	Examples of individual products within the groups to which the MRLs apply	Scientific name (2)	Melliferous capacity[2]
260050		Lentils (fresh)	*Lens culinaris* syn. *L. esculenta*	**Yes**
260990		Other legume vegetables (fresh)		**No data available**
270000	(vii) Stem vegetables (fresh)	Stem vegetables (fresh)		
270010		Asparagus	*Asparagus officinalis*	**Yes**
270020		Cardoons	*Cynara cardunculus*	**No**
270030		Celery	*Apium graveolens* var. *dulce*	**No**
270040		Fennel	*Foeniculum vulgare*	**Yes**
270050		Globe artichokes	*Cynara scolymus*	**Yes**
270060		Leek	*Allium porrum*	**No (Yes for seed production)**
270070		Rhubarb	*Rheum x hybridum*	**No**
270080		Bamboo shoots	*Bambusa vulgaris*	**No data available**
270090		Palm hearts	*Euterpa oleracea, Cocos nucifera, Bactris gasipaes, daemonorops schmidtiana*	**No data available**
270990		Other stem vegetables		**No data available**
280000	(viii) Fungi	Fungi	(viii) Fungi	
280010	Cultivated	Cultivated fungi		**No**
280020	wild	Wild fungi		**No**
280990		Other fungi		**No**
290000	(ix). Sea weeds	Sea weeds		**No**
300000	3. PULSES, DRY	PULSES, DRY		
300010		Beans	*Phaseolus vulgaris*	**Yes**
300020		Lentils	*Lens culinaris* syn. *L. esculenta*	**Yes**

Code no. (1)	Groups to which the MRLs apply	Examples of individual products within the groups to which the MRLs apply	Scientific name (2)	Melliferous capacity[2]
300030		Peas	*Pisum sativum*	**Yes**
300040		Lupins	*Lupinus* spp.	**Yes**
300990		Other pulses, dry		**No data available**
400000	4. OILSEEDS AND OILFRUITS	OILSEEDS AND OILFRUITS		
401000	(i) Oilseeds	Oilseeds		
401010		Linseed	*Linum usitatissimum*	**Yes**
401020		Peanuts	*Arachis hypogaea*	**No**
401030		Poppy seed	*Papaver somniferum*	**No**
401040		Sesame seed	*Sesamum indicum* syn. *S. orientale*	**Yes**
401050		Sunflower seed	*Helianthus annuus*	**Yes**
401060		Rape seed	*Brassica napus*	**Yes**
401070		Soya bean	*Glycine max*	**Yes**
401080		Mustard seed	*Brassica nigra*	**Yes**
401090		Cotton seed	*Gossypium* spp.	**Yes**
401100		Pumpkin seeds	*Cucurbita pepo* var. *oleifera*	**Yes**
401110		Safflower	*Carthamus tinctorius*	**Yes**
401120		Borage	*Borago officinalis*	**Yes**
401130		Gold of pleasure	*Camelina sativa*	**No data available**
401140		Hempseed	*Cannabis sativa*	**Yes**
401150		Castor bean	*Ricinus communis*	**Yes**
401990		Other oilseeds		**No data available**
402000	(ii) Oilfruits	Oilfruits		
402010		Olives for oil production	*Olea europaea*	**No**
402020		Palm nuts (palmoil kernels)	*Elaeis guineensis*	**No data available**
402030		Palmfruit	*Elaeis guineensis*	**No data available**

Code no. (1)	Groups to which the MRLs apply	Examples of individual products within the groups to which the MRLs apply	Scientific name (2)	Melliferous capacity[2]
402040		Kapok	*Ceiba pentandra*	**No data available**
402990		Other oilfruit		**No data available**
500000	5. CEREALS	CEREALS		
500010		Barley	*Hordeum* spp.	**No**
500020		Buckwheat	*Fagopyrum esculentum*	**Yes**
500030		Maize	*Zea mays*	**No**
500040		Millet	*Panicum* spp.	**No**
500050		Oats	*Avena fatua*	**No**
500060		Rice	*Oryza sativa*	**No**
500070		Rye	*Secale cereale*	**No**
500080		Sorghum	*Sorghum bicolor*	**No**
500090		Wheat	*Triticum aestivum*	**No**
500990		Other cereal		**No**
600000	6. TEA, COFFEE, HERBAL INFUSIONS AND COCOA	TEA, COFFEE, HERBAL INFUSIONS AND COCOA		
610000	(i) Tea *(dried leaves and stalks, fermented or otherwise of Camellia sinensis*)	Tea *(dried leaves and stalks, fermented or otherwise of Camellia sinensis*)		**No data available**
600010		Tea	*Camellia sinensis*	**No data available**
620000	(ii) Coffee beans	Coffee beans		**Yes**
630000	(iii) Herbal infusions *(dried)*	Herbal infusions *(dried)*		
631000	(a) Flowers	Herbal infusions (dried flowers)		**No data available**
631010		Camomille flowers	*Matricaria recutita*	**No data available**
631020		Hybiscus flowers	*Hibiscus sabdariffa*	**No data available**
631030		Rose petals	*Rosa* spec.	**No data available**

Code no. (1)	Groups to which the MRLs apply	Examples of individual products within the groups to which the MRLs apply	Scientific name (2)	Melliferous capacity[2]
631040		Jasmine flowers	*Jasminum officinale*	**No data available**
631050		Lime (linden)	*Tillia cordata*	**Yes**
631990		Other herbal infusions (dried flowers)		**No data available**
632000	(b) Leaves	Herbal infusions (dried leaves)		
632010		Strawberry leaves	*Fragaria x ananassa*	**Yes**
632020		Rooibos leaves	*Aspalathus* spec.	**No data available**
632030		Maté	*Ilex paraguariensis*	**No data available**
632990		Other herbal infusions (dried leaves)		**No data available**
633000	(c) Roots	Herbal infusions (dried roots)		**No data available**
633010		Valerian root	*Valeriana officinalis*.	**Yes**
633020		Ginseng root	*Panax ginseng*	**No data available**
633990		Other herbal infusions (dried roots)		**No data available**
639000	(d) Other herbal infusions	Herbal infusions (other herbal infusions)		**No data available**
640000	(iv) Cocoa *(fermented beans)*	Cocoa *(fermented beans)*	*Theobroma cacao*	**Yes**
650000	(v) Carob (st johns bread)	Carob (st johns bread)	*Ceratonia siliqua*	**Yes**
700000	7. HOPS *(dried)* , including hop pellets and unconcentrated powder	HOPS *(dried)*, including hop pellets and unconcentrated powder	*Humulus lupulus*	**No**
800000	8. SPICES	SPICES		
810000	(i) Seeds	Spices (seeds)		
810010		Anise	*Pimpinella anisum*	**Yes**
810020		Black caraway	*Nigella sativa*	**Yes**
810030		Celery seed	*Apium graveolens*	**No data available**

Code no. (1)	Groups to which the MRLs apply	Examples of individual products within the groups to which the MRLs apply	Scientific name (2)	Melliferous capacity[2]
810040		Coriander seed	*Coriandrum sativum*	**No data available**
810040		Cumin seed	*Cuminum cyminum*	**Yes**
810060		Dill seed	*Anathum graveolens*	**Yes**
810070		Fennel seed	*Foeniculum vulgare*	**Yes**
810080		Fenugreek	*Trigonella foenum-graecum*	**Yes**
810090		Nutmeg	*Myristica fragans*	**No data available**
810990		Other spices (seeds)		**No data available**
820000	(ii) Fruits and berries	Spices (fruits and berries)		
820010		Allspice	*Pimenta dioica*	**No data available**
820020		Anise pepper (Japan pepper)	*Zanthooxylum piperitum*	**No data available**
820030		Caraway	*Carum carvi*	**No data available**
820040		Cardamom	*Elettaria cardamomum*	**Yes**
820050		Juniper berries	*Juniperus communis*	**No data available**
820060		Pepper, black and white	*Piper nigrum*	**No data available**
820070		Vanilla pods	*Vanilla fragrans* syn. *Vanilla planifolia*	**No data available**
820080		Tamarind	*Tamarindus indica*	**Yes**
820990		Other spices (fruit and berries)		**No data available**
830000	(iii) Bark	Spices (bark)		**No data available**
830010		Cinnamon	*Cinnamonum verum* syn. *C. zeylanicum*	**No data available**
830990		Other spices (bark)		**No data available**
840000	(iv) Roots or rhizome	Spices (roots or rhizome)		
840010		Liquorice	*Glycyrrhiza glabra*	**No data available**
840020		Ginger	*Zingiber*	**No data**

Code no. (1)	Groups to which the MRLs apply	Examples of individual products within the groups to which the MRLs apply	Scientific name (2)	Melliferous capacity[2]
			officinale	**available**
840030		Turmeric (Curcuma)	*Curcuma domestica* syn. *C. longa*	**Yes**
840040		Horseradish, root spices	*Armoracia rusticana*	**No data available**
840990		Other spices (roots)		**No data available**
850000	(v) Buds	Spices (buds)		**No data available**
850010		Cloves	*Syzygium aromaticum*	**No data available**
850020		Capers	*Capparis spinosa*	**No data available**
850990		Other spices (buds)		**No data available**
860000	(vi) Flower stigma	Spices (flower stigma)		
860010		Saffron	*Crocus sativus*	**Yes**
860990		Other spices (flower stigma)		**No data available**
870000	(vii) Aril	Spices (aril)		**No data available**
870010		Mace	*Myristica fragrans*	**No data available**
870990		Other spices (aril)		**No data available**
900000	9. SUGAR PLANTS	SUGAR PLANTS		
900010		Sugar beet (root)	*Beta vulgaris*	**No (Yes for seed production)**
900020		Sugar cane	*Saccharum officinarum*	**No**
900030		Chicory roots	*Cichorium intybus*	**No (Yes for seed production)**
900990		Other sugar plants		**No data available**
1000000	10. PRODUCTS OF ANIMAL ORIGIN- TERRESTRIAL ANIMALS	PRODUCTS OF ANIMAL ORIGIN - TERRESTRIAL ANIMALS		**Not applicable**
-	11. FORAGE PLANTS	FORAGE		

Code no. (1)	Groups to which the MRLs apply	Examples of individual products within the groups to which the MRLs apply	Scientific name (2)	Melliferous capacity[2]
	(i) Gramineous			**No**
		Rye grass for forage and silage		**No**
	(ii) Legumes/Leguminous for silage			**Yes**
		Alfalfa		**Yes**
		Birdsfoot		**Yes**
		Chick pea		**Yes**
		Clover (for forage and silage)		**Yes**
		Cow peas		**Yes**
		Esparcette		**Yes**
		Kudzu		**Yes**
		Lespedeza		**Yes**
		Sainfoin		**Yes**
		Sesbania		**Yes**
		Sulla		**Yes**
		Trefoil		**Yes**
		Turnip, especially cultivated for fodder		**Yes**
		Vetches		**Yes**
	12 AGROFORESTRY AND ORNAMENTALS	TREES		
	(i) Flowering trees			**Yes**
	(ii) Conifers			**Yes**
	13. FALLOW	FALLOW		
	(i) flowering plants			**Yes**
	(ii) Non flowering plants			**No**

Code no. (1)	Groups to which the MRLs apply	Examples of individual products within the groups to which the MRLs apply	Scientific name (2)	Melliferous capacity[2]
	14. PERFUME AND MEDICINAL PLANTS	PERFUME AND MEDICAL PLANTS		
	(i) Perfume plants	Perfume plants		
		Lavender		**Yes**
		Rose		**No**
	(ii) Medical plants	Medical plants		
		Poppy	*Papaver somniferum*	**No**
		Sage		**Yes**

APPENDIX III

Experimental studies via syrup feeding

1 Objectives

2 Test principles

2.1 Application of test substance(s)
2.2 Design of trials sites
2.3 Honeybee colony preparation
2.4 Duration of the trial
2.5 Sampling, method of analysis

3 Report

3.1 Summary
3.2 Objectives
3.3 Study setup and study details
3.4 Sample preparation
3.5 Extraction, clean-up, determination, evaluation
3.6 Results and conclusion

4 References

1 Objectives

The objective of these studies is to determine the inadvertent residues in honey arising from plant protection products (PPP) use, by determining a worst case transfer of pesticides into honey, in order to allow a dietary risk assessment and to establish scientifically-based MRLs.

2 Test principles

Principle of the trial is to provide sugar solution to honey bees with the aim that bees consume, process and store the sugar solution in cells on combs as "artificial honey". As no other food stores will be in hives before feeding, all food stores will consist of the given food solution, processed to "artificial honey" stores.

2.1 Application of test substance(s)

The residue of concern should be added to an aqueous sugar solution (at least 50% (w/v (weight/volume))), which is then called the feeding solution. The feeders filled with the feeding solutions should be implemented on top of each colony according to good beekeeping practice. A quantity of 2 L freshly prepared feeding solution should be placed in each hive once per day or as soon as the previously offered feeding solution is fully consumed (in case, feeding solution is not consumed). The administration of the spiked feeding solution should be done on four consecutive days, i.e. in sum an amount of 8 L feeding solution will be fed per colony. After the first 4 L of the original feeding solution have been fully consumed (which is ideally after 2 days), on the following two days, the remaining 4 L (2 L per day) will be administered with half concentration of the original feeding solution in 50% (w/v) aqueous sugar solution Thereafter, pure 50% (w/v) aqueous sugar solutions will be administered (approximately 3-5 times a week, about 2 L per feeding) until the first capped "artificial honey" cells are observed. The feed uptake should be measured and documented daily.
The concentration in the original feeding solution should ideally be based on the residues that are found in honey sacs from homing foragers on the day of application in a tunnel trial (the highest application rate according to Good Agricultural Practice (GAP) should be used). If no tunnel study is available, the concentration in the original feeding solution should be based on the residues that are likely in aerial parts of the treated crop according to the plant residue definition.

2.2 Design of trials sites

Beehives are placed in tunnels protected with an insect-proof net so that no residue dilution will occur in honey due to bee foraging on another nectar source. The covered tunnel area is empty of melliferous plants.

Each trial should consist of one control tunnel and four tunnels per tested item group. Each tunnel should contain one colony. The colonies should be placed in the tunnels approximately three days before start of spiked feeding solution in order to give the bees the possibility to acclimatise to the new environment inside the tunnels.

Bees should always have access to water.

The tunnel size should be at least 40 m^2.

Products containing the tested active substance must not be used as maintenance chemicals, both on treated and untreated plots. In the same way, products likely to cause ill effects on honeybees must be avoided. It must be ensured that active substance for which MRL is to be determined has not been used for veterinary treatment of the bees.
Capped honey needs to be obtained.

2.3 Honeybee colony preparation

Artificial swarm technique ("shook swarm method") is used. Therefore, at least about 10,000 bees are used. Worker bees are obtained from healthy colonies which are free of symptoms of diseases. A mated, egg-laying queen is added. All frames are made of new wax foundations. Next to pure bee wax foundations, it is also possible to use pre-built plastic frames; if necessary also a honey super can be added (queen excluder necessary). No combs with food stores are provided. The only available food source is the feeding solution.
Protein supplements/pollen (between 50 and 100 g/day) needs to be supplied to the colonies to avoid a drastic drop in protein sources; this step is essential that new larvae can be raised and the colony develop normally. Pollen can be administered inside (e.g. as patties or milled pollen), or outside the hives (e.g. milled pollen or

pollen from untreated flowering plants. If a pollen comb is provided, it is recommended to take all further honey samples from the other side of the colony.

No residue analyses are needed for pollen combs and patties as this steps serves to reduce unnecessary stress for colonies only, and it can be assumed that it is unlikely that relevant cross-contamination occurs. However, from each used pollen batch a retain sample should be taken, in order to be able to analyze the pollen for residue, while this is only considered necessary in case of any unexplainable residues in e.g. the control samples.

2.4 Duration of the trial

The honeybee colonies will remain in the tunnels until the "artificial honey" reached commercial maturity (comb-closure or the water content in the "artificial honey" is below 20%, to be measured with refractometer), which is usually after one to two weeks.

2.5 Sampling, method of analysis

The sample should be taken at 3 different spots on at least 2 different combs if possible and combined as one pooled sample per colony. The sample size for each sample should be 100 g or as close as possible to this. If a honey super is used, it is recommended to sample from the brood chamber only. The pooled samples per colony/tunnel will be divided into A- and B-samples. The B-samples will be stored until finalisation of the test.

According to the laboratory recommendations, honey can be sampled with a sharp tool such as plastic spoons. For each colony/sample a new tool will be used.

The control sample and the four replicates of the treated samples (from the four treated colonies) should be prepared and analysed separately.

To analyse honey for the relevant residue, a suitable validated analytical method is required. It is necessary to achieve an appropriate limit of quantification as low as possible. A value of 0.05 mg/kg or lower is favoured.

3 Report

A report on residues in honey should include all relevant data in a suitable format. The report for an entire residue study could, for example, be sub-divided into the following sections:

- Summary
- Objectives
- Study setup and study details
- Sample preparation
- Extraction, clean-up, determination, evaluation
- Results and conclusion.

3.1 Summary

This summarises the key results, the evaluation of these results and any anomalies of the study, with reference to the objective.

3.2 Objectives

The objectives section of the report again describes the aims of the study in detail and formulates the questions to be dealt with in the study.

3.3 Study setup and study details

This section of the report summarises the key points documented in the log book. The documentation should include information on

- Site parameters,
- Application details,
- Weather data during the entire trial periods,
- Duration of trials, incl. period prior to feeding,
- Number of replicates,

Reference should be made to the critical points of the trial.

3.4 Sample preparation

This section should be used to describe sampling techniques including nature, number and size of samples taken and, where appropriate, intermediate storage, processing of samples and the storage and dispatch of these.

3.5 Extraction, clean-up, determination, evaluation

This essentially describes the method used to prepare and measure the samples. This section of the report presents the residue levels in honey and, where needed, pollen.

3.6 Results and conclusion

This section of the report discusses and evaluates the reported measurements in the light of the questions outlined in the objectives section. The relevance of results should be discussed in relation to the proposed uses of the PPP, including a critical appraisal of the study and its results. In particular the following points must be addressed:

- A residue at or above the LOQ (a value of 0.05 mg/kg or lower is favoured) in control samples.
- MRL proposal, with reasoning.

4. References

Oomen PA, 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.

Regulation (EC) No. 396/2005 of the European Parliament and of the Council on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC

APPENDIX IV

Field residue trials for MRL setting in honey

1 Objectives

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.

It is necessary to clearly establish:

- that colonies used are well defined, as homogeneous as possible, in good health and not affected by foraging in the treated area and no or only marginal honey flow from other sources within 2-3 km in the surrounding,
- as the bees are flying freely, that they have chiefly foraged plants treated according to critical GAPs (critical considering honey contamination so that it is a realistic indication of the highest bee exposure),
- that honey produced from treated plants is clearly identified,
- that dosing of residues has been achieved on “mature” and marketable honey and in conditions that allow full confidence in the analytical results.

2 Test procedure

2.1 Application of test substance(s)

The test substance should be applied in a realistic worst-case scenario with respect to residues in honey, as described for the design, preparation and realisation of residue trials in plants. The residue trials should be based on the highest authorised or proposed rate of application consistent with Good Agricultural Practice in a melliferous crop in the region(s) concerned.

2.2 Design of trials sites

As the bees are flying freely, the field size must be adapted to conditions of the surroundings to achieve results that are not influenced by these conditions. In the case of an isolated field with no other melliferous crops/production of honey dew around the trial site, a field size of 1 ha may be sufficient but larger fields are recommended as the chance of sufficient honey production increases with field size. As this may not normally be achieved, a field size of 3 ha with no other flowering crops within a 2 to 3 km radius should be sought (minimum 500 m radius in the case of less-attractive flowering crops compared to the treated crop).

The treated crop area in these trials is very large compared to standard supervised crop field trials. It is necessary to ensure that the bees are exposed to the plant protection product according to "realistic worst-case" conditions.

Products containing the tested active substance must not be used as maintenance chemicals, both on treated and untreated plots. In the same way, products likely to cause ill effects on honeybees must be avoided. It must be ensured that active substance for which MRL is to be determined has not been used for veterinary treatment of the bees.
Capped honey needs to be obtained.

2.3 Honeybee colony preparation

Healthy queen-right colonies are used with enough worker honey bees to cover all combs (at least 20 000 honey bees, depending on beehive types and on the season).
Each colony presents brood with all the different stages: eggs, larvae, capped brood as well as natural bee bread and honey stored by bees.
The colony will have at least seven brood frames containing all brood stages and food store frames.
Put the supers up not more than 2 days before application. 2-3 empty but built combs with cells that can immediately be used by bees to store honey should be provided. It is possible to use pre-built frames in plastic.
Before the application, all combs in the super containing fresh nectar can be removed but super should contain 2-3 built but empty combs at application.
Bees should always have access to water.

2.4 Number of trials

To achieve the objectives a minimum of four trial sites is necessary. In each trial site, for MRL determination purposes, two beehives per field should be used in order to collect sufficient number of honeycombs.

Trials from one growing season are sufficient but trials should be conducted in different geographical areas.

2.5 Duration of trials

For direct to crop spray applications the bee hives should be brought onto the field on the day of application of the plant protection product. For other application types application should be timed to ensure bees have foraged when residues are highest in aerial parts of the plant. After application of the plant protection product at the critical GAP the bee hives should be left within the field until the honeycombs are closed, i.e., the honey is mature (honey from the treated crop reached commercial maturity (comb-closure or the water content in the "artificial honey" is about 20%, to be measured with refractometer; normally 7-21 days after application or start of flowering).

2.6 Sampling, method of analysis

Beneath the general requirements concerning sampling and methods of analysis as described elsewhere, the following points should be taken into consideration:

At each site pollen traps should be used to collect pollen in order to analyse for pollen types. 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 sample should be taken at 3 different spots on at least 2 different combs per hive if possible and combined as one pooled sample per colony. According to the laboratory recommendations, honey can be sampled with a sharp tool such as plastic spoons. For each colony/sample a new tool will be used.
In case full honey supers are obtained, the honey samples can also be extracted according to normal bee keeping practice.

Honey can also be extracted by centrifuging de-capped broodless combs. The laboratory sample should contain at least 0.5 kg of honey.

2.7 Health effects on honey bees

The health of the colonies will be assessed prior to introduction to the fields and at the end of the trial when the honey has been collected.

The following parameters will be assessed:
- Strength of the colony (number of frames covered with bees),
- Presence of a healthy queen (i.e., presence of eggs or presence of queen cells),
- Visual assessment – percentage of frames containing pollen, nectar, and brood (eggs, larvae and capped cells). For these assessments, one frame of comb (both sides) will equal 100% and from this the percentages area of brood, pollen and nectar will be estimated. All frames in each colony will be assessed and the mean values for each colony will be calculated.

3 Report

A report on residues in honey should include all relevant data in a suitable format. The report for an entire residue study could, for example, be sub-divided into the following sections:

- Summary
- Objectives
- Field part
- Sample preparation
- Extraction, clean-up, determination, evaluation
- Results and discussion.

3.1 Summary

This summarises the key results, the evaluation of these results and any anomalies of the study, with reference to the objective.

3.2 Objectives

The objectives section of the report again describes the aims of the study in detail and formulates the questions to be dealt with in the study.

3.3 Field part

This section of the report summarises the key points documented in the log book. The documentation should include information on

- Site parameters, including crops growing in the surroundings,
- Application parameters,
- Weather data for the application and sample collection period,
- Duration of trial, incl. period prior to application,
- Number of beehives,
- Health effects.

Reference should be made to the critical points of the animal trial component, and special techniques and events should be described.

3.4 Sample preparation

This section should be used to describe sampling techniques including nature, number and size of samples taken and, where appropriate, intermediate storage, as well as the production of the laboratory or analysis samples and the storage and dispatch of these.

3.5 Extraction, clean-up, determination, evaluation

This essentially describes the method used to prepare and measure the samples. This section of the report presents the residue levels in honey and, where desirable, in pollen and the treated crop.

3.6 Results and discussion

This section of the report discusses and evaluates the reported measurements in the light of the questions outlined in the objectives section. The relevance of results should be discussed in relation to the proposed uses of the plant protection product, including a critical appraisal of the study and its results. In particular the following points must be addressed:

- A residue at or about the LOQ (a value of 0.05 mg/kg or lower is favoured) in control samples
- Adverse effects on health of the honey bees

4 References

BORNEMANN V. Personnel communication, 2003 (from Germany proposal).

Council of the European Communities. Council Regulation (EEC) No 2377/90 of 26 June 1990 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. OJ N° L 10 of 18.8.1990, p. 1.

Council of the European Communities. Council Directive 2001/110/EC of 20 December 2001 relating to honey. OJ N° L 224 of 12.1.2002, p. 47.

European Commission. Proposal for a Regulation of the European Parliament and of the Council on maximum residue levels of pesticides in products of plant and animal origin, COM, 2003, 117 final, Brussels.

APPENDIX V

Tunnel residue trials for MRL setting in honey

1 Objectives
2 Trial design
- 2.1 Application of test substance(s)
- 2.2 Design of trials sites
- 2.3 Number of trials
- 2.4 Honeybee colonies
- 2.5 Duration of field trials
- 2.6 Sampling, method of analysis
- 2.7 Health effects on honeybees

3. Report
- 3.1 Summary
- 3.2 Objectives
- 3.3 Tunnel part
- 3.4 Sample preparation
- 3.5 Extraction, clean-up, determination, evaluation
- 3.6 Results and discussion

1 Objectives

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.

It is necessary to clearly establish:

- that colonies used are well defined, as homogeneous as possible, in good health and not affected by foraging in the treated area,•
- that honey produced from treated plants is clearly identified,
- that dosing of residues has been achieved on "mature" and marketable honey and in conditions that allow full confidence in the analytical results.

2 Trial design

2.1 Application of test substance(s)

The test substance should be applied in a realistic worst-case scenario with respect to residues in honey. By confining them within tunnels, the proposed trial design ensures that bees are allowed to forage only on the treated crop, mimicking commercial situations in which large areas of crop may be grown and treated more or less simultaneously.
The residue trials should be based on the highest authorised or proposed rate of application consistent with Good Agricultural Practice in a melliferous crop in the region(s) concerned.
Application(s) should be made within the tunnels the day after introducing the hives.

2.2 Design of trials sites

The study should be conducted in tunnels placed in crop fields, to maximise exposure of the bee colonies to treated plants. Each trial site should consist of a control plot and one "treated" plot: one tunnel with one bee colony placed in a field treated with the relevant plant protection product and one tunnel with an untreated control.
The trial site must then be large enough to accommodate two tunnels.

The tunnel size should be at least 120 m^2 with one path of approximately 50 cm width in the middle, necessary for the application of the test substance. Smaller tunnel sizes are not recommend as the chance of sufficient honey production decreases with field size.
Bees should always have access to water.

Products containing the tested active substance must not be used as maintenance chemicals, both on treated and untreated plots. In the same way, products likely to cause ill effects on honeybees must be avoided. It must be ensured that active substance for which MRL is to be determined has not been used for veterinary treatment of the bees.
Capped honey needs to be obtained.

2.3 Number of trials

To achieve the objectives a minimum of four trial sites is necessary. Trials sites must be situated at different locations, at a minimum of 10 km apart. Trials from one growing season are sufficient.

2.4 Honeybee colonies

The colonies will be queen-right and contain enough bees to produce the requisite amounts of honey. The colonies can be either made as shook swarms with minimal food stores or normal, queen-right small colonies or of normal small colonies. Optionally it can be considered to remove some brood frames to reduce consumption of the nectar and to confine the queen to one brood frame. The colony will contain three to five empty frames. The colony will be kept in one brood chamber. Optionally, a super may be added in case the bees collect a volume of honey greater than that available in the storage area in the lower body.

For direct to crop spray applications the colonies should be brought in one brood chamber to the test site on the evening before the application, to avoid the collection of untreated nectar and reduce the duration of confinement

and, hence, bee stress. Applications should be timed before noon to ensure a maximum amount of hours of honey collection during the first day. For other application types application should be timed to ensure bees have foraged when residues are highest in aerial parts of the plant. In the evening prior to the application, or in the morning prior to the application, two to three empty combs should be placed in the brood body on places which were blocked with barriers. Although this measure is not in keeping with normal commercial bee-keeping practice, it will reflect the worst case, since all the honey taken afterwards will result from nectar collected from the treated plants.

After application, the bee hives should be left within the tunnels until the honey is ripe, or honey cell-closure (normally 7-14 days after introduction of the colonies in the tent), or the end of flowering, whichever is the earliest.

2.5 Duration of tunnel trials

Bee colonies will remain in the tunnels until honey cell-closure or the end of flowering until sampling is performed. If comb-closure occurs first or the water content in honey is below 20% (measured with refractometer), the residue samples should be collected and the trial ended. If comb-closure has not occurred or the water content in honey is above 20% by the time the crop has finished flowering, it will be necessary to move the colonies to remote locations (away from any crops treated with the active substance) and allow the bees to continue foraging until comb-closure occurs or the honey is mature (<20% water content) and the honey samples can be collected.

2.6 Sampling, method of analysis

Honey will be sampled when it has reached commercial maturity (comb closure or the honey water content is below 20%). Sufficient honeycombs must be collected to provide the required sample weight for analysis. For each sample, 100 g of honey will be taken, or as close as possible to this.

Honey should be removed from the sampled honeycomb by extraction of the de-capped broodless comb by each field phase.
The four replicates of the treated samples should be prepared and analysed separately. The replicates of the control can be prepared and analysed together.

To analyse honey (and the treated crop, if desired) for the relevant residue, a suitable validated analytical method should be chosen. It is therefore desirable to achieve a limit of quantification as low as possible. A value of 0.05 mg/kg per analyte is favoured.

2.7 Health effects on honeybees

The health of the colonies will be assessed prior to introduction to the tunnels and at the end of the trial when the honey has been collected.
The following parameters will be assessed:

- Strength of the colony (number of frames covered with bees),
- Presence of a healthy queen (i.e., presence of eggs or presence of queen cells),
- Visual assessment – percentage of frames containing pollen, nectar, and brood (eggs, larvae and capped cells). For these assessments, one frame of comb (both sides) will equal 100% and from this the percentages area of brood, pollen and nectar will be estimated. All frames in each colony will be assessed and the mean values for each colony will be calculated.

3 Report

A report on residues in honey should include all relevant data in a suitable format. The report for an entire residue study could, for example, be sub-divided into the following sections:

- Summary
- Objectives
- Tunnel part
- Sample preparation
- Extraction, clean-up, determination, evaluation
- Results and discussion.

3.1 Summary

This summarises the key results, the evaluation of these results and any anomalies of the study, with reference to the objective.

3.2 Objectives

The objectives section of the report again describes the aims of the study in detail and formulates the questions to be dealt with in the study.

3.3 Tunnel part

This section of the report summarises the key points documented in the log book. The documentation should include information on

- Site parameters,
- Application parameters,
- Weather data for the application and sample collection period,
- Duration of trial, incl. period prior to application,
- Health effects.

Reference should be made to the critical points of the animal trial component, and special techniques and events should be described.

3.4 Sample preparation

This section should be used to describe sampling techniques including nature, number and size of samples taken and, where appropriate, intermediate storage, as well as the production of the laboratory or analysis samples and the storage and dispatch of these.

3.5 Extraction, clean-up, determination, evaluation

This essentially describes the method used to prepare and measure the samples. This section of the report details the residue levels in honey and, where desirable, in pollen and the treated crop.

3.6 Results and discussion

This section of the report discusses and evaluates the reported measurements in the light of the questions outlined in the objectives section. The relevance of results should be discussed in relation to the proposed uses of the plant protection product, including a critical appraisal of the study and its results. In particular the following points must be addressed:

- A residue at or about the LOQ (a value of 0.05 mg/kg or lower is favoured) in control samples
- Adverse effects on health of the honey bees